CHAPTER I

INTRODUCTION AND LITERATURE REVIEWS

Diabetes mellitus is a chronic metabolic syndrome characterized by hyperglycemia, a relative or absolute deficiency of insulin, and deranged metabolism of carbohydrates, fats and proteins. As a function of time, this chronic metabolic abnormalities will lead to the developments of various chronic complications namely macroangiopathy, cardiomyopathy, neuropathy, and nephropathy.

Diabetic cardiomyopathy

The existence of a diabetic cardiomyopathy was first suggested by Rubler et al. in 1972 based on postmortem findings in four diabetic adults who suffered from congestive heart failure in the absence of atherosclerotic, valvular, congenital, hypertensive, or alcoholic heart diseases. All patients demonstrated electrocardiographic evidence of left ventricular hypertrophy and at autopsy Kimmelstiel-Wilson's disease was found in association with myocardial enlargement, hypertrophy, and fibrosis. The causal role of diabetes mellitus in the development of congestive heart failure was delineated more conclusively in the Framingham Heart Study. In 1974, this prospective study of 5000 individuals over an 18-year follow-up period, diabetic men had more than twice the frequency of congestive heart failure than nondiabetic cohorts,while diabetic women had a fivefold increased risk. This excessive risk persisted after accounting for age, hypertensive, obesity, hypercholesterolemia, and coronary artery disease, factors that commonly coexist with long-standing diabetes. Diabetic women were especially vulnerable, having twice the frequency of congestive heart failure as men

irrespective of coronary artery disease status. This excessive risk of heart failure was confined to those with hyperglycemia severe enough to warrant insulin therapy, whereas patients treated with diet or oral hyperglycemic agents had no increased risk of congestive failure. Further support for the existence of a diabetic cardiomyopathy was provided by Hamby et al., (1974) who noted an increased incidence of diabetes in patients with idiopathic cardiomyopathy. Sixteen of 73 patients (22%) with only 11% in an age and sex-matched cohort without cardiomyopathy. Autopsy findings of four diabetic subjects revealed patent epicardial coronary arteries, but pathologic changes compatible with diabetic vasculopathy were noted in smaller intramyocardial vessels.

Preclinical cardiac abnormalities have long been regcognized in diabetic patients. As early as in 1966, Karlefors noted that asymtomatic diabetic men had lower cardiac outputs during supine exercise than did controls. These findings appeared to be independent of the duration of diabetes. In a subsequent study, Calstroms et al.,(1970) newly diagnosed juvenile onset diabetic patients were noted to have lower stroke volumes during exercise than did controls. Cardiac output was maintained, a result of higher heart rates. Interestingly, a reversal of these hemodynamic derangements was noted after 1 year of insulin therapy.

The noninvasive evaluation of cardiac performance utilizing systolic time intervals, phonocardiography, M-mode and two-dimentional echocardiography and Doppler echocardiography has also documented subclinical left venticular dysfunction in diabetic individuals. Systolic time intervals have long been used to assess left venticular contractility. A prolonged preejection period (PEP) and shortened left venticular ejection time (LVET),which correlate with reduced resting ejection fraction, were uniformly noted in the studie in both type I and type II diabetic patients without evidence of clinical heart disease (Ahmed SS et al., 1975).

Shapiro et al. (1980,1981,1982) extended the evidence for increased PEP/LVET ratios, impared isovolumic relaxation, and other diastolic and systolic

left ventricular abnormalities in a large number of diabetic patients in a series of investigations. Asymptomatic diabetic subjects were consistently found to have impaired left ventricular relaxation by digitized M-mode echocardiography as compared with control subjects. These abnormalities correlated to a large degree with the duration of diabetes and the extent of microvascular complication. Left ventricular dysfunction occurred in both insulin-dependent and non-insulin-dependent subjects, but were more frequent in the former. Interestingly, Shapiro et al. also observed that left ventricular hypertrophy was noted only in diabetic subjects with concomitant hypertension. Hypertensive diabetic patients had a greater incidence of clinical congestive failure 28% versus 3% of normotensive diabetics) and profoundly abnormal PEP/LVET ratios and diminished fractional shortening.

