HOMOCYSTEINE AND MALONDIALDEHYDE AS PREDICTORS OF RESTENOSIS FOLLOWING PERCUTANEOUS CORONARY INTERVENTION

A Thesis Submitted to

The College of Graduate Studies & Research

In Partial Fulfillment of the Requirements

For The Degree of Master of Science

In The Department of Pathology

University of Saskatchewan

Saskatoon

By

Erick Donnell McNair

© Copyright Erick Donnell McNair, April 2006. All rights reserved.

PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a Master of Science degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. Furthermore, I agree that permission for copying this thesis in any manner, in whole or in part, for scholarly purposes may be granted by Professor Qureshi who supervised my thesis work, or in his absence, by the Head of the Department of Pathology or the Dean of the College of Medicine. It is understood that any copying or publication or any other use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis.

Requests for my permission to copy or to make any use of material in this thesis, in whole or in part should be addressed to **EITHER:**

Professor A. Mabood Qureshi

Department of Pathology
College of Medicine
University of Saskatchewan
103 Hospital Drive
Royal University Hospital
Saskatoon, Saskatchewan S7N OW8

or

Head of Department of Pathology
College of Medicine
University of Saskatchewan
103 Hospital Drive
Royal University Hospital
Saskatoon, Saskatchewan S7N OW8

ABSTRACT

Restenosis is one of the major adverse outcomes of Percutaneous Coronary Intervention (PCI). Previous studies have shown conflicting reports for homocysteine as a predictor of restenosis following PCI. The conflicting reports may be due to oxidative factors (stimulation of polymorphonuclear leukocyte [PMNL]-induced reactive oxygen species generation, xanthine- xanthine oxidase, and arachidonic acid metabolism) other than homocysteine which could cause endothelial cell dysfunction leading to restenosis. Malondialdehyde (MDA), a lipid peroxidation product, is a marker for oxidative stress and is related to all oxidative factors. Therefore, it is possible that serum MDA may be a better predictor of restenosis than plasma homocysteine. The purpose of this study is to determine whether or not the pre-procedural serum MDA and plasma homocysteine levels are elevated in patients who develop restenosis post PCI.

The study included fifty-one patients undergoing elective PCI who consented to participate in a protocol that was approved by the Ethics Committee of the University of Saskatchewan. Homocysteine and malondialdehyde were measured in the plasma and serum respectively. Blood samples were collected pre-procedural, 0 time, 8 hours, 24 hours, and 6 months post-procedure. Exercise tolerance tests were performed at two weeks, and six months post-procedure to determine if there was any evidence of restenosis.

The results of the study showed that pre-procedural values of plasma homocysteine in the restenosis and non-restenosis groups were 10.37 ± 0.46 and 10.73 ± 0.49 respectively. These values were not significantly different ($p=0.60$) between the groups. The pre-procedural levels of plasma homocysteine were not significantly

different (p=0.08) from the post-PCI values of those patients who did not develop restenosis at the 6-month time interval. However, the pre-procedural levels of plasma homocysteine were significantly different from the post-PCI values of those patients in the restenosis group at the 24hr (p=0.04) and 6-month (p=0.002) time intervals. In the restenosis group there was a significant increase (24%) after six months in the values of homocysteine from the pre-procedural levels. Thus, this indicates that restenosis is associated with higher post-PCI levels of homocysteine.

The pre-procedural levels of serum MDA in the restenosis and non-restenosis groups were 0.124± 0.16 and 0.147± 0.02 respectively. There was no significant difference (p=0.60) between the two groups. There was also no significant difference (p=0.053) between the pre-procedural values and the 6-month post-PCI values in those patients who did not develop restenosis. However, there was a significant difference (p=0.001) between the pre-procedural values and the 6-month post-PCI values in those patients who developed restenosis. The levels of serum MDA in patients with restenosis at 6-months increased by 109% and were significantly different (p=0.001) in the restenosis group.

The results suggest that pre-procedural levels of plasma homocysteine and serum MDA were not predictors of restenosis following PCI. However, the post-PCI six-month levels of both homocysteine and MDA are predictors of restenosis. Moreover, the post-PCI levels of MDA were better predictors of restenosis than the post-PCI levels of homocysteine because the increase in MDA levels were greater at six months than the rise in homocysteine levels at the same time interval.

ACKNOWLEDGEMENTS

I would like to thank God for allowing me this opportunity to complete this work. I dedicate this thesis to my father Reuben J. McNair, Sr. who always believed in my abilities and motivated me to strive toward success through discipline and hard work. I would like to express my sincerest appreciation to Dr. Calvin Wells for providing his patient's for the study, time, equipment, advice, and unwavering support without which this research would not have been conducted. I am greatly thankful to my supervisor Professor Mabood Qureshi for his guidance, suggestions and advice throughout my graduate studies. I would also like to thank my co-supervisor Dr. Kailash Prasad for taking the time to instruct, guide, and nurture my understanding of the various concepts involved in this thesis. I would like to thank Dr. Taras Mycyk for his assistance in editing this manuscript.

I would like to thank the technical staff from the Hemodynamics Laboratory for their support. Many thanks to Heather Neufeld, Michelle Hesson, Margaret Pashovitz, Edna Messett, Todd Reichert, and Barbara Raney for their technical expertise generously provided to me throughout the study. Many thanks are due to Mark Rosin and the Perfusion staff for their patience and support throughout the years.

Finally, I would thank Dr. Joel Gajadharsingh for his consistent advice and support in the writing of this manuscript. I would like to express my deepest gratitude to my wife Jill for her support, encouragement, and patience throughout the years. To my loving children Erick Jr., Kali, and Lauryn, I love you because you bring joy to my life.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF ABBREVIATIONS

Symbol	Full Name
ACAT	acyl: cholesterol acyltransferace
Ach	acetylcholine
ACS	acute coronary syndrome
ADP	adenosine diphosphate
AHA	American Heart Association
ALS	amyotrophic lateral sclerosis
AMP	adenosine monophosphate
ATP	adenosine triphosphate
bFGF	basic fibroblast growth factor
Br^-	bromide
BP	blood pressure
C_3	complement 3
C_5	complement 5
Ca^{2+}	calcium ion
CAD	coronary artery disease
CCl_4	carbon tetrachloride
CD 11/ CD18	integrin adhesion molecules
CHD	coronary heart disease
Cl^-	chloride anion
CMV	cytomegalovirus
C. pneumonia	Chlamydophila pneumonia
CVD	cardiovascular disease

CRP	C-reactive protein
Cu_{2+}	cuprous
DD	double distilled
DES	drug eluting stent
DM	diabetes mellitus
DNA	deoxyribonucleic acid
DTT	dithiothreitol
ECF	extracellular fluid
ECG	electrocardiogram
EDTA	ethylenediamine tetraacetic acid
EDRF	endothelial derived relaxing factor
ELAMS	endothelial-leukocyte adhesion molecules
eNOS	endothelial nitric oxide synthetase
ETT	exercise tolerance test
Fe^{2+}	ferrous fumarate
Fe^{3+}	ferric fumarate
FPIA	fluorescence polarization immunoassay
G_1	growth cycle 1: phase for preparation of chromosomes
G_2/M	growth $cycle_2$/M: phase for preparation of mitosis
GM-CSF	granulocyte-monocyte-colony stimulating factor
GSH	reduced glutathione
GSH-P_x	glutathione peroxidase
H^+	hydrogen ion
HB-EGF	heparin-binding growth factor

HCl	hydrochloric acid
HDL-C	high-density lipoprotein-cholesterol
H_2O	water
H_2O_2	hydrogen peroxide
HOCl	hypochlorous acid
$HOO^{\cdot}$	hydroperoxyl radical
HSP	heat shock proteins
HUVEC	human umbilical vein endothelial cell
I^-	iodide
IHD	ischemic heart disease
ICAM-1	intracellular adhesion molecule-1
IgA	immunoglobulin A
IGF-I	insulin growth like factor I
IgG	immunoglobulin G
IL-1	interleukin-1
IL-1β	interleukin-1β
IL-6	interleukin-6
$L^{\cdot}$	lipid radical
LDL-C	low-density lipoprotein-cholesterol
LOOH	lipid hydroperoxides
Lp (a)	lipoprotein (a)
LPL	lipoprotein lipase
LTB_4	leukotriene B_4
MACE	major cardiac events

MCP-1	monocyte chemotactic protein-1
M-CSF	macrophage-colony stimulating growth factor
MDA	malondialdehyde
MI	myocardial infarction
MMLDL	minimally modified low-density lipoprotein
MPO	myeloperoxidase
mRNA	messenger ribonucleic acid
NADH	nicotinamide adenine dinucleotide hydrogenase
NADP	nicotinamide adenine dinucleotide phosphate
NADPH	reduced nicotinamide adenine dinucleotide phosphate
$NO^{\cdot}$	nitric oxide
NO_2	nitrogen dioxide
N_2O_3	nitrogen trioxide
$^1O_2$	singlet oxygen
O_2	molecular oxygen
$O_2^{\cdot -}$	superoxide anion
OCl	hypochlorite
OFR	oxygen free radical
$OH^{\cdot}$	hydroxyl radical
OH	hydroxide
OR	oxygen radical
$ONOO^-$	peroxynitrite
OXLDL	oxidized low-density lipoprotein
PAF	platelet activating factor

PAI-1	plasminogen activator inhibitor-1
PC	phosphocholine
PCI	percutaneous coronary intervention
PCR	polymerase chain reaction
PDGF	platelet-derived growth factor
PGG_2	prostaglandin G_2
PGH_2	prostaglandin H_2
PMNL	polymorphonuclear leukocyte
PTCA	percutaneous coronary angioplasty
PUFA	polyunsaturated fatty acid
RASMC	rat aortic smooth muscle cell
RNA	ribonucleic acid
$RO^{\cdot}$	alkoxyl radical
$ROO^{\cdot}$	peroxyl radical
ROS	reactive oxygen species
RPM	revolutions per minute
SAH	S-adenosyl-L-homocysteine
SDS	sodium lauryl sulphate
SOD	superoxide dismutase
S phase	growth cycle phase for synthesis of DNA
SPSS	statistical program for the social sciences
SEM	standard error of the mean
TxA_2	thromboxane A_2
TBA	thiobarbituric acid

tHcy	total homocysteine
TNF-α	tumor necrosis factor-alpha
TG	triglycerides
TGF-α	transforming growth factor-alpha
TGF-β	transforming growth factor-beta
tPA	transplasminogen activator
VCAM-1	vascular adhesion molecule-1
VLA-4	very late antigen-4
VLDL	very low-density lipoprotein
VLDL-TG	very low-density lipoprotein-triglyceride
V/V	volume to volume

1.0 REVIEW OF THE LITERATURE

1.1 INTRODUCTION

Myocardial infarction (MI) consequent to atherosclerosis of a coronary artery is one of the principal causes of death in western civilization. As outlined by Prasad (1999) within this high-risk population, hyperhomocysteinemia, smoking, hypercholesterolemia, diabetes and hypertension are independent risk factors for coronary artery disease (CAD). Percutaneous Coronary Intervention (PCI) is one of the procedures performed to reduce the ischemic effects of CAD. Although much progress has been made in the field of interventional cardiology, restenosis remains as one of the adverse outcomes of PCI. The relationship between plasma levels of homocysteine and restenosis in patients with CAD who have undergone PCI remains unsolved. Kojoglanian et al. (2003) demonstrated that elevated levels of homocysteine strongly correlate with increased risk for restenosis. However, Ambrosi (2003) showed that hyperhomocysteinemia is probably a weak risk factor for the development of restenosis. This discrepancy may be due to factors other than homocysteine, which can generate oxygen radicals during CAD. Polymorphonuclear leukocytes (PMNLs), xanthine-xanthine oxidase, arachidonic acid metabolism, homocysteine and cytokines generate oxygen radicals (ORs), which induce oxidative stress in patients with CAD (Chambers et al. 1985; Kapoor and Prasad, 1996; Prasad et al. 1993). Furthermore, ORs cause endothelial damage by attacking polyunsaturated fatty acids with the formation of lipid hydroperoxides and conjugated dienes (Rumley and Paterson, 1998). Malondialdehyde (MDA) is a lipid peroxidation product, which provides an indirect measure of oxidative

stress. The present investigation was undertaken to determine: (1) if serum MDA and plasma homocysteine are predictors of post-PCI restenosis; and (2) if serum MDA is a better predictor of post-PCI restenosis than homocysteine.

1.1.1 Atherosclerosis and Coronary Artery Disease

The term atherosclerosis is derived from the Greek "athero" (gruel or porridge) and "sclerosis" (hardening). It is a chronic inflammatory disease of the arteries in which macrophages and oxidized lipids are the primary agents, resulting in intimal fibro-fatty plaque formation and progression. Atherosclerosis begins in childhood and slowly progresses over many years before it manifests itself with clinical symptoms (Ross and Glomset, 1976). The arteries generally affected by atherosclerosis are the thoracic aorta, coronary, internal carotids, femoral, basilar, vertebral, and circle of Willis. Myocardial infarction and stroke are two of the major clinical consequences of diminished or halted blood flow created by atherosclerosis (Ross and Glomset, 1976).

1.1.1.1 Epidemiology of Atherosclerosis

In North America, atherosclerosis continues to be one of the prominent causes of morbidity and mortality. "The incidence of disease related to atherosclerosis is as follows: ischemic heart disease (IHD), 7 million; peripheral vascular disease, 3 million; and stroke, 0.75 million per year", as described by Prasad (2003a). It is a ubiquitous disease among developed countries and has geographical variations. For instance, coronary artery disease in North America is six times higher than in Japan (Ross, 1986).

1.1.1.2 Pathology of Atherosclerosis

Intimal focal lesions of atherosclerosis are visible fatty streaks with raised yellow areas that are slender and longitudinally oriented. They are made up of lipid laden foam cells, which are macrophages and or smooth muscle cells. Fibrous plaques lesions are

raised off white lesions with three layers. The first layer is a fibrous cap consisting of smooth muscle cells, and leukocytes, along with dense connective tissue. This connective tissue consists of a basement membrane, collagen fibrils, proteoglycans, and elastin. The second layer is a cellular region under and adjacent to the fibrous cap. This layer is composed of a combination of smooth muscle cells, T lymphocytes and macrophages. The final lower necrotic layer is composed of immense foam cells, cellular debris, lipids, and calcium crystals (Duff and McMillian, 1951). However, most patients with atherosclerosis present with a combination and/ or variations of each of these characteristics. The organization of lipid and connective tissue in these lesions determines whether or not they are complicated lesions that are stable or unstable with the possibility of rupture and/ or thrombosis as outlined by Prasad, (2003a). Nevertheless, the clinical event that arises from coronary atherosclerotic plaque formation is myocardial ischemia (decreased coronary perfusion). According to Preuss et al. (1987), there are three major types of ischemia: (1) tolerable ischemia has a low degree of reduced blood flow; (2) critical ischemia reflects a modestly severe flow reduction; and (3) lethal ischemia has a severe flow reduction and is the limit of myocardial survival. Lethal ischemia, if left untreated, can lead to irreversible cell necrosis [myocardial infarction (MI)].

1.1.1.3 Theories of Atherosclerosis

Although there are several theories of atherogenesis they all have a central concept centered on the following factors: (1) lipid involvement in the formation of a lesion (2) monocyte migration and proliferation, and (3) smooth muscle proliferation.

Response to Injury Hypothesis

The most commonly accepted theory of atherosclerosis is the Response to Injury Hypothesis. It was first proposed by Virchow in 1956, was later modified and expanded by Ross and Glomset (1976), and was updated subsequently by Ross in 1986. Their theory of atherosclerosis is based upon endothelial damage caused by chemical, mechanical, immunological or toxicological insult. Endothelial damage leads to endothelial dysfunction, which initiates a lesion and causes exposure of the subendothelial tissue. The previous events may be responsible for the insudation of plasma lipoproteins into the arterial wall. Furthermore, the progression of the lesion involves monocyte and platelet chemotaxis and adherence to the endothelium (Faruqui et al. 1994). The formation of endothelial gaps (20-60 nm) may be due to structural changes in the microvasculature where there is endothelial cell contraction or intercellular endothelial cell retraction and cytoskeletal reorganization under the influence of interleukins, tumour necrosis factor (TNF) and interferon. Successively, monocytes, and T lymphocytes diapedes through the endothelium into the extravascular space where monocytes are subsequently converted into mature macrophages. Tissue macrophages phagocytize lipids, and become immobile foam cells, which are the early atherosclerotic fatty streak lesions (Ross, 1993).

The adhesion of white blood cells to the endothelial surface is accomplished through endothelial-dependent counter receptor mechanisms. The increase in recruitment and adhesion of monocytes and T lymphocytes is due to endothelial-dependent counter receptor mechanisms, which form specific sets of adhesion molecules. Monocyte expression of very late antigen-4 (VLA-4) unites with its vascular

endothelial counter receptor, vascular cell adhesion molecule (VCAM-1) to provide selective adhesion (Gimbrone, 1995). There are various other endothelium-dependent counter receptor systems contributing to the increase in adherence of monocytes to the endothelium, such as the selectin and integrin varieties: (1) intercellular adhesion molecule 1 which interacts with CD11/CD18-integrin complex, (2) endothelial derived monocyte chemotactic protein-1 (MCP-1), (3) interleukin 1 (IL-1), (4) TNF, and (5) hypercholesterolemic serum lipoproteins (Gimbrone, 1995; Denholm and Lewis, 1987).

Prasad (2003a) described several growth modulators produced by macrophages, smooth muscle cells, endothelial cells, and T lymphocytes which have paracrine and or autocrine effects. Paracrine effects are expressed when growth modulators act upon other cell types in the vicinity. However, an autocrine effect is manifest when growth modulators act upon the cells that have produced them (Denholm and Lewis, 1987). Macrophages produce the following paracrine acting growth modulators: (1) platelet-derived growth factor (PDGF); (2) heparin-binding epidermal growth factor (HB-EGF); (3) cytokines (IL-1, TNF); (4) oxidized low density lipoproteins (OX-LDL); and (5) basic fibroblast growth factor and transforming growth factors α and β (bFGF, TGF-α, TGF-β) which all modulate the action of adjacent cells (Gimbrone, 1995). Furthermore, TGF-β which is also produced by T lymphocytes has two main functions: (1) inhibition of smooth muscle cell replication; and (2) initiation of the production of connective tissue and matrix such as elastic fiber proteins, collagens and proteoglycans. In addition, smooth muscle cells form oxidized LDL (OXLDL), macrophage colony stimulating factor (M-CSF) and granulocyte-monocyte colony stimulating factor (GM-CSF) all of which induce monocyte differentiation into mature macrophages (Denholm and Lewis, 1987). Colony stimulating factor plays an important part in macrophage immobility and

replication. According to Prasad (2003a), smooth muscle and endothelial cells also control growth by the production of PDGF, bFGF, TGF, insulin like growth factor I (IGF-I), and cytokines (IL-1, TNF-α). Likewise, cytokines exert an autocrine effect on smooth muscle by stimulating smooth muscle proliferation, and a paracrine effect by eliciting endothelial activation (Prasad, 2003a).

In summary, the response to injury hypothesis is based upon insult by hyperlipidemia or other risk factors that induce endothelial injury and dysfunction causing platelet and monocyte adhesion, and liberation of cytokines, chemo-attractants, and growth regulators. Consequently, this sequence of events triggers smooth muscle cell proliferation, chemotaxis and emigration of more monocytes with eventual macrophage lipid retention creating a vicious cycle that leads to the genesis of the atherosclerotic fatty streak lesion.

Oxidative Hypothesis

In the oxidative hypothesis the production of the atherosclerotic fatty streak lesion is due to low-density lipoprotein cholesterol (LDL-C) oxidation, which then accumulates in immobile tissue macrophages termed foam cells (Henriksen et al. 1981). Plasma lipoprotein and LDL-C set the stage for atherogenesis by being oxidized by smooth muscle cells, macrophages and endothelium (Keany and Vita, 1995). The oxidation of LDL-C to minimally modified LDL-C (MMLDL) stimulates endothelial and smooth muscle cells to produce monocyte chemotactic protein 1 (MCP-1) which in turn increases monocyte migration to the subendothelial area (Chisolm, 1991). Furthermore, MMLDL is likewise oxidized to OXLDL which can directly cause endothelial damage, trigger additional monocyte migration, and stimulate the release of MCP-1 and M-CSF from endothelial cells. Conjointly, OXLDLs also serve as ligands

for the scavenger receptor of macrophages (Steinberg, 1991) as outlined by Prasad (2003a). Thus, macrophage engulfment of OXLDL renders them to become OFR releasing, immobile foam cells, stationary at the focus of the lesion as described by Prasad (2003a). Finally, the endothelial adhesion of monocytes depends upon cytokines and chemoattractants such as IL-1β and M-CSF which effect surface expression of endothelial–leukocyte adhesion molecules (ELAMS) (Schwartz et al 1993).

1.1.2 Risk Factors for Atherosclerosis

As reviewed by Prasad (2003a) the risk factors for atherosclerosis are divided into two general categories (1) major risk factors (which are further subdivided into nonmodifiable and potentially controllable categories), and (2) minor risk factors.

1.1.2.1 Major Risk Factors

Nonmodifiable Risk Factors

As described by Prasad (2003a), nonmodifiable risk factors are those inherent factors, which cannot be changed. They include genetic abnormalities, increasing age, positive family history of atherosclerosis, and male gender.

Potentially Controllable Risk Factors

1.1.2.1.1 Hypertension.

Hypertension is an independent risk factor for CAD and other atherosclerotic disease (Kannel, 1975). In addition, greater risks of atherosclerotic disease complications arise when the diastolic pressure is greater than 115mm Hg. However, other studies have demonstrated that a better indicator for hypertension-induced CAD is an elevated systolic pressure (Rosenman et al. 1976). Interestingly, atherosclerosis in hypertension may occur because of induced vascular wall growth, particularly medial smooth muscle cell hypertrophy and intimal hyperplasia. In addition, excitation from

the sympathetic nervous system and vasoactive substances can elicit smooth muscle hyperplasia and replication (Owens, 1989). Common vasoactive substances such as serotonin, endothelin, and thrombin can cause smooth muscle replication, whereas angiotensin II can cause smooth muscle hyperplasia in patients with hypertension (Chobanian, 1990). Similarly, hypertension may be induced by variability between oxidants and antioxidants. Prasad (2003a) has summarized oxidative stress and consequent hypertension-induced atherosclerosis. The vasoconstriction elicited by the superoxide anion could have a major function in essential hypertension (Nakazano et al 1991). Conversely, the administration of the antioxidant superoxide dismutase (SOD) has been reported to decrease hypertension in rats and hypertensive patients (Sagar et al. 1992). In addition, three antioxidants (vitamin C, glutathione, and thiopronine) have elicited an acute antihypertensive effect in patients with hypertension (Ceriello et al. 1991). Panza et al. (1990) have also reported that if hypertensive patients have elevated levels of superoxide anions then the effect of nitric oxide (NO) is inhibited. Therefore, the vasodilatory effect of NO is suppressed, and hypertension becomes marked. It is also speculated that elevated serum OFR levels observed in hypertensive patients could result in endothelial dysfunction, and induce atherogenesis (Prasad, 1997b).

1.1.2.1.2 Hypercholesterolemia

Hypercholesterolemia is a strong independent risk factor for CAD and atherosclerosis. Consequently, mortality is high in populations with elevated levels of cholesterol. Elevated plasma cholesterol levels have been associated with increased probability of the development of MI (Castelli, 1988). According to the follow-up of the Framingham Heart Study (Anderson KM et al. 1987), the range for total cholesterol which correlates with borderline elevated risk for the development of CAD is from 5.15

to 6.18 mmol/L, and the range for high risk total cholesterol is ≥ than 6.20 mmol/L. In addition, the risk for the development of CAD increases by 2 to 3% for each 1% increase in total cholesterol (Castelli, 1986).

1.1.2.1.3 Triglycerides

Many prospective studies have indicated that elevated triglyceride levels are associated with increased risk for the development of CAD (Steinberg and Wiztum, 1990). Atherogenesis is induced by the metabolism of high levels of triglycerides. Specifically, elevated levels of very low-density lipoprotein triglycerides (VLDL TG) result in the formation of an array of low-density lipoproteins (LDL-C) and low concentrations of high-density lipoproteins (HDL-C) both of which are atherogenic in nature (Austin, 1989). Similarly, high triglyceride levels may induce a procoagulant state, which could lead to thrombosis and MI. The mechanism by which elevated levels of triglycerides produce a procoagulant state is due primarily to their ability to induce: (1) increases in factors VII and X; (2) activation of phospholipid complexes; (3) production of fibrin and thrombin; and (4) inhibition of transplasminogen activator (tPA) (Castelli, 1986; Castelli, 1988; Austin, 1989).

1.1.2.1.4 LDL-Cholesterol (LDL-C)

It is postulated that LDL are correlated with increased atherogenesis because of their small size and increased susceptibility to be oxidized. An LDL of less than 3.6 mmol/L is considered to be normal; 3.36 to 4.11 mmol/L is borderline; and greater than 4.14 mmol/L represent high-risk values for the development of CAD (Austin et al. 1990).

Under physiological circumstances LDL are responsible for the transport of endogenous triglycerides to the periphery for storage. The endothelial LDL receptor

allows sequestration by endocytosis of LDL into the cytoplasm with the subsequent release of cholesterol for tissue use (Austin et al. 1990).

1.1.2.1.5 HDL-Cholesterol (HDL-C)

Numerous reports have demonstrated an inverse relationship between HDL levels with risk of development of CAD. Hence, the prevention of atherosclerosis is closely related to the reverse cholesterol transport from the tissues to the liver where it is cleared by a hepatic HDL receptor (Anderson et al. 1978). The two subgroups of HDL are HDL-2, which is composed of small particles, and HDL-3, which consists of larger particles. In addition, elevated HDL levels exert a cardio protective effect and delay the rate of atherogenesis (Montali et al. 1994) perhaps by retarding the permeability, transport, and retention of LDL in the arterial wall (Khoo et al. 1990). Additionally, elevated HDL concentrations may exert a cardioprotective effect by inhibiting the oxidation of LDL (Parthsarathy et al. 1990). As outlined by Prasad (2003a), elevated HDL levels are observed in people who exercise; consume moderate amounts of alcohol, and patients with hyperthyroidism. Yet reduced HDL levels are seen in patient administered thiazides or β blockers, cigarette smokers, obese patients, and those who do not exercise. Thus, patients with low levels of HDL may have an increased risk of atherogenesis. “For every 1mg/dL decrease in HDL cholesterol concentration, the risk for CAD is increased by 2-3%” (Grundy, 1995).

1.1.2.1.6 Lipoprotein (a)

Lipoprotein (a) [LP (a)] is a form of LDL-C which consists of two large glycoproteins, apolipoprotein (a) and apolipoprotein (b) that are covalently bonded by a disulfide bridge (Grundy, 1995). LP (a) is structurally similar to the plasma protein plasminogen which, when activated, functions to dissolve fibrin stands and decrease clot

formation. Essentially, LP (a) competes with plasminogen for binding sites and impairs fibrinolysis (Loscalzo et al. 1990). The mechanisms of LP (a) participation in the genesis of atherosclerosis are varied. For example, macrophages in atherosclerotic plaques express a VLDL receptor that can bind to, and mediate the catabolism of LP (a) leading to increases in cholesterol retention, LDL oxidation, and foam cell formation (Palabrica et al. 1995; Hansen et al. 1994). Macrophage foam cell formation could be due to LP (a) induction of intracellular adhesion molecule -1, which may act to immobilize macrophages (Takami et al. 1998), thus contributing to the fatty streak atherosclerotic lesion. Additionally, LP (a) may cause damage to endothelial constituents that respond to vasodilatory stimuli (Schachinger et al. 1997). Consequently, a decreased response to vasodilatory stimuli may induce hypertensive related atherosclerosis.

There have been conflicting reports concerning the relationship between LP (a) and the development of CAD. Several studies have suggested that LP (a) is an independent predictor of risk for CAD (Bostrom et al. 1994, and 1996; Schaefer et al. 1994; Austin MA et al. 1990; Ariyo et al. 2003). Other reports have indicated that there is no correlation between LP (a) and CAD. Despite this conflict, there appears to be agreement that LP (a) has a capacity in predicting the risk of development of CAD in patients with concurrently elevated cholesterol levels. According to Von Eckardstein et al. (2001), male patients with LP (a) concentrations $\geq$ 20 mg/dL were 2.7 times more likely to experience a coronary episode (nonfatal or fatal myocardial infarction and/ or sudden death) than those with lower levels.

1.1.2.1.7 Apolipoproteins

Lipoproteins are composed of protein, phospholipids, triglycerides, and esterified and unesterified cholesterol. The apolipoproteins are the protein constituents of the lipoprotein, and they function as cofactors for enzymes and ligands for receptors. There are several different apolipoproteins, and derangements in their metabolism lead to abnormalities in lipid metabolism and atherogenesis (Rader et al. 1994).

1.1.2.1.8 Diabetes Mellitus (DM)

Diabetes mellitus is an absolute risk factor for the development of atherogenesis. Specifically, in diabetic patients, the occurrence of myocardial infarction is twofold that of non-diabetics (Lyons, 1993). As outlined by Prasad (2003a), the exact mechanism of atherogenesis in diabetics is unclear; however there is growing evidence that there could be several ways that diabetes could induce atherosclerosis. For instance, diabetes can stimulate LDL-hypercholesterolemia. Prasad et al. (1994) described the probability that hypercholesterolemia could increase the concentration of oxygen free radicals and induce endothelial cell injury thereby stimulating the development and maintenance of atherogenesis. In type II diabetics, insulin levels are independent of the hyperglycemic state. Thus, normal insulin and IGF-I levels can increase the proliferation of growth factor, and induce the insudation of LDL by smooth muscle cells (Sonio et al. 2004). Diabetics also exhibit glycation, which is the nonenzymatic binding of glucose to proteins resulting in augmented uptake of LDL by macrophages (Lyons, 1993). Similarly, glycoxidation products, the free radical stimulated oxidation of lipoproteins are thought to be atherogenic. Likewise, glycosylation end products are postulated to be atherogenic due to their chemotaxis of monocytes. Finally, the ORs produced by

hyperglycemia in diabetics may induce dysfunctional endothelium which is involved in the genesis of atherosclerosis (Prasad and Kalra, 1993; Baynes, 1991).

1.1.2.1.9 Cigarette Smoking

Smoking is a major independent risk factor for the development of atherosclerosis, and patients who smoke one or more packs of cigarettes per day have an elevated risk of 2.5 to 3.2 times higher than non-smoking patients (Bazzano et al. 2003). Men in particular who smoke one pack of cigarettes per day have 3 to 5 times the risk of the development of ischemic heart disease (IHD) than non smokers (Fielding, 1985). Similarly, women over the age of thirty-five who smoke cigarettes and take oral contraceptives have an elevated risk of the development of IHD (Friedman et al. 1979). However, the risk in the development of CAD decreases abruptly with the discontinuance of smoking (Friedman et al. 1979). The mechanisms of atherogenesis from cigarette smoking are complicated. The by products of smoking such as peroxyl radicals may induce endothelial injury which in turn stimulates and maintains endothelial dysfunction and atherogenesis. Moreover, the levels of ORs are increased by smoking through augmented PMNL stimulation from complement activation (Kalra et al. 1991).

