Journal of Clinical Laboratory Analysis 25 : 317–323 (2011)

Reference Interval and Determinants of the Serum Homocysteine Level in a Korean Population

Hee-Won Moon,[1] Dong Hee Whang,[2] Young Jin Ko,[1] Shin Young Joo,[2] Yeo-Min Yun,[1]* Mina Hur,[1] and Jin Q Kim[1]

[1]*Department of Laboratory Medicine, Konkuk University School of Medicine, Hwayang-Dong, Gwangjin-Gu, Seoul, South Korea*

[2]*Department of Laboratory Medicine, Inje University Seoul Paik Hospital, Jeo-Dong, Jung-Gu, Seoul, South Korea*

In this study, we estimated the reference intervals of the serum homocysteine (Hcy) level using two automated immunoassays, and we demonstrated the effects of various factors on the Hcy level in a Korean population. We calculated the gender- and assay-specific reference intervals using the data from 809 healthy Koreans, and we assessed the effects of physiologic and lifestyle factors on the Hcy level. The upper limit was higher in males (19.21 and 19.76 μmol/l) than that in females (14.99 μmol/l and 15.16 μmol/l, AxSym and ADVIA centaur, respectively); the upper limits were comparable between the two assays. Smokers, vitamin nonusers, and persons without regular exercise showed a lower folate level and a higher Hcy level. The risk of hyperhomocysteinemia was significantly associated with the male gender (adjusted OR: 5.705, *P*-value: 0.008) and with the low folate level group (adjusted OR: 10.412, *P*-value: 0.002) on the multivariate analysis. The Hcy level was significantly different according to various factors, especially in the gender and folate level. The reference interval should be determined for each ethnic population and for each assay. The appropriate cutoff for assessing the risk for cardiovascular disease or stroke should also be validated in each population. J. Clin. Lab. Anal. 25:317–323, 2011.

Key words: homocysteine; immunoassay; reference interval; Korean

INTRODUCTION

Hyperhomocysteinemia is a sensitive marker of folate and vitamin B12 deficiency and a well-established risk factor for cardiovascular disease (1–4). An increased total homocysteine (Hcy) level is also reported to be associated with pregnancy complications, cognitive impairment, and stroke (4,5). Meta-analyses of the prospective studies have reported that lowering the Hcy level, by 3 μmol/l or a reduction of 25%, would reduce the risk of heart disease and stroke by 11–16% and 19–24%, respectively (4,6). The Hcy levels are influenced by physiologic factors, lifestyles, hereditary factors, and ethnicity (5,7–11). In addition, the Hcy concentrations can be changed according to the types of specimens and the collection or handling of samples. Because the variations among different methods and laboratories are also considerable (5,12), each laboratory needs to establish a reference interval for an individual population. Moreover, demonstrating a separate reference range according to physiologic factors or the nutritional status could be helpful for modifying the lifestyle of patients (5). However, there are very few reported large-scale studies on the reference interval of Hcy in an Asian population. In this study, we estimated the reference interval of serum Hcy in a Korean population using the two most commonly used automated immunoassays. We also demonstrated the effects of various factors on the Hcy level in a large sample, and this data could be helpful for the accurate interpretation of Hcy levels.

Grant sponsor: Konkuk University.

Hee-Won Moon and Dong Hee Whang contributed equally to this manuscript.

*Correspondence to: Yeo-Min Yun, Department of Laboratory Medicine, Konkuk University Hospital, 4-12, Hwayang-Dong, Gwangjin-Gu, Seoul, South Korea. E-mail: ymyun@kuh.ac.kr

Received 31 January 2011; Accepted 19 May 2011
DOI 10.1002/jcla.20476
Published online in Wiley Online Library (wileyonlinelibrary.com).

MATERIALS AND METHODS

Subjects

We included 809 healthy individuals who visited two university hospitals located in Seoul (Konkuk University Medical Center and Inje University Seoul Paik Hospital), Korea, for a general examination. All participants were within the normal ranges on their routine laboratory tests and they had no medical history of any specific disease.

