Circ J 2007; **71:** 266–270

Plasma Homocysteine Concentration in Patients With Poor or Good Coronary Collaterals

Nurten Sayar, MD; Sait Terzi, MD; Tuba Bilsel, MD; Hale Yaka Yilmaz, MD; Lutfullah Orhan, MD; Nazmiye Cakmak, MD; Ismail Erdem, MD; Burak Tangurek, MD; Figen Ciloglu, MD*; Ismail Peker, PhD**; Kemal Yesilcimen, MD

Background Elevated plasma homocysteine (Hcy) concentrations are associated with an increased risk of vascular disease. Hcy is known to inhibit endothelial cell proliferation in vitro. The purpose of the present study was to investigate the role of plasma Hcy concentrations on development of collateral circulation in single-vessel chronic total occlusion.

Methods and Results Collateral status was determined by Rentrop's classification. Of 817 patients, 56 cases of pure single-vessel chronic total occlusion were studied. Plasma Hcy concentrations in patients with single-vessel total coronary occlusion were higher compared with controls (17.3±12.6 μmol/L vs 10.9±4.9 μmol/L, p=0.015). There was no significant difference in plasma Hcy concentrations of the good and poor collateral groups (17.2± 13.7 μmol/L vs 15.3±9.3 μmol/L, p=0.834). Plasma Hcy concentrations in individual Rentrop subclasses 0, 1, 2 and 3 were as follows: 15.9±9.1, 16.3±12.4, 17.1±14.1 and 20.1±13.5 μmol/L (p=0.893). There was a positive linear correlation between Rentrop subclass and angina pectoris duration (r=0.41, p=0.003). Angina pectoris duration was the only independent variable affecting the development of coronary collaterals in the present study (odds ratio [confidence interval]: 1.85 [1.12–2.91], p=0.014).

Conclusion Patients with single-vessel chronic total occlusion had higher plasma Hcy concentrations than controls, but similar Hcy concentrations when compared according to the presence of poor or good coronary collaterals. There is a lack of association between plasma Hcy concentration and coronary collateral status in the current study. (*Circ J* 2007; **71:** 266–270)

Key Words: Collateral circulation; Coronary artery disease; Homocysteine; Total coronary occlusion

Homocysteine (Hcy) is a sulfur-containing amino acid derived from the demethylation of methionine. It is used either by trans-sulfuration to cysteine or by remethylation of methionine[1,2] A variety of nutritional and genetic factors can cause hyperhomocysteinemia: mutations in the genes for enzymes involved in Hcy metabolism, 5,10-methylenetetrahydrofolate reductase 677C-T mutation, and deficiencies of vitamins B6, B12 and folic acid (cofactors for Hcy metabolism) are associated with hyperhomocysteinemia[3] The atherosclerotic role of hyperhomocysteinemia was first established in 1969 by McCully;[4] who found premature extensive atherosclerosis from the autopsy of children who had died of homocysteinuria. However, the best evidence for Hcy as a risk factor for coronary artery disease (CAD) are prospective studies that measured plasma Hcy concentrations before the clinical manifestations of CAD[5–7] It is now well known that raised plasma Hcy is an independent risk factor for peripheral vascular, cerebrovascular and coronary heart disease[8,9] Hcy concentrations exceeding 15 μmol/L (upper limit of normal) are common and are found in 30% of patients with vascular disease[3] Hcy is known to inhibit endothelial cell proliferation. In several in vitro studies, the addition of Hcy to the culture medium caused endothelial cell damage in a dose-dependant manner[10–12]

Coronary collateral circulation is crucial in ischemic heart disease. Extensive previous studies in animals and in humans have confirmed the potential role of the collateral circulation in terms of limiting the extent of myocardial ischemia and limiting infarct size in areas of the myocardium supplied by the totally occluded vessels[13] Therefore, we investigated whether poor collateral formation is associated with high plasma Hcy concentration.

