CORRELATION OF HOMOCYSTEINE CONCENTRATION WITH PLASMA FIBRINOGEN AND PHYSICAL ACTIVITY IN MALES WITH CORONARY ARTERY DISEASE

by

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(Abstract)

Elevated homocysteine (Hcy) concentration has been identified as an independent risk factor for premature CAD. Associations between Hcy concentrations and established cardiovascular risk factors have occasionally, but not consistently, been demonstrated. Plasma fibrinogen and total Hcy concentrations, along with other risk factors, folate and B-vitamin supplements, and medications, were recorded for 40 males (mean age ± SD: 65 ± 9.8 yr) with CAD. Physical activity was assessed using the Modifiable Activity Questionnaire (MAQ), a written questionnaire which appraises leisure and occupational activities by recall for a 12 month period. Univariate analyses revealed those subjects on beta-blocker therapy (n = 12) had lower fibrinogen concentrations than those not on these medications (n = 28) (277.7 ± 16.7 vs. 316.1 ± 10.9 mg/dl , respectively, p = 0.04). A trend existed for those on beta-blockade to also have lower Hcy concentrations (8.3 ± 0.66 vs 9.7 ± 0.43 μmol/L, respectively, p = 0.058). Subjects in the upper tertile of physical activity had significantly lower fibrinogen concentrations than those in the lower tertile (274.7 ± 38 mg/dl vs. 320.2 ± 63, respectively, p = 0.05). Homocysteine concentration was found to be positively associated with age (p = 0.0008). No significant associations were established with multivariate analyses among fibrinogen, Hcy, physical activity, age, BMI, B-vitamin and folate supplements, beta-blocker therapy, total cholesterol, HDL, LDL, triglycerides, and TC/HDL ratio. These results support the hypothesis that hyperhomocysteinemia is an independent risk factor for CAD. Future studies should

consider the favorable effects of beta-blockade, which may be a confounding factor, on Hcy and fibrinogen concentrations. Knowledge of associations may contribute toward understanding of the pathogenesis of CAD.

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I dedicate this thesis to my deceased father, who gave me the ambition to reach for the stars and the opportunity to do whatever I wanted to do.

Special thanks to my mother, for her relentless support, and my brother, for his encouragement in my interest in the medical/health field. All of my professors, from kindergarten to college, have helped guide me to where I am today in my educational path. Friends and classmates have made this journey more enjoyable and fulfilling and have taught me to work hard and play hard.

My committee, and especially my co-chairmen, deserve recognition for the time and effort they spent on my behalf. Their guidance, knowledge and experience, though frustrating at times, have prepared me for the “next level”!

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Chapter I

INTRODUCTION

Cardiovascular disease is the number one killer of adults in the Western hemisphere. Associated modifiable risk factors include hypertension, hypercholesterolemia, obesity, physical inactivity and smoking. Fibrinogen has emerged as an independent risk factor of importance equal to or greater than that of other previously described cardiovascular risk factors (Ernst & Schmid, 1993; Folsom, 1995; Heinrich & Assman, 1995; Kannel, Wolf, Castelli, & D'Agostino, 1987; Lip, 1995; Stratton et al., 1991; Thompson, Kienast, Pyke, Haverkate, & Loo, 1995). Fibrinogen is a large plasma glycoprotein, involved in blood clotting, and in the rheologic characteristics of blood flow. It is a cardiovascular risk factor that manifests its effects in coronary occlusive events through its participation in both the atherogenic and thrombogenic processes (Ernst, Koenig, Lowe, & Meade, 1992; Lip & Beevers, 1994). Fibrinogen concentration is significantly and directly related to the incidence of coronary events (Heinrich, Balleisen, Schulte, Assman, & Loo, 1994; Kannel et al., 1987; Meade et al., 1986; Thompson et al., 1995; Yarnell et al., 1991). Thompson *et al* (1995) reported that, in a population with coronary artery disease (CAD), relative risk of subsequent coronary events tripled from that for patients in the bottom quintile in terms of fibrinogen concentration to that for those in the top quintile. Despite these recent advances in the understanding of fibrinogen and its relationship to CAD, information is required on whether reduction of plasma fibrinogen improves patient outcome and should therefore be incorporated into clinical practice.

Also playing a role in thrombosis and atherogenesis is homocysteine (Hcy), a sulfhydryl-containing amino acid formed by demethylation of methionine. Multiple studies have shown elevated Hcy concentrations in patients with CAD (Alfthan, Pekkanen, Jauhiainen, & al, 1994; Arnesen et al., 1995; Boushey, Beresford, Omenn, & Motulsky,

1995; Clarke et al., 1991; Genest et al., 1990; Malinow, 1990; Robinson et al., 1995; Stampfer et al., 1992; von Eckardstein et al., 1994). This finding is all the more convincing given the variety of methods used in these studies. The strength and independence of Hcy as a risk factor for vascular disease has been established by Clarke *et al* (1991), Pancharuniti *et al* (1994), Arnesen *et al* (1995), Robinson *et al* (1995), and Stampfer *et al* (1992). A linear relationship between Hcy concentrations and CAD risk was reported by Clark *et al* (1991), Pancharuniti *et al.* (1994) and Arnesen *et al.* (1995). In the studies of Arnesen and Pancharuniti, the relative risk for a 4 μmol/l increase in serum Hcy was 1.32. A generalized additive model, developed by Robinson *et al* (1995), demonstrates that persons at the upper end of the "normal range" for Hcy concentrations may have odds of coronary disease risk three to four times higher than persons at the lower end of the range. Stampfer *et al.* (1992), in contrast, found that the risk of acute coronary events was apparent only among those in the top fifth percentile of the Hcy distribution and the relative risk was equal to 3.4.

Few studies have been reported that analyze the relation between Hcy and fibrinogen. It would appear to be logical to investigate the association between these two metabolic factors as Hcy has been shown to have a deleterious effect on the normal prothrombolytic and anticoagulant activities of endothelial cells (Fryer, Wilson, Gubler, Fitzgerald, & Rodgers, 1993; Hajjar, 1993; Lentz & Sadler, 1991; Rodgers & Conn, 1990) and fibrinogen plays a key role in coagulation, platelet aggregation, and fibrinolysis, all which have a role in thrombosis and hypercoagulation (Lip, 1995; Teger-Nilsson, Larsson, Hjemdahl, & Olsson, 1991). Harker and colleagues (1974) investigated this association in patients with homocystinuria and found higher fibrinogen concentrations in patients compared with control subjects. Homocysteine values were found to be unrelated to plasma fibrinogen in stroke survivors (Brattstrom et al., 1992). A more recent study conducted by von Eckardstein *et al.* (1994) showed that Hcy is positively related to fibrinogen concentrations. Their results indicated that the statistical significance of the

difference of Hcy concentrations between patients and controls disappeared when adjusted for fibrinogen concentrations. Additional studies in this area are clearly indicated due to lack of data and the conflicting results of these studies. If the correlation between Hcy and fibrinogen is positive, the effects on hemostasis would result in a proaggregatory and procoagulatory state.

Usually, lifestyle changes are preferable to medication in primary or secondary prevention of chronic diseases. Exercise is a commonly recommended lifestyle intervention for individuals at risk for, or diagnosed with, CAD. While it is generally accepted that regular physical activity reduces the risk of cardiovascular disease, the physiologic effects are only partially understood. Long-term exercise favorably modifies several of the conventional CAD risk factors including blood lipids, obesity, blood pressure, and glucose intolerance, however, the magnitude of change in each of these factors by themselves is moderate, 14 - 26 percent as reported by Thompson *et al.* (1988). The inverse association between physical activity and CAD remains after controlling for the previously listed variables (Blair et al., 1996). Regular physical activity has been shown to reduce plasma fibrinogen in healthy subjects, as well as those with CAD (Connelly, Cooper, & Meade, 1992; Elwood, Yarnell, Pickering, Fehily, & O'Brien, 1993; Moller & Kristensen, 1991; Stratton et al., 1991; Wosornu, Allardyce, Ballantyne, & Tansey, 1992). No such association has been consistently shown between exercise and Hcy concentrations (Murphy-Chutorian et al., 1985; Nygard et al., 1995; Pancharuniti et al., 1994).

The purpose of this cross-sectional study is to investigate the association between fibrinogen and Hcy concentrations in male patients with CAD and to determine if regular physical activity is associated with altered plasma Hcy concentrations in this same population.

Significance of the Study

The concentration of plasma fibrinogen is regulated by both genetic and environmental influences. Factors that are positively associated with plasma fibrinogen include age, female gender, smoking, stress, obesity, menopause, oral contraceptives, and LDL cholesterol (Folsom, 1995; Folsom et al., 1991; Moller & Kristensen, 1991). Those with a negative association include estrogen replacement, HDL cholesterol, and Japanese ethnicity (Folsom, 1995; Folsom et al., 1991; Moller & Kristensen, 1991). These findings indicate that the same variables which modulate the risk of CAD (exercise and maintaining proper weight, smoking cessation, stress reduction, and dietary fat restriction) may also lower fibrinogen concentrations.

Plasma Hcy increases with age and is higher in men and smokers (Alfthan et al., 1994; Arnesen et al., 1995; Nygard et al., 1995; Robinson et al., 1995; von Eckardstein et al., 1994). The effect of high concentrations of Hcy on risk of CAD is not mediated by known risk factors. The determinants of elevated Hcy are likewise poorly understood. It seems likely that a genetic component is involved in at least some cases (Genest et al., 1991). In particular, several metabolic defects involved in the metabolism of Hcy can lead to elevations in its concentration (Kang, Wong, Bock, Horwitz, & Grix, 1991; Rubba et al., 1990). In addition, vitamins B_6, B_{12}, and folate are involved as cofactors in this metabolic process, and inadequate amounts of these vitamins, either through a deficiency in intake or through other conditions, can also lead to high concentrations of Hcy (Malinow, 1990; Pancharuniti et al., 1994; Robinson et al., 1995). Nutritional surveys suggest that suboptimal intake of vitamin B_6 and folate are not uncommon in the United States (Subar, Block, & James, 1989).

Fibrinogen and its derivatives have been shown to be involved in the initiation and growth of atherosclerotic lesions via effects on the coronary endothelium (Bini & Kudryk, 1992; Smith, Thompson, Crosbie, & Stirk, 1992), in addition to its role in the coagulation cascade and thrombosis development. Moderate elevation in plasma Hcy concentrations is

significantly associated with hemostatic changes that favor thrombosis, namely inhibited protein C activation (an anticoagulant) by decreasing thrombomodulin cell-surface expression (Lentz & Sadler, 1991). Thrombomodulin is an endothelial cell surface glycoprotein which binds thrombin, thereby reducing the amount available to catalyze the conversion of fibrinogen to fibrin. Homocysteine also has been shown to induce vascular smooth muscle cell proliferation consistent with development of premature atherosclerotic lesions and it impedes regeneration of endothelial cells (Tsai et al., 1994).

Normal vascular endothelium provides an antithrombogenic barrier that is selectively permeable to a variety of plasma constituents. Following damage to the arterial wall that involves exposure of the subendothelial collagen, platelets adhere to the site of injury, aggregate, and release a number of active substances which promote further platelet aggregation, vasoconstriction, and growth factor release. Eventually there is deposition of calcium and the development of a complicated atherosclerotic plaque. Through examination of the relationship between two factors believed to play a role in endothelial damage, fibrinogen and Hcy, a better understanding of CAD risk factor interactions may be gained.

Research Hypotheses

1) There will be no association between plasma fibrinogen and Hcy concentrations in males with diagnosed CAD.

2) There will be no association between plasma homocysteine concentrations and physical activity in males diagnosed with CAD.

Delimitations

1) Males with CAD were selected for this study because elevated concentrations of fibrinogen and Hcy are associated with CAD. Females were excluded because concentrations of these variables are significantly different in men compared with women.

2) Subjects involved in a phase III cardiac rehabilitation program were selected due to access to descriptive data and medical information for these participants.

3) The Modified Activity Questionnaire (MAQ) was chosen as the instrument to assess physical activity. It is comprehensive, allows activities to be weighted by intensity, and has accepted published evidence of reliability and validity. The MAQ was developed by and received from Andrea M. Kriska, Ph.D (Kriska et al., 1990).

Limitations

1) Physical activity questionnaires tend to overestimate actual physical activities and the MAQ depends on the ability of the subject to recall activities over a one year period.

Basic Assumptions

1) Subjects provided accurate information on the health/risk factor appraisal form and physical activity questionnaire.

2) Subjects were compliant with providing a fasting blood sample.

Definitions

Phase III Cardiac Rehabilitation Program: Community or hospital based exercise program in which participants have progressed through hospital inpatient and/or outpatient programs or may have been referred without previous participation. These programs include patients who are approximately 6 - 12 weeks post-hospital discharge, have clinically stable or decreasing angina, medically controlled dysrhythmias during exercise, a knowledge of symptoms, and the ability to self-regulate their exercise.

Summary

Fibrinogen can be considered an independent risk factor for CAD. While Hcy appears to be independent of other CAD risk factors, there is some evidence to suggest that it may be associated with plasma fibrinogen concentrations. Both fibrinogen and Hcy exert effects on endothelial cells which promote platelet aggregation and hypercoagulation. These changes contribute to risk of thrombosis and atherosclerotic disease. Regular physical activity is commonly recommended to patients with CAD due to its favorable effects on CAD risk factors. While exercise is associated with a reduction in plasma fibrinogen concentrations, no such association has been shown with Hcy. If fibrinogen is correlated with Hcy, plasma Hcy concentrations may also decline with regular exercise. Additional studies are necessary which examine the relation between Hcy and CAD risk factors and the effects of physical activity on plasma Hcy concentrations.