Among them, 307 had available data about lifestyle factors, including smoking, exercise, and vitamin supplements. The mean age of the 483 men was 51.0 ± 12.25 and that of the 326 women was 48.6 ± 9.8 years. The major characteristics of the study population are described in Table 1. Because there were significant differences of demographic or lifestyle factors between the men and women, most of the analyses were performed separately for each gender group. The study protocol was approved by the Institutional Review Boards of Konkuk University Medical Center and Inje University Seoul Paik Hospital. We obtained written informed consent from all the subjects.

Blood Sample Collection and Determination of Hcy

After venous blood collection using gel separator tubes, the samples were centrifuged at 2,500–3,000 rpm for 10 min within 1 hr of collection (8) and the serum fraction was used for measuring the Hcy levels. The serum was stored at 4°C in a refrigerator until measurement, and Hcy testing was performed on the day of collection. The serum samples for measuring the folate levels were stored at −70°C with protection from light. The serum Hcy levels were measured with the AxSym assay (Abbott Diagnostics, Chicago, IL), which is based on fluorescence polarization immunoassay technology, and the ADVIA Centaur Hcy assay (Siemens Healthcare Diagnostics Inc., Tarrytown, NY), which is based on competitive inhibition using direct chemiluminescent technology. The folate level was determined using Elecsys E170 (Roche Diagnostics, Mannheim, Germany).

TABLE 1. Demographic Characteristics and Lifestyle Factors in the Study Population

Characteristics	Male	Female	Total
No. (%)	483 (59.7)	326 (40.3)	809
Age, yrs	51.0 ± 12.3	48.6 ± 9.8	50.1 ± 13.4
Folate level[a] (nmol/l)	10.1 ± 6.0	12.1 ± 4.0	10.7 ± 3.9
Creatinine[a] (mg/dl)	1.2 ± 0.2	1.0 ± 0.1	1.0 ± 0.2
Hcy[a] (μmol/l)	12.4 ± 3.4	9.3 ± 2.6	11.1 ± 3.5
Vitamin supplement[a], Yes	24.7%	35.2%	28.9%
Current smoking[a], Yes	38.9%	5.7%	25.8%
Regular exercise[a], Yes	64.3%	49.2%	58.3%
Body mass index[a] (kg/m^2)	24.4 ± 2.9	22.4 ± 3.2	23.5 ± 3.2

Values are presented as the mean ± SD unless otherwise indicated. Hcy, homocysteine.
[a] $P < 0.001$ between the men and women.

Determination of Reference Interval

In this study, the reference interval of the serum Hcy was determined for healthy adult individuals. The elderly subjects were >65 years old and subjects with folate deficiency were excluded. The reference interval was calculated according to the guidelines of the Clinical and Laboratory Standards Institute. After excluding outliers with a ratio D/R method, the nonparametric method (2.5–97.5th percentile interval) was used when the data did not fit a normal distribution, and a parametric method was used for the normally distributed data before and after logarithmic transformation (13).

Statistics

The relationships between the continuous variables of the different groups were analyzed using Student's *t*-test, the Mann–Whitney test, and ANOVA. The significance of the categorical variables was assessed by the chi-square test. Regarding the folate level, we classified the participants into three groups (low, medium, and high) by the tertiles of the folate levels in our study group. Multivariate logistic regression was used to examine the odds ratios (OR) of hyperhomocysteinemia in association with the demographic and various lifestyle factors. Hyperhomocysteinemia was defined as a plasma tHcy concentration ≥15.0 μmol/l (5,11). The statistical analysis was performed using SPSS software, version 12.0 (SPSS Inc., Chicago, IL) and MedCalc Statistical software 9.3.9.0 (Mariakerke, Belgium). *P*-values less than 0.05 were considered to be statistically significant.