Methods

A total of 817 consecutive patients who were referred for coronary angiography were initially enrolled. Patients with unstable angina pectoris (AP), history of myocardial infarction (MI) within 3 months before the study and plasma creatinine >1.5 mg/dl were excluded. The angiographic distributions of normal and non-critical lesions (<50% stenosis), single-vessel and multivessel disease were 155, 286 and 376, respectively. Of the 286 single-vessel cases, we excluded patients with left ventricular ejection fraction (LVEF) <40% or clinical signs of heart failure, because heart failure and chronic diuretic usage increase the plasma Hcy concentration[14,15] The resulting 56 cases had 1-vessel total occlusion and no other artery with >50% stenosis. None of the patients had taken drugs, such as folate or vitamin B compounds, that would affect plasma Hcy con-

(Received May 8, 2006; revised manuscript received November 20, 2006; accepted November 27, 2006)

*Siyami Ersek Cardiovascular and Thoracic Surgery Research Hospital, Department of Cardiology, *Genlab Medical Diagnostics and Research Laboratory and **Marmara University, Faculty of Engineering, Department of Chemical Engineering, Istanbul, Turkey*
Mailing address: Nurten Sayar, MD, Inonu Cad. Sumko Sit. K2 blok Daire 11, PK:34736 Kozyatagi, Istanbul, Turkey. E-mail: sayarnurten@gmail.com

Table 1 Characteristics of the Study Population

	Good collateral (n=35)	*Poor collateral (n=21)*	*p value*
Age (years)	*56.1±12.1*	*54.9±10.7*	*0.728*
M/F	*29/6*	*14/7*	*0.134*
BMI (kg/m²)	*26.9±3.9*	*25.6±2.2*	*0.304*
Hypertension	*16 (46%)*	*10 (48%)*	*0.554*
Hyperlipidemia	*14 (40%)*	*12 (57%)*	*0.186*
Smoking	*14 (40%)*	*9 (43%)*	*0.920*
Diabetes	*5 (14%)*	*2 (10%)*	*0.646*
History of MI	*16 (45.7%)*	*7 (33.3%)*	*0.412*
Duration of AP (months)	*8.3±4.4*	*5.8±3.9*	*0.03*
Creatinine (mg/dl)	*0.9±0.1*	*0.9±0.3*	*0.483*
LVEF (%)	*49.7±11.9*	*50.8±14.3*	*0.589*

BMI, body mass index; MI, myocardial infarction; AP, angina pectoris; LVEF, left ventricular ejection fraction.

centrations. Patients were aged 27–78 years.

Thirty-five age- and sex-matched patients who had normal coronary angiography and no history of MI or chronic illness were taken as controls out of 155 cases of coronary angiography.

For Hcy measurement, blood samples were taken from the antecubital vein and collected into ethylenediaminetetraacetic acid-containing tubes before coronary angiography. The samples were immediately placed on ice and centrifuged within 30 min at 2,000 G for 10 min. The plasma was separated and stored at –80°C until analysis. The total (free plus bound) Hcy concentration was determined using Microplate Enzyme Immunoassay Homocysteine kit (Bio-Rad). Hyperhomocysteinemia was defined as fasting Hcy concentration >15 μmol/L.

Coronary angiography was performed by the Judkins' or Sones' technique and before imaging all patients had 200 μg nitroglycerin injected into the right and left coronary circulation.[16,17] Two experienced cardiologists who had no knowledge of the patients' medical and Hcy status reviewed the angiographic images. Coronary collaterals were graded from 0 to 3 according to the Cohen-Rentrop method, in which 0=no opacification; 1=filling of side branches of the artery perfused by way of collateral vessels without visualization of the epicardial segment; 2=partial filling of the epicardial segment by way of collateral vessels; and 3=complete filling of the epicardial segment by way of collateral vessels.[18] Rentrop 0 and 1 were regarded as poor collateral and Rentrop 2 and 3 as good collateral. In cases of discrepancy between the 2 reviewers on the collateral status, a third cardiologist was called in for a decision.

The study was approved by the institutional Ethics Committee and written informed consent was given by all patients before cardiac catheterization.

Statistical Analysis

Data are expressed as the mean±SD. Continuous variables were compared by unpaired Mann-Whitney U test or Kruskal-Wallis. Chi-square test was used for categorical data. The relationship between Rentrop subclasses and AP duration was tested by Spearman correlation. Multiple logistic analysis was used to determine the independent variables affecting the development of collaterals. A value of $p<0.05$ with 95% confidence interval (CI) was considered statistically significant. All tests were done with SPSS (Statistical Package for Social Sciences) for windows 10.0.