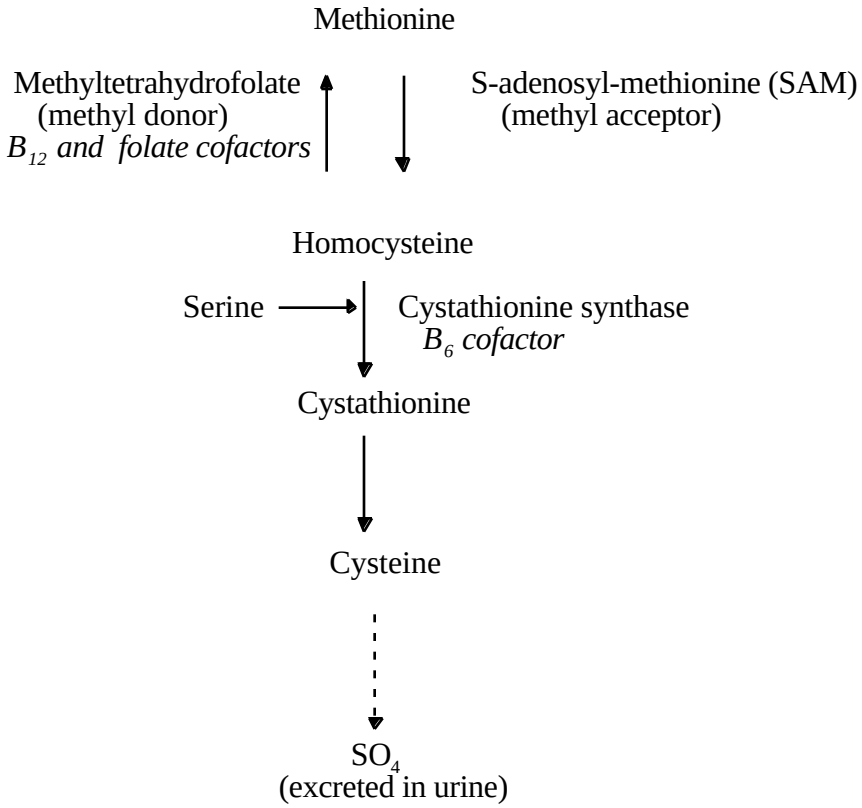
Chapter II

LITERATURE REVIEW

All the major identified cardiovascular risk factors predict CAD, including cigarette smoking, elevated cholesterol, blood pressure, fibrinogen, and physical inactivity. Data from 30 years of follow-up on the Framingham Study indicate that in males, blood pressure (systolic and diastolic), serum cholesterol, and smoking each exert an independent effect such that the risk for CAD development increases by 33 percent for each standard deviation increase ($p < 0.001$) (Kannel & Larson, 1993). In the European Concerted Action Project (COMAC) case-control study, which included 1550 participants, fasting and post-load plasma Hcy concentrations were found at least to match in strength conventional risk factors such as cholesterol level (Graham, 1994). A meta-analysis performed by Boushey *et al* (1995) found that the odds ratio for CAD for an increase of 5 µmol/L Hcy was of the same order (1.6) as a 20 mg/dl increase in total cholesterol for CAD and that the arteriosclerotic vascular response to Hcy is graded (similar to cholesterol) rather than based on a threshold effect. An association between Hcy concentrations and established cardiovascular risk factors has occasionally, but not consistently, been demonstrated. Knowledge of such associations is needed to identify potential confounders in studies of Hcy and disease, and may contribute toward understanding of the pathogenesis of CAD.

Homocysteine is a sulphur amino acid produced by the demethylation of the essential amino acid methionine (Fig. 1). Cystathionine synthase, which requires pyridoxal phosphate as a cofactor, catalyses the condensation of Hcy and serine to irreversibly form cystathionine. This is ultimately converted to sulphur dioxide and water and is excreted in the urine. Alternatively, Hcy may be remethylated to form methionine. Remethylation is catalyzed by methionine synthase, which requires vitamin B_{12} and folate as cofactors. Hyperhomocysteinemia may therefore arise from genetic defects of enzymes

Figure 1. Pathways for the trans-sulphuration and remethylation of homocysteine.



controlling Hcy degradation and remethylation, from vitamin B_{12}, B_6, or folic acid deficiency, or from other disease states such as renal failure.

The scope of this review is threefold: (1) to examine the role of plasma Hcy in the CAD risk profile, including its relation with other risk factors; (2) to explore the correlation between fibrinogen and Hcy; and (3) to study the effects of physical activity on plasma Hcy concentrations.

Homocysteine and the CAD risk profile

The association between coronary artery disease (CAD) and the factors which predispose to it are based on observational studies and, therefore, these predisposing conditions are termed 'risk factors'. Epidemiological research has delineated multiple factors associated with the subsequent development of disease, and no single risk factor has been found to be essential or sufficient in the evolution of the disease. Five major classes of risk factors which promote CAD have been suggested: (1) living habits; (2) atherogenic biologic attributes; (3) signs of active lesion degeneration; (4) indicators of a compromised circulation; and (5) host susceptibility (Kannel & Larson, 1993). Included in host susceptibility are inborn errors of metabolism. Impaired metabolism of methionine caused by partial or complete deficiencies in any of several enzymes or cofactors leads to the accumulation of the atherogenic amino acid, homocysteine (Hcy). Hyperhomocysteinemia has been associated with premature CAD and several studies suggest that it is an independent risk factor for CAD development (Arnesen et al., 1995; Clarke et al., 1991; Genest et al., 1990; Murphy-Chutorian et al., 1985; Pancharuniti et al., 1994; Robinson et al., 1995; Stampfer et al., 1992; von Eckardstein et al., 1994).

Genest *et al* (1990) studied plasma concentrations of Hcy in men with angiographically documented premature CAD (n = 170, mean age ± SD 50 ± 7 yr) and in those who were clinically free of cardiovascular disease (n = 255, 49 ± 6 yr) from the Framingham Heart Study. The relationship between CAD risk factors and Hcy

concentrations was analyzed. Plasma concentrations of Hcy were significantly higher in patients with CAD than in control subjects (13.7 ± 6 vs. 10.9 ± 4.9 nmol/ml, $p < 0.001$). There were no statistically significant associations between cholesterol, triglycerides, low-density lipoproteins (LDL), or high-density lipoproteins (HDL) and Hcy. The presence of hypertension, smoking, or diabetes mellitus did not significantly alter Hcy concentrations in the patient or the control group. Data from this study revealed that patients with four or more CAD risk factors had higher Hcy concentrations than those with two risk factors ($p < 0.04$). No significant differences in Hcy concentrations were found between subjects aged 40 to 49 yeas and those aged 50 to 60 years in either the control or the CAD group. This study did not examine the relation between Hcy and fibrinogen or physical activity.

While an investigation reported by Clarke *et al* (1991) did not examine the association of Hcy with other CAD risk factors, including fibrinogen and physical inactivity, it did demonstrate in a sample of the Irish population, that hyperhomocysteinemia was present in 30 percent of patients with premature CAD (72 males and 33 women, < 55 yr) compared with an estimated 2 percent of the Irish population. Coronary vascular disease was confirmed by demonstration of a myocardial infarct or by coronary arteriography. The unadjusted odds ratio for development of CAD among the patients with elevated Hcy concentrations was 23.9. This odds ratio is higher than those for patients with hypercholesterolemia (3.1), hypertension (7.8), and cigarette smoking (3.5). Patients with hyperhomocysteinemia had a higher risk-factor profile than those without it, making it possible that the increased risk in patients with this abnormal finding could be explained by the higher frequency of other risk factors. Red-cell folate concentrations and serum vitamin B_{12} concentrations were significantly lower ($p < 0.01$) in patients with hyperhomocysteinemia, indicating that dietary supplements of folic acid, with or without pyridoxine, may reduce Hcy concentrations.

A total of 14,916 male physicians (40 - 84 yr) participating in the Physicians' Health Study with no prior myocardial infarction (MI) provided plasma samples at baseline and

were followed up for 5 years (Stampfer et al., 1992). Samples from 271 men who subsequently developed MI were analyzed for Hcy concentrations together with paired controls, matched by age and smoking status. No pre-sample criteria, such as fasting, were described. The patients had a higher mean concentration of Hcy as compared with controls (11.1 ± 4.0 [SD] vs 10.5 ± 2.8 nmol/ml, $p = 0.03$). This difference between the means was similar to the differences for several established coronary risk factors such as cholesterol and blood pressure. Hypertensives were found to have significantly higher Hcy concentrations than normotensives ($p < 0.01$), but little association was observed between plasma Hcy concentrations and other coronary risk factors (cholesterol, HDL's, diabetes). Significant inverse correlations between Hcy and intake of vitamins B_1, B_2, B_6, B_{12}, niacin, retinol, vitamin C, vitamin E, and folate ($p < 0.001$) were found. Correlations with fibrinogen and physical activity were not considered in this study. The key observation of this investigation was that men with Hcy concentrations above the 95th percentile (Hcy > 15.8 nmol/ml) had a three-fold increase in risk of myocardial infarction compared with those with values in the bottom 90% of the control distribution. This risk remained unchanged after adjusting for other coronary risk factors.

A case-control study of early-onset CAD was performed by Pancharuniti *et al* (1994) with 101 blindly selected patients with CAD and 108 blindly selected controls. All subjects were white males, 30 - 50 y of age. Coronary artery disease was defined as 1) chest pain consistent with CAD, 2) electrocardiogram changes consistent with CAD, 3) positive exercise test or radionuclide arteriography, or 4) myocardial infarction. Information obtained from subjects at baseline included Body Mass Index (BMI), physical activity, diabetes, smoking, blood pressure, HDL and total cholesterol, triglycerides, glucose, LDL cholesterol (estimated), Hcy, and dietary intake by food-frequency questionnaire. Patients had significantly higher plasma Hcy concentrations than control subjects (13.5 vs 11.9 nmol/ml, $p < 0.0005$). Significant inverse correlations were found between plasma Hcy and folate among controls and patients ($p = 0.0001$) and between plasma Hcy and vitamin B_{12} among

controls and patients ($p = 0.0001$ and $p = 0.02$, respectively). The intake of multivitamin B supplements was inversely correlated with plasma Hcy in both groups, although it was significant only among the control subjects (control subjects: $p = 0.02$; patients: $p = 0.22$). Daily intakes of thiamin, riboflavin, niacin, and vitamins A, C, D, and E were not significantly correlated with Hcy. Low-density lipoprotein cholesterol was significantly positively correlated with plasma Hcy among the control subjects ($p = 0.008$) but not among the patients. None of the other factors examined (smoking, diabetes, blood pressure, age, BMI, and physical activity) were significantly correlated with plasma Hcy. Fibrinogen concentration was not an examined variable in this study. The odds ratio (OR) for both quartiles above the median of Hcy concentration in control subjects were significantly increased compared with the lowest quartile, 6.7. The OR increased 60 percent per quartile increase ($p < 0.001$). The authors concluded that increased plasma Hcy concentrations was an independent risk factor for onset of CAD in white males aged ≤ 50 years, which may be reduced through an increase in folate intake.

Murphy-Chutorian and colleagues (1985) interviewed 99 male patients (mean age 53 ± 8) with a diagnosis of CAD and 39 age-matched males (mean age 52 ± 8) known to have angiographically normal coronary arteries. Patients had a preangiography diagnosis of atherosclerotic heart disease, atypical chest pain, valvular heart disease, or cardiomyopathy. Data were obtained on smoking history, hypertension, family history of atherosclerotic heart disease, diabetes mellitus, hyperlipidemia, previous MI, dietary history, exercise habits, and current medications. Laboratory values were obtained for fasting plasma Hcy, blood glucose, serum cholesterol, and triglycerides. There were no significant correlations among plasma Hcy and any of the above mentioned variables. In addition, 16 percent of the subjects with CAD had Hcy concentrations above the 95th percentile (11 nmol/ml, 1.64 SD above the mean) compared with 2 percent of the subjects without CAD ($p < 0.04$). Univariate analysis of Hcy concentration yielded an OR of 7.3, indicating a sevenfold increased risk for CAD. A multivariate analysis showed that none of the known risk factors

were important confounding factors because there was little change in the OR (6.7) of elevated Hcy concentrations. This OR is higher than that of all other variables including cigarette smoking (3.5) and hypertension (4.0).

In a prospective nested case-control study, in which both cases and controls were selected from the same population-based cohort, non-fasting blood samples from 122 subjects with CAD (51.3 ± 7.3 yr, 90% male) and 478 controls (51.2 ± 7.3 yr, 90% male) were analyzed to determine Hcy concentrations and lipid profiles (Arnesen et al., 1995). Cases had a diagnosis of coronary heart disease (ICD-9 410 - 412). Other risk factor information, age, gender, state of health, and number of cigarettes smoked per day, was obtained via interview. Cases had a significantly higher mean concentration of Hcy than the controls (12.7 ± 4.7 vs 11.3 ± 3.7 umol/L, $p = 0.0002$). Serum Hcy correlated with total cholesterol ($p = 0.0031$), systolic blood pressure ($p = 0.019$) and the number of cigarettes smoked per day ($p = 0.0001$), but not with HDL cholesterol or triglycerides. Associations between Hcy and fibrinogen and physical activity were not examined. For each 4 µmol/l (approximately 1 SD) increase in serum Hcy concentration, a relative risk of 1.4 was calculated. This finding supports the author's hypothesis that within the normal range of serum Hcy, there is no threshold level below which Hcy is not associated with risk of myocardial infarction. They conclude that a considerable proportion of the population may therefore be at risk.

The objective of the Hordaland Homocysteine Study (Nygård et al 1995) was to estimate the relations between established cardiovascular risk factors and total plasma Hcy. The 16,176 participants (7591 males, 8585 females; 40 - 67 yr) in this study had no history of hypertension, diabetes, CAD, or cerebrovascular disease. Data which were collected through questionnaires, examinations, and blood sampling included smoking habits, physical activity, intake of vitamin supplements, fruits, and vegetables, blood pressure, lipid profile, height, weight, and Hcy. It should be noted that these subjects had not fasted prior to sampling and the plasma fraction, once separated, was not frozen and was analyzed 1 to 4

days after collection. Plasma Hcy concentration was higher in men than in women and increased progressively with age. Current smokers had a significantly higher plasma Hcy concentration ($p < 0.001$) that increased almost linearly with the daily number of cigarettes smoked. Plasma Hcy concentration showed a positive linear association with diastolic and systolic blood pressure ($p < 0.001$). There was also a positive linear association between Hcy and serum cholesterol ($p < 0.001$) and a weaker positive relation between triglycerides and Hcy ($p = 0.005$ in males and $p = 0.06$ in females). The vitamin and the fruit-vegetable scores were both highly significantly related to plasma Hcy concentration, while between plasma Hcy and BMI, there was only a weak relation which disappeared in the multivariate analysis. Physical activity was found to be negatively correlated with Hcy and the results will be discussed later in this review. In the final model, sex, age, cigarette smoking, and the vitamin supplement score were the strongest determinants of Hcy concentration.

