CME CREDIT

RAJ S. BALLAL, MD
Department of Cardiology, Cleveland Clinic.

DONALD W. JACOBSEN, PHD
Department of Cell Biology, Cleveland Clinic; Professor of Chemistry, Cleveland State University. His research interests are the relationship between homocysteine and cardiovascular disease and the effects of homocysteine on the vascular endothelium

KILLIAN ROBINSON, MD
Department of Cardiology, Cleveland Clinic. He specializes in research on the relationship between homocysteine and vascular disorders.

Homocysteine: Update on a new risk factor

ABSTRACT

A high fasting plasma homocysteine level is an independent risk factor for atherosclerosis and venous thrombosis. Vitamin therapy can lower homocysteine levels, but no benefit has yet been demonstrated; studies using clinical outcomes as endpoints are now in progress.

KEY POINTS

The normal range for plasma homocysteine is 5 to 15 µmol/L, although the upper limit of normal may need to be lowered.

Plasma homocysteine concentrations increase with age and in a number of congenital and acquired conditions, notably renal failure and vitamin deficiency.

Several studies have shown high plasma homocysteine levels to be a strong, independent risk factor for cardiovascular disease. There is now broad agreement that a high plasma homocysteine concentration is also a risk factor for venous thrombosis.

Folic acid supplements reduce elevated homocysteine levels; the dosage should be higher for patients with renal insufficiency than for patients with normal renal function.

IN THE PAST FEW YEARS, considerable advances have been made in our understanding of the relationship between homocysteine and vascular disorders. This review focuses on important recent findings in:

- The clinical epidemiology of hyperhomocysteinemia and vascular disorders.
- The potential mechanism of vascular damage in hyperhomocysteinemia.
- The causes of high homocysteine levels in patients with vascular disease.
- The elevated homocysteine concentrations in patients with end-stage renal disease, organ transplants, and recurrent deep venous thrombosis.
- The effect of supplementation with B vitamins on homocysteine concentrations in patients with vascular disorders.

WHAT IS HOMOCYSTEINE?

Homocysteine, a sulfur-containing amino acid derived from methionine, is essential for a number of vital biochemical processes. It is metabolized by two pathways: remethylation and transsulfuration.

Remethylation

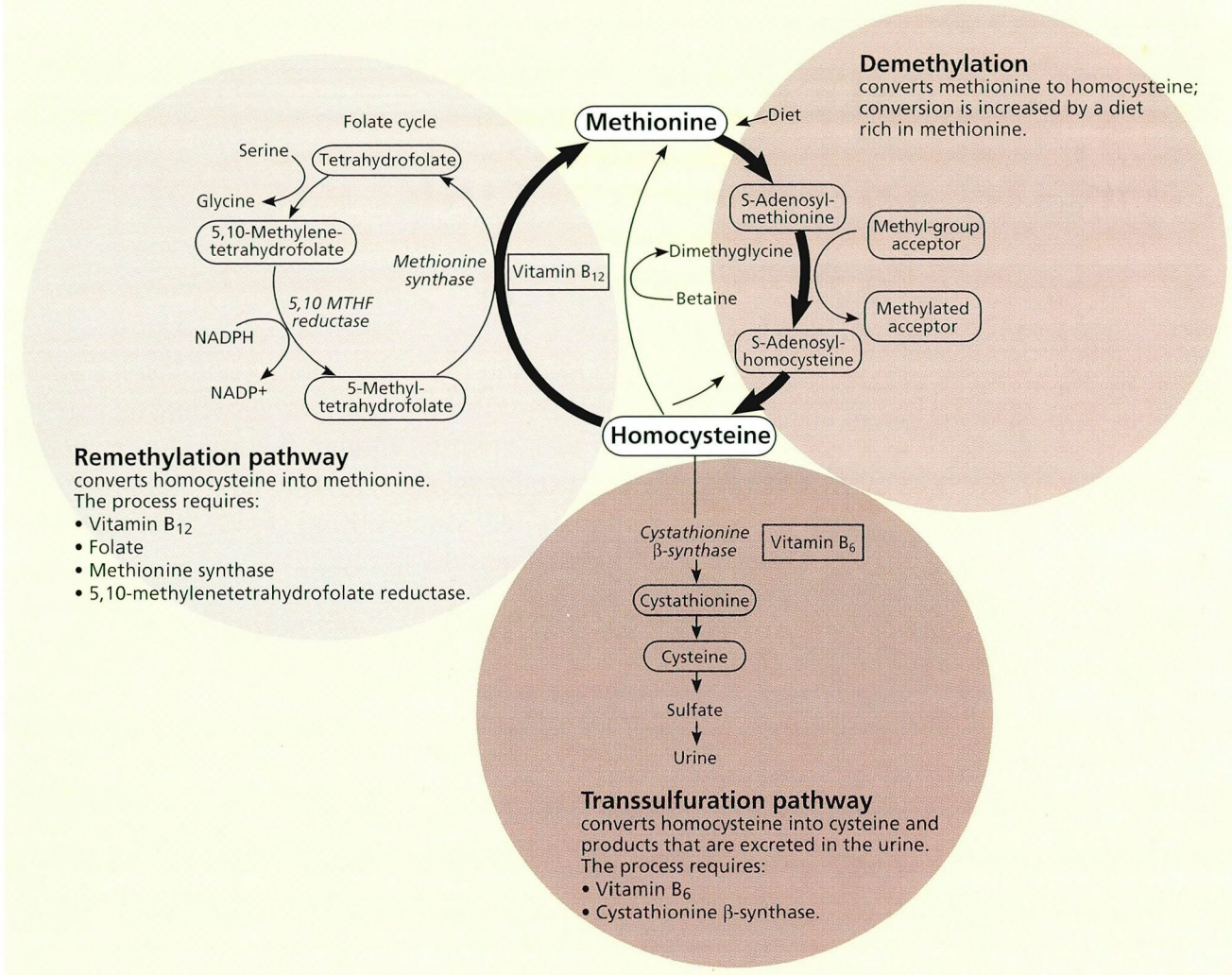
The body conserves adequate quantities of methionine through remethylation of homocysteine (FIGURE 1). This process requires a number of enzymes and an adequate supply of folic acid and vitamin B_{12}.

