Serum homocysteine is related to food intake in adolescents: the Child and Adolescent Trial for Cardiovascular Health[1–3]

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ABSTRACT

Background: An understanding of the relation in adolescents between serum homocysteine and foods rich in vitamin B-6, vitamin B-12, and folate is important because high homocysteine concentrations in childhood and adolescence may be a risk factor for later cardiovascular disease. However, little is known about the relation between food intake and homocysteine in adolescents.

Objective: Five years after national folic acid fortification of enriched grain products, cross-sectional relations between food intake and serum homocysteine concentrations were examined in 2695 adolescents [$\bar{x}$ age: 18.3 (range: 15–20) y] enrolled in the Child and Adolescent Trial for Cardiovascular Health.

Design: A nonfasting blood specimen was analyzed for serum homocysteine, folate, and vitamins B-6 and B-12. Dietary intake was assessed by using a food-frequency questionnaire. Multiple regression analyses were used to evaluate the relation of intakes of whole grains, refined grains, fruit, vegetables, dairy products, red and processed meats, and poultry with serum homocysteine concentrations after adjustment for demographic characteristics, lifestyle factors, and food intake.

Results: Serum homocysteine concentrations were lower with greater intakes of whole grains (P for trend = 0.002), refined grains (P for trend = 0.02), and dairy foods (P for trend $<$0.001); were higher with greater intake of poultry (P for trend = 0.004); and were not related to intakes of fruit, vegetables, or red or processed meat. After additional adjustment for serum B vitamins, the relations of serum homocysteine with most food groups were attenuated.

Conclusion: These observational findings suggest a beneficial effect of whole-grain, refined-grain, and dairy products on serum homocysteine concentrations in an adolescent population. *Am J Clin Nutr* 2006;83:1380–6.

KEY WORDS Homocysteine, serum folate, cardiovascular disease, adolescents, food groups, whole grain

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death in the United States (1). Although the primary cause of this disease is well understood, additional risk factors such as hyperhomocysteinemia are emerging as novel contributors. Several studies suggested that the concentration of plasma homocysteine may be an independent and modifiable risk factor for CVD in adults (2–7). Results from a meta-analysis of 20 prospective studies indicated that, for every 5-μmol/L increase in homocysteine, the odds ratio for risk of ischemic heart disease was 1.32 (95% CI: 1.19, 1.45), and the odds ratio for risk of stroke was 1.59 (95% CI: 1.29, 1.96) (8).

Early stages of atherosclerosis and other physiologic CVD risk factors are present in childhood and adolescence (9–12) and may predict CVD risk in adults (10–14). The distribution of homocysteine is significantly lower in children than in adults (15), but some children may be at risk of high homocysteine concentrations. Several studies showed that elevated homocysteine concentrations are associated with elevated systolic blood pressure (16, 17) and intimal-medial thickness of the coronary artery (18) in children and adolescents.

Folate, vitamin B-6, and vitamin B-12 are essential to the metabolic conversion of homocysteine to either cystathionine or methionine (19). Not surprisingly, epidemiologic evidence from both experimental and observational studies in adult populations showed inverse relations of homocysteine concentrations with nutrient intake and serum concentrations of vitamin B-6, vitamin B-12, and folate (3, 20–25). Multiple feeding trials that evaluated single food groups or fortified food items in relation to homocysteine concentrations in adults found reductions in homocysteine that resulted from greater consumption of fruit and vegetables (26–29), fortified cereals (30, 31), cereals manufactured before national folic acid fortification (29), whole grains (32), and soy products (33–35). However, the relation of major food groups that influence homocysteine concentrations in adolescents has not been explored. Therefore, we examined the relation of food intake to serum concentrations of homocysteine in previously studied adolescents (15, 36) 5 y after national folic acid fortification of enriched-grain products.

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SUBJECTS AND METHODS

Study population

The Child and Adolescent Trial for Cardiovascular Health (CATCH study) was a multicenter intervention trial, conducted from 1991 to 1994, to evaluate the effectiveness of an elementary school–based cardiovascular health promotion program in 56 intervention and 40 control schools in California, Louisiana, Minnesota, and Texas (37). More than 5100 ethnically diverse students in grades 3–5 enrolled in the study at baseline, and follow-up surveys were conducted in 1996–1997 (15) and 2000–2001 (36).

