Plasma homocysteine concentrations in a Belgian school-age population[1–3]

Corinne De Laet, Jean-Claude Wautrecht, Daniel Brasseur, Michèle Dramaix, Jean-Marie Boeynaems, Jean Decuyper, and André Kahn

ABSTRACT

Background: Total plasma homocysteine (tHcy) is an independent risk factor for cardiovascular disease in adults. Data for children and adolescents are lacking.

Objective: The aim of this study was to provide a reference range for tHcy and to explore the relation between tHcy and nutritional indexes in a Belgian pediatric population.

Design: tHcy, folate, and vitamin B-12 were measured in 647 healthy children (353 girls and 294 boys) aged 5–19 y.

Results: The tHcy distribution was, as in adults, skewed to the right [geometric mean (−1 SD, +1 SD): 7.41 μmol/L (5.51, 9.96)]. Concentrations were lowest in younger children and increased with age. After the tHcy distribution was examined according to age, 3 age ranges were distinguished: 5–9 y [6.21 μmol/L (5.14, 7.50)], 10–14 y [7.09 μmol/L (5.69, 8.84)], and 15–19 y [8.84 μmol/L (6.36, 12.29)]. We observed no significant differences in tHcy values between girls and boys in children aged <15 y; in postpubertal children, however, concentrations were higher in boys than in girls. In the 3 age groups, folate was inversely correlated with tHcy; the negative relation between tHcy and vitamin B-12 was less strong. Familial cardiovascular disease was more frequent in children who had hyperhomocysteinemia.

Conclusions: These observations suggest that in children, as in adults, genetic, nutritional, and endocrine factors are determinants of the metabolism of homocysteine. The significance of tHcy values in childhood and young adulthood in terms of predicting cardiovascular risk in adulthood should be investigated. Am J Clin Nutr 1999;69:968–72.

KEY WORDS Homocysteine, children, cardiovascular diseases, vitamin B-12, folate, Belgium

INTRODUCTION

Homocysteine is a sulfur amino acid derived from methionine during transmethylation. It is either salvaged back to methionine in a folate- and cobalamin-dependent remethylation reaction or transformed into cysteine via the vitamin B-6–dependent enzyme cystathionine β-synthase. Homocystinuria refers to a group of rare inborn errors of metabolism resulting in high concentrations of circulating homocysteine and urinary homocystine. A characteristic feature of this disorder is premature vascular disease. If the homocystinuria is left untreated, ≈50% of patients have thromboembolic events before the age of 30 y (1).

Observations in patients with homocystinuria led McCully (2) to suggest that homocysteine may be involved in the pathogenesis of arteriosclerosis. Clinical and epidemiologic studies showed a relation between total plasma homocysteine (tHcy) concentrations and coronary artery disease as well as peripheral artery disease, stroke, and venous thromboembolism (3–9). Because the prevalence of hyperhomocysteinemia ranges from 20% to 40% in different populations with coronary artery disease, the therapeutic control of elevated homocysteine concentrations may be important in the prevention of premature vascular disease. It is not known now whether hyperhomocysteinemia is already present during infancy and whether it represents the same risk then as in adulthood.

Plasma homocysteine concentrations are controlled by an interplay of genetic and nutritional factors. A C-to-T substitution at nucleotide 677 in the methylenetetrahydrofolate reductase gene is associated with reduced activity and increased thermolability of this enzyme. Persons homozygous for this mutation (5–10% of whites) often have mildly elevated tHcy values and low plasma folate status (10). On the other hand, cofactors (folic acid and vitamins B-6 and B-12) required for homocysteine metabolism may be important determinants of circulating tHcy concentrations. Subclinical deficiencies of these cofactors have been shown to result in hyperhomocysteinemia (11–13).