RESULTS

Characteristics and Lifestyle Factors in the Male and Female Groups

The general characteristics of the study population are described in Table 1. In our population, the proportion of males was higher (59.7%) than that of females (40.3%) and the mean age of the females (48.6 ± 9.8) was younger than that of males (51.0 ± 12.3). The creatinine, Hcy, and body mass index were significantly higher and the folate level was significantly lower in males. The proportions of current smokers and individuals who regularly exercise were higher for males, but vitamin supplement users were more common for females (Table 1).

Reference Interval of the Serum Hcy in Korean Adults

We calculated the reference interval as the 2.5–97.5th percentile of healthy reference adults. After logarithmic transformation, 95% confidence interval (CI) was also calculated using parametric methods. The separate reference intervals of serum Hcy according to gender and both assays are shown in Table 2. The upper limit (97.5th percentile) was higher in males (19.21 μmol/l and 19.76 μmol/l for AxSym and ADVIA centaur, respectively) than that in females (14.99 μmol/l and 15.16 μmol/l, for AxSym and ADVIA centaur, respectively). The upper cutoff was slightly higher for the ADVIA Centaur than that for the AxSym assay.

Comparison of Hcy Levels Between the Two Automated Immunoassays

Because the gender proportions of each population that were analyzed by the two immunoassays could influence the Hcy levels, comparison between the two assays was performed separately in the male and female groups. As shown in Table 3, the mean Hcy levels were significantly higher in the population analyzed by the ADVIA Centaur than those mean levels assessed by the AxSym assays in both the male and female groups (12.70 vs. 11.59 and 9.93 vs. 8.50, respectively, P-value <0.001). In contrast, the folate levels between the two populations (ADVIA Centaur vs. AxSym) were not significantly different in both the male and female groups.

Serum Hcy Levels in Relation to Nutritional and Lifestyle Factors

Regarding the folate level, the serum Hcy levels were significantly different between the low, medium, and high folate groups, and the Hcy level was inversely associated with the folate level. These findings were consistent in both gender groups and in both immunoassay groups, but the difference was more prominent in the male group (P-value <0.001 in all groups; Table 4). The Hcy level was higher and the folate level lower for current smokers of both genders despite there being statistical insignificance. Vitamin supplement users showed significantly higher folate levels than did the nonusers (P-value 0.026 for males and 0.015 for females) and vitamin supplement users also showed lower Hcy levels, although this was not statistically significant. People performing regular exercise showed a significantly higher folate level and lower Hcy level, especially for males (P-value 0.028 and 0.029, respectively) (Table 5).

TABLE 2. Reference Intervals of the Serum Hcy Level in Korean Adults According to Gender and the Assays

	ADVIA Centaur			AxSym		
Reference interval (μmol/l)	Male	Female	Overall	Male	Female	Overall
≤65yrs	$N=193$	$N=149$	$N=342$	$N=220$	$N=160$	$N=380$
Percentile[a]	8.43–19.76	6.72–15.16	7.0–18.19	7.26–19.21	5.50–14.99	6.00–17.35
95% CI[b]	7.93–19.23	6.50–14.49	6.85–18.06	7.26–19.21	5.13–13.17	5.51–17.59
Total	$N=198$	$N=152$	$N=350$	$N=279$	$N=174$	$N=453$
Percentile[a]	8.45–19.76	6.73–15.48	7.02–18.27	7.67–20.09	5.65–15.61	5.95–19.22
95% CI[b]	7.97–19.18	6.73–15.48	6.87–18.11	7.67–20.09	5.03–13.89	5.59–18.62

Hcy, homocysteine; CI, confidence interval.
[a]2.5–97.5th percentile using the non-parametric method.
[b]After logarithmic transformation.

TABLE 3. Comparison of the Hcy Levels Between the Two Automated Immunoassays and the Folate Levels in Each Group

	Male			Female		
Variable	AxSym ($N=220$)	ADVIA Centaur ($N=193$)	P-value	AxSym ($N=160$)	ADVIA Centaur ($N=149$)	P-value
Hcy (μmol/l)	11.59±3.25	12.70±3.36	0.001	8.50±2.58	9.93±2.19	0.000
Folate (nmol/l)	9.89±3.33	10.28±8.42	0.537	12.49±4.00	11.66±3.99	0.087

Values are presented as the mean±SD unless otherwise indicated. Comparison was performed in only an adult population (≤65yrs). Abbreviations: Hcy, homocysteine; addition, Folate levels were measured by Elecsys E170 (Roche diagnostics).

