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DIABETIC CARDIOMYOPATHY

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I. INTRODUCTION

Cardiovascular disease is one the major causes of morbidity and mortality in patients with diabetes mellitus. It is well known that diabetics are predisposed to accelerated atherogenesis. Myocardial infarction and congestive heart failure (CHF) due to extramural coronary artery disease (CAD) are commonly seen in diabetics. Other types of heart disease are also encountered in diabetic patients. In 1972, Rubler et al (1) examined at autopsy four diabetic patients with Kimmelsteil-Wilson's disease that also had cardiomegaly and CHF in the absence of hypertension, CAD, congenital, valvular or other heart disease. Since then it has become increasingly apparent from epidemiologic and clinical studies that heart disease, especially CHF, can occur in the absence of atherosclerosis and suggests a specific "diabetic cardiomyopathy." Using diabetic animal models, it has been possible to characterize better some of the functional and metabolic abnormalities found in the diabetic heart. It is the purpose of this grand rounds to review the evidence for a diabetic cardiomyopathy.

II. EPIDEMIOLOGY

One method of establishing the specificity of a diabetic cardiomyopathy is by the use of epidemiologic studies. Two such studies have been reported. In 1974 Kannel et al (2) reported on the role of diabetes in CHF using the large Framingham population base. Of 5192 men and women aged 30 to 62 years followed for 18 years, diabetes was present or developed in 292 patients. Diabetic men and women had relative risks of developing CHF of 2.4 and 5.1 times that of the nondiabetic patients. When patients with prior CAD or rheumatic heart disease (RHD) were excluded, the same relationships held with diabetic men 3.8 and diabetic women 5.5 times more likely to develop CHF. Multivariate analysis in patients without CAD or RHD demonstrated an increased incidence of CHF in diabetics independent of age, blood pressure, serum cholesterol, and relative weight. The only subgroup of diabetic patients with an increased risk of CHF was the insulin-treated group; risk was not increased in patients treated with diet alone or oral hypoglycemic drugs. The data was insufficient to analyze the effect of severity or duration of diabetes on the occurrence of CHF. To exclude patients with CAD, the diagnosis of CAD was made clinically and not by more objective testing, such as angiography. Since diabetics are more likely to have silent ischemia and/or infarction, some diabetics with CHF may have had extramural CAD and been incorrectly placed in the group without CAD (3). Similarly, only those patients with symptoms or clinical signs of CHF were included in the study. Therefore patients with subclinical left ventricular (LV) dysfunction would have been omitted.

In a smaller study, Hamby et al (4) followed 73 patients with idiopathic cardiomyopathy for six years and detected 16 patients with diabetes for an incidence of 22%. This was a statistically significant increase compared to an age and sex matched control group of 300 patients without cardiomyopathy in whom the incidence of diabetes was only 11%. All 16 patients had normal extramural coronary arteries by selective coronary arteriography and 13 had abnormal resting hemodynamics. Only those patients with symptoms or clinical signs of CHF were included in the study. In contrast to the Framingham study 94% of the diabetics were treated by diet or oral hypoglycemic drugs. The duration of diabetes was from 6 months to 11 years.

III. CLINICAL EVIDENCE

The cardiac status of diabetic patients has been studied both invasively and noninvasively and a subgroup of patients with preclinical asymptomatic left ventricular dysfunction and presumably early cardiomyopathy has been tentatively identified.

A. Noninvasive Studies

Systolic time intervals, echocardiography (Echo), and more recently Doppler and radionuclide techniques have been used to characterize left ventricular (LV) systolic and diastolic function in diabetics without clinical evidence of cardiac disease. The major limitation with all of these studies in supporting the presence of a "diabetic cardiomyopathy" is the lack of definite exclusion of extramural (large vessel) CAD by coronary angiography. Ischemia can cause abnormalities of systolic and diastolic function identical to those described by following studies for diabetic cardiomyopathy (5). Estimation of the contribution of CAD is hindered by the absence of data on the prevalence of significant coronary disease in a group of young, relatively uncomplicated diabetic patients. It is difficult to justify cardiac catheterization in asymptomatic patients for screening purposes. Many investigators have used young patients with no risk factors for CAD other than diabetes; some used exercise testing either with or without thallium scintigraphy to exclude patients with CAD, certainly those with severe disease. Evidence against CAD based on young age or clinical history is insensitive and nonspecific since it is well known that diabetes is associated with an increased frequency and an accelerated appearance of CAD in young diabetics (6), which often can be asymptomatic (3,7). Although thallium scanning increases the sensitivity and specificity of routine exercise testing, it still is not 100% sensitive or specific and the effect of myocardial abnormalities on the thallium scan is not clear. Fixed and reversible exercise thallium perfusion defects have been demonstrated in diabetic patients without significant CAD documented by cardiac catheterization (8).

