Total homocysteine and its predictors in Dutch children[1–3]

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ABSTRACT

Background: Vitamin status, methylenentetrahydrofolate reductase (*MTHFR*) genotype, age, sex, and lifestyle factors are all predictors of total homocysteine (tHcy) concentrations in adults. Limited data are available about the influence of these factors on tHcy in children.

Objective: The objective was to describe tHcy and its predictors in Dutch children.

Design: A sample of 234 white children aged 0–19 y was analyzed cross-sectionally.

Results: The geometric mean tHcy concentrations were 5.1 (95% CI: 4.6, 5.6), 4.6 (4.2, 5.1), 6.2 (5.6, 6.9), 7.3 (6.7, 8.0), and 8.7 (7.9, 9.6) μmol/L in the 0–1, 2–5, 6–10, 11–14, and 15–19 y groups, respectively. Plasma folate and vitamin B-12 concentrations decreased markedly with age. The inverse association between tHcy and plasma folate seen at all ages was stronger than that between tHcy and plasma vitamin B-12. A negative association of plasma folate with tHcy was confined to folate concentrations $<$20 nmol/L. Homozygosity for the *MTHFR* 677C$\rightarrow$T polymorphism was identified in 8.2% of the children. The homocysteine concentration did not differ significantly between the *MTHFR* genotypes.

Conclusions: This study provided age-specific data regarding tHcy concentrations and their predictors in the whole range of childhood. The tHcy concentration increased as a function of age in both sexes. Plasma folate was a concentration-dependent predictor of tHcy. The *MTHFR* 677C$\rightarrow$T polymorphism played a minor role in determining tHcy concentrations in children. *Am J Clin Nutr* 2005;81:1110–6.

KEY WORDS Children, creatinine, folate, total homocysteine, methylenetetrahydrofolate reductase, MTHFR, vitamin B-12

INTRODUCTION

Homocysteine is a sulfur-containing amino acid formed from methionine during *S*-adenosylmethionine-dependent methylation reactions. Further metabolism of this amino acid is dependent on several B vitamins. Vitamin B-6 is the cofactor of cystathionine β-synthase, the enzyme that irreversibly converts homocysteine to cystathionine. Folate in the 5-methyltetrahydrofolate form donates its methyl group to homocysteine by methionine synthase and requires vitamin B-12 as a cofactor. The enzyme methylenetetrahydrofolate reductase (MTHFR) reduces 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. The 677C$\rightarrow$T polymorphism in the *MTHFR* gene decreases its enzyme activity (1).

Hyperhomocysteinemia, defined as a moderately elevated total homocysteine (tHcy) concentration, is an established independent risk factor for arterial vascular disease and venous thrombosis in adults (2–4). Data on this relation in childhood are rare, but Cardo et al (5, 6) and our group (7, 8) found that hyperhomocysteinemia is a risk factor for ischemic stroke in children. Koch et al (9) linked elevated tHcy to an increased risk of venous thrombosis in children. tHcy and its predictors have been studied more extensively in adult populations than in children. The tHcy concentration is influenced by several determinants, such as age; sex; plasma folate, vitamin B-6, vitamin B-12, and creatinine concentrations; and the use of hormones, vitamin supplementation, and anti-folate medications (10). The MTHFR 677C$\rightarrow$T polymorphism is the most prevalent genetic cause of hyperhomocysteinemia, particularly under conditions of impaired folate status (11). tHcy is elevated in pathologic conditions such as renal failure, thyroid dysfunction, and malignancy (12).

