28

Lifestyle Factors Associated with Hyperhomocysteinemia

STEIN EMIL VOLLSET
HELGA REFSUM
OTTAR NYGÅRD
PER MAGNE UELAND

Hyperhomocysteinemia has somewhat arbitrarily been divided into *mild* (15 to 30 μmol/L), *intermediate* (30 to 100 μmol/L), or *severe* (≥ 100 μmol/L) forms. Causes of severe hyperhomocysteinemia include inborn errors of metabolism (homocystinuria) and severe cobalamin deficiency. Intermediate hyperhomocysteinemia is often caused by severe renal failure, moderate cobalamin deficiency, cobalamin antagonists (e.g., nitrous oxide), severe folate deficiency, or thermolabile 5,10-methylenetetrahydrofolate reductase (MTHFR) combined with low folate status. Finally, mild hyperhomocysteinemia is associated with drug use (antiepileptic drugs, methotrexate), renal failure, hypothyroidism, hyperproliferative disorders, thermolabile MTHFR, and subtle to moderate folate or cobalamin deficiencies (76).

A series of lifestyle factors, such as dietary habits, smoking, coffee drinking, alcohol consumption, and physical activity, are also associated with total homocysteine level differences and may predispose to hyperhomocysteinemia. This chapter reviews the epidemiological evidence regarding the relationship between these lifestyle factors and total homocysteine levels.

The Distribution of Total Homocysteine

The typical distribution of total homocysteine in the general adult population is skewed with a long thin tail toward high values. The largest data set on total homocysteine is the Hordaland Homocysteine Study, which collected data in Hordaland County, Norway, in 1992–1993 (66). Summary measures of the distribution of homocysteine in this population-based sample of 18,044 men and women aged 40 to 67 years are

Table 28.1 Characteristics of the Plasma Total Homocysteine Distribution in the Hordaland Homocysteine Study, 1992–1993

		Proportion with Hyperhomocysteinemia (%)			Percentiles of the Plasma Total Homocysteine (μmol/L) Distributions					
	Number of Subjects	Mild (15–29.99 μmol/L)	Intermediate (30–99.99 μmol/L)	Severe (≥100 μmol/L)	2.5th	5th	50th (median)	95th	97.5th	99th
All	18,044	8.5	0.80	0.017	6.2	6.7	10.2	17.1	20.3	28.3
Women										
40–42 y	6,485	4.3	0.52	0	5.6	6.0	8.9	14.8	17.7	24.5
43–64 y	347	5.2	1.15	0	5.8	6.5	9.5	16.5	19.4	[a]
65–67 y	2,639	11.6	0.80	0	6.8	7.3	10.8	17.7	20.8	26.7
Men										
40–42 y	6,110	8.1	0.98	0.016	7.0	7.5	10.5	17.0	20.9	29.9
43–64 y	336	8.0	1.19	0	7.3	7.7	11.0	17.9	21.4	[a]
65–67 y	2,127	18.9	1.03	0.094	8.0	8.4	12.1	19.7	23.3	33.1

[a] Number of observations too low to allow precise estimate of 99th percentile.

Ralph Carmel and Donald W. Jacobsen, eds. *Homocysteine in Health and Disease.*

given in Table 28.1 and show that concentrations increase with age and are higher in men than in women. Mild hyperhomocysteinemia was present in 8.5% of the subjects, intermediate hyperhomocysteinemia in 0.8%, and severe in 0.02%. Whereas the prevalence of mild hyperhomocysteinemia was higher in men than in women, and more than twice as high in those 65 to 67 years old compared with those 40 to 42 years old, the prevalence of intermediate hyperhomocysteinemia showed a weaker relation to gender and almost no relation to age. Detailed data on the distribution of total homocysteine have also been reported from the Third US National Health and Nutrition Examination Survey (43) and in a large study of American schoolchildren (69).

Lifestyle Factors and Total Homocysteine Levels

This section concerns the epidemiological evidence regarding the associations between lifestyle factors and serum/plasma total homocysteine levels. Results are presented separately for each factor. The review of homocysteine and diet is confined to studies that estimated intake of nutrients, foods, or food groups and does not include vitamin trials or correlation studies based on blood levels of vitamins or nutrients only, which are presented in other chapters. Table 28.2 lists the design features of large cross-sectional and other studies that have reported on lifestyle factors and homocysteine.

Smoking

A rationale for assessing the relationship between smoking and total homocysteine levels is provided by the association between B-vitamin status and smoking (68, 72, 94). Some (6, 10, 19, 28, 54, 57, 66, 69, 80, 82, 85, 87, 91), but not all (1, 3, 4, 13, 26, 29, 32, 52, 71), studies have shown an association between smoking and homocysteine. Only a few of these studies have accounted for potential confounding by folate intake, age, gender, and other factors that are associated with both homocysteine and smoking.

Table 28.2. Studies of Total Homocysteine and Lifestyle Factors

Study Description	n[a]	Key Characteristics	Key Variables	References
The Atherosclerosis Risk in Communities Study. The cohort was established in 1987–1989 to investigate atherosclerotic disease in middle-aged individuals. The study recruited 15,792 men and women from four US communities. Diet was assessed by a 66-food item, semiquantitative food frequency questionnaire (98). Total homocysteine was measured in subsamples in connection with a case-control and a case-cohort study.	318[b] 537[c]	M+F 45–64 y	Diet Smoking Nutrient intakes Coffee	82 26 62
The Boston Area Health Study. A case-control study included 340 cases of first myocardial infarction in 1982–1983 and 339 matched controls. Homocysteine and related metabolites were determined in 130 cases and 118 matched controls in 1993. Diet was assessed by a 116-item food frequency questionnaire (98).	118[a]	M+F <76 y	Diet Nutrient intakes	93
The Caerphilly cohort. The cohort of 2,512 participants was established in 1979–1983 by inviting all men of Caerphilly and surrounding villages in South Wales. Willing subjects were seen again in 5 years, and the cohort was augmented with men who had moved to the area, totaling 2,398 men in 1988. A detailed food frequency questionnaire (100) was used.	2,290	M adults	Diet Smoking Alcohol Nutrient intakes	91
The Child and Adolescent Trial for Cardiovascular Health. The cohort of 5,016 children was established in 1991–1992 from 96 public elementary schools in four US states. Total homocysteine was measured in a subsample of children.	3,524	M+F 13–14 y	Smoking Vitamins	69
European Atherosclerosis Research Study II. The study recruited male university students in 1993 whose fathers had suffered myocardial infarction before the age of 55, along with age-matched control subjects.	788	M 22–25 y	Smoking Alcohol Physical activity	32

Study Description	n[a]	Key Characteristics	Key Variables	References
Children with familial hypercholesterolemia study. Children with familial hypercholesterolemia were studied and diet assessed with a 190-food item quantitative food frequency questionnaire (27).	154	M+F 7–17 y FH	Diet Nutrient intakes	89
The Framingham Heart Study. The cohort consisted initially (1948–1950) of 5,209 men and women aged 30–62 years in Massachusetts. The participants were examined biennially. In 1988–1989, new blood samples were drawn and diet was assessed in 887 individuals by a 126-item food frequency questionnaire (77).	1,160	M+F 67–96 y	Diet Nutrient intakes	78 90
As part of a case-control study of premature coronary artery disease, control subjects were recruited by random selection from telephone numbers in Georgia. Diet was assessed by a food-frequency questionnaire (7).	108	M 30–50	Smoking Alcohol Physical activity Diet	71
The Hordaland Homocysteine Study. The cohort was established in 1992–1993 as part of a population-based cardiovascular screening in the county of Hordaland, Norway. Diet was assessed by questions on frequency of use of 41 food items/food groups.	18,044	M+F 40–67 y	Age/gender Smoking Coffee Alcohol Physical activity Diet	66 34 75 64 95 65
A sample of 380 men and 204 women was selected among white-collar employees of Hydro-Quebec in Montréal.	584	M+F 23–59 y	Smoking Alcohol Physical activity Diet	52
The Malmø study recruited a population-based sample of adults in the southern Swedish cities of Malmø and Lund.	244	M+F 35–95 y	Vitamins Smoking	13
The UK National Diet and Nutrition Survey. Participants, aged 65 years and over, were recruited in 1994–1995 from randomly selected postal code sectors with substratification by age and gender. Homocysteine was measured in a subgroup who gave diet information (4-day weighted dietary record with data on supplemental vitamin intake).	972	M+F 65 y and older	Diet Smoking Nutrient intakes	3
The National Health and Nutrition Examination Survey III. During 1988–1994, nationally representative information was obtained on health and nutritional status from about 40,000 individuals in the United States. Homocysteine was measured in surplus sera obtained from a subset in a later phase.	8,585	M+F 12 y and older	Age/gender Race/ethnicity Educational level Smoking	28 43
The New Mexico Aging Process Study. Participants were selected from the study cohort. Food frequency information was assessed by a slight modification of the Health Habits and History Questionnaire (8).	88	M+F 68–96 y	Diet Nutrient intakes	48
The North Sea Study. A cross-sectional study of oil production platform workers in the Norwegian sector of the North Sea assessed diet by the 24-hour recall method and a questionnaire on frequency of intake both during the periods the the men lived at home and on the platform.	310	M 21–59 y	Diet Nutrient intakes Smoking	70
The Ontario Study. Adolescent females were recruited from various sources to study relations between B-vitamin/homocysteine status and use of oral contraceptives, alcohol, and smoking. Diet was assessed using a 3-day weighed food record.	229	F 14–20 y	Diet Nutrient intakes Smoking Alcohol	29
The Oslo Hyperlipidemic Smokers Trial. Male hyperlipidemic smokers were recruited to a randomized double-blind	42 × 2	M 40–60 y	Diet Nutrient intakes	15