TABLE 4. Serum Hcy Levels in Relation to the Folate Level by Each Automated Immunoassay

	ADVIA Centaur ($N = 304$)		AxSym ($N = 383$)	
Folate level	Male ($N = 185$)	Female ($N = 119$)	Male ($N = 223$)	Female ($N = 160$)
Low	14.08±3.42	10.78±2.39	12.72±3.83	9.67±4.31
Medium	12.34±2.62	9.92±2.33	10.49±2.10	8.38±1.75
High	11.44±2.12	9.52±1.93	10.42±1.85	7.66±1.24
P-value	0.000	0.029	0.000	0.002

Age was not significantly different between the groups divided by the folate level.

TABLE 5. Serum Hcy and Folate Levels in Each Group According to Lifestyle Factors

	Male ($N = 185$)			Female ($N = 122$)		
Lifestyle factors	Yes ($N = 73$)	No ($N = 113$)	*P*-value	Yes ($N = 7$)	No ($N = 114$)	*P*-value
Current smoking						
Hcy	13.15±3.72	12.64±2.63	0.273	10.55±4.09	9.93±2.09	0.479
Folate	8.91±3.35	9.89±3.44	0.057	9.64±5.29	11.72±3.83	0.176
	Yes ($N = 46$)	No ($N = 140$)		Yes ($N = 43$)	No ($N = 79$)	
Vitamin supplement						
Hcy	12.53±2.30	13.05±3.58	0.353	9.66±1.78	10.14±2.42	0.254
Folate	10.50±3.45	9.18±3.37	0.026	12.94±4.58	10.94±3.44	0.015
	Yes ($N = 119$)	No ($N = 66$)		Yes ($N = 60$)	No ($N = 62$)	
Regular exercise						
Hcy	12.46±2.69	13.51±3.65	0.028	10.13±2.24	9.82±2.21	0.440
Folate	9.92±3.56	8.77±3.07	0.029	12.18±4.00	11.15±3.94	0.158

Values are the mean±SD unless otherwise indicated. Hcy, homocysteine.

Assessment of the Risk for Hyperhomocysteinemia

The prevalence of hyperhomocysteinemia was higher in the male gender group, lower folate group, smokers, no regular exercise group, and vitamin supplement nonusers (Table 6). Using multivariate logistic regression analysis to control confounding factors, the risk of hyperhomocysteinemia was significantly associated with the male gender (adjusted OR: 5.705, 95% CI: 1.568–20.761, *P*-value: 0.008) and the low folate level group (adjusted OR: 10.412, 95% CI: 2.326–46.606, *P*-value: 0.002).