In a case-control study conducted by von Eckardstein *et al* (1994), 199 male CAD patients (age 50.3 ± 5.2 yr) and 156 age-matched controls subjects (age 49.0 ± 7.4 yr) were studied to examine the role of Hcy as a cardiovascular risk marker in the context of traditional risk factors (blood pressure, total cholesterol, triglycerides, HDL's, LDL's, and fibrinogen). Coronary artery disease was angiographically diagnosed (at least one vessel being stenosed by 50 percent), or was determined by previous myocardial infarction or aortocoronary bypass surgery. Blood samples were not specified as fasting samples and centrifugation was performed within 2 hours after sampling. Plasma was then frozen until assays were performed. Linear regression analyses revealed positive relations between age and Hcy concentrations in both patients and control subjects ($p < 0.001$). Fibrinogen and HDL- and LDL-cholesterol were also significantly correlated with age ($p < 0.001$). Homocysteine was not significantly correlated with blood pressure, total cholesterol, triglycerides, HDL's or LDL's. Patients had a higher concentration of Hcy compared with control subjects (8.9 µmol/L vs 7.8 µmol/L, respectively, $p < 0.001$). The importance of Hcy as a marker for CAD was revealed when its serum concentration was related to the

extent of coronary vessel disease. Besides fibrinogen, only Hcy was significantly associated with the number of stenosed coronary artery vessels (1 vessel: 8.64 µmol/L, 2 vessel: 8.69 µmol/L, 3 vessel: 9.61 µmol/L, $p < 0.05$). In this age-matched study, hyperhomocysteinemia was an independent cardiovascular risk factor after adjustment for the traditional risk factors. This independence disappeared when fibrinogen was considered. The relationship between Hcy and fibrinogen will be discussed in the following sub-heading.

Homocysteine exists in numerous forms in plasma, as a small percentage in the free form, as disulfides with itself and cysteine, and as disulfides with albumin (~70%). In quantifying Hcy concentration, reducing agents are used to reduce disulfide bonds and to yield free Hcy. The next step in quantification is chromatography. Only a few methods are totally automated and the throughput is relatively low, 60 - 80 samples per day. Total Hcy is the clinically relevant measure, with reference values in fasting subjects of 5 to 15 µmol/L (Ueland et al., 1993). All of the studies discussed above utilized a form of high pressure liquid chromatography (HPLC), most with electrochemical detection. The precision of this assay, also known as the coefficient of variation (CV), was reported as ≤ 3.2% by Nygard *et al* (1995), Stampfer *et al* (1992), Arnesen *et al* (1995), and Murphy-Chutorian *et al* (1985). Two studies reported a correlation coefficient between replicate measurements. Nygard *et al* (1995) and Clarke *et al* (1991) obtained an intraclass coefficient of $r = 0.99$. A rapid and automated immunoassay for measuring plasma Hcy has been recently developed and validated (Malinow, 1994). A processor in the Abbott IMx Analyzer (Abbott Laboratories, Abbott Park, IL) reads the signal, corrects for the background, calculates the concentration, and prints the results. Twenty 70 µL samples can be processed per hour and instrument standardization can be as frequent as every two weeks. The high precision and very low inter- and intraassay CVs (3.6, 3.4, 2.7, 1.8 and 2.5, 3.5, 2.5, 1.8 at 7.5, 15, 30, and 60 µmol/L respectively) may eliminate the need to make duplicate or more

determinations. Correlations with HPLC and electrochemical detection yielded an r value of 0.98 and r values of greater than 0.99 were obtained with other HPLC methods.

Homocysteine and fibrinogen

The results obtained by von Eckardstein *et al* (1994) were further analyzed to study the association between Hcy and fibrinogen concentrations. Multiple logistic function analysis was used to test the independence of fibrinogen and Hcy as predictors of CAD. In this model, Hcy was significantly different between patients and control subjects only when fibrinogen was not considered. In contrast, fibrinogen remained significantly different ($p < 0.001$) even when adjusted for Hcy. These results were repeated when a bivariate model was run which considered only fibrinogen and Hcy. The authors concluded that moderately elevated Hcy concentrations represent a cardiovascular risk factor and that this risk association was independent of most coronary risk factors except fibrinogen.

There have been few other studies which have examined the relation between Hcy and fibrinogen. Harker *et al* (1974) designed a study to clarify the nature of the thrombotic process. Since thrombosis represents the response of hemostatic components to altered endovascular surfaces under variable flow conditions, the relative participation of platelets, coagulation factors and fibrinolysis in the thrombotic process was measured. Four patients (13 - 24 yr, 2 male and 2 female) with homocystinuria were investigated along with 35 control subjects. Homocystinuria is an inborn error of metabolism due to a deficiency of the enzyme cystathionine synthase, which along with pyridoxal phosphate, is responsible for the conversion of Hcy to cystathionine. Abnormally high concentrations of Hcy are observed and this disorder manifests clinically by progressive cardiovascular disease and a high frequency of thromboembolism. The patients were found to have higher fibrinogen levels compared with controls ($p < 0.01$). The patients also demonstrated a threefold increase in platelet consumption with a corresponding 20 percent increase in fibrinogen and plasminogen utilization ($p < 0.05$). It was concluded that the underlying process of

homocystinemic thrombosis probably involves formation of platelet thrombus on altered, nonendothelialized endarterial surfaces.

In an investigation conducted on 142 stroke survivors, analyses were performed to determine whether relationships exist between plasma Hcy and other stroke risk factors (Brattstrom et al., 1992). Due to the high average age, the stroke patients were divided into two groups by age: 38 - 72 (54 males, 16 females) and 73 - 90 (49 males, 23 females) years old. A control group was comprised of 34 males and 32 females aged 56 to 68 years old. Values for plasma Hcy were significantly higher in the younger patient group than in their respective controls (15.9 ± 5.9 umol/L vs 11.9 ± 3.0 umol/L, respectively, $p < 0.001$) but not as high as in the older age group of stroke patients (20.8 ± 8.3 umol/L, $p < 0.01$). In all, 57 out of 142 patients (40%) had Hcy values exceeding the mean plus two standard deviations for the controls. No relationship was found between Hcy and conventional occlusive arterial disease risk factors (hypertension, smoking, and cholesterol). No significant correlation between plasma homocysteine and plasma fibrinogen concentrations, body mass index, LDL cholesterol, triglycerides or blood glucose was observed. In patients, Hcy concentrations were significantly correlated with plasma pyridoxal 5'-phosphate and blood folate ($p < 0.001$) while in the control group it was only associated with pyridoxal 5'-phosphate ($p < 0.01$). The authors conclude that moderate hyperhomocysteinemia is an independent risk factor for vascular disease and is partly related to insufficient concentrations of cofactors for Hcy metabolism.

The strong and independent association between fibrinogen concentrations in the upper third of its distribution with the onset and progression of clinical atherothrombotic disease has been well established in at lease six epidemiological studies which have been reviewed recently (Ernst & Resch, 1993). It is not clear whether the predictive value of fibrinogen is solely related to the degree of its increased plasma concentration or whether qualitative changes in this complex molecule also occur. A variety of assay methods, based on different principles, have been used in different epidemiological studies. The most

frequently used method is that of Von Clauss (Clauss, 1957): the time between the addition of thrombin and clotting is recorded. The amount of thrombin chosen ensures that the clotting time is dependent on the fibrinogen concentration of the plasma sample. Usually, serial dilutions of a pool of normal plasmas are used to make a calibration curve. In the development of the first International Standard for plasma fibrinogen, the widely adopted Clauss technique was utilized to determine whether or not the standard could be used in the calibration of four lyophilized plasmas with fibrinogen concentrations ranging from low to high on a variety of automated coagulometers. The results show clearly that the current International Standard for plasma fibrinogen is valuable and reliable (Mannucci, 1995). It also appears that there is no need for a special 'high' fibrinogen standard for the measurement of concentrations found in individuals at risk of atherothrombotic disease. The study of von Eckardstein *et al* (1994) used this method for determining fibrinogen concentration. Harker *et al* (1974) used spectrophotometry where the optical density of thrombin-clottable protein is determined after collection on a glass rod and subsequent solution in alkaline urea. The methods of Brattstrom *et al* (1992) did not indicate which fibrinogen assay was utilized. Despite all the data in favor of using the Clauss assay as the method of choice for screening individuals at risk of atherothrombotic disease, most of the epidemiological studies that established the predictive value of fibrinogen concentrations used assays other than Clauss' (Kannel et al., 1987; Meade et al., 1986; Stone & Thorp, 1985; Wilhelmsen et al., 1984). These methods include the above mentioned spectrophotometry, gravimetry (which is also based on the clottable protein principle), and nephelometry (which is based on heat-precipitable protein). In addition, the Caerphilly and Speedwell epidemiological studies found that the nephelometric measurement of fibrinogen is a much better predictor of ischemic heart disease than fibrinogen assayed according to Clauss (Sweetnam, Thomas, Yarnell, & Elwood, 1994; Yarnell et al., 1991).

Homocysteine and physical activity

There are similarly few data on the association between Hcy concentrations and physical activity. Regular aerobic exercise causes cardiovascular, neural, humoral, and metabolic changes. Many of these are likely to influence cardiovascular risk and the changes vary according to the level and duration of increased physical activity. The case for exercise exerting beneficial effects derives from epidemiological data showing that sedentary subjects have, on average, double the risk of cardiovascular disease of active individuals (Ekelund et al., 1988; Paffenbarger et al., 1993; Powell, Thompson, Caspersen, & Kendrick, 1987; Sandvik et al., 1993). The major mechanisms that may be involved in prevention of CAD by regular exercise are either effects on classical risk factors such as obesity, blood pressure, and lipid profile and/or beneficial effects on novel risk factors such as fibrinolytic activity and Hcy concentrations. In the previously mentioned study conducted by Murphy-Chutorian *et al* (1985), there was no significant correlations between plasma Hcy and exercise patterns in a population of 99 angiographically diagnosed CAD patients and 36 control subjects. Similar results were obtained by Pancharuniti *et al* (1994) in their investigation with 101 CAD patients and 108 control subjects. No statistically significant associations were found between Hcy concentrations and physical activity. The Hordaland Homocysteine Study (Nygard et al., 1995) found an inverse relation between mean Hcy concentration and the amount of exercise in leisure time ($p < 0.001$). The most pronounced difference was found in the older age group (65 - 67 yr) between the physically inactive and active subjects (12.8 umol/L vs 11.2 umol/L, respectively). Moderate and active exercise were associated with almost identical mean Hcy concentrations. Heavy physical activity conferred a further reduction in mean Hcy concentration and a marked reduction in the odds ratio for hyperhomocysteinemia (OR 0.45 for heavy training vs 0.77 for moderate exercise). With increasing activity levels, a reduction in skewness of Hcy distribution was observed. This suggests that exercise, especially heavy physical activity, exerts its most favorable effect in subjects with hyperhomocysteinemia.

No details were given in the Murphy-Chutorian *et al* (1985) study on how physical activity information was obtained. Pancharuniti *et al* (1994) rated physical activity by asking participants to describe their physical activity level during work and leisure as little or none, occasional, or regular (three or more times per week). A similar approach was utilized by Nygård *et al* (1995) in the Hordaland Homocysteine Study. The subjects were asked to mark one of the following categories that best fit their average degree of activity in leisure time for the last year: (1) sedentary or no activity, (2) walking, cycling, or other type of moderate activity for at least 4 hours a week, (3) exercise, gardening with physical exertion, or similar degree of physical activity for at least 4 hours a week (active exercise), or (4) regular heavy training or competitive sport several times a week (heavy training). Such methods of ascertaining physical activity status, while providing categorical data, are not the preferred instruments in collecting research data for assessing physical activity status. The use of standardized questionnaires which are clear and precise in defining activities performed, as well as their intensity, duration, and frequency, are routinely used in studies involving assessment of leisure and occupational activities. Currently there are at least 10 questionnaires found routinely in the literature, with none representing the "gold standard" of reliability and validity. In 1996, the International Consensus Group on Physical Activity Measurement met for the first time during the American College of Sports Medicine conference with the goal of establishing criteria for this purpose. The results of their work in this area is much anticipated.

The use of a validated and reliable instrument such as the Modifiable Activity Questionnaire (MAQ) in these type of studies should yield more accurate and convincing data. The MAQ was designed for easy modification to maximize feasibility and appropriateness of physical activity assessment in a variety of populations and age groups (Kriska & Bennett, 1992). One important feature of the MAQ is its comprehensiveness in that it assesses current occupational and leisure activities, as well as extreme levels of inactivity due to disability over the past year. This format is less likely to be affected by

seasonal variation in activity level, acute health conditions, or sudden time/schedule changes. Another feature is the ability of the MAQ to weight activities by estimates of their relative intensity. An estimate of the individual's physical activity level is determined over the past year and expressed as hours per week, or can be weighted by an estimate of the metabolic cost of each activity (METs) and expressed as MET-hours per week. The MAQ has been shown to be both reliable and valid (through comparisons with activity monitors, fitness testing, and the double labeled water technique) in adults and adolescents (Aaron et al., 1993; Kriska et al., 1990; Schultz, Harper, Smith, Kriska, & Ravussin, 1994). Test-retest reliability of this instrument was shown to be quite good with Spearman rank-order correlations ranging from 0.62 to 0.96 for individuals aged 10 - 70 yr. (Kriska et al., 1990).

Summary

Cross-sectional, case-control, and prospective studies have clearly demonstrated an association between Hcy and CAD and have concluded that Hcy is an independent risk factor for CAD. There is, however, some evidence to suggest that Hcy may be correlated with fibrinogen and, therefore, acts in concert with it to produce proaggregatory and procoagulatory effects. Because regular physical activity has been shown to reduce fibrinogen concentrations in CAD patients as well as controls, it may have similar influences on Hcy. The data available on the effects of regular exercise on Hcy concentrations are conflicting and derived from studies that only have classified physical activity levels in simple ordinal categories. To better understand the role of Hcy in the risk profile for CAD, further studies are necessary which examine the relation of Hcy to other known risk factors and include a more discriminating scaling method for physical activity assessment.