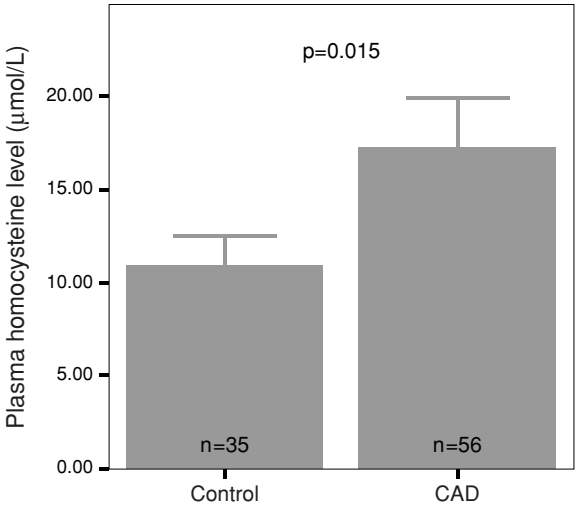

Fig 1. Comparison of plasma homocysteine concentrations of controls and the study population (p=0.015). Bars represent mean, error bars represent 95% confidence interval of mean. CAD, coronary artery disease.

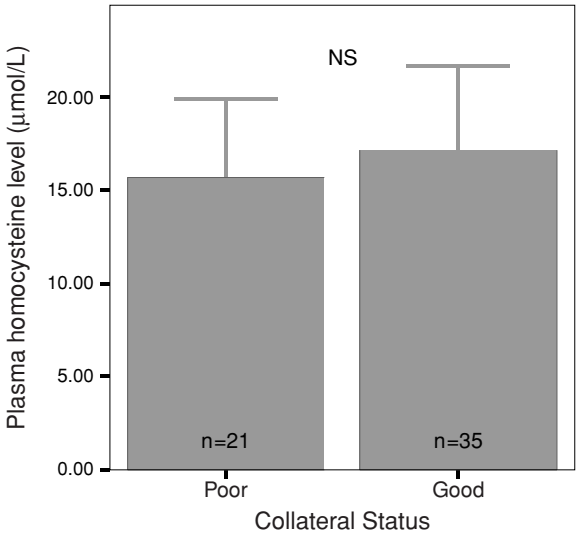

Fig 2. Plasma homocysteine concentrations in good and poor coronary collateral group. NS, not significant. Bars represent mean, error bars represent 95% confidence interval of mean.

Results

The study population consisted of 56 patients aged 27–78 (13 females). The demographic characteristics of the study population are shown in Table 1. Plasma Hcy concentrations of the study population were compared with those of age- and sex-matched controls (n=35) with angiographically normal coronary arteries, no history of MI or of chronic illness. Overall, plasma Hcy concentrations in patients with single-vessel total coronary occlusion were higher than in the controls (17.3±12.6 μmol/L vs 10.9±4.9 μmol/L, p=0.015, Fig 1).

Plasma Hcy concentrations in patients with good or poor collateral formation were 17.2±13.7 μmol/L and 15.3±9.3 μmol/L, respectively (p=0.834, Fig 2). There was no significant difference in the plasma Hcy concentration of these 2 groups. Plasma Hcy concentration in individual

Table 2 Summary of the Results in the Good and Poor Coronary Collateral Groups

	Good collateral (n=35)	Poor collateral (n=21)	p value
Hcy level (μmol/L)	17.2±13.7	15.3±9.3	0.834
%patients Hcy >15 μmol/L	36.4	36.8	0.972
Occlusion site			
LAD (%)	51.5	57.9	
RCA (%)	45.5	31.6	0.398
CX (%)	3	10.5	
Total occlusion level (%)			
Ostial-proximal	68.2	62.4	
Mid-distal	31.8	37.6	0.560
Stable AP severity (%)			
Mild	58.6	63.6	0.772
Severe	41.4	36.4	

Hcy, homocysteine; LAD, left anterior descending artery; RCA, right coronary artery; CX, circumflex artery. Other abbreviation see in Table 1.