Age- and sex-specific reference intervals for tHcy concentrations in adults have been published but data for children and adolescents are lacking (14). Tonstad et al (15) reported homocysteine concentrations in Norwegian children aged 8–12 y, a

[1] From the Laboratory of Pediatrics, University Children's Hospital Queen Fabiola, Brussels; the Medical Clinic of Vascular Diseases and the Laboratory of Medical Chemistry, Erasme Hospital, Brussels; and the Laboratory of Medical Statistics, School of Public Health, Free University of Brussels.

[2] Supported by the Danone Institute and the Foundation for Cardiac Surgery.

[3] Address reprint requests to C De Laet, Universite Libre de Bruxelles, Laboratory of Pediatrics, University Children's Hospital Queen Fabiola, Avenue JJ Crocq 15, B-1020 Bruxelles, Belgium. E-mail: pbourdou@resulb.ulb.ac.be.

Received May 21, 1998.

Accepted for publication November 3, 1998.

Am J Clin Nutr 1999;69:968–72. Printed in USA.

Spanish study reported homocysteine concentrations in 195 children aged 2 mo to 18 y (16), and Reddy (17) published reference values for tHcy in children from the New Orleans area. No data are available for other geographic areas. Our purpose was to establish the distribution of tHcy in a healthy population of school-age children and to determine the relations between tHcy and folate and vitamin B-12.

SUBJECTS AND METHODS

Study population

The study was conducted between October 1996 and May 1997 in 3 public schools in Brussels; 647 children and adolescents (353 girls and 294 boys) aged 5–19 y were included. The populations in these schools were comparable in ethnic status. The study was approved by the Ethical Committee of the University Children's Hospital Queen Fabiola, Brussels. Written, informed consent was obtained from the parents of each participating child.

Data on birth date, personal history (eg, history of chronic disease), familial cardiovascular disease (coronary artery disease, stroke, or peripheral vascular disease in at least one family member, including parents, grandparents, uncles, and aunts), and medication use (including oral contraceptives and vitamin supplements) were collected through use of a self-administered questionnaire. Weight and height were measured in 59% of children. Body mass index was calculated as weight (in kg)/height^2 (in m). Children with a severe illness (renal, heart, respiratory, endocrine, or neurologic disease) or requiring chronic treatment were not included in the study.

Blood sampling

Blood samples for the measurement of tHcy, folate, and vitamin B-12 in serum were taken after subjects had fasted overnight. Five of 647 samples were excluded from analysis because of hemolysis. Blood samples for the measurement of tHcy were collected in tubes containing EDTA. The tubes were immediately centrifuged (for 5 min at room temperature at 13000 $\times$ g) and the plasma fraction was stored at -80°C until tHcy measurement, which was performed within 5 mo.

Biochemical measurements

Plasma tHcy, which includes the sum of free and protein-bound homocysteine released by borohydride treatment, was measured by reversed-phase HPLC with fluorescence detection after precolumn derivatization (14). The precision of the assay was ≈5%. Vitamin B-12 and folate were measured by radioimmunoassay with a commercial kit (Becton Dickinson ICN, New York).

Statistics

Log_{10} transformation of tHcy was used in all analyses and parametric tests were applied. Distributions of tHcy are presented as histogram or box plots. Geometric means were calculated (inverse of the logarithmic mean) and are presented together with the interval obtained from the inverse of the logarithmic mean $\pm$ 1 SD. Ninety-fifth percentiles were derived by taking the inverse of the 95th percentile of the normal distribution with the mean and SD of the $\log_{10}$ distribution of tHcy as parameters.

After statistically analyzing the tHcy distribution according to age (by year), we established 3 age groups for whom tHcy concentrations were most significantly different: 5–9, 10–14, and 15–19 y. The number of subjects in each age group is presented in **Table 1**. The sex distribution according to age is also presented.

Student t tests and one-way and two-way analyses of variance (ANOVAs) were applied to compare means. In addition to one-way ANOVA, polynomial contrasts were used to analyze log tHcy according to age. Chi-square tests were used in the analysis of contingency tables and Pearson's correlation coefficients were computed to study the association between quantitative variables. The level of significance used was 0.05; when necessary, the Bonferroni correction was applied and a modified level of significance was considered.

