

# Diabetic Cardiomyopathy: Evidence, Mechanisms, and Therapeutic Implications

ZHI YOU FANG, JOHANNES B. PRINS, AND THOMAS H. MARWICK

University of Queensland, Brisbane, Queensland 4102, Australia

The presence of a diabetic cardiomyopathy, independent of hypertension and coronary artery disease, is still controversial. This systematic review seeks to evaluate the evidence for the existence of this condition, to clarify the possible mechanisms responsible, and to consider possible therapeutic implications.

The existence of a diabetic cardiomyopathy is supported by epidemiological findings showing the association of diabetes with heart failure; clinical studies confirming the association of diabetes with left ventricular dysfunction independent of hypertension, coronary artery disease, and other heart disease; and experimental evidence of myocardial structural and functional changes. The most important mechanisms of diabetic cardiomyopathy are metabolic disturbances (depletion of glucose transporter 4, increased free fatty acids, carnitine

deficiency, changes in calcium homeostasis), myocardial fibrosis (association with increases in angiotensin II, IGF-I, and inflammatory cytokines), small vessel disease (microangiopathy, impaired coronary flow reserve, and endothelial dysfunction), cardiac autonomic neuropathy (denervation and alterations in myocardial catecholamine levels), and insulin resistance (hyperinsulinemia and reduced insulin sensitivity).

This review presents evidence that diabetes is associated with a cardiomyopathy, independent of comorbid conditions, and that metabolic disturbances, myocardial fibrosis, small vessel disease, cardiac autonomic neuropathy, and insulin resistance may all contribute to the development of diabetic heart disease. (*Endocrine Reviews* 25: 543–567, 2004)

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## I. Introduction

OVER THE NEXT two decades, the incidence of both type II diabetes and congestive heart failure is anticipated to increase to epidemic levels in both the industrialized and developing worlds. Patients with diabetes are characterized by an increased likelihood of heart failure, largely reflecting the contribution of diabetes to coronary artery disease and its association with hypertension. Over the last three decades, a number of epidemiological, autopsy, animal, and clinical studies have proposed the presence of

diabetic heart disease as a distinct clinical entity (1–4). However, the existence of diabetic heart disease or cardiomyopathy—referring to myocardial disease in diabetic subjects that cannot be ascribed to hypertension, coronary artery disease, or any other known cardiac disease—has remained controversial. This review seeks to synthesize the existing literature for and against the existence of diabetic cardiomyopathy, its mechanisms, and its therapeutic implications.

This work was performed as a systematic review. We searched MEDLINE (from 1966 to July 2003) to include all animal and human studies of diabetic heart disease not related to hypertension, coronary artery disease, or other known causes. Experimental, pathological, epidemiological, and clinical data were included. All relevant reviews and related references were also examined. Studies were selected on the basis of a combination of the primary terms “diabetic cardiomyopathy” and “diabetic heart disease” with other specific key words related to specific topics. These included left ventricular (LV) dysfunction (e.g., “diastolic dysfunction” and “systolic dysfunction”), structural changes independently caused by diabetes (e.g., “structural changes,” “pathological,” “histological,” “morphological,” and “backscatter”), and the relationship between diabetic cardiomyopathy and diabetic control (“metabolic control,” “HbA1c,” and “glucose levels”). Young diabetic patients or children included in studies were considered to have no or less possibility of coronary artery disease. Studies about diabetic heart disease possibly caused by hypertension, coronary artery disease, and other known causes were excluded, as were those not written in the English language. There were 737 publications related to the terms diabetic cardiomyopathy or diabetic heart disease; 591 papers, including 105 reviews, were written in English and formed the basis of this review.

Abbreviations: A, Peak late filling velocity; ACE, angiotensin-converting enzyme; CAN, cardiac autonomic neuropathy; E, peak velocity of early mitral flow; FFA, free fatty acid; GLUT, glucose transporter; HbA1C, glycosylated hemoglobin; HED, hydroxyephedrine; LV, left ventricular; MIBG, metaiodobenzylguanidine; OLET, Otsuka Long-Evans Tokushima fatty; PET, positron emission tomography; SERCA, sarcoplasmic reticulum calcium.

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II. Evidence for a Diabetic Cardiomyopathy

Accumulating data from experimental, pathological, epidemiological, and clinical studies have shown that diabetes mellitus results in cardiac functional and structural changes, independent of hypertension, coronary artery disease, or any other known cardiac disease, which support the existence of diabetic cardiomyopathy.

A. Diastolic dysfunction in diabetes

The noninvasive assessment of diastolic dysfunction mainly relies on Doppler studies of transmitral inflow, measuring mitral inflow velocities, deceleration time, and isovolumic relaxation time, and assessing flow patterns (Fig. 1). As diastolic function worsens, early diastolic LV filling (E wave) is reduced, and the patient demonstrates a delayed relaxation pattern. However, as left atrial pressure increases, the E wave returns to normal, producing a mitral pattern indistinguishable from normal (pseudonormal), until the development of a restrictive filling pattern, which reflects a high left atrial pressure, usually to the extent that symptoms of left heart failure appear. The preload dependence of these techniques means that measurements in the same patient may change from one category to the next as left atrial pressure is increased or decreased. Although the assessment of pulmonary venous flow (Fig. 2) can help to identify raised filling pressure (and hence discriminate normal from pseudonormal), the combination of patterns is complicated and does not offer a parameter that is altered in a linear fashion as the heart becomes progressively abnormal. Thus, limitations in the standard techniques for the assessment of diastolic function are a significant impediment to understanding the subtle effects of diabetes and other subclinical disease on the heart. The other important impediment is the ambiguous interpretation of therapeutic effects, in particular

if the pretreatment stage is the delayed relaxation pattern as is usually observed in diabetic patients.

New techniques such as mitral annulus velocity by tissue Doppler imaging (Fig. 3) are relatively preload-independent measurements of diastolic function (5–7), which facilitate the diagnosis of diastolic dysfunction in clinical cardiac disease (8, 9). The advantages of these new technologies include excellent temporal resolution and their monodirectional relationship with progressive cardiac abnormality. This also allows more adequate quantification of therapeutic effects. Although there remains no single indicator for quantification of diastolic dysfunction and it remains important to combine several indicators together for this purpose, the development

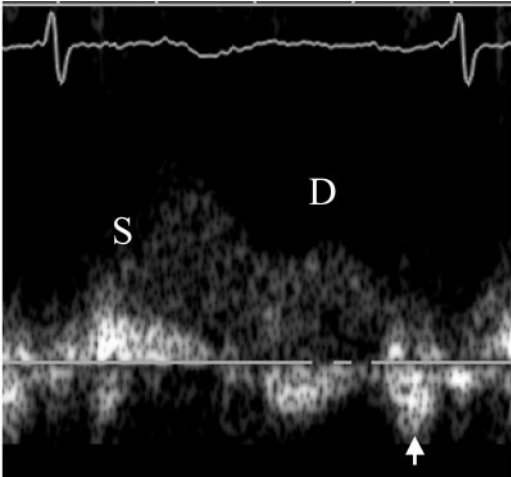


FIG. 2. Pulmonary venous flow pattern showing systolic (S) and diastolic (D) components and atrial reversal (arrow). Increasing left atrial pressure is attended by reduction of the S wave and increasing the amplitude and duration of the atrial reversal.

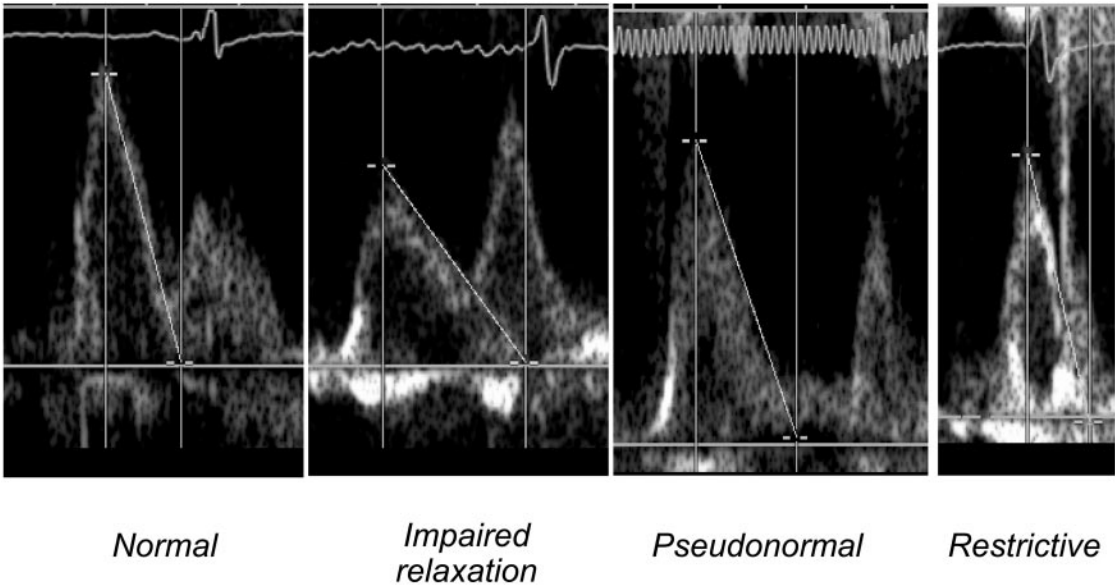


FIG. 1. LV filling patterns derived from assessment of transmitral flow. Worsening of LV function leads initially to delayed relaxation, characterized by reduction of the E wave and prolongation of the deceleration time (horizontal vector or diagonal marked on each frame). The process is reversed by elevation of LV filling pressure increasing E wave velocity and shortening deceleration time (pseudonormal and restrictive).

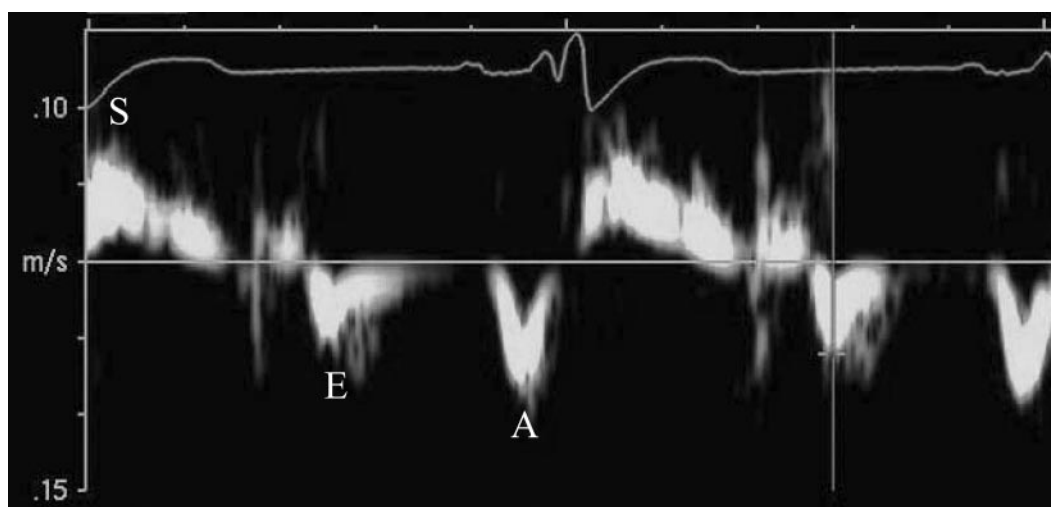


FIG. 3. Tissue Doppler signal showing systolic (S) and diastolic (E and A waves). These signals have a more linear response to LV damage than traditional transmitral flow.

of sensitive newer technologies has facilitated the recognition of subclinical disease.