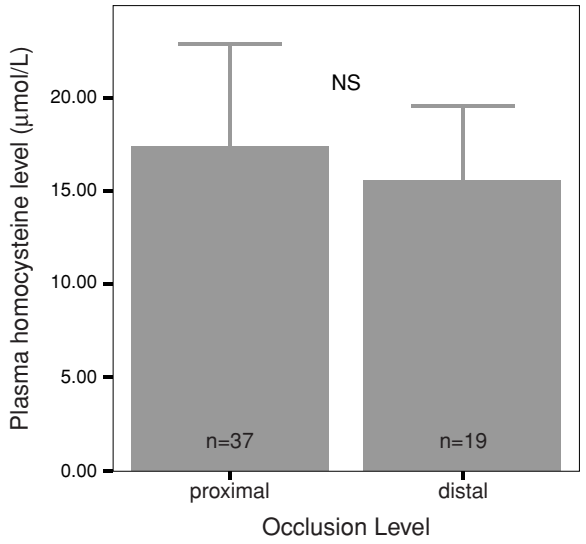

Fig 3. Plasma homocysteine concentration of patients having proximal and distal total coronary occlusions. NS, not significant. Bars represent mean, error bars represent 95% confidence interval of mean.

Rentrop subclasses 0, 1, 2 and 3 were as follows: 15.9±9.1, 16.3±12.4, 17.1±14.1 and 20.1±13.5 μmol/L (p=0.893). The percentage of patients who had plasma Hcy concentrations >15 μmol/L (upper limit of normal) in the good or poor collateral group were 36.4% and 36.8%, respectively (p=0.972). We divided patients' severity of angina into no angina-mild (CCS 0–1) group and severe angina group (CCS 2–3). As mentioned before, the Class 4 angina group was excluded from the study. There was no difference between the 2 groups in the Hcy concentration (15.2±12.1 μmol/L vs 19.8±16.6 μmol/L, p=0.669). The percentage of patients in each AP group was similarly distributed among the good and poor collateral groups (Table 2).

We investigated whether the level of total occlusion could affect Hcy concentration. Osteal and proximal occlusions were grouped together as proximal, whereas middle and distal total occlusions were grouped as distal. There was no significant difference in plasma Hcy concentration in terms of occlusion level (17.4±13.2 μmol/L vs 15.2±10.9 μmol/L, p=0.799; Fig 3). The distribution of proximal–distal occlusion in the poor and good collateral groups was similar (p=0.560, Table 2). There was no difference in the distribution of occluded vessel (left ascending artery (LAD), right coronary artery (RCA) or circumflex artery (CX), p=0.398; Table 2). Hcy concentrations in patients with totally occluded LAD, RCA, and CX were as follows: 14.8±10.4 μmol/L, 18.7±14.1 μmol/L and 22.7±17.6 μmol/L (p=0.593).

Table 3 Multivariate Predictors of Coronary Collateral Development in Chronic Total Occlusion Patients

	β	OR	95%CI	p value
AP duration	0.61	1.85	1.12–2.91	0.014
Homocysteine	0.06	1.07	0.97–1.17	0.182
Age	0.01	1.01	0.93–1.08	0.888
Male	0.12	3.7	0.15–8.48	0.903
Diabetes mellitus	–1.91	0.15	0.01–1.93	0.145
Hypertension	0.75	2.12	0.41–10.98	0.372
Hyperlipidemia	1.13	3.11	0.62–15.70	0.169
MI	1.31	3.70	0.68–20.23	0.131

OR, odds ratio; CI, confidence interval. Other abbreviations see in Table 1.

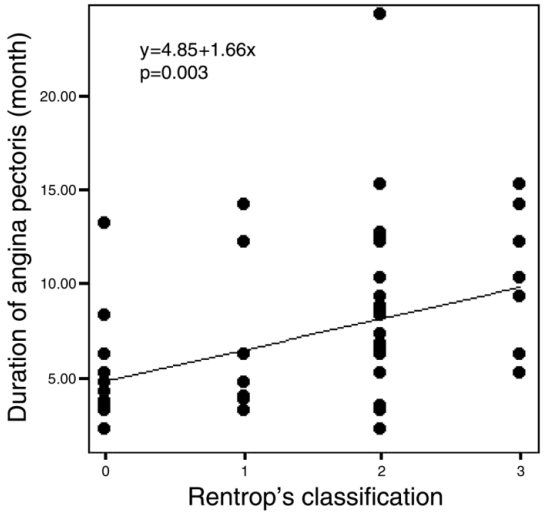

Fig 4. Relationship between angina pectoris duration and Rentrop's collateral classification (r=0.413, p=0.003).