RESULTS

The tHcy distribution was skewed to the right (**Figure 1**). The geometric mean for the total population (n = 642) was 7.41 (-1 SD, +1 SD: 5.51, 9.96) μmol/L. tHcy concentrations were progressively higher in each age group (**Figure 2**). Values in younger subjects corresponded to ≈50% of concentrations observed in adults.

For each age group (5–9, 10–14, and 15–19 y) and for both sexes, we determined the geometric means and the 95th percentiles of the tHcy distribution; these are shown in **Table 2**. Two-way ANOVA showed a significant interaction between age and sex. For both sexes, geometric means of tHcy were significantly greater with age ($P < 0.001$). Polynomial contrasts indicated that this increase was linear for girls but not for boys. The geometric means did not differ significantly between boys and girls in the first 2 age groups. However, a significant difference was observed in the oldest group, with the mean in males being significantly higher than that in females. The values in boys reached adult values after 15 y (14).

Body mass index, serum folate, and serum vitamin B-12 did not differ significantly between girls and boys; therefore, the results for both sexes were pooled in the 3 age groups (**Table 3**). As expected, body mass index was significantly greater with age, but no correlation was observed between body mass index and tHcy concentrations (**Table 4**).

A few subjects regularly took vitamin supplements containing vitamin B-6, vitamin B-12, or folate (5–9 y: 6.1% of the sample; 10–14 y: 2.6%; 15–19 y: 4.6%). Serum concentrations of folate and vitamin B-12 were comparable in children who did and did not regularly take vitamin supplements (data not shown). The mean concentrations of folate and vitamin B-12 in serum were progressively lower in each age group and mean values for each vitamin were

TABLE 1
Distribution of the children in the 3 age groups[1]

	Group 1: 5–9 y (n = 178)	Group 2: 10–14 y (n = 229)	Group 3: 15–19 y (n = 235)
Girls			
(n)	87	114	148
(% of girls)	24.9	32.7	42.4
(% of group)	49	50	63
Boys			
(n)	91	115	87
(% of boys)	31.1	39.2	29.7
(% of group)	51	50	37

[1]Sex distributions differed significantly by age, P = 0.003 (chi-square test).

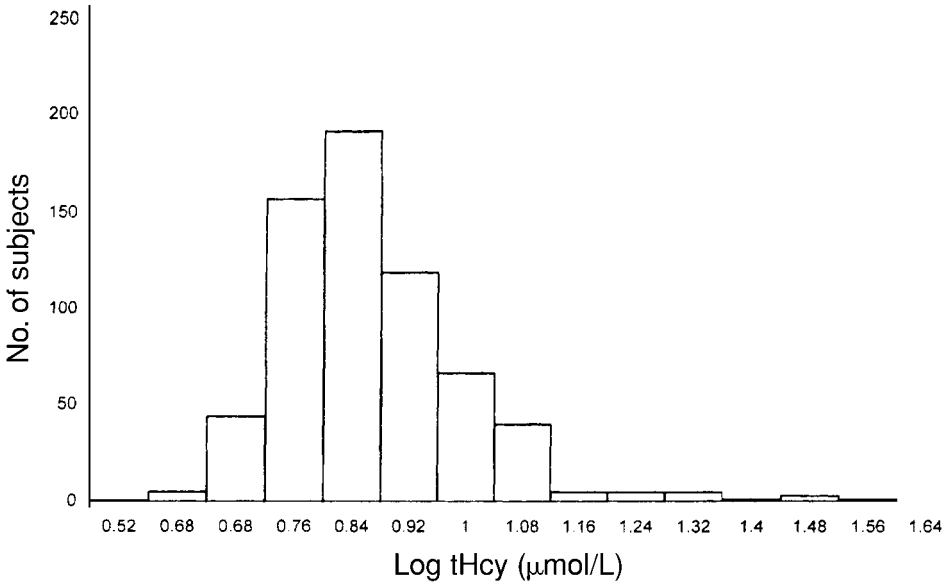

FIGURE 1. Distribution of total plasma homocysteine (tHcy) concentrations in a Belgian pediatric population (n = 642) after logarithmic transformation. Mean (±SD) tHcy concentration: 0.87 ± 0.13 μmol/L.

significantly different between age groups. Pathologic values (ie, folic acid <4.1 nmol/L or vitamin B-12 <105 pmol/L) were observed only in the oldest age group (15–19 y old).