These analyses, based on the 2001 follow-up survey data, include 2695 subjects aged 15–20 y, a total that is 53% of the original CATCH cohort. Excluded from this analysis were 60 students without a serum homocysteine value, 41 students with outlying energy intakes, 178 students with other missing data, 229 who did not return consent forms, 177 who dropped out of the study, and 1968 who either moved out of the survey area or did not participate in the 2000–2001 follow-up survey.

Written assent and written informed consent was obtained from all participants and their parents, respectively. The CATCH study was approved by the institutional review boards at all study sites.

Measurements

All data were collected by trained, certified CATCH study staff at school sites. The Health Behavior Survey was used to collect information from students about behaviors, attitudes, knowledge, and environmental influences with respect to nutrition, physical activity, smoking, alcohol, and health.

Height without shoes was measured by using a portable stadiometer (Perspective Enterprises, Portage, MI), and weight was measured by using the SECA Integra 815 portable scale (SECA, Rumilly, France). Body mass index (BMI) was calculated as weight (in kg) divided by height (in m^2).

Venipuncture

Nonfasting blood samples were obtained via venipuncture while the child was seated. The venipuncture procedure and processing have been reported (15). Total homocysteine was measured by using the fluorimetric method, except that 20% methanol in buffer B was used in the HPLC procedure (38). Serum vitamin B-12 and folate were measured by using a solid-phase, no-boil radioimmunoassay in a commercial kit (Diagnostic Productions Corp, Los Angeles, CA; 39, 40). Vitamin B-6 was analyzed by using a radioassay kit (ALPCO, Windham, NH) that measures the conversion of titrated tyrosine to tyramine by the vitamin B-6–dependent enzyme tyrosine decarboxylase (41).

Diet assessment

Diet was assessed through self-administration of the 149-item Youth/Adolescent Questionnaire. This instrument has been validated in youth and adolescent populations through the administration of 2 food-frequency questionnaires and three 24-h recalls over a period of 1 y in a sample of 261 children (42).

Foods were aggregated into 10 groups: whole grains, refined grains, fruit, vegetables, dairy, red and processed meat, poultry, fish, eggs, and nuts. The whole- and refined-grain groups were classified according to previously developed procedures (43). Whole-grain ready-to-eat breakfast cereals contained ≥25% whole grain or bran by weight, as ascertained from the package label or from records shared by cereal manufacturing companies. Other food items classified as whole-grain included cooked oatmeal, dark bread, brown rice, other grains (eg, bulgur, kasha, and couscous), bran, wheat germ, and popcorn. Refined-grain food items included ready-to-eat breakfast cereals with < 25% whole grain or bran, white bread, bagels, rolls, muffins, pasta, white rice, pancakes and waffles, doughnuts, cakes, cookies, and pie. The fruit group included 10 types of fruit (juices were not included because of their high sugar content); the vegetable group included 23 vegetables and legumes; the dairy group included milk, cheese, yogurt, and ice cream products; the meat group included red and processed meat; the poultry group included chicken or turkey as a main dish and chicken nuggets; the egg group included eggs and eggs that were used to make French toast; the nut group included peanuts and peanut butter; and the fish group included 3 fish-containing items.

Statistical analysis

Analyses were conducted with SAS statistical software (version 8.2; SAS Institute, Cary, NC). The sampling design was considered in all statistical models, and the school was treated as a random effect within the site. Because of their highly skewed distributions, serum concentrations of homocysteine, folate, vitamin B-6, and vitamin B-12 were log transformed, and values are reported as geometric means (95% CIs). Mean (±SD) and frequencies of demographics, nutrient intakes, food intakes, and other clinical characteristics were calculated, with adjustments for age, race, energy intake, and site (CA, LA, MN, or TX). We used *t* tests to evaluate sex differences for continuous variables. Spearman partial correlations adjusted for age, sex, race, site, and energy intake were calculated to describe the relation between serum concentrations and nutrient intakes. The correlations for folate, vitamin B-6, and vitamin B-12 were 0.42 ($P < 0.001$), 0.39 ($P < 0.001$), and 0.13 ($P < 0.001$), respectively.