(Continues)

Table 28.2. *(Continued)*

Study Description	n[a]	Key Characteristics	Key Variables	References
factorial intervention trial with ω-3 fatty acids and antioxidants (vitamin E,C, β-carotene and coenzyme Q10). Diet was assessed with a self-administered, quantitative, food frequency questionnaire (27). Total homocysteine was measured before and after 6 weeks of intervention.		Smokers Hyperlipidemia		
The Physicians' Health Study. During 1982–84, 22,071 US male physicians were recruited for a randomized 2 × 2 factorial trial with aspirin and β-carotene. Lifestyle assessment included information on vitamin supplements and breakfast cereals. Use of other dietary items was assessed by a limited food-frequency questionnaire focusing on vitamin A and carotene. Plasma samples were obtained from 14,916 of the participants and homocysteine levels were determined in subsamples in connection with case-control studies.	290[b] 427[b]	M 40–84 y	Diet Smoking Nutrient intakes Alcohol	83 92 54
The Vitamin, Teachers, and Longevity Study. The pilot study enrolled 297 men and women from 4,774 retired teachers who were approached for a trial of antioxidant vitamin supplements. Diet history was obtained with the 1992 version of the Health Habits and History Questionnaire, a semiquantitative food frequency questionnaire (8).	260	M+F Retired teachers	Diet Nutrient intakes Coffee	86

[a] Number of homocysteine measurements.
[b] Number of controls in case-control study.
[c] Size of cohort sample from a case-cohort study.
M, male; F, female; y, years; FH, familial hypercholesterolemia.

The first large study to address these issues was the Hordaland Homocysteine Study, where the relation between cigarette smoking and total homocysteine was studied in 16,176 men and women aged 40 to 67 years (66). There was a strong and graded association between the daily number of cigarettes smoked and homocysteine levels. The association was stronger in women than in men and stronger among elderly subjects. Former smokers had homocysteine levels similar to those of persons who never smoked. The age- and gender-adjusted estimated difference in mean total homocysteine between heavy smokers (≥20 cigarettes/day) and those who never smoked was 1.9 μmol/L. Further adjustment for intake of fruit and vegetables, use of vitamin supplements, and other possible confounders attenuated the estimated difference to 1.7 μmol/L (p-trend <0.001) (66).

A multiple regression analysis based on 293 cases of myocardial infarction and 290 control cases within the Physicians' Health Study showed a similar association between smoking and homocysteine (54). The contrast between current smokers and nonsmokers was 2.3 μmol/L before and 1.9 μmol/L after adjustment for multivitamin use and plasma folate status.

A third study was a population-based survey carried out in indigenous Australians (80). In this study of 365 men and women, the crude mean difference in total homocysteine levels between current smokers and those who never smoked was 1.5 μmol/L. The mean total homocysteine level of ex-smokers was intermediate. The smoking-homocysteine relation was strengthened by adjustment for age, sex, levels of folate and cobalamin, and other determinants of homocysteine.

In summary, although a relationship between smoking and homocysteine levels is not a consistent finding across studies, it is well supported by a series of investigations. Several detailed analyses have shown that smoking may be associated with a 1.5 to 2.0 μmol/L elevation of homocysteine levels. This is compatible with reports of low folate, vitamin B_6, and cobalamin status in smokers compared with nonsmokers (68, 72, 94). Because smokers usually eat less fruit and vegetables and use fewer vitamin supplements than do nonsmokers (5, 51, 61, 101), confounding with folate intake is likely to contribute to at least part of the smoking-homocysteine association. However, since the association remained in the studies after adjusting

for these factors, a diet-independent smoking effect on total homocysteine may exist.

Coffee Consumption

An unexpected finding in the Hordaland Homocysteine Study (64) was a strong, graded relation between the number of cups of coffee consumed per day and plasma total homocysteine. The participants were characterized by a high intake of predominantly filtered coffee. An age-gender adjusted difference of 2.5 μmol/L was observed between consumers of nine or more cups of coffee per day (974 individuals, or 6%) and subjects not drinking coffee. The association was observed among nonsmokers as well as smokers and remained strong after additional adjustments for smoking and intake of fruit, vegetables, and vitamin supplements (adjusted difference 1.7 μmol/L, p-trend < 0.005).

The coffee-homocysteine relationship was investigated in two other populations. In 537 participants of the Atherosclerosis Risk in Communities Study cohort, no association between coffee consumption and total homocysteine was found (62). In contrast, a significant trend (p = 0.01) of increasing homocysteine levels with coffee consumption was reported in a study of 260 retired schoolteachers in Baltimore (86). In a regression model accounting for folate intake, dietary protein, and serum creatinine, the contrast between the users of 3 to 9 cups/day and nonusers of coffee was 1.3 μmol/L. Like the participants of the Atherosclerosis Risk in Communities Study, these individuals were characterized by a lower consumption of coffee than the Hordaland cohort members.

In conclusion, two studies have found that high consumption of coffee is associated with a 1.3 to 1.7 μmol/L elevation in homocysteine after adjustment for folate and other lifestyle factors (64, 86). Although coffee use and smoking are highly correlated (42, 64), the latter is not an important confounder, because in the Hordaland Study, the association was also observed in nonsmokers, and very few of the Baltimore study (86) subjects smoked. However, as coffee drinking is negatively associated with intake of fruit and vegetables and vitamin supplements (5, 42, 64), residual confounding by folate intake cannot be ruled out.

Alcohol Consumption

The relationship between alcohol consumption and homocysteine levels was first studied in alcohol abusers. Total homocysteine levels were 10.5 to 11 μmol/L higher in 42 alcoholics hospitalized for alcohol detoxification than in 16 abstinent alcohol-dependent individuals or 23 control subjects (41). The findings were similar in a study of 32 chronic alcoholics and 31 healthy volunteers where mean levels were about 10 μmol/L higher in the former group (p for difference in means < 0.001) (20). The latter study found lower homocysteine levels in beer drinkers than in wine/spirits consumers (mean of 13.8 μmol/L vs 21.2 μmol/L, p = 0.05). B-vitamin status was assessed, and, whereas red blood cell folate and serum vitamin B_6 levels were lower in alcoholics than in control subjects, serum folate and cobalamin levels were higher (20).

The large population-based Caerphilly cohort showed a highly significant but decreasing trend in total homocysteine levels as alcohol consumption increased (91). After adjustment for age, social class, body mass index, smoking, total energy intake, and prevalent heart disease, mean homocysteine level was 1.8 μmol/L higher in alcohol abstainers than in men in the highest quartile of alcohol intake (p-trend < 0.0005). Standardization for folate intake, which was 0.13 mg/day lower among abstainers than among those in the upper intake quartile, greatly diminished the alcohol-homocysteine relationship. The authors attributed the association to the folate content of beer (0.09 mg/L), which was the predominant alcoholic beverage consumed by the cohort members.

The Hordaland Study found weak and quite complex associations between alcohol consumption and homocysteine levels (95). Overall, a shallow U-shaped relation was seen with a maximum homocysteine difference of 0.5 μmol/L between nondrinkers and consumers of 7 to 13 drinks of alcohol per week. After additional adjustment for smoking and intake of fruit and vegetables, the U-shape disappeared and homocysteine was 0.7 μmol/L lower in the maximum alcohol intake group (≥ 14 drinks per week) than among nondrinkers (p-trend < 0.0001). The inverse relationship between alcohol use and homocysteine was significantly stronger among smokers than nonsmokers.

A Canadian study of young women found 13% higher total homocysteine concentrations among alcohol users (29). Other studies have reported either a weak positive (26, 92) or no association (32, 52, 82) between alcohol intake and homocysteine levels.

To summarize, the conflicting results among studies (inverse, no, U-shaped, or positive associations) indicate that the relationship between alcohol consumption and total homocysteine is complex. Two small studies have shown that homocysteine levels are higher in chronic alcohol abusers than in control subjects (20, 41). Beer contains folate and two studies have shown that beer consumption is associated with lower homocysteine levels (20, 91). The latter observation is supported by a Dutch study showing a positive association between alcohol intake and serum folate

levels (16). Future studies need to carefully assess the potential confounding by folate intake and nutritional deficiencies that may accompany alcohol abuse.

B-Vitamin Intake (see also Chapters 23–25)

The vitamins folate, B_6 and cobalamin play a key role in homocysteine regulation and metabolism. Most studies with diet information have quantified the associations between total homocysteine levels and the estimated intakes of these vitamins.

Among 1,160 participants, aged 67 to 96 years, in the Framingham study, plasma levels of folate, vitamin B_6, and cobalamin showed significant inverse relationships with total homocysteine (78). On the other hand, the estimated intakes of folate and vitamin B_6, but not cobalamin, were inversely associated with homocysteine. The analyses of the relation between the intake of each vitamin and homocysteine were adjusted for the intakes of the other two of the key B vitamins. After additional adjustment for age and sex, differences in mean homocysteine levels between the lower and upper intake deciles were 3.3 μmol/L for both folate and vitamin B_6.

