CHAPTER 1

LITERATURE REVIEW
1. Review of Literature

1.1 CARDIOVASCULAR DISEASES – Major factor leading to mortality and morbidity worldwide

Cardiovascular disease (CVD) remains the principle cause of death in both developed and developing countries, accounting for roughly 20% of all worldwide deaths per year. Basic Health Information In The Western Pacific Region (Revised as of 11 November 2004) published by World Health Organisation (WHO) Western Pacific Region office states that diseases of the circulatory system mainly stroke and coronary heart diseases are among the major causes of death in 32 countries and areas of the Region. No less than 3 million deaths due to diseases of the circulatory system occur each year in the Region. In China, the estimated mortality from this disease is 2.4 million. The mortality rate is estimated at 114 per 100 000 population in 9 countries (Australia, Brunei Darussalam, China, Hong Kong, Japan, Malaysia, New Zealand, Philippines, and Singapore). In Australia, Japan, and New Zealand, death rates range from 250 to 300 per 100 000 while in the remaining countries, death rates range from 100 to 150 per 100 000. The prevalence of cardiovascular diseases in various ethnic groups with mortality rate is given below.

1.1.1 European Origin

People of European origin include those who originate from diverse backgrounds in Northern Europe (Nordic countries), Western Europe (e.g., United Kingdom and France), Southern Europe (e.g., Spain and Italy), and Eastern Europe (e.g., Poland and Ukraine). Differences in the Age-Standardized Mortality Rates (ASMR) vary widely between European populations. Data from the World Health Organization (WHO) indicate that the cardiovascular disease mortality rate is 6-fold higher among men and women in the Russian Federation compared with people in France. Although the CVD mortality rates are much lower among women compared with men, similar variations among women between countries also exist. Eastern European countries such as the Ukraine, the Russian Federation, Hungary, and the Czech Republic have among the highest and increasing CVD rates in the world, which is in marked contrast to most economically stable countries.
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1.1.2 Hispanics

The term Hispanic includes Americans of Cuban, Mexican, and Puerto Rican descent. There are approximately 35.3 million Mexican-Americans living in the US, and they comprise approximately 12.5% of the US population (US Bureau of the Census; 2000). CVD is the leading cause of death among Hispanic males (28%) and females (34%) (US Bureau of the Census; 2000). Although earlier studies suggested that the age-adjusted mortality rates for major CVD among Mexican-Americans (28.8 and 26.6 per 100,000 men and women, respectively) were lower than those of African-Americans and whites (Becker et al., 1988). Recent data from the Corpus Christi Heart Project, reported a greater incidence of myocardial infarction (MI) in Mexican-Americans compared with non-Hispanic whites (Goff et al., 1997). The age-adjusted MI incidence was higher among Mexican-American men and women compared with non-Hispanic whites (Stern and Gaskill, 1997) with greater MI case fatality rate among Mexican-Americans than non-Hispanic whites. Under the age of 60 years, Hispanics have a significantly elevated CVD death rate compared with non-Hispanic whites (32 versus 19 and 36 versus 18 per 100,000 in men and women, respectively). However, in older age categories the CVD rate in Hispanics is substantially lower than whites (589 versus 765 and 535 versus 847, respectively) (Gillium, 1995a). Although declines in CHD and CVD mortality have occurred in Mexican-Americans over the past 20 years, this decline has been less than that which has occurred among non-Hispanic whites (Stern and Gaskill, 1997).

1.1.3 Aboriginal Populations

The rates of chronic degenerative diseases such as CVD, diabetes, cancer, and mental illness are increasing among Aboriginal peoples. These trends parallel the epidemiological transition that is occurring in other developing populations throughout the world. Mortality rates for CVD among aboriginal populations had been reported to be lower than people of European ancestry; however, CHD is the leading cause of death in North American Indians and Alaskan Natives (US Bureau of the Census; 2000). The Strong Heart Study (Howard et al., 1995), which was initiated in 1988, studied 4549 American Natives aged 45 to 74 years from 13 tribes in the Southern US. The prevalence estimates of definite MI in those aged 45
to 74 years was 2.8% in men without diabetes and 5.3% in men with diabetes, and 0.4% in women without diabetes and 1.4% in women with diabetes. In the US, the CBVD mortality rate under the age of 65 years is similar in Native Americans and white Americans, and substantially lower than rates in African Americans (Gillium, 1995 b). In Canada, CVD is the leading cause of death among Aboriginal peoples. Although the CHD mortality rates among Aboriginal and Canadian males are similar, the CHD mortality among Aboriginal women is 61% higher compared with Canadian women. In addition, the stroke mortality rate is 44% and 93% higher among Aboriginal men and women, respectively, compared with the general Canadian population (Anand et al., 2001). However, all the above data are based on studies conducted 10 or more years ago. A recent prevalence study in Canada indicates a 2.5-fold higher rate of CVD among aboriginal peoples compared with Canadians of European origin (Anand et al., 2001). As more Aboriginal people give up their traditional lifestyles and adopt “urban” lifestyles, the prevalence of CVD and its risk factors will likely increase. Comparing Canadian data from 1979 to 1983 to data from 1984 to 1985 reveals a 25% decline in CHD among Native men, but a 5% increase among Native women (Mao et al., 1992). In the US, the age-adjusted rates of CBVD mortality declined by about 20% between 1980 and 1990 in Native Americans, which is similar to the 26% decline in white Americans (Gillium, 1995 b).

In a Canadian study, Aboriginal people had a higher prevalence of CVD, atherosclerosis, glucose abnormalities, obesity, and poverty compared with European Canadians. There is a clear inverse relationship between higher incomes and lower rates of risk factors and CVD. However, at each income level, aboriginals had higher risk factors and CVD compared with European Canadians (Anand et al., 2001).

1.1.4 West Indies
In Trinidad, data from 1989 reveal that the age-adjusted incidence of CHD in people of African origin was 6.8 and 5.4 per 1000 person years among men and women, respectively. The rates among black men approximated those of European descent (6.8 versus 6.5/1000 person years at risk), whereas the rates among black women were higher (5.4 versus 2.9/1000) (Miller et al., 1985).
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1.1.5 United States

African-Americans are the largest nonwhite population in the US and represent 12.9% of the population. CVD is the leading cause of death among African-Americans. Although the CHD mortality rate in African-American men is similar to that among white men (224 versus 236/100 000), it is higher among African-American women (160 versus 140/100 000) (US Bureau of the Census; 2000). Sudden cardiac death is more common among African-Americans (Gillium, 1985) In addition, the CBVD mortality rate is substantially higher among African American men (89 versus 62/100 000) and women (76 versus 58/100 000) compared with whites (US Bureau of the Census; 2000).

Since the 1960s, CV mortality has been declining among Americans of all races (Sempos et al., 1988, Pickle and Gillium, 1999). However, since the mid 1970s, the rate of decline has slowed down among African Americans (Akinkugbe, 1985), (Pearson et al., 1993) leading to a widening disparity in CVD death rates between blacks and whites.

ASIANS

1.1.6 Japanese

In parallel with a rise in economic prosperity, CVD rates in Japan have declined more markedly than those of western countries, and the life expectancy in Japan is among the highest in the world. Mortality rates from CHD have traditionally been much lower in Japan than in western countries (1997–1999 World Health Statistics Annual, WHO, 2000).

In Japan, the ASMR for CHD in males is 43/100 000 and in females is 22/100 000, which is one-fourth the rate of CHD in North America, and for CBVD is 72/100 000 and 46/100 000 among males and females, respectively (1997–1999 World Health Statistics Annual, WHO, 2000). This pattern of higher CBVD compared with CHD among Japanese differs from western populations (Shimamoto et al., 1989).

1.1.7 Chinese

Death rates from CVD (particularly CHD) have been increasing in China in recent decades (Woo and Donnan, 1989). Although the CVD mortality rate in China is
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approximately the same as that in the US, the CHD mortality rates are approximately 50% lower than the rates observed in most western countries, and the CBVD rate is significantly higher. In 1996, in urban China, the ASMR for CHD for men and women aged 35 to 74 was 100/100 000 in men and was 69/100 000 in women (World Health Statistics Annual. WHO; 1998). However, the ASMR for CBVD in men and women aged 35 to 74 was 251/100 00 in men and 170/100 000 in women (World Health Statistics Annual. WHO; 1998). Intracerebral haemorrhage occurs between 2 and 3 times more frequently in the Chinese than in white Caucasians. Only 6% to 12% of strokes in European populations are reported as intracerebral haemorrhages compared with 25% to 30% of hemorrhagic strokes in Chinese (Thorvaldsen et al, 1995).

1.1.8 South Asians

South Asian (SA) refers to people who originate from India, Sri Lanka, Bangladesh, Nepal, and Pakistan. The WHO and the World Bank estimate that deaths attributable to CVD have increased in parallel with the expanding population in India, and that CVD now accounts for a large proportion of disability adjusted life years (DALY) lost. Of all deaths in 1990, approximately 25% were attributable to CVD, compared with 10% from diarrheal diseases, 13% from respiratory infections, and 8% from tuberculosis (Murray and Lopez, 1996) SA migrants to the United Kingdom, South Africa, Singapore, and North America experience 1.5 to 4.0 times higher CHD mortality compared with indigenous populations) (Enas et al., 1992)

In India, the CHD rate is expected to rise in parallel with the increase in life expectancy secondary to increases in per capita income and declining infant mortality. The average life expectancy has increased from 41 years in the years 1951 to 1961, to 61.4 years in the years 1991 to 1996 and is projected to reach 72 years by 2030, which could lead to large increases in CVD prevalence (Reddy and Yusuf, 1998) By contrast, in the UK and Canada, although the CHD mortality rate of SAs compared with other populations remains high, a decline in CHD rates has been observed over the past 10 years (Sheth et al., 1999) These data indicate that the high rates of CHD with economic changes are reversible and perhaps even
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avoidable. Therefore, lessons learnt from migrant SAs may be helpful in developing prevention strategies for the Indian subcontinent.