Chapter III

JOURNAL MANUSCRIPT

Correlation of Homocysteine Concentration with Plasma Fibrinogen and Physical Activity in Males with Coronary Heart Disease

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Abstract

Elevated homocysteine (Hcy) concentration has been identified as an independent risk factor for premature CAD. Associations between Hcy concentrations and established cardiovascular risk factors have occasionally, but not consistently, been demonstrated. Plasma fibrinogen and total Hcy concentrations, along with other risk factors, folate and B-vitamin supplements, and medications, were recorded for 40 males (mean age ± SD: 65 ± 9.8 yr) with CAD. Physical activity was assessed using the Modifiable Activity Questionnaire (MAQ), a written questionnaire which appraises leisure and occupational activities by recall for a 12 month period. Univariate analyses revealed those subjects on beta-blocker therapy (n = 12) had lower fibrinogen concentrations than those not on these medications (n = 28) (277.7 ± 16.7 vs. 316.1 ± 10.9 mg/dl , respectively, p = 0.04). A trend existed for those on beta-blockade to also have lower Hcy concentrations (8.3 ± 0.66 vs 9.7 ± 0.43 µmol/L, respectively, p = 0.058). Subjects in the upper tertile of physical activity had significantly lower fibrinogen concentrations than those in the lower tertile (274.7 ± 38 mg/dl vs. 320.2 ± 63, respectively, p = 0.05). Homocysteine concentration was found to be positively associated with age (p = 0.0008). No significant associations were established with multivariate analyses among fibrinogen, Hcy, physical activity, age, BMI, B-vitamin and folate supplements, beta-blocker therapy, total cholesterol, HDL, LDL,

triglycerides, and TC/HDL ratio. These results support the hypothesis that hyperhomocysteinemia is an independent risk factor for CAD. Future studies should consider the favorable effects of beta-blockade, which may be a confounding factor, on Hcy and fibrinogen concentrations. Knowledge of associations may contribute toward understanding of the pathogenesis of CAD.

key words: homocysteine, coronary artery disease, beta-blockers

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Introduction

Cardiovascular disease is the number one killer of adults in the Western hemisphere. Associated modifiable risk factors include hypertension, hypercholesterolemia, obesity, physical inactivity and smoking. Fibrinogen has emerged as an independent risk factor of importance equal to or greater than that of other previously described cardiovascular risk factors (Ernst & Schmid, 1993; Folsom, 1995; Heinrich & Assman, 1995; Kannel et al., 1987; Lip, 1995; Stratton et al., 1991; Thompson et al., 1995). It is a cardiovascular risk factor that manifests its effects in vascular occlusive disease and in coronary events through its participation in both the atherogenic and thrombogenic processes (Bachmann et al., 1995; Ernst et al., 1992; Lip & Beevers, 1994).

Also playing a role in thrombosis and atherogenesis is homocysteine (Hcy), a sulfhydryl-containing amino acid formed by demethylation of methionine. Impaired metabolism of methionine caused by partial or complete deficiencies in any of several enzymes or cofactors leads to the accumulation of this atherogenic amino acid. Hyperhomocysteinemia has been associated with premature CAD and several studies have suggested that it is an independent risk factor for CAD development (Arnesen et al., 1995; Clarke et al., 1991; Genest et al., 1990; Murphy-Chutorian et al., 1985; Pancharuniti et al., 1994; Robinson et al., 1995; Stampfer et al., 1992; von Eckardstein et al., 1994).

Usually, lifestyle changes are preferable to medication in primary or secondary prevention of chronic diseases. Exercise is a commonly recommended lifestyle intervention for individuals at risk for, or diagnosed with, CAD. Long-term exercise favorably modifies several of the conventional CAD risk factors including blood lipids, obesity, blood pressure, and glucose intolerance, however, the magnitude of change in each of these factors by themselves is moderate, i.e., 14 - 26 percent, as reported by Thompson *et al.* (1988). The inverse association between physical activity and CAD remains after controlling for the previously listed variables (Blair et al., 1996). Regular physical activity has been shown to reduce plasma fibrinogen in healthy subjects, as well as those with CAD (Connelly et al.,

1992; Elwood et al., 1993; Moller & Kristensen, 1991; Stratton et al., 1991; Wosornu et al., 1992). No such association has been consistently shown between exercise and Hcy concentrations (Murphy-Chutorian et al., 1985; Nygard et al., 1995; Pancharuniti et al., 1994).

Few studies have been reported that analyze the relation between Hcy and fibrinogen. It would appear to be logical to investigate the association between these two metabolic factors since Hcy has been shown to have a deleterious effect on the normal prothrombolytic and anticoagulant activities of endothelial cells (Fryer et al., 1993; Hajjar, 1993; Lentz & Sadler, 1991; Rodgers & Conn, 1990) and fibrinogen plays a key role in coagulation, platelet aggregation, and fibrinolysis, all which have a role in thrombosis and hypercoagulation (Lip, 1995; Teger-Nilsson et al., 1991). The purpose of this cross-sectional study is to investigate the association between fibrinogen and Hcy concentrations in male patients with CAD and to determine if regular physical activity is associated with altered plasma Hcy concentrations in this same population.

Methods

Patients and Samples

Forty males diagnosed with CAD were interviewed for this study. These subjects were recruited from cardiac rehabilitation maintenance programs and each signed an informed consent. Subjects were considered to have CAD if they had suffered a previous myocardial infarction, had angioplasty, coronary artery bypass grafting, and/or had angiographic documentation of stenosis ≥ 70% of at least one major epicardial coronary vessel. A health/risk factor questionnaire was also completed by each subject. Exclusion criteria included chronic inflammatory conditions, unstable angina, and low functional capacity due to congestive heart failure, chronic obstructive pulmonary disease, etc. All current medication and vitamin dosages were also recorded. Permission to proceed with this investigation was provided by the Human Subjects Committee of the Division of Human

Nutrition, Foods, and Exercise and from the Institutional Review Board for Research Involving Human Subjects of Virginia Polytechnic Institute and State University.

Body mass index (BMI = Wt.[kg] / Ht.[m^2]) was determined by obtaining each subject's height and weight using a calibrated medical beam balance scale. A blood lipid profile information (total cholesterol, HDL- cholesterol, triglycerides, LDL-cholesterol [calculated], and total cholesterol/HDL ratio) was extracted from each patient's most recent blood analysis. If blood values were more than 6 months old, they were not included in the data reported. Physical activity status was determined using the Modifiable Activity Questionnaire. This instrument lists all leisure and occupational activities, as well as periods of inactivity longer than one week, for a 12 month period. The MAQ has been shown to be both reliable and valid (through comparisons with activity monitors, fitness testing, and the double labeled water technique) in adults and adolescents (Aaron et al., 1993; Kriska et al., 1990; Schultz et al., 1994). Test-retest reliability of this instrument was shown to be quite good with Spearman rank-order correlations ranging from 0.62 to 0.96 for individuals aged 10 - 70 yr. (Kriska et al., 1990). The MET levels of all leisure and occupational activities were determined from a compendium of physical activities (Ainsworth et al., 1993) and physical activity was converted and reported as MET hours per week.

Laboratory methods

Participants were instructed to fast, consume no alcohol, or engage in physical activity for 12 h prior to blood sampling. Whole blood was drawn from the antecubital vein by a certified phlebotomist and aspirated into two 4.5 ml evacuated tubes containing sodium citrate . Tubes were mixed to avoid coagulation, chilled, and centrifuged at 2000 g for 20 min within 1 hour after sampling. The plasma fractions from both tubes were each transferred to a plastic vial and frozen at -20° C. Homocysteine and fibrinogen concentrations were measured within 6 weeks after sample collection.

Vials with plasma for Hcy analysis were packed in dry ice and sent by overnight shipping (Federal Express) to Abbott Laboratories (Abbott Park, IL). An automated assay

method on the Abbott IMxâ analyzer was utilized. This analyzer uses the fluorescence polarization immunoassay (FPIA) methodology. Correlations with high pressure liquid chromatography (HPLC) methods ranged from 0.98 to 0.997 (Shipchandler & Moore, 1995). Fibrinogen analyses were provided by the laboratory at Columbia Montgomery Regional Hospital (Blacksburg, VA). The TOA Medical Electronics Model CA-1000 is a fully automated, computerized blood plasma coagulation analyzer. The CA-1000 employs the photo-optical clot detection method, which is a modification of the Clauss method (Clauss, 1957).

Statistical analyses

Common log transformation was performed on Hcy values since the distribution of values was found to be positively skewed. Univariate analysis was used to determine relations between the potential explanatory variables and Hcy and fibrinogen concentrations. Chi-squared test was used to examine differences between subjects on beta-blockers and those not on beta-blockers among tertiles of fibrinogen and Hcy concentrations. All continuous variables were then entered into a multivariate analysis of correlations to determine the effect of multiple risk factors and physical activity, on plasma fibrinogen and Hcy concentrations. The Pearson's product moment correlation coefficient was used to test interrelation between the variables. Data was analyzed using JMP (Version 3.1.7, Statistical Analysis Systems, Cary, NC). Findings were considered statistically significant if the p-value was ≤ 0.05.

Results

The characteristics of the subject population are found in Table 1. In this sample, 26 subjects had a previous myocardial infarction, 17 previously had coronary artery bypass grafting (CABG), while 10 had undergone percutaneous translumenal coronary angioplasty (PTCA). Aspirin was being administered therapeutically to 82.5% (n = 33) of the cohort, while administration of warfarin for therapy was reported by 7.5% (n = 3). Subjects on beta-

blocker therapy (n = 12) had significantly lower fibrinogen concentrations than those who were not currently taking beta-blockers (n = 28) (277.7 ± 16.7 vs. 316.1 ± 10.9 mg/dl, respectively, $p = 0.04$). Those subjects in the most active third of this cohort had significantly lower fibrinogen concentrations than those in the lowest third ($p = 0.05$, Table 2). The most active subjects reported significantly more usage of beta-blockers than those in the least active tertile ($p < 0.005$) (Table 3). No other examined variables were associated with fibrinogen concentrations. A trend also existed for subjects on beta-blocker therapy to have lower Hcy concentrations than those not on such medication (8.3 μmol/L vs 9.7, respectively, $p = 0.058$). Homocysteine was significantly positively associated with age ($r = 0.52$, $p = 0.0008$) (Figure 2). No other associations were found with the examined variables. Multivariate correlation analysis revealed no significant relations among the continuous variables (Table 4).

Table 1. Descriptive data for 40 male coronary artery disease patients, age 35 to 79 years

	Mean ± SD	N
Age, y	65 ± 9.8	40
BMI, kg/m^2	27 ± 3.2	40
Total Cholesterol, mg/dl	187 ± 30.8	34
HDL, mg/dl	38 ± 11.8	29
LDL, mg/dl	124 ± 34	31
Triglycerides, mg/dl	140 ± 91	33
TC/HDL ratio	5.1 ± 1.5	28
Fibrinogen, mg/dl	303.9 ± 60.1	40
Homocysteine, µmol/L	9.53 ± 2.4	40
Physical Activity Index (MET hr/wk)	34.4 ±16.8	40

Table 2. Plasma fibrinogen concentrations (mg/dl) and physical activity tertiles in a group of 40 males with coronary artery disease

Least active third	320.2 ± 63
Middle third	314.7 ± 67
Most active third	274.7 * ± 38

• $p = 0.05$

Table 3. Chi-squared contingency table on the use of beta-blocker therapy on fibrinogen concentrations (mg/dl) in tertiles of physical activity (1 = least active, 2 = middle tertile, 3 = most active).

		Beta-blocker use		
	Count Col % Cell χ^2	No	Yes	n
Fibrinogen in tertiles of physical activity	1	11	2	13
		39.29	16.67	
		0.3967	0.9256	
	2	8	6	14
		28.57	50.00	
		0.3306	0.7714	
	3	9	4	13
		32.14	33.33	
		0.0011	0.0026	
	n	28	12	40

Figure 2. Correlation between age (yr) and $\log_{10}$ Hcy (r = 0.52, p = 0.0008). Confidence intervals are 95%.

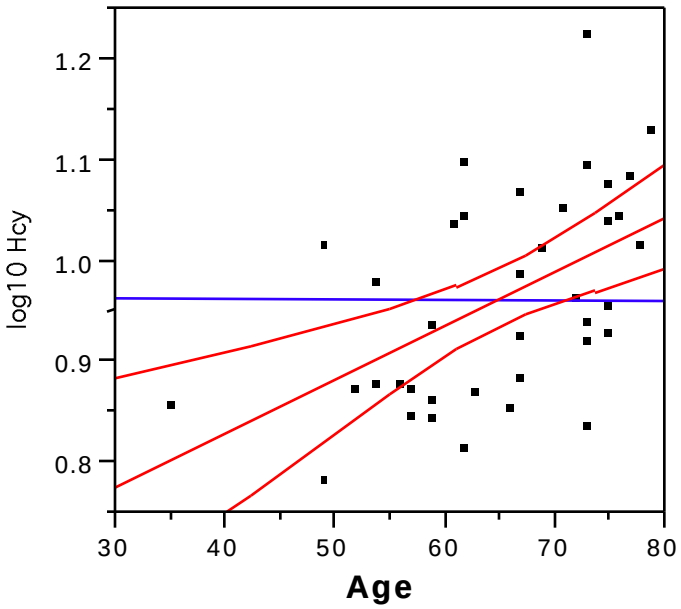

Table 4. Pearson product moment correlation coefficients for continuous variables.