Changes in diastolic function are a widely reported finding in diabetic animals (10, 11) and diabetic patients without evidence of heart disease caused by other factors (12–24). In experimental diabetes, papillary muscles from animal hearts have shown prolongation of relaxation and considerable slowing in relaxation velocity (25, 26), and isolated perfused hearts from type II diabetic rats showed prolonged isovolumic relaxation time and increases in late mitral inflow velocity and LV end-diastolic pressure (27). In an *in vivo* animal study with prodromal type II diabetes characterized by postprandial hyperglycemia and hyperinsulinemia, 54 Otsuka Long-Evans Tokushima fatty (OLETF) rats with normal average fasting serum glucose and without premature atherosclerosis and hypertension demonstrated prolonged deceleration time and reduced peak velocity of early filling (3), which represent an early manifestation of abnormal LV diastolic function. A similar study of 30 male diabetic and 30 male control rats showed significant differences in early to late diastolic mitral inflow velocity ratio and isovolumic relaxation time, but not in shortening fraction, deceleration time, and myocardial collagen content. These findings suggest that the presence of diastolic dysfunction in diabetic hearts may relate to uncoupling of the contractile apparatus (which drives early relaxation), without concomitant increases in chamber stiffness (which produces more late diastolic changes) (28). Furthermore, isoproterenol administration to hearts from 4-wk-old diabetic rats reduced the peak rate of relaxation, although the rate of contraction increased normally (29).

Diastolic function parameters in diabetic patients are analogous to those in animal studies. LV ejection time is often reduced, and the length of the preejection period and the ratio of preejection period to LV ejection time are often increased. Diastolic inflow patterns are frequently abnormal, reflecting underlying abnormalities in relaxation and/or reduced myocardial compliance. LV diastolic dysfunction appears to be quite common in well-controlled type II diabetic

patients without clinically detectable heart disease (30); in 46 well-controlled type II diabetic patients who had no evidence of diabetic complications, hypertension, coronary artery disease, congestive heart failure, or thyroid or overt renal disease, and no overt systolic dysfunction, LV diastolic dysfunction was present in 28 subjects (60%), of whom 13 (28%) had a pseudonormal pattern of ventricular filling (indicating raised filling pressure), and 15 (32%) had impaired relaxation (a milder form of diastolic dysfunction). A similar study of 87 young type I diabetic patients without known cardiac disease demonstrated reduced early peak mitral velocity, increased late peak mitral velocity, and prolonged deceleration time and isovolumic relaxation time compared with 87 controls, despite normal LV dimensions and systolic function (31). These findings are concordant with earlier studies in both type II (32) and type I diabetes, compared with healthy control subjects matched for sex, age, and body surface area (33). These findings have been confirmed with newer, less load-dependent techniques; recent results from our laboratory have shown diabetic patients with normal or comparable diastolic function had decreased myocardial diastolic tissue Doppler velocities (34).

Several studies have examined the clinical correlates of diastolic function changes in diabetes. In a study of 49 diabetic patients (26 type I and 23 type II) without known heart disease, these abnormalities were unrelated to sex, age, duration of diabetes, or the presence or extent of complications (35).

Studies that have examined both systolic and diastolic dysfunction in both type I and type II diabetes suggest that the latter is more susceptible to preclinical changes. In a study of 27 type I and 25 type II diabetic patients without hypertension and coronary artery disease, mitral early peak diastolic velocity and its ratio to late peak velocity were more severely decreased in type II than type I diabetics (36). These findings have been confirmed in subsequent work; in 20 type I and 10 type II diabetic patients, systolic function parameters were normal, but diastolic function was clearly impaired in diabetic patients without overt cardiovascular disease com-



pared with 12 healthy controls. Moreover, ventricular filling was impaired more significantly in the type II diabetic patients than in the type I diabetic patients, especially the peak early filling velocity E (37). However, more sensitive systolic parameters, such as preejection period/LV ejection time may be abnormal when ejection fraction is unaltered (35).

However, not all studies show the presence of diastolic dysfunction in diabetic patients. In a comparison of 61 type I diabetic children (average age, 13 yr; diabetes duration, 6 yr) on no medication other than insulin, with 23 healthy subjects without other cardiovascular risk factors, there were no significant differences in systolic and diastolic dimensions, systolic time intervals, fractional shortening, mean velocity of circumferential fiber shortening, percentage relaxation of the LV posterior wall at 50% of diastole, peak velocity of early (E) and late atrial (A) mitral flow, E/A ratio, deceleration time, or isovolumetric relaxation time. Moreover, there was no relation observed between duration of diabetes and any of the parameters analyzed (38). The lack of an association between diabetes and LV diastolic dysfunction in young diabetic subjects (<35 yr) (39) may relate to the prevalence of type I diabetes. Nonetheless, another comparison of both type I and type II adult diabetic patients also showed no significant difference in mean rate-corrected preejection period, LV ejection time, electromechanical systole, and preejection period/LV ejection time ratio compared with those of age- and sex-matched normal subjects (40). The mechanism of protection of type I diabetic patients may relate to protective effects of insulin therapy and lack of insulin resistance. Indeed, animal data suggest correction of abnormal function with insulin therapy, with indices of cardiac performance significantly greater in insulin-treated rats when compared with control rats (41).

### B. Systolic dysfunction in diabetes

Animal studies have shown diabetes to also be associated with systolic dysfunction (27, 42, 43). Similar findings were reported in intact animals; heart rate, systolic blood pressure, and fractional shortening were significantly reduced in diabetic animals compared with control animals (44). In murine isolated papillary muscle preparations, systolic LV pressure was reduced by 15%, and active force was reduced by 61% (25). These changes take some time to develop; systolic function was unchanged in 6-wk-old db/db mice, but fractional shortening and velocity of circumferential fiber shortening were reduced in 12-wk-old db/db mice relative to db/+ control mice (10). These studies suggest that diabetes is the cause of systolic dysfunction.

These findings in animals are supported by both epidemiological and clinical studies. There is a significant association of idiopathic dilated cardiomyopathy with diabetes (45). Conversely, Hamby *et al.* (1) found that the incidence of diabetes was greater than expected in patients with dilated cardiomyopathy, with 16 of 73 (22%) patients with idiopathic cardiomyopathy being diabetic in contrast to only 33 of 300 (11%) control patients. Although the 16 diabetic patients showed normal or no significant coronary artery obstruction by coronary angiography, they were found to have LV dilatation and hypertrophy, elevated LV end-diastolic pres-

sure, and decreased ejection fraction (1). However, gender plays a role in this association; in an analysis of 292 diabetics and 4900 nondiabetics in the Framingham study, there was a 2.4-fold increased incidence of congestive heart failure in diabetic men, compared with a 5.1-fold increase in diabetic women over 18 yr (46). Patients without prior coronary or rheumatic heart disease demonstrated an increased incidence of congestive heart failure in diabetes, independent of age, systolic blood pressure, serum cholesterol, and weight. These epidemiological studies indicate that diabetic patients have a greater risk of developing heart failure independent of coronary artery disease, hypertension, serum cholesterol, and age, suggesting that diabetes might be a cause of dilated cardiomyopathy or heart failure.

In clinical practice, the existence of a diabetic cardiomyopathy was first recognized by Rubler *et al.* (4) based on a study with four adult diabetic patients with congestive heart failure that could not be explained by coronary artery disease, hypertension, valvular or congenital heart disease, or alcoholism. In an echocardiographic comparison of 33 children with known diabetes for an average of 4.5 yr with 51 normal children without increased myocardial mass, Friedman *et al.* (47) demonstrated that diabetic patients had an increased end-systolic diameter and volume, a diminished ejection fraction, and a decreased minor axis shortening and velocity of circumferential fiber shortening. In a similar study of 40 type II normotensive diabetic patients, 22 (55%) patients had systolic dysfunction, but only three (7.5%) had electrocardiographic changes compatible with cardiac ischemia; 16 (40%) patients were also found to have LV hypertrophy (48). Regan *et al.* (2) provided more definitive evidence for cardiomyopathy in four adult diabetic patients without coronary artery disease. After ruling out large vessel disease by coronary angiography and small vessel disease by showing an absence of lactate production during atrial pacing, these patients were found to have modestly increased LV end-diastolic pressure, normal LV end-diastolic volume, and decreased LV compliance. Three patients even had a low ejection fraction with diffuse hypokinesis (2).

Although a number of studies have confirmed the association of LV systolic dysfunction with diabetes mellitus, this finding has not been uniformly reported (2, 4, 17, 18, 47–57). However, many of those who have normal LV systolic function at rest may show abnormalities during exercise or dobutamine stress (53, 55, 56), indicating that LV systolic reserve is reduced in these patients. Diabetic patients have been shown to have a lower cardiac output during supine exercise than controls, with no difference at rest. This lower cardiac output was the result of a lower stroke volume and was independent of the duration of diabetes (56). In a study of juvenile diabetics shortly after diagnosis, stroke volume during exercise was diminished, but cardiac output remained normal due to a higher heart rate (55). Evaluation of cardiac response to dynamic exercise in a group of otherwise healthy insulin-dependent older children and adolescents has shown diabetic patients to have similar LV function at rest compared with controls but reduced systolic function, indicated by fractional shortening and rate-corrected velocity of circumferential fiber shortening after exercise (51). This is not restricted to the young; in 30 diabetic men with normal

resting LV ejection fraction and without coronary or any other cardiovascular disease, LV ejection fraction decreased after exercise in five of the 30 diabetic patients (17%), remained unchanged in eight (27%), and increased normally in only 17 patients (53). However, this is also not uniformly reported; for example, Nugent *et al.* (58) showed no evidence of impairment of the exercise response in subjects with long-standing diabetes. Other studies have shown that an abnormal ejection fraction response during exercise may be attributable to alterations in ventricular loading conditions or cardiac autonomic innervation, or both, rather than to abnormalities in intrinsic ventricular systolic function (contractility). Indeed, despite subgroups showing an abnormal ejection fraction response to exercise, all patients with diabetes have a normal response to afterload manipulation, normal baseline ventricular contractility as assessed by load- and heart rate-independent end-systolic indexes and normal contractile reserve, as assessed with dobutamine challenge (59).

Although many studies have shown that diabetic patients have abnormal diastolic dysfunction but preserved systolic function, we suspect that this relates to the techniques used for systolic function evaluation being less sensitive than those used for assessment of diastolic dysfunction. Recently, we have shown that more sensitive techniques for systolic assessment such as strain, strain rate, and myocardial tissue Doppler velocity can detect preclinical systolic abnormalities in diabetic patients (49).