The whole-grain, refined-grain, fruit, vegetable, dairy, poultry, and meat groups were categorized into quintiles; the lowest quintile of intake for each food group formed the reference category for that food group. Eggs, nuts, and fish were categorized into tertiles, because of the low level of consumption. Linear regression was used to evaluate the relation between serum homocysteine as the dependent variable to the quintiles of food intake as the independent variables. Other models were developed to evaluate the relations of the serum vitamin B variables to food intake.

Three models were developed to evaluate the relation between serum variables and food intake. Model 1, which ascertained whether these relations were independent of demographic characteristics, was adjusted for age, sex, race, site, and energy intake. Model 2, which further adjusted for the lifestyle factors of smoking (yes if ≥ 1 cigarette/wk or no), vitamin supplement use (yes or no), BMI, and intake of the following food groups: whole grains, refined grains, fruit, vegetables, dairy, red or processed meat, and poultry. To ascertain whether homocysteine was related to food intake independent of biologic mechanisms, further adjustment in model 3 included serum folate and vitamins B-6 and B-12. Finally, interaction terms were included in the models to ascertain whether differences—including differences in vitamin supplement use and sex—existed between subgroups. However, because interaction terms were not significant, the models were adjusted for these covariates.

TABLE 1
Demographic characteristics and dietary intake of adolescent boys and girls in the Child and Adolescent Trial for Cardiovascular Health

	Boys (n = 1318)	Girls (n = 1377)	p^1
Demographic characteristics[2]			
Age (y)	18.3 ± 0.5[3]	18.2 ± 0.5	< 0.001
Race [n (%)]			
White	1014 ± 77	969 ± 70	0.001
Black	133 ± 10	190 ± 14	
Hispanic	120 ± 9	161 ± 12	
Other	49 ± 4	57 ± 4	
Multivitamin use [n (%)]	305 ± 23	416 ± 31	< 0.001
Current smoker [n (%)]	463 ± 33	402 ± 31	0.22
Physical measures[2,4]			
Blood pressure (mm Hg)			
Systolic	120.1 ± 0.3	111.9 ± 0.3	< 0.001
Diastolic	56.6 ± 0.2	57.6 ± 0.2	0.001
BMI (kg/m^2)	24.8 ± 0.1	24.2 ± 0.1	0.007
Daily nutrient intake[2,4]			
Energy (kcal)	2131 ± 21	1786 ± 21	< 0.001
Total fat (g)	70.0 ± 0.3	67.2 ± 0.3	< 0.001
Protein (g)	71.7 ± 0.4	70.1 ± 0.3	0.002
Carbohydrates (g)	263.4 ± 0.8	272.1 ± 0.8	< 0.001
Folate (μg)	269 ± 4	290 ± 3	< 0.001
Vitamin B-6 (mg)	1.58 ± 0.01	1.67 ± 0.01	< 0.001
Vitamin B-12 (μg)	6.7 ± 0.1	6.6 ± 0.1	0.49
Fiber (g)	13.6 ± 0.1	15.3 ± 0.1	< 0.001
Daily food intake (servings)[2,4]			
Whole grain	0.69 ± 0.02	0.75 ± 0.02	0.03
Refined grain	4.27 ± 0.04	4.35 ± 0.03	0.13
Fruit[5]	0.70 ± 0.02	0.96 ± 0.02	< 0.001
Vegetables	1.77 ± 0.03	2.16 ± 0.03	< 0.001
Dairy	2.50 ± 0.03	2.32 ± 0.03	0.003
Meat	0.80 ± 0.01	0.63 ± 0.01	< 0.001
Poultry	0.22 ± 0.01	0.27 ± 0.01	< 0.001

[1] Calculated by using linear regression.
[2] Adjusted for age, race, energy intake, and site (ie, CA, LA, MN, or TX).
[3] $\bar{x}$ ± SD (all such values, unless indicated otherwise).
[4] $\bar{x}$ ± SE (all such values).
[5] Daily servings of fruit do not include fruit juice.