1.1.8.1 Geographic Variations

Marked increases in both CHD prevalence and risk factors is observed in urban India compared with rural settings (Gupta and Gupta, 1996) A recent overview of prevalence surveys conducted over 2 decades in India reported a 9-fold increase of CHD in urban centers, compared with a 2-fold increase in CHD rates among rural populations (Gupta and Gupta, 1996) This increase in CHD rates in urban areas is associated with an increase in the prevalence of lipid and glucose abnormalities as well as hypertension and obesity. By contrast, the rates of tobacco smoking are higher in rural compared with urban populations. (Although these studies used somewhat different methods of sampling and varying definitions for CHD, collectively, they suggest that there is likely a real increase in CHD; however, the magnitude of the increase remains uncertain).

Since cardiac disease is typically progressive and often associated with inter-related disease states, these conditions also represent significant social costs requiring years of therapy and extensive health care costs. In 2000 alone, the total cost (direct and indirect) of CVD in the United States was estimated to be more than $275 billion. As the population ages and costs rise, this annual estimate is expected to increase (American Heart Association, 2000). Although these statistical data are intimidating, important advances in the understanding of CVD initiation and progression have emerged, and therapies continue to evolve as mechanisms are defined.

The data indicates that there is steep rise in the prevalence of cardiovascular diseases because of several factors. Fortunately, the mortality rate is decreasing due to availability of several interventions. The ability of surgical interventions to minimize myocardial loss following myocardial infarction has advanced dramatically over the past two decades. Since 1989, the death rate due to acute myocardial infarctions has declined 24%, while the actual number of deaths declined only 7% (American Heart Association: Heart Disease and Stroke Statistics—2002 Update). Over the last 40 years, especially during the 1980s, new pharmacologic agents, interventional cardiology procedures, and coronary artery
bypass surgical techniques have advanced and have led to a decrease in the overall morbidity and mortality associated with acute myocardial infarction (Lavie and Gersh, 1990; Goldberg et al., 1991). Despite this overall improvement, mechanical and electrical complications such as cardiogenic shock, rupture of the ventricular septum or free wall, acute mitral regurgitation, pericarditis, tamponade, and arrhythmias challenge the medical community caring for patients presenting with acute myocardial infarction on a daily basis (Lavie and Gersh, 1990; Goldberg et al., 1991). Of these complications, cardiogenic shock complicating acute myocardial infarctions has the most significant impact on in-hospital mortality and long-term survival. Loss of more than 40% of functioning left ventricular mass is the major cause of cardiogenic shock and is determined by both the degree of preinfarction ventricular dysfunction and the size of the infarcted vessel (Page et al., 1971; Alonso et al., 1973). Restoration of blood flow to the threatened myocardium offers the best chance of survival following acute coronary occlusion, but the means and timing of revascularization continue to be a highly debated and studied topic. Thrombolytics, percutaneous transluminal coronary angioplasty, and coronary artery bypass surgery have decreased the mortality associated with acute myocardial infarctions. At the same time the complications arising out after restoration of blood flow are the major concerns as most of these complications are believed to be because of reperfusion injury.

1.2. MYOCARDIAL INFARCTION AND PATHOPHYSIOLOGY

Myocardial ischemia due to coronary occlusion for as little as 60 seconds causes ischemic zone changes from a state of active systolic shortening to one of passive systolic lengthening (Tennant and Wiggers, 1935). Occlusions for less than 20 minutes usually cause reversible cellular damage and depressed function with subsequent myocardial stunning. Furthermore, reperfusion of the infarct leads to variable amounts of salvageable myocardium. After 40 minutes of ischemia followed by reperfusion, 60% to 70% of the ultimate infarct is salvageable, but this decreases dramatically to 10% after 3 hours of ischemia (Jennings and Reimer, 1983; Schaper, 1978) Animal model evidence has also demonstrated that 6 hours of regional ischemia produces extensive transmural necrosis (Reimer and Jennings, 1979). The exact timing in humans is even more difficult to analyze because of
collateral flow, which is a major determinant of myocardial necrosis in the area at risk in humans (Schaper, 1978). The collateral blood supply is extremely variable, especially in patients with long-standing coronary disease. However, collateral flow is jeopardized with arrhythmias, hypotension, or the rise of left ventricular end-diastolic pressure above tissue capillary pressure (Jennings and Reimer, 1983). Thus loss of collateral flow to the infarct area may lead to the cellular death of salvageable myocardium. Control of blood pressure and prevention of arrhythmias are vital during this immediate time after infarction.

Many clinical trials have shown the beneficial effects of early reperfusion within (Rahimtoola, 1989) hours after acute myocardial infarction (Sadanandan and Hochman, 2000). Although benefits of late reperfusion beyond (Rahimtoola, 1989) hours, particularly in asymptomatic patients, have yet to be shown in large clinical studies, advocates for aggressive management believe that reperfusion is warranted to preserve the border areas that may be underperfused during the early days after an infarction. While some of these patients may develop objective evidence of ischemia, the clinical assumption that a hypotensive patient with a suddenly dilated and pressure-overloaded ventricle is prone to losing more muscle mass in border zones of the infarct is reasonable. This is true even in patients who have had complete revascularization. Conservative measures, such as nitroglycerin and intra-aortic balloon pumps, have demonstrated their efficacy in this population of patients without clearly salvageable myocardium by improving coronary blood supply and reducing the work demand of the left ventricle. More radical approaches such as insertion of a left ventricular assist device (LVAD) have been advocated as well (Mancini et al, 1998; Chen et al, 1999).

Anatomically, the location of the coronary obstructive lesion and additional diseased vessels and the presence of collateral flow will determine the extent of early injury, especially for borderline areas. However, ventricular remodeling of the infarct has important consequences influencing ventricular function after myocardial infarction (Eaton et al, 1979). Thus appropriate and aggressive invasive therapies such as PTCA, IABP, CABG, controlled reperfusion, and LVAD insertion can mitigate myocardial injury and salvage borderline areas. However, since last two decades it has been noticed that the beneficial effects of restoration
of blood supply comes with consequences leading to injury known as reperfusion injury.

1.2.1 Reperfusion injury

It is major contributor to myocardial damage as free oxygen radicals are released and destroy endothelial cells and produce interstitial edema. The timing and management of reperfusion effects on myocardial damage may have an impact on both survival and functional recovery of individuals following acute myocardial infarction (Buckberg, 1986). Some centers have argued convincingly that controlled reperfusion with specially designed perfusate and a decompressed, energy-conserving ventricle resting on cardiopulmonary bypass is the best means to preserve muscle mass (Allen et al, 1993).

1.2.2 Cardiogenic Shock

Cardiogenic shock is defined clinically as a systolic blood pressure below 80 mm Hg in the absence of hypovolemia, peripheral vasoconstriction with cold extremities, changes in mental status, and urine output of less than 20 mL/h. Hemodynamic parameters for cardiogenic shock include cardiac index less than 1.8 L/min/m², stroke volume index less than 20 mL/m², mean pulmonary capillary wedge pressure greater than 18 mm Hg, tachycardia, and a systemic vascular resistance of over 2400 dyn·sec/cm⁵. These patients are defined as type IV by the Killip classification, a widely used system to classify myocardial infarctions (Killip and Kimball, 1972).

1.2.3 Prevalence

Shock is the most common cause of in-hospital mortality following myocardial infarction (Goldberg et al, 1991). The in-hospital mortality associated with cardiogenic shock has remained unchanged at approximately 80% despite the development of new treatment modalities (Goldberg et al, 1991). Cardiogenic shock occurs in 2.4% to 12.0% of patients with acute myocardial infarction (Gacioch et al, 1992). Since 1975, the incidence of cardiogenic shock complicating acute myocardial infarctions has remained constant at 7.5%, ranging between 5% and 15% (Goldberg et al, 1991). One reason these figures may have remained constant is the increasing efficiency of emergency medical systems in resuscitating patients in the community and bringing them to the hospital. Previously, these
patients would have died before reaching the hospital. Similarly, there has been a decrease in the incidence of out-of-hospital deaths due to coronary disease between 1975 and 1988 (Goldberg et al, 1991). The key to success in patients in shock is early intervention and revascularization. In a prospective randomized study, Hochman et al showed that revascularization within 6 hours of diagnosis of cardiogenic shock confers survival benefits, particularly in those patients under 75 years of age (Hochman et al, 1999 and 2001). Use of mechanical circulatory support also may play a role by resting stunned myocardium to allow its recovery and to prevent the irreversible end-organ injury that may result from prolonged shock (Mancini et al, 1998 and Chen et al, 1999).

1.2.4 Infarct Size and Shock

Shock is directly related to the extent of the myocardium involved. Myocardial infarctions resulting in loss of at least 40% of the left ventricle have been shown to result in cardiogenic shock (Page et al, 1971; Alonso et al, 1973 and Wackers et al, 1976). Autopsy findings also revealed marginal extension of the recent infarct and focal areas of necrosis in patients with cardiogenic shock (Page et al, 1971). Extensive three-vessel disease is usually found in individuals with cardiogenic shock, and extension of the infarct is an important determinant in those individuals (Page et al, 1971; Alonso et al, 1973 and Wackers et al, 1976). Limiting the size of the infarct and its extension is the key to therapeutic interventions in patients with myocardial infarction. By following creatinine phosphate kinase (CPK) levels, Gutovitz et al (1978) showed that the progression/extension of myocardial damage results in cardiogenic shock. Patients who develop shock have higher peak values.