1.1.2.2 Minor Risk Factors

1.1.2.2.1 Alcohol

Overindulgence of substantial amounts of alcohol is absolutely correlated with the development of CAD (Schmidt and deLint, 1972). On the other hand, reasonable consumption of alcohol has been correlated with a decreased risk of the development of CAD (Hennekens et al. 1978) due to the protective effect of elevation in HDL levels and lowering of LDL concentrations in the serum. Prasad (2003a) has summarized the

oxidative stress with alcohol consumption. Alcohol increases triglyceride and OR levels which have been shown to be atherogenic. The potential propagation of ORs from alcohol abuse are metabolism of acetaldehyde (via the metabolism of ethanol), microsomal ethanol oxidizing, and the acetaldehyde-induced stimulation of the plasma membrane of hepatocytes to stimulate PMNLs to produce superoxide anions (Cederbaum et al. 1977; Klein et al. 1983; Muller, 1987; Puig and Fox, 1984; Williams and Barry, 1986; Winston and Cederbaum, 1982).

1.1.2.2.2 Coffee

Prasad (2003a) summarizes the effect of coffee on the development of atherosclerosis. Atherosclerosis has not been correlated to coffee consumption in past prospective studies (Kannel and Dawber, 1973). Yet, present studies have shown a correlation between coffee consumption and elevated risk of CAD. Furthermore, myocardial infarction has been related to coffee consumption in a retrospective case control study (Jick et al. 1973). In animal models where coffee consumption was studied, the results demonstrated increased myocardial irritability and a decreased ventricular fibrillation threshold, which suggests a probable cause of sudden death in human coffee consumption (Bellet et al. 1972). Similarly, coffee related atherogenesis has been correlated to increased serum TG and cholesterol levels (Thelle et al. 1987). Essentially, an elevated LDL is a consequence of the consumption of ground coffee beans boiled directly with water. The hypercholesterolemia resulting from boiled coffee is due to diterpine alcohols. The diterpine alcohol cafestol has been demonstrated to lower bile acid synthesis and down regulate the LDL receptor, which can cause an increase in serum cholesterol in boiled coffee drinkers. Conversely, the

hypercholesterolemic effect is not seen when coffee is purified through a filter because the diterpines are removed (Thelle et al. 1987).

1.1.2.2.3 Infection

Prasad (2003a) has summarized oxidative stress and atherosclerosis with infection. Recent reports have suggested that specific types of infections may have a part in atherogenesis by creating a low-grade constant state of acute or chronic inflammation that can result in endothelial dysfunction, which is the initial step in proatherogenic properties (Gabay and Kushner, 1999). Interestingly, animal models have demonstrated that continuous susceptibility to circulating endotoxins produces a risk factor for atherosclerosis (Lehr et al. 2001). Infections can also stimulate the production of heat shock proteins (HSP), which are stress proteins that respond to injurious stimuli. Those induced subsequent to injurious excitation; presumably act in refolding denatured polypeptides to re-establish their function before cell dysfunction or death (Xu et al. 2000; Zhu et al. 1999; Burian et al. 2001; and Pockley, 2002). Specifically, HSP 60 causes human macrophages to produce TNF-α and matrix metalloproteinase's. Earlier in the section on "Theories of Atherosclerosis", the cytokine TNF-α and its role in growth modulation in the development of early fatty streak lesion was discussed. Moreover, HSP 60 may also trigger endothelial cell expression of monocyte adhesion molecules E- selectin, ICAM-1, VCAM-1, and IL-6. Finally, HSP 60 has been positively associated with other markers of inflammation and with the existence of CAD (Xu et al. 2000; Zhu et al. 1999; Burian et al. 2001; and Pockley, 2002).

The principal organisms studied which exhibited a relationship between acute or chronic inflammatory infection and risks of developing atherosclerosis are Chlamydophila

pneumonia (C. pneumonia), human cytomegalovirus (CMV), and Helicobacter pylori. However, numerous other viruses have been associated with the proatherosclerotic events (Smeeth, 2004).

Chlamydophila Pneumonia

C. pneumonia, a known and classified human pathogen from respiratory tract infection is transferred from the lungs to the vascular wall by circulating monocytes. It can be sustained within the monocyte without lysis for up to ten days (Grayston et al. 1986 and Gieffers et al. 2001). In animal models and in human subjects, C. pneumonia induced atherosclerosis may be produced by alternative methods. First, C. pneumonia may stimulate the expression of the procoagulant plasminogen activator inhibitor-1 (PAI-1) protein and the proinflammatory cytokine interleukin-6 which could potentially increase atherogenesis by influencing thrombus formation (Dechend, 1999). Secondly, the observation of aortic tissues from normocholesterolemic and hypercholesterolemic groups of mice with manipulated LDL receptor deficiency in the presence of C. pneumonia resulted in amplified atherosclerosis in only the hypercholesterolemic group. Thus, it is possible that an atherosclerotic lesion induced by hypercholesterolemia is present prior to infection by C. pneumonia (Hu et al. 1999). Chronic infection can be detected by serology or polymerase chain reactions (PCR) when there is an appearance of elevated titers of immunoglobulin G (IgG), or immunoglobulin A (IgA) antibodies to C. pneumonia in the blood (Wong et al. 1999). In human subjects serologic and PCR evaluations show that positive and negative correlations exist between circulating C. pneumonia antibody titers and CAD. Specifically, meta-analysis prospective studies of IgG and IgA titers revealed no significant associations and weak correlations between CAD, and these titers respectively (Danesh et al. 2000; Danesh et al. 2002). On the

other hand, elevated titers of C. pneumonia antibody have been strongly correlated with a high probability of the development of adverse cardiac events in case control studies that have differentiated serologic results for C. pneumonia in patients with CAD with matched controls (Siscovick et al. 2000; Ridker et al. 1999). Overall, there is a positive correlation between C. pneumonia and atherosclerosis. It is still debatable however, as to whether the virus is a stimulus for inflammation and chronic infection or a component of atherosclerotic tissue. Most viruses respond to antibiotic therapy thus, many antibiotic trials have been undertaken. Even though C. pneumonia may not be able to be removed from circulating monocytes with antibiotics, they may provide an anti-inflammatory effect (Gieffers et al. 2001). Nevertheless, meta-analysis of random control studies using the antibiotics azithromycin and roxithromycin to treat patients with stable CAD, acute MI, an acute coronary syndrome (ACS), coronary restenosis, and peripheral artery disease against patients receiving a placebo has revealed that there was no significant difference between groups in attenuating cardiovascular episodes or death from three months to five years (Wells et al. 2004; Anderson et al. 1999). Thus, there was no benefit in prescribing antibiotic therapy to patients with atherosclerosis.

Human Cytomegalovirus

Infection with cytomegalovirus has been correlated with the development of atherosclerosis in an abundance of studies. However, there are some studies that show no relationship between the two (Adler et al. 1998; Blum et al. 1998; Sorlie et al. 2000). The mechanism of action in which CMV induces the development of atherosclerosis may be through various pathways. According to Muhlestein et al. (2000), the combination of increased serum levels of inflammatory indicators such as C- reactive protein (CRP), and interleukin-6 (IL)-6 with CMV seropositivity is an absolute predictor

of mortality. Consequently, it is suggested that certain patients who develop atherosclerosis have had an inflammatory response from CMV, and those patient's in whom the inflammatory response was absent were resistant (Zhu et al. 1999). Another concept of how CMV infection may motivate the formation of atherosclerosis is by promoting the expression of the Type A scavenger receptor for OXLDL on vascular smooth muscle cells (Zhou et al. 1996). Thus, an increased expression of this receptor contributes to the formation of foam cells and the genesis of the early atherosclerotic fatty streak lesion. Finally, it is also postulated that CMV infection may accelerate neointimal reaction to vascular injury whether the virus exists in the vascular wall or not (Zhou et al. 1999). Consequently, endothelial injury is known to lead to endothelial dysfunction, which greatly increases the likelihood of the development of atherosclerosis.

Other Pathogens

Dental infections of the teeth and gingivae such as peridontitis and caries have been associated with the development of CAD. Lactobacillus, Haemophilus influenzae, Streptococci pneumonia, viridans and nonhemolytic streptococci are a few of the oropharyngeal flora implicated in the development of atherosclerosis (Mattila et al. 1993; Prasad, 2003a). In addition, Helicobacter pylori infection has been associated with atherosclerosis and MI. However, this correlation is unclear at this time (Patel et al. 1995; Gunn et al. 2000). Finally, Prasad (2003a) reported that the total amount of pathogens to which a patient is exposed is termed the pathogen burden, and it is perhaps related to the existence and complexity of CAD, and is possibly a better indicator of endothelial dysfunction than any one particular pathogen.

1.1.2.2.4 C-reactive protein (CRP)

Prasad (2003c) gave an account of C-reactive protein as being an acute phase reactant protein that serves as a novel biomarker for inflammation and atherosclerosis. Generally, it is a risk factor for cardiovascular disease and may specifically predict the development of MI and stroke in healthy individuals (Pearson et al. 2003). According to Gauldie et al. (1987), the stimulation of monocytes and macrophages during inflammation induces them to release cytokines such as IL-6, IL-1 β, TNF-α, interferon λ and transforming growth factor β. Cytokines in turn stimulate hepatocytes to release the major positive acute phase proteins CRP and serum amyloid A. The major negative acute phase proteins are albumin, transferrin and transthyretin (Kushner and Feldmann, 1978). Those proteins whose plasma levels increase by at least twenty–five percent during inflammatory circumstances are termed positive acute phase proteins. Conversely, those proteins whose plasma concentrations decrease by at least twenty-five percent are termed negative acute phase proteins (Morley and Kushner, 1982). As specified by Pearson et al. (2003) CRP elicits numerous reactions such as complement activation, accelerated neutrophil phagocytosis and release of ORs, stimulation of adhesion molecules, production of tissue factor, and increases in platelet aggregation. A postulated primary proinflammatory function of CRP is related to its affinity for phosphocholine (PC), a host microbe ligand that is expressed on the phospholipid components of damaged cells and pathogens. Thus, through an innate immune response the binding of CRP to PC allows the detection of foreign pathogens by activation of the classical complement pathway, thereby inducing the elimination of these target cells (Volanakis, 1997; Hoffmann et al. 1999). CRP has anti-inflammatory roles as well. For

instance, one study demonstrated that increased CRP levels caused a detachment of L-selectin thereby decreasing endothelial neutrophil adhesion (Zouki et al. 1997). Equivocally, Prasad (2003c) detailed that CRP may enhance the expression of adhesion molecules by increasing oxidative stress and decreasing antioxidant production. Nevertheless, Gershov et al. (2000) have shown that CRP stimulated macrophage engulfment of apoptotic cells, and the attachment of CRP to lymphocytes subjected to apoptosis inhibited complement activation and lysis.

As previously alluded to by Prasad (2003c) CRP may cause atherogenesis by the production of oxygen free radicals and expression of adhesion molecules. The oxidative theory of atherosclerosis is based upon the pathophysiological generation of ORs. CRP directly stimulates monocytes and neutrophils to produce ORs, and indirectly induces OR production by complement activation, stimulation of platelet activating factor and expression of cytokines. Moreover, CRP is also thought to induce the production of MCP-1 by arterial endothelial cells (Ridker et al. 1998).

There appears to be a body of evidence that levels of CRP are correlated with serum lipids. Elevated levels of CRP have been shown in LDL- hypercholesterolemia resulting from cholesterol-enhanced diets. Conversely, low CRP levels are observed in dietary cholesterol deficient diets (Verhamme et al. 2002). In addition, elevated plasma Lp (a) is correlated with increased concentrations of CRP. Several studies have demonstrated that hydroxymethylglutaryl coenzyme A reductase inhibitors (statins) may be used to reduce plasma CRP levels in patients with hyperlipidemia (Moon and Kashyap, 2002; Verhamme et al. 2002; Ridker et al. 1998). Moreover, the incorporation of niacin and lovastatin administered to patients with hyperlipidemia lowers serum LDL and TG, and decreases LP (a) and CRP by 25 and 24% respectively (Moon and Kashyap, 2002).

1.1.2.2.5 Homocysteine (tHcy)

Hyperhomocysteinemia (>15 μmoles/L) has been identified as an independent risk factor for the development of atherosclerosis and deep vein thrombosis (Lee and Prasad, 2002; Gaustadnes et al. 2000). Prospective and cross sectional investigations have positively correlated tHcy with elevated risk for the development of coronary heart disease (Stampfer et al. 1992). Homocysteine is a sulphur containing intermediary amino acid which is derived by the demethylation of methionine (Shipchandler et al. 1995). The primary source of methionine is animal protein (Hankey and Eikelboom, 1999). Trans-sulphuration is the biochemical reaction in which homocysteine is catabolized to sulphate under the influence of vitamin B_6 and excreted in the urine. However, during transmethylation folate and vitamin B_{12} catalyze the remethylation of homocysteine to methionine. Total homocysteine (tHcy) exists in plasma in the following four forms (1) circulating as a free thiol {1%}; (2) bound to proteins {80-90%} (i.e. albumin) by disulphides; (3) as a dimer {5-10%}(bound with itself); and (4) bound with different thiols {5-10 %} (i.e. cysteine) (Hankey and Eikelboom, 1999). Increases in the plasma concentration of tHcy may be due to genetic insufficiencies of the enzymes needed for its metabolism, to nutritional deficits in vitamin cofactors, or to other circumstances such as drugs and medical conditions (diabetes) (Kang, 1995; Kang and Wong, 1996; Mezzano et al. 1999; Sonio et al. 2004). Similarly, low intake and plasma concentrations of folate and vitamins B_6 and B_{12} have been associated with increased plasma tHcy levels (Lee and Prasad, 2002).

One major property of tHcy is its ability to induce atherogenesis (Clark et al. 1991). The pathological hallmarks of homocysteine induced endothelial injury are the

formation of platelet saturated thrombi, smooth muscle hypertrophy, elastic lamina separation, intimal expansion and the formation of S-nitroso-homocysteine (MuCully, 1969; Harker et al. 1976; Prasad, 1999). Homocysteine also causes atherogenesis by increasing DNA synthesis of a gene called Cyclin A which in turn stimulates an uncontrollable replication of cells in one region within the lining of blood vessels. In addition, tHcy may stimulate atherosclerosis by deranging the endothelium vasomotor regulation of nitric oxide by the formation of S-nitroso-homocysteine through a process of nitrosation (NO reacting with homocysteine in the presence of water). S-nitroso-homocysteine causes vascular injury by decreasing the protective effect of NO reserves, thus inhibiting physiological smooth muscle vasodilation and membrane stability (Hankey and Eikelboom, 1999; Langman, 2000; Fonseca et al. 1999; D'Angelo and Selhub, 1997; Ling and Hajjar, 2000; Tsai et al. 1994 and 1996; Prasad, 1999). Finally, according to Prasad (1999), homocysteine has been postulated to promote atherosclerosis by the stimulation of oxidative injury to the endothelium, which is a crucial step in the response to injury hypothesis.

1.1.3 Oxygen Radicals and Atherosclerosis

Prasad (2003b) outlined ORs and their relationship to atherosclerosis. Within an atom there is a nucleus, which is surrounded by orbitals that contain spinning electron pairs. Prasad (2003b) defined a free radical as an extremely toxic intermediate reactive atom or molecule with a short half-life that has one or more unpaired electrons in its outer orbital. However, an oxygen free radical (OFR) is a compound that is produced from molecular oxygen and has gained less than four electrons. Reactive oxygen species (ROS) also termed oxygen radicals (ORs) such as hydrogen peroxide and hyperchlorous acids are derived from molecular oxygen, and they contain an even

quantity of electrons in their outer shell (Lindqvist and Nordstrom, 2001). Many ROS exist, and they have been shown to contribute to the development of various human diseases such as hypercholesterolemic atherosclerosis, ischemia-reperfusion injury, peripheral vascular disease, heart failure, diabetes mellitus, Parkinson's disease, Alzheimer disease, multiple sclerosis, amyotrophic lateral sclerosis (ALS), hemorrhagic and endotoxic shock, burns, cataracts, rheumatoid arthritis, asthma and cancer (Prasad, 1999; Prasad et al. 1994; Pattanaik and Prasad, 1998; Kapoor and Prasad, 1996; Babior, 1984; Baynes, 1991; Lindquist and Nordstrom, 2001). The oxidative hypothesis of atherosclerosis is based upon endothelial injury from oxidation of LDL (Henriksen et al. 1981). The oxidation of LDL could be due to the production of ORs (Keany and Vita, 1995). Interestingly, endothelial injury activates complement (C_3 and C_5) (Webster et al. 1980). Activated complement, platelet-activating factor (PAF), leukotrienes B_4 (LTB_4) and TNF stimulate PMNLs to release oxygen free radicals (Ford-Hutchinson et al. 1980; Braquet et al. 1989; Zoratti et al. 1991; Paubert-Braquet et al. 1988). This evidence suggests that there could be increased OR production contributing to the genesis of atherosclerosis.

1.1.3.1 Formation of Oxygen Radicals

Prasad (2003b), particularized, the five common oxygen free radicals are the superoxide anion (O_2^-), the hydroxyl radical (OH^-), the peroxyl radical (ROO^-), the alkoxyl radical (RO^-), and the hydroperoxyl radical (HOO^-). Oxygen species that have an even number of electrons in their outer orbital, for instance hydrogen peroxide (H_2O_2), and hypochlorous acid (HOCl) are termed reactive oxygen species (ROS) not oxygen free radicals. The magnitude of ORs generated is dependent upon the severity of tissue injury (i.e. ischemia), the activation and recruitment of generators of ORs (i.e.

PMNLs), the level of oxygen in solution, and the presence of endogenous or exogenous scavengers or inhibitors (Prasad, 1998; Kapoor and Prasad, 1996; Babior, 1984; Baynes, 1991).

1.1.3.2 Superoxide Radical (O_2^-)

During physiological respiration within the internal mitochondrial membrane, superoxide radicals may be formed from the reduction of molecular oxygen by the addition of four electrons to produce water. This reactive oxygen metabolite is further reduced to form hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-). Specialized phagocytic cells such as macrophages produce (O_2^-) by means of an oxidative burst to destroy engulfed pathogens (Fantone and Ward, 1982; McCord and Fridovich, 1973; McCord, 1985). Moreover, the ubiquitous flavin cytosolic enzyme xanthine oxidase catalyzes the formation of superoxide anions from hypoxanthine and oxygen (Babior et al. 1973). According to Warren and Ward (1986), the superoxide radical oxidizes catecholamines, vitamin C, homocysteine, and sulphates. It also functions in the inhibition of many enzyme systems. The superoxide radical may produce toxicity but it has restricted reactivity with lipids. Thus, the majority of the injurious effects may be due to the secondary production of extremely toxic $^{\cdot}OH$ through Haber-Weiss reactions (Fantone and Ward, 1982; McCord and Fridovich, 1973; McCord and Roy, 1982; McCord, 1985).

1.1.3.3 Hydroxyl Radical ($^{\cdot}OH$)

As reviewed by Prasad (2003b) the $^{\cdot}OH$ is an extremely reactive oxygen metabolite produced by the metallic (copper or iron) induced disunion of O_2^- and H_2O_2 by the Fenton or Haber-Weiss reactions. In addition, superoxide anion reacts with nitric oxide (NO) to form peroxynitrite ($ONOO^-$) in endothelial cultures in human neutrophils

(Kooy and Royall, 1994; Carreras et al. 1994). During normal cellular metabolism peroxynitrite forms the very volatile peroxynitrous acid, a potent oxidant that quickly decomposes to hydroxyl radical and nitrogen dioxide (Beckman et al. 1990; Koppenol et al. 1992). Interestingly, hydroxyl radicals have been demonstrated to induce relaxation of isolated aortic strips in animals (Bharadwaj and Prasad, 1994). Prasad and Bharadwaj (1996) also introduced scavengers of superoxide anion and hydroxyl radical to isolated rabbit aortic preparations and showed that acetylcholine (Ach) increases levels of $NO^{\cdot}$ which reacts with superoxide anion to form hydroxyl radical that induces vascular relaxation. These results demonstrated that Ach-induced relaxation is mediated by hydroxyl radical. Thus, the $^{\cdot}OH$ produced in physiological concentrations may not damage endothelial cell function, however the generation of high concentrations of $^{\cdot}OH$ under pathological conditions may promote the harmful effects of perioxidation of lipid membranes that may cause damage in biological systems and induce atherogenesis (Prasad and Bharadwaj, 1996).

1.1.3.4 Nitric Oxide (NO)

Nitric oxide functions as a weak oxidant as well as an antioxidant under physiological conditions (up to 100nM). Moreover, it plays a role of vasodilation and membrane stability in vascular endothelium. Acetylcholine-induced relaxation of rabbit aortic strips is induced by endothelium-derived relaxing factor (EDRF) (Furchgott and Zawadzki, 1980; Furchgott, 1983). Palmer et al. 1987 and Ignarro et al. 1987 discovered that EDRF is the free radical NO. Thus, the vascular relaxation by EDRF is actually due to $NO^{\cdot}$ mediated by $^{\cdot}OH$ (Kooy and Royall, 1994; Prasad and Bharadwaj, 1996). Nitric oxide is produced by stimulated macrophages and endothelial cells, is strongly correlated to inflammation, and it may play a role in the genesis of atherosclerosis.

Nitric oxide reacts with oxygen to form nitrogen dioxide ($^{\cdot}NO_2$), which can further react with more $^{\cdot}NO$ to form nitrogen trioxide (N_2O_3). Peroxynitrate ($ONOO^-$), a regulator of oxidant reactions such as nitrosation, oxidation, and nitration reactions is formed from the reaction of superoxide anion and nitric oxide (Beckman et al. 1990; Koppenol et al. 1992).

1.1.3.5 Singlet Oxygen ($^1O_2$)

Molecular oxygen is not a radical. However, singlet oxygen, which is a more reactive form of oxygen, was first observed in 1924 and is a higher energy state molecular oxygen species (Wasserman, 1979). In addition, its reactivity may be enhanced by specific reactions, which involve light of visible or ultraviolet wavelengths. The existence of molecular oxygen with a photosensitizer (photoexcitable compounds) reverses the spin of one of the electrons in the outer orbital thus catalyzing an oxidative reaction to produce singlet oxygen (Ogryzlo, 1978). Tetrapyrroles, flavins, chlorophyll, hemoproteins, and nicotinamide adenine dinucleotide (NADH) are examples of photosensitizers that catalyze oxidative reactions in the presence of molecular oxygen to produce ($^1O_2$) (Ogryzlo, 1978). Singlet oxygen may also be produced by three other known reactions: (1) the dismutation of ($O_2^{\cdot -}$); (2) the transition metal catalyzed Haber-Weis reaction; and (3) the oxidation of halides by H_2O_2 (Southorn and Powis, 1988). The production of singlet oxygen from phagocytic PMNLs is due to the reaction of hydrogen peroxide and hypochlorite (OCl) ions under the enzymatic influence of myeloperoxidase. The activated PMNLs undergo a respiratory burst to release cytotoxic ORs ($^1O_2$, $^{\cdot}OH$, H_2O_2, HOCl, and O_2^-), which cause lipid peroxidation and protein oxidation resulting in cell death (Freeman and Crapo, 1982; Prasad, Kalra and Bharadwaj, 1993; Prasad et al. 1990; Kapoor and Prasad, 1996).

As described by Prasad (2003b) the two forms of singlet oxygen are sigma and delta. Sigma singlet oxygen has a short half-life and is more reactive in comparison to delta singlet oxygen. On the other hand, delta singlet oxygen has a longer half-life and increases lipid membrane peroxidation (Southorn and Powis, 1988). Many studies have demonstrated that singlet oxygen is a crucial intermediate species in the detrimental oxidation of biological molecules. Specifically, it has been shown that it may react with many different biological molecules such as proteins, lipids and DNA and is involved in several disease processes including heart disease (Halliwell and Gutteridge, 1982).

1.1.4 Important Radicals and Atherosclerosis

1.1.4.1 Hydrogen Peroxide (H_2O_2)

Prasad (2003b) gave an account of the oxidant activity of hydrogen peroxide. Hydrogen peroxide is a strong, lipophilic (soluble in cell membranes) oxidant constantly formed primarily from enzymatic reactions of microsomes, peroxysomes and mitochondria. The dismutation of the $O_2^{\cdot -}$ is catalyzed by the enzyme superoxide dismutase (SOD) to produce H_2O_2 in plant and animal cells. Cellular antioxidant activity is demonstrated by the conversion of $H_2O_2 \rightarrow H_2O + O_2$ and is catalyzed by the enzymes catalase or glutathione peroxidase (Freeman and Crapo, 1982; Prasad, Kalra and Bharadwaj, 1993; Prasad et al. 1990; Kapoor and Prasad, 1996).

1.1.4.2 Hypochlorous Acid and Halogenated Amines

Hypochlorous acid (HOCl) and halogenated amines are not oxygen free radicals. They are, however, extremely strong oxidants. In addition, neutrophil phagocytosis induces the production of hydroxyl radicals through a reaction where myeloperoxidase and Cl^- are the major participants. Hypochlorous acid is 100 times more reactive than

O_2^- or H_2O_2. Moreover, it forms hydroxyl radicals as in the following reaction (Freeman and Crapo, 1982; Prasad, Kalra and Bharadwaj, 1993; Prasad et al. 1990; Kapoor and Prasad, 1996):

$$O_2^- + HOCl \rightarrow {}^{\cdot}OH + O_2 + Cl^- \quad (1)$$

1.1.4.3 Peroxyl Radicals ($ROO^{\cdot}$)

Peroxyl radicals have a short half-life of nanoseconds, are produced from hydroxyl radical reactions with lipids, proteins, nucleotide bases and carbohydrates, causing cellular damage as outlined by Prasad (2003b). However, they are less reactive than hydroxyl radicals (Southorn and Powis, 1988).

1.1.5 Sources of Oxygen Radicals

ORs are produced under physiological and pathophysiological conditions throughout the body.

1.1.5.1 Mitochondria

The primary location for O_2^- formation is the mitochondrial ubiquinone-cytochrome b region of the electron transport chain. In this univalent process, oxygen is reduced to O_2^- and receives one electron at a time (Southorn and Powis, 1988). Under physiological conditions only 5% of the inhaled oxygen is metabolized via a univalent process into highly reactive ORs (Fridovich, 1978). Yet, a tetravalent pathway producing no reactive oxygen species metabolizes the other 95%. The production of H_2O_2 (formed from the dismutation of O_2^-) is via the Fenton reaction:

$$(Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\cdot}OH + OH^{-}) \quad (1.1)$$

and or the two part Haber-Weiss reaction:

$$(Fe^{3+} + O_2^{-} \rightarrow Fe^{2+} + O_2, \; Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\cdot}OH + OH^{-}) \quad (1.2)$$

resulting in the formation of hydroxyl radicals and hypochlorous acid (HOCL) all of which may occur in the mitochondria (Fridovich, 1978).

1.1.5.2 Polymorphonuclear Leukocytes (PMNL's)

Polymorphonuclear leukocytes contain the NADPH oxidase system, which is a membrane bound flavoprotein complex that functions as an electron transport chain. The NADPH oxidase system is quiescent in uninduced PMNLs. However, upon PMNL receptor stimulation by specific ligands (activated complement, N-formyl- Met-Leu-Phe, immune complexes, and LTB_4) this system catalyzes the reduction of molecular oxygen to superoxide anion through the following reaction:

$$2O_2 + NADPH \xrightarrow[\text{oxidase}]{\text{NADPH}} 2O_2^{-} + NADP^{+} + H^{+} \quad (1.3)$$

The NADPH in this reaction originates from the pentose phosphate pathway (Freeman and Crapo, 1982). In addition, the dismutation of O_2^{-} to H_2O_2 provides a key component for the synthesis of hypophalous acid (a potent oxidizing agent). According to Kapoor and Prasad (1996), azurophilic granules, located within the PMNL contain myeloperoxidase (MPO), which is released into the phagocytic vacuole upon excitation of PMNLs. Myeloperoxidase catalyzes the oxidation of halides including: chloride

(Cl^-), bromide (Br^-) and iodide (I^-) to their related hypophalous acids. HOCl is the most potent and abundant hypophalous acid and it is produced primarily by PMNLs. HOCl can induce the chlorination of an abundance of oxidizing species such as endogenous and exogenous amino acids, amines, thioesters and aromatic carbon group (Pattanaik and Prasad, 1998; Prasad et al. 1990). The enzyme substrate complex (MPO + H_2O) oxidizes Cl^- by the following reaction (Fantone and Ward, 1982; Freeman and Crapo, 1982; Prasad et al. 1993; Prasad et al. 1990; Kapoor and Prasad, 1996):

$$H_2O_2 + Cl^- + H^+ \rightarrow HOCl + H_2O \qquad (1.3)$$

1.1.5.3 Enzymes

Prasad (2003b) delineated the enzymatic sources of ORs. Under physiological conditions 80% of the metabolized molecular oxygen is transformed into H_2O_2 through a divalent reaction. However, 20% is transformed into superoxide anions through univalent reduction. Xanthine oxidase and aldehyde oxidase are important enzymes that produce ORs from the metabolism of O_2 during ischemic tissue injuries (Engerson et al. 1987; McCord and Roy, 1982; McCord, 1985)

1.1.5.4 Xanthine Oxidase and Ischemia

As specified by Prasad (2003b) the biochemical reactions that occur during ischemia induce the formation of ORs. In non-ischemic tissue, xanthine dehydrogenase is an enzyme that is responsible for catalyzing the reduction of NAD^+ to NADH without the production of any ORs. However, during myocardial ischemia there are increases of xanthine and xanthine oxidase, which could lead to the formation of oxygen radicals (Jennings and Reimer, 1982; Chambers et al. 1985). Specifically, during ischemia-

reperfusion xanthine dehydrogenase is transformed into xanthine oxidase by proteases and sulfhydryl group oxidation. As described by Prasad (2003b) ischemic induced adenosine triphosphate (ATP) catabolism to adenine nucleotides (adenosine diphosphate {ADP}, adenosine monophosphate {AMP}, inosine) result in hypoxanthine and xanthine formation (McCord, 1985; McCord et al. 1985). The combination of xanthine and xanthine oxidase in the presence of molecular oxygen produces the superoxide anion and hydrogen peroxide in the following reaction (Engerson et al. 1987; McCord and Roy, 1982; Fantone and Ward, 1982):

$$\text{Xanthine} + O_2 \xrightarrow[\text{oxidase}]{\text{xanthine}} O_2^- + H_2O_2 + \text{uric acid} \qquad (1.4)$$

Vanden (1980) outlined that the decrease in pH during ischemia stimulates phospholipase A_2 which would increase the production of arachidonic acid from membrane phospholipids and synthesis of prostaglandins and leukotrienes. Moreover, oxygen radicals are formed during the synthesis of prostaglandins and leukotrienes (Panganamala et al. 1976: Murota et al. 1990). It has been shown by Rao et al. (1983) that ischemia without reperfusion may produce moderate quantities of ORs from oxygen located within the lipid bilayer of cell membranes. However, the burst of OR generation is not observed until reperfusion is strengthened by direct and indirect evidence. The direct evidence has been demonstrated by electron spectroscopy of serum levels of ORs obtained prior to, and after reperfusion of myocardial tissue, which showed that ORs are generated to a limited extent during ischemia, but an increase in OR production is observed in the beginning period of reperfusion followed by a decrease in production for many hours afterwards (Zweier et al. 1987; Bolli et al. 1989). Similarly, the indirect

demonstrative support for the reperfusion oxidative burst may be evidenced by numerous studies, which show that inhibitors or scavengers of ORs and anti-neutrophil agents administered during reperfusion, attenuated serum levels of OR's (Simpson et al. 1988; Barroso-Aranda et al. 1988; Barroso-Aranda and Schmidt-Schonobein, 1989; Kapoor and Prasad, 1996). Furthermore, endogenous antioxidants such as SOD, catalase, glutathione peroxidase and glutathione are depleted, thus decreasing the inherent myocardial antioxidant defense mechanisms. Consequently, ischemia promotes the biochemical circumstances for tissue damage due to OR production (Kapoor and Prasad, 1994).