RESULTS

The cohort of 2695 participants had a mean age of 18 y, and 51% were female. More than 70% of the participants were white, 12% were black, 10% were Hispanic, and 4% were classified as "other." Demographic characteristics and mean dietary intakes of the boys and girls are shown in **Table 1**. The clinical characteristics of participants by sex are shown in **Table 2**. Compared with the girls, the boys had significantly ($P < 0.001$ for all) higher serum concentrations of homocysteine (6.4 and 5.1 μmol/L, respectively), vitamin B-6 (44.2 and 36.2 nmol/L,

TABLE 2
Clinical characteristics of adolescent boys and girls in the Child and Adolescent Trial for Cardiovascular Health[1]

Clinical characteristics	Boys (n = 1318)	Girls (n = 1377)	P^2
Total cholesterol (mg/dL)[3]	158 (156, 160)	168 (167, 170)	< 0.001
Serum homocysteine (μmol/L)[4]	6.4 (6.3, 6.6)	5.1 (5.0, 5.3)	< 0.001
Serum B vitamins[4]			
Folate (ng/mL)	18.9 (18.5, 19.4)	20.4 (19.9, 20.8)	< 0.001
Vitamin B-6 (nmol/L)	44.2 (42.4–46.2)	36.2 (34.8–37.8)	< 0.001
Vitamin B-12 (pg/mL)	460 (449–471)	428 (418–438)	< 0.001

[1] Values were adjusted for age, race, site (ie, CA, LA, MN, or TX), and energy intake.
[2] Calculated by using linear regression.
[3] $\bar{x}$; 95% CI in parentheses.
[4] Geometric $\bar{x}$; 95% CI in parentheses.

TABLE 3
Serum homocysteine concentrations across quintiles (Q) of intake of each food group in adolescent boys and girls in the Child and Adolescent Trial for Cardiovascular Health[1]