i. Systolic Time Intervals

Systolic time intervals (STI) are ejection phase indices that have been shown to correlate with angiographic parameters of LV function, e.g. ejection fraction (9), when appropriate factors are taken into account, especially heart rate, conduction abnormalities (LBBB), and loading conditions of the ventricle. The three principle intervals used are the pre-ejection period (PEP), the LV ejection time (LVET), and the ratio of PEP to LVET. These intervals are obtained from simultaneous recordings of the EKG, the phonocardiogram, and the carotid artery pulse tracing. LV failure from any cause is associated with a lengthening of the PEP and a shortening of the LVET leading to an increase in the PEP/LVET ratio. Prolongation of PEP is principally due to a reduction in the rate of LV pressure development during isovolumic contraction(LV dP/dt) and reflects impaired contractility and alteration of the myocardial force-velocity relationship. The shortening of the LVET is multifactorial but depends significantly on the rate and extent of fiber shortening, and therefore preload, as well as prolongation of the PEP. Several investigators have demonstrated a significant 13 to 50% increase in the PEP/LVET ratio in up to 30% of asymptomatic diabetic patients compared to control patients well-matched for

age and sex (7,10-14). None of the patients had hypertension or clinical evidence (history, exam, EKG, or CXR) of heart disease. Seneviratne (11) and Shapiro (12) demonstrated these abnormalities only in those diabetics with other evidence of microvascular disease (proliferative retinopathy, nephropathy) while others (10,13,14) have shown them in uncomplicated diabetics as well. None of the studies were able to correlate the impairment LV function to type of treatment, degree of hyperglycemic control, age, sex, or age of onset and duration of diabetes.

ii. Echocardiography

a. M-mode Echo

The greatest utility of M-mode Echo has been in the assessment of diastolic dysfunction. Normal relaxation and subsequent filling of the LV is reflected in the closely coordinated timing and movement between outward wall movement and mitral valve opening. These events can be readily visualized and timed by this technique. Using computer analysis of digitized M-mode echocardiograms Sanderson et al (15) and Shapiro et al (16) showed these relationships are often disturbed in diabetics in the presence of normal systolic function. Sanderson evaluated 23 diabetics aged 15-39 years with a mean duration of diabetes of 17 years (range 2-28) and no history of hypertension or clinical evidence of heart disease. Two thirds had proliferative retinopathy. Seventeen (74%) patients had one (or more) abnormality of diastolic function compared to 15 age and sex matched controls. Similarly, Shapiro and his group evaluated 142 diabetics with a mean age of 41 $\pm$ 9 and mean duration of diabetes of 9 years (range 0 - 25) without clinical heart disease or hypertension. Abnormalities were found in 92% of the diabetic patients. The abnormalities reported in these two studies and others were a significantly delayed mitral valve opening relative to either minimal systolic LV dimension or aortic valve closure and a prolonged period of isovolumic relaxation (7,12,15-17). The isovolumic relation interval is the period of time when the aortic and mitral valves are closed during which LV pressure is falling without a change in volume. The duration and rate of posterior wall thinning and LV dimension change (- dP/dt) were significantly prolonged and slowed. In the Shapiro study (16), the abnormalities in diastolic function correlated significantly with the extent of other microvascular complications (nephropathy, retinopathy) and the duration of diabetes in those with juvenile onset of their disease.

b. 2D/Doppler Echo

More recently two dimensional (2D) and Doppler echocardiography have been used to evaluate asymptomatic diabetic patients for evidence of LV dysfunction. Measurement of transmitral flow characteristics and velocity by Doppler has been shown to be useful in identifying abnormal LV filling in diastole and to correlate with angiographic (18) and radionuclide (19) techniques. Takenaka et al (20) investigated 60 diabetic patients aged 60 $\pm$ 12 years with diabetes for a mean of 11 $\pm$ 9 years and compared them to 19 age and sex matched controls. None of the patients had hypertension, angina, MI, an ETT positive for ischemia, CHF, other heart disease, or an abnormal EKG. The diabetics were divided into three groups based on the presence or absence of wall motion abnormalities (WMA) on 2D Echo and on the presence or absence of retinopathy. Patients with LV WMA with or without retinopathy had significantly prolonged PEP/LVET ratios and lower percent fractional shortening of the LV compared to diabetics without WMA or control patients. These data

suggested decreased LV systolic function in this group but no other assessment, e.g. by MUGA, was done to confirm this finding. Isovolumic relaxation times were prolonged 23-50% in all diabetic patients compared to controls but reached significance only in the group with LV WMA. Abnormal patterns of diastolic filling were also noted. Patients with WMA and those with retinopathy but without WMA had higher peak mitral flow during atrial systole (late diastole) and an increased ratio of late to early diastolic peak mitral flow compared to normals and diabetics without retinopathy. Zarich et al (21) studied 21 insulin-dependent diabetics aged 21-49 with a mean disease duration of 17 years and 21 age and sex matched healthy patients. No patient had hypertension, angina, MI, CHF, abnormal EKG, WMA or other cardiac disease. All patients over age 25 had a negative Bruce exercise test to a mean heart rate of 165 and duration of 12.2 min. All patients had a normal ejection fraction. Seventy percent of diabetics had at least one and 30% had a least two independent abnormal indices of diastolic function which were similar to the previous study. Early diastolic peak velocity was decreased and late diastolic or atrial peak velocity was increased resulting in an elevated early to late peak velocity ratio compared to controls. In addition the percent of total diastolic filling due to atrial systole was significantly elevated in the diabetic patients (35% vs 27%, $P<0.001$) with less filling occurring during early diastole. The findings did not correlate with duration of diabetes or other microvascular complications. These studies indicated there is a greater contribution of atrial systole to LV filling and stroke volume in diabetics. This is likely due to an altered pressure-volume relationship as has been described in other cardiomyopathic states.