1.1.5.5 Arachidonic Acid

The production of arachidonic acid by phospholipases from cell membrane phospholipids and its subsequent metabolism diverges into two distinct pathways. The lipoxygenase pathway producing leukotrienes and the cyclooxygenase pathway forming prostaglandins both generate ORs. Specifically, during the transformation from prostaglandin G_2 (PGG_2) to prostaglandin H_2 (PGH_2), and from PGH_2 to thromboxane A_2 (TxA_2), OH^- and O_2^- are produced. Likewise, OH^- is produced from the lipoxygenase pathway of arachidonic acid metabolism (Kontos et al. 1980; Salvador et al. 1977).

1.1.5.6 Other Sources of Oxygen Radicals

According to Prasad (2003b) there are various other sources of OR production such as the auto-oxidation of small molecules (catecholamines, flavins, hydroquinones and thiols) and sites such as the endoplasmic reticulum, nuclear membrane, macrophages and peroxisomes (Freeman and Crapo, 1982; Muller and Sies, 1987).

1.1.6 Oxygen Radicals and Cellular Membrane Damage

Prasad (2003b) detailed that oxygen free radicals with their differing reactivities not only generate local oxidizing effects, but also produce damaging pathological repercussions at regions away from the original area of production. In addition, ORs have the ability to initiate and propagate free radical chain reactions that can damage many biological substrates (Rice-Evans and Baurdon, 1993; Southorn and Powis, 1988). Even though hydroxyl radicals have a half-life of nanoseconds it forms toxic products that can migrate to great distances to cause lipid peroxidation to vulnerable polyunsaturated fatty acids (PUFAs) in plasma membranes and organelles (Southorn and Powis, 1988). The chain reaction and propagation of lipid peroxidation by OH^- and probably singlet oxygen is due to their ability to attack double bonds of PUFAs in cell membranes as long as oxygen stores and unoxidized fatty acid chains are accessible. The resultant bond modification forms conjugated dienes, which react with more O_2 to produce peroxyl radicals, lipid radicals ($L^{\cdot}$), and lipid hydroperoxides (LOOH) all of which can continue chain propagation (Rice-Evans and Baurdon, 1993). However, the discontinuance of propagation may be caused by antioxidants such as alpha tocopherol, which scavenge peroxyl radicals in the presence of vitamin C (Rice-Evans and Baurdon, 1993). The metabolites formed from lipid peroxidation are alkanals (MDA), alkenals (4-hydroxynonenal) and alkanes. Intracellular and plasma membrane injury can be due to MDA's ability to induce cross-linking of structural proteins, enzymes, DNA, RNA and phospholipids. In addition, MDA changes membrane permeability, ion transport, alters LDL degradation, causes loss of PUFAs, is mutagenic, cytotoxic and carcinogenic (Rice-Evans and Baurdon, 1993; Freeman and Crapo, 1982). An increase in MDA levels implies an increased level of oxygen radicals, which may be caused by an

increased production of ORs and or decreased antioxidant reserve. An increased formation of ORs may be through sources such as xanthine-xanthine oxidase system, auto-oxidation of catecholamines and homocysteine, prostaglandin synthesis and PMNL stimulation (Prasad et al. 1996).

1.1.7 Homocysteine and Oxygen Radicals

The oxidative hypothesis of atherosclerosis requires oxidative damage as a prerequisite for the development of atherosclerosis (Henriksen et al. 1981). Homocysteine may promote atherosclerosis by damaging endothelial cells by auto-oxidation leading to the production of oxygen radicals (Welch et al. 1996). The free sulfhydryl group of homocysteine expresses strong redox properties allowing homocysteine to react with another homocysteine molecule or with ferric or cupric ions to form disulfide, hydrogen peroxide, oxygen radicals and homocysteine radicals (homocysteine thiolactate) (Olszewski and McCully, 1993; Welch et al. 1996). In addition, homocysteine may also generate oxygen radicals by forming a mixed disulfide with other sulfhydryl containing proteins through a similar oxidation mechanism. Vascular endothelial injury has also been demonstrated by observing homocysteine auto-oxidation to cytotoxic superoxide anion, hydrogen peroxide and hydroxyl radicals (Starkebaum and Harlan, 1986; Welch et al. 1996; Hultberg et al. 1995; Misra, 1974; Rowley and Halliwell, 1982). Impaired endothelial function could be due to homocysteine oxidation where the generated oxygen radicals induce lipid peroxidation in circulating lipoproteins and in the cell membranes (Heinecke et al. 1987; Tsai et al. 1994). As outlined by Prasad (1999) the oxidation of LDL is a critical step in the genesis of atherosclerotic lesion. The superoxide anion formed by homocysteine auto-oxidation could cause vascular injury by inducing the oxidation of LDL (Heinecke et al.

1987). As described by Prasad (1999) numerous studies have correlated thiol-containing amino acids like homocysteine in the presence of cupric or ferric ions with increased oxidation of LDL (Heinecke et al. 1984; Heinecke et al. 1987; Parthasarathy, 1987). Moreover, homocysteine-mediated oxidation of LDL may generate lipid products that could stimulate platelet activation and the liberation of growth factors may induce smooth muscle proliferation and vascular hypertrophy (Tsai et al. 1994).

1.1.7.1 Homocysteine and Vascular Dysfunction

Endothelial injury is a requirement for the response to injury hypothesis of atherosclerosis (Ross, 1993). Homocysteine causes endothelial damage at several levels. Numerous studies have implicated that the cytotoxic effects of homocysteine on endothelial cells grown in tissue culture may be responsible for endothelial dysfunction and the genesis of atherosclerosis (Blundell et al. 1996; and Jones et al. 1994). Lentz et al. (1996) demonstrated that monkeys that were fed a diet that effectuated moderate hyperhomocysteinemia experienced an increase in vascular dysfunction analogous to that observed in atherosclerotic monkeys. Similarly, Harker et al. (1976), upon studying baboons that were administered uninterrupted intravenous infusions of homocysteine, concluded that homocysteine was a causative agent for the development of patchy vascular endothelial injury and intimal smooth muscle cell lesions which correlated to the early atherosclerotic fatty streak lesion observed in humans.

1.1.7.2 Homocysteine and Atherosclerosis

According to Ross (1986) the response to injury hypothesis of atherosclerosis requires endothelial dysfunction/damage for the progression of atherosclerosis. Homocysteine-induced damage is primarily due to its toxicity to endothelial cells (Blundell et al. 1996; Jones et al. 1994; Wall et al. 1980; Harker et al. 1974; Starkebaum

and Harlan 1986; Weimann et al. 1980). The toxicity to endothelial cells created by homocysteine may be due to homocysteine's formation of oxygen radicals (Blundell et al. 1996; Jones et al. 1994; Wall et al. 1980; Harker et al. 1974; Starkebaum and Harlan 1986; Weimann et al. 1980). Jones et al. (1994), demonstrated that homocysteine generated oxygen free radicals by observing the toxic effects of homocysteine independently and in the presence of Cu^{2+}, and their correlation with increased lipid peroxidation, which was inhibited by catalase and attenuated by desferal. Oxygen radicals have been shown to cause endothelial injury (Weis et al. 1981; Sacks et al. 1978; Crapo, 1986; Kapoor and Prasad, 1994). The oxidative hypothesis of atherosclerosis is based upon oxidative stress induced endothelial injury (Henriksen et al. 1981).

The endothelial cell cytotoxicity induced by homocysteine may be related to its ability to produce ORs (Jones et al. 1994; Crapo, 1986). Endothelial injury by superoxide anions, hydroxyl radicals, hydrogen peroxide and homocysteine radicals is produced when the sulfhydryl group of homocysteine is reduced by copper or iron ions (transition metals) (Olszewski and McCully, 1993). Moreover, in cultured cell mediums, which have the presence of transition metals, reduced homocysteine is oxidized by thiols to generate toxic ORs (Wall et al. 1980). Active endothelial cells contain the enzymes necessary for homocysteine metabolism (Anderson et al. 1995). Consequently, auto-oxidation of homocysteine in plasma generates ORs, mixed disulfides, and homocysteine thiolactate (a by-product of homocysteine oxidation) (Anderson et al. 1995). Homocysteine thiolactate (also termed homocysteine thiolactone) has also been postulated to cause endothelial injury by mediating the oxidation of LDL (Heinecke et al. 1987). According to Prasad (1999), macrophage and smooth muscle foam cell

formation is induced by the incorporation of LDL with homocysteine thiolactate (Naruszewicz et al. 1994). In addition, oxidized LDL and oxygen radicals have been associated in the genesis of hypercholesterolemic atherosclerosis (Steinberg, 1991; Prasad and Kalra, 1993; Prasad et al. 1994; Prasad et al. 1997; Prasad et al. 1998). The production and accumulation of immobile lipid laden foam cells in the vascular wall is central to the genesis of ROS and the atherosclerotic fatty streak lesion (atheromatous plaques) (Schwartz et al. 1993). The chemotaxis of monocytes to the area of injury is under the influence of endothelial derived MCP-1. Homocysteine has been shown to increase the expression of MCP-1 in human aortic endothelium (McCully, 1994). Thus, an increase of the transmigration of monocytes to the area of injury may accelerate the development of the atherosclerotic fatty streak lesion. Finally, homocysteine thiolactate may cause damage to oxidative metabolism in a microenvironment, which would induce overproduction of ORs that cause endothelial injury (McCully, 1994). Particularly, in resting cells the prevention of mitochondrial free radical damage is mediated by the conversion of homocysteine thiolactone to thioretinaco ozonide. Thioretinaco ozonide functions as an electron acceptor in molecular oxygen metabolism and as the binding site for ATP synthesis by mitochondria. During cell division methionine is converted to homocysteine thiolactone, which in turn converts thioretinaco ozonide to thioco. Thioco increases OR generation, and decreases cellular defensive antioxidants glutathione and ascorbate. Thus, an impairment of homocysteine thiolactone metabolism results in a attenuation of the protective effect of thioretinaco ozonide and may lead to derangement of oxidative phosphorylation with keratinization, squamous metaplasia, and dysplasia of the endothelium and smooth muscle (Prasad, 1999; Fiddian-Green, 2002; McCully, 1993).

Homocysteine may also induce atherosclerosis by damaging endothelial cells through decreasing the plasma concentrations of antioxidants. According to Toboreck et al. (1995), methionine-induced atherosclerotic rabbits exhibited a decrease in the activity of antioxidant enzymes such as: superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSH-P_x). However, in red blood cells the concentration of SOD increased, catalase was unchanged, and GSH-P_x was attenuated. Additionally, the activity of aortic antioxidant enzymes increased with this treatment. Thus, the variations in antioxidant status were correlated with aortic atherosclerosis. The data suggests that methionine-induced atherosclerosis may be related to oxidative stress. Moreover, this treatment may also increase plasma and aortic malondialdehyde, which increases membrane permeability with a loss of membrane integrity (Toboreck et al. 1995). Hultberg et al. (1995) described the mechanism of endothelial cell damage by the metabolism of homocysteine. As described by Prasad (1999) hyperhomocysteinemia may cause an increase in intracellular reduced homocysteine that may enter the transulfuration pathway of homocysteine metabolism, producing cysteine, glutathione, and sulfate (Hultberg et al. 1995). The imbalance created by elevated levels of reduced homocysteine causes a ratio favoring decreased glutathione and NAD^+ (Blundell et al. 1996). This imbalance may induce a decrease in antioxidant reserve, which would increase the likelihood of lipid peroxidation and endothelial damage leading to the genesis of atherosclerosis (Steinberg, 1992; Prasad, 1994).

1.1.7.3 Homocysteine and CAD

Wilcken D and Wilcken B (1976) published the first report, correlating patients with coronary artery disease with abnormal homocysteine metabolism. The increase in studies in the 1990s relating homocysteine to coronary artery disease is significant

(Boushey et al. 1995; Mayer et al. 1996; Angeline et al. 2005; Clarke et al. 1991; Stampfer et al. 1992; Glueck et al. 1995). Stampfer et al. (1992) described an association between homocysteine levels and myocardial infarction. They observed, after correction for other conventional risk factors, that men with plasma homocysteine levels 12% above the upper limit of the normal range experienced a three-fold increase in the risk of a myocardial infarction as compared with those with lower levels. In another study conducted by Alfthan et al. (1994) it was observed that baseline homocysteine concentrations were correlated with the evolution of myocardial infarction and death during the follow-up period. In addition, Boushey et al. (1995) in a retrospective study of 4000 patients came to the conclusion that homocysteine is an independent risk factor for coronary artery disease by observing that 10% of the risk of coronary artery disease in the general population may be caused by homocysteine. They also determined that an increase of the plasma homocysteine concentration of 5μmol/L raises the risk of coronary artery disease to a similar level as that of an increase of 0.52 μmol/ L cholesterol concentration. However, a study conducted by Nygard et al. (1997), which consisted of 587 patients with coronary artery disease, concluded that total plasma homocysteine concentration had a weak correlation with the degree of coronary artery disease. On the other hand, they discovered that there was a firm relationship between homocysteine concentration and overall mortality, particularly when the homocysteine concentration was above 15 μmol/ L.

1.1.7.4 Homocysteine and Smooth Muscle

Two notable hallmarks of atherosclerosis are smooth muscle cell proliferation and migration and collagen deposition. The relationship of homocysteine to increases in

smooth muscle cell proliferation has been investigated. According to Majors et al. (1997) homocysteine enhances cultured arterial smooth muscle cell density and stimulates collagen production. Furthermore, homocysteine thiolactate, a by-product of homocysteine oxidation, has been implicated in fibrosis and proliferation of smooth muscle (McCully, 1993). In addition, the effect of homocysteine on the growth of both vascular smooth muscle and endothelial cells at physiological concentrations demonstrated that the incorporation of 0.1mM of homocysteine produced a 25% increase in DNA synthesis in rat aortic smooth muscle cells (RASMC). Similarly, homocysteine concentration of 1mM increased DNA synthesis by 4.5 times in RASMC and analogous increases in DNA synthesis were observed with homocysteine assimilation into human aortic smooth muscle cells. However, homocysteine attenuated DNA synthesis in human umbilical vein endothelial cells (HUVEC) (Tsai et al. 1994). According to Lubec et al. (1996) and Tsai et al. (1994) homocysteine stimulates the mRNA of cyclins (regulators of cell cycles) necessary for the return of inactive cells into the cell cycle. Specifically, cyclin D1 and cyclin A induced by growth factors or serum function as growth regulators during the early G_1 phase and S and G_2/M phase of the cell cycle respectively (Tsai, 1996). Therefore, the interactions of homocysteine and growth factors or serum induce the replication of vascular smooth muscle cells lining the blood vessels and facilitate the mechanism for homocysteine-induced atherosclerosis (Tsai et al. 1994; Tsai, 1996).

1.1.7.5 Homocysteine and Nitric Oxide

Homocysteine-induced endothelial injury and dysfunction may predispose the endothelium to decrease the synthesis and release of nitric oxide (Wall et al. 1980; Stamler et al. 1993). The functions of the endothelial-derived relaxing factor, nitric

oxide include: (1) regulation of the following; endothelial–leukocyte interactions, smooth muscle proliferation, vascular tone (via vasodilation); and (2) inhibition of platelet adhesion and aggregation (Luscher et al. 1996). Homocysteine chemically reacts with NO in the presence of water (nitrosation) to produce S-nitroso-homocysteine, which is similar to NO in that it inhibits platelet aggregation and causes vasodilation but does not produce H_2O_2. The nitrosation of homocysteine has been suggested to act as a protective mechanism to offset homocysteine's deleterious effects. Nevertheless, the time and concentration of tissue exposed to homocysteine likely determines its effect on nitric oxide production. For instance, in a dose dependent approach, the early consequence of exposure of bovine aortic endothelial cells to homocysteine was an increase in NO synthesis (Upchurch et al. 1997). However, 15 minutes of exposure of bovine aortic endothelial cells to homocysteine resulted in the production of NO and S-nitroso-homocysteine (Upchurch et al. 1997). Even further, chronic exposure and elevated concentrations of homocysteine attenuated NO formation (Stamler et al. 1993; Upchurch et al. 1996). Therefore, increasing levels of homocysteine may damage endothelium, indirectly induce atherosclerosis by decreasing NO bioavailability, and diminish the protective effects of S-nitroso-homocysteine (Stamler et al. 1993; Upchurch et al. 1996; Hultberg et al. 1995; Stuhlinger et al. 2001).

1.1.7.6 Homocysteine and Thrombosis

Homocysteine-induced thrombosis may be due to its procoagulant activity as well as its ability to attenuate the anticoagulation mechanism (Lee and Prasad, 2002). The endothelial synthesis of clotting factors helps to maintain the delicate balance between procoagulant and anticoagulant pathways that is crucial to vascular homeostasis (Thambyrajah and Townsend, 2000; Thrambyrajah et al. 2001). The endothelium

creates an antithrombogenic surface through the following mechanisms: (1) inhibition of platelet activation by ecto–ADPase, prostacyclin, and NO; (2) production of plasminogen activators (limit plug formation and fibrin deposition) and; (3) regulation of antithrombin III (heparin sulfate), thrombomodulin, and protein C activity (Rogers, 1988). The mechanisms of accelerated vascular atherothrombosis due to hyperhomocysteinemia may be multi-factorial. Platelet dysfunction, coagulation abnormalities, and endothelial impairment (through the release of ROS) are possibly the results of elevated plasma homocysteine concentrations (Lee and Prasad, 2002).

1.1.7.7 Homocysteine and Platelets

Homocysteine-induced endothelial injury and dysfunction exposes the subendothelial matrix, which stimulates platelet activation and thrombus formation (Harker et al. 1976). In addition, platelet adhesiveness may be increased in hyperhomocysteinemia due to a modification of platelet arachidonic acid metabolism (Shattil et al. 1975). Arachidonic acid metabolism through the cycloxygenase pathway produces the proaggregant vasoconstrictor thromboxane A_2 (TxA_2) (Di Minno et al. 1993). According to Durand et al. (1997) rats in which hyperhomocysteinemia were induced by folate deficiency developed an increase in methionine concentrations, TxA_2 synthesis, and macrophage factor activity. Since elevated plasma homocysteine concentrations may cause an increase in platelet TxA_2 synthesis with concurrent increased platelet adhesion, then this may strongly contribute to the prothrombotic effect observed in hyperhomocysteinemia. Antiplatelet therapy administered to baboons with hyperhomocysteinemia exhibited a decrease in intimal lesion expression implying that platelets are involved in smooth muscle proliferation (Harker et al. 1976). On the other

hand, Di Minno et al. (1993) reported that in in-vitro platelet function tests there were no significant differences between homocysteinuric patients and healthy controls.

1.1.7.8 Homocysteine and Procoagulant Factors

Factor V is an important cofactor in the common pathway of the coagulation cascade. Homocysteine induces a protease endothelial cell activator of factor V, and provides a pathway for the augmentation of coagulation in the absence of thrombin (Rogers and Kane, 1986). They reported that subsequent to administration of 0.5 $mmol.1^{-1}$ of homocysteine, human umbilical vein endothelial cells demonstrated an increase in Factor V activity. Moreover, after administration of homocysteine at levels of 0.3-0.6 $mmol.1^{-1}$, the activity and transcription of tissue factor is increased (Fryer et al. 1993). Homocysteine also activates factor XII of the intrinsic pathway of the coagulation cascade, and inhibits the activity of thrombomodulin. Thrombomodulin is an endothelial surface glycoprotein that binds with thrombin to activate protein C. Activated protein C causes the inhibition of factors Va, and VIIIa (Bertina et al. 1994). Homocysteine influences thrombomodulin by attenuating its surface expression on endothelial cells. It has been demonstrated by Lentz and Sadler (1991) that homocysteine precisely and irreversibly deactivates thrombomodulin and protein C. In addition, a study involving the in-vivo thoracic aorta of monkeys with diet-induced hyperhomocysteinemia reported a thrombomodulin dependent protein C activation decrease by 35% compared to controls (Lentz et al. 1996). Therefore, the ability of homocysteine to inhibit thrombomodulin and thus protein C activity may have the effect of endothelial thrombus formation (Lee and Prasad, 2002; Griffin et al. 1995).

Homocysteine is also engaged in the deactivation of fibrinolysis by the inhibition of transplasminogen activator (t-PA) binding to human endothelial cells.

Transplasminogen activator is responsible for the conversion of plasminogen to plasmin, which lyses fibrin (Hajjar, 1993). Finally, homocysteine blocks the expression of the anticoagulant heparin sulfate, which binds to thrombin and causes its deactivation (Port et al. 1996). Homocysteine-induced inhibition of heparin sulfate is accomplished by the production of hydrogen peroxide (Nishinaga et al. 1993). Thus, by inhibiting heparin sulfate homocysteine contributes to thrombus formation.

1.1.8 Percutaneous Coronary Intervention (PCI)

Percutaneous coronary intervention is a medical procedure in which a high-grade coronary stenosis (plaque) is dilated with a balloon tipped catheter system (percutaneous coronary angioplasty {PTCA}), and, or supported by metal scaffolds (stents) to diminish, and, or prevent the ischemic effects of coronary atherosclerosis (Serruys et al. 1994; Sigwart et al. 1987). Inflation of the balloon tipped catheter to a desired atmospheric pressure results in intra-luminal radial forces. These forces produce plaque compression. However, the primary change in lumen geometry is caused by endothelial denudation, fracture and separation of plaque from the underlying medial and adventitial layers (Fischman et al. 1994). Although balloon inflation provides an increased lumen for blood flow, the endothelial injury that results may induce two important adverse outcomes of PCI – acute vessel closure and restenosis. The angiographic restenosis rate for balloon PCI alone is approximately 30- 40 %; however the clinical restenosis rate is 20 to 30% (Bengtson et al. 1990; Ruygrok et al. 2001; Cannan et al. 1999). Acute vessel closure is usually a result of vessel dissection, and, or thrombus formation in about three to five percent of cases and it typically occurs within the first twenty-four hours of the procedure (O' Meara and Dehmer, 1997). The clinical events produced from acute

vessel closure are myocardial infarction, emergency coronary artery bypass surgery, and death (O' Meara and Dehmer, 1997).

1.1.8.1 PCI and Coronary Restenosis

Restenosis is an adverse outcome following balloon angioplasty and stenting for atherosclerosis in which there is a re-narrowing of the artery. The elevated incidence of restenosis following PCI progressively limits long term benefits of the procedure. Restenosis is most likely the result of physical damage to the endothelial and subintimal layers of the vessel from the PCI procedure itself. Consequently, the artery attempts to repair itself through the stimulation of smooth muscle cell migration from the media of the vessel wall into the subintimal region where they proliferate (Serruys et al. 1994; Sigwart et al. 1987). The inability of the smooth muscle cells to regulate their own growth effectively results in neointimal hyperplasia, and vascular remodeling that may eventually hinder adequate blood flow due to luminal size reduction ultimately inducing major cardiac events (MACE) i.e. myocardial ischemia, infarction, thrombosis, or death (Serruys et al. 1991; Serruys et al. 1994; Sigwart et al. 1987). The two major categories of restenosis for balloon angioplasty are angiographic restenosis, which is defined as a 50% reduction of luminal diameter at follow-up angiography, which is 25 to 50% of all cases, and clinical restenosis, which is defined as recurrent angina (chest discomfort), which occurs in 20 to 30% of patients (Hansrani, 2002). Clinical restenosis usually occurs within the first six to nine months following the procedure (Hansraniet al. 2002).

1.1.8.2 Coronary Stenting

Coronary stents are metallic mesh conduits which are implanted over the area of stenosis and deployed by balloon inflation to function as a structure to maintain the lumen of the vessel wall with the expectation of circumventing abrupt closure and

restenosis (Schatz, 1988). Coronary stents have been utilized in over 85% of the PCIs since the late 1990s (Lane, 1999). Intracoronary stents are indicated for: (1) the prevention of coronary restenosis; (2) the treatment of an acute myocardial infarction; (3) the treatment of saphenous vein graft stenosis; and (4) the prevention of acute vessel closure (Lane, 1999). Clinical in-stent restenosis remains a major problem with bare metal stents occurring in 30- 40% of patients following PCI (Erbel et al. 1996; Cultip et al. 2002).

There are two basic types of stents: bare metal (uncoated stents) and drug eluting stents (DES). Drug eluting stents are impregnated with a polymeric drug coating which has anti-inflammatory and anti-proliferative actions through continuous local release (Schwartz et al. 2004). The two major drug eluting stents, the Taxus (Boston Scientific), and the Cypher (Johnson and Johnson) are coated with paclitaxel and sirolimus respectively (Herrmann, 2003; Goy et al. 2005).

1.1.8.3 Drug Eluting Stents

Paclitaxel Drug Eluting Stents

Paclitaxel is a drug which decreases cell division and migration through inhibition of microtubule formation (Herrmann, 2003). Studies have been conducted on the release formulations of paclitaxel-eluting stents compared to bare metal stents in humans with low risk coronary lesions undergoing PCI. The TAXUS II trial used two different paclitaxel release formulations to show the safety and efficacy in a large group of patients. The stents were coated with a polymer designed to control paclitaxel release with a fundamental burst phase over the initial 48 hours following implantation, and then a low level release for ten days. The low-level release formulation was either slow release, where 90% of the total dose loaded (paclitaxel eluting stents were coated with a

dose of 1μg/mm^2) remained sequestered within the polymer formulation or moderate release where 75% remained sequestered within the polymer formulation. Coronary angiography after six months revealed that the biphasic polymer managed delivery of low levels of paclitaxel produced substantial decreases in neointimal proliferation as compared to controls (Colombo et al. 2003). However, other studies measuring the effects of paclitaxel delivery using different dosing and delivery systems revealed that the ability to deliver large quantities of paclitaxel through acrylate polymer coatings were correlated with high incidences of acute, subacute, and late in-stent thrombosis (SCORE Trial; Sousa et al. 2003). In addition, paclitaxel release from stents with nonpolymer-based delivery systems did not translate into patient clinical benefit (Park et al. 2003). Moreover, the patients in the TAXUS II trial had low risk lesions and it is not clear whether a slow release formula would be applicable to high risk patients or patients which present with complex lesions. Furthermore, the attenuation of restenosis has been demonstrated with drug eluting stents in large arteries with short lesions. However, there is not a substantial body of evidence to prove a decrease in the frequency of clinical restenosis in patients with disease bifurcation, chronic total occlusions, saphenous vein graft disease, or multivessel disease (Moses et al. 2003).

Sirolimus Eluting Stents

Sirolimus (rapamycin), a naturally occurring product that was discovered in a soil sample from Easter Island, is isolated from the species Streptomyces hygroscopicus. It is a lipophilic macrolide that has potent immunosuppressive effects on mammals, and has an impact on the care of patients with coronary artery disease because of its ability to inhibit vascular smooth muscle proliferation, and thus in-stent restenosis (Marx et al. 1995; Poon et al. 1996). The process by which sirolimus retards cell growth is related to

cell cycle arrest at the transition from the G_1 to S phases (Poon et al. 1996; and Luo et al. 1996). A study in pig models revealed that systemic sirolimus administration could inhibit restenosis in a model of angioplasty (Gallo et al. 1998). In addition, the Sirolimus-Eluting Bx Velocity Balloon- Expandable Stent utilized in a randomized study (RAVEL) exhibited attenuation in the frequency of in-stent restenosis (Fajadet et al. 2005; Windecker et al. 2005). Moreover, the (SIRIUS) trial evaluated the frequency of in-stent restenosis among patients with complex coronary artery disease that had sirolimus coated stents implanted, and found that the restenosis rate decreased to 18 % in the sirolimus group compared to a 51% restenosis rate in the bare metal stent group (Lemos et al. 2003; Schampaert et al. 2004; Schofer et al. 2003). However, it is not clear as to the duration of inhibitory effects induced by sirolimus or, whether or not there are subgroups of patients who are more resistant than others to therapeutic consequences of sirolimus (Schwartz et al. 2004; Moses et al. 2003).

1.1.9 Homocysteine and Restenosis

Restenosis following successful PCI continues to be an adverse outcome of this procedure. The numerous data that correlate homocysteine with the risk of the development, and the severity of CAD has stimulated interest in its possible function in restenosis (Prasad, 1999; Schnyder et al. 2002b). Homocysteine-induced smooth muscle cell proliferation, loss of endothelial function, coagulation abnormalities and plasma lipoprotein oxidation (from OR generation) associated with atherogenesis may contribute to restenosis as well. Thus the investigation of the governed release of intracellular homocysteine release into the plasma may provide a feasible biochemical marker for restenosis (Schnyder et al. 2001; Woo et al. 1997).

In a prospective study conducted by Schnyder et al. (2001), including 205 patients who underwent successful PCI of at least one coronary stenosis (≥50%), plasma homocysteine samples were drawn between 8 and 12 hours following the procedure. Of the 200 patients, 89% underwent angiography at 6 months for direct visualization of angiographic restenosis. The results demonstrated that patients with plasma homocysteine concentrations below 9.0 μmol/ L had a restenosis rate 49% lower than those patients who had homocysteine levels above 9.0 μmol/ L. This research offered one of the initial prospective indications that plasma homocysteine concentrations predict restenosis following PCI in small vessels treated with balloon angioplasty alone. Likewise, Morita et al. (2000) conducted a case controlled study of 112 males who underwent successful PCI in which they measured plasma homocysteine concentration as a possible risk factor for coronary restenosis at follow-up angiography (3-6 months). They discovered that plasma homocysteine concentrations were significantly higher in patients who developed restenosis. In addition, Schnyder et al. (2001) further investigated homocysteine in a prospective, randomized, double blind interventional trial to study the effect of lowering plasma homocysteine levels on restenosis following PCI. They administered a combination of folic acid (1mg), vitamin B_{12} (400 μg), and pyridoxine (10mg) or placebo to 205 patients for six months after successful PCI. The primary and secondary end points were restenosis within six-months (determined by angiography), and the presence of MACE. Their results demonstrated that treatment with an anti-homocysteine formula significantly attenuated homocysteine concentrations and the rate of restenosis following PCI. In contrast, Bennoitt et al. (1999) performed a prospective trial of 222 patients who underwent PCI and were followed up clinically for six months. Their findings suggested that there was no significant difference in

homocysteine concentrations between patients with multiple restenosis, and those without restenosis. Similarly, Miner et al. (2000) conducted a small prospective study using follow-up angiographic data of less than six months were also unable to find a significant correlation between homocysteine and the development of restenosis after PCI. Wong et al. (2004) performed a prospective single center observational study on 134 patients that electively underwent their first PCI without stent implantation and determined that there was no association between baseline plasma homocysteine levels, and the rate of restenosis after six months. Finally, it is apparent that there still exists much controversy on the role of homocysteine and restenosis in patients undergoing PCI.