DISCUSSION

Hcy is a naturally occurring amino acid and metabolized by enzymatic pathways that either convert Hcy into cysteine or remethylate it back into methionine (14). Enzymatic defects or vitamin deficiencies cause the inhibition of these metabolic pathways, and so Hcy is accumulated. Numerous studies have shown that an increased Hcy concentration is an independent risk factor for cardiovascular disease and stroke (4,6,11). However, the effects of reasonable doses of folic acid supplementation on diseases such as CVD and stroke remain controversial, and this requires further investigation (4,6,11,15,16), although folic acid intervention is known to reduce the risk of neural tube defects (17). Importantly, the reference interval or cutoff should be determined and validated in individual populations because many factors, including genetic variation, demographic and physiologic factors, and lifestyles, have an influence on the Hcy levels. In general, the reference intervals are calculated as the 2.5–97.5th percentile interval for a healthy, reference population (5,13). In this study, the 97.5th percentile as the upper cutoff was 19.21 and 19.76 μmol/l in males and 14.99 and 15.16 μmol/l in females (AxSym and ADVIA centaur, respectively). Although several studies have reported on the Hcy levels and affecting factors in Asian populations (18–22), most of these studies just demonstrated the Hcy level and they did not calculate the reference interval using a reference population. They included some individuals with specific diseases, folate and vitamin deficiency, or increased creatinine. Moreover, the methods for determining the Hcy concentration were variable among the studies. Also, most studies demonstrated the mean concentration with the standard deviation and not the percentiles, and the age of the study population was also very variable. Thus, direct comparison of Hcy levels between the previous studies seems difficult; this requires caution owing to the presence of many variations between the previous studies. Nevertheless, the mean level of serum Hcy (11.1 µmol/l in males and 8.1 µmol/l in females, AxSym immunoassay) was very similar to that of a similar age group in the previous Asian studies (Singapore Chinese, Chinese in the south area of China, and rural Koreans) using HPLC or automated immunoassay (18,19,23). The Hcy levels reported in rural Japanese and Taiwanese populations were higher than those of this study; however, the populations of those studies were elderly subjects and the mean ages were much higher than those of other studies (Table 7). Compared with the data of a large western study, the mean Hcy level in the males in our study (11.1 µmol/l) was higher than that of non-Hispanic white males (9.9–10.9 µmol/l) and lower than that of black males (10.8–12.3 µmol/l) for similar age groups. In contrast, female Koreans (8.1 µmol/l) showed a slightly lower mean Hcy level than did the non-Hispanic white females (8.8 µmol/l) (8).

Regarding physiologic factors, a significant high Hcy level in males was observed in this study, and this is a consistent finding in almost all the studies (5,8,18,19,24–26). In this study, the folate level was also lower in males and lifestyle factors that are known to increase the Hcy level, such as smoking, were also associated with a low folate level. Hcy is also increased throughout life according to an increase in age (5,8,11). However, the prevalence of hyperhomocysteinemia was not significantly different according to the age groups and, this might be because we included mainly middle-aged adults and we compared within this age group without including younger or elderly subjects.

TABLE 6. The Risk of Hyperhomocysteinemia According to Various Physiologic and Lifestyle Factors in a Korean Population

Variable	Hyperhomocyteinemia[a]	Adjusted OR (95% CI)	*P*-value
Age, yrs			
20–39	15/109 (13.8)	1 (Ref)	
40–59	19/202 (8.6)	0.688 (0.294–1.612)	0.390
60–79	3/26 (11.5)	0.473 (0.051–4.355)	0.508
P for trend	0.305		
Gender			
Male	32/204 (15.7)	5.705 (1.568–20.761)	0.008
Female	5/152 (3.3)	1 (Ref)	
Folate level			
Low	23/116 (19.8)	10.412 (2.326–46.606)	0.002
Medium	6/64 (9.4)	5.163 (0.987–27.013)	0.052
High	2/124 (1.6)	1 (Ref)	
P for trend	0.000		
Smoking			
Yes	12/79 (15.2)	1.156 (0.493–2.712)	0.739
No	20/227 (8.8)	1 (Ref)	
Regular exercise			
Yes	17/179 (9.5)	1 (Ref)	
No	15/128 (11.7)	1.551 (0.663–3.630)	0.311
Vitamin supplement			
Use	5/89 (5.6)	1 (Ref)	
Nonuse	28/219 (12.8)	1.656 (0.574–4.772)	0.351

Multivariate logistic regression analysis was used for the statistical analysis. OR, odds ratio; CI, confidence interval.
[a]Hyperhomocyteinemia was defined as a serum homocysteine level $\geq$15 µmol/l.