Variable	log10 Hcy	Fibrinogen	PAI	Age	BMI	TC	HDL	LDL	Trig	TC/HDL
log10 Hcy	1.00	0.01	0.02	0.51	-0.04	-0.02	0.44	-0.01	-0.26	-0.27
Fibrinogen	0.01	1.00	-0.17	0.07	0.11	-0.11	-0.14	0.03	0.09	0.11
PAI	0.02	-0.17	1.00	-0.08	-0.09	0.10	0.05	0.18	-0.03	0.10
Age	0.51	0.07	-0.08	1.00	-0.41	0.06	0.34	0.02	-0.03	-0.25
BMI	-0.04	0.11	-0.09	-0.41	1.00	-0.07	-0.22	0.12	0.05	0.25
TC	-0.02	-0.11	0.10	0.06	-0.07	1.00	0.12	0.84	0.46	0.33
HDL	0.44	-0.14	0.05	0.34	-0.22	0.12	1.00	-0.07	-0.34	-0.76
LDL	-0.01	0.03	0.18	0.02	0.12	0.84	-0.07	1.00	0.18	0.42
Trig	-0.26	0.09	-0.03	-0.03	0.05	0.46	-0.34	0.18	1.00	0.49
TC/HDL	-0.27	0.11	0.10	-0.25	0.25	0.33	-0.76	0.42	0.49	1.00

PAI indicates physical activity index; TC, total cholesterol; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; Trig, triglycerides; TC/HDL, total cholesterol/high-density lipoprotein cholesterol ratio.

Discussion

In the only study found which analyzes the relation between Hcy and fibrinogen in a population with CAD, the authors reported that Hcy was independent of most coronary risk factors except fibrinogen (von Eckardstein et al., 1994). Four patients with homocystinuria were shown to have higher fibrinogen concentrations compared with control subjects (Harker, Slichter, Scott, & Ross, 1974), however, this is an extremely small sample size and those study patients had a genetic form of hyperhomocysteinemia as opposed to an acquired form. The differences in etiology may influence other hemostatic factors. In an investigation which examined these variables in 154 stroke survivors (Brattstrom et al., 1992), no correlation was found between Hcy and fibrinogen concentrations. Stroke and CAD are both forms of cardiovascular disorders with similar etiologies. The results from our study suggest that, in patients with CAD, no interrelation exists between fibrinogen and Hcy.

Likewise, there was no correlation found between physical activity and Hcy in our study. Physical activity was assessed by a 12-month recall instrument which accounts for leisure and occupational activities. Levels of physical activity are further quantified by assigning MET values to each activity and expressing total activity in MET hrs/wk. Other studies which considered physical activity used general categorization (Murphy-Chutorian et al., 1985; Nygard et al., 1995; Pancharuniti et al., 1994), which does not accurately quantify the amount of habitual activity. Nygard *et al* (1995) reported that there was an inverse relationship between mean Hcy concentration and amount of exercise in leisure time ($p < 0.001$). The subjects in the Nygard *et al* (1995) study were apparently healthy. Blood samples obtained were nonfasting and determination of Hcy concentrations were made on unfrozen serum within 7 days of collection. Both Pancharuniti *et al* (1994) and Murphy-Chutorian *et al* (1985) examined the relation between Hcy and physical activity in a population with CAD. Homocysteine determinations were made from frozen plasma

samples. Neither investigation reported a significant association between physical activity and Hcy.

A protective effect of regular exercise on the incidence and the mortality of CAD has been reported in many studies (Blair et al., 1989; Folsom et al., 1985; Leon, Connett, Jacobs, & Rauramaa, 1987; Morris, Clayton, Everitt, Semmence, & Burgess, 1990; Salonen, Slater, Tuomilehto, & Rauramaa, 1988). The mechanisms through which it exerts this influence are not clear though several have been suggested (Jennings, 1995). Favorable modification of conventional CAD risk factors, including blood pressure, lipoprotein profiles, and glucose intolerance, are generally accepted effects of chronic exercise. Significant improvements in hemostatic parameters, including fibrinogen concentrations, have been reported in patients with CAD who engage in regular aerobic and leisure activity (Elwood et al., 1993; Moller & Kristensen, 1991; Wosornu et al., 1992). Our data show that subjects in the most active tertile have significantly lower plasma fibrinogen concentrations than those in the least active tertile ($p = 0.05$). In a prospective study by Wosornu *et al* (1992) conducted over 6 mo, change in fibrinogen concentration was compared among a control group, an aerobic training group, and a power training group from a cohort of men 6 wk to 12 mo from coronary artery surgery. Their results showed a 12% reduction in fibrinogen ($p = 0.01$) compared to 2% reduction in the power group and 4% reduction in the control group. This represents a 42 mg/dl change in fibrinogen concentration which is similar to the difference between the upper and lower tertiles of physical activity in the investigation reported here. Data from the Caerphilly and Speedwell heart studies (Yarnell et al., 1991) estimate that a 25 mg/dl reduction in fibrinogen concentration could result in a 7% to 8% reduction in the risk of an ischemic heart disease event, independent of all other CAD risk factors. Because fibrinogen is a key component of the coagulation cascade and exerts effects on platelet aggregation, lowering its concentration may inhibit thrombogenesis and reduce atherogenesis.

The finding that beta-blocker therapy may have an effect on fibrinogen and Hcy concentrations ($p = 0.056$ and 0.058, respectively) reinforces its use as a first line drug for treatment of CAD. Use of these therapeutic agents have been shown to exert beneficial effects in secondary prevention after myocardial infarction (1982; Olsson, Rehnquist, Sjogren, Erhardt, & Lundman, 1985). In addition to their antihypertensive and antianginal effects, they may exert effects on atherogenesis (Kaplan, Manuck, Adams, & Clarkson, 1987). Physical stress, such as exercise, is known to influence fibrinolysis via β_2-receptors. The increased fibrinolytic response to sympathetic activation has been shown to be due to elevated tissue plasminogen activator (t-PA) with no corresponding increase in plasminogen activator inhibitor (PAI-1) (Teger-Nilsson et al., 1991). These responses tend to be enhanced by beta-blocker treatment. Effects of beta-blockade treatment on fibrinogen are not well documented. In a study of young survivors of myocardial infarction, patients without beta-blockade had higher fibrinogen concentrations than those treated with beta-blockers (Berglund, Wallentin, & Schenck, 1988). No documentation could be found on the relation between Hcy and beta-blockers. Genest *et al* (1990) reported a trend for lower Hcy concentrations in patients on beta-blockade but did not comment on this finding. Homocysteine was found to be positively significantly correlated with t-PA in 50 subjects with venous or arterial thrombosis ($p < 0.001$) (Bienvenu, Ankri, Chadefaux, Montalescot, & Kamoun, 1993). It is suggested that elevated Hcy causes endothelial damage, which releases t-PA as a regulatory mechanism intrinsic to the body's natural defense in response to thrombosis. Beta-blockade could potentially augment this response. Regulation of fibrinolysis by Hcy has also been proposed. This regulation involves lipoprotein (a) [Lp(a)], which may compete with plasminogen for fibrin binding (Harpel, Chang, & Borth, 1992). This would result in diminished fibrinolysis and promote thrombosis. Homocysteine promotes Lp(a) binding to fibrin. This direct interaction between Lp(a) and fibrin provide a rationale for the relationship between increased blood concentrations of Lp(a), thrombosis, and atherogenesis because binding of Lp(a) to fibrin may provide a

mechanism for incorporating Lp(a) into the vessel wall. Because this action appears to be mediated by Hcy, a link is provided between thrombosis, atherogenesis, and Hcy metabolism.

The regulation of hemostasis and coagulation factors is tightly controlled and very complex. There appear to be many contributing factors to the pathogenesis of CAD which act synergistically in its development. This study examined but two of the many variables involved in this process. From the results presented we conclude that regular physical activity is associated with lower fibrinogen concentrations in males with CAD. A trend exists for those patients on beta-blocker therapy to have lower fibrinogen and Hcy concentrations than those not taking these drugs. Future studies of fibrinogen and Hcy should consider the possible confounding effects of subjects taking beta-blockers. In summary, Hcy appears to be an independent risk factor for CAD in a group of male CAD patients. It likely interacts with other risk factors to increase the risk of cardiovascular disease and may be modified by therapies currently used to modify these risk factors.

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Chapter IV

RESULTS AND DISCUSSION

Results

The characteristics of the subject population are found in Table 1. Twenty-six subjects had previous myocardial infarction, 17 had coronary artery bypass grafting (CABG), and 10 had undergone percutaneous translumenal coronary angioplasty (PTCA). (The total is greater than the number of subjects due to multiple procedures and/or a combination of an event and procedure.) Aspirin was being taken therapeutically by 82.5% (n = 33) of the cohort, while warfarin therapy was reported by 7.5% (n = 3). There was a trend for subjects on beta-blocker therapy (n = 12) to have lower fibrinogen concentrations than those who were not currently taking beta-blockers (n = 28) (277.7 mg/dl vs. 316.1, respectively, $p = 0.056$). Those subjects in the most active third of this cohort had significantly lower fibrinogen concentrations than those in the lowest third ($p = 0.05$, Table 2). The most active subjects reported significantly more usage of beta-blockers than those in the least active tertile ($p < 0.005$) (Table 3). No other examined variables were associated with fibrinogen concentrations. A trend also existed for subjects on beta-blocker therapy to have lower Hcy concentrations than those not on such medication (8.3 µmol/L vs 9.7, respectively, $p = 0.058$). Homocysteine was significantly positively associated with age ($r = 0.52$, $p = 0.0008$) (Figure 2). No other associations were found with the examined variables. Multivariate correlation analysis revealed no significant relations among the continuous variables (Table 4).

Discussion

In the only study found which analyzes the relation between Hcy and fibrinogen in a population with CAD, the authors reported that Hcy was independent of most coronary risk factors except fibrinogen (von Eckardstein et al., 1994). Four patients with homocystinuria

were shown to have higher fibrinogen concentrations compared with control subjects (Harker et al., 1974), however, this is an extremely small sample size and these patients have a genetic form of hyperhomocysteinemia as opposed to an acquired form. The differences in etiology may influence other hemostatic factors. In an investigation which examined these variables in 154 stroke survivors (Brattstrom et al., 1992), no correlation was found between Hcy and fibrinogen concentrations. The results of this study suggest that in patients with CAD, a form of cardiovascular disorder which also includes strokes, no interrelation exists between fibrinogen and Hcy.

Likewise, there was no correlation found between physical activity and Hcy. Physical activity was assessed by a 12-month recall instrument which accounts for leisure and occupational activities. Levels of physical activity are further quantified by assigning MET values to each activity and expressing total activity in MET hrs/wk. Other studies which considered physical activity used general categorization (Murphy-Chutorian et al., 1985; Nygard et al., 1995; Pancharuniti et al., 1994) which is not the preferred method for accurately determining the amount of habitual activity. Nygard *et al* (1995) reported that there was an inverse relationship between mean Hcy concentration and amount of exercise in leisure time ($p < 0.001$). The subjects in this study were apparently healthy. Blood samples obtained were nonfasting and determination of Hcy concentrations were made on unfrozen serum within 7 days of collection. Both Pancharuniti *et al* (1994) and Murphy-Chutorian *et al* (1985) examined the relation between Hcy and physical activity in a population with CAD. Homocysteine determinations were made from frozen plasma samples. Neither investigation found a significant association between physical activity and Hcy.

A protective effect of regular exercise on the incidence and the mortality of CAD has been reported in many studies (Blair et al., 1989; Folsom et al., 1985; Leon et al., 1987; Morris et al., 1990; Salonen et al., 1988). The mechanisms through which it exerts this influence are not clear. Favorable modification of conventional CAD risk factors, including blood pressure, lipoprotein profiles, and glucose intolerance, are generally accepted effects

of chronic exercise. Significant improvements in hemostatic parameters, including fibrinogen concentrations, have been reported in patients with CAD who engage in regular aerobic and leisure activity (Elwood et al., 1993; Moller & Kristensen, 1991; Wosornu et al., 1992). Our data show that subjects in the most active tertile have significantly lower plasma fibrinogen concentrations than those in the least active tertile ($p = 0.05$). A prospective study conducted over 6 mo compared the change in fibrinogen concentration among a control group, an aerobic training group, and a power training group from a cohort of men 6 wk to 12 mo from coronary artery surgery (Wosornu et al., 1992). Their results showed a 12% reduction in fibrinogen ($p = 0.01$) compared to 2% reduction in the power group and 4% reduction in the control group. This represents a 42 mg/dl change in fibrinogen concentration which is similar to the difference between the upper and lower tertiles of physical activity in this investigation. Data from the Caerphilly and Speedwell heart studies estimate that a 25 mg/dl reduction in fibrinogen concentration could result in a 7% to 8% reduction in the risk of an ischemic heart disease event, independent of all other CAD risk factors (Yarnell et al., 1991). Because fibrinogen is a key component of the coagulation cascade and exerts effects on platelet aggregation, lowering its concentration may inhibit thrombogenesis and reduce atherogenesis.

The finding that beta-blocker therapy may have an effect on fibrinogen and Hcy concentrations ($p = 0.056$ and 0.058, respectively) could have clinical implications. Use of these therapeutic agents have been shown to exert beneficial effects in secondary prevention after myocardial infarction (Beta-blocker Heart Attack Trial Research Group, 1982; Olsson et al., 1985). In addition to their antihypertensive and antianginal effects, they may exert effects on atherogenesis (Kaplan et al., 1987). Physical stress, such as exercise, is known to influence fibrinolysis via β_2-receptors. The increased fibrinolytic response to sympathetic activation has been shown to be due to elevated tissue plasminogen activator (t-PA) with no corresponding increase in plasminogen activator inhibitor (PAI-1) (Teger-Nilsson et al., 1991). These responses tend to be enhanced by beta-blocker treatment. Effects of beta-

blockade treatment on fibrinogen is not well documented. In a study of young survivors of myocardial infarction, patients without beta-blockade had higher fibrinogen concentrations than those treated with beta-blockers (Berglund et al., 1988). No documentation could be found on the relation between Hcy and beta-blockers. Genest *et al* (1990) reported a trend for lower Hcy concentrations in patients on beta-blockade but did not comment on this finding. Homocysteine was found to be significantly correlated with t-PA in 50 subjects with venous or arterial thrombosis ($p < 0.001$) (Bienvenu et al., 1993). It is suggested that elevated Hcy causes endothelial damage, which releases t-PA as a regulatory mechanism prerequisite for the prevention of thromboembolic disease. Beta-blockade could potentially augment this response. Regulation of fibrinolysis by Hcy has also been proposed. This regulation involves lipoprotein (a) [Lp(a)], which may compete with plasminogen for fibrin binding (Harpel et al., 1992). This would result in diminished fibrinolysis and promote thrombosis. Homocysteine promotes Lp(a) binding to fibrin. This direct interaction between Lp(a) and fibrin provide a rationale for the relationship between increased blood concentrations of Lp(a), thrombosis, and atherogenesis because binding of Lp(a) to fibrin may provide a mechanism for incorporating Lp(a) into the vessel wall. Because this action appears to be mediated by Hcy, a link is provided between thrombosis, atherogenesis, and Hcy metabolism.