1.2.5 States of Impaired Myocardium

Coronary insufficiency can result in three states of impaired myocardium: infarcted, hibernating, and stunned. Each state requires separate clinical interventions and carries different prognostic implications. Infarcted myocardium is irreversible myocardial cell death due to prolonged ischemia. Hibernating myocardium is a state of impaired myocardial and left ventricular function at rest due to reduced coronary blood flow that can be restored to normal if a normal myocardial oxygen supply-demand relationship is reestablished (Rahimtoola, 1989
Hibernating myocardium is defined as contractility-depressed myocardial function secondary to severe chronic ischemia that improves clinically immediately following myocardial revascularization. Stunned myocardium is left ventricular dysfunction without cell death that occurs following restoration of blood flow after an ischemic episode. If a patient survives the insult resulting from a temporary period of ischemia followed by reperfusion, the previously ischemic areas of cardiac muscle eventually demonstrate improved contractility.

1.2.6 Hibernating Myocardium

Hibernation may be acute or chronic. Carlson et al (1989) showed that hibernating myocardium was present in up to 75% of patients with unstable angina and 28% with stable angina. The entity also occurs after myocardial infarction. Angina after myocardial infarction commonly occurs at a distance from the area of infarction (Schuster and Bulkley, 1981). In fact, mortality is significantly higher in patients with ischemia at a distance (72%) compared with ischemia adjacent to the infarct zone (33%) (Schuster and Bulkley, 1981). It is the hibernating myocardium that may be in jeopardy and salvageable, although its presence is usually incidental to the occurrence of the acute infarction. By distinguishing between hibernating myocardium and irreversibly injured myocardium, a more aggressive approach to restoring or improving blood flow to the area at risk is reasonable. Function often improves immediately after revascularization of appropriately selected regions.

1.2.7 Stunned Myocardium

In the 1970s it was observed that after brief episodes of severe ischemia, prolonged dysfunction with gradual return of contractile activity occurred. In 1982 Braunwald and Kloner (1982) coined the phrase stunned myocardium. Stunning is a fully reversible process despite the severity and duration of the insult if the cells remain viable. However, myocardial dysfunction, biochemical alterations, and ultrastructural abnormalities continue to persist after return of blood flow. Within 60 seconds of coronary occlusion, the ischemic zone changes from a state of active shortening to one of passive shortening (Tennant and Wiggers, 1935). Coronary occlusion lasting less than 20 minutes is the classic model reproducing the stunning phenomenon (Braunwald and Kloner, 1982; Heyndrickx et al, 1975).
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The most likely mechanisms of myocardial stunning are calcium overload, generation of oxygen-derived free radicals, excitation-contraction uncoupling due to sarcoplasmic reticulum dysfunction, or a combination thereof. Other mechanisms that may contribute to the stunning phenomenon include insufficient energy production, impaired energy use by myofibrils, impaired sympathetic neural responsiveness, impaired myocardial perfusion, damaged extracellular collagen matrix, and decreased sensitivity of myofilaments to calcium (Bolli, 1990; Conti, 1991 and Marban, 1991).

Stunned myocardium can occur adjacent to necrotic tissue after prolonged coronary occlusion and can be associated with demand-induced ischemia, coronary spasm, and cardioplegia-induced cardiac arrest during cardiopulmonary bypass. Clinically these regions are edematous and even hemorrhagic. They also have a propensity for arrhythmias, which can lead to more extensive ventricular stunning and hypotension with subsequent infarction of these regions.

In summary, infarcted myocardium is nonviable myocardium, while hibernating myocardium is viable myocardium that is chronically dysfunctional due to impaired blood supply. Stunned myocardium is viable myocardium that is acutely dysfunctional after adequate blood supply has been restored.

1.3. INVOLVEMENT OF FREE RADICALS IN CARDIOVASCULAR DISEASES (CVDs)-

Several recent studies have demonstrated that altered oxygen utilization and/or increased formation of reactive oxygen species (ROS) contribute to CVD progression. Many recent studies have suggested that oxygen-derived free radicals may be important participants in a wide array of cardiac conditions, and several clinical trials evaluating the use of antioxidants as therapeutics either have already been conducted or are underway.

The initial suggestions of oxidative mechanisms during CVD were described in the acute settings of ischemia/reperfusion injury and MI. These conditions are associated with a sudden reduction of coronary perfusion and oxygen availability, leading to altered myocardial metabolism, ROS production, and cell death. Interestingly, ROS production and associated cellular damage is
higher in cardiac tissue during tissue reperfusion relative to ischemic conditions. The acute (and relatively severe) paradigm of cardiac ischemia and reperfusion has provided insight into the mechanisms of ROS-induced alteration of cardiac function and disease progression. In fact, it is now recognized that ROS may contribute to the progression of other more chronic cardiovascular conditions that are not related to acute oxygen deprivation. In addition to cellular and/or tissue evidence of oxidative damage, elevated levels of oxidative stress markers are detected in several pathologic conditions of cardiovascular disorders, including hypertension, ventricular hypertrophy, atherosclerosis, and CHF (Carlos et al., 1998; Gokce et al., 1999; Harjai, 1999; Keith et al., 1998; Miller et al., 1998; Suzuki et al., 1998).

In summary, despite the diverse etiology of cardiovascular conditions, the enhanced production of ROS and altered oxygen utilization is apparently a common phenomenon and a participant in disease progression. Further understanding of the events that contribute to these changes, and the cellular adaptations involved, may provide new opportunities for rational therapeutic strategies.

1.3.1 Sources of reactive oxygen/nitrogen species during cardiovascular disease

The general phenomenon of increased ROS production in CVD is becoming increasingly evident, but the actual sources of these species and mechanisms involved may not be identical in all conditions. Important ROS in mammalian cells include superoxide anion (O$_2^-$), hydroxyl radical (OH$^-$), and hydrogen peroxide (H$_2$O$_2$). Recent investigations have suggested that the reactive nitrogen species peroxynitrite (ONOO$^-$) also plays an important role in cardiovascular dysfunction. As described elsewhere, there are several potential contributors to cellular ROS and ONOO$^-$ increases during CVD, but the major sources of oxidants can be different in various settings, such as in acute ischemia/reperfusion (e.g., MI) and chronic conditions (e.g., heart failure). While direct evidence of ROS-induced cardiac injury during hypoxia or ischemia/reperfusion in humans is lacking (due to inadequate methodology), many studies have shown increases in biomarkers of oxidant production and/or decreases
in antioxidant capacity during myocardial ischemia (Buffon et al., 2000; Miwa et al., 1999). Furthermore, the administration of antioxidants reduces cardiac cell injury and dysfunction in acute MI (Singh et al., 1996), coronary angioplasty (Rajakumar et al., 1999), and open heart surgery that mimics myocardium ischemia/reperfusion (Fabiani et al., 1993).

1.3.1.1 Uncoupling of mitochondrial electron transport

This is a classical mechanism of intracellular oxidant production. Under normal physiological conditions, oxygen is essential for mitochondrial oxidative phosphorylation reactions and for production of ATP. The potentially toxic species (i.e., ROS) are formed intracellularly during mitochondrial electron transport, and are controlled by intracellular antioxidant defense. The lack of oxygen supply by either hypoxia (reduction of arterial oxygen partial pressure, but sufficient perfusion) or ischemia (reduction or interruption of coronary blood flow) disrupts mitochondrial electron transport chain, resulting in an accumulation of toxic metabolites, acidosis, ATP depletion, intracellular Ca\textsuperscript{2+} overload, mitochondrial membrane depolarization, matrix swelling, and cell death (Lemasters et al., 1997). Interestingly, it is now well established that although restoration of blood flow prevents progression of ischemic cell necrosis, it also causes "reperfusion injury" in surviving cells (Ambrosio & Tritto, 1999). This phenomenon is associated with a massive production of ROS due to the resumption of oxygen supply to mitochondrial respiration. Several experimental hypoxia or ischemia/reperfusion models, both in vitro and in vivo, have suggested that injury in the myocardium is caused by oxygen radicals from mitochondrial electron transport (Lesnefsky et al., 1997; Vanden Hoek et al., 1997 a, 1998). The generation of large amounts of ROS can overwhelm the intracellular antioxidant defense network, causing activation of neutrophils, lipid peroxidation,

1.3.1.2 Immune cell infiltration and cytokines

It is now well established that many immune cells produce free radicals as a means of host defense and pathogen killing. As such, infiltration of activated immune cells into cardiac muscle is a potential mechanism of cardiac oxidant production. The ROS burst during posts ischemic reperfusion is accompanied by cell death and tissue injury, which can trigger an acute inflammatory response. The
expression of immune cell chemo-attractants and surface cell adhesion molecules leads to the infiltration of the tissue by immune cells (particularly neutrophils). These activated neutrophils may cause more damage to the tissue by the secretion of several mediators, including ROS, proteolytic enzymes, and pro-inflammatory cytokines.