iii. Radionuclide Studies

a. Systolic function

Assessment of LV systolic and diastolic function by radionuclide techniques has been done in diabetic patients by several groups of investigators. Mildenberger et al (22) studied 20 insulin-dependent diabetics aged 29 $\pm$ 6 years with a mean duration of diabetes of 12.4 $\pm$ 7.3 years and 18 age and sex matched controls by rest and exercise MUGA. No patient had a clinical history of hypertension or any heart disease and all had a normal exam, CXR, and EKG. Diabetic patients had a normal resting ejection fraction (EF) of 63.7% $\pm$ 6.5% which increased to 67.6% $\pm$ 9.7% with exercise. For the control group the resting EF was 65.4% $\pm$ 6.2% and increased to 77.1% $\pm$ 7.3% with exercise. The resting EF's were not significantly different but the exercise EF's and the percent change in EF (4.0% $\pm$ 11% vs 11.7% $\pm$ 7.3%) were significantly different ($p < 0.001$ and 0.05, respectively). The exercise test was limited by leg fatigue without ischemic EKG changes or angina. Both groups achieved similar workloads, maximal heart rates, and double products. There were no WMA at rest or exercise in either group. Overall, 35% of the diabetics and 5% of the controls had an abnormal response when normal was defined as either no change or any increase in EF with exercise. Nearly identical results were obtained in a similar controlled study by Vered et al (23) who studied 30 diabetic men aged 21-35 without clinical heart disease or hypertension by history or exam. With a normal response for their lab defined as a 4% or more increase in EF with exercise, 43% of the diabetics and none of the controls had either no change or a decrease in EF. In 4 patients with a decrease in EF, exercise thallium scanning showed normal perfusion. No patient exhibited chest pain, ischemic EKG changes or WMA at rest or with exercise. Neither study demonstrated any correlation with duration of

diabetes, type of antidiabetic therapy, or the presence or absence of other microvascular complications.

b. Diastolic function

Kahn et al (24) assessed diastolic filling in diabetics using computer analysis of high temporal resolution time-activity curves from gated radionuclide ventriculograms. Patients were 19-44 years old with insulin-dependent diabetes for 10-28 years and no history of heart disease. All patients had an EF > 50%, normal Bruce ETT, and normal exercise thallium study. Twenty-one percent of their 28 patients had evidence of abnormal diastolic filling with either decreased peak filling rate (PFR) or time to PFR, or both. Interestingly, 5 of the 6 diabetics with abnormal filling had evidence of cardiac autonomic neuropathy (CAN) and the time to PFR corrected for heart rate (24.3 + 2.2 sec) was significantly longer than in diabetics without CAN (16.2 + 1.5 sec, $p < 0.01$). This corrected time to PFR correlated significantly with the clinical presence of orthostatic hypotension. Serum norepinephrine levels were significantly lower by a factor of about 3 in these patients compared to those diabetics without CAN. Although serum levels may not reflect abnormal cardiac catecholamine metabolism they may represent the degree of overall sympathetic nerve function (25). Since catecholamines have been shown to improve ventricular relaxation (26), the diastolic abnormalities were postulated to be related to catecholamine depletion in these patients (27,28). Ganguly et al (29) have shown that cardiac norepinephrine concentration, norepinephrine turnover, and norepinephrine uptake are markedly increased in chronically diabetic rats. This indicates that the conclusions based on serum determinations of catecholamines may not be relevant.

iv. Summary of Noninvasive Studies

In summary, numerous studies using various noninvasive techniques indicate that diabetic patients often have abnormal cardiac function even when they are asymptomatic and have no clinically apparent heart or other disease that can alter LV performance. Abnormalities of systolic and diastolic function have been demonstrated in 20 to 95% of diabetic patients. These findings, although frequently observed in the diabetics patient, are not specific for diabetes and are typical also of those found in CAD and other cardiomyopathic states. All of these cross-sectional studies involved patients of various ages, durations and types of diabetes, and treatments. Serial noninvasive assessments in longitudinal studies of these patient populations have not been reported. Whether these abnormalities predict eventual progression to clinically apparent CHF, due to either systolic or diastolic dysfunction (30) or both, is not known.

B. Invasive Studies

Since it is difficult to justify cardiac catheterization in asymptomatic patients, only a few such studies have been done and have evaluated patients with symptoms of chest pain, dyspnea, or frank CHF. In an uncontrolled study, Hamby et al (4) in 1974 studied 16 nonhypertensive patients aged 19-59 years with diabetes for 0-9 years. All had symptoms of CHF and/or cardiomegaly for up to 6 years and normal coronary angiograms. Hemodynamic assessment revealed a depressed EF, an elevated LV end diastolic pressure (LVEDP), and an elevated LV end diastolic volume index (LVEDV) compared to previously established normals. LV mass was increased in all patients. Regan et al (31) studied 12 nonhypertensive adult-onset diabetics aged 49+4 years who had symptoms of dyspnea or chest discomfort but had normal coronary arteriography. Four of these patients had had episodes of clinical CHF and on hemodynamic assessment had depressed ejection fraction, decreased stroke volume (SV), and an elevated LVEDP with relatively little change in LVEDV compared to controls. However, the ratio of LVEDP/LVEDV was significantly elevated suggesting increased LV wall stiffness. Of more interest were the other 8 patients who had no clinical evidence of CHF. These patients also had significantly lower LVEDV, increased LVEDP, decreased SV, and elevated LVEDP/LVEDV ratio consistent with decreased compliance compared to controls. Since ejection fraction was normal the reduced stroke volume was attributed to impaired diastolic filling. When afterload was augmented by the infusion of angiotensin, diabetic patients exhibited a subnormal increase in stroke volume in response to the increased LVEDP consistent with impaired ventricular compliance. To exclude ischemia on a microvascular basis as a cause of the diastolic abnormalites (5) in the patients without CHF, atrial pacing with arterial and coronary sinus sampling was performed in 6 patients (4 without and 2 with CHF). There was no evidence of increased lactate production or coefficient of lactate extraction to suggest impaired myocardial perfusion.