Transsulfuration

Breakdown of homocysteine by the transsulfuration pathway requires both cystathionine β-synthase and vitamin B_6 and results in

FIGURE 1

HOW HOMOCYSTEINE IS PRODUCED AND METABOLIZED



Homocysteine levels tend to rise with age

the conversion of homocysteine into cystathionine and cysteine, which are further broken down into products excreted in the urine.

CAUSES OF HIGH PLASMA HOMOCYSTEINE LEVELS

Plasma levels of homocysteine tend to rise with age, possibly due to inadequate intake of B vitamins, renal insufficiency, and perhaps also due to decreases in the activity of enzymes necessary for homocysteine metabolism; TABLE 1 shows the principal causes of high homocysteine levels.

Enzymatic defects

High plasma homocysteine levels may be seen with enzymatic defects in the transsulfuration and the remethylation pathways.

Transsulfuration enzyme disorders are rare and unlikely to account for the hyperho-

mocysteinemia frequently seen in patients with vascular disorders.

Remethylation enzyme disorders. A number of mutations of methylenetetrahydrofolate reductase have been reported. Most are rare, but a thermolabile variant caused by a single amino acid substitution (alanine to valine) due to a C-to-T point mutation at position 677 has an allele frequency of 30% to 45% in the general population. This variant, which may result in decreased enzyme activity in vivo, is associated with modest elevations in homocysteine concentrations and has been cited as a risk factor for coronary artery disease, although not consistently.[1–4] Folic acid supplements may lower the high homocysteine levels seen in this disorder.[5]

Vitamin deficiency

Because folic acid and vitamin B_{12} are essential for the remethylation pathway, and vitamin B_6 is essential for the transsulfuration pathway, a lack of these vitamins can cause impaired metabolism of homocysteine.[6–8]

CLINICAL EPIDEMIOLOGY OF HOMOCYSTEINE AND VASCULAR DISORDERS

The normal total plasma homocysteine level ranges from 5 to 15 µmol/L. However, the upper limit of normal may need to be lowered, because the risk of vascular disease appears to begin increasing with plasma homocysteine levels well within the normal range (FIGURE 2).[9]