Another thorough analysis was done within the Caerphilly cohort (91). After adjustment for age, social class, body mass index, smoking, total energy intake, and heart disease, differences in total homocysteine between lower and upper quintiles of folate, cobalamin, and vitamin B_6 intakes were 3.1, 1.8, and 2.5 μmol/L, respectively, all with highly significant trends ($p < 0.0005$).

Associations between total homocysteine levels and B-vitamin intakes have also been studied in a control group from the Atherosclerosis Risk in Communities Study cohort (82). Among nonusers of vitamin supplements, the difference in homocysteine (after adjustment for age, gender, and smoking) between the lower and upper intake tertiles was 0.9 μmol/L for folate ($p = 0.04$), 1.0 μmol/L for vitamin B_6 ($p = 0.02$), 1.4 μmol/L for cobalamin ($p < 0.01$), 1.1 μmol/L for vitamin B_1 ($p = 0.01$), and 2.1 μmol/L for vitamin B_2 ($p < 0.01$).

The UK National Diet and Nutrition Survey reported on a nationally representative sample of 972 elderly men and women (3). In a multiple regression model with nutrient intakes, age, gender, and domicile category (whether or not institutionalized), significant inverse relationships were found between total homocysteine and both folate and vitamin B_6 intake. The magnitude of the relationship was reported only for folate. The adjusted difference between lower and upper intake deciles was about 4.4 μmol/L after adjustment for confounders including creatinine and cobalamin levels (3).

In the Hordaland study, folate and cobalamin scores were constructed from data on the frequency of use of foods and vitamin supplements. Both combined food and supplement intake scores were significantly and inversely correlated with plasma homocysteine ($r = -0.20$ for the folate score, and $r = -0.12$ for the cobalamin score). Homocysteine differences between the lowest and highest decile of the folate score were 1.7 and 1.8 μmol/L in 40- to 42-year-old men and women, respectively, and 2.4 and 2.7 μmol/L, respectively, in men and women aged 65 to 67 years (65). The difference in total homocysteine between the extreme deciles of the cobalamin score was 0.8 to 2.3 μmol/L in the four main age-gender groups (65).

A laboratory-based study of 95 elderly individuals with abnormal or marginal cobalamin status and 78 control subjects included estimation of cobalamin intake using food frequency questionnaires (40). Although the study failed to show a significant association between cobalamin intake and serum homocysteine levels, the difference in total homocysteine was 2.5 μmol/L between the lower and upper quintiles of cobalamin intake.

Unadjusted analyses in 271 controls in the Physicians' Health Study (83) showed significant negative correlations ranging from –0.27 to –0.36 between homocysteine and the estimated intakes of vitamins B_1, B_2, B_6, cobalamin, folate, and niacin (all $p < 0.001$). Among 118 control subjects in a Boston area case-control study, homocysteine showed a weaker relationship with folate intake and no association with vitamin B_6 and cobalamin intakes (93). A study of 154 children with familial hypercholesterolemia found homocysteine to be inversely associated with folate intake ($r = -0.23$, $p = 0.007$) (89).

In summary, the observed differences in total homocysteine between contrasting intakes of the key B vitamins, folate, B_6, and cobalamin, range from 1 to 4 μmol/L and are strongest for folate and weakest for cobalamin. The homocysteine-lowering effects of folate and cobalamin, but not vitamin B_6, have been established in intervention trials (39). Intakes of the vitamins B_1, B_2, and niacin have also been shown to correlate with homocysteine levels (82, 83). Strong correlations between intakes of the various B vitamins (47, 93) should be taken into account when considering homocysteine associations with B vitamins other than folate and cobalamin.

Intake of Non-B Vitamins and Other Nutrients

Several studies have reported inverse association between homocysteine levels and intakes of vitamin A (15, 82, 83), vitamin C (15, 83, 89), and vitamin E (83). No associations between the intakes of any of these vitamins and homocysteine were seen in another study (71). Two studies have reported an inverse asso-

ciation between phosphorus intake and homocysteine levels (3, 82). We are aware of only single reports of associations between homocysteine levels and intakes of calcium (82), magnesium (3), and sodium (3). The reported association with calcium was inverse, and the positive associations between homocysteine and magnesium and sodium were adjusted for folate intake.

Intake of non-B vitamins and other nutrients may correlate with the intakes of folate and cobalamin, which have an established homocysteine lowering effect. Most studies did not account for this possible confounding, and the results have not been supported by intervention studies. Neither a trial with antioxidants (vitamin E, vitamin C, β-carotene, and coenzyme Q10) (15) nor a trial with high-dose vitamin C (9) showed any effect on homocysteine levels.

Protein Intake

Homocysteine in humans is formed from the essential amino acid methionine (23), and methionine or a protein-rich meal causes an acute increase in total homocysteine (33). Therefore, it was suspected that a diet rich in protein or methionine would be associated with elevated homocysteine levels.

The most careful study on long-term dietary protein intake and total homocysteine reported a strong inverse relation between energy-adjusted protein intake and homocysteine levels (86). The difference in total homocysteine levels between the lower and upper quintiles of protein intake was about 3.1 μmol/L (p = 0.008) after adjustment for energy-adjusted dietary folate intake, use of vitamin supplements, serum creatinine, dietary energy, and coffee use.

Other studies have also showed an inverse association between the usual dietary intake of protein or methionine and homocysteine levels. Among 49 individuals not taking B-vitamin supplements, an inverse association between protein intake and homocysteine existed that was nonsignificant overall (r = –0.23, p = 0.13) but highly significant among women (r = –0.45, $p < 0.01$) (48). A negative association between dietary methionine intake and homocysteine was also seen among 118 control subjects in a Boston area case-control study of myocardial infarction (93). The correlation between homocysteine levels and methionine intake was –0.27 (p = 0.003) before and –0.22 (p = 0.01) after adjustment for intake of vitamin B_6, cobalamin, and folate. Homocysteine levels also decreased across groups with increasing methionine and protein intake in nonusers of vitamin supplements among control subjects in the Atherosclerosis Risk in Communities Study (82). For both estimated methionine and total protein intake, homocysteine was 0.8 μmol/L lower in the upper compared with the lower tertile (p = 0.07 for both comparisons after adjustment for age, race, gender, and cigarette smoking). Finally, intake of protein was inversely associated with homocysteine levels among 108 population-based control subjects in a case-control study of premature coronary artery disease (r = –0.20, p = 0.03) (71).

In summary, several studies have reported an inverse relation between dietary intake of protein and homocysteine levels. Whether dietary protein induces a more efficient homocysteine metabolism or the association is due to confounding with other homocysteine-lowering factors is not clear (86).

Consumption of Individual Food Items

Relatively few studies have addressed the relationships between the consumption of individual food items and total homocysteine levels. Such studies are important because the bioavailability of folate (2, 30) and, therefore, the homocysteine response, may vary substantially depending on the food source of folate.

Relations between food items and homocysteine were studied among 885 elderly subjects in the Framingham Heart Study (90). In this study, the three major sources of folate were cold cereals, multivitamins, and orange juice, each contributing 13.3%, 12.8% and 12.4%, respectively, of the total folate intake. The strongest associations with homocysteine were found for breakfast cereals and fruit and vegetables. Differences in mean homocysteine levels between the lower and upper intake quintiles (adjusted for age, sex, energy intake, and vitamin supplement use) were 1.4 μmol/L for breakfast cereals and 1.1 μmol/L for fruit and vegetables. Despite being a major source of dietary folate and significantly associated with plasma folate, consumption of orange juice was not associated with differences in homocysteine levels (78).

In a control set of the Atherosclerosis Risk in Communities study, the strongest associations with homocysteine were found for cereals and milk (82). Among nonusers of vitamin supplements, the difference between the lower and upper intake tertiles was 1.3 μmol/L ($p < 0.01$) for cold cereals and 1.4 μmol/L ($p < 0.01$) for milk. These analyses were adjusted for age, race, gender, and cigarette smoking.