In the past decade, information on plasma tHcy in children has begun to emerge (13–36). Although age and sex were taken into account, those studies tended to target specific subgroups such as neonates (13–17), groups of smaller age ranges (18–25), or older children (26–31). Four studies included subjects whose age ranged from 0 to 19 y, but one of those studies measured only tHcy concentrations (32). Another study investigated the effect of *MTHFR* polymorphism on tHcy, but data on vitamin B status were unavailable (33). In 2 other studies, the *MTHFR* genotyping was missing (34, 35). In most studies, a negative correlation of tHcy with plasma folate and with vitamin B-12 concentrations was found (13–15, 19, 20, 22–27, 29, 30). The correlation between tHcy and plasma folate was significantly stronger than that between tHcy and vitamin B-12; the correlation between tHcy and plasma folate also varied among the age groups. Data on the effect of *MTHFR* genotype on tHcy and the interference between vitamin status and *MTHFR* genotype were limited to one study of 127 children (36). Therefore, we designed a study to investigate

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tHcy concentrations and their possible predictors in Dutch children over the whole range of childhood.

SUBJECTS AND METHODS

Subjects and data collection

Over a 3-mo period during 1997, 234 white children were recruited to participate in this study. All participants were aged 0–19 y. We included apparently healthy volunteers from secondary schools (aged 11- 19 y) in the Nijmegen area. Children aged <11 y were recruited in the Pediatric Clinic of the University Hospital in Nijmegen. When blood was drawn for diagnostic or follow-up investigations, the children or their parents (or both) were asked to donate some blood in the same venipuncture session for use in this study. The exclusion criteria were overt liver, thyroid, and renal dysfunction; hormonal therapy; anti-folate medication; neoplastic disease; closure defects such as cleft lips and spina bifida; and occlusive arterial and venous disease. Additional information about medical history, use of medication, smoking behavior, and puberty features (in girls, the occurrence of menarche; in boys, the growth of beard hair) was obtained from medical records or was collected by written questionnaire.

Informed consent (sometimes written, sometimes oral) was obtained from all the children's parents and from children who were old enough to provide it. The study protocol was approved by the local medical ethics committee.

Blood sampling and biochemical determination

Blood samples for total tHcy measurement were drawn by venipuncture into 3-mL evacuated tubes containing EDTA. In neonates, a capillary blood sample was obtained in 1-mL microtainers containing EDTA. The EDTA sample was immediately placed on ice and centrifuged within 4 h at 2000 × *g* for 10 min. The plasma was separated and stored at −20 °C until analysis. The remaining cells were stored at −20 °C and used for DNA isolation. If possible, a venous blood sample was taken into 5-mL heparin-containing evacuated tubes for plasma folate, vitamin B-12, and creatinine measurements. These plasma samples were also stored at −20 °C. The blood sample for tHcy measurement, which requires a smaller volume, had priority in the youngest children. From the 234 participants, 189 heparinized blood samples were obtained for plasma folate and vitamin B-12 measurement. For plasma creatinine measurement, 178 samples were obtained. For 220 samples, DNA isolation and genotyping were successful.

Plasma tHcy concentrations were measured by using an automated HPLC method with reverse-phase separation and fluorescence detection (Gilson 232–401 sample processor; Gilson, Middleton, WI; Spectra Physics 8800 solvent delivery system and Spectra Physics LC 304 fluorometer; Spectra Physics, San Jose, CA), as described by Fiskerstrand et al, with some modifications (37–39). Plasma folate and vitamin B-12 concentrations were measured by using the Dualcount Solid Phase Boil Radioassay (Diagnostic Products, Los Angeles, CA). The investigated mutation in the *MTHFR* gene is a C-to-T substitution at base pair 677 that alters an alanine to a valine residue. This mutation creates a *Hin*fI site, designated 677T, allowing for restriction site analysis. The prevalence of the C677T mutation was investigated by polymerase chain reaction in genomic DNA extracted from blood leukocytes, which was followed by restriction enzyme digestion with *Hin*fI and detection with the use of agarose gel electrophoresis (40).