### C. Structural changes in diabetes

A number of studies in both animals and humans have shown structural changes in parallel with the functional changes of diabetic heart disease, in the absence of hypertension, coronary artery disease, or intraventricular conduction defects (60–67).

In an experimental study with 54 OLETF (type II) diabetic rats, which show mild obesity, postprandial hyperglycemia, and hyperinsulinemia, low peak velocity of early diastolic transmitral inflow and prolonged deceleration time were associated with extracellular fibrosis and abundant TGF- $\beta$ 1 receptor II in LV myocytes. At 15 wk of age, the ratio of collagen area/visual field of LV wall in OLETF rats was greater than that in nondiabetic rats, and the collagen content/dry tissue weight ratio of the heart was significantly increased in OLETF rats compared with control rats (3). These results indicate LV fibrosis in the early stages of type II diabetes. In another study using modern stereological techniques to quantify changes in the morphology accompanying streptozotocin-induced diabetes, the results showed that the time to peak tension and relaxation of papillary muscles was prolonged, the heart weight to body weight ratio was increased, and the volume of extracellular components was increased 3-fold in diabetic rats. At the same time, this study also demonstrated that the volume, surface density, and total surface area of capillaries as well as volume fraction of myocyte mitochondria were reduced, and oxygen diffusion distance to myocyte mitochondria was increased in the diabetic animals (68). Other studies have identified ultrastructural changes in diabetic hearts (69–71). More recently, the 2-D Haar wavelet decomposition method has been used as a tool

to identify textural changes in diabetic rats, which showed increased texture energy compared with controls. These changes were detected before development of echocardiographic structural changes (72).

Similar structural alterations have been described in diabetic hearts without significant epicardial coronary disease in humans. The most prominent histopathological finding in diabetic patients is fibrosis, which may be perivascular, interstitial, or both. As the disease progresses, there is increased myocyte loss and replacement fibrosis. In an autopsy study of nine diabetic hearts (six with heart failure), Regan *et al.* (2) reported replacement fibrosis and interstitial accumulation of periodic acid Schiff-positive material (glycoprotein) in diabetic hearts. Luminal areas in diabetics were not significantly different from controls, and because perfusion and fixation were performed at normal arterial pressure levels, the authors concluded that the findings were not consistent with a microvascular basis for ischemia. However, myocardial triglyceride and cholesterol concentrations were increased in these patients (2). Similar morphological evidence for a diabetic cardiomyopathy was demonstrated by Nunoda *et al.* (73) in seven healthy controls and nine patients with mild diabetes and without hypertension or coronary artery disease. Endomyocardial biopsies were obtained from right ventricular myocardium. The mean diameter of right ventricular myocardial cells was significantly larger, and the percentage of interstitial fibrosis in diabetics was significantly greater than controls (73).

Noninvasive techniques have been used to quantitatively assess structural change in diabetic hearts. In a study using vectorcardiograms to investigate the prevalence of vectorcardiographic bites in 101 diabetic patients (35 type I and 66 type II) without hypertension, coronary artery disease, or intraventricular conduction defects in comparison with 228 normal age- and sex-matched control subjects, the prevalence of bites (expression of small areas of fibrosis, atrophy, or degeneration of the myocardium) was significantly higher in diabetic patients, being identified in 39% of diabetic patients and 10% of the control group (74). More recently, fibrosis in diabetic hearts has been quantified using new techniques such as assessment of ultrasonic backscatter, which is directly related to collagen content. In a study of 26 asymptomatic type I diabetics without hypertension or coronary artery disease, integrated backscatter in the septum and posterior wall was significantly higher in diabetics than controls, corresponding to diastolic dysfunction, although global systolic function was preserved (75). Our recent work has confirmed these results in diabetic patients without LV hypertrophy and coronary artery disease (49). The most likely explanation for the increased myocardial acoustic reflectivity of the diabetic heart is an augmentation of the connective tissue content of the myocardium. Experimental evidence has demonstrated that collagen is a primary determinant of echocardiographic scattering in myocardial tissue and there is a linear relationship between collagen deposition and backscatter magnitude (76). Positive associations were also found between heart weight and total fibrosis with the semiquantitative scale in patients with diabetes alone and with both hypertension and diabetes (77). Thus, the in-

creased myocardial tissue reflectivity in diabetics may represent an early marker of diabetic cardiomyopathy.

### III. Mechanisms of Diabetic Cardiomyopathy

#### A. Factors associated with diabetic cardiomyopathy

The development of diabetic cardiomyopathy is likely to be multifactorial. Putative mechanisms include metabolic disturbances, myocardial fibrosis, small vessel disease, autonomic dysfunction, and insulin resistance.

##### 1. Metabolic disturbances

a. *Alterations in substrate supply and utilization.* Metabolic changes in diabetes are directly triggered by hyperglycemia (78). Diabetic hearts have a primary defect in the stimulation of glycolysis and glucose oxidation (79). Increasing evidence suggests that altered substrate supply and utilization by cardiac myocytes could be the primary injury in the pathogenesis of this specific heart muscle disease (80). A significant reduction in myocardial glucose supply and utilization has been observed in isolated diabetic cardiomyocytes (81) and diabetic patients (82). A major restriction to glucose utilization in the diabetic heart is the slow rate of glucose transport across the sarcolemmal membrane into the myocardium, probably due to the cellular depletion of glucose transporters (GLUTs) 1 and 4 (83, 84), which can be corrected by insulin therapy (84, 85). A second mechanism of reduced glucose oxidation is via the inhibitory effect of fatty acid oxidation on pyruvate dehydrogenase complex due to high circulating FFA (86) (see Section III.A.1.b). This has the net effect of reducing ATP availability and may be more important in type II diabetes, in which FFA levels tend to be higher. The potential importance of this mechanism is exemplified by the observation that diabetic animals with minimal hypertriglyceridemia are resistant to the development of cardiomyopathy (87). Both of these pathological mechanisms are potentially reversible in a short time frame, and the dynamics of each mechanism is compatible with the observation that cardiac dysfunction may be improved with improved metabolic control.

Substrate metabolism affecting contractile function in diabetes has been characterized in perfused hearts from genetically diabetic mice (88). Contractile dysfunction was evident in the genetically diabetic hearts, with increased LV end-diastolic pressure and reduced LV developed pressure, cardiac output, and cardiac power. The rate of glycolysis from exogenous glucose in diabetic hearts was 48% of control, whereas glucose oxidation was depressed to 16% of control, and palmitate oxidation was increased 2-fold. Overexpression of GLUT-4 in perfused hearts from the genetically diabetic mice normalized both cardiac metabolism and contractile function. These findings strongly support a causative role of impaired glucose metabolism in the cardiomyopathy observed in genetically diabetic hearts (89). Similar findings were obtained in an echocardiographic study to determine whether contractile function in diabetic db/db mice was reduced *in vivo* and restored in mice where the transgenic db/db-human GLUT4 had been added to normalize cardiac metabolism. In this model, both systolic and diastolic func-

tion were unchanged in 6-wk-old db/db mice, but fractional shortening and velocity of circumferential fiber shortening and the ratio of E and A transmitral flows were reduced in 12-wk-old db/db mice, indicating the development of a cardiomyopathy. These cardiac functional changes were normalized in transgenic db/db-human GLUT4 mice (10), confirming that the *in vitro* findings that altered cardiac metabolism can cause contractile dysfunction in db/db hearts and that the process is associated with substrate supply and utilization. However, the major derangement in carbohydrate metabolism in diabetic myocardium was not in glycolysis but in pyruvate oxidation (90, 91).

b. *FFA metabolism.* Elevated FFA levels are believed to be one of the major contributing factors in the pathogenesis of diabetes. FFAs enhance peripheral insulin resistance and trigger cell death. Disturbances of FFA metabolism may be an important contributor to abnormal myocardial function in diabetes. These changes are characterized by elevation of circulating FFAs caused by enhanced adipose tissue lipolysis, as well as high tissue FFAs caused by hydrolysis of augmented myocardial triglyceride stores. Moreover, in addition to the FFA-induced inhibition of glucose oxidation (which may contribute to the above effects by limiting the entry of glucose into the cell), high circulating and cellular FFA levels may result in abnormally high oxygen requirements during FFA metabolism and the intracellular accumulation of potentially toxic intermediates of FFA, all of which lead to impaired myocardial performance and severe morphological changes (80, 92). Abnormalities in FFA metabolism have been demonstrated in idiopathic dilated cardiomyopathy in which the rate of FFA uptake by myocardium is inversely proportional to the severity of the myocardial dysfunction (93). It is possible that similar defects contribute to the development of diabetic cardiomyopathy. The FFA-induced impairment of glucose oxidation may be a major factor in the development of diabetic cardiomyopathy, and would explain why cardiac function tends to improve upon metabolic improvement. Furthermore, the availability of carnitine, an essential substance for myocardial FFA metabolism, is usually reduced in diabetes. Evidence of a cardiomyopathy in streptozotocin-induced diabetic rats with no evidence of coronary vascular occlusion and normal serum cholesterol correlates with reduced serum and myocardial carnitine levels and abnormal-appearing mitochondria, consistent with carnitine deficiency (94).

c. *Abnormalities in regulation of calcium homeostasis.* Oxidative stress caused by toxic molecules may play a critical role in subcellular remodeling and abnormalities of calcium handling that lead to subsequent diabetic cardiomyopathy. Alterations in regulatory proteins and contractile proteins, sarcoplasmic (endoplasmic) reticulum  $\text{Ca}^{2+}$ -ATPase and  $\text{Na}^{+}$ - $\text{Ca}^{2+}$  exchanger function may be important contributors to abnormal myocardial carbohydrate and lipid metabolism in diabetes. These changes likely result from accumulation of toxic molecules such as long-chain acylcarnitines, free radicals, and abnormal membrane lipid content. The consequences of these changes include alterations to the calcium sensitivity of regulatory proteins involved in the regulation of the cardiac



actomyosin system, possibly due to phosphorylation of sarcomeric protein troponin I (95). The diminished calcium sensitivity, along with shifts in cardiac myosin heavy chain (V1→V3) (96), reduction of sarcoplasmic reticulum  $\text{Ca}^{2+}$ -ATPase, and decreased sarcoplasmic reticulum calcium (SERCA2a) pump protein may all contribute to impaired LV function (97). Indeed, abnormal systolic and diastolic function normalizes after overexpression of SERCA2a in streptozotocin-induced diabetic rat hearts (25). Similarly, investigation of steady-state and transient changes in stimulus frequency on the intracellular  $\text{Ca}^{2+}$  transient and cell shortening show a slower decay of the  $\text{Ca}^{2+}$  transient and longer times for maximum cell shortening and relengthening. This is most likely due to an accompanying reduction in  $\text{Ca}^{2+}$  efflux from the cell, due to either depressed  $\text{Na}^+/\text{Ca}^{2+}$  exchanger activity or an elevation in intracellular  $\text{Na}^+$  levels (98). Finally, alterations in the expression of myosin isoenzymes and regulatory proteins as well as myosin phosphorylation have been demonstrated to contribute to the development of myofibrillar remodeling in the diabetic heart (99).