It has been shown that inhibition of neutrophil migration reduces reperfusion cardiac injury in feline (Buerke et al., 1994) and ischemic human hearts (Fabiani et al., 1993). Furthermore, clinical studies in cardiopulmonary bypass (which mimics global cardiac ischemia/reperfusion) patients have demonstrated that neutrophil activation, leukocyte-platelet aggregation, and cytokine release from cardiac tissue are the primary contributors of an acute inflammatory reaction during post-ischemic reperfusion (Zahler et al., 1999). These findings suggest that neutrophils (and other immune cells, including monocytes/macrophages) are key participants in at least the early events in acute hypoxia/ischemia. This mechanism may be an early response to tissue injury, and may further accentuate cardiac dysfunction and remodeling. It is important to note that immune cell infiltration during chronic settings of cardiac disease is less well-established.

1.3.1.3. Induction of oxidative enzymes

While "escape" of ROS from the mitochondria has been a classical mechanism of intracellular oxidation, many recent studies suggest that non-mitochondrial sources may be equally or perhaps more important in some cardiovascular conditions. Activation and/or induction of cytosolic oxidases (such as NADH/NADPH oxidase, xanthine oxidase [XO], and nitric oxide synthase [NOS] isoforms) have been demonstrated during several physiological stress conditions.

The potential significance of these processes is described in the following sections.

1.3.1.3.1. Xanthine oxidase

Under normal physiological conditions, XO is a key enzyme in the purine degradation pathway. The enzyme generates the final product, uric acid (excreted by urine), and the byproduct, superoxide anion. Under normal physiological
conditions, this enzyme is localized almost exclusively in the liver and the mucosa of the small intestine. However, excess ROS production, during chronic hypoxia or in the presence of increased inflammatory cytokines, can enhance XO activity and its release into the plasma. It has been demonstrated that significantly increased XO activity (and superoxide production) in the mesenteric tissue may be responsible for escalating vascular tone in an animal model of essential hypertension (Suzuki et al., 1998). Recent studies suggest that the elevated levels of circulating XO can be concentrated several-fold in the vascular tissue, and may be a significant participant in endothelial dysfunction in hypercholesterolemic rabbits and atherosclerotic humans (Houston et al., 1999). In addition to plasma increases in XO, induction of XO in cardiac tissues may also contribute to excess production of superoxide. It is important to note that the abundance and activity of XO, as well as endogenous antioxidant defense in the myocardium, is species-specific (Janssen et al., 1993). For example, XO mean activity (mU/g protein) is considerably higher in mice (33), rats (28.5), and guinea pigs (14.4) than in cows (3.7), rabbits (0.59), humans (0.31), and pigs (< 0.1) (de Jong et al., 1990). Despite the differences in basal XO levels, it has been shown that pretreatment with the XO inhibitor allopurinol improved cardiac function in isolated ischemic/reperfused rat and rabbit hearts (Brown et al., 1988; Terada et al., 1992) and in human trials during coronary bypass surgery (Bochenek et al., 1990; Castelli et al., 1995; Coghlan et al., 1994).

Thus, XO may play a role in some conditions of cardiac oxidative stress, and the clinical value of its inhibition awaits further large-scale trials.

### 1.3.1.3.2. NADH/NADPH oxidase

The enzyme NADPH oxidase generally is found in phagocytic cells. It plays an important role in nonspecific host defense during infection by generating a large quantity of superoxide millimolar. Recently, it has been demonstrated that vascular NADH/NADPH oxidase significantly contributes to superoxide production in all components of the vasculature, i.e., the endothelium, the medial smooth muscle, and the adventitia (Bayraktutan et al., 1998; Di Wang et al., 1999). Participants in cardiac disease such as angiotensin II (AngII) may activate superoxide production in vascular tissue via this enzyme. This enhanced
production of superoxide has been implicated in the pathogenesis of AngII-induced vascular hypertrophy and endothelial cell dysfunction (Griendling et al., 1994; Rajagopalan et al., 1996; Ushio-Fukai et al., 1998; Wattanapitayakul et al., 2000a).

1.3.1.3.3. Nitric oxide synthases

It recently has been recognized that several cytokines (e.g., interleukin [IL]-1b, IL-6, interferon-g, and tumor necrosis factor [TNF-α] and growth factors (e.g., insulin-like growth factor-1 and transforming growth factor-b1) are pro-oxidants, and elevated levels are commonly found in the plasma of patients with heart disease (Djurovic et al., 1999; Ruotolo et al., 2000; Torre-Amione et al., 1996; Ueda et al., 1999). Increased nitric oxide (NO) production via induction of NOS has been suggested as a major mechanism by which cytokines mediate cardiac contractile dysfunction and development of heart disease (Sawyer & Colucci, 1998; Schulz et al., 1995; Wildhirt et al., 1995).

The cytokine-induced increases in oxidants are regulated by various stimuli in several cell types engaged in tissue repair and restoration of homeostasis, as well as immune response. For example, increased cytokine release was found in acute hypoxia followed by reperfusion and in myocardium stunning. These cytokines enhance the expression of a variety of cell adhesion molecules (e.g., intercellular adhesion molecule-1, vascular cell adhesion molecule, and monocyte chemoattractant protein-1) in the myocardium, leading to transient leukocyte sequestration and its transmigration to the areas of cardiac injury (Kacimi et al., 1998; Matsumori et al., 1997). In addition to acute activation of cytokines in MI, elevated plasma levels of proinflammatory cytokines, along with high oxidase activities, are commonly observed in a more chronic pathologic condition of the cardiovascular disorders such as in heart failure patients (Katz et al., 1994; Leyva et al., 1998; Milani et al., 1996; Torre-Amione et al., 1996). Recent studies suggest that the heart per se is capable of synthesizing biologically active TNF-α, which may be responsible for the progression of heart diseases (Bergman & Holycross, 1996; Kapadia et al., 1995). Additionally, other potent oxidants can be generated from reactions of NO with other ROS. For example, NO avidly reacts with superoxide anion at diffusion-limited rates to form the potent oxidant ONOO⁻ (Beckman, 1996). The presence of ONOO⁻ and its biological marker 3-
nitrotyrosine has been associated with several oxidant-related pathologic conditions, including the atherosclerotic lesion, endothelial cell dysfunction, ischemia/reperfusion injury, MI, and heart failure (Bauersachs et al., 1999; Buttery et al., 1996; Kooy et al., 1997; Wattanapitayakul et al., 2000a). Clearly, the actions of NO in vivo may be governed not only by production capabilities, but also by the setting and chemical environment in which it is formed.

Reactions of ONOO⁻ with pathologically relevant molecules have been implicated in oxidative-related cardiovascular disorders (see Section 4). Additionally, researchers have found evidence that the ONOO⁻ biomarker 3-nitrotyrosine (free amino acid) is toxic to endothelial cells (Mihm et al., 2000).

1.4. CONSEQUENCES OF CARDIAC OXIDATIVE EVENTS

1.4.1. Cardiovascular dysfunction

Not only are oxidants associated with CVD, they actually may mediate some aspects of cardiac and vascular dysfunction. For example, in both experimental and pathologic conditions, impaired vascular function and decreased cardiac performance are mediated by ONOO⁻ and other ROS (BouloumieÂ et al., 1997; Ferdinandy et al., 1999; Matsuura & Shattock, 1991a, 1991b; Miller et al., 1998; Rubanyi & Vanhoutte, 1986; Terada, 1996). ROS have direct impact on myocardial function through inhibition of the sarcoplasmic reticulum (SR) Ca\(^{2+}\) pump in the cardiac contraction-relaxation cycle (Matsubara & Dhalla, 1996; Temsah et al., 1999). Cardiac depression (cardiac stunning) during ischemia/reperfusion and tissue damage in acute MI was prevented by using superoxide dismutase (SOD) and the antioxidant, vitamin E (Carrasquedo et al., 1999; Nagel et al., 1997). Benefits from inhibition of superoxide production have also been shown in cytokine-induced cardiac dysfunction (Cheng et al., 1999).

Pro-inflammatory cytokines and immune cell activation are modulators of cardiovascular function by a variety of mechanisms, including the generation of oxygen-derived free radicals and the production of NO (Cheng et al., 1999; Ing et al., 1999). Elevated plasma and tissue levels of the cytokine TNF-\(\alpha\) are commonly observed in severe cardiac depression, along with increased NO production (Fukuchi et al., 1998; Torre-Amione et al., 1996; Wildhirt et al., 1995).
In contrast, it has been suggested that NO plays a protective role in ischemia and reperfusion by quenching the effects of superoxide (and other ROS) on mitochondrial Ca\(^{++}\) homeostasis (Hotta et al., 1999). NO is important in reducing ischemia/reperfusion-induced adhesion of monocytes to post-ischemic endothelium and in inhibiting induction of pro-inflammatory cytokine and adhesion molecule synthesis (Grisham et al., 1998). Endothelial derived NO plays a key role in the local regulation of vasomotor tone and the prevention of thrombus formation. Studies from our laboratory and others have suggested that reduction in the bioavailability of NO and endothelial cell dysfunction may be initial events in atherosclerosis and cardiovascular disorders (Ferrari et al., 1998; Lyons, 1997; Wattanapitayakul et al., 2000a). Additionally, endothelial cell dysfunction is one of the earliest events in the pathogenesis of myocardial reperfusion injury, and NO plays an important role in cardioprotection during reperfusion by directly enhancing coronary blood flow and by preventing adhesion of immune cells (Kupatt et al., 1996). The early decline in coronary NO release occurs simultaneously with the oxygen-derived free radical burst observed in the ischemic/reperfused heart (Zahler et al., 1999), suggesting that superoxide-induced reductions in NO may play a role in disease initiation and/or progression.