These hemodynamic findings represent only one point on a pressure-volume curve and therefore represent a very limited assessment of changes in LV function. Nevertheless they clearly demonstrate that LV hypertrophy, diastolic dysfunction, and impaired contractility can occur in diabetic patients in the absence of hypertension, CAD, or other disease that could cause LV dysfunction. In addition, the findings of impaired contractility and diastolic filling are entirely consistent with the abnormalities demonstrated noninvasively.

IV. PATHOLOGICAL STUDIES

A number of autopsy studies have evaluated myocardial histopathology in diabetic patients but no lesion or lesions pathognomonic for or specific to a diabetic cardiomyopathy has been identified. Lesions reported in the myocardium include abnormal intramyocardial arteries and arterioles (20-500 microns) (1,4,32-36), interstitial and perivascular fibrosis with accumulation of connective tissue (collagen) and periodic acid-Schiff (PAS) staining material (glycoprotein) (4,31,36), capillary microaneurysms (37) and basement membrane thickening (38,39) and, myofiber hypertrophy (4,31,32,36), and increased myocardial triglyceride and cholesterol contents (31).

A. Small Vessel Disease

The most controversial of these findings is the presence and significance of small vessel or microvascular changes. Abnormalities of the myocardial intramural vessels include subendothelial and perivascular fibrosis, proliferation of medial muscle cells, intimal hyaline deposits, endothelial cell proliferation, and concentric PAS staining of the vessel wall. The presence of endothelial cell proliferation with focal projections and luminal bridging are thought to be potentially occlusive and to lead to ischemia, fibrosis, and hypertrophy. Whether this view is correct remains to be proven conclusively either histologically (40,41) or functionally (31) in diabetic patients. Reservations concerning the studies evaluating small vessel disease in diabetics include: 1) the absence of controlled (31-35,40) or even uncontrolled (1,36) population, 2) the failure to use quantitative techniques (1,4,36,40) or perfusion fixation to avoid artifacts caused by vascular contraction (1,4,31-36,40), and the inclusion of patients with extramural CAD or hypertension (32,33,35,40,41). In a recent study (41) in which the above factors were controlled, no differences in vascular pathology were found among comparable groups of patients with: a) diabetes only, b) hypertension only, c) both hypertension and diabetes, and d) neither hypertension nor diabetes. All patients, including controls, had had myocardial infarctions and had a similar amount of extramural CAD. Recently, however, small vessel disease has been implicated as a causative factor in patients with normal coronary arteries and classsical angina (42).

In an attempt to correlate small vessel disease with abnormal LV dysfunction, Das (36) recently evaluated 16 diabetic patients by endomyocardial biopsy in an uncontrolled study. No clinical data, reasons for biopsy, or attempts at exclusion of other important diseases (CAD,HTN) were given. Group I patients had cardiomegaly and CHF, Group II had normal heart size without signs or symptoms of CHF but had abnormal LV function assessed by an elevated PEP/LVET ratio, and Group III was the same as Group II except they had a normal PEP/LVET ratio. Half of the patients in Group I, one third in Group II, and none in Group III had small vessel disease indicated by vessel wall thickening, intimal proliferation and hyaline membrane deposition, and basement membrane thickening. They concluded that diabetics with symptomatic and objective LV dysfunction had the most severe small vessel disease and those without symptoms and normal LV function the least; those without symptoms but with abnormal function had intermediate disease.

B. Interstitial/Metabolic Changes

Perhaps more interesting in the study by Das (36) noted above were the extravascular changes, especially PAS-positive staining, which were very prominent in all three groups (100%, 85%, and 75% in Groups I, II, and III, respectively). In fact, common to most of the pathological studies seems to be the finding of the large accumulation of vascular, perivascular, and interstitial fibrosis and glycoprotein-like material. Regan et al (31) performed histochemical and biochemical analyses on the hearts from a group of 9 diabetic patients who at autopsy had normal coronary arteries (death due to CHF in 6, renal failure, stroke, and septicemia in one each). The walls of the small intramural arteries were mildly thickened without luminal narrowing. Compared to nondiabetic controls there were significant increases ($p<0.002$) in interstitial PAS-positive material, presumably glycoprotein, and in periarterial and interstitial fibrosis, which often penetrated between muscle bundles with myodegenerative changes. Also demonstrated were significant increases in the myocardial content of triglycerides and cholesterol in multiple sites of the LV. The accumulation of these materials and the diffuseness of the process were considered consistent with a cardiomyopathy. Such infiltration could potentially contribute to enhanced wall stiffness observed in a comparable group of diabetic patients (31). The extent of such changes in a group of patients with idiopathic cardiomyopathy but without diabetes was not addressed so that it was not possible to determine their specificity for diabetes.