Araksinen et al., (1984) studied 36 young (mean age 25 years) insulin-dependent women free of clinical cardiac disease or hypertension by digitized M-mode echocardiography. Abnormal diastolic function manifest primarily as a prolongation of early diastolic rapid filling was noted in 19 of these subjects (53%). Interestingly, a relationship was found between the duration of early filling and serum triglyceride concentration. Diabetic subjects with severe microvascular complications had thicker left ventricular walls and smaller end diastolic diameters and stroke volumes compared with healthy control individuals.

Experimental studies can address more directly many of the issues involving the role of hyperglycemia in the development of a true diabetic cardiomyopathy. The majority of experimental work has been performed in animals with drug-induced diabetes mellitus. Alloxan has been used primarily for this purpose, as low doses produce a chronic diabetic state without other significant organ toxicity. In this experiment, the prevention or reversibity of myocardial dysfunction with hypoglycemic treatment can be more fully investigated.

Regan et al.,(1974) utilized the alloxan diabetic dog model in pioneer study. Mild non-insulin-requiring diabetes was produced without fasting hyperglycemia in

mongrel dogs after low doses of alloxan were administered. Hemodynamic studies after 11 months revealed diminished left venticular end diastolic volumes and stroke volumes in diabetic dogs, despite similar end-diastolic pressures compared with control animals. This diminished left ventricular compliance was attributed to the accumulation of periodic acid-Shiff (PAS) positive material (presumably glycoprotein) in the interstitium. Similar findings were observed in dogs with spontaneously acquired diabetes, excluding a direct myocardial effect of alloxan.

Pogatsa et al., (1979) found similar evidence of diminished left ventricular compliance in chronically diabetic dogs with severe hyperglycemia. Although microscopic interstitial changes were not noted, cardiac structural proteins displayed enhanced thermal stability suggestive of increased cross-linking as the mechanism for diminished left ventricular compliance. Structural and hemodynamic parameters were prevented in part by treatment with insulin or oral hypoglycemic agents, though optimal metabolic control was not achieved. In a latter study(Palik et al., 1982) using this same model , coronary artery occlusion produced significantly larger infarcts in the diabetic cohort compared with control animals , confirming the apparent additive effects of ischemia and diabetes on ventricular function.

Numerous studies have also been carried out in rats with experimentally induced diabetes. Fein and Sonnenblick., (1980) have neatly summarized the effects of acute and chronic diabetes in rats. Acute diabetes results in a diminished response of cardiac output to increased filling pressure, while superimposed ischemia causes accelerated cardiac failure with prolonged recovery of cardiac performance. Additionally, acutely diabetic rats appear extremely sensitive to alterrations in afterload, with diminished systolic response to high levels of afterload.

Chronically diabetic rats show similar alteration in cardiac performance. Relaxation as well as the velocity of circumferential shortening is impaired. Similarly, cardiac output was reduced in diabetic rats utilizing isolated perfused

heart techniques. There was no evidence of myocardial ischemia by coronary flow or lactate production measurements. Long-term insulin therapy, but not short-term therapy, completely reversed these abnormalities.

Biochemical and pathologic correlates for altered myocardial mechanics were also examined in dibetic rats. Myocardial adenosinetriphosphatase (ATPase) activity is diminished and myosin isoenzymes with lower ATPase activity predominate. Additionnally, calcium binding and uptake by sarcoplasmic reticulum are reduced in diabetic rats. These findings which may well account for the altered systolic function and diminished ventricular relaxation in diabetic rats. However, microscopy reveals minimal parenchymal alterration in the myocardium of diabetic rats, as compared with dog and human models. accumulation of collagen and PAS-positive material in the interstitium is uncommon, myocyte integrity is preserved, and capillary basement membrane thickening is modest.

In contrast, Giacomelli and Wiener in 1979 showed progressive damage to myocytes, capillary basement membranes, and intramural small vessels in mice with genetically determined diabetes. As myocyte alterations developed initially, myocardial damage did not appear to be due to vascular abnormalities, which usually occur later. Subsequently, Factor et al.,(1981) demonstrated the effects of combined hypertension and diabetes in rats that produced marked myocytolysis, perivascular and interstitial fibrosis, and numerous saccular and fusiform microaneurysms after only 8 weeks of both stresses. Thus while diabetes alone does not appear to lead to structural heart disease in rats (except for genetically diabetic mice), coexistent hypertention results in abnormalities of myocardial architecture that may potentially culminate in systolic disfunction.