Statistical analysis

We performed statistical analysis with SPSS software (version 11.5; SPSS Inc, Chicago, IL). The distributions of the plasma concentrations of tHcy, folate, and vitamin B-12 appeared to be skewed toward higher values. Logarithmic transformations were applied to normalize these distributions. Inverse transformations were performed to provide geometric means and 95% CIs. Because of the significant age dependency of tHcy, plasma folate, vitamin B-12, and creatinine concentrations, we tabulated these variables in 5 different age groups (ie, 0–1, 2–5, 6–10, 11–14, and 15–19 y); there was an equal number of subjects in each group for tHcy measurement. Males and females were tabulated separately, although the interaction between age and sex was not significant. The difference in tHcy concentration between males and females was expressed in a ratio of males to females, with 95% CIs, calculated in a linear regression model. Correlations were calculated and expressed in Spearman's ρ coefficients.

A multiple linear regression analysis was performed to evaluate the association between various predictors and tHcy concentration. We investigated the possible interaction between the plasma folate, vitamin B-12, and creatinine concentrations and age by including an interaction term between age and the variables in a multiple linear regression model. In this model, tHcy was the dependent variable, and age, plasma folate, vitamin B-12, and creatinine concentrations were the independent variables. The β coefficients express the changes in log-transformed plasma tHcy (μmol/L) that are associated with a 1-unit change in both log-transformed plasma folate (nmol/L) and plasma vitamin B-12 (pmol/L). Because of this logarithmic transformation of both the x variable and the y variable, the interpretation of these coefficients is as follows: a 1% change in the x variable corresponds to a β% change in the y variable. To test whether the relation between plasma folate and tHcy was significantly modified by age—ie, that the differences between the β coefficients across the age groups were significant—we added the folate × age group interaction term to the regression model.

Seven children in the youngest group (aged 0–1 y) were tested for plasma folate, vitamin B-12, and creatinine concentrations. No firm conclusions could be drawn from this small subset of samples, and consequently those 7 subjects were not included in this analysis. The association of tHcy with plasma folate and vitamin B-12 was also shown graphically for evaluation of the slope direction as a function of concentration range. To test whether the slope directions were significantly different below and above the plasma folate and vitamin B-12 concentration cutoffs, we added to the linear regression model a dichotomic variable for high or low folate and high or low vitamin B-12 as an interaction term.

Age × genotype interaction was investigated in a linear regression model by adding an interaction term age × genotype describing the relation between *MTHFR* 677C→T polymorphism and tHcy concentration. The geometric mean concentration of tHcy was calculated by *MTHFR* genotypes (*CC*, *CT*, and *TT*).

RESULTS

Characteristics of the subjects

A total of 234 white children participated in this study—115 males and 119 females. The mean age of the study group was 8.4 y (range: 0–19 y). Geometric means (and 95% CIs) for tHcy,

TABLE 1
Plasma total homocysteine (tHcy), folate, vitamin B-12, and creatinine concentrations according to age in 0–19-y-old children[1]

	0–1 y	2–5 y	6–10 y	11–14 y	15–19 y	r^2
tHcy (μmol/L)[3]	5.1 (4.6, 5.6)	4.6 (4.2, 5.1)	6.2 (5.6, 6.9)	7.3 (6.7, 8.0)	8.7 (7.9, 9.6)	0.6
Males	5.1 (4.4, 5.8)	5.0 (4.5, 5.7)	6.1 (5.4, 7.0)	7.1 (6.1, 8.3)	9.3 (8.1, 10.8)	
Females	5.1 (4.5, 5.8)	4.1 (3.5, 4.8)	6.3 (5.4, 7.3)	7.4 (6.6, 8.3)	8.2 (7.3, 9.4)	
Males: females[4]	0.99 (0.82, 1.19)	1.22 (1.01, 1.49)	0.97 (0.80, 1.19)	0.96 (0.79, 1.16)	1.13 (0.93, 1.37)	
Folate (nmol/L)[5]	79 (60, 104)	24 (22, 27)	18 (16, 20)	16 (15, 18)	16 (14, 18)	−0.4
Vitamin B-12 (pmol/L)[5]	439 (326, 591)	497 (441, 560)	389 (345, 438)	318 (284, 355)	242 (216, 272)	−0.6
Creatinine (μmol/L)[5]	41 (33, 49)	47 (43, 50)	62 (59, 66)	70 (67, 73)	81 (78 = 84)	0.8