V. EXPERIMENTAL EVIDENCE

The clinical and pathological studies just reviewed are not definitive but provide strong support for the idea that a cardiomyopathy specific to diabetes exists in the absence of hypertension, coronary artery disease, or other diseases that can cause LV dysfunction. A large amount of experimental work has attempted to elucidate the possible mechanisms underlying the disorder. The effects of both acute and chronic diabetes have been investigated. Since clinical cardiomyopathy occurs over years, the studies looking at chronic diabetes are the more relevant. The subsequent discussion will focus on these studies. From this work, there has evolved a substantial body of evidence that comprises two schools of thought: a) enhanced wall stiffness leading to poor compliance and impaired filling, and b) depressed contractile protein and sarcoplasmic reticular function leading to impaired contractility and relaxation.

A. Enhanced Wall Stiffness

Regan and his colleagues have studied the effects of a chronic nonketotic diabetic state induced by alloxan in dogs and monkeys (43-45). After 11 to 18 months of mild untreated hyperglycemia, the animals were studied hemodynamically, histologically, and metabolically.

i. Hemodynamic Findings

In the dog studies steady-state baseline hemodynamic values did not differ between non-diabetic and diabetic groups (43,45). After angiotensin infusion to increase afterload, the nondiabetic group showed a small elevation in LVEDP and a significant rise in LVEDV and SW. In contrast, the diabetic dogs responded with a small increase in LVEDP but a significant decrease in LVEDV and SW. Since there was no change in EF, this was interpreted as a decrease in diastolic compliance. Using acute saline loading to increase preload, the nondiabetic dogs exhibited a proportionate rise in LVEDP and LVEDV whereas diabetic dogs showed a significantly greater rise in LVEDP for very small changes in LVEDV. Since indices of contractility and EF were unchanged this was again interpreted as a change in diastolic compliance. Similar hemodynamic findings and response to preload manipulations were observed in diabetic monkeys (44).

ii. Histologic Findings

Histologically the LV showed moderate to heavy PAS staining of amorphous glycoprotein-like material and collagen in the interstitium of the myocardium in both dogs and monkeys (43-45). The nondiabetic animals had minimal staining.

iii. Metabolic Findings

In dogs the LV showed a significant increase in the content of triglycerides and cholesterol but no change in the free fatty acid or phospholipid levels (43,45). The incorporation of radiolabeled fatty acid (^{14}C-1-oleic acid) showed a significant increase in triglyceride labeling and a decrease in phospholipid labeling. There were no differences in de novo lipid or cholesterol synthesis assessed by ^{14}C-acetate labeling. Total collagen content (measured as hydroxyproline) was increased by 50 to 70% in dogs and monkeys (44,45). Nearly all of the increase was in the acid insoluble form (55%); the acid soluble form actually decreased (44).

iv. Summary of Evidence for Enhanced Wall Stiffness

Taken together these data were interpreted by Regan and his colleagues as showing that the hemodynamic LV dysfunction was due to decreased diastolic compliance probably caused by accumulation of collagen, glycoprotein-like material, and possibly lipid (triglyceride and cholesterol) in the myocardium. Ischemia and decreased contractility were considered unlikely since myofibrils were histologically normal as were the sarcoplasmic reticulum (SR), mitochondria, and small vessels. Coronary blood flow and lactate extraction at rest and with pacing were normal as well. Furthermore the ventricular dysfunction and metabolic alterations in the dogs and monkeys were very similar to those found in the human studies by the same group, which suggests a similar disease process.

v. Potential Biochemical Mechanisms for Enhanced Wall Stiffness

Clinically and experimentally diabetes is characterized by chronic hyperglycemia and hyperlipidemia with a shift toward fatty acid metabolism and triglyceride accumulation (46). One of the consequences of hyperglycemia that may pertain to diabetic cardiomyopathy is the formation of nonenzymatic glycosylation products, the most well-known of which is hemoglobin (A_{1C}). This topic has recently been reviewed extensively and the reader is referred there for more details (47,48).

a. Nonenzymatic glycosylation products

Briefly, glucose easily forms chemically reversible Schiff bases with amino groups of proteins (an aldimine: protein-$^{+}$NH=CH-$(CHOH)_4$-CH_2OH) at a rate proportional to glucose concentration. These then rearrange to form more stable, but also chemically reversible, Amadori-type early glycosylation products (a ketoamine: protein-NH-CH_2-CO-$(CHOH)_3$-CH_2OH). Nonenzymatic glycosylation has been shown to occur in vitro and in vivo in a large of number of biological proteins such as red cell membranes, antithrombin III, fibrinogen, lens capsule and crystalline, tubulin, glomerular basement membrane, collagen, coronary artery proteins, high and low density lipoproteins, albumin, and multiple enzymes (47). Functional abnormalities of these glycosylated proteins have been demonstrated. Changes in enzyme activity and cofactor binding, possiblly induced by alteration of the catalytic and binding sites, have been reported (47).