The regulation of hemostasis and coagulation factors is tightly controlled and very complex. There appear to be many contributing factors to the pathogenesis of CAD which act synergistically in its development. This study examined but two of the many variables involved in this process. From the results presented we conclude that regular physical activity is associated with lower fibrinogen concentrations in males with CAD. A trend exists for those patients on beta-blocker therapy to have lower fibrinogen and Hcy concentrations than those not taking these drugs.

Practical and clinical applications

A practical implication of this study is the continued referral of CAD patients to exercise programs supervised by properly trained staff. Studies of the effect of physical activity on secondary prevention has been described (O'Connor, Buring, & Yusuf, 1989). The overall effect of cardiac rehabilitation programs after myocardial infarction was a significant 24% reduction in all cause mortality in the more active groups. Due to clustering of CAD risk factors, regular exercise may improve the risk profile via ameliorations of dyslipidemia, increased insulin sensitivity, body weight reduction, and vascular control mediated by exercise-induced sympathetic stimulation. As previously discussed, both fibrinogen and Hcy have been associated with beta-adrenergic activity.

Continued use of beta-blocker therapy as a preferred drug choice in the management of CAD is also supported by the results of this study. Two large trials, which included approximately 5000 patients, showed convincing evidence that mortality and risk of reinfarction are significantly decreased (23% and 21%, respectively) (Beta-blocker Heart Attack Trial Research Group, 1982; Norwegian Multicenter Study Group, 1981). The proposed mechanisms of these reductions may include the increased fibrinolytic response enhanced by beta-blocker use.

Future research

Prospective studies on aerobic exercise programs of varying intensity and duration are necessary to accurately evaluate the effects of physical activity on plasma Hcy concentrations. These studies should control for the use of beta-blockers and age, as well as include an assessment of specific intake of folate, vitamin B_6, and B_{12}, and/or measurement of plasma concentrations of these nutrients. These investigations are needed in diseased populations and those who are apparently healthy to determine if a modification in this risk factor can occur with regular physical activity, and if so, what level of activity (i.e. frequency, intensity, and duration) is optimal for such modification.

Additional epidemiological studies in primary and secondary prevention populations could help to establish or reject a correlation between fibrinogen and Hcy. Knowledge of associations with other risk factors and treatments are needed to identify potential confounders in studies of Hcy. To date, there is no consistent evidence which suggests a relation between any of the known CAD risk factors and Hcy. Hyperhomocysteinemia seems to be a risk factor for thrombotic events via its effects on platelets, vascular endothelium, and coagulation proteins. Atherogenic mechanisms promoted by Hcy include altered Factor V formation, which catalyzes prothrombin to thrombin, decreased protein C activation, diminished fibrinolysis, and platelet aggregation. A closer look is needed at the interaction between Hcy and clotting factors, fibrinolytic parameters, and endothelial-derived factors.

Prospective trials utilizing treatments for lowering fibrinogen and Hcy (e.g. fibrates and folate/B-vitamins) are needed to evaluate the merit of clinical evaluation of these variables. A reduction in risk of CAD accompanying the reduction of these concentrations must also be demonstrated before clinical determination of these values is put into practice. Several drugs have been identified which lower fibrinogen concentrations, however these drugs have additional pharmacological effects making it difficult to attribute any benefits on improving CAD risk to reduction of fibrinogen. Agents for the selective lowering of fibrinogen concentrations are under development. Treatment of homocysteinemia and its vascular complications includes folate, vitamins B_{12} and B_6, betaine, and anticoagulants. The nutrients are necessary for complete methionine metabolism. They serve as cofactors in the re-methylation of Hcy to methionine or the conversion of Hcy to cystathionine and eventually cysteine. Higher levels of these cofactors are associated with lower concentrations of Hcy. No information is available as to whether a reduction in Hcy concentrations by increased intake of supplements or foods high in these vitamins reduces CAD risk.

Conclusions

In summary, Hcy appears to be an independent risk factor for CAD. It likely interacts with other risk factors to increase the risk of cardiovascular disease and may be modified by therapies currently used to modify these risk factors. Elevated plasma Hcy can be modified with vitamin supplements and/or increased intake of fruits, vegetables, and fortified foods. Whether this regimen would affect evolution of arterial occlusive diseases remains to be established, however, in the interim this therapy is harmless at worst and has other health benefits at best.

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Appendix A

METHODOLOGY

Subjects

Permission to proceed with this investigation was provided by the Human Subjects Committee of the Division of Human Nutrition, Foods, and Exercise and from the Institutional Review Board for Research Involving Human Subjects of Virginia Polytechnic Institute and State University.

Forty males diagnosed with CAD were interviewed for this study. These subjects were recruited from cardiac rehabilitation maintenance programs and each signed an informed consent (Appendix B). Subjects were considered to have CAD if they had suffered a previous myocardial infarction, had angioplasty, coronary artery bypass grafting, and/or had angiographic documentation of stenosis ≥ 70% of at least one major epicardial coronary vessel. A health/risk factor questionnaire (Appendix C) was also completed by each subject. Exclusion criteria included chronic inflammatory conditions, unstable angina, and low functional capacity due to congestive heart failure, chronic obstructive pulmonary disease, etc. All current medication and vitamin dosages were also recorded.

Experimental Design

Body mass index (BMI = Wt.[kg] / Ht.[m^2]) was determined by obtaining each subject's height and weight using a calibrated medical beam balance scale. Lipid profile information (total cholesterol, HDL- cholesterol, triglycerides, LDL-cholesterol [calculated], and total cholesterol/HDL ratio) was extracted from each patient's most recent blood analysis. Physical activity status was determined using the Modifiable Activity Questionnaire (Appendix D). This instrument lists all leisure and occupational activities, as well as periods of inactivity longer than one

week, for a 12 month period. The MET levels of all leisure and occupational activities were determined from a compendium of physical activities (Ainsworth et al., 1993) and physical activity was converted and reported as MET hours per week.

Participants were instructed to fast, consume no alcohol, or engage in physical activity for 12 h prior to blood sampling. Whole blood was drawn from the antecubital vein in two 4.5 ml evacuated tubes containing sodium citrate by a certified phlebotomist. Tubes were mixed to avoid coagulation, chilled, and centrifuged at 2000 g for 20 min within 1 hour after sampling. The plasma fractions from both tubes were each transferred to a plastic vial and frozen at -20° C. All recommended precautions and procedures, including blood-born pathogen safety precautions, were observed during sample taking and handling.

Vials with plasma for Hcy analysis were packed in dry ice and sent by overnight shipping to Abbott Laboratories (Abbott Park, IL). An automated assay method on the Abbott IMxâ analyzer was utilized. This analyzer uses the fluorescence polarization immunoassay (FPIA) methodology, a commonly used sensitive and precise method for measuring small molecules. An indirect approach, based on the highly selective enzyme conversion of Hcy to S-adenosyl-L-homocysteine (SAH) provided a target for a monoclonal antibody. This format is comprised of three steps: 1) reduction of the disulfide bonds and enzyme treatment (SAH hydrolase) of the sample to convert Hcy to SAH, 2) addition of the antibody, and, after a 10 min incubation period, 3) addition of the tracer for fluorescence reading. The fluoresceinated tracer and a second aliquot of the reaction mixture are added, and after another 10 min incubation period, a second reading is obtained. A microprocessor calculates the polarization in mP units, corrects for background, and constructs a calibration curve from the six points. The microprocessor -stored curve is then used to calculate the values for unknown plasma samples run in the same or later cycles. Results for as many as 20 samples are available in approximately 60 min. The high selectivity of the enzyme for Hcy as substrate is reflected by the absence of measurable cross-reactivity with the closely

related amino acids cysteine and methionine, which are present in plasma in much higher concentrations (Shipchandler & Moore, 1995) Comparison of the FPIA with four well-established chromatographic methods yielded r values ranging from 0.98 to 0.997.

Fibrinogen analyses were provided by the laboratory at Columbia Montgomery Regional Hospital (Blacksburg, VA). The TOA Medical Electronics Model CA-1000 is a fully automated, computerized blood plasma coagulation analyzer. The CA-1000 employs the photo-optical clot detection method, which is a modification of the Clauss method (Clauss, 1957). By using a red light (660 nm) to illuminate the sample plasma/reagent mixture, the CA-1000 detects the change in scattered light intensity due to increased turbidity as fibrinogen changes to fibrin. Immediately after mixing and warming the plasma and reagent, scattered light intensity is low. As coagulation proceeds, the sample becomes turbid due to fibrin clot formation and the scattered light intensity increases drastically. When coagulation is complete, the scattered light intensity stabilizes. The CA-1000 stores this change in scattered light intensity in memory, and constructs a coagulation curve. The coagulation curve is drawn by taking the time and the scattered light intensity as the X and Y axes respectively. The coagulation time is determined by a percentage detection method from a preset point on the curve (50% for example). Because the clotting time endpoint is always expressed as a relative value in comparison to the final or full turbidity scale, this method allows determination of the coagulation time even on those specimens demonstrating only a slight change in scattered light intensity.

Analytical Procedures

Homocysteine and fibrinogen concentrations and physical activity (expressed as MET-hours per week) were tested for departure from normality using the Shapiro-Wilks Test. Homocysteine was positively skewed and was transformed to its common logarithm

($\log_{10}$). This distribution was normally distributed. Univariate analysis was used to determine relations between the potential explanatory variables and Hcy and fibrinogen concentrations. Chi-squared test was used to examine differences between subjects on beta-blocker therapy and those not on these medications among tertiles of fibrinogen and Hcy. All continuous variables were then entered into a multivariate analysis of correlations to determine the effect of multiple risk factors and physical activity, on plasma fibrinogen and Hcy concentrations. Pearson's product moment correlation coefficient was used to test interrelation between the variables. Non-observable confounding variables were controlled by sampling from a homogeneous population and exclusion criteria. Known confounding variables, such as age, were tested for influence on correlation with Hcy and fibrinogen. If there was evidence of an influence, the factor was adjusted for and a partial correlation was considered. Data were analyzed using JMP (Version 3.1.7, Statistical Analysis Systems, Cary, NC) software. Findings were considered statistically significant if the p-value was ≤ 0.05.

Appendix B

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY

Informed consent for participants of investigative projects

Title of Project: Correlation of homocysteine concentration with plasma fibrinogen and physical activity in males with coronary artery disease.

Principle Investigators: Monica Prerost, Bernard Feldman, D.V.M., Ph.D., and William Herbert, Ph.D.

Purpose of this research project: This study will involve 50 males diagnosed with coronary artery disease who have been screened for specific exclusion criteria. Associations between two risk factors for coronary artery disease, fibrinogen and homocysteine concentrations, will be analyzed. We will measure the levels of a protein derivative in your blood. We also will ask you to provide information on your physical activity status to see if a correlation exists between these variables.

PROCEDURES

All invited participants will be screened through use of a health/risk factor questionnaire. If you meet the study criteria you will be asked to complete two additional steps. First, you will be interviewed to determine physical activity status. This is accomplished through the two-page Modifiable Activity Questionnaire which takes approximately 20 - 30 minutes. At this time resting blood pressure, height, and weight will be obtained. The second step will be to have a fasting blood sample taken by a trained medical technician. You will be asked to not consume any food, drink any beverages (except water), or perform moderate physical activity for 12 hours prior to this sample. Blood draws will be taken from 6:15 am to 7:15 am on Monday, Wednesday, or Friday in the Human Performance Lab, 230 War Memorial Hall, on the Virginia Tech campus. A single sample will be required and will take approximately 15 minutes to draw. All blood samples will be sent to a certified laboratory for determination of cholesterol profile, fibrinogen, and homocysteine.

RISKS

Possible risks during blood draws include infection, physical discomfort, and bruising. These risks will be minimized by having an experienced phlebotomist conduct the blood draws. This phlebotomist will use universal precautions against blood-borne pathogens which includes, but is not limited to, wearing gloves. Your blood may be

screened for these pathogens (hepatitis, HIV) if any person handling the sample is exposed to it. You will be advised of these procedures, should they occur, and will receive the results of such screenings.

BENEFITS OF THIS PROJECT

While no financial compensation will be provided to you, your participation will enable the investigators to contribute to the understanding of coronary artery disease risk factors. Benefits to you for participating in this study include the evaluation of your cholesterol profile, physical activity status, and blood pressure. These results may be released to your physician upon request. Risk factor counseling and exercise guidelines may be provided, if you desire. A summary of the research results may be obtained from the investigators upon completion of this investigation.

EXTENT OF ANONYMITY AND CONFIDENTIALITY

The information obtained, including results of your blood analyses, will be kept confidential. Each participant will be assigned a subject number which will identify you during analyses and any written reports of this research. Only the principal investigators will have access to the number that is coded with your results to protect your identity.

FREEDOM TO WITHDRAW

You are free to withdraw from this study at any time if you choose to do so. You are free to not answer any questions that are asked of you, however, the investigator may choose to exclude you from this study if an accurate response is necessary for data analyses.

APPROVAL OF RESEARCH

This research project has been approved by the Institutional Review Board for Research Involving Human Subjects at Virginia Polytechnic Institute and State University and by the Department of Human Nutrition, Foods, and Exercise.

SUBJECT'S RESPONSIBILITIES

I voluntarily agree to participate in this study. I have the following responsibilities:

* Accurately complete the Health/Risk Factor Questionnaire

* Allow myself to interviewed by the investigator to determine physical activity status, height, weight, and blood pressure

* Consume no food or beverages, except water, and refrain from exercise for 12 hours prior to blood draw.

SUBJECT'S PERMISSION

I, (print name) _____ have read and understand the informed consent and conditions of this project. I have had all my questions answered. I hereby acknowledge the above and give my voluntary consent for participation in this project.

If I participate, I may withdraw at any time without penalty. I agree to abide by the rules of this project.