[1] All values are geometric $\bar{x}$; 95% CI in parentheses. All variables were measured in plasma.
[2] Spearman's correlation test was used to establish the relation between tHcy, plasma folate, vitamin B-12, and creatinine concentrations and age. *P* for trend was significant ($P < 0.0001$) for all correlation coefficients.
[3] $n = 48$, 45, 44, 51, and 46 for the age groups from left to right. Sex × age interaction was not significant ($P = 0.7$).
[4] Calculated in a linear regression model.
[5] $n = 7$, 43, 43, 50, and 46 for the age groups from left to right.

plasma folate, vitamin B-12, and creatinine concentrations in the age groups are shown in **Table 1**. The geometric mean tHcy concentration for the total population ($n = 234$) was 6.2 μmol/L (95% CI: 5.9, 6.6). We observed a wide range of tHcy concentrations in the newborns (aged 0 y).

For both boys and girls, the geometric mean tHcy concentrations increased significantly as a function of age. For the whole cohort, no significant interaction between age and sex was present ($P = 0.7$). The concentrations in boys reached adult values at age 15 y.

Both plasma folate and vitamin B-12 concentrations decreased significantly with age. The plasma creatinine concentration increased with age (Table 1). No difference was seen between the boys and the girls in these variables. Very high concentrations of plasma folate were measured in 7 of the youngest children (aged 0–1 y), whose mean value of 79 nmol/L (95% CI: 60, 104) was 3 to 5 times that in the older children. The plasma vitamin B-12 concentration measured in the youngest children was 439 pmol/L (95% CI: 326, 591 pmol/L), which was not significantly lower than that in the 2–5-y-old children (497 pmol/L; 95% CI: 441, 560 pmol/L) but was twice that in the oldest children (aged 15–19 y).

Change in tHcy concentrations in relation to plasma folate, vitamin B-12, and creatinine

Predictors of tHcy concentration were estimated by multiple linear regression analysis (**Table 2**). In the regression model, the plasma folate × age interaction for the continuous relation between tHcy and plasma folate concentration was significant ($P = 0.003$); the plasma vitamin B-12 × age interaction for the relation between tHcy and plasma vitamin B-12 and the plasma creatinine × age interaction for the relation between tHcy and creatinine were not significant (P = 0.2 and 0.6, respectively). Because of the significant interaction, β coefficients were stratified to age for plasma folate. The relation between tHcy and plasma folate, after adjustment for plasma vitamin B-12 and creatinine, was negative for all age groups (Table 2). The slopes of the tHcy and plasma folate relation and those adjusted for vitamin B-12 were significantly different across the age groups. After adjustment for vitamin B-12 and creatinine, the differences in slope between the 2–5-y-old group and the 11–14- and 15–19-y-old groups and between the 6–10-y-old group and the 15–19-y-old group remained significant. The β coefficient of the relation between tHcy and plasma vitamin B-12, after adjustment for age, plasma folate, and creatinine, was −0.16 (95% CI: −0.26, −0.05) for the whole group.

To evaluate the slope direction as a function of concentration range, we plotted the tHcy concentrations against plasma folate (**Figure 1**) and plasma vitamin B-12 (**Figure 2**) for the whole group. The plots show that elevated tHcy concentrations seemed to be most frequent when the plasma folate concentration was <20 nmol/L and when the plasma vitamin B-12 concentration was <200 pmol/L. At higher concentrations of plasma folate and vitamin B-12, the dose-response relation between vitamins and tHcy appeared to plateau. We tested the significance of this observation by adding these cutoffs to the regression model. The additional plasma folate × low or high (< or > 20 nmol/L) folate interaction was significant ($P = 0.03$), but the additional plasma

TABLE 2
Linear regression model for the concentration of plasma total homocysteine (tHcy) associated with a change in plasma folate stratified by age[1]