*d. Correlation of metabolic changes with LV dysfunction.* If cardiac changes were triggered by hyperglycemia in diabetes, functional or structural changes in diabetic heart disease would be closely related with diabetic control. In isolated papillary muscle from rat hearts, resting and developed tension in animals with short-term streptozotocin-induced diabetes was similar with isometric contraction, but time to peak tension and time to half relaxation were prolonged, and the peak rate of tension rise and tension fall was depressed. Myocyte diameters were similar with all disease durations, although slightly increased interstitial fibrosis and disarrangement of myocytes were found after 12 wk in the diabetic hearts. However, myocardial functional changes did not worsen in parallel with histological changes but correlated with the blood glucose level, suggesting that short-term functional abnormalities in the experimental diabetic rat heart result from the metabolic disorder itself at an early stage (100). In a study of 50 type I diabetic children free of cardiovascular symptoms (mean age, 13 yr; diabetic duration, 5.9 yr), Cerutti *et al.* (16) reported a significant delay in LV filling (pressure half time), those with longer diabetic duration and poor glycemic balance having more disturbed filling. Other studies in type I diabetics have also demonstrated similar results (101, 102). In type II diabetes, there is a close relationship between glycemic control and serum IGF-I level, with worse control being associated with lower IGF-I levels (103). IGF-I has been shown to suppress myocardial apoptosis and improve myocardial function in various models of experimental cardiomyopathy. In a study of both type I and type II diabetic patients without overt systolic dysfunction and known heart disease, diastolic function was clearly impaired in both groups of patients, with ventricular filling impaired more significantly in the type II patients. There was a significant inverse correlation between glycosylated hemoglobin (HbA1C) and peak late filling velocity (A) in both groups of patients, and there was a direct correlation between diastolic velocity time integral and age, duration of diabetes, and HbA1C (37). Finally, a study of ultrastructural changes in diabetic myocardium using myo-

cardial integrated backscatter in 20 diabetic patients has shown that myocardial integrated backscatter was significantly greater in diabetic patients than in normal subjects, and there was a significant correlation between HbA1c and myocardial integrated backscatter in diabetic patients. Moreover, the greatest myocardial integrated backscatter was shown in patients with hypertension (65).

*e. Response to therapy.* The response to hypoglycemic therapy further confirms the correlation of myocardial functional and structural changes with glycemic control. Pogatsa *et al.* (104) evaluated the effects of hypoglycemic therapy on chronically diabetic dogs with marked hyperglycemia. They found untreated diabetic animals had a higher LV passive elastic modulus (a measure of stiffness) and LV end-diastolic pressure, and a lower cardiac output. There was also a close inverse relationship between cardiac output and passive elastic modulus (104). An equivalent study in rats showed that diabetes caused significant decreases in resting LV systolic pressure, developed pressure, maximal  $+dP/dt$ , and the overall chamber stiffness constant, whereas LV end-diastolic pressure, LV cavity/wall volume, and end-diastolic volume were increased, and the time constant of LV relaxation was prolonged after 26 d of diabetes. All of these abnormalities were reversed by insulin treatment (105). In an experimental study of hearts in mild diabetic rats, there was a 36% reduction in glucose utilization, mainly caused by a 55% reduction in glucose uptake in the diabetic heart. This reduced carbohydrate metabolism was accompanied by a 37% reduction of oxygen uptake as well as a significant reduction in cardiac output. Diabetic hearts obtained 46% of their energy requirements from endogenous glycogen compared with 9% from this source in the control hearts. Both islet transplantation and insulin therapy led to a complete reversal of the hemodynamic and metabolic alterations (106).

Several studies have examined the effects of therapy on structural changes. A study in diabetic animals demonstrated a significant decrease in myocyte cross-sectional area during the first 12 wk of diabetes and then stabilization, accompanied by decreases in the relative volume densities of myofibrils and mitochondria and interstitial and perivascular deposition of extracellular matrix. Capillary density and diameter also exhibited progressive decreases of more than 20% over 26 wk of diabetes. These structural changes were prevented by insulin treatment begun 3 d after induction of diabetes. When delayed for 12 wk, insulin reversed the changes in myocyte and capillary relative volume densities, and in capillary diameter within 6 wk, ultrastructural changes within 12 wk, and myocyte cross-sectional area after 26 wk. However, even after 26 wk of treatment, the extracellular matrix remained more than twice that observed in nondiabetic animals, with a consequent decrease in the number of capillaries per unit volume of tissue. This study suggests that diabetes results in progressive, marked changes in the myocardium that can be prevented by early insulin treatment but only selectively reversed by delayed insulin treatment (62). A similar study examined alloxan-induced diabetic rats for the effects of diabetes and insulin treatment on contractile and supporting elements of myocardium. Diabetes caused a focal, progressive loss of myofibrils, transverse

tubules, and sarcoplasmic reticulum and separation of the fasciae adherens at the intercalated disks. These changes were accompanied by interstitial and perivascular deposition of connective tissue, thickening of the endothelial cytoplasm with pinocytotic hyperactivity, and characteristic basal laminar changes. Most, but not all, of these changes were reversed after 6–12 wk of insulin treatment (64). Interestingly, experimental studies have also demonstrated normalization of the collagen alteration by endurance training, begun relatively early in the disease process (107). The improvement may be related to improved diabetic control due to increased insulin sensitivity caused by exercise training.

In diabetic patients without known cardiac disease, abnormalities of LV function primarily reflect a diastolic abnormality. This diastolic abnormality appears related to interstitial collagen deposition, although LV hypertrophy may eventually appear in the absence of hypertension. Reversibility of this process can be achieved with chronic insulin therapy. Sykes *et al.* (108) found that the preejection period was shortened in a group of 19 diabetics before treatment with either diet or oral hypoglycemic agents and LV ejection time was shortened. These abnormalities were reversed after 3 months of therapy (108). Shapiro *et al.* (109) studied 69 type II diabetics before and after hypoglycemic therapy using both systolic time intervals and M-mode echocardiography. The preejection period/LV ejection time ratio was increased in the untreated group, and this ratio correlated well with blood glucose concentration. Treatment resulted in a fall in preejection period/LV ejection time ratio in 54 patients with a modestly increased initial ratio but no response in the remaining 15 patients with a markedly elevated initial ratio after 4 months of therapy. Isovolumetric relaxation was prolonged in diabetics, but it was not affected by hypoglycemic therapy (109). In another study of 15 type I diabetic subjects without known heart disease and diabetic complications, systolic time intervals were evaluated at rest and after dynamic exercise during poor and good metabolic control, obtained by means of insulin therapy. Resting systolic time intervals were normal during poor and good metabolic control. After exercise, a greater increase in preejection period/LV ejection time ratio as a result of an increased preejection period was found during poor control, and a smaller increase in preejection period/LV ejection time ratio occurred during good metabolic control, suggesting that good diabetic control is associated with the improvement in LV function (101).

However, a discordant relationship between diabetic control and functional changes has also been found in some studies. In a study of type II diabetes without evidence of hypertension, coronary artery disease, and other known cardiac diseases, the results showed there was no correlation between LV diastolic dysfunction and indices of metabolic control in those with normal systolic function and abnormal diastolic function (30). Friedman *et al.* (47) demonstrated an increased LV end-systolic diameter and volume, a diminished ejection fraction, minor axis shortening and velocity of circumferential fiber shortening in type I diabetic children. However, no relationship between ventricular function and either the duration or the severity of diabetes was observed

(47). The impact of diabetic treatment is also associated with discordant results. Regan *et al.* (110) demonstrated a lower stroke volume in animal models of diabetes mellitus in dogs despite normal LV end-diastolic pressure, normal coronary arteries, and coronary blood flow. Chamber stiffness was increased in diabetic dogs compared with control dogs, presumably related to the deposition of interstitial glycoprotein and collagen. However, these changes could not be reversed with correction of hyperglycemia or prevented by insulin (110).

**2. Myocardial fibrosis.** Myocardial fibrosis and myocyte hypertrophy are the most frequently proposed mechanisms to explain cardiac changes in diabetic cardiomyopathy. Studies in dogs, monkeys, and rabbits have shown that experimentally induced diabetes causes defects in cellular calcium transport (111), defects in myocardial contractile proteins (112), and an increase in collagen formation (110), which result in anatomic and physiological changes in the myocardium.

*a. Myocyte cell death.* Myocyte cell death may be caused by apoptosis or necrosis or both. Apoptosis is an active genetically controlled process that removes unwanted or damaged cells, whereas myocyte necrosis refers to myocyte destruction due to biochemical damage. Apoptosis can be evaluated by the identification of double-strand DNA cleavage with single base or longer 3' overhangs. In contrast, myocyte necrosis can be assessed by detection of DNA damage with blunt end fragments (113).

Both apoptosis and necrosis have been identified in diabetic heart disease. In a study of diabetic and diabetic-hypertensive hearts, myocyte necrosis was 1.4-fold more prevalent in patients with diabetes and hypertension than with diabetes alone, whereas myocyte apoptosis was not affected by the addition of hypertension (114). These two distinct forms of cell death also cause different consequences. Apoptosis does not cause scar formation or significant interstitial collagen accumulation (115), with nuclear fragmentation and cell shrinkage being replaced by the surrounding cells (116, 117). Conversely, myocyte necrosis results in widening of the extracellular compartments among myocytes and increased deposition of collagen in a diffuse or scattered manner (118, 119), resulting from both replacement fibrosis due to myocyte necrosis and connective tissue cell proliferation (120).

*b. Process of myocardial fibrosis.* Collagen accumulation in the diabetic myocardium may be due in part to impaired collagen degradation resulting from glycosylation of the lysine residues on collagen. Hyperglycemia also results in the production of reactive oxygen and nitrogen species, which increases oxidative stress and causes abnormal gene expression, alters signal transduction, and activates the pathways leading to programmed myocardial cell death or apoptosis. This process is associated with the glycosylation of p53, which results in an increment in angiotensin II synthesis; this in turn leads to p53 phosphorylation, increased Bax expression, and also to myocyte apoptosis. These changes parallel the concentrations of glucose in the medium and the duration of the culture. Inhibition of the p53 glycosylation prevents



the initial synthesis of angiotensin II and consequent p53 activation and apoptosis (121). Evidence *in vivo* has shown that hyperglycemia directly induces apoptotic cell death and myocyte necrosis in the myocardium, triggered by reactive oxygen species derived from high levels of glucose (122). Interestingly, cardiomyocytes incubated for 3 d with medium containing 25 mM glucose showed less hypoxia-induced apoptosis and necrosis than cells exposed to medium containing 5 mM glucose, suggesting that glucose treatment renders the cardiomyocyte resistant to hypoxia-induced apoptosis and necrosis (123).

The increased angiotensin II and angiotensin receptor levels have been shown in an *in vivo* study in streptozotocin-induced diabetic rats, in which changes in angiotensin II quantity, the fraction of angiotensin II positive cells, and the number of angiotensin II receptor sites per myocyte paralleled the change in myocyte death (124). The change in angiotensin II and angiotensin II receptors in diabetic hearts appears to be local and independent of the circulating renin-angiotensin system (125, 126). Up-regulation of the local renin-angiotensin system in diabetes may enhance oxidative damage, activating cardiac cell apoptosis and necrosis (114). Thus, either increased angiotensin II or increased angiotensin II receptor density enhances the effect of angiotensin II. Whichever the mechanism, angiotensin II has dose-dependent effects on collagen secretion and production in rat adult cardiac fibroblasts (128). On the other hand, alterations of endothelin-1 and its receptors were also associated with increased focal fibrous scarring with apoptotic cardiomyocytes in diabetic rats, and the fibrotic process was completely prevented by treatment with bosentan, suggesting that hyperglycemia-induced up-regulation of the endothelin system in the diabetic heart may also play an important role in myocardial fibrosis (129).