_____ _____

Signature Date

Should I have any questions about this procedure or its conduct, I can contact:

Monica Prerost
Master's Candidate
Department of Human Nutrition, Foods, and Exercise
231-8209

William Herbert, Ph.D.
Professor
Department of Human Nutrition, Foods, and Exercise
231-6565

Bernard Feldman, D.V.M., Ph.D.
Professor
Virginia-Maryland Regional College of Veterinary Medicine
231-4684

Tom Hurd
Chairman of the IRB at Virginia Tech
231-5281

Appendix C

Health and Risk Factor Questionnaire

Name **Phone #**

Physician Physician phone #

Age Ht Wt BMI Resting BP

Medical History / Risk Factors (Check all that apply)

Hypertension ( ) Family history of cardiovascular disease ( ) Diabetes ( ) Smoker ( )

COPD ( ) Arthritis - rheumatoid or osteo ( ) Angina ( ) Hyperlipidemia ( ) Shortness of breath with exertion ( ) Congestive Heart Failure ( ) Fainting or dizzy spells ( ) Claudication ( )

Previous: MI ( ) CABG ( ) PTCA ( ) Dates: _____

Orthopedic limitations (list) _____

_____

Medications and dosages: _____

_____

Vitamins and dosages: _____

_____

I hereby attest that the above information is correct and accurate to the best of my knowledge.

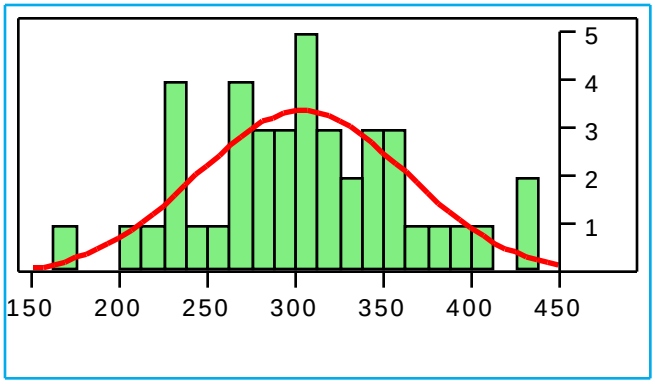
Signature Date

Appendix D

Appendix E

Statistical Procedures & Tables

Fibrinogen (mg/dl)



Quantiles

maximum	100.0%	427.90
	99.5%	427.90
	97.5%	427.90
	90.0%	392.76
quartile	75.0%	341.70
median	50.0%	307.70
quartile	25.0%	267.55
	10.0%	226.80
	2.5%	168.28
	0.5%	166.50
minimum	0.0%	166.50

Moments

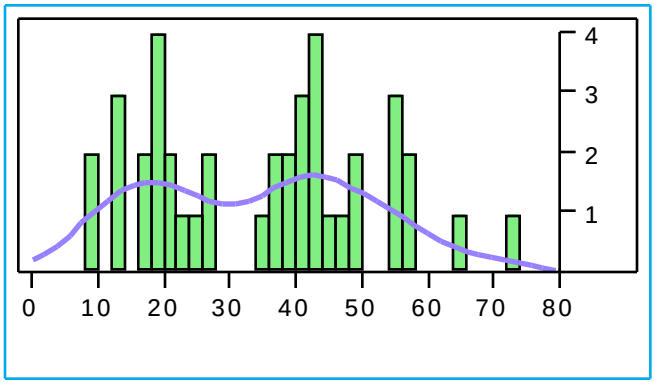
Mean	303.9073
Std Dev	60.1100
Std Error Mean	9.3876
Upper 95% Mean	322.8803
Lower 95% Mean	284.9344
N	41.0000
Sum Weights	41.0000
Sum	12460.2
Variance	3613.2077
Skewness	0.0581
Kurtosis	-0.1626
CV	19.7790

Test for Normality

Shapiro-Wilk W Test

W	Prob<W
0.984644	0.9058

Physical Activity Index (MET hr/wk)



Quantiles

maximum	100.0%	72.400
	99.5%	72.400
	97.5%	72.228
	90.0%	56.010
quartile	75.0%	46.250
median	50.0%	37.950
quartile	25.0%	18.850
	10.0%	12.330
	2.5%	8.438
	0.5%	8.400
minimum	0.0%	8.400

Moments

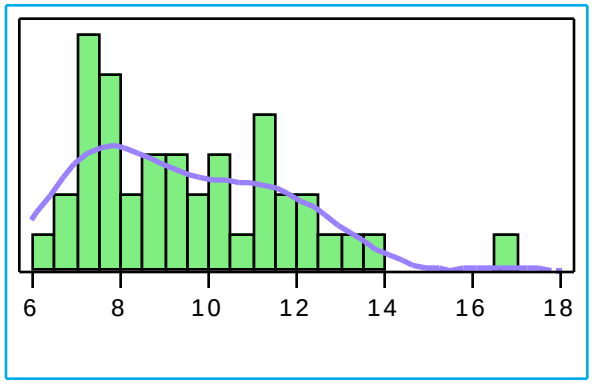
Mean	34.86250
Std Dev	16.71478
Std Error Mean	2.64284
Upper 95% Mean	40.20812
Lower 95% Mean	29.51688
N	40.00000
Sum Weights	40.00000

Test for Normality

Shapiro-Wilk W Test

W	Prob<W
0.946888	0.0839

Homocysteine (µmol/L)



Quantiles

maximum	100.0%	16.790
	99.5%	16.790
	97.5%	16.709
	90.0%	12.534
quartile	75.0%	11.120
median	50.0%	9.125
quartile	25.0%	7.500
	10.0%	7.023
	2.5%	6.122
	0.5%	6.110
minimum	0.0%	6.110

Moments

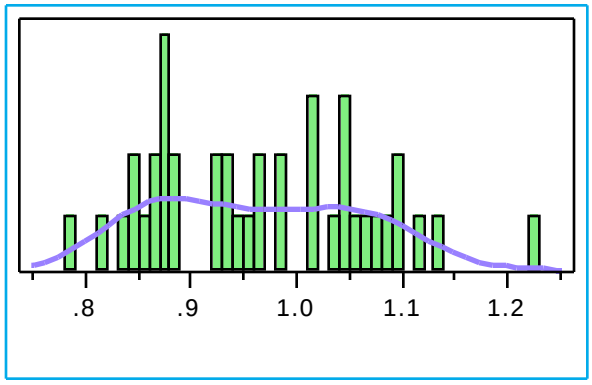
Mean	9.52675
Std Dev	2.35046
Std Error Mean	0.37164
Upper 95% Mean	10.27846
Lower 95% Mean	8.77504
N	40.00000
Sum Weights	40.00000

Test for Normality

Shapiro-Wilk W Test

W	Prob<W
0.932544	0.0253

log10 Hcy



Quantiles

maximum	100.0%	1.2251
	99.5%	1.2251
	97.5%	1.2227
	90.0%	1.0981
quartile	75.0%	1.0461
median	50.0%	0.9602
quartile	25.0%	0.8751
	10.0%	0.8465
	2.5%	0.7868
	0.5%	0.7860
minimum	0.0%	0.7860

Moments

Mean	0.96678
Std Dev	0.10297
Std Error Mean	0.01628
Upper 95% Mean	0.99972
Lower 95% Mean	0.93385
N	40.00000
Sum Weights	40.00000

Test for Normality

Shapiro-Wilk W Test

W	Prob<W
0.961412	0.2615

Response: Fibrinogen (PAI)

Summary of Fit

RSquare	0.094596
RSquare Adj	0.07138
Root Mean Square Error	57.92491
Mean of Response	303.9073
Observations (or Sum Wgts)	41

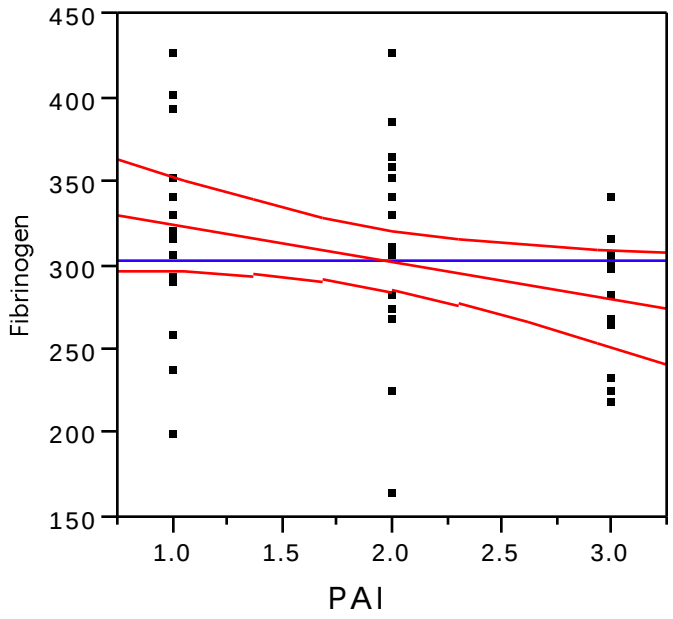
Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Column 1	1	1	13671.773	4.0747	0.0504

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	13671.77	13671.8	4.0747
Error	39	130856.54	3355.3	**Prob>F**
C Total	40	144528.31		0.0504

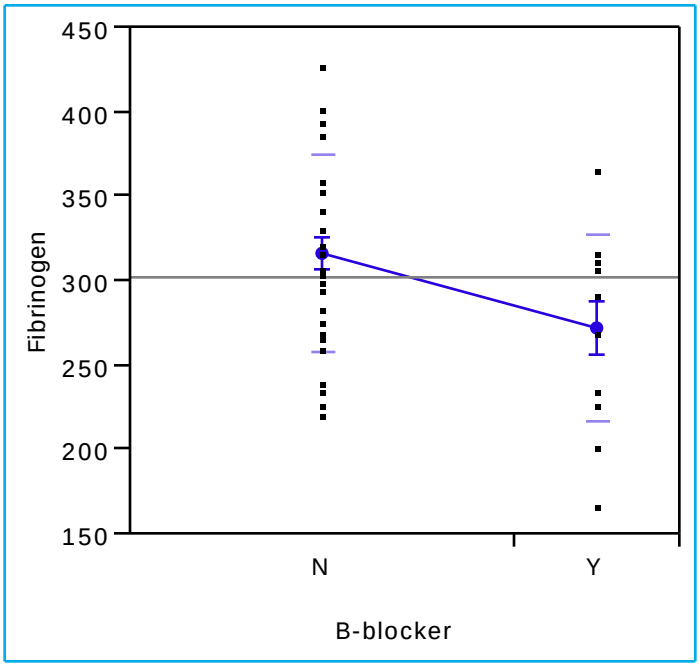
PAI and Fibrinogen



Effect Test

Sum of Squares	F Ratio	DF	Prob>F
13671.773	4.0747	1	0.0504

Fibrinogen By B-blocker



Oneway Anova

Summary of Fit

RSquare	0.11217
RSquare Adj	0.088806
Root Mean Square Error	57.81477
Mean of Response	302.9625
Observations (or Sum Wgts)	40

t-Test

	Difference	t-Test	DF	Prob>|t|
Estimate	43.7083	2.191	38	0.0346
Std Error	19.9480			
Lower 95%	3.3259			
Upper 95%	84.0907			

Assuming equal variances

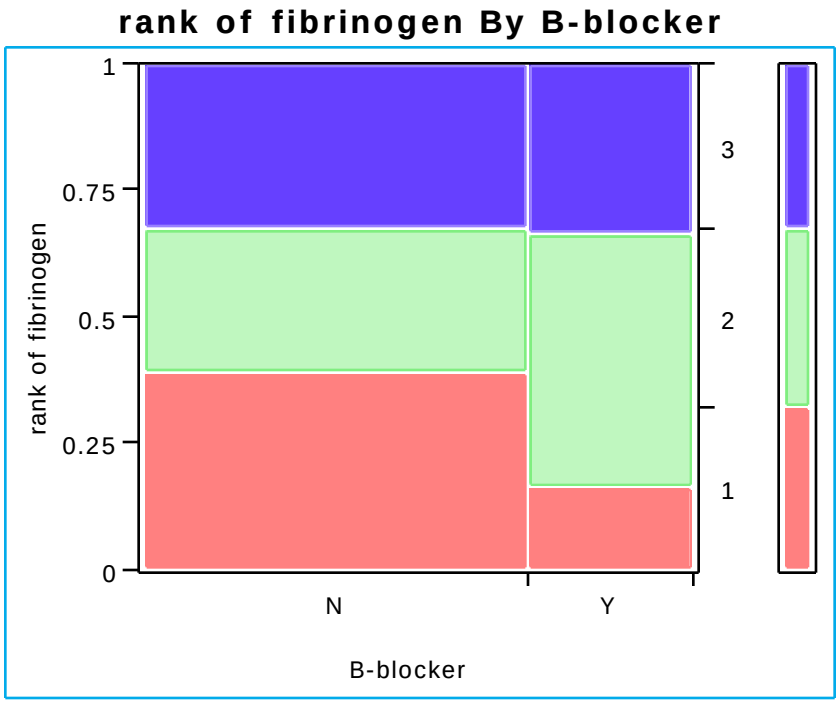
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	16047.51	16047.5	4.8010
Error	38	127016.80	3342.5	Prob>F
C Total	39	143064.31	3668.3	0.0346

Means for Oneway Anova

Level	Number	Mean	Std Error
N	28	316.075	10.926
Y	12	272.367	16.690

Std Error uses a pooled estimate of error variance



Crosstabs

rank of fibrinogen/ B-blocker			
Count	N	Y	
Col %			
Cell Chi^2			
1	11	2	13
	39.29	16.67	
	0.3967	0.9256	
2	8	6	14
	28.57	50.00	
	0.3306	0.7714	
3	9	4	13
	32.14	33.33	
	0.0011	0.0026	
	28	12	40

Tests

Source	DF	-LogLikelihood	RSquare (U)
Model	2	1.268516	0.0289
Error	36	42.651176	
C Total	38	43.919692	
Total Count	40		

Test	ChiSquare	Prob>ChiSq
Likelihood Ratio	2.537	0.2812
Pearson	2.428	0.2970

Warning: 20% of cells have expected count less than 5, Chi-squares suspect

Response: log10 Hcy (age)

Summary of Fit

RSquare	0.27072
RSquare Adj	0.250462
Root Mean Square Error	0.088965
Mean of Response	0.962887
Observations (or Sum Wgts)	38