	2–5 y ($n = 43$)	6–10 y ($n = 43$)	11–14 y ($n = 50$)	15–19 y ($n = 46$)
tHcy versus plasma folate	−0.42 (−0.52, −0.30)	−0.36 (−0.48, −0.24)	−0.31 (−0.43, −0.18)	−0.25 (−0.37, −0.12)
Adjusted for plasma vitamin B-12	−0.39 (−0.50, −0.29)	−0.34 (−0.46, −0.22)	−0.30 (−0.42, −0.18)	−0.25 (−0.37, −0.13)
Adjusted for plasma vitamin B-12 + creatinine	−0.32 (−0.42, −0.21)[a]	−0.29 (−0.40, −0.18)[a,b]	−0.27 (−0.38, −0.15)[b,c]	−0.24 (−0.36, −0.12)[c]

[1] 95% CI in parentheses. Because of skewed distribution, tHcy, plasma folate, and vitamin B-12 were log transformed. The β values express the change in log tHcy associated with a 1-unit change in log folate. The β coefficient of the relation between tHcy and plasma vitamin B-12, adjusted for age, plasma folate, and creatinine, was −0.16 (95% CI: −0.26, −0.05) for the whole group. In the regression model, the folate × age interaction was significant ($P = 0.003$); the vitamin B-12 × age and creatinine × age interactions were not significant ($P = 0.2$ and 0.6, respectively). All values in the first and second rows were significantly different across the 4 age groups, and the values in the third row with different superscript letters were significantly different ($P < 0.05$ for both).

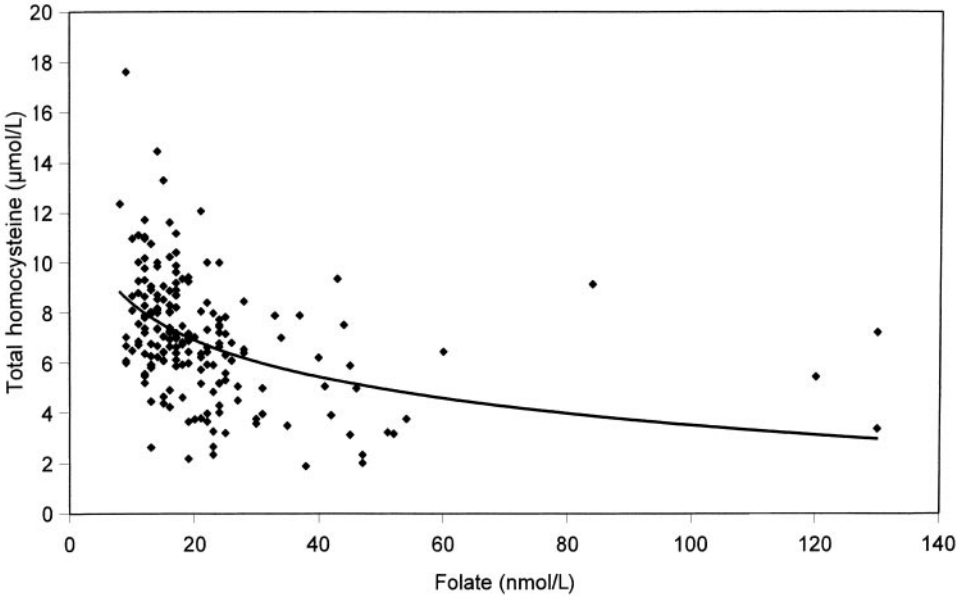

FIGURE 1. Relation between plasma total homocysteine and plasma folate concentrations in children aged 0–19 y ($n = 189$). Higher tHcy concentrations were confined to folate concentrations less than ≈20 nmol/L. The plasma folate × low or high (< or >20 nmol/L) folate interaction was significant ($P = 0.03$).

vitamin B-12 × low or high (< or > 200 pmol/L) vitamin B-12 interaction was not significant ($P = 0.4$).