We observed a negative correlation between tHcy and folate concentrations. The correlation was present in all 3 age groups but was most striking in the youngest and oldest (5–9 y and 15–19 y). The correlation between tHcy and vitamin B-12 concentrations was also negative, but weaker than the correlation between tHcy and folate (Table 4).

Forty girls (27%) in the 15–19-y-old age group took oral contraceptives. The mean tHcy concentrations of girls taking an oral contraceptive [8.51 μmol/L (6.86, 10.84)] and of other girls of the same age [8.26 μmol/L (6.29, 11.02)] were not significantly different.

Cardiovascular disease in the family was reported more frequently when the child had a tHcy concentration above the 95th percentile. Among children with a tHcy concentration above the 95th percentile, 2/6 (33.3%) in the 5–9-y-old age group, 3/12 (25%) in

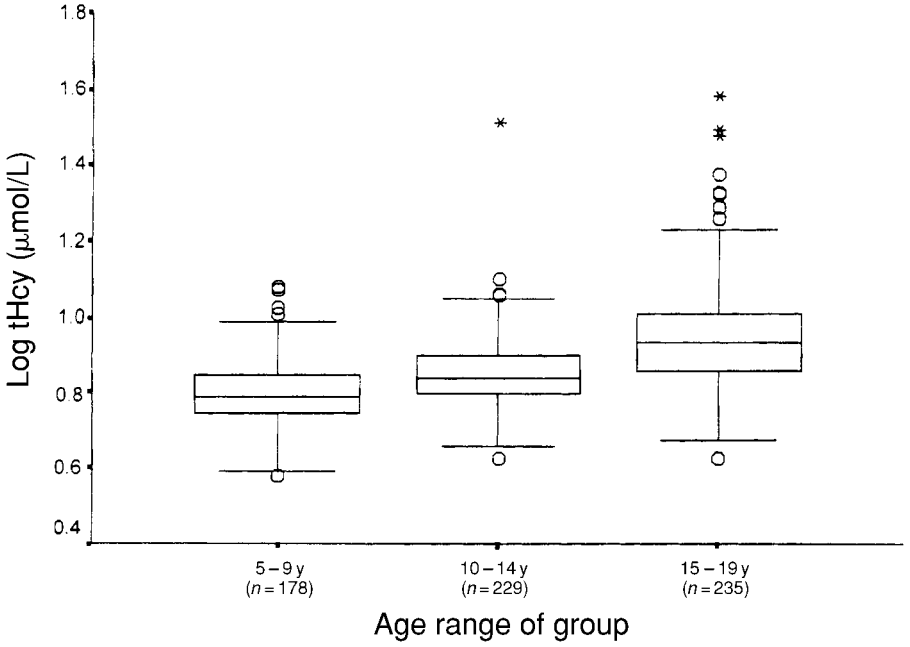

FIGURE 2. Distribution of total plasma homocysteine (tHcy) concentrations in 3 age groups after logarithmic transformation. The lower and upper lines of the box correspond to the 25th and 75th percentiles, respectively. The line in the middle of the box represents the median. The error bars are situated at ≈1.5 times the interquartile range (ie, 75th percentile to 25th percentile) from the upper and lower lines of the box. ○, outliers; *, extreme values.