1.4.1.1. Too much or too little nitric oxide?

Involvement of NO in CVD is different in specific organs/tissues and pathologic environments. Depending on tissue availability, NO can act as a "good guy" (inhibit platelet aggregation, prevent immune cell infiltration, maintain vascular tone, etc.) or a "bad guy" (induce cardiac dysfunction, activate apoptosis, generate a potent oxidant ONOO\(^{-}\), etc.). Since NO is highly regulated (i.e., produced and acts locally), its bioavailability is dictated by the surrounding chemical environment (e.g., destroyed by superoxide or protected by SOD or antioxidants) and the amounts produced (e.g., induction of enzyme NO synthases). Thus, the net concentrations of NO at the tissue level may predict its protective effects or toxic effects. Furthermore, several recent investigations suggest that genetic polymorphisms of the NOS3 isoform (also known as endothelial NOS) occur in humans and that these molecular variations may play a role in NO control and CVD risk (Wattanapitayakul et al., 2000b).
1.4.2. Cell death

Two types of cell death—necrosis and apoptosis—are both implicated in the oxidative-related cell loss in cardiovascular tissue. Generally, necrotic cell death is associated with inflammatory cell infiltration and subsequent collagen deposition and scar formation, while apoptotic cell death is differentiated by ultrastructural and biochemical features, such as cytoplasmic and nuclear condensation, formation of the membrane-bound apoptotic body, and DNA fragmentation (180 bp). Although recognized since 1972, the apoptotic phenomenon has only been described in CVD over the last decade. It appears that cardiac and vascular cell loss observed during the remodeling process occurs in the absence of necrosis. This event may be exclusively controlled via apoptotic signalling pathways. Discussed in the following sections is the involvement of ROS in both types of cell death in the models of CVD.

1.4.2.1. Necrosis

Prolonged ischemic conditions may cause irreversible myocardial cell injury and cell death via necrosis. Transmigration of immune cells from the vasculature into the myocardium results in the release of toxic mediators that induce myocardial cell dysfunction and necrotic cell death (Grisham et al., 1998). Unlike apoptosis, necrosis generally does not appear in more chronic oxidative conditions, but occurs primarily in prolonged ischemic conditions (Taimor et al., 1999).

1.4.2.2. Apoptosis

Recently, the recognition of a different cell death phenomenon, "apoptosis," has become of major clinical interest. It accounts for a great proportion of cell death associated with MI and/or myocardial ischemia/reperfusion. Cell loss through apoptosis contributes to the impairment of cardiac performance, and also plays an important role in the myocardial and vascular remodeling processes. Induction of apoptosis is implicated in atherogenesis and cardiac dysfunction. Not only ROS per se, but also their oxidative products and other secondary messenger molecules generated by ROS can trigger the programmed cell death. For example, oxidized low-density lipoprotein (oxLDL) has been shown to induce DNA fragmentation and apoptosis in macrophages (Kinscherf et al., 1998).
antioxidant N-acetylcysteine prevented apoptosis induced by oxLDL, ceramide, TNF-α, and H₂O₂ (Kinsherf et al., 1998; Inserte et al., 2000).

ROS-induced cardiac apoptosis is mediated through several signalling systems, including intracellular Ca²⁺, cytokines, lipid oxidation, and proto-oncogene activation. The perturbation of intracellular Ca²⁺ homeostasis by the cellular redox state can aggravate free radical reactions and can activate endonucleases via activation of caspases, key enzymes in the apoptotic pathway. Additionally, many recent studies have demonstrated that the intrinsic degree of oxidant production regulates cellular susceptibility to apoptosis through both p53-dependent and p53-independent pathways (Lotem et al., 1996; von Harsdorf et al., 1999). Different ROS activate distinct signalling pathways for programmed cell death in cardiac myocytes (von Harsdorf et al., 1999).

It has been shown that the saturated fatty acids palmitate and stearate induce apoptosis in neonatal rat myocytes (de Vries et al., 1997). Both palmitate and stearate are precursors of de novo synthesis of ceramide—a second messenger of the sphingomyelin signalling pathway. This signalling pathway is initiated by the hydrolysis of the plasma membrane sphingomyelin to ceramide. Ceramide and sphingolipid metabolites are involved in the antiproliferative responses and apoptosis in several cell types, including cardiac myocytes. Several stimuli known to trigger sphingolipid-induced apoptosis include TNF-α, interferon-γ, ionizing irradiation, and ischemia/reperfusion (Sparagna & Hickson-Biek, 1999). The cytokine TNF-α is pro-apoptotic via several signalling pathways, including activation of the Fas ligand and the binding of TNF-α to the death domain of TNF receptors. Binding of TNF-α and the Fas ligand to their receptors results in degradation of sphingomyelin to ceramide, which mediates apoptosis through induction of c-Jun N-terminal kinase (JNK)/stress-activated protein kinase (SAPK).

NO can mediate both pro-apoptotic and anti-apoptotic signals, depending on the level of oxidants/antioxidants and predominant regulatory pathways endowed in the cell types (Kim et al., 1999; Hotta et al., 1999). For example, it appears that NO-induced apoptosis in vascular smooth muscle cells is mediated by...
cyclic GMP accumulation, while intracellular elevation of cyclic GMP, in response to NO activation, inhibits apoptosis in PC12 cells and hepatocytes. The formation of ONOO' from the interaction of NO and superoxide may account for the pro-apoptotic effects of NO. ONOO' is a potent oxidant that induces DNA fragmentation and p53-dependent apoptosis. In addition, the accumulation of the tumor suppressor protein p53 is a crucial and early event in NO-mediated apoptosis (Kim et al., 1999).

In summary, ROS-induced changes in cellular signalling and gene expression, leading to apoptosis, involve multiple pathways. There is not one unifying pathway in all pathologic conditions of CVD. Rather, it is a complex and intertwined process, requiring several selective participants in each pathologic condition or cell type.

1.4.3. Altered endogenous antioxidant defenses

Oxidative stress is a reflection of excess intracellular concentrations of oxidants, such as H$_2$O$_2$ and O$_2^-$, as well as antioxidants defense molecules such as glutathione (GSH). The tripeptide GSH (consisting of glutamate, cysteine, and glycine) is important for cellular defense against ROS toxicity. It is the key cellular reductant to maintain the redox state of cysteine-thiol linkages in proteins. The intracellular levels of oxidized GSH (GSSG) is increased by the metabolism of H$_2$O$_2$ and by GSH peroxidase, but decreased by extracellular GSSG export and GSH reductase. Depletion of the protective form of GSH by excess amounts of ROS leads to increased protein oxidation at the cysteine-thiol linkage. This modification has significant impact on protein conformation and protein function. Additionally, these alterations can occur in important proteins that function as receptors, enzymes, or signal transducers, thus impairing normal cellular processes. Additionally, GSH participates in maintaining ascorbic acid (vitamin C), an antioxidant, in its reduced form (the active form). Low levels of GSH are associated with a number of disease conditions known to generate high amounts of ROS, such as observed in atherosclerosis, heart failure, diabetes, neurodegenerative disorders, and acquired immunodeficiency syndrome. Increased oxidative stress in CHF patients is associated with increased GSH peroxidase and decreased plasma antioxidant vitamins, e.g., vitamins C and E (Keith et al., 1998).
In addition to GSH, the most important endogenous antioxidant defense against superoxide is the enzyme SOD. Three isoforms of SOD have been cloned and identified: mitochondrial manganese-containing SOD (Mn-SOD), cytosolic copper/zinc SOD (Cu/Zn-SOD), and extracellular SOD (EC-SOD). Cu/Zn-SOD plays an important role in protecting NO from destruction by superoxide in the endothelium (Harrison, 1997).

An important question is whether the long-term consequences of oxidative stress are due to excess production of ROS or depletion of antioxidant defenses. While a myriad of evidence supports increased ROS production during CVD, depletion of endogenous chemical antioxidants and some reductions in antioxidant enzyme capacity have also been reported (albeit, at a less frequent rate). For example, reductions in cardiac antioxidant enzyme function, including GSH peroxidase, catalase, and SOD isoforms, have all been observed in a variety of animal models of cardiac disease (Singal et al., 1993; Kapoor et al., 1997; Lin et al., 1997; Haramaki et al., 1998). Interestingly, the potent oxidant ONOO⁻ can inactivate the Mn-SOD via nitration of a specific tyrosine residue at its active site (Yamakura et al., 1998; MacMillan-Crow & Thompson, 1999), further promoting ROS availability. Whether deficient enzyme function is consistently involved in human cardiac disease is not clear, but recent studies also suggest that genetic variation in enzyme expression may be an important contributor to disease risk. For instance, polymorphic variations in the Mn-SOD isoform have been linked to increased risk of dilated cardiomyopathy (Hiroi et al., 1999). Several recent studies have also suggested that a high degree of EC-SOD genetic variant is associated with coronary artery disease and other risk factors (Wang et al., 1998, 1999). Clearly, excess production of ROS is not the only predictor of CVD risk; depletion of antioxidant reserve is also critical. Further research in the areas of defining oxidant and antioxidant balance during disease and the participation of genetic variations in the antioxidant defense network is warranted.

1.4.4. Altered lipid and protein metabolism/function
1.4.4.1. Lipid metabolism and lipid peroxidation

Lipids, both in free and bound forms, are vulnerable and represent one of the immediate targets of ROS. The overall effects of lipid peroxidation include
diminishing membrane fluidity, increasing membrane permeability, destabilizing membrane receptors, and inducing immune response to altered phospholipids. Described in the following sections are three major lipid categories that contribute to the pathogenesis of CVD.