Some early glycosylation products, especially those on collagen and other long-lived structural proteins, do not dissociate but undergo a slow chemical transformation to form irreversible advanced glycosylation end products (AGEP) whose levels don't return to normal when hyperglycemia is controlled (48). Their accumulation is proportional to the time-integrated concentration of glucose and the "incubation time." These AGEP are capable of cross-linking extravasated plasma proteins, insoluble matrix proteins, and collagen by forming strong covalent bonds with amino groups on these other proteins. Once formed, these cross-linked proteins become very resistant to degradation by proteolysis. Furthermore, they are able to continue to form cross-links and to trap potentially harmful soluble proteins like albumin, IgG, and lipoproteins, even in the absence of glucose (49). These compounds have been shown to be bound 3-5 times higher in vivo and in vitro to collagen cross-linked with AGEP than to identical amounts of collagen without AGEP (50,51). This

accumulation of albumin and IgG could explain the basement membrane thickening and immune complex deposition seen in diabetic kidney disease (52) as well as the increased triglyceride and cholesteral levels described by Regan (31,43). Levels of ACEG on collagen have been measured in tissues such as skin (53) and tendon (54) and correlate with the severity of diabetic retinopathy (55). They have not been measured in heart but increased amounts of heat-stable cardiac structural proteins have been reported in chronically diabetic dogs (56).

B. Depressed Contractile Mechanism

Fein, Penpargkul, Sonnenblick and their associates have done animal experiments which have implicated abnormal function of myocardial contractile apparatus rather than increased wall stiffness as an etiology for the diabetic cardiomyopathy (57,58) These well controlled experiments utilized the isolated working rat heart and papillary muscle preparations obtained from chronic (8 week) streptozotocin-induced diabetic rats (57,58).

i. Hemodynamic Findings in Working Hearts

In these experiments aortic impedance was held constant while preload was varied (57). Multiple hemodynamic variables were measured at each level of preload. As left atrial pressure was increased the peak LV systolic pressure, maximum -dP/dt (rate of relaxation) and flow acceleration (rate of change of aortic flow) were significantly depressed compared to control. Maximum +dP/dt (contractility) tended to be low but not significantly. In contrast coronary flow, cardiac output (CO), and stroke work (SW) tended to be higher in the diabetic hearts at low LA pressures. In the hearts from diabetic animals CO and SW fell rapidly compared to controls as the LA pressure increased, much as would be seen in the failing heart. Systolic performance also could be normal under resting conditions but abnormal on exposure to stress (ischemia, hypertension, exercise) in the intact animal (59,60). When LVEDP was plotted against LVEDV, the diabetic curve was shifted to the right. Thus to obtain a given LVEDP a larger LVEDV was required. These results were opposite to those observed by Regan (31,43).

ii. Isolated Muscle Findings

In a companion study, these investigators used isolated rat heart papillary muscles to study further the contractile properties of diabetic muscle (58). In isometric studies resting and developed tensions were similar in nondiabetic and diabetic preparations. However the time to peak tension was prolonged and the peak rate of tension rise was slightly less in the diabetic muscle. More striking were the differences during relaxation. The time for peak developed tension to fall by 50% was prolonged, peak rate of tension fall was depressed, and time from peak developed tension to peak rate of tension fall were significantly increased in diabetic muscle. Isotonic studies revealed that the extent of muscle shortening did not differ but the time to peak shortening was prolonged and the peak velocities of shortening and relaxation were lower. The time to peak relaxation was increased also. Shortening velocity was depressed significantly under all loading conditions of the muscle. Changes in

calcium and glucose bath concentration, stimulus frequency, the presence of norepinephrine, or duration of diabetes did not alter the results. Thus they demonstrated:

1) delayed onset of relaxation
2) slowed rate of relaxation
3) prolonged time to peak relaxation rate
4) depressed force-velocity relation

Resting tension, peak developed tension and peak shortening appeared to be maintained at control levels in the face of decreased contractility by increasing the time to peak tension or peak shortening. Whether these abnormalities in relaxation are important in the intact animal is not known. At physiological heart rates, such a relaxation defect might impair diastolic filling as was seen by Regan (31,43).

iii. Summary of Evidence for Depressed Contractile Mechanism

Scheuer et al (57,58) concluded that the mechanical defects related to depressed LV pressure development, decreased velocity of ejection, and impaired relaxation were possibly due to abnormalities of the contractile mechanism. Since there were no histological changes it was postulated that the alterations in contractility and relaxation might result from a disorder of the contractile proteins and/or the handling of calcium by the sarcoplasmic reticulum.

iv. Biochemical Studies

a. Contractile proteins

Several groups of investigators have demonstrated depressed Ca^{2+}-activated myosin and actomyosin ATPase activities (61-64). This decrease may be related to a shift in the myosin isozyme pattern (65). Normally about 70% of the total myosin is in the most active form V_1 with 15% each in the less active V_2 and V_3 forms. In diabetes the pattern shifts to be predominantly (70%) in the V_3 form which is the slowest with respect to ATP hydrolysis and crossbridge formation. The mechanism for this shift has not been defined but may be due in part to diabetes-induced hypothyroidism which is seen in animals(rat) but infrequently in humans (58,62,66,67). Support for this comes from data indicating that thyroidectomy causes a similar isozyme shift in nondiabetic rats (68) and that in diabetic rats thyroid (and insulin) replacement prevents the isozyme shift (65).

b. Sarcoplasmic reticulum function

Significantly depressed SR Mg^{2+}-ATPase activity, Ca^{2+}-Mg^{2+}-ATPase, and Ca^{2+} uptake by isolated SR from diabetic rat hearts may act as a factor contributing to impaired relaxation and contractility (69-71). If less calcium is taken up then less is available for release and initiation of contraction. Decreased reuptake of the released calcium then impairs relaxation. The mechanism for this inhibition is unknown but long chain fatty acyl CoA (LCFAC) and long chain acyl carnitine (LCAC), both of which are increased in diabetes (59,71), can inhibit these activities in isolated SR preparations (72). LCAC are derivatives of long chain fatty acids (LCFA) which are formed on the outer mitochondrial membrane and are responsible for transport of fatty acids into the mitochondria for oxidative metabolism and production of ATP by the electron transport chain. LCFAC have been shown in mitochondrial suspensions to inhibit the adenine nucleotide translocase which transports the ATP formed from the mitochondria back into the cytosol. Whether this in important physiologically in the intact animal is not known.