The relationship between diet and homocysteine was studied in 310 oil workers in the North Sea (70). A multiple regression analysis showed that intake of bread (p = 0.01) and vegetables (p = 0.04) was negatively associated with total homocysteine, but intake of fat (p = 0.05) was positively correlated. After adjustment for age and smoking, differences in mean homocysteine levels between the no-intake group and the group with intake above the median were 1.0 μmol/L (p = 0.10) for bread, 0.7 μmol/L (p = 0.04) for skimmed milk, and 2.5

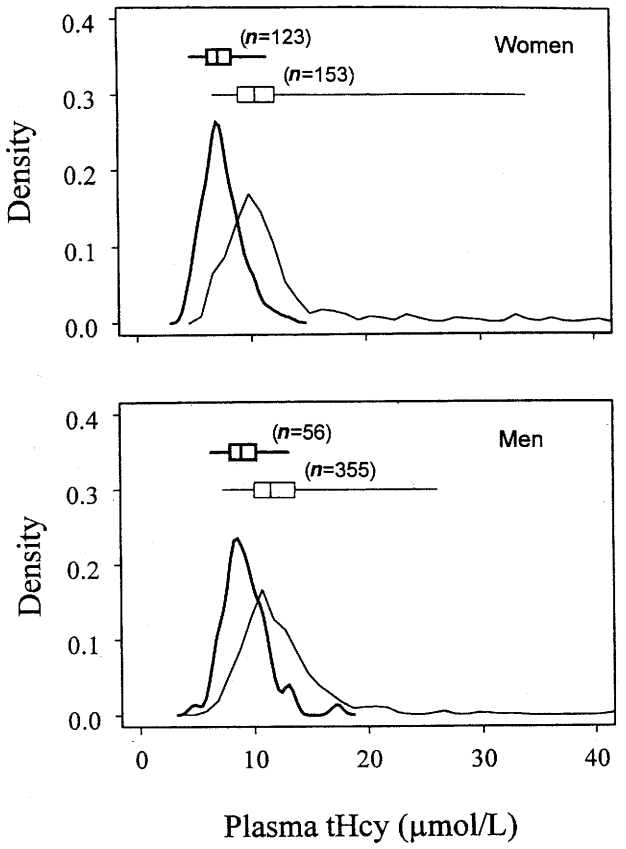

Fig. 28.1. The density distribution of plasma total homocysteine (tHcy) according to contrasting lifestyle groups in 40- to 42-year-old women (top) and men (bottom). The distributions of homocysteine (continuous curves) are shown in the main part of each figure and the same data are presented as box plots at the top of the figure. The curve in boldface represents nonsmokers with high folate intakes and consumption of <1 cup of coffee/day, whereas the thin line represents smokers drinking ≥5 cups of coffee/day and with low folate intakes. Each box plot shows the 25th to the 75th percentile interval, the vertical line inside the box is the median value, and the horizontal line indicates the 2.5th and 97.5th percentile interval. (Reproduced from Nygård et al. [65], with permission.)

μmol/L for vegetables (p = 0.008). The UK National Diet and Nutrition Survey observed significant, inverse associations between total homocysteine and consumption of breakfast cereals and liver but did not report the magnitude of the relationships (3).

The impact on homocysteine levels of the compulsory folate fortification of enriched grain products that started in the United States in 1997 was assessed in the Framingham Offspring Study (44). Among nonusers of B-vitamin supplements, mean homocysteine levels were 0.7 μmol/L (p < 0.001) lower after folate fortification, whereas among supplement users an increase of 0.6 μmol/L (p = 0.006) was observed.

To summarize, individual food items associated with homocysteine levels include breakfast cereals, fruit and vegetables, milk, liver, and bread. These foods are all important sources of folate (16, 90), and differences in homocysteine levels between low and high intakes ranged from 0.7 to 2.5 μmol/L. Orange juice is an example of a folate-rich food item that seems not to be associated with lower homocysteine levels. This may be related to poor bioavailability of folate in orange juice resulting from inhibition of folate deconjugation by organic acids (31). The strongest and most consistent association with homocysteine was found for breakfast cereals, which are commonly fortified with folate and other B-vitamins in the countries where the studies were conducted. Furthermore, a randomized, blinded trial of fortified cereals has shown that daily portions of cereals containing 0.13, 0.50, or 0.67 mg of folic acid taken for 5 weeks reduced homocysteine levels by 0.5 (p = 0.24), 1.7 (p < 0.001), and 2.8 μmol/L (p = 0.001), respectively (56).

Use of Multivitamins

Multivitamins often contain all three key B vitamins involved in homocysteine metabolism (vitamin B_6, cobalamin, and folic acid), along with several other B vitamins and vitamins A, C, D, and E. The composition and doses show considerable variation among products and countries, often as a consequence of legislation regulating the maximum doses of individual vitamins. For example, in the United States, the folate content of multivitamins is usually 0.4 to 0.8 mg, whereas in Norway, multivitamins contain no or, at most, 0.1 to 0.2 mg of folic acid.

In the Framingham Study, the strongest association between homocysteine and any single dietary component was reported for vitamin supplements containing folic acid. After adjustment for age, gender, and total energy intake, the difference in mean homocysteine levels between nonusers and users was 2.7 μmol/L (p < 0.0001) (90). In a Swedish population-based sample of 244 men and women, homocysteine levels were 3.0 μmol/L lower among 31 regular users of multivitamins (with folic acid) than among nonusers (13). In the New Mexico Aging Process Study, the difference in homocysteine levels between nonusers and users of vitamin supplements containing vitamin B_6, cobalamin, and folate was 1.7 μmol/L (48). The Atherosclerosis Risk in Communities study observed homocysteine levels about 1.5 μmol/L lower in users of vitamin supplements than in nonusers (82). In the main age and gender groups of the Hordaland Study, mean homocysteine levels were 1.5 to 2.2 μmol/L lower in nonusers compared with daily users of multivitamin

supplements (65). The difference was larger in 65 to 67 year olds (2.0 in men and 2.2 μmol/L in women) than in the 40 to 42 year olds (1.5 μmol/L in both men and women) (65).

To summarize the data from observational studies, multivitamin supplement users have a mean total homocysteine level that is 1.5 to 3.0 μmol/L lower than among nonusers. This difference is slightly smaller than the typical reduction from 12 μmol/L to 8 to 9 μmol/L estimated from the combined evidence of placebo-controlled trials of folic acid alone or combined with other B vitamins (39).

Fish Oil Intake

A small, cross-over trial first suggested that fish oil may lower homocysteine levels in hyperhomocysteinemic men (67). Another small, cross-over trial reported a significant 10% decrease in homocysteine levels after administration of a mixture of fish oil and evening primrose oil (36). However, the participants also received folic acid and vitamin B_6. There was no difference in homocysteine levels between Greenland Inuits, who have higher intakes of fish oil, and Danes on a standard Western diet (60). A Norwegian trial in hyperlipidemic smokers found no effect of intervention with omega-3 fatty acids on homocysteine levels (15) despite an inverse, pretreatment association between omega-3 fatty acids in serum phospholipids and homocysteine levels ($r = -0.37$, $p < 0.05$). Thus, the current evidence for an effect of fish oil on homocysteine levels is limited and has not been supported by later research.

Weight Reduction

A mean increase of 0.8 μmol/L ($p < 0.0001$) in homocysteine levels was observed during a 3-week period with moderate weight reduction in 11 subjects. The same authors later showed that supplementation with B-vitamins prevented such an increase (37, 38). This evidence for a relationship between weight loss and homocysteine must be regarded as preliminary.

Physical Activity

The relationship between leisure-time physical activity, assessed by a four-category question, and homocysteine was studied in the Hordaland Homocysteine Study (66). A 1.2 μmol/L contrast between the no-activity and heavy-training groups was observed (p-trend < 0.001; adjusted for age and gender). This difference was reduced to 0.4 μmol/L (p-trend < 0.001) after additional adjustment for smoking, intake of fruit and vegetables, and vitamin supplements. However, two other studies found no relation between physical activity and homocysteine (32, 52). Likewise, no significant relation was found between the maximum oxygen uptake capacity, a more objective measure of fitness, and homocysteine levels in a controlled study of 20 healthy men (99).

Lifestyle Patterns and the Homocysteine Distribution

The Hordaland Study compared homocysteine levels in individuals with contrasting lifestyle patterns. The homocysteine differences between individuals characterized by low folate intake, smoking, and high coffee consumption, on the one hand, and nonsmoking individuals with a high folate intake and low coffee consumption, on the other, ranged from 3.2 to 4.8 μmol/L in the four main gender and age groups (65). Similarly, among the elderly Framingham subjects, combining intake of the three main B vitamins—folate, cobalamin, and B_6—produced a contrast of 5.3 μmol/L between the low and high B-vitamin intake groups (78).

The large sample size of the Hordaland study has permitted study of lifestyle-associated differences in the shape of the homocysteine distribution. Figure 28.1 shows the distribution of homocysteine levels among 40- to 42-year-old subjects who smoked and who had high consumption of coffee and low folate intake. For such individuals, the distribution was skewed with a long tail toward high values (Figure 28.1; thin line). In contrast, in nonsmoking subjects with high folate intake and low or no coffee consumption, the homocysteine distribution not only was shifted toward lower values but was almost symmetrical with a much smaller upper tail (thick line).