1.1.10 Oxygen Radicals and Restenosis

The endothelial injury induced by the PCI itself can activate platelets and neutrophils that may produce ROS. Reactive oxygen species formed at the site of damage may stimulate chain reactions of endothelial dysfunction and LDL oxidation leading to restenosis. OXLDLs activate macrophages, which in turn liberate numerous growth factors that induce smooth muscle proliferation. Smooth muscle cell proliferation, migration, and collagen accumulation are major determinants of neointimal growth. Mediators of smooth muscle cell proliferation are numerous, including ROS (Teirstein and King, 2003; Marumo et al. 1997; Sundaresen et al. 1995). Smooth muscle cell proliferation and stenosis after vascular injury coincide with elevations in ROS (Szocs et al. 2002; Shi et al. 2001). Reactive oxygen species have been implicated in growth signaling pathways and in smooth muscle cell proliferation (Colavitti et al. 2002; Abe and Berk, 1999; Bhunia et al. 1997). In addition, Jacobson et

al. (2003) have implicated that superoxide anions are involved in neointimal development after vessel injury based on the administration of the neointimal inhibitor gpg1ds-tat (the chimeric peptide inhibitor of NAD(P)H oxidase assembly). Moreover, ROS have been correlated with the development of hypercholesterolemic atherosclerosis (Prasad and Kalra, 1993; Prasad, 1999; Prasad, Kalra and Lee 1994; Prasad, 2005; Prasad, 1997b). Tardif et al. (1997) investigated the oxidative stress produced by PCI with subsequent restenosis. In a double blind randomized trial they studied whether drugs with antioxidant qualities attenuate the occurrence and severity of restenosis following angioplasty. Their work suggested that antioxidants may have inhibited endothelial dysfunction, LDL oxidation, and attenuated neointimal formation all of which may be involved in the mechanism of restenosis.

The sources of oxygen radicals during PCI could be numerous including xanthine-xanthine oxidase, mitochondria, polymorphonuclear leukocytes, arachidonic acid metabolites, homocysteine and C-reactive protein (Prasad et al. 1996; McCord and Roy, 1982; Prasad, 1999; Misra, 1974; Kanellakis et al. 2004). Thus, it is plausible that ORs may be involved in restenosis following PCI.

1.1.11 Exercise Tolerance Testing (ETT)

An exercise tolerance test (ETT) evaluates myocardial function during physical or pharmacological exertion to examine cardiac reaction to increased demand for oxygen and thus offers crucial information that a normal electrocardiogram (ECG) will not provide (Bruce, 1956; Bengtson et al. 1990). Exercise tolerance testing has proven to be one of the most practical non-invasive methods of discovering latent coronary artery disease (Bruce, 1956; Bengtson et al. 1990). Generally, patients with coronary lesions

of up to 50% obstruction in one vessel may be asymptomatic even though there may be a reduction in blood flow (Astrand, 1965). Moreover, Dagenais et al. (1982) demonstrated that a decrease in blood flow must be at least 75% to elicit changes in the ECG. Work by Bengtson et al. (1990) suggested the usefulness of this diagnostic tool by illustrating a positive correlation between stress tests and coronary angiograms in 200 patients where they were able to detect lesions as low as 25%.

The hallmark of exercise stress testing is based on the discovery that exercise in patients with coronary artery disease induces ST-segment depression (Bruce, 1956; Bruce, 1967; Bengtson et al. 1990). Angina, ST segment and T wave changes in the ECG are evidence of ischemia due to a lesion that produces a decreased blood flow in a coronary artery (Dagenais et al. 1982; Goldschlager et al. 1976). In patients who experience atypical chest pain, a maximum stress test may often establish the presence or absence of coronary artery disease. In terms of assessing prognosis and severity of coronary artery disease, several studies have suggested that exercise tolerance testing is accurate in determining these parameters (Dagenais et al. 1982; Goldschlager et al. 1976). In addition, the exercise tolerance test is instrumental in identifying recurrent ischemia in patients post- PCI that may be at risk for restenosis (Bengtson et al. 1990).

1.2 HYPOTHESIS

The long-term benefit of PCI is hampered by the possibility of restenosis of the treated segment, which occurs in 20-50% of patients (Nobuyoshi et al. 1988; Hirshfeld et al. 1991; Waller, 1989). Intracoronary stent implantation along with balloon angioplasty is highly effective in the treatment of acute vessel closure (Sigwart et al. 1987; Roubin et al. 1992; de Jaegere et al. 1993a; Fischman et al. 1994). However, bare metal stents are thrombogenic, and the benefit achieved at the expense of high-risk

vascular complications (Schatz et al. 1991; Serruys et al. 1994; de Jaegere et al. 1993b; Serruys et al. 1991) must be considered. The use of stents has significantly improved the outcome of PCI. Despite major advances in PCI, in-stent restenosis remained a major limitation until recently. Drug eluting stents have emerged as a very promising approach in preventing restenosis and improving clinical outcome (Fischman et al. 1994; McKeage et al. 2003; Grube et al. 2003; Gershlick et al. 2004; Waugh and Wagstaff, 2004). However, the possibility of restenosis exists. In-stent restenosis is now the most common form of restenosis. The incidence is between 20-40% with bare metal stents and less than 10% with drug eluting stents (Macaya et al. 1996; Stone et al. 2004). Up to half of restenosis (20-50%) is quiescent, but it may result in myocardial ischemia as with symptomatic patients. While stent based therapeutics in clinical trails have shown markedly reduced rates of restenosis, the scope of clinically proven anti-restenotic agents is still extremely limited, requiring additional strategies (Morice et al. 2002; Teirstein and King, 2003).

Restenosis can be caused by numerous factors including inflammation, platelet mediated thrombus formation, proliferation of smooth muscle cells and vascular remodeling (Waller, 1989). Smooth muscle cell proliferation, migration and collagen accumulation are major determinants of neointimal growth. Mediators of smooth muscle cell proliferation are abundant, including ROS (Teirstein and King, 2003; Marumo et al. 1997; Sundaresen et al. 1995). Smooth muscle cell proliferation and stenosis after vascular injury coincide with elevations in ROS (Szocs et al. 2002; Shi et al. 2001). Reactive oxygen species have been demonstrated in growth signaling pathways and in smooth muscle cell proliferation (Colavitti et al. 2002; Abe and Berk, 1999; Bhunia et al. 1997). Reactive oxygen species have been suggested in the

development of hypercholesterolemic atherosclerosis (Prasad and Kalra, 1993; Prasad, 1999; Prasad et al. 1994; Prasad, 2005; Prasad, 1997b; Prasad, 1998). Antioxidants attenuate/ prevent the development of atherosclerosis in cholesterol-fed rabbits and in humans (Prasad and Kalra, 1993; Prasad, 1999; Prasad et al. 1994; Prasad, 2005; Prasad, 1997a). The sources of oxygen radical production during PCI could be numerous including xanthine-xanthine oxidase, mitochondria, polymorphonuclear leukocytes, arachidonic acid metabolites, homocysteine, and C reactive protein (Prasad, 1999; Prasad, 1997a; McCord, 1985; Prasad, 2003a; Prasad, 1999; Misra, 1974; Prasad, 2004). It is possible that ORs may be involved in restenosis following PCI. In this context Kojoglanian et al. (2003) have demonstrated that elevated concentrations of plasma homocysteine are well correlated with increased risk of the development of coronary restenosis. However, Koch et al. (2003) reported no association between plasma levels of homocysteine and restenosis.

Based on the above background knowledge it is therefore hypothesized that plasma levels of homocysteine and serum levels of MDA may be predictors of restenosis following percutaneous coronary intervention.

1.3 RATIONALE FOR THE STUDY

The relationship between pre-PCI plasma homocysteine concentrations and coronary restenosis following a successful PCI remains controversial. It has been shown that homocysteine can cause the endothelial damage to induce atherogenesis, and possibly restenosis. It has also been demonstrated that the auto-oxidation of homocysteine produces ORs, and these reactive oxygen species may induce atherogenesis and perhaps restenosis. The controversy of whether or not homocysteine can predict the development of restenosis may be better understood if the oxidative

stress produced by homocysteine and by other sources are investigated. Perhaps the varying sources that produce ORs increase oxidative stress to the extent that they are primarily involved with the formation of coronary restenosis. Total oxidative stress can be indirectly quantitated by measuring serum malondialdehyde (MDA), a lipid peroxidation product related to lipid membrane damage. It is possible that pre-procedural serum MDA and plasma homocysteine levels are elevated in patients who develop restenosis. It is also plausible that high levels of homocysteine and low levels of MDA in post-PCI patients may show no restenosis. This study will elucidate the understanding of the relationship between plasma homocysteine, serum MDA, and restenosis in patients following PCI.

1.4 OBJECTIVES

The objectives of this study are to determine:

(1) Whether pre-procedural levels of plasma homocysteine are elevated in patients who develop restenosis following PCI.

(2) Whether pre-procedural levels of oxidative stress (MDA) are elevated in patients who develop post-PCI restenosis.

(3) Which of the two (MDA or Homocysteine) is a better predictor of restenosis.

(4) Whether plasma levels of homocysteine and, or, serum MDA levels are pre-procedural predictors of restenosis following PCI.

To achieve the above objectives specific parameters were measured in the blood serum and plasma. Oxidative stress was assessed by measuring lipid peroxidation products or malondialdehyde (MDA) in serum. Homocysteine was measured from plasma samples using fluorescence polarization immunoassay technology.

2.0 PATIENTS AND METHODS

2.1 PATIENT SELECTION

Fifty-one patients with coronary artery disease signed a consent form to participate in the study. The University of Saskatchewan Biomedical Research Ethics Board approved the study protocol (EC # 2003-800). The Royal University Hospital Ethics Committee also approved the study. Clinical characteristics and demographics of the patients were obtained through direct interviews with the patients prior to the procedure or from patients' files with permission. The demographics and clinical characteristics are summarized in Tables 1-3. All patients in the study met the following criteria: (1) patients' with discrete denovo localized lesions in single or multiple vessels; (2) patients that were non-diabetic; (3) patients who experienced stable angina; (4) patient's that were non-acute MI or non-acute coronary syndrome; (5) patients that were ambulatory and capable of performing an exercise tolerance test (ETT); and (6) patients that were willing to return for follow-up. The patients were divided into two groups: twenty-two who developed post-PCI restenosis (group I) and twenty-nine who did not develop post-PCI restenosis (group II).

All of the patients that underwent PCI were scheduled to return for a follow-up ETT at two weeks and six months post-PCI or at anytime within six months if they developed recurrent angina.

2.1.1. Exercise Tolerance Test Timetable

The exercise tolerance test timetable for study patients was as follows: (1) two weeks post-PCI; (2) 6 months post-PCI; and (3) at any point within the six month follow-up period where the patient experienced recurrent angina.

2.1.2 Criteria for Restenosis

The criteria for clinical coronary restenosis were as follows: (1) angina; (2) failed ETT; and (3) angina and angiographic evidence.

2.2 METHODS

The most common approach for the measurement of free radical activity is to measure the end products of lipid peroxidation (MDA), by means of a thiobarbituric acid reaction (TBA Test) as described by Prasad (2001).

2.2.1 Serum Preparation for MDA Assay

Serum preparation: Seven milliliters of arterial blood was collected and transferred into labeled serum vacutainer tubes (EM Science, Merck KgaA, Germany). Blood samples were then kept refrigerated at 4 °C and left to clot at until centrifugation within six hours. The blood was centrifuged at 3000 RPM for 15 minutes and serum was pipetted into labeled Eppendorf tubes and stored at -80°C.

2.2.2 Materials for MDA Assay

The MDA standard solution consisted of (~ 97% in solution) 1, 1, 3, 3,-tetraethoxypropane (Sigma Aldrich Canada, LTD). A 100 µM stock solution of MDA was prepared by adding 6 µL of MDA standard to 250 ml of double distilled (DD) water in a volumetric flask.

The sodium lauryl sulphate solution (SDS) (8.1% w/v) was prepared by dissolving 8.1 g SDS in 100 ml of DD water.

Thiobarbituric acid 0.67% w/v (TBA) (Sigma Aldrich Canada) was prepared by dissolving 3.35 g of TBA dissolved in 50 ml of 2.0 N NaOH. The pH was adjusted to 5.9 with the addition of concentrated HCL, and the volume increased to 500 ml with the addition of DD water.

Glacial acetic acid (20%) was prepared in a fume hood by adding 200 ml of glacial acetic acid to 800 ml of DD water.

The pyridine/butanol solution was also prepared in a fume hood by adding 50 ml of pyridine to 450 ml butanol in a graduated cylinder.

2.2.3 Method for MDA Assay

A 10μM working MDA standard solution was composed before assay by adding 100μL of MDA stock solution to 900 μL of distilled water. Ten-milliliter glass tubes were used to perform the assays in duplicate. In order to zero the spectrophotometer, blank tubes were prepared; using 800 μL of distilled water each. Next, standard tubes were prepared by adding 25 μL of the working MDA solution to 775 μL of distilled water to bring the total volume to 800 ml. Consecutively, 25 μL of each serum sample was added to the other tubes each containing 775 μL of distilled water to bring the total volume to 800 ml. All tubes had the addition of the following in sequence, using auto repeat pipettes: 200 μL of SDS; 1.5 ml of TBA; and 1.5 ml of glacial acetic acid solutions, succeeded by mixing with a vortexer. The test tubes were then placed in a 95°C glycol bath for one hour after which they were allowed to cool for ten minutes. Then, 1 ml of DD water was added to each, followed by the addition of 5 ml of pyridine: butanol solution in order to extract the thiobarbituric acid reactive substances. Subsequently, the tubes were capped and vortexed for 60 seconds followed by centrifugation at 3000 RPM for 15 minutes. Lastly, the supernant was individually

pipetted and placed in a fluorescence spectrophotometer and read at an excitation of 515 nm and an emission of 553 nm.

2.2.3.1 Calculation of MDA

The concentration of MDA in serum can be calculated as follows:

$$\frac{0.25\ \text{nmol}}{F} \times \frac{f}{\text{Vol. of Sample}} \times \text{Dilution factor} \qquad (4.0)$$

$$\text{Where: } F = \text{Standard} - \text{Blank} \qquad (4.1)$$

$$f = \text{Sample} - \text{Blank} \qquad (4.2)$$

* Serum MDA concentration is reported in nmol / ml (µmol/L)

2.2.4 Plasma Preparation for Homocysteine Assay

Arterial blood was drawn and transferred into tripotassium EDTA vacutainer tubes (EM Sciences, Merck KgaA, Germany). Samples were refrigerated at 4°C for up to six hours prior to centrifugation at 3000 RPM for 10 minutes to separate the plasma from the cellular constituents of the blood. Plasma was then pipetted into labeled Eppendorf Tubes and stored at -80°C until assayed. Samples were mixed thoroughly after thawing to ensure consistency.

2.2.5 Materials for Homocysteine Assay

The homocysteine reagent pack (Abbot Laboratories, USA) was refrigerated at 4°C until utilized for assay. The kit was composed of sample cartridges, cuvettes, and four bottles of the following solutions: (1) 4.5 ml pretreatment solution (containing dithiothreitol {DTT} and adenosine in citric acid); (2) 10.5 ml S-adenosyl-L-

homocysteine Hydrolase solution; (3) 10.5 ml Anti-S-adenosyl-L-homocysteine Antibody (mouse monoclonal); and (4) 10.5 ml S-adenosyl-L-cysteine Fluorescein Tracer.

The calibrators for the homocysteine assay were stored at 2-8°C and consisted of five different types: calibrator A, 3.5 ml phosphate buffer solution, and calibrators B-F consisting of 2.5 ml of varying concentrations of S-adenosyl-L-homocysteine (SAH). The concentrations of S-adenosyl-L-homocysteine in bottles A-F were 0.0, 2.5, 5.0, 10.0, 20.0, and 50.0 µmol/L respectively. The calibrators functioned to check the accuracy of the instrument by measurement of its variation from the standard. The instrument was calibrated with each new homocysteine reagent pack.

The three controls (Low, Medium and High) have a volume of 2.5 ml, each consisting of IMx homocysteine. The low, medium and high controls had concentrations of 7 µmol/L with a range of 5.25 - 8.75 µmol/L; 12.5 µmol/L with a range of 10.0 - 15.0 µmol/L; and 25.0 µmol/L with a range of 20.0 - 30.0 µmol/L respectively. Essentially, they are used as standards against which the sample results may be evaluated. All three controls were incorporated into each run of plasma samples.

2.2.6 Method for Homocysteine Assay

An Abbot IMx instrument utilizing fluorescence polarization (FPIA) technology was used to measure total homocysteine in the plasma samples. A 50 µL aliquot of plasma is the minimum volume required to perform the assay. The biological properties of the procedure involve the reduction of homocysteine, mixed disulphide, and protein bound forms of homocysteine in the sample to form free homocysteine under the influence of the enzyme dithiothreitol. Next, the total free homocysteine is transformed to S-adenosyl-L-homocysteine (SAH) by the enzyme SAH Hydrolase and residual

adenosine. Successively, the unified chemical form of homocysteine (SAH) and the labeled Fluorescein Tracer (both of which are antigens) contend for sites on the monoclonal antibody molecule. The polarization from the fluorescein-antigen conjugate is determined by its rate of rotation during the lifetime of the excited state in solution. Thus, the binding of the antigen to the antibody (Anti-S-adenosyl-L-homocysteine Antibody) changes the polarization. Finally, the intensity of the polarization is measured by the FPIA optical assembly and related to the known antigen concentration (Shipchandler et al. 1995).

2.3 STATISTICAL ANALYSIS

Statistical analysis was performed utilizing the Statistical Program for the Social Sciences version 13.0 (SPSS). The results were expressed as mean ± standard error mean (SEM). In addition, an unpaired "T" test was used to compare data between groups. Finally, a value of $p < 0.05$ was considered significant.

3.0 RESULTS

3.1 DEMOGRAPHIC CHARACTERISTICS OF PATIENTS

Demographic and clinical features of patients who developed restenosis and those who did not following PCI are shown in Table I. Seventy-seven percent of the patients who underwent PCI and developed restenosis had a positive ETT at 2 weeks post-PCI. However, 23% of the patients who underwent PCI and developed restenosis had a negative ETT at 2 weeks post-PCI. Fourteen percent of the patients who did not developed post PCI restenosis had a positive 2 week ETT. Whereas, 86% of the patients who did not develop post PCI restenosis had a negative ETT at 2 weeks. All of the patients that developed post-PCI restenosis had a positive ETT at 6-months. None of the patients that developed post-PCI restenosis had a negative ETT at 6-months. Seven percent of the patients who did not develop post-PCI restenosis had a positive ETT at 6-months. However 93% of the patients who did not develop post-PCI restenosis had a negative ETT at 6-months. The number of diseased vessels and number of coronary lesions were similar in the two groups and are summarized in Table 2. The results of the lipid profile were divided into those patients who developed restenosis following PCI (Group I) and those who did not (Group II). Eighty-six percent of the patients in Group I and 55% of the patients Group II were hypercholesterolemic (above 6.2 mmol/L for patients above 65 years of age) respectively. In addition, 14% of the patients from Group I and 45% of the patients from Group II had total cholesterol levels within the normal range (4.2-6.2 mmol/L). Fifty-five percent of the patients that developed post-PCI restenosis had a history of hypertension (>150/90 mmHg) while 45% did not. Moreover, 52% of the patients that did not develop post-PCI restenosis had a history of hypertension while 48% did not. In patients that developed post-PCI restenosis 32%

were smokers while 50% were ex-smokers. In patients who did not develop post-PCI restenosis 17% were smokers and 34% were ex-smokers. Age and sex were similar in both groups, the patients being mainly males. The age of patients who developed post-PCI restenosis (n=22) ranged from 43 to 76 (mean 60.5 ± 2.0) years. The age of patients who did not develop post-PCI restenosis (n=29) also ranged from 43 to 76 (mean 59.9 ± 2.0) years. There was no significant difference in age between the two groups (p=0.85). There were 51 patients enrolled in the study. There were 45 males and 6 females. Forty-four percent of the males developed post-PCI restenosis while 56% of the males did not. Thirty-three percent of the females developed post-PCI restenosis while 67% did not. From the demographic data it appears that a significant number of patients who developed restenosis were hypercholesterolemic. In addition, there were more nonsmokers in the group that did not develop post-PCI restenosis.

TABLE 1: Demographic and clinical characteristics for patients with and without post-PCI coronary restenosis.

NO.	Characteristic	Restenosis	Non-restenosis
1	Hypercholesterolemia	86%	55%
2	Normal Cholesterol Range	14%	45%
3	History of Hypertension	55%	52%
4	Normotensive	45%	48%
5	Smoker	32%	17%
6	Ex-smoker	50%	34%
7	2 week positive ETT	77%	14%
8	6 month positive ETT	100%	7%
9	2 week negative ETT	23%	86%
10	6 month negative ETT	0%	93%
11	Age in years (mean ± SE)	60.5 ± 2.0	59.5 ± 2.0
12	# of Males	20	25
13	# of Females	2	4

Table 2: Diseased Vessel and Number of Lesions in Patients with and without post-PCI Restenosis

Patients with Restenosis			Patients without Restenosis		
Patient Number	**Diseased Vessel**	**Number of Lesions**	**Patient Number**	**Diseased Vessel**	**Number of Lesions**
1	LAD	Single	1	CX	Single
2	CX	Double	2	RCA	Triple
3	RCA	Single	3	LAD/DIAG	Double
4	RCA/CX	Double	4	LAD	Double
5	LAD	Triple	5	RAMUS/RCA	Double
6	LAD	Triple	6	CX	Triple
7	RCA	Single	7	RCA	Single
8	LAD/DIAG	Triple	8	LAD	Single
9	RCA	Single	9	LAD	Triple
10	RCA	Single	10	CX	Single
11	RCA	Triple	11	RCA	Double
12	RCA/CX	Double	12	LAD	Double
13	LAD/CX	Triple	13	DIAG	Single
14	LAD	Single	14	RCA	Double
15	CX	Single	15	RCA	Double
16	CX	Double	16	LAD	Double
17	RCA	Single	17	LAD	Double
18	RCA	Triple	18	LAD	Double
19	LAD	Single	19	RCA	Single
20	LAD	Single	20	RCA	Single
21	OM	Single	21	LAD/DIAG	Double
22	DIAG	Double	22	LAD	Single
			23	CX	Single
			24	LAD	Single
			25	OM	Double
			26	LAD	Double
			27	RCA	Double
			28	Ramus/RCA	Single
			29	LAD/CX	Double

LAD, left anterior descending; CX, circumflex; RCA, right coronary artery; Diag, diagonal; OM, obtuse marginal; Ramus, Ramus Intermedius

3.2 SERUM MALONDIALDEHYDE

The changes in serum malondialdehyde for the five observed time intervals are summarized in Figure 1. The pre-procedural serum MDA levels in patients with (Group I) and without (Group II) restenosis were 0.124 ± 0.016 and 0.147 ± 0.016 nmol/mL respectively. There was no significant difference (p= 0.60) between these two groups.

At the 0 time interval the values of serum MDA of those patients who developed restenosis and those patients who did not were 0.082 ± 0.009 and 0.135 ± 0.014 nmol/mL respectively. There was a significant difference (p=0.001) in the values of serum MDA between the two groups. In addition, the values of serum MDA decreased significantly (p=0.03) at the 0 time interval from the pre-procedural levels in the restenosis group. In the non-restenosis group there was no significant difference (p=0.73) between the preprocedural levels and the values from the 0 time interval.

At the 8 hr time interval the values of serum MDA of those patients who developed post-PCI restenosis and those who did not were 0.165 ± 0.42 and 0.158 ± 0.02 nmol/mL respectively. There was no significant difference (p= 0.88) between the two groups. There was also no significant difference between the 8 hr values of serum MDA as compared to the pre-procedural values of those patients who developed (p=0.36) or those patients who did not develop (p=0.67) post-PCI restenosis.

At the 24 hr time interval the values of serum MDA for those patients who developed post-PCI restenosis and those who did not were 0.12 ± 0.001 and 0.140 ± 0.017 nmol/mL respectively. There was a significant difference (p=0.033) between the two groups. In addition, there was no significant difference between the pre-procedural

values as compared to the 24 hr values in those patients who developed (p=0.17) restenosis and those who did not (p=0.78).

At the 6-month time interval the values of serum MDA for those patients who developed restenosis and those who did not were 0.224 ± 0.016 and 0.198 ± 0.017 nmol/mL respectively. There was no significant difference (p=0.28) between the two groups. There was also no significant difference (p=0.053) between the pre-procedural values and the 6-month post-PCI values in those patients who did not develop restenosis. However, there was a significant difference (p=0.001) between the pre-procedural values as compared to the 6-month post-PCI values in those patients who developed restenosis.

The percentage of pre-procedural percutaneous coronary interventional serum MDA values for the five observed time intervals are summarized in Figures 2 and 3. There was a significant increase of 109% (p=0.001) in the 6-month post-PCI values as compared to the pre-procedural levels in the restenosis groups. However, there was not a significant increase (41%, p=0.09) in the 6-month post-PCI values as compared to the pre-procedural levels in the non-restenosis group.

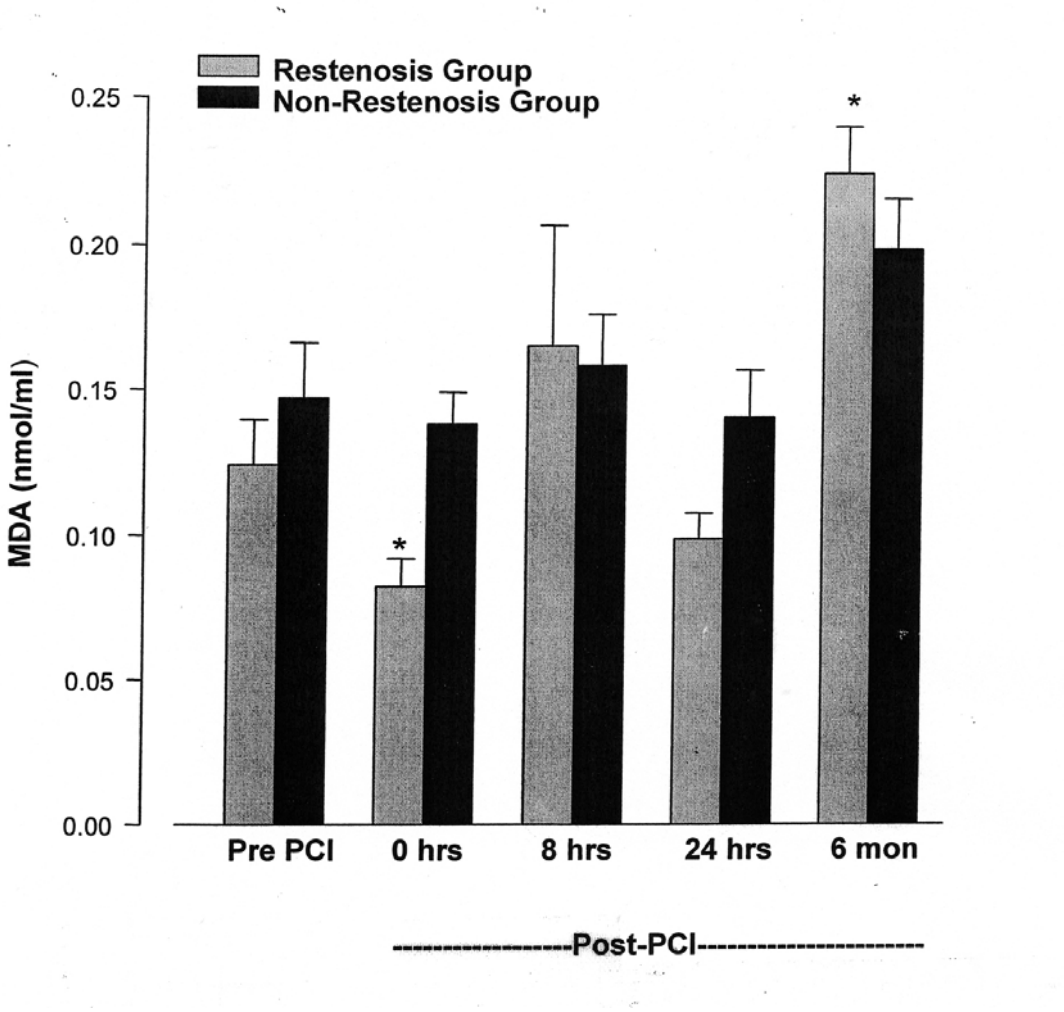

Figure 1 The levels of malondialdehyde (MDA) before percutaneous coronary intervention (pre-PCI), and at 0 hrs, 8 hrs, 24 hrs and 6-months post-PCI in patients with and without restenosis. Results are expressed as mean ± SE. *$P<0.05$, pre-PCI vs. 0 hrs or 6 months in the restenosis group.

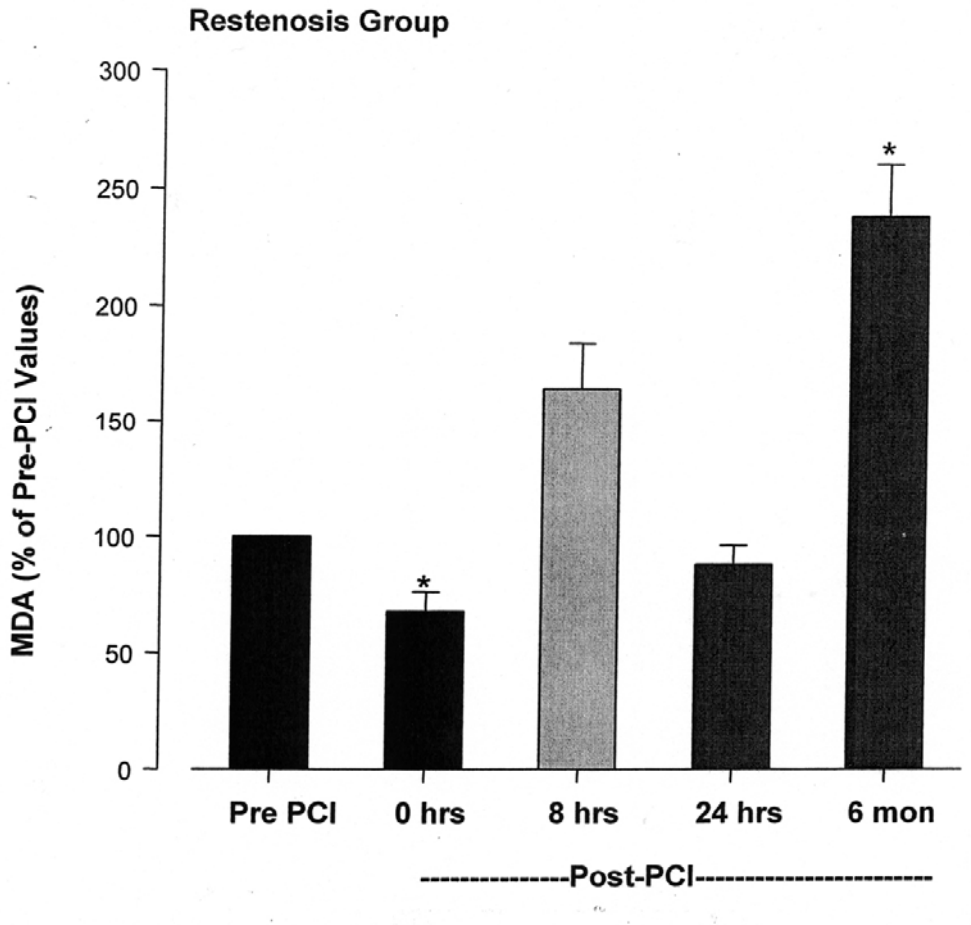

Figure 2 The percentage of pre-procedural percutaneous coronary interventional (pre-PCI) malondialdehyde (MDA) values at 0 hrs, 8 hrs, 24 hrs and 6-months post-PCI in patients with restenosis. Results are expressed as mean ± SE. *P<0.05, pre-PCI vs. 0 hrs or 6 months.