	Q1	Q2	Q3	Q4	Q5	*P* for trend[2]
			servings/d			
Whole grains	0.00–0.20[3]	0.20–0.40	0.40–0.64	0.64–1.07	1.07–6.14	
Model 1	5.91 (5.68, 6.14)[4]	5.88 (5.66, 6.10)	5.68 (5.46, 5.90)	5.63 (5.42, 5.84)	5.44 (5.23, 5.66)	0.006
Model 2	5.93 (5.70, 6.17)	5.89 (5.67, 6.12)	5.68 (5.46, 5.91)	5.58 (5.38, 5.80)	5.42 (5.20, 5.65)	0.002
Model 3	5.78 (5.57, 6.00)	5.81 (5.60, 6.02)	5.69 (5.48, 5.91)	5.69 (5.49, 5.90)	5.67 (5.45, 5.90)	0.35
Refined grains	0.07–2.49	2.49–3.39	3.39–4.28	4.28–5.82	5.82–15.81	
Model 1	5.90 (5.63, 6.17)	5.81 (5.58, 6.05)	5.59 (5.38, 5.80)	5.69 (5.47, 5.91)	5.56 (5.29, 5.85)	0.13
Model 2	5.99 (5.71, 6.28)	5.82 (5.59, 6.06)	5.61 (5.40, 5.83)	5.67 (5.45, 5.89)	5.43 (5.15, 5.72)	0.02
Model 3	5.80 (5.55, 6.07)	5.73 (5.52, 5.96)	5.65 (5.45, 5.86)	5.81 (5.60, 6.03)	5.65 (5.37, 5.94)	0.65
Fruit[5]	0.00–0.28	0.28–0.43	0.43–0.71	0.71–1.29	1.29–5.29	
Model 1	5.73 (5.51, 5.96)	5.64 (5.44, 5.85)	5.88 (5.67, 6.11)	5.67 (5.46, 5.88)	5.62 (5.40, 5.84)	0.56
Model 2	5.74 (5.50, 5.98)	5.61 (5.40, 5.82)	5.90 (5.68, 6.13)	5.67 (5.46, 5.90)	5.61 (5.38, 5.85)	0.61
Model 3	5.71 (5.49, 5.94)	5.62 (5.42, 5.83)	5.88 (5.67, 6.10)	5.73 (5.53, 5.95)	5.71 (5.49, 5.94)	0.76
Vegetables	0.00–1.00	1.00–1.42	1.42–1.96	1.96–2.80	2.80–9.43	
Model 1	5.62 (5.40, 5.85)	5.65 (5.44, 5.86)	5.66 (5.46, 5.88)	5.80 (5.58, 6.02)	5.84 (5.61, 6.08)	0.13
Model 2	5.66 (5.43, 5.91)	5.65 (5.44, 5.88)	5.65 (5.44, 5.87)	5.77 (5.56, 6.00)	5.76 (5.52, 6.01)	0.44
Model 3	5.67 (5.45, 5.90)	5.68 (5.47, 5.89)	5.70 (5.49, 5.91)	5.83 (5.62, 6.05)	5.76 (5.53, 6.00)	0.39
Dairy	0.00–1.11	1.11–1.68	1.68–2.43	2.43–3.66	3.66–9.72	
Model 1	6.19 (5.94, 6.46)	5.86 (5.64, 6.09)	5.70 (5.49, 5.91)	5.55 (5.35, 5.77)	5.29 (5.07, 5.52)	< 0.001
Model 2	6.21 (5.95, 6.48)	5.86 (5.63, 6.09)	5.70 (5.48, 5.93)	5.56 (5.34, 5.77)	5.22 (4.99, 5.46)	< 0.001
Model 3	5.99 (5.75, 6.25)	5.82 (5.61, 6.05)	5.71 (5.50, 5.92)	5.69 (5.48, 5.90)	5.44 (5.21, 5.68)	0.004
Meat	0.00–0.32	0.32–0.52	0.52–0.71	0.71–1.04	1.04–3.22	
Model 1	5.78 (5.55, 6.03)	5.73 (5.51, 5.96)	5.55 (5.35, 5.77)	5.63 (5.42, 5.85)	5.85 (5.60, 6.12)	0.94
Model 2	5.93 (5.68, 6.19)	5.76 (5.53, 5.99)	5.57 (5.36, 5.79)	5.56 (5.34, 5.78)	5.69 (5.43, 5.96)	0.15
Model 3	6.02 (5.77, 6.27)	5.78 (5.56, 6.00)	5.64 (5.44, 5.85)	5.56 (5.36, 5.77)	5.66 (5.41, 5.91)	0.04
Poultry	0.00–0.10	0.10–0.14	0.14–0.21	0.22–0.43	0.43–1.00	
Model 1	5.57 (5.37, 5.78)	5.49 (5.29, 5.69)	5.82 (5.62, 6.03)	5.70 (5.46, 5.95)	5.98 (5.76, 6.21)	0.003
Model 2	5.54 (5.33, 5.76)	5.44 (5.24, 5.65)	5.80 (5.60, 6.02)	5.73 (5.48, 5.98)	6.02 (5.79, 6.26)	0.004
Model 3	5.55 (5.35, 5.75)	5.45 (5.25, 5.64)	5.86 (5.66, 6.07)	5.76 (5.52, 6.01)	6.06 (5.84, 6.29)	< 0.001

[1] Model 1 is adjusted for age, sex, race, energy intake, and site; model 2 is additionally adjusted for smoking, vitamin intake, BMI, and intakes of the other food groups; and model 3 is additionally adjusted for serum folate and vitamins B-6 and B-12.
[2] Test for trend for each statistical model was calculated by using linear regression.
[3] Upper and lower limits of daily intake in the quintile (all such values).
[4] Geometric $\bar{x}$; 95% CI in parentheses (all such values).
[5] Daily servings of fruit do not include fruit juice.

respectively), and vitamin B-12 (460 and 428 pg/mL, respectively) and significantly ($P < 0.001$) lower serum concentrations of folate (18.9 and 20.4 ng/mL, respectively).