Local angiotensin II effects are modulated by the function of IGF-I, a key factor for cardiac growth and function. Angiotensin II and IGF-I are generated by cardiomyocytes and exert pleiotropic effects in an autocrine/paracrine fashion. Both angiotensin II and apoptosis are reduced by IGF-I. IGF-I is decreased in diabetes, and exogenous IGF-I treatment has been shown to ameliorate contractile disturbances in cardiomyocytes from diabetic animals, suggesting that IGF-I also plays an important role in myocardial fibrosis and development of diabetic cardiomyopathy. This was demonstrated in streptozotocin-induced diabetic mice, wherein diabetes progressively depressed ventricular performance but had no hemodynamic effect on those with IGF-I overexpression. Myocyte apoptosis measured at 7 and 30 d after the onset of diabetes was 2-fold higher in diabetic mice without than with IGF-I overexpression. Myocyte necrosis was apparent only at 30 d and was more severe in diabetic nontransgenic mice, which lost 24% of their ventricular myocytes and showed a 28% myocyte hypertrophy, both of which were prevented by IGF-I (130). Therefore, resistance to actions of IGF-I and insulin could explain the abnormalities of both diastolic and systolic function and LV hypertrophy.

The effects of angiotensin II may also be promoted by the production and release of TGF- $\beta_1$  by cardiac fibroblasts (131, 132). TGF- $\beta_1$  plays a critical role in organ morphogenesis, development, growth regulation, cellular differentiation,

gene expression, and tissue remodeling. TGF- $\beta_1$  induced by metabolic abnormalities (chronic postprandial hyperglycemia, hyperinsulinemia, insulin resistance) has also been implicated in the development of diabetic cardiomyopathy. In the rat heart, TGF- $\beta$  increases fibrous tissue formation and up-regulates collagen expression during tissue repair by binding to the TGF- $\beta$  type II receptor. TGF- $\beta_1$  receptor II expression has been shown to be significantly increased in the left ventricle of OLETF (type II diabetes model) rats, and the ratio of collagen content/dry weight of the left ventricle was significantly higher in OLETF rats than in control rats at 15 wk of age (3). Thus, this cytokine may participate in the onset of cardiac fibrosis by stimulating extracellular matrix synthesis.

*c. Consequences of myocardial fibrosis.* Fibrosis is attributed to replacement fibrosis caused by focal myocyte necrosis (133, 134) and increased interstitial fibrosis, in part due to the reaction of connective tissue cells to pathological loads (120).

A biopsy study in patients with diabetes mellitus has shown that hypertrophy of myocardial cells and interstitial fibrosis of the myocardium are present in mild diabetes mellitus (73). Indeed, diabetic heart disease may simply reflect increased interstitial fibrosis in the heart, because collagen accumulation occurs mainly as a result of an increase in type III collagen in the diabetic heart (135). Cell death in the diabetic myocardium is not only necrotic in nature but is also mediated by apoptosis—thus, interstitial fibrosis may not be severe. In a longitudinal study of cardiac performance in streptozotocin-induced type I diabetic rats for 56 d using noninvasive echocardiographic techniques, significant reductions in diastolic performance (transmitral flow velocities and slopes) and systolic dysfunction (LV fractional shortening, cardiac output) developed in the absence of fibrosis (136), suggesting that abnormal heart function in this model may be of metabolic rather than structural origin. This is also supported by a similar study to investigate the chronic effects of streptozotocin-induced diabetes on contraction in rat ventricular myocytes, which showed that time to peak contraction was significantly longer at 2 months but appeared to normalize at 10 months, and the time to half relaxation of contraction was not significantly different after 2 months but was significantly reduced at 10 months. The ultrastructure of cardiac muscle and sarcomere lengths were not greatly altered after streptozotocin treatment, also indicating that morphological defects in contractile myofilaments and associated structures do not explain contractile dysfunction seen in this model (137).

Another question is whether the fibrosis and/or dysfunction in the diabetic heart are a result of small vessel disease. At present, this seems unlikely; several studies have shown decreased LV function without vascular lesions (138). Similarly, our recent work shows no increment of abnormal function (measured by sensitive tissue Doppler indices) after dobutamine stress (34). The up-regulation of the local renin-angiotensin system suggests that cardiac structural and functional changes in diabetes are not a result of change in the circulating renin-angiotensin system, but are relatively specific to the heart, leading to a specific diabetic cardiomyopathy.

*d. Correlation of structural changes to LV dysfunction.* The functional abnormality in diabetic myocardium is considered to be associated with myocardial structural changes, and indeed, these structural changes might play a role in progressive deterioration of cardiac hemodynamics.

Experimental data strongly support the connection between structural changes and heart muscle dysfunction in diabetes. After 2 months of streptozotocin-induced diabetes, *in vitro* study of myocytes showed a 30% increase in time to peak shortening, which corresponded to a significant reduction in resting sarcomere length and a change in the microtubular cytoskeleton (60), suggesting that myocardial structural change may be the basis for cardiac dysfunction. Another study showed that rats with streptozotocin-induced non-insulin-dependent diabetes had prolonged isovolumic relaxation time, elevated LV end-diastolic pressure, and increased chamber stiffness; these functional changes were accompanied with increased LV mass (27). A similar experimental study in animals also showed that functional changes (*e.g.*, reduced LV compliance) after 1 yr of diabetes were associated with increased interstitial connective tissue (110). A clear relationship between functional and structural changes is indicated by a study showing that diabetic rats exhibited prolonged deceleration time and low peak velocity of early diastolic transmitral flow, which is associated with extracellular fibrosis in LV myocytes, and higher ratio of collagen area/visual field of LV wall and ratio of collagen content/dry heart weight compared with control rats (3).

In diabetic patients, noninvasive studies revealed abnormal systolic and diastolic function present in many diabetic patients, particularly in the presence of hypertension. Pathology studies show that myocardial hypertrophy and fibrosis are commonly present in these patients. Das *et al.* (139) have found that there was a correlation between histological and clinical features in a study of endomyocardial biopsies in 16 diabetics, with myocardial changes more pronounced in the symptomatic group and less so in asymptomatic patients (139), suggesting that myocardial dysfunction in diabetics might be secondary to accumulation of glycoprotein within the interstitium together with mild interstitial fibrosis. Zoneraich (140) also showed increased myocardial fibrosis in diabetics, particularly in those with cardiomegaly, and suggested that changes in cardiac interstitial collagen might increase myocardial wall stiffness that is usually associated with functional changes.

Systolic dysfunction may be more dependent on the degree of myocyte loss and myocyte injury. Myocyte cell death and injury may impair the ability of the myocardium to develop force, and they account for reduced contractility, decreased pump function, and ejection fraction. The development of systolic dysfunction during exercise in some patients may reflect loss of contractile reserve related to limited myocyte loss, insufficient to influence resting function.

Abnormal LV systolic function in diabetic patients may be transient, reversible, and related to changes in diabetic control within a certain range and need not indicate structural myocardial disease (141). This has been well illustrated in a study of LV ejection fraction by nuclear angiography in nine newly diagnosed type I diabetic patients at diagnosis and after a period of stable control, after which five showed a

significant change in LV ejection fraction. In contrast, a control group of 10 type I diabetic patients whose control was stable showed no significant change in LV ejection fraction.

In contrast, diastolic dysfunction is likely the result of both accumulation of collagen and myocyte injury in the heart. This may explain the greater prevalence of diastolic dysfunction in type II diabetes, because aging-related increments in cardiac collagen are likely additive, although less satisfactory glycemic control may be an important factor as well. The role of fibrosis is supported by a study showing reversal of cardiac fibrosis by short-term pirfenidone and spironolactone treatment and attenuation of increased diastolic stiffness without normalizing cardiac contractility in streptozotocin-induced diabetic rats (142). Nonetheless, myocyte injury does affect diastolic function; diabetes mellitus can produce a stiff myocardium before the development of myocardial fibrosis due to formation of advanced glycosylation end products (143). However, the contribution of myocyte injury to diastolic dysfunction appears to be smaller than that due to accumulation of collagen. Alterations in myocardial structure are usually small at an early stage of diabetes, and these may be mainly related to myocyte injury, which may be reversible or partially reversible. As diabetes progresses, accumulation of collagen becomes obvious and may play a major role in the development of diastolic dysfunction. These chronic alterations are believed to result from repeated acute cardiac responses to suddenly increased glucose levels at the earlier stage of diabetes.

The relationship between metabolic disturbance, fibrosis, and diastolic dysfunction may be superimposed on the three stages of diastolic dysfunction. Stage 1 represents impaired myocardial relaxation (both myocardial and mitral inflow  $E/A < 1$  and impaired relaxation mitral inflow pattern). Early relaxation is an active process; thus, this stage is characterized by metabolic disturbance more than fibrosis. Stage 2 represents moderate diastolic dysfunction (myocardial  $E/A < 1$ , mitral inflow  $E/A > 1$ , pseudonormal mitral inflow pattern); this stage is characterized by moderate fibrosis and increased left atrial pressure. Stage 3 represents severe diastolic dysfunction (myocardial  $E/A < 1$ , mitral inflow  $E/A > 1.5$ , restricted mitral inflow pattern); this stage features severe fibrosis and significantly increased left atrial pressure.

*3. Small vessel disease.* Structural and functional alterations of the small vessels in diabetes have been incriminated in the development of diabetic cardiomyopathy, although this remains controversial. This section will examine structural and functional abnormalities in diabetic vessels, and then review the evidence in support of a connection with diabetic myocardial disease.

*a. Structural abnormalities of vessels.* The morphological changes of small vessels seen in diabetic myocardium are characterized by a microangiopathy involving arterioles, capillaries, and venules, and by hyaline arteriosclerosis. These changes usually include basement membrane thickening, arteriolar thickening, capillary microaneurysms, and reduced capillary density, which may be the results of periarterial fibrosis and focal subendothelial proliferation and fibrosis, possibly due to abnormal permeability of diabetic capillaries. Thus, in a biopsy study, diabetic patients had

nearly normal or mildly depressed systolic LV function but significantly greater thickening of the capillary basement membrane, accumulation of toluidine blue-positive materials (*i.e.*, materials showing metachromasia), interstitial fibrosis, and smaller myocytes (cell atrophy) compared with the control subjects, and the presence of hypertension was synergistic for these changes (144). This suggests that alterations in capillaries due to diabetes may lead to myocardial cell injury and interstitial fibrosis and, ultimately, to diabetic cardiomyopathy. The association of microvascular disease with diabetic cardiomyopathy is further supported by a study in two models of congestive cardiomyopathy including the hereditary cardiomyopathic Syrian hamster and the hypertensive-diabetic rat. Histopathological study revealed microvascular spasm in both the genetic and the acquired disease models early in the disease associated with small areas of myocytolytic necrosis that undergo subsequent fibrosis. The combination of cell loss and slowly decreasing contractility resulting from the reactive hypertrophy due to a compensatory response to myocellular necrosis culminates in a cardiomyopathy (145). All of these features have been described in diabetic hearts, suggesting a similar disease process in the cardiac microcirculation and the presence of diffuse myocardial small vessel disease in diabetes.