High homocysteine levels are a risk factor for coronary artery disease

Several studies have shown high plasma homocysteine levels to be an independent risk factor for cardiovascular disease.[6–8] This association was first suspected from studies of the natural history of homocystinuria, a rare inborn error of metabolism complicated by occlusive arterial disease and thromboembolism. This autosomal recessive disease is caused by cystathionine β-synthase deficiency and is characterized by extremely high homocysteine concentrations (> 100 µmol/L). Subsequent studies revealed more modestly elevated homocysteine levels (>15 to 20 µmol/L) in patients with coronary artery disease, stroke, and peripheral vascular disease.[6–8]

TABLE 1

CAUSES OF HYPERHOMOCYSTEINEMIA

Inherited causes

Disorders of transsulfuration
- Cystathionine β-synthase deficiency

Disorders of remethylation
- Defective vitamin B_{12} transport
- Defective vitamin B_{12} coenzyme synthesis
- Defective methionine synthase
- 5,10-methylenetetrahydrofolate reductase deficiency (rare) and a thermolabile variant (common in most populations)

Acquired causes

Diseases
- Chronic renal failure
- Acute lymphoblastic leukemia
- Psoriasis

Vitamin deficiencies
- Vitamin B_{12}
- Folate
- Vitamin B_6

Drugs
- Methotrexate (an inhibitor of dihydrofolate reductase)
- Phenytoin and carbamazepine (antagonists of folate)
- Nitrous oxide (an inactivator of methionine synthase)
- Theophylline (an antagonist of vitamin B_6)
- 6-azauridine triacetate (an antagonist of vitamin B_6)

Folate, vitamin B_6 levels are lower in patients with coronary artery disease

A growing body of epidemiologic evidence shows that low levels of both folic acid and vitamin B_6 may be risk factors for vascular disorders. In 1994, Pancharuniti et al[10] showed an association between lower folate levels and angiographic evidence of coronary artery disease. Recently, Morrison et al[11] reported that persons with lower folate levels had a higher 15-year coronary mortality rate. In a large European case-control study of 750 patients and 800 controls, homocysteine correlated negatively with folate and vitamin B_6 levels. Low folate status was associated with an increased risk of vascular disease.[12] In the fol-

Higher doses of folic acid are needed to lower homocysteine in renal failure

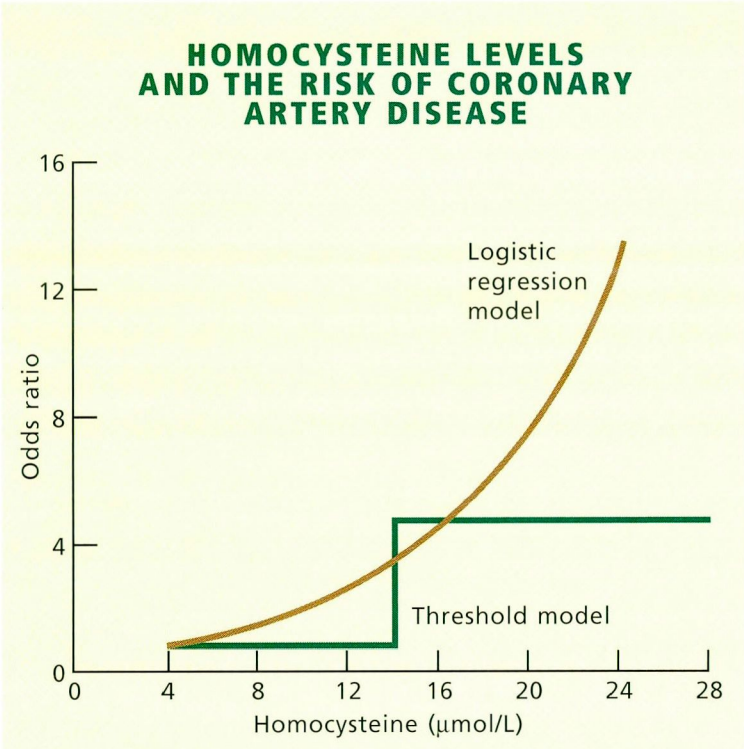


FIGURE 2. Odds ratios for coronary artery disease in relation to total plasma homocysteine concentrations using two different models. The simple threshold model uses a cut-point at 14 µmol/L to yield an odds ratio of 4.8 in persons with levels greater than this value. When logistic regression is used, increased odds ratios for coronary artery disease are seen at total plasma homocysteine concentrations below 14 µmol/L and within a "normal" range.