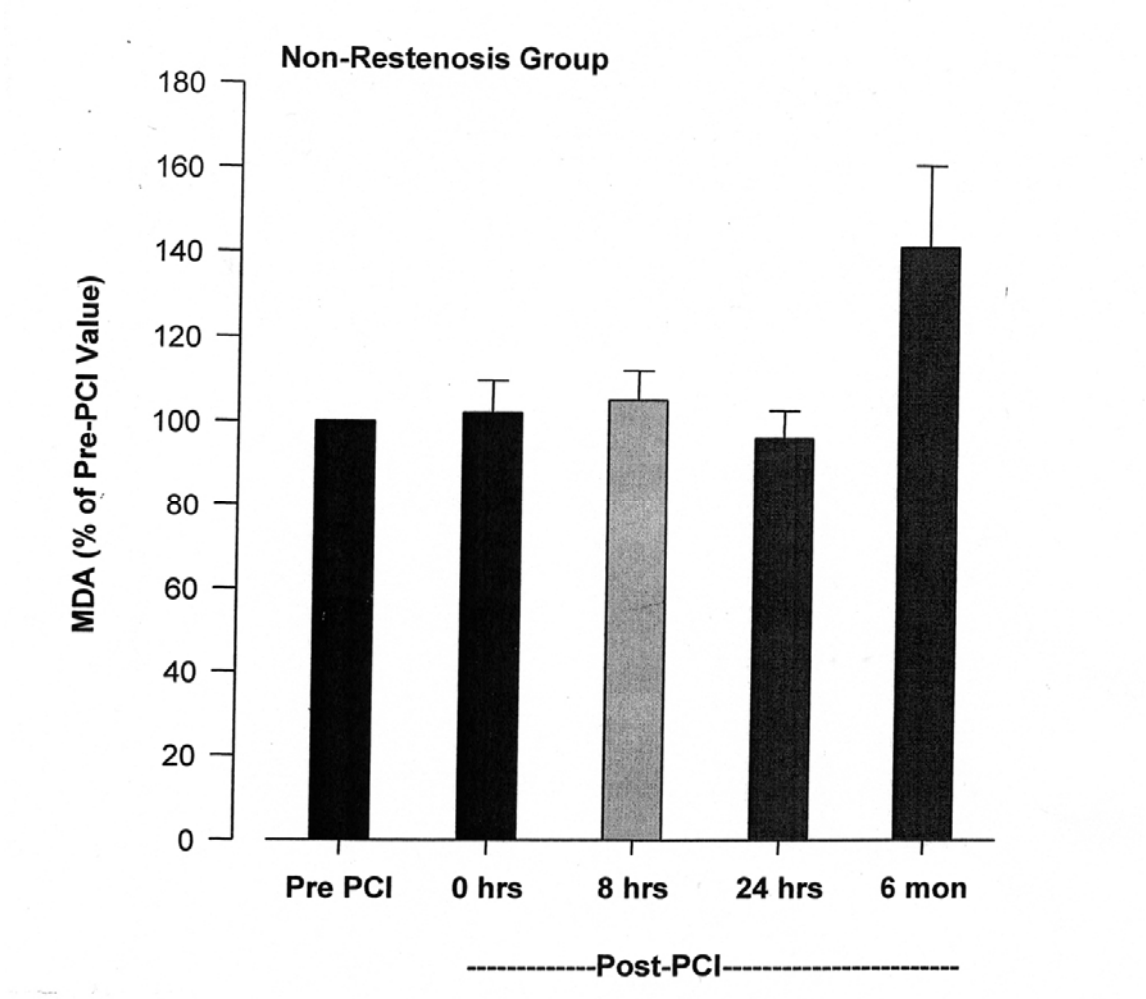

Figure 3 The percentage of pre-procedural percutaneous coronary interventional (pre-PCI) malondialdehyde (MDA) values at 0 hrs, 8 hrs, 24 hrs and 6-months of post-PCI in patients without restenosis. Results are expressed as mean ± SE.

3.3 PLASMA HOMOCYSTEINE

The changes in the plasma homocysteine levels for the four observed time intervals are summarized in Figure 4. The pre-procedural plasma homocysteine levels in patients with and without post-PCI restenosis were 10.37 ± 0.46 and 10.73 ± 0.49 μmol/L respectively. There was no significant difference (p=0.60) between the two groups.

At the 8 hr time interval the values of plasma homocysteine of those patients who developed restenosis and those who did not were 10.40 ± 0.43 and 10.16 ± 0.47 μmol/L respectively. There was no significant difference in the values (p=0.71) between the two groups. There was also no significant difference between the pre-procedural values of plasma homocysteine as compared to the 8 hr values of the restenosis (p=0.95) or the non-restenosis group (p=0.40).

At the 24 hr time interval the values of plasma homocysteine of those patients who developed restenosis and those who did not were 9.01 ± 0.42 and 8.92 ± 0.43 μmol/L respectively. There was no significant difference (p= 0.88) between the two groups. However, there was a significant decrease in the values of plasma homocysteine in both the restenosis (p=0.04) and non-restenosis (p= 0.01) groups at 24 hrs as compared to the pre-procedural levels.

At the 6-month time interval the values of plasma homocysteine of those patients who developed post-PCI restenosis and those who did not were 12.45 ± 0.43 and 11.40 ± 0.80 μmol/L respectively. There was no significant difference (p=0.96) in the values between the two groups. The pre-procedural levels of plasma homocysteine were not significantly different (p=0.82) as compared to the 6-month post-PCI values of homocysteine in the non-restenosis group. However, the pre-procedural values of

plasma homocysteine were significantly different (p=0.002) as compared to the 6-month post-PCI values of plasma homocysteine in those patients who developed restenosis.

The percentage of pre-procedural percutaneous coronary interventional plasma homocysteine values for the four observed time intervals are summarized in Figures 5 and 6. In patients who developed restenosis, there was a significant (p=0.001) increase in the values of plasma homocysteine from the pre-procedural levels of 24% at the 6-month time interval. In patients who did not develop restenosis there was an increase of 16% in the values of plasma homocysteine at the 6-month interval as compared to the pre-procedural levels, however this increase was not significant (p=0.80).

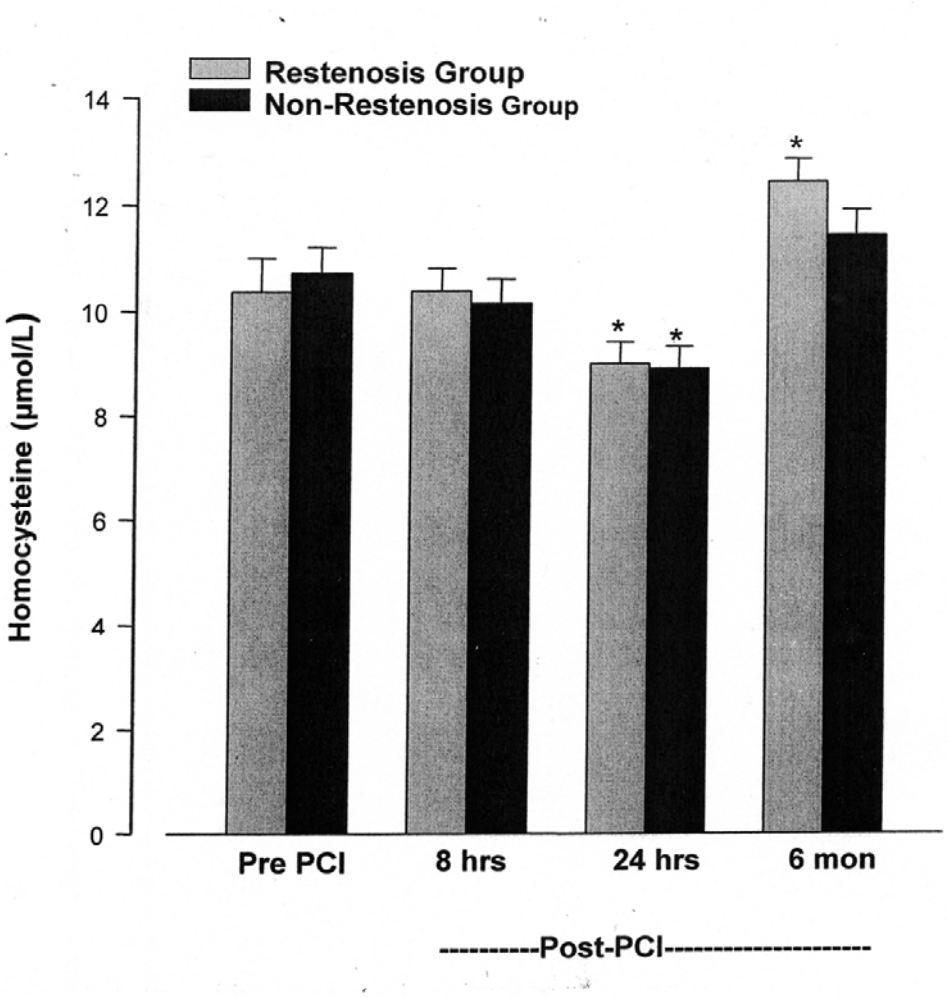

Figure 4 The levels of plasma homocysteine before percutaneous coronary intervention (pre-PCI), and at 8 hrs, 24 hrs and 6-months post-PCI in patients with and without restenosis. Results are expressed as mean ± SE. *P<0.05, pre-PCI vs. 24 hrs or 6 months in the restenosis group. *P<0.05, pre-PCI vs. 24 hrs in the non-restenosis group.

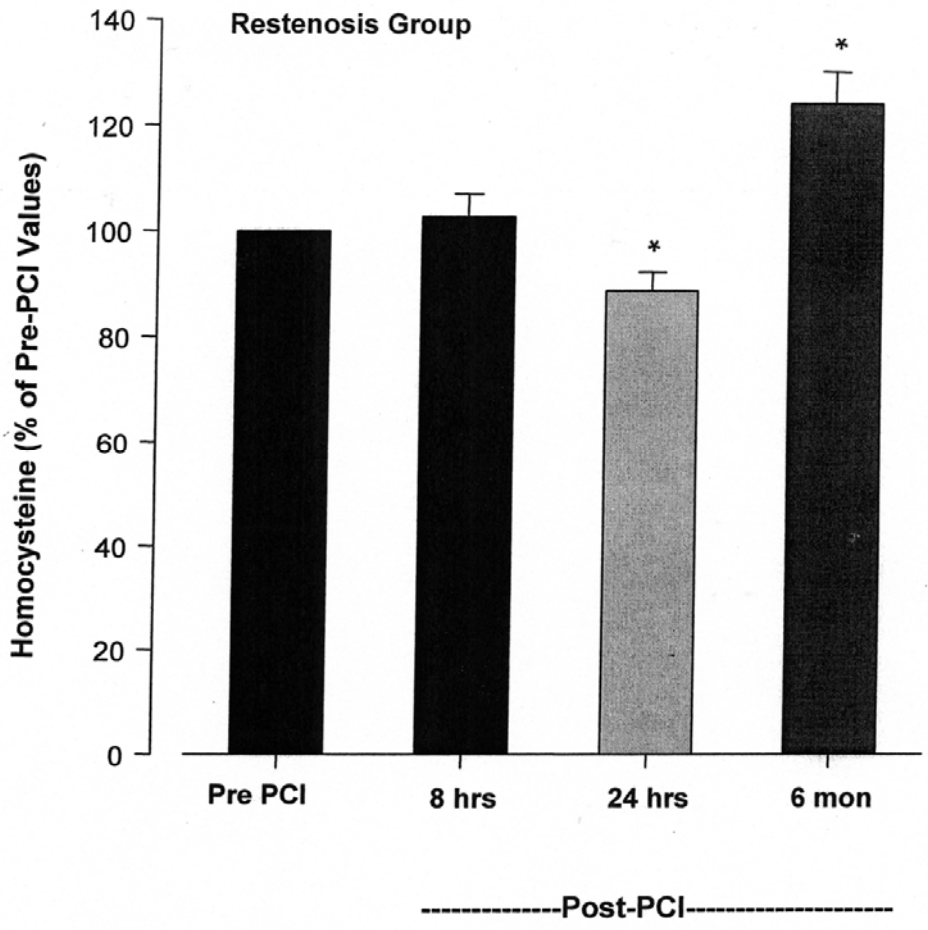

Figure 5 The percentage of pre-procedural percutaneous coronary interventional (pre-PCI) homocysteine values at 8 hrs, 24 hrs and 6-months post-PCI in patients with restenosis. Results are expressed as mean ± SE. *P<0.05, pre-PCI vs. 24 hrs or 6 months.

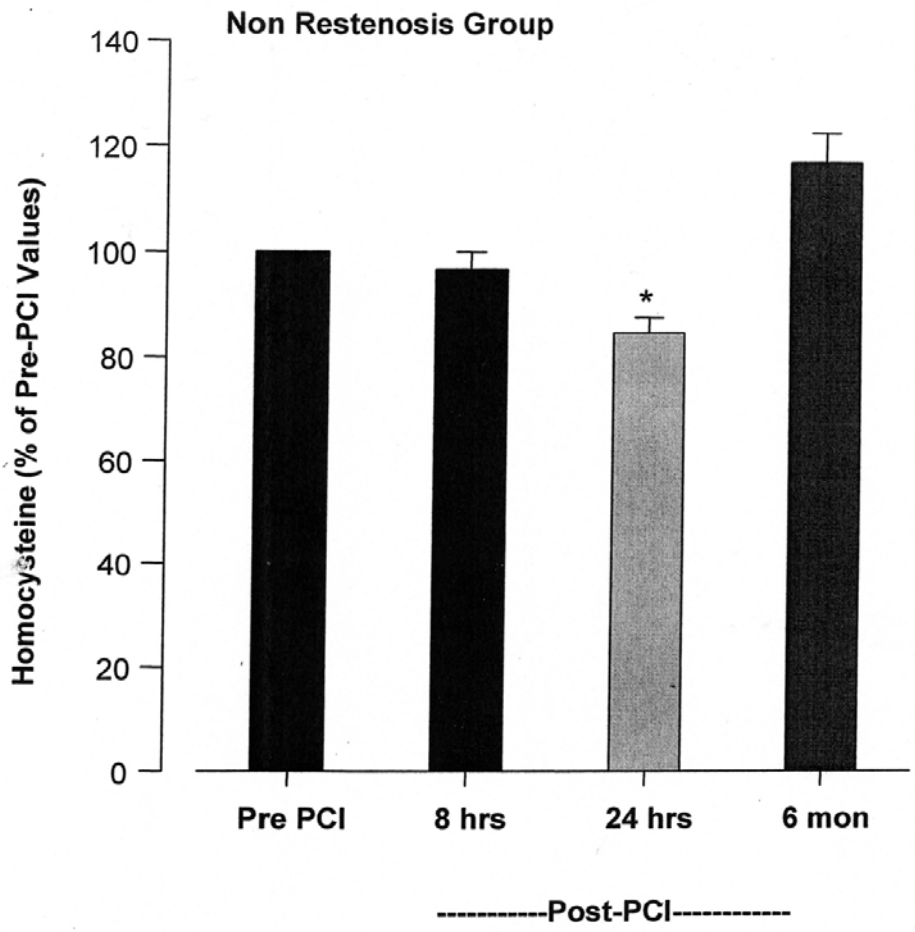

Figure 6 The percentage of pre-procedural percutaneous coronary interventional (pre-PCI) homocysteine values at 8 hrs, 24 hrs and 6-months post-PCI in patients without restenosis. Results are expressed as mean ± SE. *$P<0.05$, pre-PCI vs. 24 hrs.

3.4 CHOLESTEROL

A lipid profile (total cholesterol, triglycerides, HDL-C and LDL-C) was determined on all patients entering the study. The results of the lipid profile were divided into those patients who developed restenosis following PCI (Group I) and those who did not (Group II).

3.4.1 Serum Total Cholesterol

The values of total cholesterol are summarized in Figure 7. The patients in Group I had a serum total cholesterol range from 2.85 to 8.24 (mean 5.05 ± 0.31) mmol/L while those patients in Group II had a serum total cholesterol range from 2.25 to 5.24 (mean 3.88 ± 0.12) mmol/L. The values were significantly ($p=0.001$) higher in Group I as compared to Group II.

3.4.2 Serum Triglycerides

The values of serum triglyceride are summarized in Figure 8. The serum triglyceride levels of those patients in Group I ranged from 1.29 to 4.18 (mean 2.40 ± 0.18) mmol/L while those patients in Group II had a serum triglyceride range from 0.61 to 3.91 (mean 1.89 ± 0.17) mmol/L. The values of the two groups were not significantly different from each other ($p=0.058$).

3.4.3 Serum HDL-C

The values of serum HDL-C are summarized in Figure 9. The serum HDL-C levels of those patients in Group I ranged from 0.30 to 1.48 (mean 0.86 ± 0.07) mmol/L while those patients in Group II ranged from 0.65 to 1.86 (mean 1.18 ± 0.06) mmol/L. The values were significantly ($p=0.001$) lower in Group I as compared to Group II.

3.4.4 Serum LDL-C

The values of serum LDL-C are summarized in Figure 10. The serum LDL-C levels of those patients in group I ranged from 1.3 to 5.47 (mean 2.90 ± 0.23) mmol/L while the levels of those patients in group II ranged from 0.43 to 4.91 (mean 2.17 ± 0.20) mmol/L. The values were significantly ($p=0.02$) higher in Group I as compared to Group II.

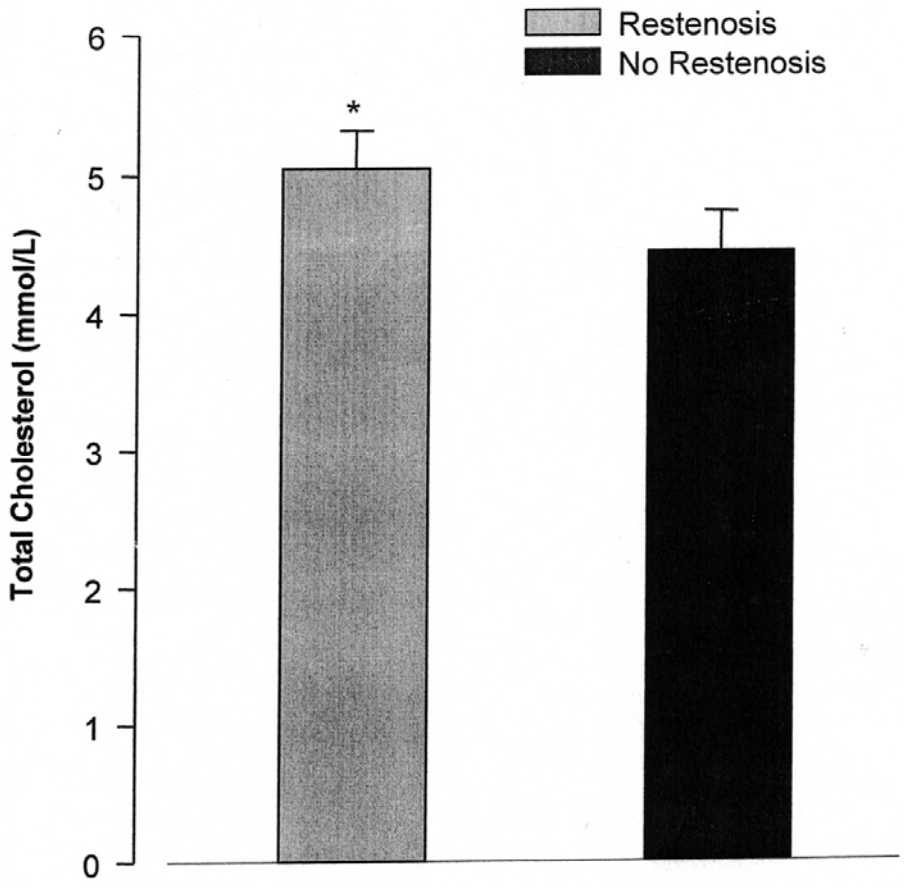

Figure 7 The pre-procedural percutaneous coronary interventional (pre-PCI) levels of serum total cholesterol in patients with and without post-PCI restenosis. Results are expressed as mean ± SE. *P<0.05, restenosis vs. non-restenosis.

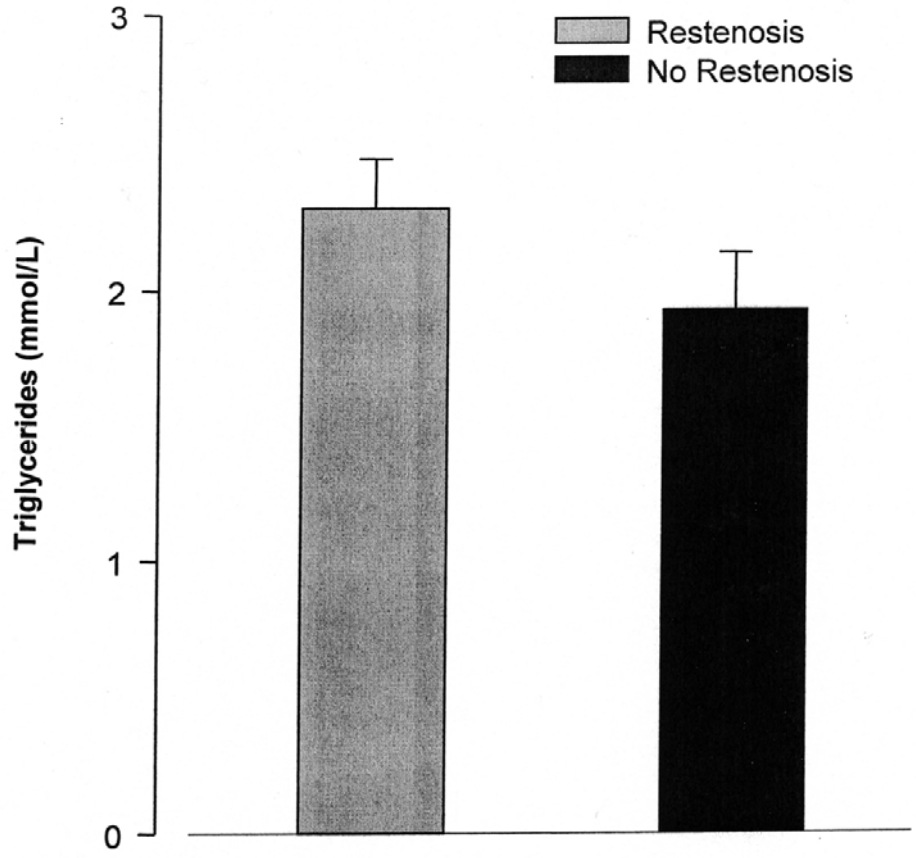

Figure 8 The pre-procedural percutaneous coronary interventional (pre-PCI) levels serum triglyceride in patients with and without post-PCI restenosis. Results are expressed as mean ± SE.

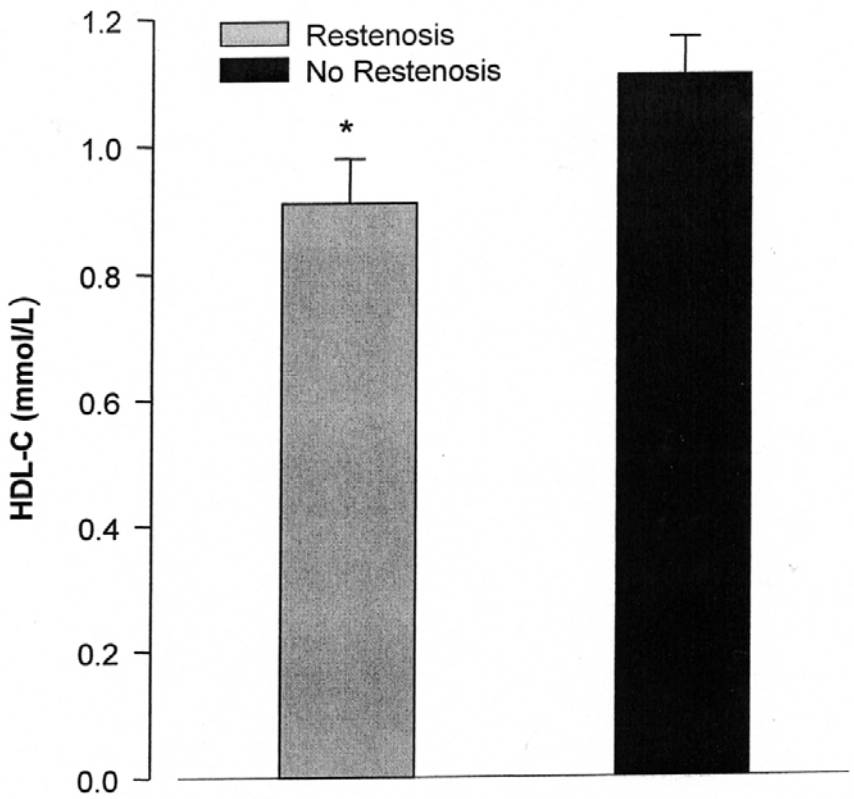

Figure 9 The pre-procedural percutaneous coronary interventional (pre-PCI) levels of serum HDL-C in patients with and without post-PCI restenosis. Results are expressed as mean ± SE. *P<0.05, restenosis vs. non-restenosis.

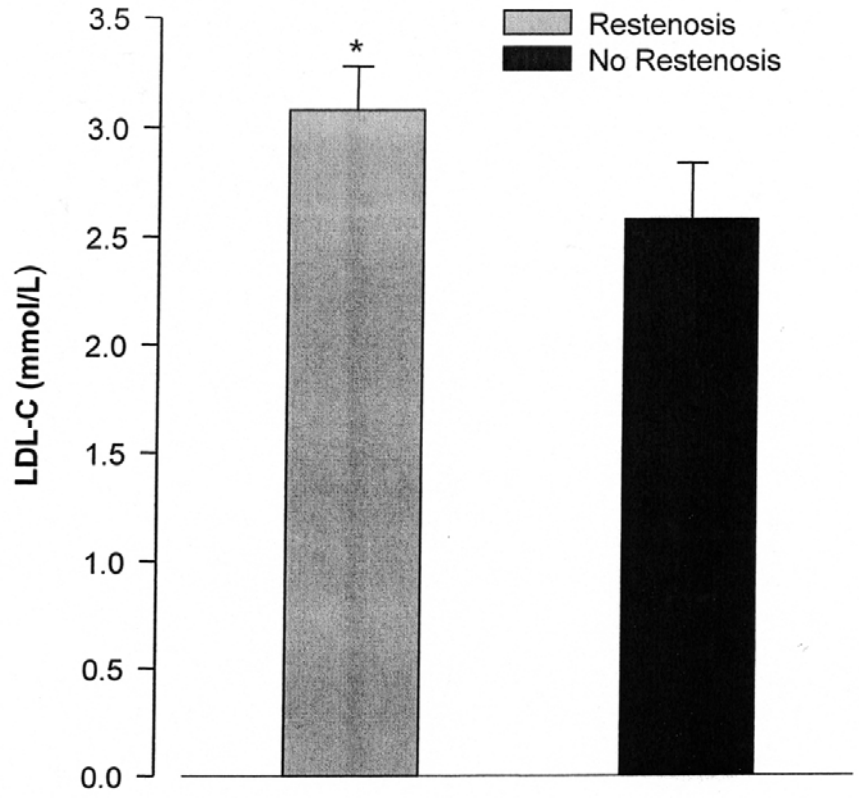

Figure 10 The pre-procedural percutaneous coronary interventional (pre-PCI) levels of serum LDL-C in patients with and without post-PCI restenosis. Results are expressed as mean ± SE. *P<0.05, restenosis vs. non-restenosis.

3.6 HOMOCYSTEINE, SMOKING AND RESTENOSIS

The values of plasma homocysteine in smokers and non-smokers are summarized in Figures 11 and 12. The plasma homocysteine levels were analyzed for patients who developed restenosis and were divided into smokers and non-smokers. The pre-procedural plasma homocysteine concentrations for those patients who developed post-PCI restenosis and smoked and those who developed post-PCI restenosis and were non-smokers were 9.60 ± 0.51 and 10.72 ± 1.3μmol/L respectively. The values were not significantly different (p=0.46) between the groups.

At the 8 hr time interval the levels of plasma homocysteine of smokers and non-smokers who developed post-PCI restenosis were 10.34 ± 0.60 and 11.21 ± 1.6 μmol/L respectively. The values were not significantly different (p=0.63) between the groups. There was also no significant difference between the pre-procedural values of plasma homocysteine as compared to the 8 hr values of smokers (p=0.37) or non-smokers (p=0.82) who developed restenosis.

At the 24 hr time interval the levels of plasma homocysteine of smokers and non-smokers who developed post-PCI restenosis were 8.91 ± 0.35 and 8.32 ± 1.20 μmol/L respectively. The values were not significantly different (p=0.65) between the groups. There was also no significant difference between the pre-procedural values of plasma homocysteine as compared to the 24 hr values of smokers (p=0.29) or non-smokers (p=0.21) who developed restenosis.

At the 6-month time interval the values of plasma homocysteine for smoking and non-smoking patients who developed post-PCI restenosis were 11.45 ± 0.56 and 13.89 ± 1.8 μmol/L respectively. The values were not significantly different (p=0.26)

between the groups. There was also no significant difference (p=0.21) between the pre-procedural levels of plasma homocysteine as compared to the 6-month values in non-smokers who developed restenosis. However, there was a significant difference (p=0.03) between the pre-procedural levels of plasma homocysteine as compared to the 6-month concentrations in smokers who developed restenosis.

3.7 MDA, SMOKING AND RESTENOSIS

The values of serum MDA for cigarette smokers and non-smokers are summarized in Figures 13 and 14. The serum MDA levels were analyzed for smokers and non-smokers who developed post-PCI restenosis. The pre-procedural serum MDA concentrations for smokers and non-smokers who developed post-PCI restenosis were 0.127 ± 0.031 and 0.120 ± 0.04 nmol/mL respectively. The values were not significantly different (p=0.83) between the groups.

At 0 time interval the levels of serum MDA for smoking and non-smoking patients who developed post-PCI restenosis were 0.08 ± 0.02 and 0.11 ± 0.04 nmol/mL respectively. The values were not significantly different (p=0.47) between the groups. There was also no significant difference between the pre-procedural values of serum MDA as compared to the 0 time interval values in the smoking (p=0.18) or non-smoking (p=0.86) groups.

At the 8 hr time interval the levels of serum MDA for smoking and non-smoking patients who developed post-PCI restenosis were 0.11 ± 0.03 and 0.252 ± 0.22 nmol/mL respectively. The values were not significantly different (p=0.35) between the groups. There was also no significant difference between the pre-procedural values of serum MDA as compared to the 8 hr time interval values in the smoking (p=0.71) or non-smoking (p=0.36) groups.

At the 24 hr time interval the levels of serum MDA for smoking and non-smoking patients who develop post-PCI restenosis were 0.10 ± 0.02 and 0.12 ± 0.04 nmol/mL respectively. The values were not significantly different (p=0.92) between the groups. There was also no significant difference between the pre-procedural values of serum MDA as compared to the 24hr time interval values in the smoking (p=0.39) or the non-smoking (p=0.10) groups.