Examination of the myocardium in diabetic animals shows that the volume of extracellular components is increased 3-fold and the volume of capillaries is reduced. The surface density and total surface area of capillaries was reduced, and oxygen diffusion distance to myocyte mitochondria increased (68). An *in vivo* animal study of diabetic rats also revealed numerous areas of microvascular tortuosity, focal constrictions, and microaneurysm formation, although these changes were most prominent in rats with both hypertension and diabetes (146).

Evidence for the association of small vessel disease with myocardial disease is supported by an autopsy study of three diabetic patients, in whom both endothelial and subendothelial proliferation with fibrosis was observed in the small coronary arteries (1). This was further supported by a post-mortem study of intramural coronary arteries in 116 diabetic patients compared with 105 nondiabetic patients. The results showed that endothelial proliferation with interspersed peroxidase acid Schiff material was found more commonly in vessels of all sizes in diabetics than in those of nondiabetics. Small arteries and arterioles displayed hyaline thickening in 50% of diabetics compared with 21% of nondiabetics. These changes were not related to systolic hypertension (147). Furthermore, a biopsy study during coronary bypass surgery by Fischer *et al.* (148) found capillary basement membrane thickening in diabetics, which was quantitatively greater in patients with overt diabetes compared with those with only glucose intolerance.

Despite these findings, it has been proposed that such focal changes in microvessels are insufficient to account for the diffuse myocardial degeneration with interstitial fibrosis in diabetic cardiomyopathy. Another substantial argument against the contribution of microangiopathy was shown in a study of patients with diabetes compared with control patients with hypertension, both hypertension and diabetes mellitus, and neither hypertension nor diabetes mellitus. Us-

ing vascular perfusion fixation and sampling tissue blocks in three different planes, Sunni *et al.* (66) showed no significant differences in the extent of small vessel disease or the density distribution of vessels of various size categories between the groups. No significant differences were found in intramyocardial arteries in diabetic cardiomyopathy and arterial lesions of diabetes compared with controls (66). Although most of these patients with diabetes mellitus also had myocardial infarction and the effects of large vessel ischemia may have affected any difference between the groups, there is no direct proof that microvasculopathy is an underlying cause of diabetic cardiomyopathy. In a similar study comparing endomyocardial biopsies from seven symptom-free type I diabetic patients with biopsies from seven age- and sex-matched nondiabetic subjects, arteriolar hyalinization was found in three patients and arteriolar thickening was observed in five patients. Morphometry performed on electron micrographs showed no significant difference in the thickness of the capillary basal lamina between diabetics and controls. These findings further indicate that the abnormality of cardiac function described in diabetes is not associated with thickening of the myocardial capillary basal lamina (138).

*b. Functional abnormalities of vessels.* The association of small vessel disease with diabetic cardiomyopathy is supported by the observation that similar abnormalities in coronary small vessel function occur in both diabetes and dilated cardiomyopathy, maximal pharmacological coronary flow reserve is reduced, and endothelium-dependent coronary vasodilation is impaired in both dilated cardiomyopathy (149, 150) and diabetes mellitus (32, 151).

Recent studies have directed more attention to the role of functional alterations in small vessels such as impaired coronary vascular reserve and abnormal endothelium-dependent vasodilation in diabetic heart disease. Metabolic substrates or products such as adenosine play an important role in regulating microvascular tone to maintain constant coronary blood flow for a given level of metabolic demand. Increase of coronary blood flow induced either by pacing or inotropic agents (to increase myocardial oxygen demand) was reduced in spontaneously diabetic rats compared with nondiabetic rats (152).

Reduced coronary flow reserve may lower the threshold for myocardial ischemia, particularly when coronary stenoses are present. It has been proposed that diabetic cardiomyopathy is a consequence of repeated episodes of myocardial ischemia resulting from both structural and functional abnormalities in small vessels during increased myocardial demand or from microvascular spasm due to changes in calcium distribution. Such a process would lead to focal cell loss due to microvascular spasm and reperfusion injury, with the subsequent development of focal fibrosis and reactive hypertrophy in response to the myocardial necrosis.

These findings are, however, outweighed by a larger body of work that shows no association between vascular and myocardial disease in diabetes. A study using dipyridamole in diabetic patients with normal global systolic function and impaired diastolic function has shown maximal coronary flow to be significantly reduced and minimal coronary re-



sistance to be increased, although there was no difference in myocardial oxygen consumption compared with controls (32). Similarly, a 29% reduction of myocardial blood flow and significant increase in total coronary resistance during hyperemia and consequent impairment of coronary flow reserve have been reported in type I young adult diabetic patients with no or minimal microvascular complications and without any evidence of coronary heart disease (153). Another study in normotensive type II diabetes demonstrated that myocardial blood flow was not only significantly reduced in diabetic patients but also correlated significantly with average fasting glucose concentration and average HbA1c (154). Although a further study confirmed reduction of flow reserve, this was ascribed to a significantly higher resting myocardial blood flow (155).

Similarly, diabetic patients did not exhibit lactate production during atrial pacing (2, 156), and our studies of the dobutamine response show no further decrement in tissue velocity with increasing stress (as might be expected with ischemia) (34). A number of other studies contest the association of a diabetic cardiomyopathy with stenosis of small coronary arteries (138, 156). Finally, myocyte alterations have been shown to develop before the detection of vascular lesions in genetically diabetic mice (112).

*c. Endothelial dysfunction.* Endothelial dysfunction associated with diabetes mellitus has recently been reviewed in *Endocrine Reviews* (157) and may in part explain the reduced coronary flow reserve observed in diabetic patients. Endothelium-dependent responses of both small and large vessels are impaired in diabetic rats (158, 159). Diabetic patients with an otherwise low likelihood of atherosclerosis also have impaired endothelium-dependent dilatation in the epicardial coronary arteries (151) and in forearm arteries (160). Several mechanisms have been implicated in the abnormal endothelium-dependent vasodilation in diabetes. The half-life of nitric oxide is reduced due to increased oxidative stress (27, 161–163), and nitric oxide activity is attenuated by accumulated glycosylation end products (164). On the other hand, synthesis of vasoconstrictor prostanoids by the endothelium was increased, so that vasoconstriction is enhanced in diabetic subjects (165). In addition, protein kinase C activity is increased in hyperglycemia and may also play a role in development of endothelial dysfunction in diabetes (166). Protein kinase C activation is associated with abnormal retinal and renal hemodynamics in diabetic animals, and overexpression of the  $\beta$ -isoform in myocardium is associated with cardiac hypertrophy and failure (167), implying that this may play a role in the development of diabetic cardiomyopathy by affecting vascular cells.

*d. Summary: abnormal microvascular structure and function and diabetic cardiomyopathy.* In the acute diabetic heart, metabolic derangements in both fuel supply and utilization by heart tissue could serve as the biochemical lesion initiating disease. Over a chronic period, a number of subsequent vascular changes develop and involve an abnormal vascular sensitivity and reactivity to various ligands, depressed autonomic function, increased stiffness of the vascular wall, and abnormalities of various proteins that control ion move-

ments, particularly intracellular calcium. Although it is still unclear how coronary microvascular abnormalities in diabetes lead to diabetic cardiomyopathy, the association of microvascular disease with diabetic cardiomyopathy is supported by the observation that similar abnormalities in coronary microvascular function occur in both diabetes and dilated cardiomyopathy, maximal pharmacological coronary flow reserve is reduced, and endothelium-dependent coronary vasodilation is impaired in both dilated cardiomyopathy (149, 150) and diabetes mellitus (32, 151). Thus, diabetic cardiomyopathy can be caused by focal cell loss due to microvascular spasm and reperfusion injury, with the subsequent development of focal fibrosis and reactive hypertrophy in response to myocardial necrosis.

*4. Cardiac autonomic neuropathy (CAN).* CAN may also play a role in the development of diabetic cardiomyopathy.

*a. Evaluation of CAN.* CAN can be assessed by conventional methods, including the heart rate response to the Valsalva maneuver, standing up, or deep breathing; the blood pressure response to standing up or exercise; and corrected QT measurements (168–170). Heart rate variability determined in either the time or frequency domain reflects parasympathetic, mixed sympathetic, and parasympathetic and circadian rhythms (171). Heart rate variability is a good indicator of CAN in diabetic patients without cardiac disease in response to exercise stress (172) or dipyridamole stress (173), although the development of autonomic symptoms in asymptomatic patients with abnormal heart rate variability is uncommon over a long period (174).

Sympathetic denervation is an important feature of CAN in diabetes. Recently, direct assessment of cardiac sympathetic integrity has become possible. Scintigraphic studies have provided unique insights into the effects of diabetes on cardiac sympathetic integrity and the pathophysiological consequences of LV sympathetic denervation. Quantitative scintigraphic assessment of cardiac sympathetic innervation is possible with either  $^{123}\text{I}$ -metaiodobenzylguanidine ( $^{123}\text{I}$ -MIBG) imaged by single photon emission computed tomography (169, 175–177) or  $^{11}\text{C}$ -hydroxyephedrine (HED) with positron emission tomography (PET) (178–180).

In diabetic patients with CAN, global myocardial uptake of  $^{123}\text{I}$ -MIBG is decreased, indicating the presence of cardiac sympathetic dysfunction (181). Cardiac sympathetic denervation is common in long-term type I diabetes without ischemic heart disease even in patients without other evidence of CAN. The posterior myocardium is predominantly affected, indicating the presence of regional heterogeneity of cardiac sympathetic denervation (182). Defects of both global and regional cardiac  $^{123}\text{I}$ -MIBG uptake have been shown in newly diagnosed metabolically stabilized type I diabetes patients without myocardial perfusion abnormalities. The uptake of  $^{123}\text{I}$ -MIBG in diabetic patients was reduced more in the posterior myocardial region compared with the lateral and apical region, and the septal myocardial region exhibited a smaller uptake than the lateral myocardial region. These results suggest that cardiac sympathetic denervation with regional differences was present in newly diagnosed metabolically stabilized type I diabetes patients without myocardial perfusion defects (170). Furthermore, reduced myocar-

dial MIBG uptake was more severe in type II diabetes patients than that in type I patients, particularly involving inferoposterior segments. These findings suggest that regional sympathetic damage is relatively common in type II diabetes (183).