A significant positive linear association was observed between tHcy and plasma creatinine ($r = 0.57$, $P < 0.0001$). In the multiple linear regression model, after adjustment for age, plasma folate, and vitamin B-12 concentrations, the effect of plasma creatinine on the tHcy concentration was small but significant ($\beta = 0.005$, 95% CI: 0.001, 0.01).

The influence of *MTHFR* 677C→T polymorphism on tHcy concentration

Of the 220 subjects, 18 (8.2%) were homozygous (TT), 104 (47.3%) were heterozygous (CT), and 98 (44.5%) were wild-type (CC) for the 677C→T polymorphism in the *MTHFR* gene. The genotype × age interaction in the regression model was not significant ($P = 0.13$). For all ages, the geometric mean tHcy concentrations were 6.2 (95% CI: 5.8, 6.7), 6.3 (5.8, 6.8), and 6.8 μmol/L (5.2, 8.5 μmol/L) for the *CC*, *CT*, and *TT* genotypes, respectively. The *P* values for the effect of *MTHFR* 677*CT* and *TT* genotypes on tHcy concentrations were 0.7 and 0.3, respectively.

To investigate the effect of the *MTHFR* 677C→T polymorphism on tHcy concentrations in relation to plasma folate concentrations, we included plasma folate × genotype as independent variables in the linear regression model. The genotype × plasma folate interaction, adjusted for age, was significant ($P =$

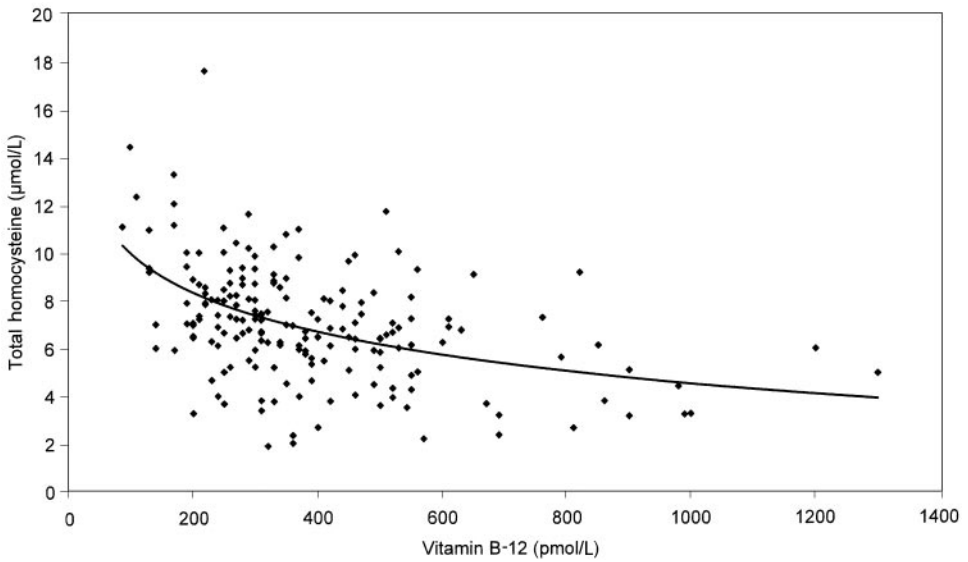

FIGURE 2. Relation between plasma total homocysteine and plasma vitamin B-12 concentrations in children aged 0–19 y ($n = 189$). Higher tHcy concentrations seemed to be confined to vitamin B-12 concentrations less than ≈200 pmol/L. The plasma vitamin B-12 × low or high (< or >200 pmol/L) vitamin B-12 interaction was not significant ($P = 0.4$).

0.05), which suggested that tHcy concentration seemed to be higher in persons with the *TT* genotype and low plasma folate concentration than in those with the *TT* genotype and high folate concentration.

Medication, puberty, and smoking

None of the children in this study used medication that could interfere with folate metabolism. We investigated the possible effect of puberty on tHcy concentrations in adolescents. As the criterion for exposure to endogenous sex hormones in children aged 10–19 y, we used the occurrence of menarche or the growth of beard hair. In the relatively small subgroups, no significant difference in the tHcy concentration was found between children with or without these features (data not shown).