Average homocysteine concentrations are shown across quintiles of dietary intake of whole grains, refined grains, fruit, vegetables, dairy, red and processed meat, and poultry (**Table 3**). After adjustment for demographic characteristics (model 1) and further adjustment for lifestyle factors, BMI, and other food groups (model 2), homocysteine concentrations were significantly lower across greater quintiles of whole-grain intake. In model 2, homocysteine concentrations ranged from 5.9 μmol/L in quintile 1 to 5.4 μmol/L in quintile 5 (P for trend = 0.002). However, in model 3, the relation was attenuated after further adjustment for serum folate and B vitamins (P for trend = 0.35). Refined grains had a weak relation to serum homocysteine in the base model (P for trend = 0.13), which was strengthened after adjustment for lifestyle factors, BMI, and food groups (P for trend = 0.02). Additional adjustment for serum folate and B vitamins in model 3 attenuated the relation (P for trend = 0.65).

Intakes of fruit and vegetables were not significantly related to homocysteine. Dairy intake was significantly and inversely related to homocysteine in all of the models. Serum homocysteine concentrations were not significantly associated with red and processed meat intakes, but they were positively associated with poultry intake ($P = 0.004$). Intakes of eggs, nuts, and fish were not significantly related to homocysteine in any of the models (data not shown).

Serum folate concentrations were positively associated with dietary intakes of whole grains (P for trend < 0.001), refined grains (P for trend < 0.001), fruit (P for trend = 0.008), and dairy (P for trend < 0.001) and inversely associated with dietary intakes of red and processed meat ($P = 0.008$), but they were not associated with the intake of vegetables or poultry (data not shown). Serum vitamin B-6 was positively associated with intakes of whole grains (P for trend < 0.001), fruit (P for trend = 0.04), vegetables (P for trend = 0.002), dairy (P for trend < 0.001), and poultry (P for trend = < 0.001) but not with those of refined grains or red and processed meat (data not shown). In

comparison, serum vitamin B-12 was positively associated with intakes of whole grain (P for trend = 0.001), dairy (P for trend < 0.001), and red and processed meat (P for trend = 0.001) but not with intakes of refined grains, fruit, vegetables, or poultry (data not shown).

DISCUSSION

Few, if any, observational studies of adolescents have examined the relation between food intakes and homocysteine concentrations. In this cohort of 2695 adolescents, boys had significantly greater mean homocysteine concentrations than did girls—6.4 and 5.1 μmol/L, respectively. After control for demographic and lifestyle factors and other food group intakes, there was an inverse dose-response relation between homocysteine and intakes of whole-grain foods, refined-grain foods, and dairy products. However, poultry intake was positively associated, and intakes of fruit, vegetables, red and processed meat, eggs, nuts, and fish were not associated with serum homocysteine.

Consistent with our study results, clinical studies conducted in adults also show an inverse relation between whole-grain intake and homocysteine concentrations. Several food-based feeding trials have shown reductions in homocysteine that resulted from greater consumption of fortified cereals (30, 31), cereals manufactured before national folic acid fortification (29), whole grains (32), and a combination diet rich in fruit, vegetables, and low-fat dairy products but low in saturated fat (44). Another study found elevated homocysteine concentrations after the cessation of habitual consumption of breakfast cereals (30).

The inverse relation between homocysteine and whole-grain intake and the positive relation between serum folate and whole-grain intake found in this study are consistent with the biological mechanisms by which homocysteine is created and with the results from previously conducted food-based feeding trials (29–31). In our analyses, the relation between whole grain and homocysteine was attenuated after adjustment for serum folate, which suggested that folate drives the relation between whole grain and homocysteine. Whole grains are inherently rich sources of folate (25), which acts in the conversion of homocysteine to methionine (19) and has been found to lower homocysteine in numerous nutrient-based feeding trials (32).