SOURCE: ROBINSON ET AL, REFERENCE 9

Folate or vitamin B_{12} deficiency can cause high plasma homocysteine

low-up of subjects in the National Health and Nutrition Examination Survey, a rising risk of stroke was observed in persons with lower folate levels, especially in blacks.[13] Low folate levels may predispose to vascular disease by causing elevated homocysteine concentrations.

Patients with coronary artery disease and other forms of atherosclerosis also have lower levels of vitamin B_6 than do controls,[9,14] and low vitamin B_6 levels confer an independent risk of vascular disease. Lower levels of this vitamin are also associated with higher homocysteine concentrations and could promote atherosclerosis in this manner. In addition, lower vitamin B_6 levels may also predispose to atherosclerosis by effects on serum cholesterol. In one study, supplementation with pyridoxine for 8 weeks decreased plasma LDL cholesterol concentrations by up to 17%, perhaps by enhancing the catabolism of LDL. In the same study, antithrombin III activity rose, perhaps by inhibition of its glycosylation.[15] In vitro studies have also shown that platelet aggregation may be abolished by pyridoxal phosphate, suggesting an additional possible mechanism for thrombosis in persons with low vitamin B_6 levels.[16,17]

Homocysteine levels are high in end-stage renal disease

The kidney is an important organ in homocysteine metabolism,[18] and end-stage renal disease is associated with greatly increased homocysteine levels and possibly lower levels of B vitamins.

Recent studies showed that a high homocysteine concentration in patients with renal failure is an independent risk factor for vascular complications and that this risk rises with increasing plasma homocysteine levels.[19]

Homocysteine levels are high after organ transplantation

Centers in Europe and North America have reported that homocysteine levels increase after organ transplantation—and transplant recipients have a high risk of vascular events.

In patients at the Mayo clinic,[20] homocysteine concentrations increased by 70% after cardiac transplantation and remained elevated 12 months later. Vitamin B_{12} and folic acid levels decreased, as did the glomerular filtration rate.

At the Cleveland Clinic,[21] homocysteine concentrations were higher in 189 heart transplant recipients than in controls. Folate and pyridoxal 5′-phosphate levels were lower, and deficiencies of these vitamins were seen in 10% to 20% of transplant recipients. Hyperhomocysteinemia was seen more often in patients with vascular complications following transplantation than in those without.

Homocysteine levels are also higher in recipients of transplanted kidneys, lungs, and

livers. The role, if any, of homocysteine in the genesis of the vascular complications that occur in these patients requires further study.

High homocysteine increases the risk of deep venous thrombosis

In a pooling study of 629 patients with homocystinuria, Mudd et al[22] found that deep venous thrombosis was the most frequent thrombotic complication, accounting for approximately 50% of all such events. Several recent studies have therefore examined the relationship between high homocysteine concentration and venous thromboembolism.

A study by den Heijer et al[23] measured the plasma homocysteine concentrations of patients with recurrent venous thrombosis, and also administered a methionine loading test. (This is a test similar to a glucose tolerance test, in which patients receive a standardized dose of methionine. Homocysteine concentrations are measured at intervals afterward; this test is sometimes used to expose a tendency to high homocysteine levels in patients in whom fasting values are normal).

The researchers found that 25% of patients with thrombosis had fasting homocysteine concentrations greater than the 95th percentile for values in normal controls; these high concentrations carried an odds ratio of 3.1 for thrombosis. A similar relationship was seen for abnormal results on the methionine loading test.

In 60 patients with unexplained thrombotic episodes studied at the Cleveland Clinic, hyperhomocysteinemia was the only abnormality detected on a hypercoagulation profile.[24] In general, there is now broad agreement that a high plasma homocysteine concentration is also a risk factor for venous thrombosis.

HOW HIGH HOMOCYSTEINE LEVELS MAY LEAD TO VASCULAR DAMAGE

Although high circulating levels of homocysteine are associated with atherosclerosis and thromboembolic disorders, the underlying mechanism by which it might cause vascular damage remains unknown (TABLE 2). It is possible that the sulfhydryl group of homocysteine could damage the endothelium.