At the 6-month time interval the levels of serum MDA for smoking and non-smoking patients who develop post-PCI restenosis were 0.19 ± 0.03 and 0.24 ± 0.04 nmol/mL respectively. The values were not significantly different (p=0.37) between the groups. There was also no significant difference between the pre-procedural values of serum MDA as compared to the 6-month time interval values in the smoking (p=0.14) or the non-smoking (p=0.07) groups.

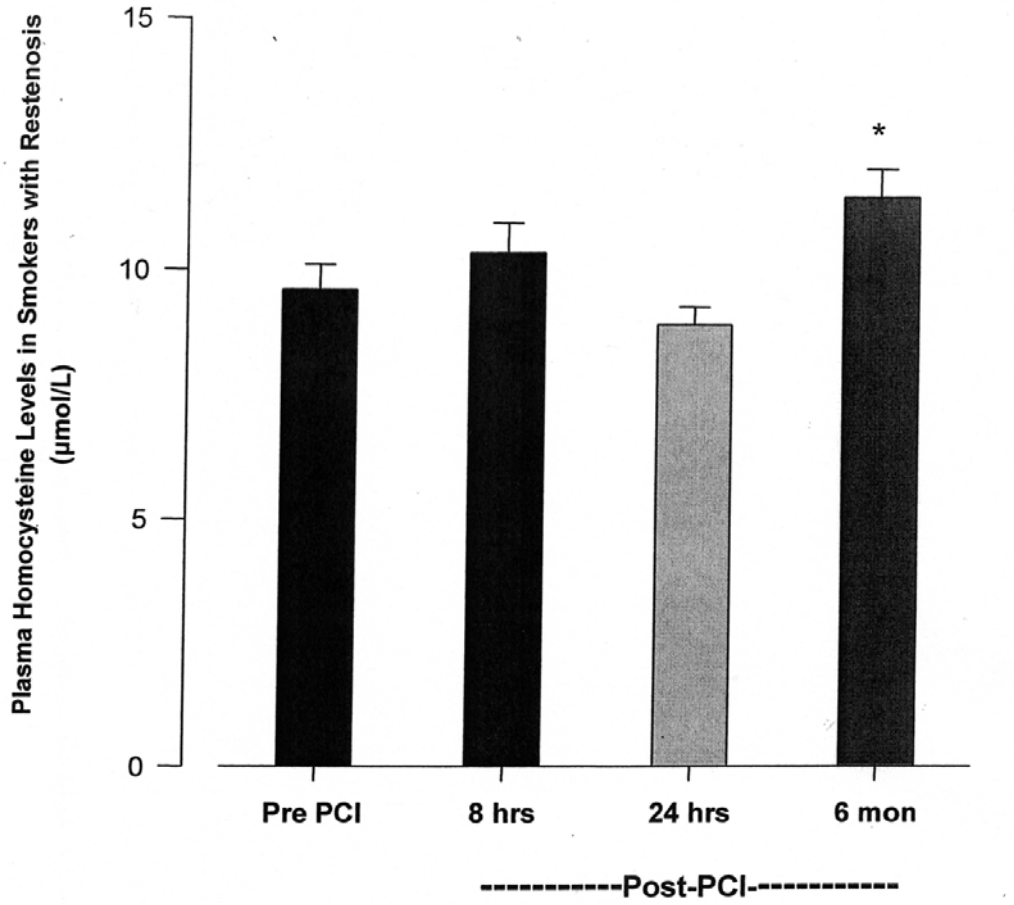

Figure 11 The levels of plasma homocysteine before percutaneous coronary intervention (pre-PCI), and at 8 hrs, 24 hrs and 6-months post-PCI in smokers who developed restenosis. Results are expressed as mean ± SE. *P<0.05, pre-PCI vs. 6 months in the restenosis group.

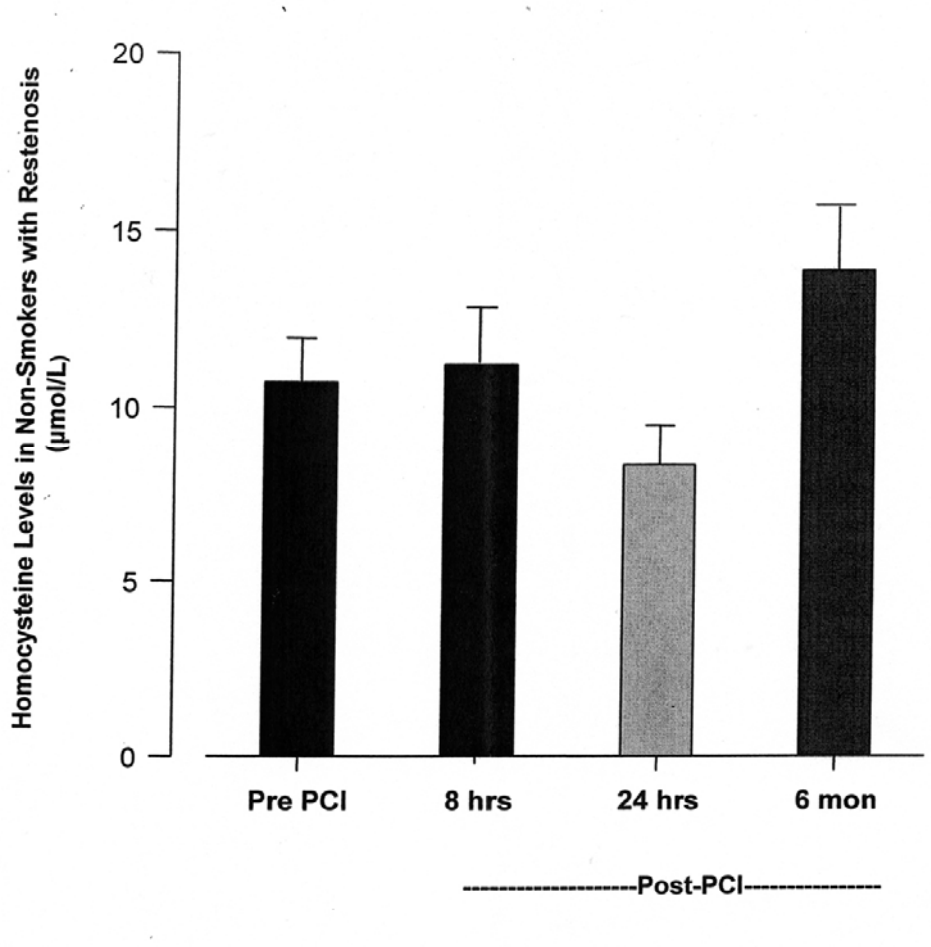

Figure 12 The levels of plasma homocysteine before percutaneous coronary intervention (pre-PCI), and at 8 hrs, 24 hrs and 6-months post-PCI in non-smokers who developed restenosis. Results are expressed as mean ± SE.

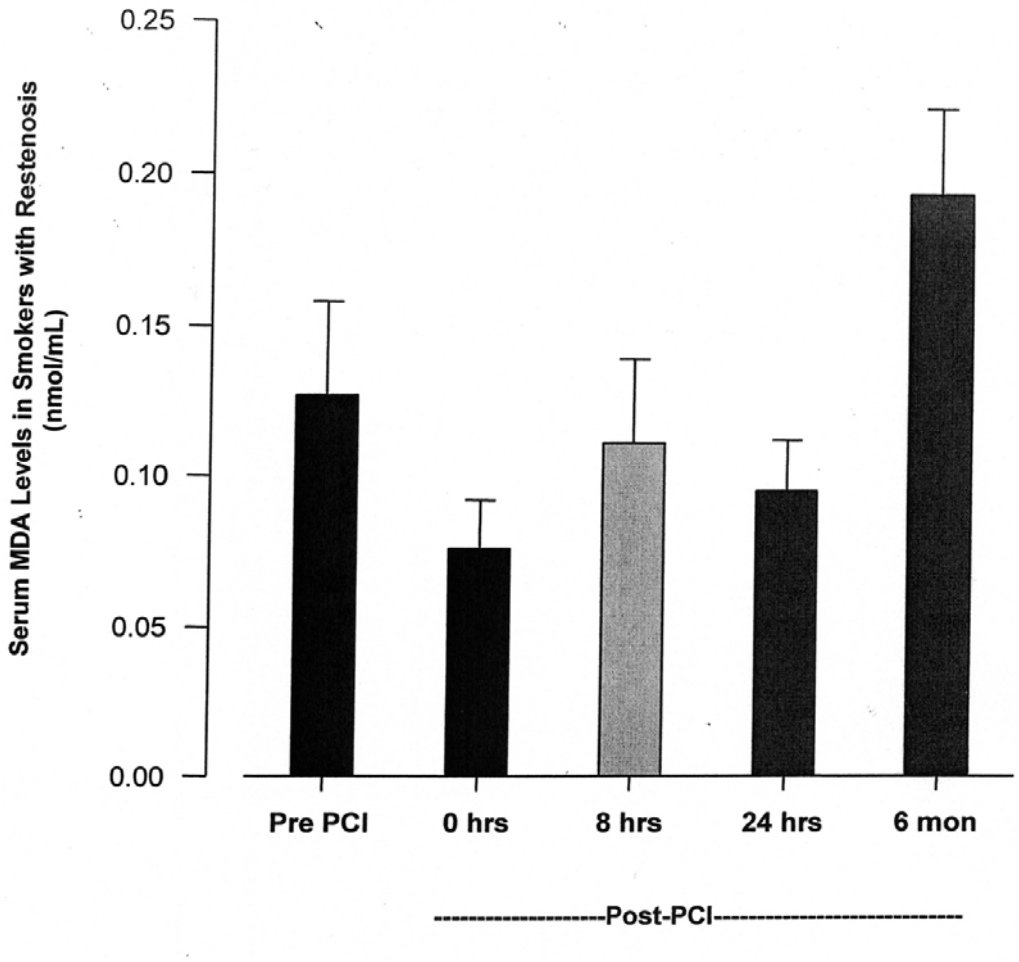

Figure 13 The levels of serum malondialdehyde (MDA) before percutaneous coronary intervention (pre-PCI), and at 0 hrs, 8 hrs, 24 hrs and 6-months post-PCI in smokers who developed restenosis. Results are expressed as mean ± SE.

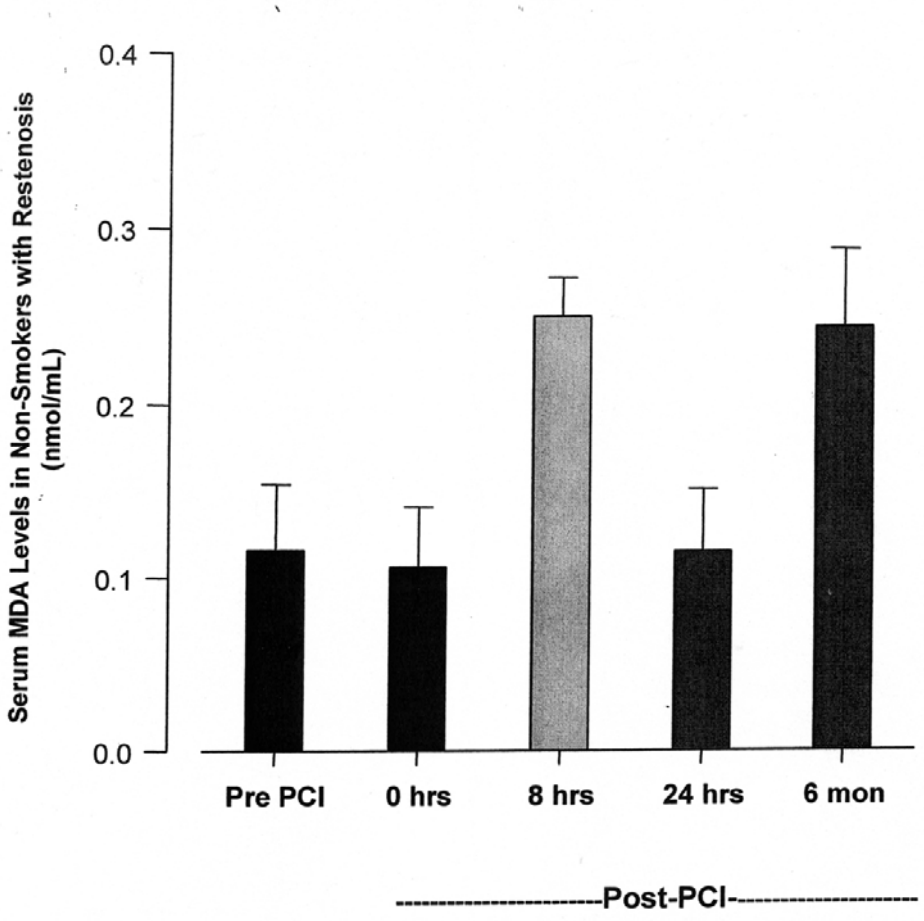

Figure 14 The levels of serum malondialdehyde (MDA) before percutaneous coronary intervention (pre-PCI), and at 0 hrs, 8 hrs, 24 hrs and 6-months post-PCI in non-smokers who developed restenosis. Results are expressed as mean ± SE.

4.0 DISCUSSION

The results of the present study suggest that the pre-procedural level of plasma homocysteine is not a predictor of restenosis. However, the post-PCI levels of homocysteine are associated with restenosis. Earlier reports on the pre-procedural plasma level of homocysteine as a predictor of restenosis are controversial. Total plasma homocysteine has been suggested as a factor of post-PCI restenosis (Schnyder et al. 2001; Kumbasar et al. 2001; Morita et al. 2000). In addition, Schnyder et al. (2002b) demonstrated that lowering plasma homocysteine levels attenuates post-PCI restenosis. SoRelle (2001) reported that folic acid consumption reduces plasma homocysteine levels and decreases post-PCI restenosis. On the contrary, there are reports which suggest that elevated levels of plasma homocysteine were not predictors of post-PCI restenosis (Hodish et al. 2002; Miner et al. 2000). In addition, Genser et al. (2002) reported that there is no correlation between plasma levels of homocysteine and in-stent coronary restenosis. Nevertheless, it has been demonstrated that homocysteine is toxic to endothelial cells (Blundell et al. 1996; Wall et al. 1980; Jones et al. 1994). This controversy could be due to dominant factors other than homocysteine (i.e. OR generation) that could damage endothelial cells and initiate the development of atherosclerosis and restenosis. It is plausible that the presence of elevated levels of plasma homocysteine may exist with low levels of OR's (measured indirectly by MDA levels) and increased levels of antioxidants in the tissue. Under these circumstances the coronary artery may not undergo post-PCI restenosis. However, it is also plausible that hyperhomocysteinemia could induce endothelial cell damage through oxygen free radical production (auto-oxidation of homocysteine) and possibly set the stage for

restenosis (Blundell et al. 1996; Jones et al. 1994; Wall et al. 1980; Harker et al. 1974; Starkebaum and Harlan 1986; Weimann et al. 1980; Anderson et al. 1995).

4.1 HOMOCYSTEINE AND RESTENOSIS

Restenosis following successful PCI continues to be an adverse outcome of this procedure. All patients of the present study (n=51) were implanted with bare metal stents. Our post-PCI restenosis rate was 43% which is consistent with the reported restenosis rates in the literature (Erbel et al. 1996). The numerous data that correlate homocysteine with the risk of the development, and the severity of CAD has stimulated interest in its possible function in restenosis (Prasad, 1999; Christen et al. 2000; Schnyder et al. 2002a). Homocysteine-induced smooth muscle cell proliferation, loss of endothelial function, coagulation abnormalities and plasma lipoprotein oxidation (from OR generation) associated with atherogenesis may contribute to restenosis as well. Thus the investigation of the governed release of intracellular homocysteine release into the plasma may provide a feasible biochemical marker for restenosis (Schnyder et al. 2001b). This study has demonstrated that patients who developed restenosis had a significant increase in the values of plasma homocysteine from the pre-procedural levels as compared to the 6-month post-PCI values. This suggests that the post-PCI values are predictors of restenosis. Perhaps this would explain why SoRelle (2001) found that lowering plasma homocysteine levels with folic acid consumption decreased post-PCI restenosis. In addition, Schnyder et al. (2002) demonstrated that lowering plasma homocysteine levels attenuates post-PCI restenosis; however the levels of homocysteine were only measured pre-procedurally and at 6-months follow-up.

In the present study, the significant increase in the plasma levels of post- PCI homocysteine as compared to pre-procedural levels in patients who developed restenosis

could be attributed to the greater number of patients in the restenosis group (55%) who had a history of cigarette smoking. The patients who developed restenosis post-PCI demonstrated a significant difference (p=0.031) between the pre-procedural values of plasma homocysteine in the smokers group (9.60 ± 0.51 μmol/L) as compared to the 6-month post-PCI plasma homocysteine levels of the same group (11.45 ± 0.056 μmol/L**).** This observation is supported by the study conducted by Nygard et al. (1995) in which they showed that cigarette smoking increases plasma homocysteine levels. Nygard et al. (1995) compared current smokers to non-smokers and found that current smokers had a higher plasma homocysteine level that rose linearly with the number of cigarettes smoked/day. Specifically, cigarette smokers with a plasma homocysteine level above 12 mmol/L had a 12 times the risk of developing cardiovascular disease compared to non-smokers with homocysteine levels with the normal range (O'Callaghan et al. 2002; El-Khairy et al. 1999). The risk of developing cardiovascular disease among ex-smokers was similar to that of current smokers (O'Callaghan et al. 2002; El-Khairy et al. 1999). Incidentally, in the present study, over half of the patients who developed restenosis post-PCI were ex-smokers. However, information pertaining to when the patients quit smoking is not available. Nevertheless, vitamins B_6, B_{12}, and folate were higher in non-smokers than in those who smoked (O'Callaghan et al. 2002; El-Khairy et al. 1999). The explanation for the increased plasma homocysteine levels among patients with a history of smoking is not clear. However, smoking is associated with differing plasma thiol redox status, perhaps due to the formation of ORs (Mansoor et al. 1995). According to Kalra et al. (1991) the serum concentration of ORs is increased in cigarette smokers. Cigarette smoking has been associated with increased risk of the development of atherosclerosis (Friedman et al. 1979). However, the present study was unable to

establish a positive correlation between MDA, restenosis, and smoking. The lack in correlation of the results from the present study in comparison to the Kalra et al. (1991 could be due to the small sample size. The results of the present study revealed that the patients who developed restenosis post-PCI showed no significant difference ($p=0.137$) between the pre-procedural values of serum MDA in the smokers group compared to the 6-month post-PCI serum MDA levels of the same group. There was also no significant difference between the pre-procedural levels of MDA and the 6-month post-PCI levels of MDA in the restenosis nonsmoker group.

As outlined by Prasad (1999), during smoking polymorphonuclear leukocytes are stimulated by nicotine-induced C_{5a} generation which in turn may lead to the production of peroxyl radicals (by-products of cigarette smoke) that could injure endothelial cells (Kalra et al. 1991). ORs can induce endothelial injury and can propagate and maintain atherogenesis and restenosis. In addition, the ORs produced from smoking may induce atherosclerosis by stimulating clotting factors and cause smooth muscle cell proliferation. Smooth muscle cell hyperplasia (proliferation) is central to the development of post-PCI restenosis.

4.2 OXIDATIVE STRESS AND RESTENOSIS

Eighty-six percent of the patients in this study who developed restenosis had a history of hypercholesterolemia. Hypercholesterolemia may increase serum concentrations of ORs through multiple mechanisms. Hypercholesterolemia has been demonstrated to amplify the cholesterol content of PMNLs, platelets and endothelial cells (Gorg and Kakkar, 1987; Prisco et al. 1986; Stuart et al. 1980; Prasad, 2001). Platelets that are high in cholesterol liberate adenosine diphosphate (ADP), thrombin, and histamine (Henry, 1977; Shattil et al. 1975). Histamine and ADP stimulate

phospholipase A_2 that acts on membrane phospholipids causing the release arachidonic acid (Ruzicka and Printz, 1984; Vanden, 1980). Hypercholesterolemia is associated with increased intracellular Ca^{2+} concentrations which can also increase phospholipase A_2 (Quan-sang et al. 1987; Vanden, 1980). The arachidonic acid stimulates an increase in the synthesis of leukotrienes and prostaglandins through the lipoxygenase and cyclooxygenase pathways respectively. ORs are produced during biosynthesis of leukotrienes and prostaglandins from arachidonic acid (Egan et al. 1976; Marnett et al. 1975; Murota et al. 1990; Panganamala et al. 1976). Hypercholesterolemia may also indirectly produce ORs and hypochlorous acid (HOCl) from leukocytes that are activated by platelet activating factor (PAF), leukotriene B_4, (LTB_4) and activated complement components (C_{3a} and C_{5a}) (Ford-Hutchinson et al. 1980; Hanahan, 1986; Fantone and Ward, 1982; Prasad et al. 1989; Vanden, 1980; Whatley et al. 1989; Steward et al. 1990). LTB_4 could be produced by the aforementioned mechanism of arachidonic acid metabolism, while PAF formation and release is dependent on intracellular Ca^{2+} concentrations and plasma thrombin which are elevated during hypercholesterolemia. Oxygen radicals (superoxide anion, hydroxyl radical, hydrogen peroxide and HOCl) are associated with tissue injury and atherogenesis (Gupta and Prasad, 1992a; Jolly et al. 1984; McCord, 1985). ORs exert their effect on unsaturated fatty acids of the lipid membrane to generate lipid peroxides (MDA) which in turn increases membrane permeability with a loss of membrane integrity (Freeman and Crapo, 1982). An increase in serum MDA suggests the participation of ORs in tissue damage. In the present study there was a significant increase ($p=0.001$) in serum MDA from the pre-procedural values as compared to the 6-month post-PCI values in the patients who developed restenosis. Therefore, it is possible that hypercholesterolemia

may increase the levels of ORs and may be responsible for endothelial injury (Prasad et al. 1994) possibly mediating initiation of restenosis. Endothelial injury constitutes a crucial step in initiating the development in the pathogenesis of atherosclerosis and perhaps coronary restenosis (Ross, 1986; Thambyrajah and Townsend, 2000). Prasad et al. (1994) demonstrated that hypercholesterolemic atherosclerosis is correlated with an increase in aortic tissue MDA and decreased antioxidant reserve. These results also suggest that increased aortic tissue MDA is related to an increased level of OR production and or decreased antioxidant reserve. Prasad et al. (1994) have shown that ORs are involved in the development and maintenance of hypercholesterolemic atherosclerosis. Cholesterol-lowering agents combined with niacin therapy have been reported to increase plasma homocysteine levels (Blankenhorn, 1991). Sixty-eight percent of the patients in the present study were on cholesterol lowering agents with niacin therapy which may explain the post-PCI six-month increase in plasma homocysteine levels as compared to baseline in those patients who developed restenosis. On the other hand, two studies which investigated the administration of lipid lowering agents' post-PCI and their ability to reduce restenosis found that there was no uniform significant difference in angiographic or clinical restenosis as compared to those patients who received placebo (Serruys et al. 1999; Serruys et al. 2002). However, their role in limiting the development and progression of atherosclerosis is of importance (Kinlay et al. 1996). For instance, many primary and secondary prevention trials using 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) have shown reductions in mortality and morbidity in patients with CAD (Maron et al. 2000; Schwartz et al. 2001). The results of the present study show that the serum levels of total cholesterol ($p=0.001$) and LDL-C ($p=0.02$) were significantly higher and serum HDL-C levels ($p=0.001$) were

significantly lower in patients who developed post-PCI restenosis than in patients who did not develop post-PCI restenosis. These results suggest that there is an association between hypercholesterolemia and post-PCI restenosis. It is possible that the ORs generated from hypercholesterolemia play a role in the genesis of post-PCI restenosis.

Rodrigo et al. (2003) reported that there was an association between essential hypertension and elevated levels of plasma homocysteine. The results of the present study show that 55% percent of the patients who developed restenosis had a history of hypertension. As described by Prasad et al. (1991) homocysteine impairs vascular smooth muscle and endothelial cell function by altering endothelial dependent vasomotor regulation. The mechanism by which elevated concentrations of plasma homocysteine contributes to hypertension may be multi-factorial. Homocysteine decreases vasodilation by the inhibition of nitric oxide. It increases oxidative stress by the generation of ORs, thereby inducing vascular smooth muscle cell proliferation and alterations of the elastic qualities of the vascular wall leading to hypertension-induced atherosclerosis and possibly restenosis. According to Stamler and Slivka (1996), endothelial relaxations are dependent on the bioavailability of endothelial NO synthetase (eNOS) to produce nitric oxide. Nitric oxide is critical for sustaining vascular homeostasis (Rodrigo et al. 2003). The effect of preservation of the endothelium by NO is weakened by hyperhomocysteinemia (Prasad, 1999). Thus, vascular injury is due to a disproportional between NO production and the concentrations of homocysteine. In addition, the lipid peroxidation produced by elevated levels of homocysteine may attenuate the expression of eNOS and catabolize NO (Blom et al. 1995; Chin et al. 1992; Liao et al. 1995). Homocysteine is also involved in essential hypertension through its production of ORs. Oxygen radicals may cause the release of Ca^{2+} from the

sarcoplasmic reticulum and mitochondria resulting in increased vascular tone (Tsai et al. 1994). Lastly, it has been suggested by Tsai et al. (1994) that homocysteine may play a role in the development of smooth muscle cell proliferation. Moreover, elevated plasma homocysteine concentrations have been observed in patients with carotid artery intimal thickening suggesting an increase in vascular tone (Willinek et al. 1997). Thus, the relationship between homocysteine–induced hypertension and atherosclerosis is strong and it is possible that there may be an association between homocysteine-induced hypertension and post-PCI restenosis.

Studies have demonstrated a correlation between restenosis and the fibrinolytic system. Huber et al. (1992) found an increase in the procoagulant protein plasminogen activator inhibitor-1 (PAI-1) concentrations post-PCI in patients with restenosis and a decrease in PAI-1 from baseline in those patients who did not develop restenosis. Lang et al. (2000) showed that exposure of cultured endothelial cells to homocysteine led to an increase in the intracellular production of superoxide anion. It has also been demonstrated that superoxide anion and singlet oxygen produce thrombus formation (Jourdan et al. 1995). It is possible that the oxidative stress produced by the auto-oxidation of homocysteine is related to thrombus-induced restenosis post-PCI. Superoxide anion, hydroxyl radicals and hydrogen peroxide are produced during the auto-oxidation of homocysteine (Welch et al. 1996; Misra, 1974; Rowley and Halliwell, 1982). Other studies conducted by Montalescot et al. (1995) demonstrated that patients with restenosis showed elevations in several procoagulant factors such as PAI-1, tissue plasminogen activator antigen, fibrinogen, and factors VII and VIII. In addition, Ishiwata et al. (1997) identified depression of the fibrinolytic response in patients who developed restenosis post-PCI as compared to those patients who did not develop

restenosis. Homocysteine directly mediates the inhibition of fibrinolysis by preventing the cofactor activity of thrombomodulin and protein-C stimulation on endothelial cells (Hayashi et al. 1992; Rodgers and Conn, 1990; Lenz and Sadler, 1991). In addition, homocysteine activates factor V activity, blocks anti thrombin III and inhibits t-PA binding to human endothelial cells (Rodgers and Kane, 1986; Nishinaga et al. 1993; Hajjar, 1993). Unfortunately, procoagulant or fibrinolytic substances were not measured in the present study. It is possible however, that an association of post-PCI levels of homocysteine with restenosis is due to its procoagulant properties. As such, studies have shown that post-PCI restenosis is correlated to an increase in procoagulant and depression of fibrinolytic activity (Welch et al. 1996; Misra, 1974; Rowley and Halliwell, 1982; Ishiwata et al. 1997).

Prasad et al. (1994) have demonstrated the attenuation of the genesis of cholesterol diet-induced atherosclerosis in rabbits treated with the antioxidant probucol. Restenosis, after successful PCI, has been hypothesized to occur because of an aggressive healing process with increased intimal proliferation (hyperplasia), inflammation, thrombus formation and compression of the arterial wall (Beatt et al. 1992; Lam et al. 1986; Liu et al. 1989). Compression of the arterial wall by PCI devices causes destruction of the vasa vasorum in the arterial adventitia possibly causing arterial wall hypoxia, increased platelet activation and deep arterial wall injury (Lam et al. 1986). Animal model studies have revealed that the extent of post-stenting neointimal proliferation and restenosis is directly related to the extent of procedural coronary arterial injury (Frimerman et al. 1997; Carter et al. 1996). Similarly, increased intimal hyperplasia from deep arterial injury (medial or adventitial) was reported in a post mortem study of patients who had post-PCI restenosis (Nobuyoshi et al. 1991). Tardif et

al. (1997) reported from their Multivitamins and Probucol Trial (MVP) that the antioxidant probucol attenuates neointimal proliferation. In their study, one month before PCI, 317 patients were randomly assigned to be administered twice a day either placebo, probucol (500mg), multivitamins (30,000 IU of beta carotene, 500mg of vitamin C, and 700 IU of vitamin E) or both probucol and multivitamins. The results of their study showed that the restenosis was 40.3%, 38.9%, 28.9%, 20.7% respectively. Moreover, Prasad (1994) described the effectiveness of probucol in attenuating hypercholesterolemic atherosclerosis. Moreover, Wantanabe et al. (1996) described the preventative effects of probucol on post-PCI restenosis. In addition, Martin et al. (1997) reported that Vitamin E inhibits low-density-lipoprotein-induced adhesion of monocytes to human aortic endothelial cells in-vitro. Recently, Tardif et al. (2003) demonstrated that AGI-1067, a metabolically stable analog of probucol, has an equal antioxidant effect and attenuate restenosis following PCI. The results of the present study indicate that patients who developed post-PCI restenosis had higher levels of oxidative stress (MDA) as compared to their pre-procedural values. This suggests that post-PCI restenosis is associated with increased levels of ORs, which may be due to increased OR production and or decreased antioxidant reserve.

5.0 CONCLUSION

The results of the present study suggest that (i) The pre-procedural levels of plasma homocysteine are not predictors of coronary restenosis. However, the 6-month post-PCI levels of plasma homocysteine are associated with restenosis. The values of plasma homocysteine increased by 24% at six-months as compared to the pre-procedural levels. (ii) The pre-procedural concentrations of serum malondialdehyde are not predictors of post-PCI restenosis. However, the 6-month post-PCI levels of MDA are predictors of post-PCI restenosis. The values of serum MDA increased 109% at the six-months post-PCI in those patients who developed restenosis as compared to the pre-procedural levels (iii) The 6-month post-PCI levels of serum MDA are better predictors of restenosis than the post-PCI plasma levels of homocysteine (iv) There is a positive correlation between the pre-procedural levels of serum total cholesterol and LDL-C and post-PCI restenosis and (v) The pre-procedural concentration of HDL-C is negatively correlated with post-PCI restenosis.

6.0 REFERENCES

Abe J, Berk BC. Fyn and JAK2 mediate Ras activation by reactive oxygen species. J Biol Chem. 1999;274:21003.

Adler SP, Hur JK, Wang JB, et al. Prior infection with cytomegalovirus is not a major risk factor for angiographically demonstrated coronary artery atherosclerosis. J Infect Dis. 1998;177:209.

Alfthan G, Pekkanen J, Jauhiainen M, et al. Relation of serum homocysteine and lipoprotein (a) concentrations to atherosclerotic disease in prospective Finnish population based study. Atherosclerosis. 1994;106:9.

Ambrosi P. Homocysteine and post-angioplasty restenosis. Nutrition Metabolism & Cardiovascular Disease. 2003;13(6):391.