There was a significant difference in AP duration in patients with good collaterals vs patients with poor collaterals (8.3±4.4 months vs 5.8±3.9 months, p=0.03; Table 1). There was a positive linear correlation between Rentrop subclasses and AP duration (r=0.41, p=0.003; Fig 4). We assessed the effect of demographic variables such as age, sex, history of MI, presence of hypertension, hyperlipidemia, diabetes mellitus (DM), AP duration, and plasma Hcy concentration on the development of collaterals. The only independent variable affecting the development of collaterals was AP duration (odds ratio (OR) [CI]: 1.85 [1.12–2.91], p=0.014).

Discussion

The coronary collateral circulation is an alternative source of blood supply to a myocardial area jeopardized by ischemia. More than 200 years ago, Heberden described coronary vasodilatation with opening of collateral vessels![19] Since then there have been numerous investigations showing a protective role of well developed collaterals showing

smaller infarcts, less negative ventricular remodeling, improved ventricular function, better prognosis and improved survival[13,20,21]

Determinants of coronary collateral circulation are myocardial ischemia, pressure gradient and shear stresses and growth factors[22] Clinically, patients with myocardial ischemia, who had critical stenosis that results in significant pressure gradient or who had higher growth factors and chemokines had better collateral circulation.

Postmortem studies suggest a greater degree of collateralization in patients with a longer duration of ischemic symptoms in life[23] In 50 patients undergoing angioplasty for total chronic total occlusions, collateral vessels were associated with normal left ventricular wall motion if the occlusion had occured >3 months previously[24] In the current study, the patients with good collateral circulation had longer duration of AP and we found a positive linear correlation between Rentrop score and AP duration.

Although it is clear that high grade coronary stenosis is responsible for collateral vessel growth, it remains unclear as to which factors affect the extent of collateral growth in the presence of severe CAD[25] It has been reported that the prevalence of a well developed collateral circulation was significantly lower in the elderly group than in the younger group[26] The influence of diabetes on collateral formation is not clear. Some studies showed poorer vessel formation in DM, whereas other studies declared that DM patients develop more extensive or comparable degree of collateral formation compared with non-diabetic patients[26] Hypercholesterolemia also impairs endothelial function; therefore it is possible that the extent of collateral vessel growth is poorer in hypercholesterolemia[27] In the present study we investigated age, sex, plasma Hcy concentration, history of MI, DM, hypertension, hyperlipidemia and duration of AP as determinants of collateral development. AP duration was the only independent variable affecting the development of collaterals.

The presence of collaterals defined at coronary angiography has prognostic significance. In the 1980s, Ramirez and Fernandez de la Reguera showed that patients who had a poor collateral response had more complications at the time of MI and higher mortality[28] Furthermore, Hansen later showed that patients with a good collateral circulation had a significantly improved 10-year survival[29] Assessment of the collateral circulation using fractinal blood flow in a prospective study, showed that individuals with a high fractional blood flow index had fewer subsequent ischemic events[30] Moreover, coronary flow index (CFI) has also been used to show the important prognostic role of the collateral circulation in chronic stable angina. Patients (n=403) underwent coronary angiography and measurement of CFI and were followed up for a mean of 94 weeks. Patients with higher CFI had fewer major cardiac events (cardiac death or acute coronary syndrome); suggesting a protective role of the collateral circulation[31] Choi et al used a novel index of collateral development, studying only type 2 diabetic patients and showed that patients with poor collateral formation had higher cardiovascular death, non-fatal MI and unstable AP like the previous study[32]

Hcy is a risk factor for development of CAD. Laboratory studies suggest that an elevated Hcy concentration is both atherogenic and thrombogenic[33] A meta-analysis of 20 prospective studies of serum Hcy and risk of vascular disease involving 3,820 participants found that a 5 μmol/L increase in Hcy concentration was associated with a 32% increase in odds of CAD (OR, 1.32; 95% CI, 1.19–1.45) and a 59% increase in odds of stroke (OR, 1.59; CI, 1.29–1.96)[34] Similar to published reports, we found elevated plasma concentrations of Hcy in patients with angiographically established CAD compared with patients with normal angiography.