Age, gender, and the major lifestyle determinants of homocysteine also show different associations with very low or very high homocysteine values (64, 75). Based on data from the Hordaland study, Figure 28.2 shows the associations between extreme values of homocysteine and age, gender, smoking, coffee consumption, and use of vitamin supplements. Only cigarette smoking and nonuse of vitamin supplements showed a relationship with *intermediate* hyperhomocysteinemia, whereas these two factors plus old age and male gender were associated with *mild* hyperhomocysteinemia. The figure also shows the relationships between these five factors and low (5.5 to 6.9 μmol/L) and very low homocysteine levels (< 5.5 μmol/L). These two low ranges of homocysteine values were chosen to contain similar proportions of the population values as mild and intermediate hyperhomocysteinemia, respectively. All five factors were inversely associated with the likelihood of having a low or very low homocysteine value. It is also noteworthy that

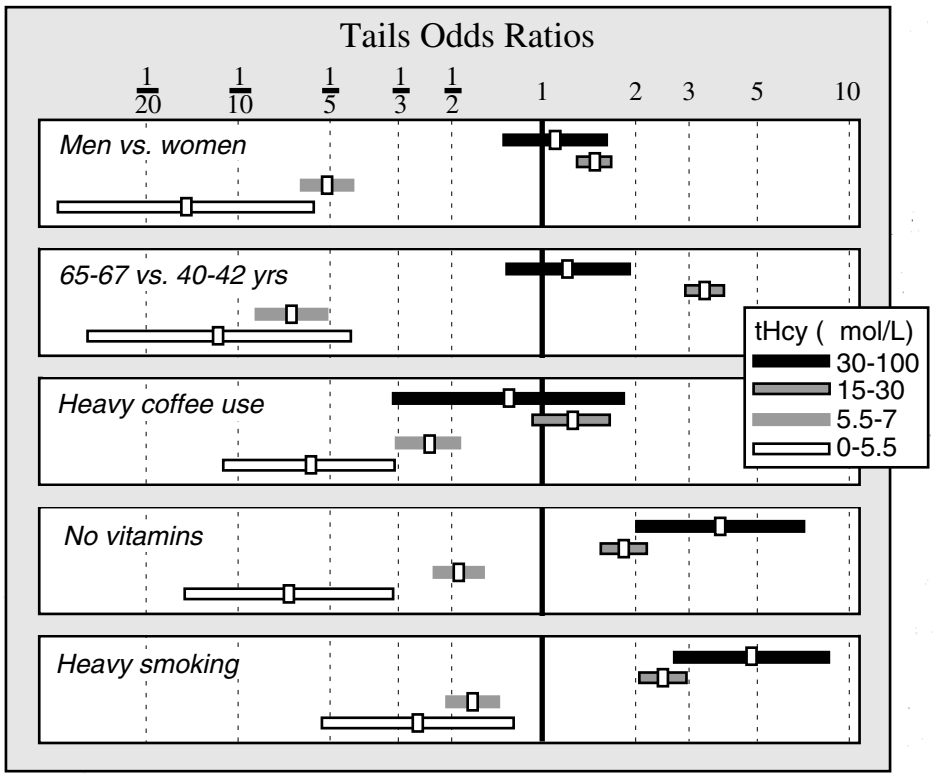

Fig. 28.2. Odds ratios (with 95% confidence limits) of having a high or low value of total homocysteine (tHcy), comparing contrasting categories of cigarette smoking (≥10/day vs. never smoked), use of vitamin supplements (no use vs. frequent use), coffee consumption (≥5 cups/day vs. no use), age (65 to 67 years vs. 40 to 42 years), and gender (men vs. women). Four definitions of high and low total homocysteine levels are used: intermediate (30 to 99.99 μmol/L) and mild hyperhomocysteinemia (15 to 29.99 μmol/L), and low (5.50 to 6.99 μmol/L) and very low (3.6 to 5.49 μmol/L) homocysteine levels, with 0.8%, 8%, 7%, and 0.7% of the population belonging to each of these four homocysteine groups, respectively. The estimated odds ratios are from logistic regression models with all factors present. These odds ratios are also denoted as tails odds ratios as they estimate the risk of having a very low (being in the lower tail of the distribution) or very high homocysteine value (being in the upper tail of the homocysteine distribution).

both old age and male gender decreased the likelihood of having a low homocysteine value more strongly than they increased the risk of hyperhomocysteinemia.

As to the lifestyle factors, the extremes of coffee use and cigarette smoking were associated with about the same contrast in mean homocysteine levels (64, 66). However, coffee use was associated only with a decreased probability of having a low value and not with high homocysteine values. On the other hand, heavy smoking was associated with both a decreased likelihood of having a low homocysteine value and an increased risk of hyperhomocysteinemia. Nonuse of vitamin supplements showed a pattern similar to cigarette smoking. Smoking and nonuse of vitamins were more strongly associated with intermediate than with mild hyperhomocysteinemia, and more strongly with very low (< 5.5 μmol/L) than with low (5.5 to 6.99 μmol/L) homocysteine values. The implications of these differential associations with low and high homocysteine values are unclear, but they suggest different underlying biological mechanisms for the various factors. Furthermore, if hyperhomocysteinemia causes disease, and the lifestyle associations are causal, these results suggest that the effect of coffee on homocysteine levels may be more benign than that of smoking or low vitamin status.

The 677C→T MTHFR Polymorphism and Lifestyle

The 677C→T polymorphism in the MTHFR gene (see Chapter 22) has been studied extensively in relation to homocysteine and folate levels, and to diseases associated with them (14, 49, 53, 79, 81). The homozygous TT genotype is generally associated with reduced MTHFR enzyme activity and with about 25% higher total homocysteine levels than the more common CC genotype (14). Subjects with the TT genotype are susceptible to hyperhomocysteinemia under conditions of impaired folate status (14, 34, 56). They also attain particularly high homocysteine levels during renal failure (24, 25) and after intake of some drugs (35, 88). Notably, elevated homocysteine levels in subjects with

the TT genotype are folate-responsive and are efficiently reduced by folate supplementation (34, 56).

In the Hordaland study, 73% of the 67 individuals found to have hyperhomocysteinemia ≥ 40 μmol/L were of the TT genotype, compared with only 10% of control subjects (33). However, the individuals with high homocysteine levels also had a different lifestyle, characterized by a higher proportion of current smokers (59% vs. 29% in control subjects) and less use of vitamin supplements (2% daily use vs. 13% in control subjects). These observations suggest that smoking may contribute to intermediate hyperhomocysteinemia in individuals with TT genotype. No studies have yet addressed the issue of differential effects by the MTHFR genotype on homocysteine response to smoking or other nondietary lifestyle factors.

Causality, Confounding, and Consequences

Diet, Other Lifestyle Factors, and Homocysteine

As discussed in Chapters 23 and 24, folate and cobalamin treatment lowers homocysteine levels. A meta-analysis of 12 randomized trials of B-vitamin therapy showed that, on average, folic acid lowers homocysteine levels by 25% and that cobalamin has an additional homocysteine lowering effect of about 7% (39). The observed associations between homocysteine and intake of individual food items that are important sources of folate (fruit, vegetables, liver, milk, bread) are likely to be causal. However, this has been proven and quantified only for fortified breakfast cereals (55).

Because of strong interrelations among age, gender, dietary patterns, smoking, coffee use, alcohol consumption, and physical activity (5, 42, 51, 61, 64, 101), efficient analytical adjustment for age, gender, and folate intake is of paramount importance to avoid confounding in the assessment of lifestyle determinants of homocysteine. Such adjustment is dependent on precise measurement of the confounders, which poses no problem with age and gender. The accurate quantification of folate intake, however, is problematic. Sources of error include both imperfect dietary assessment using food frequency questionnaires or other methods (97) and lack of standardized and precise food composition tables for folate (22).

Identification of nutritional factors that affect homocysteine levels is further complicated by the presence of strong correlations between the dietary intake of many nutrients (17, 47, 82, 86). For example, food items that provide folate frequently contain vitamin B_6 and cobalamin (47). Folate-rich vegetables are also rich in the vitamins A and C and in dietary fiber. Use of multivitamin preparations will further complicate the identification of the vitamins that are responsible for the homocysteine reduction. The difficulty in discriminating between different nutrients was well illustrated in an Australian case-control study of neural tube defects and nutrient intake in early pregnancy. Before folate was included as an adjustment variable in the analyses, equally strong, inverse, and statistically significant relationships with neural tube defects were found for intakes of fiber, calcium, vitamin C, and carotene (12). Thus, correlations with folate may explain the relatively strong associations between intakes of vitamin B_6 and vitamin C and homocysteine levels that until now have been unsupported by intervention trials (9, 15, 39).

Reliable answers to these questions may be obtained only by manipulation of each potential homocysteine-lowering factor in controlled trials. Such studies are complex and expensive but could be carried out for coffee, selected food items, and vitamins. Valuable insight into the role of smoking on homocysteine levels could be obtained by monitoring subjects in smoking cessation programs.

Clinical Implications

Contrasting levels of the various lifestyle factors are associated with 0.5 to 3 μmol/L differences in homocysteine levels; the differences are smallest for physical activity, higher for coffee and smoking, and highest for use of vitamin supplements or foods fortified with folic acid (e.g., breakfast cereals). The combination of extreme settings of several of these factors may be associated with 4 to 5 μmol/L group differences in homocysteine levels, which may be of considerable clinical relevance. Among patients with coronary heart disease, subjects with a homocysteine level of 15 μmol/L have an estimated 60% higher overall mortality compared with those with a level of 10 μmol/L (63). Similar differences in risk have been reported from meta-analyses on homocysteine and ischemic heart disease, where the combined evidence suggests a highly significant 60% to 90% risk enhancement per 5 μmol/L homocysteine increase (11, 21, 96). When only prospective nested case-control studies were considered, the corresponding risk increase was 30% (95% confidence interval: 10% to 50%) (21). Results from ongoing clinical trials with homocysteine-lowering vitamin therapy in secondary prevention of heart disease (18) will provide valuable new information on the clinical importance of B vitamins and homocysteine. This knowledge is crucial to understanding the relevance of the lifestyle-homocysteine relationships.

Homocysteine levels have also been reported to be associated with an increased risk of Alzheimer's disease (19, 58), pregnancy complications (50, 73, 74), congenital malformations (59, 84), and colon cancer

(45); there are ongoing intervention trials with folate in subjects with colorectal adenomas (46). Depending on the still unclarified role of homocysteine in these diseases, the observed lifestyle-homocysteine relationships may contribute to the understanding of the etiology and to possible prevention of some of these clinical conditions.