Anderson A, Lindgren A, Hultberg B. Effects of thiol oxidation and thiol export from the erythrocytes on determination of redox status of homocysteine and other thiols in plasma from healthy subjects and patients with cerebral infarctions. Clin Chem. 1995;41:361.

Anderson DW, Nichols AV, Pan SS, et al. High-density lipoprotein distribution: resolution and determination of three major components in a normal population sample. Atherosclerosis. 1978;29:161.

Anderson JL, Muhlestein JB, Carlquist J, et al. Randomized secondary prevention trial of azithromycin in patients with coronary disease and serologic evidence of Chlamydia pneumonia infection: The Azithromycin in Coronary Artery: Elimination of Myocardial Infection with Chlamydia (ACADEMIC) study. Circulation. 1999;99:1540.

Anderson KM, Castelli WP, Levey D. Cholesterol and mortality: 30 years of follow-up from The Framingham Study. JAMA. 1987;257:2176.

Angeline T, Aruna RM, Ramadevi K, et al. Homocysteine status and acute myocardial infarction among Tamilians. Indian J Clin Biochem. 2005;20(1):18.

Ariyo AA, Thach C, Tracy R. Lp (a) lipoprotein, vascular disease, and mortality in the elderly. N Eng J Med. 2003;349:2108.

Astrand I. Exercise electrocardiograms recorded twice within an 8-year interval in a group of 204 women and men 48-63 years old. Acta Med Scand. 1965;118:27.

Austin MA. Plasma triglycerides as a risk factor for coronary artery disease: the epidemiological evidence and beyond. Am J Epidemiol. 1989;129:249.

Austin MA, King MC, Vranizam KM, et al. Atherogenic lipoprotein phenotype: a proposed genetic marker for coronary heart disease. Circulation. 1990;82:495.

Babior BM. The respiratory burst of phagocytes. J Clin Invest. 1984;73:599.

Babior BM, Kipnes RS, Curnutte JT. Biological defense mechanisms: The production by leukocytes of superoxide anion, a potential bactericidal agent. J Clin Invest. 1973;52:741.

Barroso-Aranda J, Schmidt-Schonobein GW. Transformation of neutrophils as indicators of irreversibility in hemorrhagic shock. Am J Physiol. 1989;257:H846.

Barroso-Aranda J, Schmidt-Schonobein GW, Zweibach BW, et al. Granulocytes and no reflow phenomenon in irreversible hemorrhagic shock. Circ Res. 1988;63:437.

Baynes JW. Perspectives in diabetes, role of oxidative stress in the development of complications of diabetes. Diabetes. 1991;40:405.

Bazzano LA, He J, Muntner P, et al. Relationship between cigarette smoking and novel risk factors for cardiovascular disease in the United States. Ann Intern Med. 2003;138:891.

Beatt KJ, Serruys PW, Luijten HE, et al. Restenosis after coronary angioplasty: the paradox of increased lumen diameter and restenosis. J Am Coll Cardiol. 1992;19:258.

Beckman JS, Beckman JW, Chen J, et al. Apparent hydroxyl radical production by peroxynitrate: Implication for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci. U S A. 1990;87:1620.

Bellet S, Horstmann E, Roman LR, et al. Effect of caffeine on ventricular fibrillation threshold in normal dogs and dogs with acute myocardial infarction. Am Heart J. 1972;84:215.

Bengtson JR, Mark DB, Honan MB, et al. Detection of restenosis after elective percutaneous transluminal coronary angioplasty using the exercise treadmill test. Am J Cardiol. 1990;65:28.

Bennoitt C, Furber A, Le Bouil A, et al. Plasma homocysteine is not a predictive factor of restenosis after coronary angioplasty. Arch Mal Coeur Vaiss. 1999;92:1457.

Bertina RM, Koeleman BP, Koster T, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature. 1994;369:64.

Bharadwaj L, Prasad K. Role of oxygen-derived free radicals in modulation of vascular smooth muscle tone. Proceedings of the 36th World Congress of the International College of Angiology. New York, USA, July 2-9,1994;3.

Bhunia AK, Han H, Snowden A, et al. Redox-regulated signalling by lactosylceramide in the proliferation of human aortic smooth muscle cells. J Biol Chem. 1997;272:1564.

Blankenhorn DH, Malinota MR, Mack WJ. Colestipol plus niacin therapy elevates plasma homocyst(e)ine levels. Coron Art Dis. 1991;2:357.

Blom HJ, Kleinveld HA, Boers GH, et al. Lipid peroxidation and susceptibility of low density lipoprotein to in vitro oxidation in hyperhomocysteinemia. Eur J Clin Invest. 1995;25:149.

Blum A, Giladi M, Weinberg M, et al. High anti-cytomegalovirus (CMV) IgG antibody titer is associated with coronary artery disease and may predict post-coronary balloon angioplasty restenosis. Am J Cardiol. 1998;81:866.

Blundell G, Jones BG, Rose FA, et al. Homocysteine-mediated endothelial cell toxicity and its amelioration. Atherosclerosis. 1996;122:163.

Bolli R, Jeroudi MO, Patel BS, et al. Marked reduction of free radical generation and contractile dysfunction by antioxidant therapy begun at the time of reperfusion: evidence that myocardial "stunning" is a manifestation of reperfusion injury. Circ Res. 1989;65:607.

Bostrom AG, Cupples LA, Jenner JL, et al. Elevated plasma lipoprotein (a) and coronary heart disease in men aged 55 years and younger. A prospective study. JAMA. 1996;276:544.

Bostrom AG, Gagnon DR, Cupples LA, et al. A prospective investigation of elevated lipoprotein (a) detected by electrophoresis and cardiovascular disease in women: The Framingham Heart Study. Circulation. 1994;90:1688.

Boushey CJ, Beresford SA, Omenn GS, et al. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. J Am Med Assn. 1995;274:1049.

Braquet P, Hosfard D, Braquet M, et al. Role of cytokines and platelet activating factor in microvascular immune injury. Int Arch Allergy Appl Immunol. 1989;88:88.

Bruce RA. Evaluation of the functional capacity and exercise tolerance of cardiac patients. Concepts in Cardiovasc Dis. 1956;25:321.

Bruce RA. Comparative prevalence of segment S-T depression after maximal exercise in healthy men in Seattle and Taipei. In Simonson E(ed): Physical Activity and the Heart, Charles C. Thomas, Springfield, IL. USA. 1967;17.

Burian K, Kis Z, Virok D, et al. Independent and joint effects of antibodies to human heat-shock protein 60 and Chlamydia pneumonia infection in the development of coronary atherosclerosis. Circulation. 2001;103:1503.

Cannan CR, Yeh W, Kelsey SF, et al. Incidence and predictors of target vessel revascularization following percutaneous coronary transluminal angioplasty: A report from the National Heart, Lung, and Blood Institute Percutaneous Transluminal Coronary Angioplasty Registry. Am J Cardiol. 1999; 84:170.

Carreras MC, Pargament GA, Catz SD, et al. Kinetics of nitric oxide and hydrogen peroxide production and formation of peroxynitrite during respiratory bursts of human neutrophils. FEBS Lett. 1994;341:65.

Carter AJ, Farb A, Laird JR, et al. Neointimal formation is dependent on underlying arterial substrate after coronary stent placement (abstract). J Am Coll Cardiol. 1996;27:320A.

Castelli WP. The triglyceride issue: a view from Framingham. Am Heart J. 1986;112:432.

Castelli WP. Cholesterol and lipids in the risk of coronary artery disease: the Framingham Study. Can J Cardiol. 1988;(suppl 4A) 5A.

Cedarbaum AI, Cicker E, Rubin E, et al. The effect of dimethyl sulfoxide and other hydroxyl-radical scavengers on the oxidation of ethanol by rat liver microsomes. Biochem Biophys Res Commun. 1977;78:1254.

Ceriello A, Guigliano D, Quantraro A, et al. Antioxidants show an antihypertensive effect in diabetic and hypertensive subjects. Clin Sci. 1991;81:739.

Chambers DE, Parks DA, Patterson G, et al. Xanthine oxidase: a source of free radical damage in myocardial ischemia. J Mol Cell Cardiol. 1985;17:145.

Chin JH, Azhar S, Hoffman BB. Inactivation of endothelial derived relaxing factor by oxidized lipoproteins. J Clin Invest. 1992;89:10.

Chisolm GM. Antioxidant and atherosclerosis: a current assessment. Clin Cardiol. 1991;14:25.

Christen R, Daly R, Robinson K, et al. Blood levels of homocysteine and increased risks of cardiovascular disease: causal or casual? Arch Intern Med. 2000;160:422.

Clarke R, Daly L, Robinson K, et al. Hyperhomocysteinemia: an independent risk factor for vascular disease. N Engl J Med. 1991;324:1149.

Colavitti R, Pani G, Bedogni B, et al. Reactive oxygen species as downstream mediators of angiogenic signaling by vascular endothelial growth factor receptor-2/KDR. J Biol Chem. 2002;277:3101.

Columbo A, Drzewiecki J, Banning A, et al. Randomized study to assess the effectiveness of slow and moderate release polymer-based paclitaxel-eluting stents for coronary artery lesions. Circulation. 2003;108:788.

Crapo JD. Morphological changes in pulmonary oxygen toxicity. Annu Rev Physiol. 1986;48:721.

Cultip DE, Chauhan MS, Baim DS, et al. Clinical restenosis after coronary stenting: perspectives from multicenter clinical trails. J Am Coll Cardiol. 2002;40:2082.

Dagenais GR. Survival of patients with strongly positive exercise electrocardiogram. Circulation. 1982;65(3):452.

D' Angelo A, Selhub J. Homocysteine and thrombotic disease. Blood. 1997;90:1.

Danesh J, Whincup P, Walker M, et al. Chlamydia pneumonia IgG titers and coronary heart disease. Prospective study and meta-analysis. BMJ. 2000;321:208.

Danesh J, Whincup P, Walker M, et al. Chlamydia pneumonia IgG titers and coronary heart disease. Prospective study and meta-analysis. Eur Heart J. 2002;23:371.

Dechend R, Maass M, Gieffers J, et al. Chlamydia pneumonia infection of vascular smooth muscle and endothelial cells activates NF-kappaB and induces tissue factor and PAI-1 expression: A potential link to arteriosclerosis. Circulation. 1999;100:1369.

de Jaegere PPT, de Feyter PJ, van der Giessen WJ, et al. Endovascular stents: preliminary clinical results and future developments. Clin Cardiol.1993(a);16:369.

de Jaegere PPT, Hermans WR, Rensing BJ, et al. Matching based on quantitative coronary angioplasty as a surrogate for randomized studies: comparison between stent implantation and balloon angioplasty of native coronary artery lesions. Am Heart J. 1993(b);125:310.

Denholm EM, Lewis JC. Monocyte chemoattractants in pigeon aortic atherosclerosis. Am J Pathol. 1987;126:464.

Di Minno G, Davi G, Margaglione M, et al. Abnormally high thromboxane biosynthesis in homozygous homocystinuria. Evidence for platelet involvement and probucol-sensitive mechanism. J Clin Invest. 1993;92:1400.

Duff GL, McMillian GC. Pathology of atherosclerosis. Am J Med. 1951;11:92.

Durand P, Lussier-Cacan S, Blanche D. Acute methionine load induced hyperhomocysteinemia enhances platelet aggregation, thromboxane biosynthesis, and macrophage-derived tissue factor activity in rats. FASEB J. 1997;11:1157.

Egan RW, Paxton J, Kuehl FA. Mechanism for irreversible self-deactivation of prostaglandin synthase. J Biol Chem. 1976;251:7329.

El-Khairy L, Ueland PM, Nygard O, et al. Lifestyle and cardiovascular disease risk factors as determinants of total cysteine in plasma: The Horland Homocysteine Study. Am J Clin Nutrition. 1999;70:(6)1016.

Engerson TD, McKelvey TG, Rhyne DB, et al. Conversion of xanthine dehydrogenase to oxidase in ischemic rat tissue. J Clin Invest. 1987;79:1564.

Erbel R, Haude M, Hoop HW, et al. Restenosis Stent (REST) Study: randomized trial comparing stenting and balloon angiolplasty for treatment of restenosis after balloon angiolplasty. J Am Coll Cardiol. 1996;27:139A.

Fajadet J, Morice MC, Bode C, et al. Maintenance of long term clinical benefits with sirolimus-eluting coronary stents: Three year results of the RAVEL Trial. Circulation. 2005;111:1040.

Fantone JC, Ward PA. Role of oxygen-derived free radicals and metabolites in leukocyte-dependent inflammatory reactions. Am J Pathol. 1982;107:397.

Faruqui R, de la Motte C, DiCorleto DE. Alpha-tocopherol inhibits agonist-induced monocyte cell adhesion to cultured human endothelial cells. J Clin Invest. 1994;94:592.

Fiddian-Green RG. Homocysteine causes mitochondrial dysfunction. bmj.com. 2002.

Fielding JE. Smoking: health effects and control (pt1). N Engl J Med. 1985;313:491.

Fischman DL, Leon MB, Baim DS, et al. A randomized comparison of coronary-stent placement and balloon angioplasty in the treatment of coronary artery disease. Stent Restenosis Study Investigators. N Engl J Med. 1994;331:496.

Fonseca V, Guba SC, Fink LM. Hyperhomocysteinemia and the endocrine system; Implications for atherosclerosis and thrombosis. Endocrine Reviews. 1999;20(5):738.

Ford-Hutchinson AW, Bray MA, Doig MV, et al. Leukotriene B4, a potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. Nature. 1980;286:264.

Freeman BA, Crapo JD. Biology of disease: free radical and tissue injury. Lab Invest. 1982;47:412.

Fridovich I. The biology of oxygen radicals. Science. 1978;201:875.

Friedman GD, Dales LG, Ury HK. Mortality in middle-aged smokers and nonsmokers. N Engl J Med. 1979;300:213.

Frimerman A, Litvack F, Makkar R, et al. Neointimal in-stent proliferation is related to the degree of strut induced arterial wall injury (abstract). J Invas Cardiol. 1997;9:71C.
.
Fryer RH, Wilson BD, Gubler DB, et al. Homocysteine a risk factor for premature vascular disease and thrombosis, induces tissue factor activity in endothelial cells. Arterioscler & Thrombosis. 1993;13:1327.

Furchgott RF. Role of endothelium in responses of vascular smooth muscle. Circ Res. 1983;53:557.

Furchgott RF, Zawadski JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature (Lond). 1980;288:373.

Gabay, C, Kushner, I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med. 1999;340:448.

Gallo R, Padurean A, Chesebro JH, et al. Inhibition of intimal thickening after balloon angioplasty in porcine coronary arteries by targeting regulators of the cell cycle. Circulation. 1998;99:2164.

Gauldie J, Richards C, Harnish D, et al. Interferon b2/B-cell stimulatory factor type 2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells. Proc Natl Acad Sci. U S A. 1987;84:7251.

Gaustadnes M, Rudiger N, Rasmussen K, Ingerslev J. Intermediate and severe hyperhomocysteinemia with thrombosis: a study of genetic determinates. Throm Haemost. 2000;83:554.

Genser D, Prachar H, Hauer R, et al. Relation of homocysteine, vitamin B12, and folate to coronary in-stent restenosis. Am J Cardiol. 2002;89:495.

Gershlick A, De Scheerder I, Chevalier B, et al. Inhibition of restenosis with a paclitaxel-eluting, polymer-free coronary stent: the European evaluation of Paclitaxel Eluting Stent (ELUTES) trial. Circulation. 2004;109:487.

Gershov D, Kim S, Brot N, et al. C-reactive protein binds to apoptotic cell, protects the cells from assembly of the terminal complement components, and sustains an anti-inflammatory innate immune response: implications for systemic autoimmunity. J Exp Med. 2000;192:1353.

Gieffers J, Fullgraf H, Jahn J, et al. Chlamydia pneumonia infection in circulating human monocytes is refractory to antibiotic treatment. Circulation. 2001;103:351.

Gimbrone MA Jr. Vascular endothelium: an integrator of pathophysiologic stimuli in atherosclerosis. Am J Cardiol. 1995;75:67B.

Gluek CJ, Shaw P, Lang JE, et al. Evidence that homocysteine is an independent risk factor for atherosclerosis in hyperlipidemic patients. Am J Cardiol. 1995;75:132.

Goldschlager N, Selzer A, Cohn K. Treadmill stress tests as indicators of the presence and severity of coronary artery disease. Ann Intern Med. 1976;85:277.

Gorg P, Kakkar V. Increased uptake of monocyte-treated low-density lipoproteins by aortic endothelium in vivo. Atherosclerosis. 1987;65:99.

Goy JJ, Stauffer JC, Siegenthaler M, et al. A prospective randomized comparison between paclitaxel and sirolimus stents in the real world of interventional cardiology. The TAXI Trial. J Am Coll Cardiol. 2005;45:308.

Grayston JT, Kuo CC, Wang SP, et al. A new chlamydia psittaci strain, TWAR, isolated in acute respiratory tract infections. N Engl J Med. 1986;315:161.

Griffin JH, Evatt BL, Zimmerman TS, et al. Deficiency of protein C in congenital thrombotic disease. J Clin Invest. 1981;68(13):1370.

Grube E, Silber S, Hauptmann KE, et al. TAXUS I: six-and twelve-month results from a randomized, double blind trial on a slow-release paclitaxel-eluting stent for de novo coronary lesions. Circulation. 2003;107:38.

Grundy SM. Atherogenic dyslipidemia: lipoprotein abnormalities and implications for therapy. Am J Cardiol. 1995;(suppl 75 B):B45.

Gunn M, Stephens JC, Thompson JR, et al. Significant association of cagA positive Helicobacter pylori strains with risk of premature myocardial infarction. Heart. 2000;84:267.

Gupta JB, Prasad K. Mechanism of H_2O_2-induced modulation of airway smooth muscle. Am J Physiol. 1992;263:L714.

Hajjar KA. Homocysteine-induced modulation of tissue plasminogen activator binding to its endothelial cell membrane receptor. J Clin Invest. 1993;91:2873.

Halliwell B, Gutteridge MC. Oxygen radicals and tissue damage. In: Caldarera CM, Harris P, eds. Advances in studies on heart metabolism. Bologna: Cooperativa Libraria Universitavia Editrice, Bologna, 1982:403.

Hanahan DJ. Platelet activating factor: A biologically active phosphoglyceride. Annu Rev Biochem. 1986;55:483.

Hankey GJ, Eikelboom, JW. Homocysteine and vascular disease. The Lancet. 1999;9176:407.

Hansen PR, Kharazmi A, Jauhiainen M, et al. Induction of oxygen free radical generation in human monocytes by lipoprotein (a). J Clin Invest. 1994;24:497.

Hansrani M, Gillespie JI, Stansby G. Homocysteine and neointimal hyperplasia. Eur J Endovasc Surg. 2002;23:3.

Harker LA, Ross R, Slichter SJ, et al. Homocysteine-induced arteriosclerosis. The role of endothelial injury and platelet response in its genesis. J Clin Invest. 1976;68:731.

Harker LA, Slichter SJ, Scott CR, et al. Homocysteinemia, vascular injury and arterial thrombosis. N Engl J Med. 1974;291:537.

Hayashi T, Honda G, Suzuki K. An atherogenic stimulus homocysteine inhibits cofactor activity of thrombomodulin and enhances thrombomodulin expression in human umbilical vein endothelial cells. Blood. 1992;79:2930.

Heinecke JA, Rosen H, Suzuki LA, et al. Iron and copper promote modification of low-density lipoprotein by human arterial smooth muscle cells in culture. J Clin Invest. 1984;74:1890.

Heinecke JA, Rosen H, Suzuki LA. The role of sulphur containing amino acids in superoxide production and modification of low-density lipoprotein by arterial smooth muscle cells. J Biol Chem. 1987;262:98.

Hennekens CH, Rosner B, Cole DS. Daily alcohol consumption and coronary artery disease. Am J Epidemiol. 1978;107:196.

Henriksen T, Mahoney EM, Steinberg D. Enhanced macrophage degradation of low density lipoprotein previously incubated with cultured endothelial cells: recognition by receptor for acetylated low density lipoproteins. Proc Natl Acad Sci. U S A. 1981;78:6499.

Henry RL. Platelet function. Seminars in thrombosis and hemostasis. Science. 1977;4:93.

Herrmann HC. Paclitaxel-eluting stent prevents restenosis. J Watch Cardiol. 2003;11:1.

Hirshfeld JW, Schwartz JS, Jugo R, et al. Restenosis after coronary angioplasty: a multivariate statistical model to relate lesion and procedure variables to restenosis. J Am Coll Cardiol. 1991;18:647.

Hodish I, Matetzky S, Selah B-A, et al. Effect of elevated homocysteine levels on clinical restenosis following percutaneous coronary intervention. Cardiology. 2002;97:214.

Hoffmann JA, Kafatos FC, Janeway CA, et al. Phylogenetic perspectives in innate immunity. Science. 1999;284:1313.

Hu HE, Pierce GN, Zhong G. The atherogenic effects of chlamydia are dependent upon serum cholesterol and specific to chlamydia pneumonia. J Clin Invest. 1999;103:747.

Huber K, Jorg M, Probst P, et al. A decrease in plasminogen activator inhibitor-1 activity after successful percutaneous transluminal coronary angioplasty is associated with a significantly reduced risk of coronary restenosis. Thromb Haemost. 1992;67:209.

Hultberg B, Andersson A, Isaksson A. Metabolism of homocysteine, its relation to the other cellular thiols and its mechanism of cell damage in a cell culture line (human histiocytic cell line). Biochem Biophys Acta. 1995;1269:6.

Ignarro LJ, Buga GM, Wood KS, et al. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. Proc Natl Acad Sci. U S A. 1987;84:9265.

Ishiwata S, Tukada T, Nakanishi S, et al. Post angioplasty restenosis: Platelet activation and the coagulation-fibrinolysis system as possible factors in the pathogenesis of restenosis. Am Heart J. 1997;133:387.

Jacobson GM, Dourron HM, Liu J, et al. Novel NAD(P)H oxidase inhibitor suppresses angioplasty-induced superoxide and neointimal hyperplasia of rat carotid artery. Circ Res. 2003;92:637.

Jennings RB, Reimer KA. Lethal myocardial ischemic injury. Am J Pathol. 1982;102:241.

Jick H, Miettinen OS, Neff RK, et al. Coffee and myocardial infarction. N Engl J Med. 1973;289:63.

Jolly SR, Kane WJ, Baile MB, et al. Canine myocardial perfusion injury. Its reduction by the combined administration of superoxide dismutase and catalase. Cir Res. 1984;54:277.

Jones BG, Rose FA, Tudball N. Lipid perioxidation and homocysteine induced toxicity. Atherosclerosis. 1994;105:165.

Jourdan A, Aguejouf O, Imbault P, et al. Experimental thrombosis model induced by free radicals: application to aspirin and other different substances. Thromb Res. 1995;79:109.

Kalra J, Chaudhary AK, Prasad K. Increased production of oxygen free radicals in cigarette smokers. Int J Exp Pathol. 1991;72:1.

Kanellakis P, Nestel P, Bobik A. Angioplasty-induced superoxide anions and neointimal hyperplasia in the rabbit carotid artery: suppression by the isoflavone trans-tetrahydrodaidzein. Atherosclerosis. 2004;176:63.

Kang SS. Critical points for determining moderate hyperhomocysteinemia. Eur J Clin Invest. 1995;25:806.

Kang SS, Wong PWK. Genetic and nongenetic factors for moderate hyperhomocysteinemia. Atherosclerosis. 1996;119:135.

Kannell WB. Role of blood pressure in cardiovascular disease: the Framingham Study. Angiology. 1975;26:1.

Kannel WB, Dawber TR. Coffee and coronary disease. N Engl J Med. 1973;289:100.

Kapoor R, Prasad K. Role of oxyradicals in cardiovascular depression and cellular injury in hemorrhagic shock and re-infusion: effect of SOD and catalase. Circ Shock. 1994;43:79.

Kapoor R, Prasad K. Role of polymorphonuclear leukocytes in cardiovascular depression and cellular injury in hemorrhagic shock and re-infusion. Free Rad Biol and Med. 1996;21:609.

Keany JF, Vita JA. Atherosclerosis, oxidative stress, and antioxidant protection in endothelium-derived relaxing factor action. Prog Cardiovasc Dis. 1995;38:129.

Khoo JC, Miller E, McLoughlin P, et al. Prevention of low density lipoprotein aggregation by high density lipoprotein or apolipoprotein A-1. Lipid Res. 1990;31:645.

Kinlay S, Selwyn AP, Delagrange D, et al. Biological mechanisms for the clinical success of lipid–lowering in coronary artery disease and the use of surrogate end-points. Curr Opin Lipidol. 1996;7:389.

Klein SM, Cohen G, Lieber CS, et al. Increased microsomal oxidation of hydroxyl radical scavenging agents and ethanol after chronic consumption of ethanol. Arch Biochem Biophys. 1983;223:425.

Koch W, Ndrepepa G, Mehilli J, et al. Homocysteine status and polymorphisms of methylenetetrahydrofolate reductase are not associated with restenosis after stenting in coronary arteries. Arterioscler Thromb Vasc Biol. 2003;23:2229.

Kojoglanian SA, Jorgenson MB, Wolde-Tsadik G, et al. Restenosis in intervened coronaries with hyperhomocysteinemia (RICH). Am Heart J. 2003;146:1077.

Kontos HA, Wei EP, Povlishock JT, et al. Cerebral arteriolar damage by arachidonic acid and prostaglandin G_2 . Science. 1980;209:1242.

Kooy NW, Royall JA. Agonist-induced peroxynitrite production by endothelial cells. Arch Biochem Biophys. 1994;310:353.

Koppenol WH, Moreno JJ, Pryor WA, et al. Peroxynitrite: a cloaked oxidant from superoxide and nitric oxide. Chem Res Toxicol. 1992;5:834.

Kumbasar SD, Dincer I, Ertas F, et al. Hyperhomocysteinemia and restenosis. J Cardiovasc Risk. 2001;8:9.

Kushner I, Feldmann G. Control of the acute phase response. Demonstration of C-reactive protein synthesis and secretion by hepatocytes during acute inflammation in the rabbit. J Exp Med. 1978;148:466.

Lam JYT, Chesebro JH, Steale PM, et al. Deep arterial injury during experimental angioplasty: relation to a positive indium-111-labled scintigram, quantitative platelet deposition and mural thrombus. J Am Coll Cardiol. 1986;8:1380.

Lane DM. Dramatic increase in intracoronary stents. Am J Cardiol. 1999;84:1141.

Lang D, Kredan MB, Moat SJ, et al. Homocysteine-induced inhibition of endothelial-dependent relaxation in rabbit aorta. Arterioscler Thromb Vasc Biol. 2000;20:422.

Langman LJ. Hyperhomocysteinemia and the increased risk of venous thromboembolism. Arch of Int Med. 2000;160(7):961

Lee P, Prasad K. Hyperhomocysteinemia and venous thrombosis. Lower Extremity Wounds. 2002;(1):4.

Lehr HA, Sagban TA, Ihling C, et al. Immunopathogenesis of atherosclerosis: Endotoxin accelerates atherosclerosis in rabbits on hypercholesterolemic diet. Circulation. 2001;104:914.

Lemos PA, Saia F, Ligthart JM, et al. Coronary restenosis after sirolimus-eluting stent implantation: morphological description and mechanistic analysis from a consecutive series of cases. Circulation. 2003;108:257.

Lentz SR, Sadler JE. Inhibition of thrombomodulin surface expression and protein C activation by the thrombogenic agent homocysteine. J Clin Invest. 1991;88:1906.

Lentz SR, Sobey CG, Piegors DJ, et al. Vascular dysfunction in monkeys with diet-induced hyperhomocysteinemia. J Clin Invest. 1996;98:24.

Liao JK, Shin WS, Lee WY, et al. Oxidized low-density lipoprotein decreases the expression of endothelial nitric oxide synthetase. J Biol Chem. 1995;270:319.

Lindqvist C, Nordstrom T. Generation of hydroxyl radicals by the anti-viral compound phosphonoformic acid (Foscarnet). Pharmacology and Toxicology. 2001;89:49.

Ling Q, Hajjar KA. Inhibition of endothelial cell thrombo-resistance by homocysteine. Journal of Nutrition. 2000;130:373S.

Liu MW, Roubin GS, King SB. Restenosis after coronary angioplasty: potential biological determinants and role of intimal hyperplasia. Circulation. 1989;79:1374.

Loscalzo J, Weinfeld M, Fless GM, et al. Lipoprotein (a), fibrin binding, and plasminogen activation. Arteriosclerosis. 1990;10:240.

Lubec B, Labudova O, Hoeger H, et al. Homocysteine increases cyclin-dependent kinase in aortic rat tissue. Circulation. 1996;94:2620.

Luo Y, Marx SO, Kiyokawa H, Koff A, et al. Rapamycin resistance tied to defective regulation of p27Kipl. Mol Cell Biol. 1996;16:6744.

Luscher TF, Tanner FC, Noll G. Lipids and endothelial function: effects of lipid lowering and other therapeutic interventions. Curr Opin Lipidol. 1996;7:234.

Lyons TJ. Glycation and oxidation: a role in the pathogenesis of atherosclerosis. Am J Cardiol. 1993;17:26B.

Macaya C, Serruys PW, Ruygrok P, et al. Continued benefit of coronary stenting versus balloon angioplasty: one-year clinical follow-up of Benestent trial: Benestent study group. J Am Coll Cardiol. 1996;27:255.

Majors A, Ehrhart LA, Pezacka EH. Homocysteine as a risk factor for vascular disease. Enhanced collagen production and accumulation by smooth muscle cells. Arterioscler Thromb Vasc Biol. 1997;17:2074.

Mansoor MA, Bergmark C, Svardal AM, et al. Redox status and protein binding of plasma homocysteine and other aminothiols in patients with early onset peripheral vascular disease. Arterioscler Throm Vasc Biol. 1995;15:232.

Marnett LJ, Woldawer P, Samuelson B. Co-oxygenation of organic substrates by prostaglandin synthetase of sheep vesicular gland. J Biol Chem. 1975;250:8510.

Maron DJ, Fazio S, Linton MF. Current perspectives on statins. Circulation. 2000;101:207.

Martin A, Foxall T, Blumberg JB, et al. Vitamin E inhibits low-density-lipoprotein-induced adhesion of monocytes to human aortic endothelial cells in-vitro. Arterioscler Thromb Vasc Biol. 1997;17:429.

Marumo T, Schini-Kerth VB, Fisslthaler B, et al. Platelet-derived growth factor-stimulated superoxide anion production modulates activation of transcription factor NF-

$_{K}$B and expression of monocyte chemoattactant protein-1 in human aortic smooth muscle cells. Circulation. 1997;96:2361.

Marx SO, Jayaraman T, Go LO, et al. Rapamycin-FKBP inhibits cell cycle regulators of proliferation in vascular smooth muscle cells. Circ Res. 1995;76:412.

Mattila KJ, Valle MS, Nieminen MS, et al. Dental infections and coronary atherosclerosis. Atherosclerosis. 1993;103:205.

Mayer EM, Jacosen DW, Robinson K. Homocysteine and coronary atherosclerosis. J Am Coll Cardiol. 1996;27:17.

McCord JM. Oxygen derived free radicals in post-ischemic tissue injury. N Engl J Med. 1985;312:159.

McCord JM, Fridovich I. Production of O^{-} in photolyzed water demonstrated through the use of superoxide dismutase. Photochem Photobiol. 1973;17:115.

McCord JM, Roy RS. The pathophysiology of superoxide: notes on inflammation and ischemia. Can J Physiol Pharmacol. 1982;60:1346.