Experimentally, Hcy is known to inhibit endothelial cell proliferation[35] It is shown that there is a rapid onset of endothelial dysfunction after hyperhomocysteinemia[36] Diet-induced hyperhomocysteinemia exacerbates neointima formation in carotid arteries after balloon injury in rats[37] Nagai et al showed that increased level of Hcy inhibited angiogenesis by preventing proliferation and migration of endothelial cells[38] In Woo et al's study, impaired flow mediated brachial artery dilatation and surrogate of endothelial dysfunction, was shown in healthy individuals with high plasma Hcy compared with normohomocystemic controls[39] The effect of Hcy on coronary collateral circulation was sparsely studied.

We found only one study that investigated the association between Hcy concentration and collateral formation in humans. Nagai et al studied the effect of plasma concentration of Hcy on collateral circulation in single-vessel disease[40] The design of the study was similar and plasma concentrations of Hcy in 49 single-vessel CAD patients were studied. Nineteen patients had single-vessel total occlusion. They found significantly higher plasma Hcy concentration in poor collateral group. According to their study, the independent factors affecting the development of collateral circulation in patients with single-vessel disease were Hcy concentration, AP duration and degree of stenosis. In this present study we found that plasma concentration of Hcy was significantly higher in patients with single-vessel total coronary occlusion compared with controls with normal coronary arteries. The Hcy concentrations in poor and good collateral group and in each Rentrop subclasses did not differ significantly. AP duration was found to be the only independent variable affecting collateral formation from covariates of age, presence of DM, hypertension, hyperlipidemia, history of MI, age, sex and Hcy concentrations. As all of the cases were total occlusions we can not investigate the role of degree of stenosis on collateral development. The present study showed no significant difference in plasma Hcy concentrations between poor and good collateral group within a larger study population.

The differences between the study of Nagai and colleagues' study and the current study can be explained. In our study, all patients had single-vessel total occlusion and no other lesion >50%, not only in the donor vessel but in the entire coronary circulation, which we assessed as a whole, and any significant stenosis might alter the milieu that can affect collateral development. Previous studies have shown that patients with good collateral development are more likely to have multivessel CAD disease[25,41] The greater the atherosclerotic burden, the more angiogenic and arteriogenic factors are in play.

It is known that diuretic usage elevates plasma Hcy concentrations[15] The patients in the poor collateral group were more likely to have worse LVEF and receiving diuretic therapy. To eliminate this possible confounding effect, we did not take patients with left ventricular dysfunction or those taking diuretics. The mean LVEF in good and poor collateral groups were similar (49.7±11.9% vs 50.8±14.3%, Table 1). The left ventricular function was not stated in the aforementioned study.

There might be a racial difference in terms of plasma

Hcy concentration and collateral formation.

Study Limitations

Assessment of the collateral circulation by angiography can define only vessels >100μm in size, whereas most collateral channels are much smaller[42] Myocardial contrast echocardiography or Doppler techniques would be more accurate in deciding the collateral status of the patient.

We did not assess the vitamin B and folic acid status of the patients. We assumed that it was evenly distributed in each collateral group. Further clinical studies are needed to determine if there is any association between Hcy concentratios and coronary collateral formation.