1.4.4.1.1. Long-chain free fatty acids.

Elevated plasma levels of free fatty acids (FFA) are implicated in many pathologic conditions, as observed in myocardial ischemia, diabetes, hyperlipidemia, and cardiac hypertrophy (Sparagna & Hickson-Bick, 1999). Under normal physiological conditions of the heart, FFA are the preferred source of energy generated via β-oxidation within the mitochondrial matrix. FFA are estimated to account for 60-70% of the oxygen consumption for energy production (Grynberg & Demaison, 1996). However, β-oxidation cannot proceed under oxygen-deprived conditions, such as in hypoxia or ischemia, where FFA and their metabolites become harmful. When FFA metabolism is inhibited, metabolic intermediates are accumulated and incorporated into cell membranes, i.e., sarcolemma, SR, and mitochondrial membrane. The intermediates of lipid metabolism interfere with membrane integrity and the function of membrane-bound enzymes (by altering their conformations and affecting ion pumps). High levels of FFA and their metabolites impair Ca++ homeostasis and ion gradients, which may lead to cardiac arrhythmias during ischemia/reperfusion (Hendrickson et al., 1997).

1.4.4.1.2. Membrane lipids.

In addition to oxidation of FFA in the cytosolic compartment, ROS also react with membrane-bound lipids leading to "lipid peroxidation." Generally, reperfusion injury occurs when oxygen from the recirculating blood is exposed to reactive intermediate compounds formed during the ischemia. The interactions of those compounds lead to the generation of oxygen free radicals and highly active molecules, such as the superoxide anion, hydroxyl radical, and hydrogen peroxide. These ROS further react with polyunsaturated lipids in membranes generating lipid peroxidation products that can inhibit protein synthesis and alter enzyme activities. Oxidation of polyunsaturated fatty acid moieties of membrane phospholipids can cause membrane disintegration, mitochondrial dysfunction, and Ca++ overload. It
has been shown that altered cellular redox state and increased lipid peroxidation are associated with the transition of cardiac hypertrophy to heart failure (Dhalla et al., 1996). Elevated levels of plasma lipid peroxides were also observed in CHF patients (Keith et al., 1998; DöAaz-VeAlez et al., 1996).

1.4.4.1.3. Lipoproteins.

It is widely accepted that hypertriglyceridemia and abnormal lipoprotein profile are associated with an increase in cardiovascular risk. Polyunsaturated fatty acid residues in lipoproteins are chemically vulnerable to free radical oxidation. Unlike the superoxide anion, other oxidizing species, such as the hydroxyl, peroxyl, and alkoxy radicals, are capable of entering the hydrophobic membrane interior and initiating free radical chain reactions (Maxwell & Lip, 1997). Therefore, endogenous antioxidants in the hydrophilic cellular compartment cannot prevent propagation of the carbon-centered radical and oxidation reaction, which leads to long-chain lipid (hydro)peroxidation. These unstable peroxides are degraded rapidly and form secondary products that are toxic to cells. These adducts can interact with LDLs, resulting in oxLDLs. These modified LDLs abnormally affect cellular recognition and are chemotactic for circulating monocytes and toxic to endothelial cells (Diaz et al., 1997). After monocytes enter the arterial wall, they differentiate into macrophages and take up oxLDLs due to their immunogenicity. The unregulated uptake of LDLs leads to the formation of foam cells, and ultimately results in fatty streak-the first phase of an atherosclerotic lesion. Studies in isolated human endothelial cells has also shown cytotoxic effects of oxLDLs mediated by both GSH depletion and the GSH-independent pathway (Therond et al., 2000). oxLDLs promote production of several cytokines, immune cell chemoattractant proteins, and growth factors. In addition, they increase platelet aggregation, which aggravates the lesion and causes arterial wall thickening (Zhao & Xu, 2000; Maeno et al., 2000). The discovery of novel oxLDL receptors-oxLDL receptor-1 and lectin-like oxLDL receptor-1-has provided a more mechanistic insight into oxLDL-induced atherogenesis.

Expression of these receptors in vascular cells is sensitive to cytokine activation (Minami et al., 2000; Kume et al., 2000). Circulating oxLDLs may enhance the progress of atherosclerosis via regulation of the redox-sensitive
transcription factor natural factor (NF)-kB through ligand-receptor binding (Cominacini et al., 2000). These data suggest that oxLDLs are not merely a by-product of lipid oxidation, but may act as a signalling molecule and a key participant in the initiation and progression of atherosclerotic disease.

1.4.4.2. Post-translational protein modifications

In addition to their direct impact on cardiac function, ROS can oxidize amino acid side chains and the protein backbone, leading to protein-protein cross-linking and protein fragmentation (Berlett & Stadtman, 1997). As a consequence of these modifications, signalling proteins may not function properly, which may lead to organ malfunction and even cell death. Two major protein derivatives have been used as markers of ROS-mediated protein modifications: protein carbonyl derivatives and protein nitrotyrosine derivatives. The presence of carbonyl groups in proteins has been referred to as ROS-mediated protein oxidation, while nitrated tyrosine residues have been identified as a product of peroxynitrite-dependent nitration (ONOO\(^-\), mainly derived from the interaction of O\(_2^\cdot\) and NO). Elevated levels of nitrated proteins are observed in cardiac tissue from animals and patients under various conditions of excess oxidant production, including ischemia/reperfusion, CHF, and myocardial sepsis (Flesch et al., 1999; Kooy et al., 1997; Lopez et al., 1997; Luoma et al., 1998). However, it is not clear whether protein nitration/oxidation causes cardiovascular dysfunction or if it simply represents excess oxidant production.

1.4.5. Altered signal transduction

Recent studies have demonstrated that ROS may indeed act as signal transduction molecules. For example, cellular H\(_2\)O\(_2\) was transiently increased upon activation of platelet-derived growth factor (Sundaresan et al., 1995). Several signalling pathways are affected by the platelet-derived growth factor-induced increase in H\(_2\)O\(_2\) concentration, because of the effect of H\(_2\)O\(_2\) on tyrosine phosphorylation, mitogen-activated protein kinase (MAPK) activation, DNA synthesis, and chemotaxis. Additionally, H\(_2\)O\(_2\) activates hypertrophic and apoptotic signalling pathways in cardiac myocytes (Chen et al., 2000). ROS activate a wide variety of cellular signalling molecules and pathways, including Ca\(^{++}\), protein tyrosine kinases, serine/threonine kinases, and phospholipases (Kamata & Hirata,
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1999). Components of cell signalling pathways known to be associated with ROS activation are discussed in Sections 4.5.1 and 1.4.5.2.

1.4.5.1. Ca**++** signalling

Ca**++** is a second messenger that regulates a broad array of biological processes in cardiac tissue, including contraction, neurotransmission, gene expression, and cell growth. Two sources of Ca**++**-influx across the sarcolemmal membrane and from the sarcoplasmic reticulum (SR) of cardiac cells are critical for excitation-contraction coupling in cardiac tissue. Increases in ROS during ischemia/reperfusion induce excessive intracellular Ca**++** accumulation (Tani, 1990; Kaneko et al., 1994). Suggested mechanisms of oxidant-induced Ca**++** influx from extracellular to intracellular space include increased membrane ATP-dependent Ca**++** binding, activation of Ca**++** and K+ channels, changes in Na+, Na+ /Ca**++** exchangers and Na+ /K+ -ATPase activities, and stimulation of the adrenergic system (Chakraborti et al., 1998). Additionally, an increase in cytosolic Ca**++** is transiently enhanced by the inhibition of the Ca**++** pump of the SR, resulting in passive movement of SR Ca**++** to the cytosolic space (Suzuki et al., 1997). Other cellular components, such as the mitochondria and Ca**++**-binding proteins, have also been suggested as sources of oxidant-induced Ca**++** release.

1.4.5.2. Protein phosphorylation

Protein phosphorylation regulates a wide array of cellular signalling pathways that carry signals through different functional proteins, including enzymes, receptors, transcription factors, and contractile elements (Suzuki et al., 1997). Generally, oxidant-related stimulation of protein phosphorylation acts through the enzymes that regulate the balance of phosphorylation (kinases) and dephosphorylation (phosphatases) of specific amino acid residues of signalling proteins.

The overall increases in protein phosphorylation generally reflect stimulation of the signalling pathway. Two well defined subtypes of protein phosphorylation are discussed in forthcoming sections.

1.4.5.2.1. Tyrosine phosphorylation.

Most growth factor receptors are transmembrane tyrosine kinases, with the exception of transforming growth factor-b and insulin-like growth factor-II. These
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receptor kinases are sensitive to ROS stimulation, leading to activation of downstream signal transducers, such as the protein kinases of the MAPK cascade, phospholipase C, protein kinase C, and phosphatidylinositol-3-kinase (see the next section) (Kamata & Hirata, 1999; Yamamoto et al., 2000).