Paralleling these experimental findings in animal models are numerous studies in humans that explore the association of diabetes with histopathologic abnormalities, possibly accounting for a myopathic state. Early postmortem studies in diabetic subjects without overt congestive failure documented a higher incidence

of intramural PAS-positive material and hyaline thickening with and without endotherial cell proliferation in both intramural and extramural coronary arteries., (Blunmenthal.,1960, and ledet.,1968). Zoneraich et al.,(1980) also found evidence of intramural involvement with significant proliferative changes and wall thickening in young juvenile diabetic individuals. Small vessel disease was evident in 72% of normotensive diabetic patients, but was present in only 12% of nondiabetic subjects in the latter study.

In diabetic subjects with congestive heart failure without significant coronary atherosclerosis, similar pathology has been found. Rubler et al.,(1972) noted substantial myocardial hypertrophy and fibrosis in their original series of four patients with diabetic cardiomyopathy. Endothelial and subendothelial proliferation were also prominent, suggesting that small vessel disease may be involved in the pathogenesis of myocardial dysfunction. Zoneraich et al.,(1988) has recently reviewed an extensive body of literature concerning the role of small vessel disease in diabetic cardiomyopathy. Interestingly, small vessel disease may also be operant in diabetic patients with angina pectoris and normal coronary angiograms, as two such patients recently were found to have identical small vessel changes with right ventricular endomyocardial biopsy.

Many alterations in myocardial small vessels are qualitatively similar to those seen in other organs of diabetic patients. Such alterations include increased thickening of myocardial capillaly basement membranes and microaneurysms in autopsy specimens as well as in tissue analyzed at the time of coronary artery bypass surgery. Basement membrane thickening was more severe in those with overt diabetes compared with those with simple glucose intolerance. Such microvascular damage within the myocardium may represent part of the spectrum of vasculopathy typically found in diabetic patients.

Interstitial infiltration with PAS-positive material and fibrosis,however, may be associated with systolic and diastolic abnormalities. In this context, Regan et al.,

(1977) noted minimal changes in intramural coronary arteries in nine diabetic subjects without coronary atherosclerosis. Similar finding were noted in diabetic patients with and without overt congestive heart failure. Perivascular and interstitial fibrosis and accumulation of PAS-positive material were observed along with degeneration and fragmentation of myocytes. Factor et al.,(1980) have emphasized that the combination of diabetes and hypertention may lead to more severe interstitial fibrosis and myocellular damage than in diabetics or hypertension alone. In nine hypertensive diabetic patients with severe congestive heart failure and minimal obstructive coronary artery disease, dense interstitial connective tissue was seen throughout the myocardium. Myocytolysis and scarring were also prominent in these dilated, hypertrophied hearts. These changes were much more prominent than in patients with isolated diabetes or hypertension. Interestingly, there was a similar extent of small vessel changes among the three groups despite significant differences in interstitial scarring. Suggesting that interstitial disease is responsible for mechanical dysfunction and not "small vessel disease" which may be an incidental finding.

Conflicting evidence has been presented regarding the relative role of small vessel disease, interstitial fibrosis, microvascular changes, and metabolic derangements in the pathogenesis of diabetic cardiomyopathy. The relationship of type, duration, and severeity of diabetes to myocardial abnormalities similarly remains somewhat nebulous. Also, myocardial disease may or may not be related to the degree and extent of other microvascular complications.

Role of coronary artery disease

Although a substantial amount of evidence supports the existence of a diabetic cardiomyopathy in dependent of obstructive coronary disease, the role of coronary artery disease needs to be addressed for several reasons. First, ischemic heart disease is a leading cause of congestive heart failure. From the studies of

Garcia MJ.,1974, indicated that diabetes mellitus is clearly an independent risk factor for atherosclerosis and is responsible for the higher incidence of cardiac mortality and morbidity in diabetic subjects compared with nondiabetic individuals. Dash et al.,(1977) have shown that diabetic patients with coronary artery disease develop cardiomyopathy more commonly than nondiabetic individuals, probably due to an increased risk of proximal coronary artery disease and mutiple myocardial infarctions.