References

1. Refsum H, Ueland PM, Nygard O, Vollset SE. Homocysteine and cardiovascular disease. *Ann Rev Med* 1998; **49:** 31–62.
2. Selhub J, Miller JW. The pathogenesis of homocysteinemia: Interruption of the coordinate regulation by S-adenosylmetwhionine of the remethylation and transsulfuration of homocysteine. *Am J Clin Nutr* 1992; **55:** 131–138.
3. Chambers JC, Kooner JS. Homocysteine: A novel risk factor for coronary heart disease in UK Indian Asians. *Heart* 2001; **86:** 121–122.
4. McCully KS. Vascular pathology of homocystinemia: Implications for the development of arteriosclerosis. *Am J Pathol* 1969; **56:** 111–128.
5. Stampfer MJ, Malinow MR, Willet WC, Newcomer LM, Upson B, Ullman D, et al. A prospective study of plasma homocysteine and risk of myocardial infarction in US physicians. *JAMA* 1992; **268:** 877–881.
6. Nygard O, Vollset SE, Refsum H, Stensvold I, Tverdal A, Nordrehaug JE, et al. Total plasma homocysteine and cardiovascular risk profile: The Hordaland Homocysteine Study. *JAMA* 1995; **274:** 1526–1533.
7. Whincup PH, Refsum H, Perry IJ, Morris R, Walker M, Lennon L, et al. Serum total homocysteine and coronary heart disease: Prospective study in middle aged men. *Heart* 1999; **82:** 448–454.
8. Malinow MR, Kang SS, Taylor LM, Wong PW, Coull B, Inahara T, et al. Prevalance of hyperhomocysteinemia in patients with peripheral arterial occlusive disease. *Circulation* 1989; **79:** 1180–1188.
9. Perry IJ, Refsum H, Morris RW, Ebrahim SB, Ueland PM, Shaper AG. Prospective study of serum total homocysteine concentration and risk of stroke in middle-aged British men. *Lancet* 1995; **346:** 1395–1398.
10. Harker LA, Ross R, Slichter SJ, Scott CR. Homocysteine-induced arteriosclerosis: The role of endothelial cell injury and platelet response in its genesis. *J Clin Invest* 1976; **58:** 731–741.
11. Wall RT, Harlan JM, Harker LA, Striker GE. Homocysteine induced cell injury in vitro: A model for the study of vascular injury. *Thromb Res* 1980; **18:** 113–121.
12. Stamler JS, Osborne JA, Jaraki O, Rabbani LE, Mullins M, Singel D, et al. Adverse vascular effects of homocysteine are modulated by endothelium-derived relaxing factor and related oxides of nitrogen. *J Clin Invest* 1993; **91:** 308–318.
13. Kolibash AJ, Bush CA, Wepsic RA, Schroeder DP, Tetelman MR, Lewis RP. Coronary collateral vessels: Spectrum of physiologic capacities with respect to providing rest and stress myocardial perfusion: Maintenance of left ventricular functin and protection against infarction. *Am J Cardiol* 1982; **50:** 230–238.
14. Schofield RS, Wessel TR, Walker TC, Cleeton TS, Hill JA, Aranda JM. Hyperhomocysteiemia in patients with heart failure referred for cardiac transplantation: Preliminary observations. *Clin Cardiol* 2003; **26:** 407–410.
15. Ventura P, Panini R, Verlato C, Scarpetta G, Salvioli G. Hyperhomocysteinemia and related factors in 600 hospitalized elderly subjects. *Metabolism* 2001; **50:** 1466–1471.
16. Judkins MP. Selective coronary arteriography: Percutaneous transfemoral technique. *Radiology* 1967; **89:** 815–824.
17. Sones FM Jr. Acquired heart disease: Symposium on present and future of cineangiography. *Am J Cardiol* 1959; **3:** 710–712.
18. Rentrop Kp, Cohen M, Blanke H, Philips RA. Changes in collateral filling immediately after controlled coronary artery occlusion by an angioplasty balloon in human subjects. *J Am Coll Cardiol* 1985; **5:** 587–592.
19. Seiler C. The human coronary collateral circulation. *Heart* 2003; **89:** 1352–1357.
20. Billinger M, Kloos P, Eberli F, Windecker S, Meier B, Seiler C. Physiologically assessed coronary collateral flow and adverse cardiac ischemic events: A follow-up study in 403 patients with coronary artery disease. *J Am Coll Cardiol* 2002; **40:** 1545–1550.
21. Hansen JF. Coronary collateral circulation: Clinical significance and influence on survival in patients with coronary artery occlusion. *Am Heart J* 1989; **117:** 290–295.
22. Koerselman J, Yolanda VDG, Jaegere PP, Grobbee DE. Coronary collaterals: An important and underexposed aspect of coronary artery disease. *Circulation* 2003; **107:** 2507–2511.