TABLE 7. Comparison of the Studies on the Hcy Level in Asian Populations

No.	Subjects	Mean age (M/F, yrs)	Assay	Specimen	Folate level (M/F) (nmol/l)	Hcy level (µmol/l) M	Hcy level (µmol/l) F
1.	Rural, Japanese	64.0 ± 62.5	HPLC	Plasma	NT	12.5 (±5.5)[a]	9.8 (±2.7)[a]
2.	Rural, Chinese		HPLC	Plasma			
	South	49.5			16.7 (11.5–24.8)[b]	10.7 (10.4–11.0)[b]	7.9 (7.7–8.1)[b]
	North	49.3			8.2 (5.6–12.2)[b]	16.1 (15.4–16.9)[b]	10.6 (10.1–11.1)[b]
3.	Singapore Chinese	58.2 ± 57.1	HPLC	Plasma	12.2 (11.5–12.9)[b]/ 15.4 (14.6–16.3)[b]	11.2 (10.8–11.6)[b]	9.1 (8.8–9.4)[b]
4.	Taiwanese, elderly	71.3 ± 71.1	Immunoassay	Plasma	24.5 ± 29.5[a,c]	13.3 (±0.6)[a]	10.6 (±0.7)[a]
5.	Rural, Korean	55.8 ± 54.9	Immunoassay	Serum	11.8 (11.3–12.4)[b]/ 16.0 (15.5–16.5)[b]	11.2 (10.8–11.7)[b]	8.4 (8.1–8.7)[b]
6.	Urban, Korean (Present)	51.0 ± 43.5	Immunoassay[d]	Serum	11.0 ± 4.0[a]	11.1 (7.22–19.90)[e]	8.1 (5.49–12.99)[e]

Hcy, homocysteine; HPLC, high performance liquid chromatography.
[a]Mean (±SD).
[b]Mean (95% CI).
[c]Values were only for the subjects with a Hcy level $\leq$15 µmol/l.
[d]Only the AxSym data was presented here.
[e]Median (2.5–97.5th percentile).

In addition, there have been marked regional and seasonal variations observed for the Hcy level, as was reported in a Chinese study (19). These variations could be explained by the differences in the vitamin status, including folate, vitamin B12, and vitamin B6 (5,6,19,23). In contrast to the Chinese study, a previous Korean study revealed that the mean Hcy levels from urban subjects were comparable to the levels of rural Korean subjects using the same assay (AxSym assay), although the folate level was higher in rural Koreans (18).

In our study, the serum Hcy levels were significantly higher in the low folate group, and these findings were consistent in both genders and for both immunoassay groups. As expected, the vitamin supplement users showed significantly higher folate levels than did the nonusers (*P*-values 0.026 and 0.015 in males and females, respectively) and a lower Hcy level in both genders. Although we did not evaluate the exact composition of the vitamin supplements, it seems that people using vitamin supplements would have a healthier lifestyle, an increased folate level, and a lower Hcy level. Generally, the mean Hcy level is known to be 10–30% lower in adults eating a typical diet compared with those adults using folic acid supplements or those with healthy lifestyles (5,9,27). Similarly, the folate level was significantly higher and the Hcy level lower in the people who performed regular exercise.

For assessing the risk of hyperhomocysteinemia, we used multivariate logistic regression analysis to adjust for confounding factors. After adjusting, the risk of hyperhomocysteinemia was significantly associated with the male gender (adjusted OR: 5.705, 95% CI: 1.568–20.761, *P*-value: 0.008) and with a low folate level (adjusted OR: 10.412, 95% CI: 2.326–46.606, *P*-value: 0.008). Although we demonstrated the reference intervals of Hcy for each assays, hyperhomocysteinemia was defined according to a previously validated commonly used cutoff (a plasma tHcy concentration of $\geq 15.0\,\mu$mol/l). The definition of hyperhomocysteinemia for the assessment of the cardiovascular risk needs to be validated using clinical data.

Importantly, there are many preanalytic variables that influence the Hcy level during sample collection and handling (5,12). There is a time- and temperature-dependent release of Hcy from blood cells into the serum before separating the serum. Because the serum specimen needs to be left for a while to allow for coagulation, the serum Hcy level is usually higher (5–10%) than that of the plasma Hcy level (5,12). However, this can be prevented by using a gel separator tube, immediate centrifugation, and cool storage until centrifugation (5,28). In this study, we used gel separator tubes and the samples were centrifuged within an hour and then stored at 4°C until measurement. The mean Hcy levels using the serum samples in our study were comparable with the data using plasma samples in the previous Asian studies (Table 7).