REFERENCES

1. Alfthan, G, Pekkanen, J, Jauhiainen, M, Pitkäniemi, J, Karvonen, M, Tuomilehto, J, Salonen, JT, Ehnholm, C. Relation of serum homocysteine and lipoprotein(a) concentrations to atherosclerotic disease in a prospective Finnish population based study. *Atherosclerosis* 1994; 106: 9–19.
2. Bailey, LB. Dietary reference intakes for folate: The debut of dietary folate equivalents. *Nutr Rev* 1998; 56: 294–99.
3. Bates, CJ, Mansoor, MA, van der Pols, J, Prentice, A, Cole, TJ, Finch, S. Plasma total homocysteine in a representative sample of 972 British men and women aged 65 and over. *Eur J Clin Nutr* 1997; 51: 691–97.
4. Berg, K, Malinow, MR, Kierulf, P, Upson, B. Population variation and genetics of plasma homocyst(e)ine level. *Clin Genet* 1992; 41: 315–21.
5. Berger, J, Wynder, EL. The correlation of epidemiological variables. *J Clin Epidemiol* 1994; 47: 941–52.
6. Bergmark, C, Mansoor, MA, Swedenborg, J, de Faire, U, Svardal, AM, Ueland, PM. Hyperhomocysteinemia in patients operated for lower extremity ischaemia below the age of 50. Effect of smoking and extent of disease. *Eur J Vasc Surg* 1993; 7: 391–96.
7. Block, G. *A Data-Based Brief Diet Questionnaire for Epidemiologic Studies*. National Cancer Institute, Bethesda, MD, 1984.
8. Block, G, Hartman, AM, Dresser, CM, Carroll, MD, Gannon, J, Gardner, L. A data-based approach to diet questionnaire design and testing. *Am J Epidemiol* 1986; 124: 453–69.
9. Bostom, AG, Yanek, L, Hume, AL, Eaton, CB, McQuade, W, Nadeau, M, Perrone, G, Jacques, PF, Selhub, J. High dose ascorbate supplementation fails to affect plasma homocyst(e)ine levels in patients with coronary heart disease. *Atherosclerosis* 1994; 111: 267–70.
10. Bots, ML, Launer, LJ, Lindemans, J, Hoes, AW, Hofman, A, Witteman, JCM, Koudstaal, PJ, Grobbee, DE. Homocysteine and short-term risk of myocardial infarction and stroke in the elderly. *Arch Intern Med* 1999; 159: 38–44.
11. Boushey, CJ, Beresford, SAA, 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: 1049–57.
12. Bower, C, Stanley, FJ. Dietary folate as a risk factor for neural-tube defects: Evidence from a case-control study in Western Australia. *Med J Aust* 1989; 150: 613–19.
13. Brattström, L, Lindgren, A, Israelsson, B, Andersson, A, Hultberg, B. Homocysteine and cysteine: Determinants of plasma levels in middle-aged and elderly subjects. *J Intern Med* 1994; 236: 633–41.
14. Brattström, L, Wilcken, DEL, Öhrvik, J, Brudin, L. Common methylenetetrahydrofolate reductase gene mutation leads to hyperhomocysteinemia but not to vascular disease. The result of a meta-analysis. *Circulation* 1998; 98: 2520–26.
15. Brude, IR, Finstad, HS, Seljeflot, I, Drevon, CA, Solvoll, K, Sandstad, B, Hjermann, I, Arnesen, H, Nenseter, MS. Plasma homocysteine concentration related to diet, endothelial function and mononuclear gene expression among male hyperlipidaemic smokers. *Eur J Clin Invest* 1999; 29: 100–8.
16. Brussaard, JH, Lowik, MRH, van den Berg, H, Brants, HAM, Goldbohm, RA. Folate intake and status among adults in the Netherlands. *Eur J Clin Nutr* 1997; 51: S46–S50.
17. Chasan-Taber, L, Selhub, J, Rosenberg, IH, Malinow, MR, Terry, P, Tishler, PV, Willett, W, Hennekens, CH, Stampfer, MJ. A prospective study of folate and vitamin B6 and risk of myocardial infarction in US physicians. *J Am Coll Nutr* 1996;5:36–43.
18. Clarke, R, Collins, R. Can dietary supplements with folic acid or vitamin B6 reduce cardiovascular risk? Design of clinical trials to test the homocysteine hypothesis of vascular disease. *J Cardiovasc Risk* 1998; 5: 249–55.
19. Clarke, R, Smith, AD, Jobst, KA, Refsum, H, Sutton, L, Ueland, PM. Folate, vitamin B12, and serum total homocysteine levels in confirmed Alzheimer disease. *Arch Neurol* 1998; 55: 1449–55.
20. Cravo, ML, Glória, LM, Selhub, J, Nadeau, MR, Camilo, ME, Resende, MP, Cardoso, JN, Leitão, CN, Mira, FC. Hyperhomocysteinemia in chronic alcoholism: Correlation with folate, vitamin B-12, and vitamin B-6 status. *Am J Clin Nutr* 1996; 63: 220–24.
21. Danesh, J, Lewington, S. Plasma homocysteine and coronary heart disease: Systematic review of published epidemiological studies. *J Cardiovasc Risk* 1998; 5: 229–32.
22. Deharveng, G, Charrondière, UR, Slimani, N, Southgate, DAT, Riboli, E. Comparison of nutrients in the food composition tables available in the nine European countries participating in EPIC. *Eur J Clin Nutr* 1999; 53: 60–79.
23. Finkelstein, JD. Methionine metabolism in mammals. *J Nutr Biochem* 1990; 1: 228–37.
24. Fodinger, M, Mannhalter, C, Wolfl, G, Pabinger, I, Muller, E, Schmid, R, Horl, WH, Sunder-Plassmann, G. Mutation (677 C to T) in the methylenetetrahydrofolate reductase gene aggravates hyperhomocysteinemia in hemodialysis patients. *Kidney Int* 1997; 52: 517–23.
25. Fodinger, M, Wolfl, G, Fischer, G, Rasoul-Rockenschaub, S, Schmid, R, Horl, WH, Sunder-Plassmann, G. Effect of MTHFR 677C>T on plasma total homocysteine levels in renal graft recipients. *Kidney Int* 1999; 55: 1072–80.
26. Folsom, AR, Nieto, FJ, McGovern, PG, Tsai, MY, Malinow, MR, Eckfeldt, JH, Hess, DL, Davis, CE. Prospective study of coronary heart disease incidence in relation to fasting total homocysteine, related genetic polymorphisms and B vitamins: The Atherosclerosis