23. Fulton W. The time factor in the enlargement of anastomosis in coronary artery disease. *Scot Med J* 1964; **9:** 18–23.
24. Werner G, Ferrari M, Betge S, Gastmann O, Richartz BM, Figulla HR, et al. Collateral function in chronic total occlusions is related to regional myocardial function and duration of occlusion. *Circulation* 2001; **104:** 2784–2790.
25. Pohl T, Seiler C, Billinger M, Herren E, Wustmann K, Mehta H, et al. Frequency distribution of collateral flow and factors influencing collateral channel development: Functional collateral channel measurements in 450 patients with coronary artery disease. *J Am Coll Cardiol* 2001; **38:** 1872–1878.
26. Fujita M, Tambara K. Recent insights into human coronary collateral development. *Heart* 2004; **90:** 246–250.
27. Van Belle E, Rivard A, Chen D, Silver M, Bunting S, Ferrara N, et al. Hypercholesterolemia attenuates angiogenesis but does not preclude augmentation by angiogenic cytokines. *Circulation* 1997; **96:** 2667–2674.
28. Ramirez M, Fernandez de la Reguera G. Coronary collateral circulation: Its importance and significance in ischemic cardiopathy. *Arch Instit Cardiol Mex* 1983; **53:** 397–405.
29. Hansen JF. Coronary collateral circulation: Clinical significance and influence on survival in patients with coronary artery occlusion. *Am Heart J* 1989; **117:** 290–295.
30. Pijls NH, Bech GJ, el Gamal MI, Bonnier HJ, De Bruyne B, Van Geider B, et al. Quantification of recruitable coronary collateral flow in conscious human and its potential to predict future ischemic events. *J Am Coll Cardiol* 1995; **25:** 1522–1528.
31. Billinger M, Kloos P, Eberli Fr, Windecker S, Meier B, Seiler C. Physiologically assessed collateral flow and adverse cardiac ischemic events: A follow-up study in 403 patients with coronary artery disease. *J Am Coll Cardiol* 2002; **40:** 1545–1450.
32. Choi EK, Kim HS, Park KW, Kim HK, Cho JW, Lee MM, et al. Novel index of coronary collateral development as a useful predictor of cinical outcome in Type 2 diabetic patients with coronary artery disease. *Circ J* 2005; **69:** 786–792.
33. Hankey GJ, Eikelboom JW. Homocysteine and vascular disease. *Lancet* 1999; **354:** 407–413.
34. Wald DS, Law M, Morris JK. Homocysteine and cardivascular disease: Evidence on casuality from a meta-analysis. *BMJ* 2002; **325:** 1202–1206.
35. Tsai JC, Perrella MA, Yoshizumi M, Hsieh CM, Haber E, Schlegel R, et al. Promotion of vascular smooth muscle cell growth by homocysteine: A link to atherosclerosis. *Proc Natl Acad Sci USA* 1994; **91:** 6369–6373.
36. Chambers JC, Mc Gregor A, Jean Marie J, Obeid OA, Kooner JS. Demonstration of rapid onset vascular endothelial dysfunction after hyperhomocysteinemia: An effect reversible with vitamin C therapy. *Circulation* 1999; **99:** 1156–1160.
37. Morita H, Kurihara H, Yoshida S, Saito Y, Shindo Y, Oh-Hashi Y, et al. Diet-induced hyperhomocysteinemia exacerbates neointima formation in rat carotid arteries after balloon injury. *Circulation* 2001; **103:** 133–139.
38. Nagai Y, Tasaki H, Takatsu H, Nihei S, Yamashita K, Toyokawa T, et al. Homocysteine inhibits angiogenesis in vitro and in vivo. *Biochem Biophys Res Comm* 2001; **281:** 726–731.
39. Woo KS, Chook P, Lolin YI, Cheung AS, Chan LT, Sun YY, et al. Hyperhomocysteinemia is a risk factor for arterial endothelial dysfunction in humans. *Circulation* 1997; **96:** 2542–2544.
40. Nagai Y, Tasaki H, Miyamato M, Nihei S, Kobayashi K, Yamashita K, et al. Plasma level of homocysteine is inversely associated with the development of collateral circulation in patients with singlevesselcoronary artery disease. *Circ J* 2002; **66:** 158–162.
41. Nathoe HM, Koerselman J, Buskens E, van Dijk D, Stella PR, Plokker TH, et al. Determinants and prognostic significance of collaterals in patients undergoing coronary revascularization. *Am J Cardiol* 2006; **98:** 31–35.
42. Cohen MV. Morphological considerations of the coronary collateral circulation in man. In Coronary Collaterals: Clinical and Experimental Observations. Armonk, NY: Futura Publishing; 1985: 93–114.