TABLE 2
Distribution of total plasma homocysteine (tHcy) concentrations by age and sex[1]

	tHcy	95th percentile
	μmol/L	μmol/L
Group 1: 5–9 y		
All	6.21 (5.14, 7.50)[2]	8.46
Girls	6.11 (5.11, 7.30)	8.22
Boys	6.30 (5.18, 7.66)	8.70
Group 2: 10–14 y		
All	7.09 (5.69, 8.84)	10.20
Girls	7.07 (5.81, 8.60)	9.75
Boys	7.12 (5.58, 9.01)	10.60
Group 3: 15–19 y		
All	8.84 (6.36, 12.29)	15.19
Girls	8.33 (6.29, 11.02)	13.22
Boys	9.78 (6.70, 14.30)[3]	18.25

[1]In both sexes, geometric means of tHcy were significantly greater with age, $P < 0.001$.
[2]Geometric mean; ± 1 SD in parentheses.
[3]Significantly different from girls, $P = 0.001$.

the 10–14-y-old age group, and 4/12 (33.3%) in the 15–19-y-old age group reported cardiovascular disease in the family. The corresponding numbers in the whole study group were 6/178 (3.4%) in the 5–9-y-old age group, 13/229 (5.6%) in the 10–14-y-old age group, and 19/235 (8.1%) in the 15–19-y-old age group.

DISCUSSION

Few studies have investigated tHcy concentrations in children. Thus, the aim of the present study was to establish a reference range for tHcy and to explore the relation between tHcy and nutritional indexes in a Belgian pediatric population. In this population, the tHcy distribution was skewed to the right, as in adults. Concentrations were lowest in younger children and were progressively higher in each age group. The effect of age on basal tHcy concentrations was observed previously in adults. Selhub et al (11) found a significant age-related increase in plasma tHcy concentrations among 1060 survivors of the original Framingham Study cohort.

Three studies of tHcy measurement in children have been published. In Norway, Tonstad et al (15) studied the relation of tHcy, lipid, and apolipoprotein B concentrations in children to premature cardiovascular disease in family members. In children aged 8–12 y, the mean tHcy concentration was 5.3 μmol/L, which is lower than that measured in our study. In the second study, Vilaseca et al (16) measured tHcy concentrations in 195 Spanish children and adolescents aged 2 mo to 18 y. After statistically analyzing all age groups, the investigators established 3 age groups for whom tHcy concentrations were most significantly different. These age groups were nearly the same as ours: 2 mo to 10 y, 11–15 y, and 16–18 y. In this study, tHcy also increased significantly with age. The median tHcy concentration in the oldest age group was significantly different from that in the other 2 age groups, but there were no significant differences between boys and girls. The sample size in this study may have been too small to detect a significant difference between the sexes. In the third study, Reddy (17) measured tHcy values in children in 4 age groups (15 boys and 15 girls in each group) from the New Orleans area. They observed no significant trends either between the age groups or between the sexes. Although preanalytic conditions (whether the subjects had fasted before blood samples were collected and the time between blood sampling and centrifugation) may have contributed to the differences in tHcy values between these 3 studies and ours, the differences may also be explained by the nutritional and genetic environment of the groups in the 4 geographic areas in which the studies were carried out.

We measured 2 of the cofactors involved in tHcy metabolism, folate and vitamin B-12. The younger children had the highest concentrations of folate and vitamin B-12 and vitamin concentrations were progressively lower in each age group. Vitamin B-12 and folate concentrations correlated well with nutritional intake (18). Thus, it seems that dietary intakes of folate and vitamin B-12 were adequate in the younger children but not in the older children. Reduced nutritional intakes in adolescents may explain the decreases in serum concentrations of these vitamins. For example, McNulty et al (19) reported that boys and girls aged 12–15 y have low folate intakes compared with the estimated average requirement.

In studies of adult populations, negative correlations between tHcy and folate and between tHcy and vitamin B-12 have been shown (11). In our sample, we observed the expected inverse relations with folate and vitamin B-12. Nevertheless, the influence of vitamin B-12 on tHcy was less marked than the influence of folate. However, the correlation of tHcy with the vitamin concentrations (maximum correlation of −0.42 for folate in the 5–9-y-old age group) did not sufficiently explain the higher tHcy concentrations observed with age. Sulfur amino acid intakes may play a role in the higher tHcy concentrations with age; in this study, however, we did not study protein intakes in the different age groups.