Second, diabetic subjects have a higher incidence of asymtomatic ischemia (Chiariello M,1985). Diabetic subjects may present with symptoms of congestive heart failure as the initial clinical manifestation of severe multivessel coronary disease due to asymtomatic . Early data from the Joslin Clinic showed that angina was less severe or even absent in diabetic patients with acute myocardial infarction. Data from the Framingham Study(Margolis,1973) also indicated that diabetic subjects have more electrocardiographically documented myocardial infarctions than nondiabetic subjects (39% versus 22%). Abenavoli et al., (1981) employing thallium scintigraphy, showed that reversible defects occurred in 5 of 16 asymtomatic diabetic subjects during exercise compared with their appearance in only 1 of 12 control subjects. In1985, Rubler and Fisher confirmed these findings in a group of 38 asymtomatic patients with adult onset diabetes. Sixty percent of asymtomatic of diabetic subjects had ischemic electrocardiographic changes and infarction or ischemia on thallium testing (or both) during exercise, compared with only 7% of controls. On retesting 1 to 2 years later, 77% of diabetic subjects were abnormal.

Nesto et al.,(1988) studied 50 consecutive diabetic patients and 50 consecutive nondiabetic subjects with ischemia with thallium excercise scintigraphy. Although a similar proportion of patients complained of angina, only 28% of diabetic patients compared with 68% of nondiabetic patients experienced angina during exercise- induced myocardial ischemia. Only the presence of diabetes accounted for the difference in asymptomatic ischemia between the two groups,as

they were similar with regard to demographic and clinical factors as well as the extent of exercise induced ischemia. Murray et al.,(1988) found a similar degree of painless ischemia with exercise in diabetic subjects compared with nondiabetic subjects (76% versus 35%). Diabetic individuals with painless ischemia had a greater than two fold increases in incidence of microvascular disease (74% versus 33%).

Not only is diabetes a risk factor for the development of atherosclerosis, but it may be responsible for an accelerated rate of progression of atherosclerosis. Recent analyses (Viogorito,1980) reveal that patients with juvinile and maturity onset diabetes have significantly more coronary artery disease than age-matched non diabetic individuals. Although other studies in maturity onset diabetes patients have shown an equal incidence of coronary artery disease compared with controls, diabetic subjects usually have a greater extent of disease(Waller.,1980).

Atherosclerosis in diabetes mellitus

The major problem in diabetics is atherosclerosis, and this can affect the myocardium, brain and lower limbs. Recently, many investigations have interested to clarify the mechanisms of atherosclerosis in nondiabetes. One attractive sequence of events has been proposed by Ross (1986) and Gomset (1975), it is likely that certain aspects of this process are enchanced in diabetes. According to Ross's theory about the pathogenesis of atherosclerosis, this process starts with monocyte adherence to the endothelium, presumably at an area of endothelial damage. Endothelium may be damaged by physical means such as trauma, by a variety of biochemical mechanisms including glucose, free fatty acids, and hypercholesterolemia, as well as immune mechaisms and by drugs. Monocyte adherence may occur, and subendothelial migration, with transformation into macrophages, then follows. A fatty streak develops,representing lipid accumulation

by monocyte derived macrophages. Macrophages may release growth factors that stimulate smooth muscle cell proliferation and migration.

A second series of events may be initiated by platelet adherence to a site of endothelial injury. Platelet aggregation results in the release of thromboxane, a potent vasoconstricting arachidonic acid metabolite. Like macrophages, platelets may release growth factor that stimulate smooth muscle cell migration and proliferation. Low density (LDL), intermediate density, and very low density lipoprotien (VLDL) may deliver cholesterol to the damage area.