TABLE 2

HOMOCYSTEINE AND ATHEROTHROMBOSIS: POSSIBLE MECHANISMS

Effects on vascular endothelium

- Cytotoxic damage at high doses
- Abnormal prostacyclin synthesis
- Altered chemokine production
- Changes mediated through adhesion molecules

Effects on platelets and clotting factors

- Increased synthesis of thromboxane B_2 and other eicosanoids
- Increased levels of platelet-derived thromboxane A_2
- Increased platelet adhesion and aggregation
- Decreased platelet survival
- Activation of factor XII
- Decreased antithrombin III levels
- Factor V activation
- Decreased activation of protein C
- Inactivation of thrombomodulin production or activity
- Increased affinity of Lp(a) for plasmin-modified fibrin
- Inhibition of von Willebrand factor processing and secretion
- Blocking of t-PA binding to endothelial cells

Endothelial damage

Current studies mostly focus on the endothelium as the site where vascular damage starts, rather than on platelets or clotting factors. A number of pathogenetic mechanisms, such as reduced activity of essential enzymes or nutrients, could lead to high homocysteine levels within endothelial cells. For example, we have been unable to demonstrate the presence of cystathionine β-synthase activity in cultured human aortic endothelial cells and in human cardiovascular tissue extracts. This finding implies that endothelial cells cannot metabolize homocysteine by the transsulfuration pathway. If this is true, any increase in plasma homocysteine concentrations could then disrupt endothelial function.[25] Decreased DNA synthesis was demonstrated in a recent in vitro study,[26] in which endothelial cells exposed to homocysteine and adenosine showed reduced proliferation. The effect was dose-dependent and was observed at concentrations as low as 10 µmol/L. In addition, this effect was specific for homocysteine and was not observed with

We give 0.4 mg of folic acid a day, and recheck the homocysteine level after 4 to 6 weeks

cysteine. Very low levels of homocysteine (10 to 50 µmol/L) also enhance the expression of monocyte chemattractant protein I (MCP-1) in human aortic endothelial cells.[27]

Vascular smooth muscle cells may also be adversely affected by homocysteine. Homocysteine can stimulate proliferation of smooth muscle cells grown in culture.[28] Hydrogen peroxide, formed by oxidation of homocysteine, may cause oxidant stress and play an important role in smooth muscle cell damage before overt vascular disease develops.[29] Recently, homocysteine exposure was shown to decrease the ratio between the intracellular concentrations of reduced and oxidized glutathione, the most important intracellular redox buffer.[29]

Consequences of endothelial damage

Once initiated, endothelial damage may be expressed in various ways, including inhibited synthesis of prostacyclin, decreased platelet survival, increased factor V expression, inactivation of endothelial anticoagulant protein C, or disruption of the processing and secretion of von Willebrand factor. In monkeys, diet-induced hyperhomocysteinemia is associated with altered endothelium-dependent vascular function,[30] and a recent clinical study in humans[31] documented that homocysteine may also inhibit endothelium-dependent flow-mediated dilation, perhaps by inhibiting nitric oxide.

Check the vitamin B_{12} level before starting folic acid

VITAMIN THERAPY FOR PATIENTS WITH VASCULAR DISEASE OR RENAL FAILURE AND HIGH HOMOCYSTEINE

Folic acid lowers homocysteine concentrations in normal subjects, patients with vascular disease, and patients with established renal failure.[6–8] It can be used alone or in combination with vitamins B_6 and B_{12}. The treatment is rapid and is effective after only 2 weeks.

The dose and combination of vitamins that should be used remains unclear, although levels of 1 mg or even less may be useful in patients with coronary artery disease. Naurath et al[32] used a regimen of 1.1 mg of folic acid, 1 mg of vitamin B_{12}, and 5 mg of vitamin B_6 and normalized elevated homocysteine levels. At the Cleveland Clinic, we recently completed a placebo-controlled study of approximately 100 patients with coronary artery disease. Patients were given folic acid 0.4, 1.0, or 5 mg daily for 3 months. Homocysteine concentrations fell by approximately 30% in all treatment groups but remained unchanged in the placebo group.[33] Thus, doses of folic acid as low as those found in multivitamin pills may be of use in lowering homocysteine levels in patients with coronary artery disease.

Much higher doses of folic acid are needed to lower homocysteine concentrations in patients with renal failure—as high as 15 mg/day or more.[34]

Recommendations

Although folic acid supplementation may lower homocysteine concentrations, there is no evidence that it improves clinical outcomes. Nevertheless, if homocysteine levels are high, and no other risk factors for vascular disease are present, or if episodes of thrombosis are recurrent, vitamin therapy may be a reasonable addition to usual treatment. In such patients, we currently use 0.4 mg folic acid daily and recheck the homocysteine concentration after 4 to 6 weeks. This therapy is innocuous and inexpensive.