Risk in Communities (ARIC) study. *Circulation* 1998; 98: 204–10.
27. Frost Andersen, L, Nes, M, Lillegaard, IT, Sandstad, B, Bjørneboe, GE, Drevon, CA. Evaluation of a quantitative food frequency questionnaire used in a group of Norwegian adolescents. *Eur J Clin Nutr* 1995; 49: 543–54.
28. Giles, WH, Croft, JB, Greenlund, KJ, Ford, ES, Kittner, SJ. Total homocyst(e)ine concentration and the likelihood of nonfatal stroke: Results from the third National Health and Nutrition Examination Survey, 1988–1994. *Stroke* 1998; 29: 2473–77.
29. Green, TJ, Houghton, LA, Donovan, U, Gibson, RS, O'Connor, DL. Oral contraceptives did not affect biochemical folate indexes and homocysteine concentrations in adolescent females. *J Am Diet Assoc* 1998; 98: 49–55.
30. Gregory, JF. The bioavailability of folate. In *Folate in Health and Disease*. (Bailey, LB, ed.). Marcel Dekker, Inc, New York, 1995; pp. 195–235.
31. Gregory, JF, Wei, MM. Organic acids in selected foods inhibit intestinal brush border pteroylpolyglutamate hydrolase in vitro: Potential mechanism affecting the bioavailability of dietary polyglutamyl folate. *J Agric Food Chem* 1998; 46: 211–19.
32. Gudnason, V, Stansbie, D, Scott, J, Bowron, A, Nicaud, V, Humphries, S, on behalf of the EARS group S. C677T (thermolabile alanine/valine) polymorphism in methylenetetrahydrofolate reductase (MTHFR): Its frequency and impact on plasma homocysteine concentration in different European populations. *Atherosclerosis* 1998; 136: 347–54.
33. Guttormsen, AB, Schneede, J, Fiskerstrand, T, Ueland, PM, Refsum, HM. Plasma concentrations of homocysteine and other aminothiol compounds are related to food intake in healthy human subjects. *J Nutr* 1994; 124: 1934–41.
34. Guttormsen, AB, Ueland, PM, Nesthus, I, Nygård, O, Schneede, J, Vollset, SE, Refsum, H. Determinants and vitamin responsiveness of intermediate hyperhomocysteinemia (≥40 μmol/liter). The Hordaland Homocysteine Study. *J Clin Invest* 1996; 98: 2174–83.
35. Haagsma, CJ, Blom, HJ, van Riel, PL, van't Hof, MA, Giesendorf, BA, van Oppenraaij-Emmerzaal, D, van de Putte, LB. Influence of sulphasalazine, methotrexate, and the combination of both on plasma homocysteine concentrations in patients with rheumatoid arthritis. *Ann Rheum Dis* 1999; 58: 79–84.
36. Haglund, O, Wallin, R, Wretling, S, Hultberg, B, Saldeen, T. Effects of fish oil alone and combined with long chain (n-6) fatty acids on some coronary risk factors in male subjects. *J Nutr Biochem* 1998; 9: 629–35.
37. Henning, BF, Tepel, M, Riezler, R, Doberauer, C. Unfavourable changes in homocysteine metabolism during weight reduction. *Med Sci Res* 1997; 25: 555–56.
38. Henning, BF, Tepel, M, Riezler, R, Gillessen, A, Doberauer, C. Vitamin supplementation during weight reduction—favourable effect on homocysteine metabolism. *Res Exp Med* 1998; 198: 37–42.
39. Homocysteine Lowering Trialists' Collaboration. Lowering blood homocysteine with folic acid based supplements: Meta-analysis of randomised trials. *BMJ* 1998; 316: 894–98.
40. Howard, JM, Azen, C, Jacobsen, DW, Green, R, Carmel, R. Dietary intake of cobalamin in elderly people who have abnormal serum cobalamin, methylmalonic acid and homocysteine levels. *Eur J Clin Nutr* 1998; 52: 582–87.
41. Hultberg, B, Berglund, M, Andersson, A, Frank, A. Elevated plasma homocysteine in alcoholics. *Alcohol Clin Exp Res* 1993; 17: 687–89.
42. Jacobsen, BK, Thelle, DS. The Tromsø Heart Study: Is coffee drinking an indicator of a life style with high risk for ischemic heart disease? *Acta Med Scand* 1987; 222: 215–21.
43. Jacques, PF, Rosenberg, IH, Rogers, G, Selhub, J, Bowman, BA, Gunter, EW, Wright, JD, Johnson, CL. 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: 482–89.
44. Jacques, PF, Selhub, J, Bostom, AG, Wilson, PWF, Rosenberg, IH. The effect of folic acid fortification on plasma folate and total homocysteine concentrations. *N Engl J Med* 1999; 340: 1449–54.
45. Kato, I, Dnistrian, AM, Schwartz, M, Toniolo, P, Koenig, K, Shore, RE, Akhmedkhanov, A, Zeleniuch-Jacquotte, A, Riboli, E. Serum folate, homocysteine and colorectal cancer risk in women: A nested case-control study. *Br J Cancer* 1999; 79: 1917–22.
46. Kim, YI. Folate and carcinogenesis: Evidence, mechanisms, and implications. *J Nutr Biochem* 1999;10: 66–88.
47. Koehler, KM, Pareo-Tubbeh, SL, Liang, HC, Romero, LJ, Baumgartner, RN, Garry, PJ. Some vitamin sources relating to plasma homocysteine provide not only folate but also vitamins B-12 and B-6. *J Nutr* 1997;27: 1534–35.
48. Koehler, KM, Romero, LJ, Stauber, PM, Pareo-Tubbeh, SL, Liang, HC, Baumgartner, RN, Garry, PJ, Allen, RH, Stabler, SP. Vitamin supplementation and other variables affecting serum homocysteine and methylmalonic acid concentrations in elderly men and women. *J Am Coll Nutr* 1996; 15: 364–76.
49. Kupferminc, MJ, Eldor, A, Steinman, N, Many, A, Bar-Am, A, Jaffa, A, Fait, G, Lessing, JB. Increased frequency of genetic thrombophilia in women with complications of pregnancy. *N Engl J Med* 1999; 340: 9–13.
50. Laivuori, H, Kaaja, R, Turpeinen, U, Viinikka, L, Ylikorkala, O. Plasma homocysteine levels elevated and inversely related to insulin sensitivity in preeclampsia. *Obstet Gynecol* 1999; 93: 489–93.
51. La Vecchia, C, Negri, E, Franceschi, S, Parazzini, F, Decarli, A. Differences in dietary intake with smoking, alcohol, and education. *Nutr Cancer* 1992; 17: 297–304.
52. Lussier-Cacan, S, Xhignesse, M, Piolot, A, Selhub, J, Davignon, J, Genest, J. Plasma total homocysteine in healthy subjects: Sex-specific relation with biological traits. *Am J Clin Nutr* 1996; 64: 587–93.
53. Ma, J, Stampfer, MJ, Giovannucci, E, Artigas, C, Hunter, DJ, Fuchs, C, Willett, WC, Selhub, J, Hennekens, CH, Rozen, R. Methylenetetrahydrofolate reductase poly-

morphism, dietary interactions, and risk of colorectal cancer. *Cancer Res* 1997; 57: 1098–102.
54. Ma, J, Stampfer, MJ, Hennekens, CH, Frosst, P, Selhub, J, Horsford, J, Malinow, MR, Willett, WC, Rozen, R. Methylenetetrahydrofolate reductase polymorphism, plasma folate, homocysteine, and risk of myocardial infarction in US physicians. *Circulation* 1996; 94: 2410–16.
55. Malinow, MR, Duell, PB, Hess, DL, Anderson, PH, Kruger, WD, Phillipson, BE, Gluckman, RA, Block, PC, Upson, BM. Reduction of plasma homocyst(e)ine levels by breakfast cereal fortified with folic acid in patients with coronary heart disease. *N Engl J Med* 1998; 338: 1009–15.
56. Malinow, MR, Nieto, FJ, Kruger, WD, Duell, PB, Hess, DL, Gluckman, RA, Block, PC, Holzgang, CR, Anderson, PH, Seltzer, D, Upson, B, Lin, QR. The effects of folic acid supplementation on plasma total homocysteine are modulated by multivitamin use and methylenetetrahydrofolate reductase genotypes. *Arterioscler Thromb Vasc Biol* 1997; 17: 1157–62.
57. Mansoor, MA, Bergmark, C, Svardal, AM, Lønning, PE, Ueland, PM. Redox status and protein binding of plasma homocysteine and other aminothiols in patients with early-onset peripheral vascular disease. *Arterioscler Thromb Vasc Biol* 1995; 15: 232–40.
58. McCaddon, A, Davies, G, Hudson, P, Tandy, S, Cattell, H. Total serum homocysteine in senile dementia of Alzheimer type. *Int J Geriatr Psychiatry* 1998; 13: 235–39.
59. Mills, JL, McPartlin, JM, Kirke, PN, Lee, YJ, Conley, MR, Weir, DG, Scott, JM. Homocysteine metabolism in pregnancies complicated by neural-tube defects. *Lancet* 1995; 345: 149–51.
60. Møller, JM, Nielsen, GL, Ekelund, S, Schmidt, EB, Dyerberg, J. Homocysteine in Greenland Inuits. *Thromb Res* 1997; 86: 333–35.
61. Morabia, A, Wynder, EL. Dietary habits of smokers, people who never smoked, and ex-smokers. *Am J Clin Nutr* 1990; 52: 933–37.
62. Nieto, FJ, Comstock, GW, Chambless, LE, Malinow, MR. Coffee consumption and plasma homocyst(e)ine: Results from the Atherosclerosis Risk in Communities Study. *Am J Clin Nutr* 1997; 66: 1475–76.
63. 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: 230–36.
64. Nygård, O, Refsum, H, Ueland, PM, Stensvold, I, Nordrehaug, JE, Kvåle, G, Vollset, SE. Coffee consumption and plasma total homocysteine: The Hordaland Homocysteine Study. *Am J Clin Nutr* 1997; 65: 136–43.
65. Nygård, 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: 263–70.
66. Nygård, O, Vollset, SE, Refsum, H, Stensvold, I, Tverdal, A, Nordrehaug, JE, Ueland, PM, Kvåle, G. Total plasma homocysteine and cardiovascular risk profile. The Hordaland Homocysteine Study. *JAMA* 1995; 274: 1526–33.
67. Olszewski, AJ, McCully, KS. Fish oil decreases serum homocysteine in hyperlipemic men. *Coron Artery Dis* 1993; 4: 53–60.
68. Ortega, RM, Lopez-Sobaler, AM, Gonzalez-Gross, MM, Redondo, RM, Marzana, I, Zamora, MJ, Andres, P. Influence of smoking on folate intake and blood folate concentrations in a group of elderly Spanish men. *J Am Coll Nutr* 1994; 13: 68–72.
69. Osganian, SK, Stampfer, MJ, Spiegelman, D, Rimm, E, Cutler, JA, Feldman, HA, Montgomery, DH, Webber, LS, Lytle, LA, Bausserman, L, Nader, PR. Distribution of and factors associated with serum homocysteine levels in children. Child and adolescent trial for cardiovascular health. *JAMA* 1999; 281: 1189–96.
70. Oshaug, A, Bugge, KH, Refsum, H. Diet, an independent determinant for plasma total homocysteine. A cross sectional study of Norwegian workers on platforms in the North Sea. *Eur J Clin Nutr* 1998; 52: 7–11.
71. Pancharuniti, N, Lewis, CA, Sauberlich, HE, Perkins, LL, Go, RCP, Alvarez, JO, Macaluso, M, Acton, RT, Copeland, RB, Cousins, AL, Gore, TB, Cornwell, PE, Roseman, JM. Plasma homocyst(e)ine, folate, and vitamin B-12 concentrations and risk for early-onset coronary artery disease. *Am J Clin Nutr* 1994; 59: 940–48.
72. Piyathilake, CJ, Macaluso, M, Hine, RJ, Richards, EW, Krumdieck, CL. Local and systemic effects of cigarette smoking on folate and vitamin B-12. *Am J Clin Nutr* 1994; 60: 559–66.
73. Powers, RW, Evans, RW, Majors, AK, Ojimba, JI, Ness, RB, Crombleholme, WR, Roberts, JM. Plasma homocysteine concentration is increased in preeclampsia and is associated with evidence of endothelial activation. *Am J Obstet Gynecol* 1998; 179: 1605–11.
74. Rajkovic, A, Catalano, PM, Malinow, MR. Elevated homocyst(e)ine levels with preeclampsia. *Obstet Gynecol* 1997; 90: 168–71.
75. Refsum, H, Nygård, O, Kvåle, G, Ueland, PM, Vollset, SE. The Hordaland Homocysteine Study: The opposite tails odds ratios reveal differential effects of gender and intake of vitamin supplements at high and low plasma total homocysteine concentrations. *J Nutr* 1996; 126: 1244S–48S.
76. Refsum, H, Ueland, PM, Nygård, O, Vollset, SE. Homocysteine and cardiovascular disease. *Annu Rev Med* 1998; 49: 31–62.
77. Rimm, ED, Giovannucci, EL, Stampfer, MJ, Colditz, GC, Litin, LB, Willett, WC. Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. *Am J Epidemiol* 1992; 135: 1114–26.
78. Selhub, J, Jacques, PF, Wilson, PWF, Rush, D, Rosenberg, IH. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. *JAMA* 1993; 270: 2693–98.
79. Shaw, GM, Rozen, R, Finnell, RH, Wasserman, CR, Lammer, EJ. Maternal vitamin use, genetic variation of infant methylenetetrahydrofolate reductase, and risk for spina bifida. *Am J Epidemiol* 1998; 148: 30–7.
80. Shaw, JTE, McWhinney, B, Tate, JR, Kesting, JB, Marczak, M, Purdie, D, Gibbs, H, Cameron, DP,