In our study, the difference in tHcy concentrations between the sexes appeared in children aged ≥15 y; in these postpubertal children, tHcy concentrations were higher in boys than in girls. The effect of puberty on tHcy concentrations may be the result of increased muscle mass, sex hormones, or both. The effect of muscle mass may be related to the large amount of homocysteine formed in conjunction with creatine-creatinine synthesis. In line with this theory, a positive correlation between tHcy and serum creatinine was reported in adults and was related to the sex dif-

TABLE 3
Body mass index, serum folate concentrations, and vitamin B-12 concentrations by age group[1]

	Group 1: 5–9 y	Group 2: 10–14 y	Group 3: 15–19 y
BMI (kg/m^2)	16.28 ± 1.86 [76]	18.78 ± 2.96 [173]	21.42 ± 2.90 [127]
Folate (nmol/L)	21.06 ± 7.99 [172]	18.87 ± 6.61 [225]	15.02 ± 6.34 [229]
Vitamin B-12 (pmol/L)	517.4 ± 172.6 [172]	426.1 ± 167.3 [225]	340.8 ± 138.9 [229]

[1]$\bar{x}$ ± SD; n in brackets. Mean values for body mass index, folic acid, and vitamin B-12 are significantly different between age groups, $P < 0.001$ (one-way ANOVA).

TABLE 4
Pearson correlation coefficients between BMI, folate, and vitamin B-12 and total homocysteine by age group

	Group 1: 5–9 y	Group 2: 10–14 y	Group 3: 15–19 y
BMI	0.07	0.09	0.07
Folate	−0.42[1]	−0.19[2]	−0.40[1]
Vitamin B-12	−0.28[1]	−0.19[2]	−0.22[1]

[1] $P < 0.001$.
[2] $P < 0.01$.

ference in tHcy concentrations in adults (20). Although we did not measure creatinine in the present study, we did analyze the relation between tHcy and body mass index and found no correlation. Hormonal effects on tHcy have also been suggested in several studies in adults. For example, tHcy concentrations appear to be related to estrogen status. Premenopausal women have lower tHcy concentrations than do postmenopausal women or men. In addition, plasma tHcy was been reported to decrease during pregnancy, in postmenopausal women taking hormone-replacement therapy, and during the high-hormone phase in women taking oral contraceptives (21–23). In rats, Kim et al (24) showed a significant lowering effect of steroid hormones (cortisol and estradiol) on homocysteine concentrations.

Elevated tHcy concentrations have been observed in the offspring of hyperhomocysteinemic patients with premature cardiovascular diseases (25). In line with this observation, we observed a history of cardiovascular disease more frequently in families of children with a tHcy concentration above the 95th percentile than in families of children with a concentration below the 95th percentile. We did not measure tHcy concentrations in the parents, however.

In conclusion, we observed that tHcy concentrations were lowest in younger children and increased with age. We observed no significant differences in tHcy concentrations between girls and boys in children aged < 15 y; in postpubertal children, however, concentrations were higher in boys than in girls. Our observations suggest that in children, as in adults, genetic, nutritional, and endocrine factors play a role in the metabolism of homocysteine. Whether hyperhomocysteinemia in childhood is predictive of future cardiovascular disease cannot be established from a retrospective study. Prospective studies should be conducted to explore the significance of mild hyperhomocysteinemia in childhood and its implication in terms of prevention.

We thank M Meerseman for her valuable help in organizing the blood samplings in the 3 public schools in Brussels and P Goyens and HL Vis for their scientific contributions.