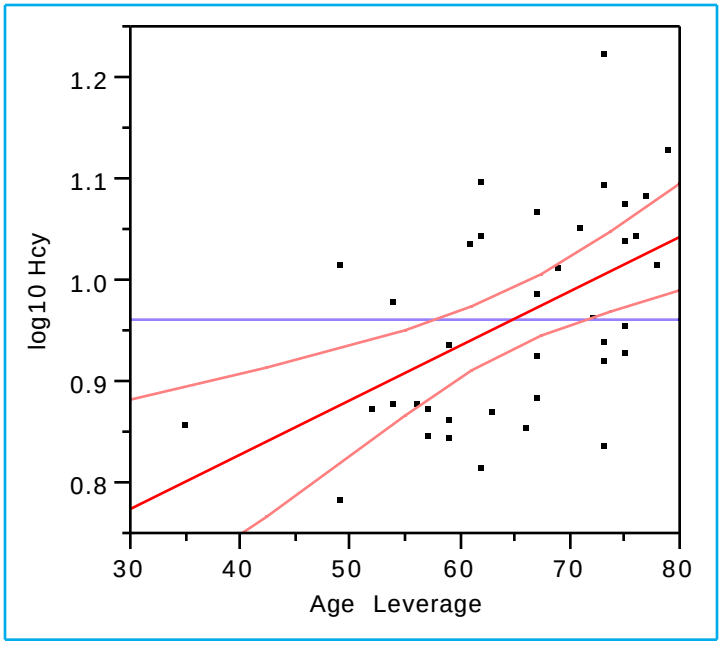
Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Age	1	1	0.10577103	13.3637	0.0008

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.10577103	0.105771	13.3637
Error	36	0.28493180	0.007915	Prob>F
C Total	37	0.39070283		0.0008

Age



Effect Test

Sum of Squares	F Ratio	DF	Prob>F
0.10577103	13.3637	1	0.0008

Response: log10 Hcy (β-block)

Summary of Fit

RSquare	0.091387
RSquare Adj	0.067476
Root Mean Square Error	0.099438
Mean of Response	0.966783
Observations (or Sum Wgts)	40

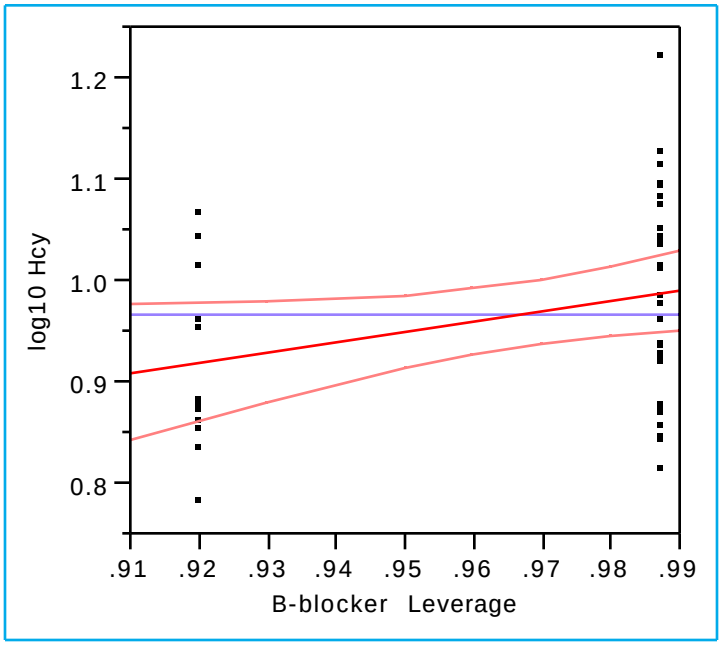
Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
B-blocker	1	1	0.03779120	3.8220	0.0580

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.03779120	0.037791	3.8220
Error	38	0.37573829	0.009888	Prob>F
C Total	39	0.41352949		0.0580

B-blocker



Effect Test

Sum of Squares	F Ratio	DF	Prob>F
0.03779120	3.8220	1	0.0580

Least Squares Means

Level	Least Sq Mean	Std Error	Mean
N	0.9869057247	0.0187919528	0.986906
Y	0.9198314963	0.0287051820	0.919831

Pearson Product Correlation Coefficients in Multivariate Analysis

Variable	log10 Hcy	Fibrinogen	PAI	Age	BMI	TC	HDL	LDL	Trig	TC/HDL
log10 Hcy	1.0000	0.0143	0.0217	0.5142	-0.0404	-0.0234	0.4411	-0.0105	-0.2606	-0.2684
Fibrinogen	0.0143	1.0000	-0.1740	0.0673	0.1148	-0.1140	-0.1409	0.0300	0.0920	0.1114
PAI	0.0217	-0.1740	1.0000	-0.0762	-0.0903	0.1025	0.0512	0.1851	-0.0346	0.1048
Age	0.5142	0.0673	-0.0762	1.0000	-0.4107	0.0656	0.3453	0.0177	-0.0289	-0.2513
BMI	-0.0404	0.1148	-0.0903	-0.4107	1.0000	-0.0697	-0.2173	0.1203	0.0504	0.2497
TC	-0.0234	-0.1140	0.1025	0.0656	-0.0697	1.0000	0.1190	0.8368	0.4600	0.3268
HDL	0.4411	-0.1409	0.0512	0.3453	-0.2173	0.1190	1.0000	-0.0716	-0.3405	-0.7644
LDL	-0.0105	0.0300	0.1851	0.0177	0.1203	0.8368	-0.0716	1.0000	0.1822	0.4216
Trig	-0.2606	0.0920	-0.0346	-0.0289	0.0504	0.4600	-0.3405	0.1822	1.0000	0.4924
TC/HDL	-0.2684	0.1114	0.1048	-0.2513	0.2497	0.3268	-0.7644	0.4216	0.4924	1.0000

Partial Correlation Coefficients

Variable	log10 Hcy	Fibrinogen	PAI	Age	BMI	TC	HDL	LDL	Trig	TC/HDL
log10 Hcy	•	0.0136	0.0411	0.4797	0.2098	-0.0098	0.2435	-0.0359	-0.1490	0.1952
Fibrinogen	0.0136	•	-0.2941	-0.0459	-0.1459	-0.4422	0.2469	0.4198	0.3719	0.1467
PAI	0.0411	-0.2941	•	-0.2541	-0.3415	-0.4046	0.3779	0.4081	0.2749	0.2902
Age	0.4797	-0.0459	-0.2541	•	-0.5392	-0.3207	0.2355	0.3420	0.3579	0.0431
BMI	0.2098	-0.1459	-0.3415	-0.5392	•	-0.5234	0.3529	0.5168	0.4196	0.2441
TC	-0.0098	-0.4422	-0.4046	-0.3207	-0.5234	•	0.6948	0.9310	0.7995	0.3538
HDL	0.2435	0.2469	0.3779	0.2355	0.3529	0.6948	•	-0.5412	-0.4844	-0.7677
LDL	-0.0359	0.4198	0.4081	0.3420	0.5168	0.9310	-0.5412	•	-0.7636	-0.1486
Trig	-0.1490	0.3719	0.2749	0.3579	0.4196	0.7995	-0.4844	-0.7636	•	-0.0557
TC/HDL	0.1952	0.1467	0.2902	0.0431	0.2441	0.3538	-0.7677	-0.1486	-0.0557	•

partialled with respect to all other variables

Appendix F

Raw Data

	AGE	BMI	PAI	FIB	HCY	log HCY	VITB /FOL	B-BLOCK	TC	HDL	LDL	TRIG	TC/ HDL
1	75	23.3	41.7	276.2	8.52	0.93044	N	N	164	46	106	58	3.5
2	73	23.5	16.3	394.3	8.73	0.941014	Y	N	207	44	113	250	4.7
3	73	23.9	42.6	427.9	8.36	0.922206	N	N	181	28	128	123	6.5
4	61	25.8	27	358.5	10.9	1.03862	N	N	220	28	160	161	7.9
5	59	29.7	18.2	352.7	8.68	0.93852	N	N	193	40	125	139	3.1
6	69	28.7	42.8	234	10.3	1.0141	Y	N	213	42	115	281	5.1
7	67	26	23.7	312.2	7.68	0.885361	N	Y	161	34	95	159	4.7
8	62	29.4	55	265.9	12.5	1.098298	Y	N	133	66	60	37	2
9	75	27.5	39.4	386.6	12	1.077368	N	N	154	40	99	74	3.9
10	73	23.2	72.4	307.7	6.87	0.836957	Y	Y	198	41	139	90	4.8
11	54	.	54.4	226.8	7.61	0.881385	N	N	206	35	153	87	5.8
12	52	31	42.9	341.7	7.5	0.875061	Y	N	171	28	120	116	6.1
13	62	25.1	41.6	331.3	11.1	1.044932	Y	N	143	40	88	74	3.6
14	78	30.1	18.4	259.4	10.4	1.016197	N	N	183	28	135	98	6.5
15	75	23.5	46.4	269.2	9.03	0.955688	Y	Y	184	50	120	68	3.7
16	57	28.7	16.2	402.2	7.05	0.848189	Y	N	113	18	70	124	6.3
17	73	26.5	18.5	321.5	12.5	1.096215	N	N	156	.	.	.	.
18	49	31.3	48.7	316.8	10.4	1.017451	N	Y	.	.	.	.	.
19	.	32.6	12.1	291.1	9.24	0.965672	Y	Y	.	.	.	.	.
20	.	30.3	9.9	331.3	13.1	1.115943	N	N	.	.	.	.	.
21	67	28.4	55.2	220.1	8.46	0.92737	Y	N	210	28	143	196	7.5
22	49	29.4	45.8	234	6.11	0.786041	Y	Y	190	32	135	114	5.9
23	67	23.2	35.6	166.5	11.7	1.068928	N	Y	151	38	107	31	4
24	67	23	65.5	269.2	9.71	0.987219	Y	N	238	35	157	230	6.8
25	57	21.8	20	202	7.5	0.875061	Y	Y	146	30	24	121	4.9
26	54	26.7	21.7	427.9	9.58	0.981366	N	N	202	.	.	120	.
27	79	24.8	12.3	295.1	13.5	1.131298	Y	N	228	78	140	48	2.9
28	73	24.4	56.1	283.5	16.8	1.225051	Y	N	191	39	138	68	4.9
29	35	31.6	49	303.4	7.25	0.860338	N	N	217	32	164	103	6.8
30	77	31.2	19.9	307.7	12.2	1.086004	N	N	184	29	126	143	6.3
31	71	25.1	37.4	352.7	11.3	1.053463	N	N	189	37	126	132	5.1
32	62	24.5	12.6	239	6.57	0.817565	Y	N	224	35	163	110	4.2
33	66	24.7	41.3	269.2	7.18	0.856124	N	Y	198	36	120	211	5.5
34	72	22	38.5	283.5	9.22	0.964731	N	N	.	45	128	.	.
35	76	23.5	24	365.2	11.1	1.046495	N	Y	181	.	.	106	.
36	59	30.5	36.8	226.8	7.33	0.865104	N	Y	242	.	199	378	.
37	56	31.6	26.9	307.7	7.57	0.879096	Y	Y	.	.	.	.	.
38	63	30	8.4	316.8	7.46	0.872739	N	N	222	.	.	445	.
39	59	29.2	42.3	341.7	7.02	0.846337	Y	N	.	.	.	.	.
40	75	26.6	57	299.2	11	1.041393	Y	N	.	.	.	.	.

	Sys BP	Dia BP	MI	PTCA	CABG	Vessel	ASA	Warfarin
1	110	60	Y	Y	N	.	N	N
2	132	92	Y	N	Y	2	Y	N
3	144	72	Y	Y	N	2	Y	N
4	112	76	Y	Y	Y	2	Y	N
5	148	78	N	N	Y	2	Y	N
6	182	90	Y	N	N	.	Y	N
7	164	88	N	N	Y	4	Y	N
8	130	84	Y	N	N	1	Y	N
9	122	82	Y	N	N	.	Y	N
10	108	72	Y	N	N	1	N	N
11	.	.	Y	N	N	.	Y	N
12	132	80	Y	N	Y	4	Y	N
13	122	82	Y	N	N	.	Y	N
14	134	84	Y	N	Y	.	Y	N
15	154	86	N	Y	N	.	Y	N
16	130	90	N	N	Y	5	Y	N
17	116	64	N	N	Y	.	Y	N
18	134	82	Y	Y	N	.	Y	N
19	.	.	Y	N	N	.	Y	N
20	116	76	N	N	Y	.	Y	N
21	118	72	Y	N	N	.	Y	N
22	112	70	Y	N	N	.	Y	N
23	102	62	N	N	Y	3	Y	N
24	132	86	N	N	Y	3	Y	N
25	138	80	N	N	Y	.	Y	Y
26	160	90	N	N	Y	.	Y	N
27	114	74	Y	N	N	.	N	N
28	160	80	Y	N	N	.	N	N
29	114	70	Y	Y	N	.	Y	N
30	130	74	N	N	Y	4	Y	N
31	162	88	N	Y	Y	.	N	N
32	132	80	N	N	Y	4	Y	N
33	114	78	Y	N	N	.	Y	N
34	128	80	Y	N	N	.	Y	Y
35	134	70	Y	N	N	.	Y	N
36	118	78	Y	N	N	.	N	N
37	106	76	Y	Y	N	.	Y	N
38	116	80	Y	N	N	.	N	Y
39	144	80	Y	Y	Y	.	Y	N
40	132	80	N	Y	N	.	Y	N

Vita

Monica Rene Prerost was born in Bayfield, WI, where she completed her secondary education. After attending the University of Wisconsin - River Falls and St. Olaf College, she received her B.A. in Physical Education from the United States International University, San Diego, CA. Monica has worked as a strength and conditioning consultant, exercise physiologist, and general manager of a hospital-based fitness center prior to entering the cardiac rehabilitation field. Before commencing her graduate studies in clinical exercise physiology at Virginia Polytechnic Institute and State University, Monica was the director of cardiac rehabilitation and health/fitness services at Lubec Regional Medical Center, Lubec, ME. After completing her doctoral program, Monica hopes to work in cardiovascular research, either in a private or governmental agency or in a medical institution.