In patients taking folic acid, it is prudent to check vitamin B_{12} levels before starting treatment, and perhaps also during follow-up, to ensure that vitamin B_{12} deficiency is not present. Oral or parenteral vitamin B_{12} supplements may be given if necessary. Intervention studies using folic acid supplements in patients with cerebrovascular disease are now in progress to assess the role of vitamin therapy in patients with atherosclerosis.

Food fortification with folic acid

Beginning in January 1998, products made with cereal grain or flour will be fortified by the addition of 140 µg of folic acid per 100 g of flour. This measure is intended to reduce the incidence of neural tube defects, but it may also reduce homocysteine concentrations and decrease the risk of atherosclerosis in the general population, although substantially higher levels of folate may be needed to achieve desirable effects in many subgroups.

REFERENCES

1. **Kang SS, Wong PW, Susmano A, Sora J, Norusis M, Ruggie N.** Thermolabile methylenetetrahydrofolate reductase: an inherited risk factor for coronary artery disease. Am J Hum Genet 1991; 48(3):536–545.
2. **Adams M, Smith PD, Martin D, Thompson JR, Lodwick D, Samani NJ.** Genetic analysis of thermolabile methylenetetrahydrofolate reductase as a risk factor for myocardial infarction. QJ Med 1996; 89:437–444.
3. **Kluijtmans LA, van den Heuvel LP, Boers GH, et al.** Molecular genetic analysis in mild hyperhomocysteinemia: a common mutation in the methylenetetrahydrofolate reductase gene is a genetic risk factor for cardiovascular disease. Am J Hum Genet 1996; 58:35–41.
4. **Wilcken DE, Xing XL, Sim AS, McCredie RM.** Distribution in healthy and coronary populations of the methylenetetrahydrofolate reductase (MTHFR) $C_{677}T$ mutation. Arterioscler Thromb Vasc Biol 1996; 16:878–882.
5. **Jacques PF, Bostom AG, Williams RR, et al.** Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. Circulation. 1996; 93:7–9.
6. **Ueland PM, Refsum H, Brattström L.** Plasma homocysteine and cardiovascular disease. In: Francis RB Jr, editor. Atherosclerotic cardiovascular disease, hemostasis, and endothelial function. New York: Marcel Dekker Inc, 1992:183–236.
7. **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.
8. **Mayer EM, Jacobsen DW, Robinson K.** Homocysteine and coronary atherosclerosis. J Am Coll Cardiol 1996; 27:517–527.
9. **Robinson K, Mayer EL, Miller DP, et al.** Hyperhomocysteinemia and low pyridoxal phosphate. Common and independent reversible risk factors for coronary artery disease. Circulation 1995; 92:2825–2830.
10. **Pancharuniti N, Lewis CA, Sauberlich HE, et al.** Plasma homocyst(e)ine, folate, and vitamin B-12 concentrations and risk for early-onset coronary artery disease. Am J Clin Nutr 1994; 59:940–948.
11. **Morrison HI, Schaubel D, Desmeules M, Wigle DT.** Serum folate and risk of fatal coronary heart disease. JAMA 1996; 275:1893–1896.
12. **Robinson K, Arheart K, Refsum H, et al.** Low circulating folate and vitamin B_6 concentrations: Risk factors for stroke, peripheral vascular disease and coronary artery disease. Circulation. In press.
13. **Giles WH, Kittner SJ, Anda RF, Croft JB, Casper ML.** Serum folate and risk for ischemic stroke. First national health and nutrition examination survey epidemiologic follow-up study. Stroke 1995; 26:1166–1170.
14. **Leklem JJ.** Vitamin B6. In: Shils ME, Olson JA, Shike M, editors. Modern nutrition in health, disease. 8th ed. Lea & Febiger, Philadelphia, 1994:383–394.
15. **Brattström L, Stavenow L, Galvard H, et al.** Pyridoxine reduces cholesterol and low-density lipoprotein and increases antithrombin III activity in 80-year-old men with low plasma pyridoxal 5-phosphate. Scand J Clin Lab Invest 1990; 50:873–877.