The degree of sympathetic damage also differs in the distal compared with the proximal left ventricle in subjects with CAN. A study using the sympathetic neurotransmitter analog  $^{11}\text{C}$ -HED showed that 6 months of streptozotocin-induced diabetes resulted in heterogeneous cardiac sympathetic denervation in the rat, with maximal denervation occurring distally (178). In another similar study evaluating the sympathetic nervous system of the heart in diabetic patients using PET imaging with  $^{11}\text{C}$ -HED, abnormal regional  $^{11}\text{C}$ -HED retention was seen in seven of eight patients with CAN. Relative tracer retention was significantly reduced in apical, inferior, and lateral segments, and absolute myocardial tracer retention index measurements showed a significant decrease in distal compared with proximal myocardial segments in CAN (184). However, a study of LV sympathetic innervation in diabetic patients using the PET sympathetic neurotransmitter analog  $^{11}\text{C}$ -HED showed that diabetes results in LV sympathetic denervation with proximal hyperinnervation complicating distal denervation (185).

Interestingly, CAN is associated with altered myocardial blood flow, with regions of persistent sympathetic innervation exhibiting the greatest deficits of vasodilator reserve. This was demonstrated in a study of 14 diabetic subjects (seven without CAN, seven with advanced CAN) and 13 nondiabetic control subjects without known coronary artery disease. PET using  $^{11}\text{C}$ -HED was used to characterize LV cardiac sympathetic innervation and  $^{13}\text{N}$  ammonia to measure myocardial blood flow at rest and after iv administration of adenosine. Persistent LV proximal wall sympathetic innervation was observed, even in advanced CAN. Global LV myocardial blood flow and coronary flow reserve were significantly less in the neuropathic subjects than in the non-neuropathic diabetic group during adenosine infusion, despite higher resting myocardial blood flow in the neuropathic subjects. Assessment of the myocardial innervation/blood flow relation during adenosine infusion showed that myocardial blood flow in neuropathic subjects was significantly lower (43%) in the proximal innervated segments, but the same in the distal denervated myocardium compared with that in the nonneuropathic diabetic subjects (186). These results may suggest alterations in LV regional functional relationships between the proximal and distal myocardial segments.

*b. Myocardial catecholamine levels.* The change in sympathetic innervation in the diabetic heart has drawn attention to alterations of catecholamine levels and adrenergic receptors in the myocardium. In streptozotocin-induced diabetic rats, ventricular norepinephrine levels were increased after 1 and 2 months of diabetes, but were at or below control levels after 4 months of diabetes. Histofluorescence studies demonstrated that the density of noradrenergic varicosities in diabetic rat hearts appeared increased, with abundant branched profiles after 1 month of diabetes (187). Similar

results were also found in spontaneously diabetic Chinese hamsters.

Cardiac norepinephrine content and  $\beta$ -adrenergic receptor density are also significantly increased in short-term diabetics. These changes preceded both the development of cardiac hypertrophy and the enhanced adenylyl cyclase activity. However, as the diabetic state developed, cardiac norepinephrine content,  $\beta$ -adrenergic receptor density, and adenylyl cyclase activity returned to control levels (188). The increased norepinephrine in the early stages of diabetes may be due to increased bradykinin-induced release of norepinephrine, which has been shown to be four times greater in diabetic than in normal preparations (189), as well as the acute effects of high glucose levels on sympathetic activity (190). However, plasma noradrenaline level has been shown to be reduced in diabetic patients in some studies; in 10 type II diabetic and eight control inpatients, blood for catecholamine measurement was collected every 4 h, and the mean 24-h plasma noradrenaline level in diabetic patients was significantly lower than that in controls. In contrast, no significant difference in adrenaline levels was observed (191). A similar study in diabetic subjects showed lower arterial levels of noradrenaline in diabetic subjects compared with control subjects during exercise but similar disappearance rates after exercise, indicating lower release of noradrenaline in diabetics (192).

These data suggest that the cardiac  $\beta$ -adrenergic system is enhanced by the alterations in cardiac sympathetic activity during the early stage of diabetes, which may induce toxic effects on the heart. Although diabetes has been shown to decrease the severity of the cardiac necrosis induced by the administration of isoproterenol (193), norepinephrine has been shown to induce apoptosis in cultured neonatal rat myocytes (194–196) via the formation of reactive oxygen species (197–199). Similar results were also demonstrated in ferrets receiving chronic norepinephrine (200). However, the activation of apoptosis is dependent on the type of adrenergic receptors stimulated. Pharmacological studies of cardiac myocytes *in vitro* demonstrate that stimulation of  $\beta_1$ -adrenergic receptor induces apoptosis that is cAMP-dependent and involves the voltage-dependent calcium influx channel. In contrast, stimulation of  $\beta_2$ -adrenergic receptor exerts an antiapoptotic effect that appears to be mediated by a pertussis toxin-sensitive G protein. Stimulation of  $\alpha_1$ -adrenergic receptors causes myocyte hypertrophy and may exert an antiapoptotic action (201). Overexpression of  $\beta_1$ -adrenergic receptors causes marked myocyte hypertrophy, interstitial fibrosis, and reduced contractile function, which was accompanied by increased myocyte apoptosis (202). A study using phenylephrine or isoproterenol in streptozotocin-induced diabetic rats has demonstrated that the *in vivo* response to  $\beta$ -adrenoceptor stimulation is well preserved, whereas the effects of  $\alpha$ -stimulation are markedly reduced, especially in the left ventricle and systemic circulation (203), suggesting that the antiapoptotic effect may be also reduced in diabetes.

*c. Relation to LV dysfunction.* Extensive evidence has demonstrated the association of autonomic dysfunction with abnormal cardiac function in diabetes. In 38 type I diabetes

patients, 56% of patients were found to have CAN, and 12% had LV diastolic dysfunction; none had LV systolic dysfunction. All diabetic patients with LV diastolic dysfunction had evidence of CAN, and there was no correlation between LV dysfunction and microvascular complications of diabetes mellitus (12). In another study of 20 type I diabetic patients who were clinically free of cardiovascular disease and had normal LV systolic function, mean E/A values of diabetics with CAN and without CAN were significantly lower than those of controls, and the CAN score correlated with worsening LV relaxation (204). A similar correlation of indexes of LV diastolic filling to CAN was also shown in 28 patients with type I diabetes without evidence of ischemic heart disease (205).

Myocardial contractility responses to stress have also been shown to be affected by CAN in four studies of diabetic patients. In the first, although baseline myocardial contractility was normal, an abnormal response of LV ejection fraction to isometric (9 of 14) or to dynamic (8 of 14) exercise was found in 14 diabetic patients with autonomic dysfunction and without ischemic heart disease based on exercise stress echo and coronary angiography. The abnormal ejection fraction at peak handgrip was completely reversed by postextrasystolic potentiation (a potent inotropic stimulation independent of the integrity of adrenergic cardiac receptors), suggesting that defective inotropic recruitment plays an important role in LV dysfunction in diabetic patients with CAN during exercise (206). Second, the effect of autonomic dysfunction on both LV systolic and diastolic dysfunction was demonstrated using radionuclide ventriculography in a study of 20 diabetic patients at rest and during handgrip exercise. The results showed that diastolic dysfunction was frequently present at rest, and systolic dysfunction became evident during exercise in patients with CAN (207). Third, in a study of 24 patients with type I diabetes without coronary artery disease excluded by 201Tl scintigraphy compared with 10 controls, cardiac innervation was evaluated by both MIBG scintigraphy (tomographic imaging) and cardiovascular reflex tests. LV systolic (ejection fraction) and diastolic (peak filling rate) function was determined by equilibrium radionuclide angiography at rest and during bicycle exercise. All control and six diabetic patients exhibited a normal myocardial MIBG distribution, and 18 diabetic patients had evidence of regional adrenergic denervation. All patients had a normal ejection fraction at rest. However, patients with regional adrenergic denervation showed an impaired response to exercise as indicated by a smaller increase in ejection fraction and a lower peak filling rate, indicating that subclinical LV dysfunction is related to derangements of adrenergic cardiac innervation (208). Finally, similar findings were reported in a study of 14 asymptomatic patients with type I diabetes in the absence of hypertensive or coronary artery disease, using LV contractile reserve assessment by postextrasystolic potentiation obtained by transesophageal cardiac electrical stimulation and dobutamine infusion, and with myocardial  $^{123}\text{I}$ -MIBG scintigraphy to assess adrenergic cardiac innervation.

More recently, the adverse effect of CAN on myocardial perfusion has been demonstrated by a pharmacological stress study. Dynamic contrast-enhanced magnetic reso-

nance perfusion imaging was performed during baseline conditions and after dipyridamole-induced vasodilatation in nine type I diabetic patients with CAN, 10 type I diabetic patients without CAN, and 10 healthy control subjects. Despite similar baseline myocardial perfusion index in the three groups, myocardial perfusion index was significantly lower in the patients with CAN than in the other groups during dipyridamole vasodilatation. A significant blood pressure decrease was only found in patients with CAN, and there was a significant correlation between blood pressure response to dipyridamole and myocardial perfusion reserve index. The decreased myocardial perfusion reserve capacity during dipyridamole vasodilatation may be caused by defective myocardial sympathetic function to maintain blood pressure during vasodilatation (209). The decreased myocardial perfusion reserve may be in part responsible for abnormal heart function at rest or during exercise in diabetic patients with CAN.

These results suggest that the abnormal response to exercise in the early phase of diabetic cardiomyopathy is strongly related to an impairment of cardiac sympathetic innervation (210). However, increments of catecholamine level in early diabetes may mask cardiac dysfunction. In an *in vivo* longitudinal study to examine the time course of development of cardiac dysfunction in streptozotocin-induced diabetic rats, overt and covert contractile dysfunction of the myocardium unmasked by isoproterenol began at 5 wk of diabetes, and the overt LV systolic and diastolic dysfunction were fully manifest after 6 wk of diabetes (43).

**5. Insulin resistance.** Insulin resistance is associated with hypertension (211), coronary artery disease (212, 213), and diabetes (214, 215). TNF- $\alpha$  is recognized as a key component in the development of insulin resistance in diabetes (216, 217). Changes in sympathetic nervous system modulation (218), cardiac parasympathetic dysfunction (219), or marked autonomic dysfunction (214) are also related to increased insulin resistance in diabetes. In addition, endothelial dysfunction may be involved in the pathogenesis of insulin resistance, and plasma soluble thrombomodulin might reflect endothelial damage better than the plasma von Willebrand factor in the state of insulin resistance in patients with type II diabetes (220).

Insulin resistance has been linked to LV early diastolic abnormalities in hypertension, independently from the influence exerted by increased blood pressure levels, overweight, and LV hypertrophy (221, 222). Although myocardial insulin resistance is not a feature of type II diabetic patients without ischemic heart disease (223), another study shows that reduced insulin sensitivity can be found even when type II diabetes is isolated and well controlled (215). A study in rats has demonstrated that insulin resistance altered cardiac contractile function at the myocyte level (224). Cardiomyocyte abnormalities in sucrose-fed rats were demonstrated in an insulin-resistant stage that precedes frank type II diabetes, and metformin prevented the development of sucrose-induced insulin resistance and the consequent cardiomyocyte dysfunction (225). These results were confirmed by a human study in type II diabetic patients without hypertension using the insulin-sensitizing drug troglitazone,



which showed that LV hypertrophy and diastolic function were associated with insulin resistance (226).