VI. REVERSIBILITY OF DIABETIC CARDIOMYOPATHY

There have been conflicting reports about whether experimental diabetic cardiomyopathy is reversible. This is probably due to the different animal models, methods of inducing diabetes, duration of diabetes before beginning therapy, length of therapy, and other factors.

A. Enhanced Wall Stiffness

Regan (45) evaluated the effect of insulin therapy in his long term diabetic dog model described above. Insulin was begun after alloxan diabetes had been present for 3 months and treatment to control postprandial hyperglycemia was continued for an additional 9 months. The abnormalities of ventricular compliance, hemodynamic responses, and accumulation of triglycerides and collagen were not reversed by this therapy. On the other hand Baandrup (73) made rats diabetic with streptozotocin and 2 days later began therapy with insulin to maintain normoglycemia for 9 months. By point counting techniques, the diabetic group had similar amounts of collagen compared to controls but significantly less than another group with poorly controlled diabetes:

Group	% Connective Tissue
Control	11.4 ± 2.8
Diabetic	
Good control	11.2 ± 2.7
Poor control	14.8 ± 2.2*

* $p<0.01$ vs control

B. Depressed Contractile Mechanism

Fein et al (74) made rats diabetic (nonketotic) for 6-10 weeks and then treated 4 groups with insulin for 2, 6, 10 , or 28 days. The mechanical performance of isolated papillary muscles and the activities of contractile proteins were then assessed. By day 10 recovery of mechanical performance was almost at baseline and by 28 days there was complete reversal of myocardial mechanics. There was concurrent gradual recovery of actomyosin and myosin ATPase activities also. Subsequently, this was shown to translate into improved myocardial hemodynamics and function using the isolated perfused working hearts from similarly treated diabetic rats (75). Peak LV pressure, + dP/dt, rate of relaxation, and the response of cardiac output to increasing preload were similar to or better than control hearts without diabetes and much better than untreated diabetic rats. More recently, this same group has obtained similar results in papillary muscles from insulin-treated or untreated rabbits with chronic diabetes (76).

C. Synthesis of the Reversibility Studies

The studies by Regan (45) and Baandrup (73) suggest that poorly controlled hyperglycemia results in the irreversible accumulation of collagen and probably other matrix components and extravasated proteins. In the Regan study diabetes was well established for 3 months prior to beginning therapy. Subsequent control of hyperglycemia failed to prevent the hemodynamic, histologic and metabolic abnormalities. On the other hand, Baandrup's study showed that when antihyperglycemic therapy was initiated very early in the course of diabetes and normoglycemia was strictly maintained, then collagen accumulation did not occur. A reasonable scenario that can be proposed to explain these findings would be the following. Early and continued control of hyperglycemia prevents the formation of early and ultimately advanced glycosylation end products. Therefore collagen does not accumulate above normal levels as in Baandrup's study. In the prsence of hyperglycemia, the ACEG have a higher tendency to form. Once ACEG are formed they are resistant to degradationand continue to accumulate and trap other soluble proteins even when normoglycemia is achieved later as described above.

If the preceding is true, then diabetic complications would develop and progress despite tight control of serum glucose concentration as observed by Regan. To prevent complications one would need to develop a different approach to therapy in addition to blood glucose control. To that end Brownlee and associates have investigated aminoguanidine, a small hydrazine compound with an amino group that is more reactive than those in proteins (48). The presence of this compound results in the formation of a substituted Amadori product which is unreactive and therefore unable to form ACEG. This compound inhibits in vitro cross-linking of soluble plasma proteins to collagen and of collagen to itself. Aminoguanidine has been shown to be effective in in vivo experiments as well. Alloxan diabetic rats were divided into two groups - one to receive aminoguanidine in saline and the other saline alone. These were compared to control rats without diabetes but treated similarly. After 16 weeks of treatment, the levels of collagen crosslinked to itself and to lipoprotein through ACEG in renal glomeruli and the aorta were identical in the treated diabetic rats and in the nondiabetic animals. The untreated diabetic group had four times as much crosslinked collagen. The potential of this drug

for preventing early and late complications in diabetes is now being evaluated in Phase I clinical trials (48).

Finally, how does one explain the reversibility of enzyme function and myocardial performance seen the studies of Fein (74,76) and Schaible (75)? These animals were chronically diabetic for 6-10 weeks before initiation of any therapy. This is probably related to control of both hyperglycemia and hyperlipidemia. With the shift away from fatty acid metabolism, LCAC and LCFAC levels would decline and allow greater activity of the SR enzymes involved in calcium handling. Alternatively the some of the depression of enzyme activities could have been due to the formation of early glycosylation products which can theoretically alter function by involvement of catalytic and binding sites of the enzymes (47). Since these reactions are reversible over days to weeks when hyperglycemia is controlled, then enzyme activity should increase once glucose is removed from the protein.