Seven children in the oldest group (aged 15–19 y) smoked cigarettes. The geometric mean tHcy concentration in the smokers group was 9.0 μmol/L (95% CI: 7.3, 11.1 μmol/L). This did not differ significantly from the 8.6 μmol/L (95% CI: 7.9, 9.4 μmol/L) seen in nonsmokers.

DISCUSSION

This study was primarily designed to explore tHcy and its predictors (ie, age; sex; plasma folate, vitamin B-12, and creatinine concentrations; and *MTHFR* genotype) in presumably normal white children aged 0–19 y. The mean tHcy concentrations in these Dutch children ranged from 4.6 to 8.7 μmol/L, which is comparable to those observed in other European children (19, 20, 23, 26, 27, 29, 30, 32, 34, 35). Studies performed in black and white children in the United States or Canada found tHcy concentrations ≈1.5 μmol/L lower than our values (21, 22, 28, 31, 33). These differences in tHcy concentrations may indicate a real difference between populations due to environmental, including nutritional and genetic, factors. Must et al (31) observed differences in tHcy concentrations among 3 racial or ethnic groups of children.

The strong association that we observed between tHcy concentration and age was also found in all earlier studies except that of Reddy et al (34). Several studies established age subgroups comparable to ours. Vilaseca et al (32) measured median tHcy concentrations of 5.8, 6.6, and 8.1 μmol/L in Spanish children aged 2 mo to 10 y, 11–15 y, and 16–18 y, respectively. De Laet et al (27) measured tHcy in school-age Belgian children and found geometric mean concentrations of 6.2, 7.1, and 8.8 μmol/L in 5–9-, 10–14-, and 15–19-y-olds, respectively. When Greenlund et al (28) examined black and white children with a positive parental history of coronary artery disease, the mean tHcy concentrations were 6.0 and 6.9 μmol/L for 5–14- and 15–17-y-olds, respectively. Bates et al (30) found that measurements of tHcy concentrations in their study varied between age groups (ie, 4–6, 7–10, 11–14, and 15–19 y old) from 4.8 to 7.8 μmol/L in girls and from 5.2 to 8.5 μmol/L in boys.

The mean tHcy concentrations in adult men and women aged 20–85 y, as determined in our laboratory, were 10.9 and 9.8 μmol/L, respectively. According to our observation, the 11% higher tHcy concentration in men than in women appeared at adolescence. Some (21, 22, 27) but not all (19, 28–30, 34, 36) studies observed sex differences in tHcy concentrations in children, with higher values becoming manifest in boys during and after puberty. Only in the study by Must et al was the sex difference in tHcy concentration found to be significant at ≈10 y of age (31). Both the higher tHcy concentration in boys than in girls and the age effect could be explained by increases in muscle mass according to age and sex. This contention is supported by studies showing a positive relation between tHcy and creatinine in healthy children without renal dysfunction (19, 29). We observed a small but significant effect of plasma creatinine on tHcy concentration. Considering the negative correlation between estrogen concentrations and tHcy concentrations, the postpubertal differences in tHcy concentration may also be explained by exposure to estrogen in pubescent girls (41).

Our observation that both plasma folate and vitamin B-12 concentrations decreased markedly with age has also been described in 3 other studies (27, 35, 42). The high plasma vitamin concentrations may be reflected in the lower tHcy concentrations at younger age. In the current study, plasma folate is inversely correlated with tHcy concentrations at all ages, which is consistent with findings in previous studies in children (13–15, 19, 20, 22–27, 29, 30, 35, 36). Elevation of tHcy was particularly seen when the plasma folate concentration was <20 nmol/L. This meant that 29, 60, 71, and 63% of the 2–5-, 6–10-, 11–14-, and 15–19-y-olds, respectively, were at risk of elevated tHcy. Osagian et al (22) showed in 13–14-y-old US children that tHcy increased at slightly higher concentrations of plasma folate, starting at ≈30 nmol/L.