McCord JM, Roy RS, Schaffer SW. Free radicals and myocardial ischemia: The role of xanthine oxidase. Adv Myocardiol. 1985;5:183.

McCully KS. Vascular pathology of homocysteinemia: implications for the pathogenesis of atherosclerosis. Am J Pathol. 1969;56:111.

McCully KS. Chemical pathology of homocysteine I. Atherogenesis. Ann Clin Lab Sci. 1993;23:477.

McCully KS. Chemical pathology of homocysteine. II. Carcinogenesis and homocysteine thiolactone metabolism. Ann Clin Lab Sci. 1994;24:27.

McKeage K, Murdoch D, Goa KL. The sirolimus-eluting stent: a review of its use in the treatment of coronary artery disease. Am J Cardiovasc Drugs. 2003;3:211.

Mezzano D, Munoz X, Martinez C, et al. Vegetarians and cardiovascular risk factors: hemostasis, inflammatory markers and plasma homocysteine. Throm Haemost. 1999;81:913.

Miner SE, Hegele RA, Sparkes J, et al. Homocysteine, lipoprotein (a), and restenosis after percutaneous transluminal coronary angioplasty: a prospective study. Am Heart J. 2000;140: 272.

Misra HP. Generation of superoxide free radicals during the autooxidation of thiols. J Biol Chem. 1974;249:2151.

Moon YS, Kashyap ML. Niacin extended-release lovastatin: combination therapy for lipid disorders. Expert Opin Pharmacother. 2002;3:1763.

Montalescot G, Ankri A, Vicaut E, et al. Fibrinogen after coronary angioplasty as a risk factor for restenosis. Circulation. 1995;92:31.

Montali A, Vega GL, Grundy SM. Concentrations of apolipoprotein A-1 containing particles in patients with hypoalphalipoproteinemia. Arteriosc Thromb. 1994;14:511.

Morice MC, Serruys PW, Sousa JE, et al. A randomized comparison of a sirolimus-eluting stent with a standard stent for coronary revascularization. N Engl J Med. 2002; 346:1773.

Morita H, Kurihara H, Kuwaki T, et al. Homocysteine as a risk factor for restenosis after coronary angioplasty. Thromb Haemost. 2000;84:27.

Morley JJ, Kushner I. Serum C-reactive protein levels in disease. Ann NY Acad Sci. 1982;389:406.

Moses JW, Leon MB, Popma JJ, et al. Sirolimus-eluting stents versus standard stents in patients with restenosis in a native coronary artery. N Eng J Med. 2003;349:1315.

Mudd Sh, Skovby F, Levy HL, et al. The natural history of homocysteinuria due to cystathione β-synthetase deficiency. Am J Hum Genet. 1985;37:1.

Muhlestein JB, Horne BD, Carlquist JF, et al. Cytomegalovirus seropositivity and C-reactive protein have independent and combined predictive value for mortality in patients with angiographically demonstrated coronary artery disease. Circulation. 2000;102:1917.

Muller A, Sies H. Alcohol, aldehydes, and lipid peroxidation: current notions. Alcohol-Alcohol. 1987;(suppl I):67.

Murota S, Morita I, Suda N. The control of vascular endothelial cell injury. Ann NY Acad Sci. 1990;598:182.

Nakazano K, Wantanabe N, Masumo K, et al. Does superoxide underlie the pathogenesis of hypertension? Proc Natl Acad Sci. U S A. 1991;88:10045.

Naruszewicz M, Mirikiewicz E, Olszewski AJ, et al. Thiolation of low-density lipoprotein by homocysteine thiolactone causes increased aggregation and altered interaction with cultured macrophages. Nutr Metab Cardiovasc Dis. 1994;4:70.

Nishinaga M, Ozawa T, Shimada K. Homocysteine a thrombogenic agent, suppresses anticoagulant heparin sulfate expression in cultured porcine aortic endothelial cells. J Clin Invest. 1993;92:1381.

Nobuyoshi M, Kimura T, Nosaka H, et al. Restenosis after successful percutaneous transluminal coronary angioplasty; serial angiographic follow-up of 229 patients. J Am Coll Cardiol. 1988;12:616.

Nobuyoshi M, Kimura T, Ohishi H, et al. Restenosis after percutaneous transluminal coronary angioplasty: pathologic observations in 20 patients. J Am Coll Cardiol. 1991;17:433.

Nygard O, Nordrehaug JE, Refsum H, et al. Plasma homocysteine levels and mortality in patients with coronary artery disease. N Engl J Med. 1997;337:230.

Nygärd O, Vollset E, Refsum H, et al. Total plasma homocysteine and cardiovascular risk profile. The Hordaland Homocysteine Study. JAMA. 1995;274:1526.

O'Callaghan P, Meleady R, Fitzgerald I, et al. Smoking and homocysteine. Eur Heart J. 2002;23:1580.

Ogryzlo EA. The nature of singlet oxygen. In: Ranby B, Rabek JF, eds. Singlet Oxygen: Reactions with Organic Compounds and Polymers. New York, NY: John Wiley & Sons;1978:4.

Olszewski AJ, McCully KS. Homocysteine metabolism and the oxidative modification of proteins and lipids. Free Radical Biol Med. 1993;14:683.

O'Meara JJ, Dehmer GJ. Care of the patient and management of complications after percutaneous coronary artery interventions. Annals Int Med. 1997;127(6):458.

Owens GK. Control of hypertrophic versus hyperplastic growth of vascular smooth muscle cells. Am J Physiol. 1989;(suppl 257):H1755.

Palabrica TM, Liu AC, Aronovitz MJ, et al. Antifibrinolytic activity of apolipoprotein (a) in vivo: Human apolipoproteinemia (a) transgenic mice are resistant to tissue plasminogen activator-mediated thrombolysis. Nat Med. 1995;1:256.

Palmer RM, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature (Lond). 1987;327:524.

Panganamala RV, Sharma HM, Heikkila JC, et al. Role of hydroxyl radical scavengers dimethyl sulfoxide, alcohols and methional in the inhibition of prostaglandin biosynthesis. Prostaglandins. 1976;11:599.

Panza JA, Quyyumi AA, Brush JE, et al. Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. N Engl J Med. 1990;323:22.

Parks S-J, Shim WH, Ho DS, et al. A paclitaxel-eluting stent for the prevention of coronary restenosis. N Engl J Med. 2003;348:1537.

Parthasarathy S. Oxidation of low-density lipoprotein by thiol compounds leads to its recognition by the acetyl LDL receptor. Biochem Biophys Acta. 1987;917:337.

Parthasarathy S, Barnett J, Fong LG. High density lipoprotein inhibits the oxidative modification of low-density lipoprotein. Biochem Biophys Acta. 1990;1044:275.

Patel P, Mendall MA, Carrington D, et al. Association of Helicobacter pylori and Chlamydia pneumonia infections with coronary heart disease and cardiovascular risk factors. BMJ. 1995;311:711.

Pattanaik U, Prasad K. Oxygen free radicals and endotoxic shock: Effect of flaxseed. J Cardiovasc Pharmacol Therapeut. 1998;3(4):305.

Paubert-Braquet M, Longchamps MO, Koltz P. Tumor necrosis factor (TNF) primes human neutrophil (PMN) platelet-activating factor (PAF)-induced superoxide generation. Consequences in promoting PMN-mediated endothelial cell (EC) damage (abstract). Prostaglandins. 1988;35:803.

Pearson TA, Menash GA, Alexander RW, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for health care professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation. 2003;107:499.

Pockley, AG. Heat shock proteins, inflammation, and cardiovascular disease. Circulation. 2002;105:1012.

Poon M, Marx SO, Gallo R, et al. Rampamycin inhibits vascular smooth muscle cell migration. J Clin Invest. 1996;98:2277.

Port SR, Rosendaal FR, Reitsma PH, et al. A common genetic variation in the 3'-untranslated region of prothrombin gene associated elevated plasma prothrombin levels and an increase in venous thrombosis. Blood. 1996;88:3698.

Prasad K. Oxygen free radicals as a mechanism of hypercholesterolemic atherosclerosis: effect of probucol. Intl J Angiol. 1994;3:100.

Prasad K. Hydroxyl radical scavenging property of secoisolariciresinol diglucoside (SDG) isolated from flaxseed. Mol Cell Biochem. 1997(a);168:117.

Prasad K. Dietary flaxseed in the prevention of hypercholesterolemic atherosclerosis. Atherosclerosis. 1997(b);132:69.

Prasad K. Reduction of serum cholesterol and hypercholesterolemic atherosclerosis in rabbits by secoisolariciresinol diglucoside isolated from flaxseed. Circulation. 1998;99:1355.

Prasad K. Homocysteine, a risk factor for cardiovascular disease. Int J Angiol. 1999;8:76.

Prasad K. Secoisolariciresinol diglucoside from flaxseed delays the development of type-2 diabetes in Zucker rat. J Lab Clin Med. 2001;138:32.

Prasad K. Pathophysiology of Atherosclerosis. In; Textbook of Angiology. J.B. Chang, E.R. Olsen, K. Prasad and B.E. Sumpio, Eds. New York, NY: Springer-Verlag. 2003(a):85.

Prasad K. Oxygen free radicals and peripheral vascular disease. In; Textbook of Angiology. J.B. Chang, E.R. Olsen, K. Prasad and B.E. Sumpio, Eds. New York, NY: Springer-Verlag. 2003(b):427.

Prasad K. C-reactive protein and cardiovascular disease. Intl J Angiol. 2003(c);12:1.

Prasad K. C-reactive protein increases oxygen radical generation by neutrophils. J Cardiovasc Pharmacol Therap. 2004;9:203.

Prasad K. Hypocholesterolemic and anti-atherosclerotic effect of flax ligan complex isolated from flaxseed. Atherosclerosis. 2005;179:269.

Prasad K, Bharadwaj L. Hydroxyl radical-a mediator of acetylcholine-induced vascular relaxation. J Mol Cell Cardiol. 1996;28:2033.

Prasad K, Gupta JB, Kalra J, et al. Oxidative stress as a mechanism of cardiac failure in chronic volume overload in canine model. J Mol Cell Cardiol. 1996;28:375.

Prasad K, Kalra J. Oxygen free radicals and hypercholesterolemic atherosclerosis: effect of vitamin E. Am Heart J. 1993;125:958.

Prasad K, Kalra J, Bharadwaj B. Cardiac depressant effects of oxygen free radicals. Angiology. 1993;44:257.

Prasad K, Kalra J, Bharadwaj L. Increased chemiluminescence of polymorphonuclear leukocytes in dogs with volume overload in heart failure. Brit J Pathol. 1989;70:463.

Prasad K, Kalra J, Bharadwaj B, et al. Increased oxygen free radical activity in patients on cardiopulmonary bypass undergoing aorto-coronary bypass surgery. Am Heart J. 1992(a);123:37.

Prasad K, Kalra J, Chaudhary AK, et al. Effect of polymorphonuclear leukocyte-derived oxygen free radicals and hypochlorous acid on cardiac function and some biochemical parameters. Am Heart J. 1990;119:538.

Prasad K, Kalra J, Lee P. Oxygen free radicals as a mechanism of hypercholesterolemic atherosclerosis: Effects of Probucol. Int J Angiol. 1994;3:100.

Prasad K, Lee P, Kalra J. Influence of endothelin on cardiovascular function, oxygen free radicals, and blood chemistry. Am Heart J. 1991;121:178.

Prasad K, Lee P, Mantha SV, et al. Detection of ischemia – reperfusion injury by cardiac muscle chemiluminescence. Mol Cell Biochem. 1992(b);115:49.

Prasad K, Mantha SV, Kalra J. Prevention of hypercholesterolemic atherosclerosis by garlic, an antioxidant. J Cardiovasc Pharmocol Therapeu. 1997;2:239.

Prasad K, Mantha SV, Muir AD, Westscott ND. Reduction of hypercholesterolemic atherosclerosis by CDC-flaxseed with very low alphalinolenic acid. Atherosclerosis. 1998;136:367.

Preuss KC, Gross GJ, Brooks HL, et al. Time course recovery of "stunned" myocardium following variable periods of ischemia in conscious and anesthetized dogs. Am Heart J. 1987;114:696.

Prisco D, Rogasi PG, Matucci M, et al. Age related changes in lipid composition. Thromb Res. 1986;44:427.

Puig JG, Fox IH. Ethanol–induced activation of adenine nucleotide turnover. Evidence for a role of acetate. J Clin Invest. 1984;74:936.

Quan-sang KH, Levenson J, Simon R, et al. Platelet cytosolic free Ca^{2+} concentration and plasma cholesterol in untreated hypertensives. J Hypertension. 1987;(suppl 5):S521.

Rader DJ, Hoeg JM, Brewer HB. Quantitation of plasma apolipoproteins in the primary and secondary prevention of coronary artery disease. Ann Intern Med. 1994;120:1012.

Rao PS, Cohen MV, Mueller HS. Production of free radical and lipid peroxides in early experimental ischemia. J Mol Cell Cardiol. 1983;15:713.

Rice-Evans C, Baurdon R. Free radical-lipid interactions and their pathological consequences. Prog Lipid Res. 1993;32:71.

Ridker PM. Evaluating novel cardiovascular risk factors: Can we better predict heart attacks? Ann Intern Med. 1999;130:933.

Ridker PM, Cushman M, Stampfer MJ, et al. Plasma concentration of C-reactive protein and the risk of developing peripheral vascular disease. Circulation. 1998;97:425.

Rodrigo R, Passalacqua W, Araya J, et al. Homocysteine and hypertension. J Clin Pharmocol. 2003;43(12):1299.

Rogers GM. Hemostatic properties of normal and perturbed vascular cells. FASEB J. 1988;2:116.

Rogers GM, Conn MT. Homocysteine an atherogenic stimulus, reduces protein C activation by arterial and venous endothelial cells. Blood. 1990;75:895.

Rogers GM, Kane WH. Activation of endogenous factor V by a homocysteine-induced vascular endothelial cell activator. J Clin Invest. 1986;77:1909.

Rosenman RH, Sholt RI, Brand RJ. A study of comparative blood pressure measures in predicting risk of coronary heart disease. Circulation. 1976;54:51.

Ross R. The pathogenesis of atherosclerosis-an update. N Engl J Med. 1986;314:488.

Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature. 1993;362:801.

Ross R, Glomset JA. The pathogenesis of atherosclerosis. N Eng J Med. 1976;295:369.

Roubin GS, Cannon AD, Agrawal SK, et al. Intracoronary stenting for acute and threatened closure complicating percutaneous transluminal coronary angioplasty. Circulation. 1992;85:916.

Rowley DA, Halliwell B. Superoxide-dependent formation of hydroxyl radicals in the presence of thiol compounds. FEBS Lett. 1982;138:33.

Rumley AG, Paterson JR. Analytical aspects of antioxidants and free radical activity in clinical biochemistry. Ann Clin Biochem. 1998;35:181.

Ruygrok PN, Webster MW, de Valk V, et al. Clinical and angiographic factors associated with asymptomatic restenosis after percutaneous coronary intervention Circulation. 2001;104:2289.

Ruzicka T, Printz MP. Arachidonic acid metabolism in skin: A review. Rev Physiol Biochem Pharmacol. 1984;100:121.

Sacks T, Moldow CF, Craddock PR, et al. Oxygen radical mediates endothelial cell damage by complement–stimulated granulocytes: an in vitro model of immune vascular damage. J Clin Invest. 1978;6:1161.

Sagar S, Kallo IJ, Kaul N, et al. Oxygen free radicals in essential hypertension. Mol Cell Biochem. 1992;111:103.

Salvador M, Bonting S, Mullane K, et al. Imidazole, a selective inhibitor of thromboxane synthetase. Prostaglandins. 1977;13:611.

Schachinger V, Halle M, Minners J, et al. Lipoprotein (a) selectively impairs receptor-mediated endothelial vasodilator function of the human coronary circulation. J Am Coll Cardiol. 1997;30:927.

Schaefer EJ, Lamon-Fava S, Jenner JL, et al. Lipoprotein (a) levels and risk of coronary heart disease in men: The Lipid Research Clinics Coronary Primary Prevention Trial. JAMA. 1994;271:999.

Schampaert E, Cohen EA, Schluter M, et al. C-SIRUS Investigators. The Canadian study of the sirolimus-eluting stent in the treatment of patients with long de novo lesions in small native coronary arteries. JACC. 2004;43:1110.

Schatz RA. Introduction to intravascular stents. Clin Cardiol. 1988;6:357.

Schatz RA, Baim DS, Leon M, et al. Clinical experience with the Palmaz-Schatz coronary stent: initial results of a multicenter study. Circulation. 1991;83:148.

Schmidt W, deLint J. Causes of death of alcoholics. QJ Stud Alcohol. 1972;33:171.

Schnyder G, Flammer Y, Roffi M, et al. Plasma homocysteine levels and late outcome after coronary angioplasty. J Am Coll Cardiol. 2002(a);40:1769.

Schnyder G, Roffi M, Flammer Y, et al. Association of plasma homocysteine with restenosis after percutaneous coronary angioplasty. Eur Heart J. 2002(b);23:726.

Schnyder G, Roffi M, Pin R, et al. Decreased rate of coronary restenosis after lowering of plasma homocysteine levels. N Engl J Med. 2001;345:1593.

Schofer J, Schluter M, Gershlick AH, et al. E-SIRIUS Investigators. Sirolimus-eluting stent for treatment of patients with long atherosclerotic lesions in small coronary arteries: double blind randomized controlled trial (E-SIRIUS). Lancet. 2003;362:1093.

Schwartz CJ, Valenta AJ, Sprauge EA. A modern view of atherogenesis. Am J Cardiol. 1993;(suppl B 71):B9.

Schwartz GG, Olsson AG, Ezekowitz MD, et al. Effects of atorvastatin on early recurrent ischemic events in acute coronary syndromes. The MIRACL Study: A randomized controlled trail. JAMA. 2001;285:1711.

Schwartz RS, Chronos NA, Virmani R. Preclinical restenosis models and drug-eluting stents. Still important, still much to learn. JACC. 2004;44:1373.

Serruys P, deFeyter P, Macaya C, et al. Fluvastatin for prevention of cardiac events following successful first percutaneous coronary intervention. A randomized controlled trial. JAMA. 2002;287:3215.

Serruys P, de Jaegere P, Kiemeneij F, et al. A comparison of balloon expandable stent implantation with angioplasty in patients with coronary disease. N Engl J Med. 1994; 331:489.

Serruys P, Foley D, Jackson G, et al. A randomized placebo-controlled trial of fluvastatin for prevention of restenosis after successful coronary balloon angioplasty; final results of fluvastatin angiographic restenosis (FLARE) trial. Eur Heart J. 1999;20:58.

Serruys P, Strauss BH, Beatt KJ, et al. Angiographic follow-up after placement of a self-expanding coronary-artery stent. N Engl J Med. 1991;324:13.

Shattil SJ, Anaya-Galindo R, Bennet J, et al. Platelet hypersensitivity induced by cholesterol incorporation. J Clin Invest. 1975;55:636.

Shi Y, Niculescu R, Wang D, et al. Increased NAD(P)H oxidase and reactive oxygen species in coronary arteries after balloon injury. Arterioscler Thromb Vasc Biol. 2001;21:739.

Shipchandler MT, Moore EG. Rapid, fully automated measurement of plasma homocysteine with the Abbott IMx analyzer. Clin Chem. 1995;41:991.

Sigwart U, Puel J, Mirkovitch V, et al. Intravascular stents to prevent occlusion and restenosis after transluminal angioplasty. N Engl J Med. 1987;316:701.

Simpson PJ, Todd FR, Fantone JC, et al. Reduction of experimental canine myocardial reperfusion injury by a monoclonal antibody (anti Mo1, anti CD-11b) that inhibits leukocyte adhesion. J Clin Invest. 1988;81:624.

Siscovick DS, Schwartz SM, Corey L, et al. Chlamydia pneumonia, herpes simplex virus type 1, and cytomegalovirus and incident myocardial infarction and coronary artery disease death in older adults: the cardiovascular health study. Circulation. 2000;102:2335.

Smeeth L, Thomas SL, Hall AJ, et al. Risk of myocardial infarction and stroke after acute infection or vaccination. N Engl J Med. 2004;351:2611.

Soinio M, Marneimi J, Laakso M, et al. Elevated plasma homocysteine levels is an independant predictor of coronary heart disease events in patients with type II diabetes mellitus. Ann Intern Med. 2004;140:94.

SoRelle R. Folate reduces homocysteine levels and lowers rate of restenosis. Circulation. 2001;104:9050.

Sorlie PD, Nieto FJ, Adam E, et al. A prospective study of cytomegalovirus, herpes simplex virus 1, and coronary heart disease: the atherosclerosis risk in communities (ARIC) study. Arch Intern Med. 2000;160:2027.

Sousa JE, Serruys PW, Costa MA. New frontiers in cardiology. Drug-eluting stents: part II. Circulation. 2003;107:2383-2389.

Southorn PA, Powis G. Free radicals in medicine, I: Chemical nature and biological reactions. Mayo Clin Proc. 1988;3:381.

Stamler JS, Osborne JA, Jaraki O, et al. Adverse vascular effects of homocysteine are modulated by endothelium relaxing factor and related oxides of nitrogen. J Clin Invest. 1993;91:308.

Stamler JS, Slivka A. Biological chemistry of thiols in the vasculature and in vascular-related disease. Nutr Rev. 1996;54:1.

Stampfer MJ, Malinow MR, Willett WC, et al. A prospective study of plasma homocysteine and risk of myocardial infarction in US physicians. J Am Med Assn. 1992;268:877.

Starkebaum G, Harlan JM. Endothelial cell injury due to copper-catalyzed hydrogen peroxide generation from homocysteine. J Clin Invest. 1986;77:1370.

Steinberg D. Antioxidant and atherosclerosis: a current assessment. Circulation. 1991;84:1420.

Steinberg D. Antioxidants in the prevention of human atherosclerosis. Circulation. 1992;85:2338.

Steinberg D, Wiztum JL. Lipoproteins and atherogenesis: current concepts. JAMA. 1990; 264:3047.

Steward AG, Dubbin PN, Harris T, et al. Platelet-activating factor may act as second messenger in the release of eicosanoids and superoxide anions from leukocytes and endothelial cells. Proc Natl Acad Sci. U S A. 1990;78:3215.

Stone GW, Ellis SG, Cox DA, et al. TAXUS-IV Investigators. A polymer-based, paclitaxel-eluting stent in patients with coronary artery disease. N Engl J Med. 2004;350:221.

Stuart MJ, Gerrard JM, White JG. Effect of cholesterol on production of thromboxane B_2 by platelets in vitro. N Engl J Med. 1980;302:6.

Stuhlinger MC, Tsao PS, Her JH, et al. Homocysteine impairs the nitric oxide synthase pathway: role of asymmetric dimethylarginine. Circulation. 2001;104:2569.

Sundaresen M, Yu-Xu Y, Ferrans VJ, et al. Requirement for generation of H_20_2 for platelet-derived growth factor signal transduction. Science. 1995;270:296.

Szocs K, Lassegue B, Sorescu D, et al. Up-regulation of nox-based NAD(P)H oxidases in restenosis after carotid injury. Arterioscler Thromb Vasc Biol. 2002;22:21.

Takami S, Yamashita S, Kihara S, et al. Lipoprotein (a) enhances the expression of intercellular adhesion molecule-1 in cultured human umbilical vein endothelial cells. Circulation. 1998;97:721.

Tardif JC, Cote G, Lesperance J, et al. Probucol and multivitamins in the prevention of restenosis after coronary angioplasty. Multivitamins and Probucol Study Group. N Eng J Med. 1997;337:365.

Tardif JC, Gregoire J, Schwartz L, et al. Effects of AGI-1067 and Probucol after percutaneous coronary interventions. Circulation. 2003;107:552.

Teirstein PS, King S. Vascular radiation in a drug-eluting stent world: it's not over till it's over. Circulation. 2003;108:384.

Thelle DS, Heyden S, Fodor JG. Coffee and cholesterol in epidemiological and experimental studies. Atherosclerosis. 1987;67:97.

Thambyrajah J, Landray MJ, Jones HJ, et al. A randomized double blind placebo-controlled trial of the effect of homocysteine-lowering therapy with folic acid on endothelial function in patients with coronary artery disease. J Am Coll Cardiol. 2001;37:1858.

Thambyrajah J, Townsend JN. Homocysteine and atherothrombosis-mechanisms for injury. Eur Heart J. 2000;21:967.

Toborek M, Kopieczna-Grzebieniak E, Drozdz M, et al. Increased lipid perioxidation as a mechanism of methionine-induced atherosclerosis in rabbits. Atherosclerosis. 1995;115:217.

Tsai J-C. Induction of Cyclin A gene expression by homocysteine in vascular smooth muscle. J Clin Invest. 1996;97(1):146.

Tsai J-C, Perrella MA, Yoshizumi M, et al. Promotion of vascular smooth muscle cell growth by homocysteine: a link to atherosclerosis. Proc Natl Acad Sci. USA. 1994;91:6369.

Upchurch GR, Jr., Welch GN, Fabian AJ, et al. Stimulation of endothelial nitric oxide production by homocysteine. Atherosclerosis. 1997;132:177.

Upchurch GR, Jr., Welch GN, Loscalzo J. Homocysteine, EDRF, and endothelial function. J Nutr. 1996;(suppl 126):1290S.

Uttara P, Prasad K. Oxygen free radicals and endotoxic shock: effect of flaxseed. J Cardiovasc Pharmacol Therapeut. 1998;3(4):305.

Vanden H. Intracellular phospholipase A. Biochem Biophys Acta. 1980;604:191.

Verhamme P, Quarck R, Hao H, et al. Dietary cholesterol withdrawal reduces vascular inflammation and induces coronary plaque stabilization in miniature pigs. Cardiovasc Res. 2002;56:135.

Volanakis JE. Acute phase proteins in rheumatic disease. In: Arthritis and Allied Conditions: A textbook of Rheumatology, Koopman WJ, (Ed), Baltimore, Maryland. Williams Wilkins, 1997.505.

Von Eckardstein A, Schulte H, Cullen P, et al. Lipoprotein (a) further increase the risk of coronary events in men with high global cardiovascular risk. J Am Coll Cardiol. 2001;37:434.

Wall RT, Harlan JM, Harker LA, et al. Homocysteine-induced endothelial cell injury in vitro: a model for the study of vascular injury. Thromb Res. 1980;18:113.

Waller BF. "Crackers, breakers, stretchers, drillers, scrapers, shavers, burners, welders and melters" – the future treatment of atherosclerotic coronary artery disease? A clinical-morphologic assessment. J Am Coll Cardiol. 1989;13:969.

Wantanabe K, Sekiya M, Ikeda S, et al. Preventative effects of probucol on restenosis after transluminal coronary angioplasty. Am Heart J. 1996;132:23.

Warren JS, Ward PA. Oxidative injury to vascular endothelium. Am J Med Sci. 1986;292:97.

Wasserman HH. Introductory remarks. In: Singlet Oxygen, Wasserman HH(Ed), New York, NY. Academic Press. 1979:100.

Waugh J, Wagstaff, AJ. The paclitaxel (TAXUS)-eluting stent: a review of its use in the management of de novo coronary artery lesions. Am J Cardiovasc Drugs. 2004;4:257.

Webster RO, Hong SR, Johnston RB, et al. Biological effects of human complement fragments C_5 and C_{5a} des Arg on neutrophil function. Immunopharmacology. 1980;2:201.

Weimann BJ, Kuhn H, Baumgartner HR. Effects of homocysteine on cultured bovine and human endothelial cells. Experimentia. 1980;36:762.

Weis SJ, Young J, LoBuglio AF, et al. Role of hydrogen peroxide in neutrophil-mediated destruction of cultured endothelial cells. J Clin Invest. 1981;68:714.

Welch GN, Upchurch GR, Keany JF, et al. Homocysteine decreases cell redox potential in vascular smooth musle cells. J Am Coll Cardiol. 1996;(Suppl 27A):163A.

Wells BJ, Mainous AG, Dickerson LM. Antibiotics for the secondary prevention of ischemic heart disease: a meta-analysis of randomized controlled trials. Arch Intern Med. 2004;164:215.

Whatley RE, Nelson P, Zimmerman GA, et al. The regulation of platelet activating factor production in endothelial cells. The role of calcium and protein kinase-C. J Biol Chem. 1989;264:6325.

Wilcken DEL, Wilcken B. The pathogenesis of coronary artery disease. A possible role of methionine metabolism. J Clin Invest. 1976;57:1079.

Williams AJK, Barry RE. Superoxide anion production and degranulation of rat neutrophils in response to acetaldehyde-altered liver cell membranes. Clin Sci. 1986;71:313.

Willineck WA, Lennarz M, Dudeck M, et al. High normal serum homocysteine concentrations are associated with increased risk for early atherosclerotic carotid artery wall lesions in normotensive subjects. Proceedings of the 4th Symposium on Multiple Risk Factors in Cardiovascular Disease: Strategies of Prevention of Coronary Heart Disease, Cardiac Failure, and Stroke. Washington, D.C. USA.1997:39.

Windeker S, Remondino A, Eberli F, et al. Sirolimus-eluting and paclitaxel-eluting stents for coronary revascularization. N Engl J Med. 2005;353:653.

Winston GW, Cedarbaum AI. A correlation between hydroxyl radical generation and ethanol oxidation by liver, lung, and kidney microsomes. Biochem Pharmacol. 1982;31:2031.

Wong C-K, Hammett, French JK, et al. Lack of association between baseline plasma homocysteine concentrations and restenosis rates after a first elective percutaneous coronary intervention without stenting. Heart. 2004;90:1299.

Wong YK, Dawkins KD, Ward ME. Circulating Chlamydia pneumonia DNA as a predictor of coronary artery disease. J Am Coll Cardiol. 1999;34:1435.

Woo KS, Chook P, Lolin YI, et al. Hyperhomocysteinemia is a risk factor for the endothelial dysfunction in humans. Circulation. 1997;96:2542.

Xu Q, Schett G, Perschinka H, et al. Serum soluble heat shock protein 60 is elevated in subjects with atherosclerosis in a general population. Circulation. 2000;102:14.

Zhou YF, Guetta E, Yu ZX, et al. Human cytomegalovirus increases modified low-density lipoprotein uptake and scavenger receptor mRNA expression in vascular smooth muscle cells. J Clin Invest. 1996;98:2129.

Zhou YF, Shou M, Guetta E, et al. Cytomegalovirus infection of rats increases the neointimal response to vascular injury without consistent evidence of direct infection of the vascular wall. Circulation. 1999;100:1569.

Zhu J, Quyyumi AA, Norman, JE, et al. Cytomegalovirus in the pathogenesis of atherosclerosis: the role of inflammation as reflected by elevated C-reactive protein levels. J Am Coll Cardiol. 1999;34:1738.

Zoratti EM, Segwich JB, Vrtis RR, et al. The effect of platelet-activating factor on the generation of superoxide anion in human eosinophils and neutrophils. J Allergy Clin Immunol. 1991;88:749.

Zouki C, Beauchamp M, Baron C, et al. Prevention of in vitro neutrophils adhesion to endothelial cells through shedding of L-selectin by C-reactive protein and peptides derived from C-reactive protein. J Clin Invest. 1997;100:522.

Zweier JL, Flaherty JT, Weisfeldt ML. Direct measurement of free radicals generated following reperfusion of ischemic myocardium. Proc Natl Acad Sci. USA. 1987;84:1404.