The recent large studies did not confirm the significant effects of lifestyle changes or folic acid/vitamin B supplementation on the risk for recurrent CVD or mortality (15,16,29–31), and the adverse effects of a higher intake of folic acid have also been reported (32). However, this does not mean there are no relationships and this might be because the folic acid status would have an influence at too early a stage of cardiovascular events to prevent them, or the previous studies lacked sufficient statistical power (6,19). Moreover, a recent study showed improved stroke mortality in the countries that have adapted folic acid fortification as compared with those countries where fortification was never implemented (19,33).

In conclusion, we demonstrated the effects of various factors on the Hcy level using a large sample of a Korean population, and we calculated the reference intervals of the serum Hcy level using two automated immunoassays. The Hcy level is significantly different according to various factors, especially gender, folate level, and employed assays. Determining the reference interval and validating the appropriate cutoff value for the risk assessment of diseases are needed in each ethnic population and for each type of assay.

ACKNOWLEDGMENTS

This work was supported by Konkuk University in 2011.

REFERENCES

1. Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. JAMA 1995;274:1049–1057.
2. Stampfer MJ, Malinow MR, Willett WC, et al. A prospective study of plasma homocyst(e)ine and risk of myocardial infarction in US physicians. JAMA 1992;268:877–881.
3. Klee GG. Cobalamin and folate evaluation: Measurement of methylmalonic acid and homocysteine vs vitamin B(12) and folate. Clin Chem 2000;46:1277–1283.
4. Homocysteine Studies Collaboration. Homocysteine and risk of ischemic heart disease and stroke: A meta-analysis. JAMA 2002; 288:2015–2022.
5. Refsum H, Smith AD, Ueland PM, et al. Facts and recommendations about total homocysteine determinations: An expert opinion. Clin Chem 2004;50:3–32.
6. McNulty H, Pentieva K, Hoey L, Ward M. Homocysteine, B-vitamins and CVD. Proc Nutr Soc 2008;67:232–237.
7. de Bree A, Verschuren WM, Blom HJ, Kromhout D. Association between B vitamin intake and plasma homocysteine concentration in the general Dutch population aged 20–65 y. Am J Clin Nutr 2001;73:1027–1033.