1.4.5.2.2. Serine/threonine phosphorylation.

The MAPK superfamily of protein serine/threonine kinases is a group of enzymes that are involved in the regulation of cell growth, proliferation, and differentiation, as well as oxidative responses. There are three major subfamilies of MAPKs, including the extracellular responsive kinases (ERKs), the c-Jun N-terminal kinases (JNKs, also known as SAPKs), and the p38-MAPKs. The ERKs appear to be associated with cell growth and differentiation, while the JNKs and the p38-MAPKs are involved in responses to cytotoxic insults. In the heart, the ERKs are primarily activated by G-protein-coupling receptor agonists, such as α-adrenergic receptor agonists, AngII, and endothelin-1, leading to activation of the phospholipase C cascade and ultimately activation of protein kinase C (Choukroun et al., 1998; Yamazaki et al., 1998). The release of ROS, proinflammatory cytokines, and humoral factors during ischemia/reperfusion activates ERKs, which are known to be responsible for cardiac hypertrophy in the non-ischemic zone of the heart to compensate for myocyte loss (ventricular remodeling). Additionally, JNKs and p38-MAPKs are activated during global cardiac ischemia and after incubation of H2O2, TNF-α, or IL-1β with cultured myocytes (Sugden & Clerk, 1997, 1998). Inhibition of p38-MAPK attenuated ischemia-induced cardiac apoptosis and decreased TNF-α production in cardiac tissue of experimental animals and humans (Cain et al., 1999; Ma et al., 1999; Mackay & Mochly-Rosen, 1999).

1.4.6. Altered gene expression

It has become increasingly evident that ROS are more than simply cellular toxicants and that they may be important modulators of cellular gene expression patterns. For example, redox cycling of cysteinyl residues is one of the important mechanisms of ROS-regulated activity of transcription factors and signalling molecules (Dalton et al., 1999). Disruption (reduction) or formation (oxidation) of disulfide bonds play a central role in determining a protein conformation.
Conformation is critical for proper protein-protein and protein-DNA interactions, which, in turn, drive specific signal transduction pathways. ROS-induced alterations in gene expression are mediated by activation of transcription activators, such as nuclear factor-kB (NF-kB) and activation protein-1 (AP-1). Changes in early response genes, such as egr-1, hsp70, c-fos, c-jun, and c-myc, are detected within 30 min after a hypertrophic stimulation (Sen & Packer, 1996). Additionally, re-expression of fetal genes, such as β-major histocompatibility complex, α-skeletal actin, and atrial natriuretic peptide, was also observed within 6-12 hr (Hefti et al., 1997). ROS-induced increases in intracellular Ca**++* homeostasis may be a significant initial step in the activation of NF-kB (Sen & Packer, 1996). Furthermore, lipid peroxidation products can activate NF-kB. Thus, ROS-induced alterations in gene expression are a consequence of changes in cellular signalling pathways through modifications of enzyme activities and alterations of molecular structures of biomolecules (e.g., proteins, lipids, glycoproteins, nucleotides). At least two important transcription factors, NF-kB and AP-1, are controlled by the intracellular redox state. These redox-regulated transcription factors bind to several promoter regions of genes that are directly responsible for the pathogenesis of atherosclerosis, complications of diabetes, cancer, and acquired immunodeficiency syndrome (Sen & Packer, 1996). Transcription activator NF-kB is important in inflammatory responses since it regulates a number of cytokine genes and their receptors, including TNF-α, IL-1, IL-2, and major histocompatibility complex Class I. NF-kB binding sites were also found in a variety of cell adhesion molecules, such as colony-stimulating factor-1, monocyte chemoattractant protein-1, and vascular cell adhesion molecule-1 (Suzuki et al., 1997).

AP-1 proteins are a family of leucine zipper transcription factors that specifically regulate gene expression upon binding to cis-acting transcriptional control DNA element. AP-1 genes are inducible by a broad range of stimuli, including ROS. ROS act as mediators of transcriptional regulators in signal transduction processes, leading to cell proliferation and transformation (Sun & Oberley, 1996). Examples of AP-1 transactivators are immediate-early response gene families, such as homodimers of c-jun proteins or heterodimers of c-fos and
c-jun. The activity of AP-1 is regulated at transcriptional, post-transcriptional, and post-translational levels. Activation of c-fos/c-jun transcription is promoted by oxidants, cytokines, and growth factors. Redox regulation of AP-1 binding appears to occur at the post-translational level. Oxidants can modify the redox state of cysteine residues located in the DNA-binding domain of each fos/jun protein that are crucial to their DNA binding activity and subject to redox regulation. Under oxidative stress, the loss of redox regulation by cysteine residues in AP-1 proteins inhibits their binding to DNA, whereas treatment with reducing agents, such as NADPH, GSH, or β-mercaptoethanol, increases AP-1 DNA binding (Sun & Oberley, 1996).

The field of oxidant-related stress genes and signalling is rapidly expanding, and a great deal of new insight into cellular responses to physiological stress has emerged. The regulation of transcriptional activators by oxidants and reductants is complex, and the ultimate biological effects are dependent upon the net results of interactions between these redox-mediated transcriptional activators.

1.5. THERAPEUTIC IMPLICATIONS

In general, the current "conventional" therapies for CVD include drugs that primarily affect vascular tone, cardiac contractility, fluid status, or lipid levels. However, given emerging evidence that oxidative processes are involved in cardiac and vascular disease, it seems reasonable to suggest that antioxidant therapeutic strategies may have value. Below is a brief review and current perspective regarding antioxidants for CVD management.

1.5.1. Currently employed pharmaceuticals

Interestingly, several of the most prominent pharmaceuticals used in cardiovascular medicine are already known to have some antioxidant properties that may contribute to their long-term efficacy. For example, the first commercially available ACE inhibitor, captopril, contains a sulfhydryl moiety in its chemical structure. While this has been linked to a unique side effect of patient coughing, the sulfhydryl structure has also been suggested as a chemical scavenger of free radicals that may contribute to captopril's actions (Anning et al., 1997; Mittra & Singh, 1998). Several recent reports also suggest that the long-term value of AngII inhibition for CVD is mediated by nonhemodynamic actions rather than acute...
vasorelaxant effects per se. It has been recognized that AngII itself is a stimulator of cardiac and vascular oxidative pathways via induction of NADH/NADPH oxidase. Therefore, inhibitors of the renin-angiotensin system (ACE inhibition or AngII receptor antagonism) may actually serve as indirect antioxidants by blocking this pathway, and some aspects of their long-term efficacy may be related to this effect. ACE inhibition recently has been shown to improve endothelial function in patients with coronary artery disease or its risk factors (TREND; Trial on Reversing Endothelial Dysfunction) (Schlaifer et al., 1999). While the mechanism of this clinical benefit is unclear, inhibition of AngII-mediated superoxide production may be a protective mechanism associated with preservation of endothelial health (Rajagopalan et al., 1996; Laursen et al., 1997).

The recently introduced vasodilating β-blocker carvedilol has also been shown to possess antioxidant effects in vitro and in vivo. This agent has emerged as a valuable therapeutic drug for long-term survival enhancement in heart failure, for prevention of diabetes-related renal failure, and in long-term hypertension control. The antioxidant actions of carvedilol (and its primary metabolite) significantly contribute to cardioprotective effects in hypertension and CHF (Moser & Frishman, 1998; Feuerstein et al., 1998). Carvedilol administration significantly increased the resistance to LDL oxidation in patients with essential hypertension (Maggi et al., 1996). Inhibition of ROS in the myocardium may prevent the consequences of oxidative damage, such as cardiac remodeling, activation of transcription factors, and apoptosis (Feuerstein & Ruffolo, 1998; Feuerstein et al., 1998).

1.5.2. L-arginine

Following the recognition of the important preventive role of NO in cardiovascular disorders (i.e., increases blood flow, inhibits key processes involved in atherogenesis), its natural precursor, L-arginine, has received attention as a therapeutic agent. As described in Section 3, the bioavailability of NO is known to be inversely related to the presence of other ROS. One approach to augment NO bioavailability is through enhanced provision of NOS substrate arginine. Clinical studies have also shown that L-arginine supplement improved coronary and peripheral blood flow in healthy and heart failure patients (Lerman et al., 1998;
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Rector et al., 1996). Although L-arginine supplement has shown some therapeutic value, its low efficacy has become a drawback in patient compliance. The lowest effective dosage ranged from 6 to 8 g/day (up to 20 large capsules/day), whereas the maximum effective dose is 18-20 g/day (Tenenbaum et al., 1998). In addition to the large dose required, L-arginine has a bitter unpleasant taste.

Therefore, the ongoing development of an L-arginine enriched nutrient bar (the HeartBar1) may promote the use of this potentially valuable nutrient (Cooke, 1998). While there has been some suggestion that this supplementation may have value, large-scale, double-blind, placebo controlled trials have not been conducted, and the available small scale data suggest only minor efficacy.

1.5.3. Antioxidant vitamin supplements
1.5.3.1. General aspects

Having recognized the significance of ROS in the pathogenesis of CVD, several antioxidants from normal and dietary supplements have been proposed to prevent or reduce disease risk. The term cardiovascular "nutriceuticals" has been introduced to describe natural agents that have been shown scientifically, through clinical trials, to enhance cardiovascular health (Cooke, 1998). The discussion in the following sections is limited to three important exogenous antioxidants—vitamin C (ascorbic acid), vitamin E (α-tocopherol), and β-carotene (provitamin A)—because little information is available on the benefits of other potential nutritional antioxidants in human subjects.

The potential value of antioxidant vitamin supplements has become an area of interest for cardiovascular and other disease management. In the last decade, several studies have been conducted using highly variant experimental designs, populations, vitamin supplements, and/or combinations. In general, targeted therapy in high-risk populations (secondary prevention trials) have shown more consistent value for antioxidants than large-scale primary prevention trials.

1.5.3.1.1. Vitamin C.

Vitamin C is the primary antioxidant water-soluble vitamin that can directly scavenge singlet oxygen, superoxide, and hydroxyl radicals. The Food and Nutritional Board of the Academy of Sciences (Washington, D.C., USA) now proposes the new recommended dietary allowance for vitamin C as 120 mg/day.
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(previously 60 mg/day), based on new biochemical, molecular, epidemiologic, and clinical data (Levine et al., 1999).