VII. EFFECT OF HYPERTENSION ON DIABETIC CARDIOMYOPATHY

The presence of hypertension is well-known to accelerate the progression of retinopathy and nephropathy in patients with pre-existing diabetes (77,78). There is clinical and pathological evidence that hypertension may accelerate the processes leading to structural heart disease and eventually CHF in diabetic patients. The experimental studies showing poorer function with increased levels of afterload may be pertinent in this regard (43,60).

A. Clinical Evidence

Shapiro (17) reported significant increases in the systolic time interval, PEP index, isovolumic relaxation time and decreased percent fraction shortening in a group of patients with diabetes and hypertension compared to a group with only diabetes. Using computerized M-mode ECHO, Venco (79) demonstrated that diabetic patients had more severe impairment of LV function than patients with a similar degree of hypertension alone. The most consistent abnormalities in this study were the reduced rate of chamber enlargement during filling and the slower wall thinning (-dP/dt), suggesting impaired LV relaxation and distensibility.

B. Pathological Evidence

i. Human Studies

Factor et al (80) reported a group of patients with CHF who had both diabetes and hypertension. At autopsy these hearts showed myocardial hypertrophy, extensive myocytolysis, focal scarring, and marked replacement, perivascular, and interstitial fibrosis. The coronary arteries were normal or had minimal disease. Hearts from patients with isolated diabetes showed little or no such changes while those with isolated hypertension exhibited hypertrophy with mild to moderate fibrotic changes. Extensive deposition of PAS-positive glycoprotein and interstitial collagen was found but only in regions of interstitial fibrosis and focal scar and only in the hypertensive and hypertensive-diabetic hearts. There were no abnormalities in the small intramural vessels.

ii. Animal Studies

Factor and his associates developed a rat model of renovascular hypertension and drug-induced diabetes which reproduced their light microscopic findings in humans (81). Later they demonstrated identical findings at the electron microscopic level (82). They concluded that the combination of diabetes and systolic hypertension leads to myocardial damage manifested as interstitial accumulations of collagen, basement membrane material, and focal replacement fibrosis in association with evidence of cellular hypertrophy. Because of the focal nature of these changes, the absence of extramural and intramural coronary atherosclerosis, and the lack of diffuse degenerative changes in the myocytes, they believed that an abnormal microcirculation might contribute to such damage. Using Microfil (silicone rubber) injection techniques, they described numerous areas of microvascular tortuosity, focal constriction, and microaneurysm formation (83) which had been previously demonstrated in diabetic human hearts (37). Microaneurysms have been described in retinal vessels and renal glomeruli in diabetics, suggesting this abnormality is a generalized phenomenon. These abnormalities were quantitatively greater in hypertensive-diabetic rats compared to normal and normotensive-diabetic rats where they were distinctly rare. Normoglycemic hypertensive rats had intermediate amounts of these lesions. Since such lesions and increased capillary permeability have been demonstrated in both diabetes and hypertension, these authors postulated that the combination of vascular insults leads to accelerated leakage of plasma constituents into the interstitial space. This extravascular material might then stimulate synthesis of basement membrane or collagen leading to fibrosis and scarring (84). Striking mechanical and electrophysiological abnormalities have been demonstrated in isolated papillary muscles from hypertensive-diabetic rats (85). These changes were much greater than those found in muscle from rats with isolated hypertension or diabetes. These animals also had acute and chronic congestive changes observed in their lungs and livers , especially those that died spontaneously during the study.

C. Summary of Effects of Hypertension

These clinical and pathological studies clearly indicate that the presence of hypertension can have a potentially serious adverse effect on cardiac function in diabetic patients. Although there is no evidence it would seem reasonable that patients with preclinical abnormalities of cardiac function may be at higher risk of developing overt heart disease when hypertension is present. Hence it would seem prudent from a clinical point of view to maintain normotension as much as possible in all patients with diabetes.

VIII: CONCLUSIONS

It is apparent from the preceding discussion that diabetes mellitus probably causes a specific cardiomyopathy in the absence of other diseases that can cause LV dysfunction. The clinicopathological work, epidemiological data, and invasive catheterization data (in dogs and humans) make this evident. Abnormalities of LV systolic and diastolic function often can be detected preclinically in asymptomatic patients by a number of noninvasive cardiac techniques. Their relationship to the type of diabetes, its duration and severity, type of treatment, and progression to overt CHF is not known. The pathogenesis of this disorder remains to be elucidated. Abnormalities of the microvasculature and small intramural arteries have been demonstrated in pathological studies but their functional significance remains unproven. However, the combination of hypertension and diabetes appears to make the microvascular lesions worse and cause considerable cardiac damage and congestion in both rats and humans. Experimental studies have shown that increased interstitial connective tissue and collagen in chronically diabetic dogs and monkeys lead to a stiffer ventricle. Similar changes have been shown in man and may account for the impaired diastolic filling seen in this disorder. Although not yet demonstrated in cardiac muscle muscle fibrosis may be related to the development of advanced glycosylation end products caused or accelerated by poor control of hyperglycemia and possibly hypertension. Functional alterations in the myocardium were clearly shown in the chronically diabetic rat and correlated with depression of contractile protein activity and impairment of intracellular calcium uptake. These changes may explain the decrease in contractility and slowing of relaxation shown invasively and noninvasively in humans. Some but not all of these abnormalities are reversible with antihyperglycemic therapy and appeared to depend on when treatment was begun in relation to the onset of diabetes. Thus although much is known about diabetic cardiomyopathy, much more work must be done to understand better its pathogenesis, treatment, and prevention.ts

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