8. Jacques PF, Rosenberg IH, Rogers G, et al. Serum total homocysteine concentrations in adolescent and adult Americans: Results from the third National Health and Nutrition Examination Survey. Am J Clin Nutr 1999;69:482–489.
9. Nygard O, Refsum H, Ueland PM, Vollset SE. Major lifestyle determinants of plasma total homocysteine distribution: The Hordaland Homocysteine Study. Am J Clin Nutr 1998;67:263–270.
10. Rasmussen K, Moller J, Lyngbak M, Pedersen AM, Dybkjaer L. Age- and gender-specific reference intervals for total homocysteine and methylmalonic acid in plasma before and after vitamin supplementation. Clin Chem 1996;42:630–636.
11. Refsum H, Nurk E, Smith AD, et al. The Hordaland Homocysteine Study: A community-based study of homocysteine, its determinants, and associations with disease. J Nutr 2006;136: 1731S–1740S.
12. Nauck M, Bisse E, Wieland H. Pre-analytical conditions affecting the determination of the plasma homocysteine concentration. Clin Chem Lab Med 2001;39:675–680.
13. Clinical and Laboratory Standards Institute. How to define and determine reference intervals in the clinical laboratory; approved guideline- second edition (CLSI document C28-A). Wayne, PA: Clinical and Laboratory Standards Institute; 2000.
14. Refsum H, Ueland PM, Nygard O, Vollset SE. Homocysteine and cardiovascular disease. Annu Rev Med 1998;49:31–62.
15. Toole JF, Malinow MR, Chambless LE, et al. Lowering homocysteine in patients with ischemic stroke to prevent recurrent stroke, myocardial infarction, and death: The Vitamin intervention for stroke prevention (VISP) randomized controlled trial. JAMA 2004;291:565–575.
16. Albert CM, Cook NR, Gaziano JM, et al. Effect of folic acid and B vitamins on risk of cardiovascular events and total mortality among women at high risk for cardiovascular disease: A randomized trial. JAMA 2008;299:2027–2036.
17. Daly S, Cotter A, Molloy AE, Scott J. Homocysteine and folic acid: Implications for pregnancy. Semin Vasc Med 2005;5: 190–200.
18. Kim HJ, Kim MK, Kim JU, Ha HY, Choi BY. Major determinants of serum homocysteine concentrations in a Korean population. J Korean Med Sci 2010;25:509–516.
19. Hao L, Ma J, Zhu J, et al. High prevalence of hyperhomocysteinemia in Chinese adults is associated with low folate, vitamin B-12, and vitamin B-6 status. J Nutr 2007;137:407–413.
20. Chen KJ, Pan WH, Yang FL, et al. Association of B vitamins status and homocysteine levels in elderly Taiwanese. Asia Pac J Clin Nutr 2005;14:250–255.
21. Lim HS, Heo YR. Plasma total homocysteine, folate, and vitamin B12 status in Korean adults. J Nutr Sci Vitaminol (Tokyo) 2002; 48:290–297.
22. Adachi H, Hirai Y, Fujiura Y, et al. Plasma homocysteine levels and atherosclerosis in Japan: Epidemiological study by use of carotid ultrasonography. Stroke 2002;33:2177–2181.
23. Saw SM, Yuan JM, Ong CN, et al. Genetic, dietary, and other lifestyle determinants of plasma homocysteine concentrations in middle-aged and older Chinese men and women in Singapore. Am J Clin Nutr 2001;73:232–239.
24. Must A, Jacques PF, Rogers G, Rosenberg IH, Selhub J. Serum total homocysteine concentrations in children and adolescents: Results from the third National Health and Nutrition Examination Survey (NHANES III). J Nutr 2003;133: 2643–2649.
25. Selhub J, Jacques PF, Bostom AG, Wilson PW, Rosenberg IH. Relationship between plasma homocysteine and vitamin status in the Framingham study population. Impact of folic acid fortification. Public Health Rev 2000;28:117–145.
26. Selhub J, Jacques PF, Rosenberg IH, et al. Serum total homocysteine concentrations in the third National Health and Nutrition Examination Survey (1991–1994): Population reference ranges and contribution of vitamin status to high serum concentrations. Ann Intern Med 1999;131:331–339.
27. Wald DS, Bishop L, Wald NJ, et al. Randomized trial of folic acid supplementation and serum homocysteine levels. Arch Intern Med 2001;161:695–700.
28. Rasmussen K, Moller J. Total homocysteine measurement in clinical practice. Ann Clin Biochem 2000;37:627–648.
29. Lonn E, Yusuf S, Arnold MJ, et al. Homocysteine lowering with folic acid and B vitamins in vascular disease. N Engl J Med 2006; 354:1567–1577.
30. Bonaa KH, Njolstad I, Ueland PM, et al. Homocysteine lowering and cardiovascular events after acute myocardial infarction. N Engl J Med 2006;354:1578–1588.
31. Husemoen LL, Linneberg A, Fenger M, Thuesen BH, Jorgensen T. Changes in lifestyle, biological risk factors and total homocysteine in relation to MTHFR C677T genotype: A 5-year follow-up study. Eur J Clin Nutr 2009;63:1233–1240.
32. Ebbing M, Bonaa KH, Nygard O, et al. Cancer incidence and mortality after treatment with folic acid and vitamin B12. JAMA 2009;302:2119–2126.
33. Yang Q, Botto LD, Erickson JD, et al. Improvement in stroke mortality in Canada and the United States, 1990 to 2002. Circulation 2006;113:1335–1343.