Renin angiotensin system interaction in cardiovascular complications

In general, the renin angiotensin system plays a central role in the regulation of systemic blood pressure, electrolyte and fluid balance, and blood volume (Cushman.,1982). Its physiological activities are mediated primarily through an effector peptide, angiotensin II (AngII). Angiotensin, a glycoprotein (molecular weight 55 to 65 kDa)[2] synthesized mainly in the liver, is transformed by renin into the biologically inactive decapeptide angiotensin I(AngI). In turn,Ang I is converted by angiotensin converting enzyme (ACE) to the physiologically active octapeptide AngII. AngII is subsequently converted by aminopeptidase to another physiologically active through less potent peptide, angiotensinIII (AngIII) . The metabolism of AngIII yields several inactive by-products.

AngII is one of the most potent vasoactive substances known. It directly affects smooth-muscle cells and stimulates the synthesis and secretion of aldosterone. The vasoconstricting properties of AngII are mediated by two receptors, AT_1 and AT_2. The former is a G-protien-coupled membrane protein for which the amino acid sequence has been characterized. Little information concerning the AT_2 receptor is available(Griendling.,1993). ACE is a zinc-containing exopeptidase similar in structure to carboxypeptidase A and identical to kinase II, a component of the kallikrein-kinnin system. Its main action is to cleave dipeptidases from the carboxy-terminal end of peptide substrates. Kininase II is protease that catalyzes the

breakdown of the vasodilator bradykinin. Kallikreins are proteases involved in the transfomation of kininogens into bradykinin(Kastis JB, and Waeber B., 1988).

Local renin angiotensin systems

Recent discoveries are challenging the traditional belief that the renin-angiotensin system is solely systemic.There is mounting evidence that most if not all of its components are produced locally and that both circulating and locally produced components of the renin angiotensin system are active. AngI may be produced intracellularly or secreted and converted to AngII on the cell surface. AngII may be synthesized intracellularly and secreted, or it may be absorbed within tissues from plasma. The observed effects are likely a composite of each mechanism (Dzau and Parder KN.,1986, Kifor.,1988).

There is biochemical evidence of reninlike activity, renin substrate, ACE, angiotensinogen, and both AngI and AngII in the brain, adrenal glands, kidneys, blood vessels (endothelium and smooth-muscle cells), testes, uterus, sympathetic ganglia, submandibular lymphatic grands,and heart. Inaddition, the genes for renin and angiotensin are expressed in several of these organ systems(Dzau.,1987). Louden et al reported that circulating renin can be taken up by blood vessels.

Rogers et al. in 1986 localized high-affinity receptors for AngII in spontaneously contracting neonatal rat myocyte. Wright et al.,(1983) was able to isolate two distinct AngII binding sites in the myocardial cells of rabbits. These cells demonstrated chronotropic stimulation by AngII, suggesting strongly that the receptors were functional. In addition, stimulation of these receptors have been shown to enhance myocardial contractility: this positive inotropic effect occurred through direct and indirect mechanisms and often was masked in intact animals by increase in afterload after AngII administration(Baker.,1984). AngII receptors are

saturable and specific, and act independently of β-adrenergic stimulation and cyclic adenine monophosphate.

Dzau and Re in 1987, identified reninlike activity and renin messenger ribonucleic acid (mRNA) in isolated mouse cardiac myocytes. The reninlike activity in these cells was neutralized by a specific antimouse renin antibody. Ludwig et al., (1985) also found evidence of renin mRNA in mice and found AngI, AngII, AngIII, and ACE activity in various primate tissues, including the brain, left atrium, right ventricle, and kidney. By showing similar hemodynamic responses to infusions of AngI, AngII, and the synthetic tetradecapeptide of renin substrate(TDCP-RS) in the absence of exogenous renin and ACE, Oliver and Sciacca.,(1984) concluded that AngII was generated locally by renin and ACE within the vasculature. An increase in blood pressure following TDCP-RS administration, similar to that caused by AngI and AngII, was attenuated by captopril and renin inhibitory peptides. Linz et al.,(1986) added AngI to the perfusate of isolated rat hearts and observed a prompt conversion to AngII, which was subsequently inhibited by the addition of captopril.