REFERENCES

1. Mudd SH, Levy HL. Disorders of transulfuration. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The metabolic and molecular bases of inherited diseases. New York: McGraw Hill, 1995:1279–327.
2. McCully KS. Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis. Am J Pathol 1969;56:111–28.
3. Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. JAMA 1995;274:1049–57.
4. The European Concerted Action Project. Plasma homocysteine as a risk factor for vascular disease. JAMA 1997;277:1775–81.
5. den Heijer M, Koster T, Blom HJ, et al. Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. N Engl J Med 1996;334:759–62.
6. Nygård O, Vollset SE, Refsum H, et al. Total homocysteine and cardiovascular risk profile. The Hordaland Homocysteine Study. JAMA 1995;274:1526–33.
7. Stampfer MJ, Malinow MR, Willett WC, et al. A prospective study of plasma homocysteine and risk of myocardial infarction in US physicians. JAMA 1992;268:877–81.
8. Nygård O, Nordrehaug JE, Refsum H, Ueland PM, Farstad M, Vollset SE. Plasma homocysteine levels and mortality in patients with coronary artery disease. N Engl J Med 1997;337:230–6.
9. Selhub J, Jacques PF, Bostom AG, et al. Association between plasma homocysteine concentrations and extracranial carotid-artery stenosis. N Engl J Med 1995;332:286–91.
10. Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995;10:111–3.
11. Selhub J, Jacques PF, Wilson PW, Rush D, Rosenberg IH. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. JAMA 1993;270:2693–8.
12. Verhoef P, Stampfer MJ, Buring JE, et al. Homocysteine metabolism and risk of myocardial infarction: relation with vitamins B6, B12 and folate. Am J Epidemiol 1996;143:845–59.
13. Guttormsen AB, Ueland PM, Nesthus I, et al. Determinants and vitamin responsiveness of intermediate hyperhomocysteinemia. J Clin Invest 1996;98:2174–83.
14. Ueland PM, Refsum H, Stabler SP, Malinow MR, Andersson A, Allen RH. Total homocysteine in plasma or serum: methods and clinical applications. Clin Chem 1993;39:1764–79.
15. Tonstad S, Refsum H, Sivertsen M, Christophersen B, Ose L, Ueland PM. Relation of total homocysteine and lipid levels in children to premature cardiovascular death in male relatives. Pediatr Res 1996;40:47–52.
16. Vilaseca MA, Moyano D, Ferrer I, Artuch R. Total homocysteine in pediatric patients. Clin Chem 1997;43:690–2.
17. Reddy MN. Reference ranges for total homocysteine in children. Clin Chim Acta 1997;262:153–5.
18. Jacques PF, Sulsky SI, Sadowski JA, Phillips JC, Rush D, Willett WC. Comparison of micronutrient intake measured by a dietary questionnaire and biochemical indicators of micronutrient status. Am J Clin Nutr 1993;57:182–9.
19. McNulty H, Eaton-Evans J, Cran G, et al. Nutrient intakes and impact of fortified breakfast cereals in schoolchildren. Arch Dis Child 1996;75:474–81.
20. Brattström L, Lindgren A, Israelsson B, Andersson A, Hutelberg B. Homocysteine and cysteine: determinants of plasma levels in middle-aged and elderly subjects. J Intern Med 1994;236:633–41.
21. Wouters MG, Moorrees MTH, Van der Mooren MJ, et al. Plasma homocysteine and menopausal status. Eur J Clin Invest 1995;25:801–5.
22. Andersson A, Hultberg B, Brattström L, Isaksson A. Decreased serum homocysteine in pregnancy. Eur J Clin Chem Clin Biochem 1992;30:377–9.
23. Lien EA, Anker G, Lonning PE, Refsum H, Ueland PM. Effects of hormones on the plasma levels of the atherogenic amino acid homocysteine. Biochem Soc Trans 1997;25:33–5.
24. Kim MH, Kim E, Passen EL, Meyer J, Kang S. Cortisol and estradiol: non genetic factors for hyperhomocysteinemia. Metabolism 1997;46:247–9.
25. Genest JJ, McNamara JR, Upson B, et al. Prevalence of familial hyperhomocysteinemia in men with premature coronary artery dis-