Hickman, PE. Plasma homocysteine levels in indigenous Australians. *Med J Aust* 1999; 170: 19–22.
81. Shields, DC, Kirke, PN, Mills, JL, Ramsbottom, D, Molloy, AM, Burke, H, Weir, DG, Scott, JM, Whitehead, AS. The "thermolabile" variant of methylenetetrahydrofolate reductase and neural tube defects: An evaluation of genetic risk and the relative importance of the genotypes of the embryo and the mother. *Am J Hum Genet* 1999; 64: 1045–55.
82. Shimakawa, T, Nieto, FJ, Malinow, MR, Chambless, LE, Schreiner, PJ, Szklo, M. Vitamin intake: A possible determinant of plasma homocyst(e)ine among middle-aged adults. *Ann Epidemiol* 1997; 7: 285–93.
83. Stampfer, MJ, Malinow, MR, Willett, WC, Newcomer, LM, Upson, B, Ullmann, D, Tishler, PV, Hennekens, CH. A prospective study of plasma homocyst(e)ine and risk of myocardial infarction in US physicians. *JAMA* 1992; 268: 877–81.
84. Steegers-Theunissen, RP, Boers, GH, Trijbels, FJ, Finkelstein, JD, Blom, HJ, Thomas, CM, Borm, GF, Wouters, MG, Eskes, TK. Maternal hyperhomocysteinemia: A risk factor for neural-tube defects? *Metabolism* 1994; 43: 1475–80.
85. Stehouwer, CDA, Weijenberg, MP, van den Berg, M, Jakobs, C, Feskens, EJM, Kromhout, D. Serum homocysteine and risk of coronary heart disease and cerebrovascular disease in elderly men: A 10-year follow-up. *Arterioscler Thromb Vasc Biol* 1998; 18: 1895–901.
86. Stolzenberg-Solomon, RZ, Miller, ER, Maguire, MG, Selhub, J, Appel, LJ. Association of dietary protein intake and coffee consumption with serum homocysteine concentrations in an older population. *Am J Clin Nutr* 1999; 69: 467–75.
87. Taylor, LM, Moneta, GL, Sexton, GJ, Schuff, RA, Porter, JM. The Homocysteine and Progression of Atherosclerosis Study Investigators. Prospective blinded study of the relationship between plasma homocysteine and progression of symptomatic peripheral arterial disease. *J Vasc Surg* 1999; 29: 8–19.
88. Tonstad, S, Refsum, H, Ose, L, Ueland, PM. The C677T mutation in the methylenetetrahydrofolate reductase gene predisposes to hyperhomocysteinemia in children with familial hypercholesterolemia treated with cholestyramine. *J Pediatr* 1998; 132: 365–68.
89. Tonstad, S, Refsum, H, Ueland, PM. Association between plasma total homocysteine and parental history of cardiovascular disease in children with familial hypercholesterolemia. *Circulation* 1997; 96: 1803–8.
90. Tucker, KL, Selhub, J, Wilson, PWF, Rosenberg, IH. Dietary intake pattern relates to plasma folate and homocysteine concentrations in the Framingham Heart Study. *J Nutr* 1996; 126: 3025–31.
91. Ubbink, JB, Fehily, AM, Pickering, J, Elwood, PC, Vermaak, WJH. Homocysteine and ischaemic heart disease in the Caerphilly cohort. *Atherosclerosis* 1998; 140: 349–56.
92. Verhoef, P, Hennekens, CH, Malinow, MR, Kok, FJ, Willett, WC, Stampfer, MJ. A prospective study of plasma homocyst(e)ine and risk of ischemic stroke. *Stroke* 1994; 25: 1924–30.
93. Verhoef, P, Stampfer, MJ, Buring, JE, Gaziano, JM, Allen, RH, Stabler, SP, Reynolds, RD, Kok, FJ, Hennekens, CH, Willett, WC. Homocysteine metabolism and risk of myocardial infarction: Relation with vitamins B6, B12, and folate. *Am J Epidemiol* 1996; 143: 845–59.
94. Vermaak, WJH, Ubbink, JB, Barnard, HC, Potgieter, GM, van Jaarsveld, H, Groenewald, AJ. Vitamin B-6 nutrition status and cigarette smoking. *Am J Clin Nutr* 1990; 51: 1058–61.
95. Vollset, SE, Nygård, O, Kvåle, G, Ueland, PM, Refsum, H. The Hordaland Homocysteine Study: Lifestyle and total plasma homocysteine in western Norway. In *Homocysteine Metabolism: From Basic Science to Clinical Medicine.* (Graham, I, Refsum, H, Rosenberg IH, Ueland PM, eds.). Kluwer Academic Publishers, Boston, 1997. pp 177–82.
96. Wald, NJ, Watt, HC, Law, MR, Weir, DG, McPartlin, J, Scott, JM. Homocysteine and ischemic heart disease. Results of a prospective study with implications regarding prevention. *Arch Intern Med* 1998; 158: 862–67.
97. Willett, WC. *Nutritional Epidemiology. Monographs in Epidemiology and Biostatistics,* 2nd ed. Oxford University Press, New York, 1998.
98. Willett, WC, Sampson, L, Stampfer, MJ, Rosner, B, Bain, C, Witschi, J, Hennekens, CH, Speizer, FE. Reproducibility and validity of a semi-quantitative food frequency questionnaire. *Am J Epidemiol* 1985; 122: 51–65.
99. Wright, M, Francis, K, Cornwell, P. Effect of acute exercise on plasma homocysteine. *J Sports Med Phys Fitness* 1998; 38: 262–65.
100. Yarnell, JWG, Fehily, AM, Milbank, JE, Sweetnam, PM, Walker, CL. A short dietary questionnaire for use in an epidemiological survey: Comparison with weighed dietary records. *Hum Nutr Appl Nutr* 1983; 37A: 103–12.
101. Zondervan, KT, Ocké, MC, Smit, HA, Seidell, JC. Do dietary and supplementary intakes of antioxidants differ with smoking status? *Int J Epidemiol* 1996; 25: 70–9.

PUBLISHED BY THE PRESS SYNDICATE OF THE UNIVERSITY OF CAMBRIDGE
The Pitt Building, Trumpington Street, Cambridge, United Kingdom

CAMBRIDGE UNIVERSITY PRESS
The Edinburgh Building, Cambridge CB2 2RU, UK
40 West 20th Street, New York, NY 10011-4211, USA
10 Stamford Road, Oakleigh, Melbourne 3166, Australia
Ruiz de Alarcón 13, 28014 Madrid, Spain
Dock House, The Waterfront, Cape Town 8001, South Africa

http://www.cambridge.org

First published 2001

Printed in the United Kingdom at the University Press, Cambridge

Typefaces Sabon 10/12 and Franklin Gothic *System* Quark XPress™ [HT]

A catalog record for this book is available from the British Library

Library of Congress Cataloging in Publication data

Homocysteine in health and disease / edited by Ralph Carmel, Donald W. Jacobsen.
p. ; cm
Includes bibliographical references and index.
ISBN 0-521-65319-3 (hardback)
1. Homocysteine—Pathophysiology. 2. Homocysteine—Metabolism—Disorders. 3. Homocysteine—Physiological effect. I. Carmel, Ralph. II. Jacobsen, Donald W. (Donald Weldon), 1939–
[DNLM: 1. Hyperhomocysteinemia—physiopathology. 2. Cardiovascular Diseases—etiology. 3. Homocysteine—metabolism. 4. Hyperhomocysteinemia—complications. WD 205.5.A5 H768 2001]
RC632.H65 H66 2001
616.1′071—dc21 00-46795

ISBN 0 521 65319 3 hardback