Hydrophilic antioxidants (e.g., ascorbic acid and GSH) appear to be the first line of antioxidant defenses against reperfusion damages during the return of blood flow, while lipophilic antioxidants (e.g., ubiquinol 9 and vitamin E) play an important role in protecting the integrity of cellular membranes from oxidative damage at later times (Haramaki et al., 1998). Several factors are known to contribute to changes in the plasma concentration of antioxidant vitamins. For instance, a significant decrease in plasma vitamin C is associated with the presence of unstable coronary syndrome in patients with coronary artery disease (Vita et al., 1998), while there was no significant association of plasma levels of vitamin C and increased risk of CVD mortality in healthy non-smokers (Loria et al., 2000). Serum ascorbic acid concentrations were associated with decreased prevalence of angina only in subjects who consumed alcohol (Simon et al., 1999).

In addition to the antioxidant activity of vitamin C, recent studies have shown that it activates NOS in endothelial cells (Heller et al., 1999; Huang et al., 2000). A single dose of oral vitamin C supplement (2 g) reduces arterial stiffness and platelet aggregation in healthy subjects (Wilkinson et al., 1999), and it improves endothelial function in CHF and angina patients (Hirashima et al., 2000; Hornig et al., 1998). Studies in young smokers demonstrated that a single dose of oral vitamin C supplement (2 g) immediately improved endothelial function, but had no beneficial long-term effect (1 g daily, 8 weeks) (Raitakari et al., 2000). Dietary vitamin C has also been shown to protect oxLDL-induced smooth muscle cell apoptosis in vitro (Siow et al., 1999). Additionally, administration of ascorbic acid increases the efficacy of estrogen in inhibiting LDL oxidation (Hwang et al., 2000).

1.5.3.1.2. Vitamin E.

Vitamin E appears to be the most effective lipid soluble antioxidant in biological systems (Nagel et al., 1997). It inhibits lipid peroxidation and regenerates reduced vitamin C and GSH. By protecting myocardial membranes and inhibiting the oxidation of lipoproteins, vitamin E inhibits membrane peroxidative damage and atherogenesis (Upston et al., 1999). The cardioprotective effect of oral
vitamin E against oxidative damage has been well recognized in both animal studies and clinical trials (Carrasquedo et al., 1999; Dhalla et al., 1996; Nagel et al., 1997). While endothelial dysfunction may be an initiating event in CVD, recent investigations have shown that plasma levels of α-tocopherol correlate well with endothelial health (Kinlay et al., 1999).

The decreases in the plasma levels of lipophilic antioxidants, such as α- and β-carotene, and vitamin E have been suggested as indicative of atherosclerosis in patients with coronary heart disease (CHD) (Kinlay et al., 1999; Kontush et al., 1999). It has been shown that administration of vitamin C or vitamin E restored endothelial function in patients with CHD (Gokce et al., 1999; Ito et al., 1998; Motoyama et al., 1998) and patients with abnormal lipoprotein profile (Kugiyama et al., 1999). Therefore, vitamin E and/or vitamin C may be important in preserving endothelial function in atherosclerotic disease.

1.5.3.1.3. β-Carotene and vitamin A.

The antioxidant properties of β-carotene and vitamin A have been demonstrated by their ability to quench singlet oxygen and to interrupt generation of ROS at a very early stage (Nagel et al., 1997; Palace et al., 1999). Most of the provitamin A carotenoids absorbed across the brush border are packaged into chylomicrons and transported into the plasma in lipoprotein particles. Their uptake, cellular distribution, and metabolism vary widely among species. Thus, studies from animal models cannot be extrapolated to humans. Despite the fact that the antioxidant mechanisms involved are not well understood, some epidemiological studies in humans provide supportive evidence of their antioxidant value.

1.5.3.2. Antioxidant supplements in cardiovascular disease

Although inconsistent, the observational epidemiologic studies provide data suggesting that consumption of foods rich in antioxidant vitamins reduces the risk of developing heart disease. The available data from large-scale, randomized, controlled trials are also inconclusive. Among the lipid-phase antioxidants studied, vitamin E shows the most preventive benefits on CHD, while β-carotene shows very little benefit or even harmful effects. Both vitamin E and β-carotene (and vitamin A) are carried within LDL particles. However, the apparent superior preventive effects of vitamin E may be explained by its higher efficiency in
protecting LDLs from oxidation in vitro and in vivo (Tribble, 1999; van het et al., 1999).

While oxidative processes have been consistently demonstrated in CVD, the use of antioxidants has not been shown to be consistently effective in all clinical trials. Several issues are likely to be important. For example, conflicting outcomes from the studies of antioxidant intake may reflect the type of preparation used and bioavailability. Since antioxidant supplements fall into the dietary supplement category, quality control of the products is not regulated by the United States Food and Drug Administration. Vitamin E provides an important example of how vitamin isomers present in commercially available preparations affect bioavailability. \( \alpha \)-Tocopherol is the predominant tocopherol that accounts for 70% of the total vitamin E intake in the United States, but its antioxidant activity and steady-state concentration in plasma and tissue is only 10-20% of the \( \alpha \)-tocopherol isomer (Cohn, 1997).

Other factors also influence plasma levels and bioavailability of each antioxidant. For example, plasma levels of \( \beta \)-carotene may not reflect the absorbability differences between natural and supplemental sources (van het et al., 1999). In a study of the effect of antioxidant vitamins on death from CHD in postmenopausal women, Kushi et al (1996) found no association between vitamin E supplements (>250 IU/day) and mortality rate, whereas an inverse correlation was observed for food-derived vitamin E (>10 IU/day). Additionally, alterations in vitamin E metabolism and antioxidant defenses in pathologic states can influence the bioavailability of tissue vitamin E. For example, tissue levels of vitamin E vary in patients with different manifestations of atherosclerosis, such as in aortic occlusion, aneurysm, and peripheral occlusive disease (Killion et al., 1996). Dosage differences may account for the incongruent outcomes of the vitamins studied. Based on kinetic studies of vitamin E transport in humans, it is recommended that the amount of vitamin E required for maintaining a steady-state level is 135-150 IU/day and that the minimum amount effective for protecting against LDL oxidation is 40 IU/day (Weber et al., 1997). It has been suggested that a 200 IU/kg/day dose of vitamin E for 5-10 days is required to achieve a 2-fold increase of the vitamin in rat myocardium (Machlin & Gabriel, 1982). Levels of
vitamin E decreased in the infarcted myocardium of rats receiving vitamin E supplements for 2 weeks, although plasma levels of the vitamin remained unchanged (Palace et al., 1999). Using antioxidant vitamins in combination may also be important in maximizing antioxidant defenses through synergistic effects and replenishment of antioxidant reservoirs in both aqueous and lipid phases. For instance, in addition to its aqueous phase antioxidant property, vitamin C serves as a reducing agent in the regeneration of antioxidant vitamin E (and vice versa) from the α-tocopheroxyl radical. A study in human subjects shows that elevated plasma levels of supplemental ascorbic acid are inversely correlated with susceptibility of lipoproteins to oxidation, while vitamin E concentrations in LDL particles were unchanged (Harats et al., 1998). Niki et al. (1995) demonstrated that during the oxidation of fatty acids, in the presence of both vitamin E and β-carotene, vitamin E was consumed first, while β-carotene was spared and rapidly used after depletion of vitamin E. Despite inconsistent data from human studies, the antioxidant vitamin C may have value when optimal plasma/tissue concentrations are achieved. Combination therapy with vitamin C and other antioxidants may provide a better antioxidant defense in body tissues.

In summary, some evidence from cohort studies suggests benefits of antioxidants for CVD. However, these benefits have not been demonstrated consistently, and additional randomized, placebo-controlled trials are needed. Several trials are underway, including continuation of Physician's Health Study, the Supplementation en Vitamines et Minéraux Antioxydants (SU.VI.MAX), Women's Health Study, Heart Protection Study, Women's Antioxidant Cardiovascular Study (WACS), etc. Furthermore, standardization and optimization of antioxidant use based on pharmacokinetic considerations are warranted. Several ongoing large-scale trials will further define the role of antioxidants in the prevention and treatment of CVD. It would be worthwhile to explore activities of various drugs as antioxidants in reperfusion injury, the most severe condition resulting in to free radical release during course of treatment of acute myocardial infarction.
CVD is prevalent and represents huge costs to the health care systems of all the nations in terms of mortality, morbidity, insurance costs, medical bills and many other factors acute myocardial infarction being the prominent cause. Fortunately the advances in interventions helped a lot to reduce mortalities. However, because of the increasing prevalence and post treatment complications it still remains major source of mortality and morbidity. Post treatment complications are believed to be because of reperfusion injury resulting during restoration of blood flow. It is well established that during reperfusion of ischemic myocardium free radicals are the most important inducers of cardiac injury and related vascular dysfunction (Verma et al, 2002) apart from other pathogenic mechanisms. Several pro-oxidant pathways have been identified, and these oxidation mechanisms may be important in the structural and functional changes that occur during the initiation and/or progression of RI. This consistency has provided the rationale for antioxidant trials in humans, but data currently available, using vitamin supplements, have been less than convincing (Cecone et al., 2002). Several ongoing trials will provide further insight regarding pharmacokinetic and pharmacologic aspects of antioxidants for RI. Additional mechanistic investigations regarding the molecular and biochemical aspects of oxidation pathways will also be valuable. It is likely that such pursuits will lead to a better understanding of these biological phenomena, and hopefully will provide new opportunities for therapeutic interventions.