Since folic acid fortification of enriched-grain products was mandated by the US government in 1998, observational studies have shown higher concentrations of serum folate in adults (45, 46) and children (37). Adults enrolled in the Framingham Offspring Study had mean serum folate concentrations after the fortification of grain products (1997-1998) that were significantly higher than those measured before folic acid fortification (1995-1996); the prevalence of adults with a high serum homocysteine concentration was 50% lower in the later than in the earlier group (47).

Serum homocysteine concentrations measured in CATCH study participants before and after fortification showed that extra dietary folate increased serum folate (from 37.8 to 43.5 nmol/L) in this cohort of adolescents and may offset an age-related increase in homocysteine (37). Our finding that refined-grain consumption was negatively associated with homocysteine concentrations and positively associated with serum folate concentrations is also consistent with our a priori hypotheses. Furthermore, the relation between refined grains and homocysteine was attenuated after adjustment for folate and vitamin B-12 concentrations, which supports the notion that folic acid fortification has a beneficial effect on homocysteine concentrations (47).

Inverse associations between homocysteine and fruit and vegetable intakes have been observed in numerous epidemiologic studies (26–29, 44). However, no association was observed between homocysteine and fruit or vegetable intake in the current study, even though fruit and vegetables are good sources of folate. The range of fruit and vegetable intakes in the adolescents enrolled in the current study may have been too limited for observation of a significant association, even though fruit and vegetable intakes were positively associated with serum folate concentrations.

Dairy products are a rich source of vitamin B-12, which is necessary for the metabolic conversion of homocysteine to methionine (19). In epidemiologic studies, dairy products have been found to have a modest effect on homocysteine (48), as has vitamin B-12, especially when considered together with folate (4, 20, 21, 49). There is evidence that a low serum vitamin B-12 concentration may prevent an optimal response to folate (50, 51).

Poultry intake has not previously been associated with homocysteine concentrations. However, most studies did not examine poultry independent of other meats. Poultry contains relatively high concentrations of methionine, which is the sole dietary precursor of homocysteine (52). Red and processed meats were not related to the homocysteine concentration in the current study, a finding that is consistent with previous research (48, 53).

The major strength of the current study is the large number of adolescents who continue to participate in the CATCH study. One limitation of the current study is that the analysis included only 53% of the original CATCH study cohort. Nevertheless, although a direct comparison has not been conducted, there is little reason to believe that the relation between food intake and serum homocysteine concentrations would differ significantly between those who participated in the current study and those who did not. The dietary intake was assessed by using a food-frequency questionnaire, an instrument that does not capture information about fortified foods (42). We do not know how much folate was in the form of folic acid rather than as natural folates. Consequently, we were unable to adjust for differences in bioavailability. After ascertaining that there was no modifying effect of supplement use on the relation between food intake and homocysteine concentrations, we adjusted for vitamin supplement use in the regression models. However, we do know that dietary folate affected homocysteine concentrations, because relations between diet and homocysteine remained significant after adjustment for multivitamin intake. In addition, although the food-frequency questionnaire was not initially designed to differentiate whole-grain from refined-grain foods, any misclassification of foods would have resulted in an attenuated association. For example, hot cereals were classified as a refined-grain food; therefore, any association would be underestimated, because some whole-grain hot cereals (other than oatmeal, a whole-grain cereal that was queried about separately) were actually included in the food group labeled "refined grains." It is also possible that dietary intakes may be confounded, because eating behaviors tend to cluster. For example, other studies report that greater whole-grain consumption is related to healthier eating behaviors, including lower red meat intake and greater fruit and vegetable intakes (43). To control for this phenomenon, we adjusted our statistical models for the other food groups, as in model

2; however, it is possible that residual confounding remains. Our findings are consistent with the recommendations in the 2005 edition of *Dietary Guidelines for Americans* (54) to eat a variety of foods, including grains, fruit, vegetables, and dairy products.

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PLL, LMS, and SKO designed the study. PLL and LMS conducted the analysis and wrote the manuscript. PLL, LMS, HAF, DHH, RVL, LAL, LSW, MZ and SKO made substantial conceptual contributions and revisions. None of the authors had a personal or financial conflict of interest.

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