16. **Krishnamurthi S, Kakkar VV.** Studies on the effect of platelet inhibitors on platelet adhesion to collagen and collagen-induced human platelet activation. Thromb Haemost 1985; 53:337–342.
17. **Zahavi M, Zahavi J, Kakkar VV.** Effect of adenylcyclase activators, phosphodiesterase inhibitors and pyridoxal-5-phosphate on platelet aggregation and adenosine-3′- 5′-cyclic monophosphate accumulation. Thromb Haemost 1984; 52:205–209.
18. **Bostom AG, Brosnan JT, Hall B, Nadeau MR, Selhub J.** Net uptake of plasma homocysteine by the rat kidney in vivo. Atherosclerosis 1995; 116:59–62.
19. **Robinson K, Gupta A, Dennis V, et al.** Hyperhomocysteinemia confers an independent increased risk of atherosclerosis in end-stage renal disease and is closely linked to plasma folate and pyridoxine concentrations. Circulation 1996; 94:2743–2748.
20. **Berger PB, Jones JD, Olson LJ, et al.** Increase in total plasma homocysteine concentration after cardiac transplantation. Mayo Clin Proc 1995; 70:125–131.
21. **Gupta A, Moustapha A, Jacobsen DW, et al.** Hyperhomocysteinemia and vascular complications in heart transplant recipients: relationships with folate and vitamin B_6. Submitted for publication.
22. **Mudd SH, Skovby F, Levy HL, et al.** The natural history of homocystinuria due to cystathionine β-synthase deficiency. Am J Hum Genet 1985; 37:1–31.
23. **den Heijer M, Blom HJ, Gerrits WBJ, et al.** Is hyperhomocysteinaemia a risk factor for recurrent venous thrombosis? Lancet 1995; 345:882–885.
24. **Kottke Marchant K, Green R, Jacobsen DW, et al.** High plasma homocysteine: a risk factor for arterial and venous thrombosis in patients with normal hypercoagulation profiles. Clin Appl Thromb Hemost. In press.
25. **Jacobsen DW, Savon SR, Stewart RW, et al.** Limited capacity for homocysteine catabolism in vascular cells and tissues: a pathophysiologic mechanism for arterial damage in hyperhomocysteinemia? (abstract) Circulation 1995; 92(Suppl I):104.
26. **Wang H, Yoshizumi M, Lai K, Tsai JC, Perrelle MA, Haber E, Lee ME.** Inhibition of growth and $p21^{ras}$ methylation in vascular endothelial cells by homocysteine but not cysteine. J Biol Chem. 1997; 272:25380–25385.
27. **Poddar R, Sivasubramanian N, Robinson K, Jacobsen DW.** Homocysteine modulates the expression of a specific cytokine (monocyte chemoattractant protein-1) in human aortic endothelial cells. (abstract) Circulation. In press.
28. **Tsai J-C, Perella MA, Yoshizumi M, et al.** Promotion of vascular smooth muscle growth by homocysteine: a link to atherosclerosis. Proc Natl Acad Sci USA 1994; 91:6369–6373.
29. **Welch GN, Upchurch GR, Keaney JF, Loscalzo J.** Homocyst(eine decreases cell redox potential in vascular smooth muscle cells (abstract). J Am Coll Cardiol 1996; 27 (Suppl 2a):163A.
30. **Lentz SR, Sobey CG, Piegors DJ, et al.** Vascular dysfunction in monkeys with diet-induced hyperhomocyst(e)inemia. J Clin Invest 1996; 98:24–29.
31. **Tawakol A, Omland T, Gerhard M, Wu JT, Creager MA.** Hyperhomocysteinemia is associated with impaired endothelial- dependent vasodilation in humans. Circulation 1997; 95:1191–1121.
32. **Naurath HJ, Joosten E, Reizler R, Stabler SP, Allen RH, Lindenbaum J.** Effects of vitamin B_{12}, folate, and vitamin B_6 supplements in elderly people with normal serum vitamin concentrations. Lancet 1995; 346:85–89.
33. **Lobo A, Naso A, Arheart K, et al.** Effect of low-dose folic acid on homocysteine levels in patients with coronary disease: a randomized, placebo-controlled study. Submitted for publication.
34. **Bostom AG, Shemin D, Lapane KL, et al.** High dose B-vitamin treatment of hyperhomocysteinemia in dialysis patients. Kidney Int 1996; 49:147–152.

ADDRESS: *Killian Robinson, MD, Department of Cardiology, F15, 9500 Euclid Avenue, Cleveland, OH 44195.*