Other studies observed an significant inverse relation between tHcy and plasma vitamin B-12 concentrations; age was not always evaluated (20, 22, 23, 25, 27, 29, 30, 35, 36). In our study, elevation of tHcy seemed to occur when plasma vitamin B-12 concentration was <200 pmol/L. Persons with a vitamin B-12 concentration significantly <200 pmol/L had higher tHey concentrations, but they did not differ significantly from those in persons with vitamin B-12 >200 pmol/L. This meant that 4, 4, 2, and 28% of the 2–5-, 6–10-, 11–14-, and 15–19-y-olds, respectively, were at risk of elevated tHcy. In the third National Health and Nutrition Examination Survey, which included adolescents (>12 y old) and adults, approximately two-thirds of the cases of high tHcy concentration were associated with low plasma folate concentrations and with vitamin B-12 concentrations < 250 pmol/L (43). Folate and vitamin B-12 supplementation, given to lower tHcy concentrations, may be less effective if plasma folate and vitamin B-12 concentrations are >20 nmol/L and >200 pmol/L, respectively. This is relevant because hyperhomocysteinemia is also a known risk factor for ischemic stroke in children (5–8). Intervention studies are needed to illuminate this issue.

We also observed a greater variation in tHcy concentrations in newborns during the first months. Newborns, in particular those who were premature, should be considered a separate group for whom separate reference ranges should be established (13–17). The reports evaluating tHcy in newborns showed that, in a significant proportion of newborns, elevated tHcy could be attributed to an impaired vitamin B-12 status, and that vitamin B-12 deficiency and higher tHcy and methylmalonic acid concentrations were frequently present in breastfed babies (13, 14, 17). In our study, the number of blood samples for vitamin concentration measurement ($n = 7$) was too low to evaluate this relation.

The proportion of children identified with the *MTHFR* 677*TT* genotype (8.2%) was lower than that reported in French Canadian children (17%) by Delvin et al (36). Balasa et al (33) reported the *MTHFR* 677*TT* genotype to be present in 11% of whites and 3% of African Americans. In our study, the *MTHFR*

677C→T polymorphism did not significantly influence the tHcy concentration overall. Higher tHcy concentrations were confined to persons with the *TT* genotype and lower plasma folate status, which was comparable to observations in adults (11, 40, 44). High plasma folate concentrations, particularly in younger children, may prevent elevated tHcy concentrations in those with the *MTHFR* 677*TT* genotype. Balasa et al (33) found that the *MTHFR* 677*TT* genotype accounted for 2.9% of the variance in tHcy in children, but data about folate status were absent. In the older children (aged >10 y) in the study by Delvin et al (36), the *MTHFR* 677*TT* genotype resulted in lower plasma folate concentrations with a trend toward higher tHcy concentrations.

In summary, we provided data on age-specific tHcy concentrations and their predictors in white children aged 0–19 y. Considering the growing interest in tHcy as a risk factor for cardiovascular disease in children (5–9) and in relation to other diseases (45), it is important to provide age-specific data and to explore the predictors of tHcy concentration. In the current study, tHcy concentrations were strongly related to age and were lower than those seen in adults in other studies. Plasma folate and vitamin B-12 concentrations were predictors of the plasma tHcy concentrations in children. The influence of plasma creatinine on tHcy was small, but present. The *MTHFR* 677C→T polymorphism did not significantly influence tHcy, except in children with low plasma folate status.

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IMvBwas responsible for data collection, data analysis, and writing of the manuscript. MdH is recipient of a VENI grant from the Netherlands Foundation of Scientific Research and was involved in the study design, data analysis and manuscript review. CMGT was responsible for vitamin analysis and manuscript review. LA performed the genotyping and data analysis. DO-vE was involved in data collection and tHcy determination. HJB is an Established Investigator of the Netherlands Heart Foundation, was the principal investigator, and was involved in all aspects of the study. None of the authors had any financial or personal interest in the Netherlands Heart Foundation or any other conflict of interest.

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