Vascular renin- angiotensin system

After ligating the coronary arteries in rats, Hirsch et al.,(1991) obsearved an increase in cardiac ACE activity,ACE mRNA,and locally produced AngII. The concentrations of pulmonary, renal, and aortic ACE remained unchanged. These findings demonstrated a selective increase in locally produced components of the renin- angiotensin system. Renin like activity also has been detected in the venous circulartory system (Rosenthal.,1984) by infusing AngI and AngII into human vascular beds, Schalekamp et al.,(1989) showed that circulating renin levels were not adequate to explain the concentration of the peptide must have been synthesized locally. Experiments with binephrectomized rats have shown that AngII and plasma renin activity persist for many hours after plasma renin is no longer detectable.

Further support of this hypothesis was provided by the isolation of angiotensin mRNA from blood vessels.

The physiologic and potential pathologic roles of the local renin-angiotensin system are under intense investigation. Several functions have been proposed,including(1) regulation of regional vascular tone and blood flow;(2) development of vascular hypertrophy;(3) contribution to the vascular response to inflammation and injury;and(4) response to pharmacologic inhibitors of the renin-angiotensin system. The potential cardiac manifestations include (1) development of cardiac hypertrophy;(2) potentiation of coronary vasoconstriction; (3) increased contractility;and (4) a propensity towards ventricular arrhythmias during myocardial ischemia and reperfusion.

Locally produced angiotensins may influence vascular tone through paracrine or autocrine effects. Vascular AngII can produce vasoconstriction by directly affecting smooth-muscle cells and by amplifying the vasoconstriction included by the sympathetic nervous system. Sympathetic activation can occur directly(in the blood vessel wall) or in the central nervous system,where locally produced angiotensins can generate AngII,leading to an activation of sympathetic outflow to the peripheral vascular beds. Conversely,by stimulating the release of prostaglandins or endothelium-derived relaxing factor, they can cause vascular smooth-muscle relaxation and vasodilation. The overall net effect depends on the relative contributions of these opposing mechanisms; however, experimental data suggest that vasoconstriction is the primary effect of AngII in the peripheral vasculature (Unger.,1987 and Mizuno.,1988).

ACE inhibitors (ACE-I) directly influence the vascular response to AngII. Kawasaki et al.,(1984) and Nakamura et al.,(1986) concluded that β-adrenergic stimulation increased catecholamine release. The resulting vasoconstriction could be blocked by ACE inhibition, or alternatively, by AngII-blocking agents. Simon et al., (1984) demonstrated an increased compliance within brachial and carotid arterises of

humans treated with captopril. The ability of ACE inhibitors to increase vascular compliance has two important implications: first, the response to vascular injury may be buffered, and second, the development of left ventricular hypertrophy may be attenuated.

The recent study demonstrated that ACE-I prevent the development of LVH in animal models of hypertension. In humans and animals with established hypertension, ACE-I induced regression of LVH (Pfeffer et al.,1988). Interestingly, ACE-I was experimentally showed to have cardioprotective effects, such as, it appeared to limit myocardial infarct size, attenuate left ventricular dilatation and hypertrophy. Recently, the cardioprotective effects of ACE-I are very well documented in the reduction of coronary vascular resistance, and increasing of myocardial blood flow associated with blunting of AngII formation. By contrast, several investigations have been reported that ACE-I could suppress the vascular response to injury by interrupting the conversion of AngI to AngII, and thus this supression mechanism could result in preventing smooth muscle cell hypertrophy, proliferation and matrix protien synthesis (Ert et al., 1982; Hock, Ribeiro and Lefer, 1985).

From the points of view that ACE-I could improve cardiac performance including increased cardiac contractility and preventing VSMC proliferation, the idea is that ACE-I may be used as a preventor of cardiovascular complications in diabetes mellitus. In this investigation, STZ-rats were used as diabetic model to study the changes of cardiac functions and effects of ACE-I on the changes during the cause of experiment, 8,20 weeks.

Therefore, the major purpose of this study are:

1) To study the changes of cardiac functions related to morphological examinations in 8, 20 weeks STZ-rats.

2) To evaluate what is the major mechanisms of angiotensin converting enzyme inhibitor (ACE-I) that can prevent the cardiovascular changes in diabetes,

due to the decrease of arterial blood pressure or the direct inhibition of trophic of AngII.