Insulin resistance has been associated with LV hypertrophy (227) or increased LV mass (228) in nondiabetic subjects. In a study including 140 consecutive diabetic patients with and without hypertension, the fasting plasma insulin level was found to be the strongest independent predictor of LV mass, both in the whole population and in the normotensive or hypertensive diabetic subgroups (229). However, other studies suggested that insulin resistance was not an independent determinant of LV mass in nondiabetic subjects when adequate account was taken of body mass and blood pressure (230). In a study in 2623 Framingham Study subjects (1514 women) free of myocardial infarction and heart failure and with different glucose tolerance categories, including normal glucose tolerance, impaired glucose tolerance, impaired fasting glucose, and newly diagnosed diabetes, insulin resistance was only associated with increased LV mass in women, and this relation was largely accounted for by obesity (231). Therefore, although insulin resistance also appears to be associated with structural changes in the diabetic heart, it may not be an independent determinant of these abnormalities.

### *B. Interaction with hypertension and ischemic heart disease*

With the addition of untreated hypertension and/or myocardial ischemia, the mild subclinical cardiomyopathy of diabetes may rapidly advance to clinically obvious diastolic and systolic dysfunction. In clinical practice, it is difficult to separate out the concurrent role of hypertension and/or ischemia in the development of diabetic cardiomyopathy. Furthermore, the presence of “silent ischemia” in diabetic patients makes the diagnosis of diabetic cardiomyopathy more complicated, and care should be taken not to identify silent ischemia as diabetic cardiomyopathy.

**1. Interaction with hypertension.** The clinical and morphological features of heart disease in hypertensive diabetic patients are more severe than those of hypertensive patients or diabetic patients alone. Studies have shown that diabetics with hypertension have greater interstitial connective tissue deposition than is present in patients with either diabetes or hypertension as isolated entities (77), and concomitant hypertension further increases the susceptibility to necrotic cell death in myocytes and endothelial cells but does not increase apoptosis (114). These differences are attributed to increased angiotensin II receptor and to oxidative stress in diabetic hearts. The histopathological myocardial damage in hypertensive diabetics may be mainly attributed to hypertension, whereas the myocellular dysfunction may be attributed to diabetes. In rats with diabetes induced by streptozotocin and hypertension due to renovascular lesions, greater replacement of myocardium by fibrosis was found in the hypertensive/diabetic rats than in control groups, and no myocardial alterations were found in rats with diabetes alone (232).

The prevalence of hypertension is approximately doubled in diabetic patients compared with nondiabetic controls (233, 234). Hypertension may be secondary to diabetes and is

associated with LV dysfunction in patients with established diabetes, because hyperglycemia has been shown to increase blood pressure in humans and in animal models of type I diabetes, and the increase has been linked to angiotensin II (235). Hyperinsulinemia and endothelial dysfunction are also related to hypertension; indeed, hyperinsulinemia may be more important than endothelial dysfunction as a cause of hypertension in fructose-fed rats (236).

The presence of hypertension in type I diabetes may be a consequence of renal disease. Nephropathy precedes the rise in blood pressure, based on the fact that with low levels of microalbuminuria, the arterial pressure remains normal (234). In a study examining the effect of hypertension on the progression of diabetic cardiomyopathy, the results showed that hypertension exacerbates the cardiac dysfunction during diabetes, especially when spontaneously hypertensive rats are injected with streptozotocin prior to the elevation of blood pressure. The presence of LV hypertrophy in the spontaneously hypertensive rats at the time of streptozotocin injection may compensate for the damaging effects of diabetes on the myocardium (237). Systolic hypertension has also been shown to be independently associated with diastolic dysfunction in diabetic patients (238). On the other hand, hypertensive patients are more predisposed to the development of diabetes than are normotensive persons. In a large prospective cohort study of 12,550 adults, the development of type II diabetes was almost 2.5 times as likely in hypertensive patients than in normotensive controls (239). Further investigations are required to compare the effects of hypertension before and after the onset of diabetes with respect to the progression of diabetic cardiomyopathy.

**2. Interaction with ischemic heart disease.** Patients with diabetes are particularly prone to early development of atherosclerosis. Endothelial dysfunction plays a pivotal role in the initial stage of atherosclerosis. The increased angiotensin II in diabetic myocardium (240) and lipid metabolism abnormalities in diabetes may play a central role in early atherogenesis and progression to atherosclerotic plaque. Insulin resistance is also associated with accelerated atherosclerosis, especially coronary heart disease. Although lipid metabolism abnormalities associated with diabetes do not have direct influence on the development of diabetic cardiomyopathy, they are at least partly responsible for enhanced coronary atherosclerosis in these patients. The atherogenic process depends on diabetic control and on the disease duration. Enhanced atherosclerosis in the coronary arteries is directly related to myocardial ischemia, increased oxidative stress, and vascular endothelial dysfunction, which may promote the progression of diabetic cardiomyopathy.

A number of studies demonstrated the association of a high level of lipoprotein (a) with the development of ischemic heart disease, myocardial infarction, or other forms of atherosclerosis even at a young age (241–243). Children and adolescents with type I diabetes mellitus, who have higher levels of lipoprotein (a) and apolipoprotein B, are prone to premature development of atherosclerosis irrespective of the degree of diabetes control (244). In particular, patients with both diabetes and hypertension have a higher incidence of coronary artery disease, and the coronary artery disease and

cardiac structural and functional abnormalities are more pronounced than those from either diabetes or hypertension alone (245).

C. Stages of diabetic cardiomyopathy

Our understanding of the progression of diabetic cardiomyopathy is summarized in Table 1. Diabetic cardiomyopathy appears to consist of two major components, the first being a short-term, physiological adaptation to metabolic alterations, whereas the second represents degenerative changes for which the myocardium has only limited capacity for repair. Thus, therapies during the early stages of diabetes can potentially delay or impede the progression of more permanent sequelae. However, it should be noted that many factors such as treatments, metabolic characteristics, lipid profile, and other individual differences may affect the process of development of diabetic cardiomyopathy, and not all diabetic patients are affected by the same factors or to the same degree, which may result in marked variability in the clinical manifestations of the diabetic cardiomyopathy.

1. *Early stage.* Diabetic cardiomyopathy is initiated by hyperglycemia at an early stage and characterized by metabolic disturbances such as depletion of GLUT4, increased FFAs, carnitine deficiency, calcium homeostasis changes, and insulin resistance. This stage of diabetic cardiomyopathy has insignificant changes in myocardial structure (such as normal LV dimensions, wall thickness, and mass) or only substructural changes in myocytes. Cardiac dysfunction usually can only be detected by sensitive methods such as strain, strain rate, and myocardial tissue velocity. Endothelial dysfunction occurs at an early stage.
2. *Middle stage.* Cellular changes such as defects in calcium transport and fatty acid metabolism may lead to increases in myocyte apoptosis and necrosis, angiotensin II, TGF- $\beta$ 1, and possibly mild CAN, resulting in myocyte injury, loss, and myocardial fibrosis and initially causing abnormal mitral inflows that may advance to low ejection fraction. This stage of diabetic cardiomyopathy is mainly characterized by myocellular hypertrophy and myocardial fibrosis. Patients at this stage may have minor changes in structure (such as LV dimension, wall thickness, or mass) and significant changes in diastolic and systolic function, which may be detected by

conventional echocardiography. Myocardial vascular structural lesions at this stage are usually insignificant.

3. *Late stage.* The further changes in metabolism and development of myocardial fibrosis result in myocardial microvascular changes. This stage of diabetic cardiomyopathy is characterized by both myocardial microvascular structural and functional changes probably accompanying recurrent microvascular spasm. Changes in cardiac structure and function are obvious. Diabetic cardiomyopathy at this stage is frequently associated with hypertension and early development of ischemic heart disease in diabetes.

IV. Therapeutic Implications of Diabetic Cardiomyopathy

The mechanisms of metabolic disturbances, myocardial fibrosis, microvascular disease, CAN, and insulin resistance in diabetic cardiomyopathy imply that various treatments might be effective for preventing or delaying the development of diabetic cardiomyopathy and its complications. These include improving diabetic control; use of calcium blockers, angiotensin-converting enzyme (ACE) inhibitors, or related drugs; exercise training; lipid-lowering therapy; and antioxidant and insulin-sensitizing drugs.

Hyperglycemia increases levels of FFA, oxidative stress, and growth factors and causes abnormalities in substrate supply and utilization, calcium homeostasis, and lipid metabolism, so diabetic control might be expected to be the most basic and important strategy for preventing development of diabetic cardiomyopathy. Unfortunately, there are scant data to support this expectation. This may be in part due to the differing pathophysiologies of type I and type II diabetes. Hansen *et al.* (246) showed that type I diabetic patients have impaired myocardial function and perfusion in the basal state, which can be improved by replacement of C-peptide. In eight type I diabetic patients, tissue velocity and perfusion were reduced compared with control subjects, and administration of C-peptide led to improvements in both function and perfusion. The role of poor diabetic control (associated with lower IGF-I levels) in diabetic cardiomyopathy is supported by experimental work showing that exogenous IGF-I treatment can restore the diabetes-induced decline in SERCA and may ameliorate contractile disturbances in cardiomyo-

TABLE 1. Three stages of diabetic cardiomyopathy

Stages	Characteristics	Functional features	Structural features	Study methods
Early stage	Depletion of GLUT4 Increased FFA Carnitine deficiency Ca <sup>2+</sup> homeostasis changes Insulin resistance	No overt functional abnormalities or possible overt diastolic dysfunction but normal ejection fraction	Normal LV size, wall thickness, and mass	Sensitive methods such as strain, strain rate, and myocardial tissue velocity
Middle stage	Apoptosis and necrosis Increased AT II Reduced IGF-I Increased TGF- $\beta$ 1 Mild CAN	Abnormal diastolic dysfunction and normal or slightly decreased ejection fraction	Slightly increased LV mass, wall thickness, or size	Conventional echocardiography or sensitive methods such as strain, strain rate, and myocardial tissue velocity
Late stage	Microvascular changes Hypertension CAD Severe CAN	Abnormal diastolic dysfunction and ejection fraction	Significantly increased LV size, wall thickness, and mass	Conventional echocardiography

AT II, Angiotensin II; CAD, coronary artery disease.

cytes from diabetic animals (247). The limited data in support of prevention of the development of diabetic cardiomyopathy by tight glycemic control has also been due to the lack of sensitive techniques allowing repetitive quantification of myocardial perfusion and diastolic and systolic function in the clinical arena.

Intracellular retention of calcium in diabetes is associated with depletion of high-energy phosphate stores and a derangement of ultrastructure and cardiac dysfunction. Calcium channel blockers are capable of reversing the intracellular calcium defects and preventing diabetes-induced myocardial changes. Verapamil has been shown to significantly improve the depressed rate of contraction and rate of relaxation, lower peak LV systolic pressure, and elevate LV diastolic pressure (248), as well as to improve the altered myofibrillar ATPase activity, myosin ATPase, myosin isoenzyme distribution, and sarcoplasmic reticular  $\text{Ca}^{2+}$ -pump activities in streptozotocin-induced diabetic rats (249). Diltiazem has been shown to suppress interstitial fibrotic changes in type II diabetic mice (63), and nifedipine increases insulin sensitivity and prevents the increase in cholesterol and triglyceride levels in streptozotocin-induced diabetic rats (250).

ACE inhibitors facilitate blood flow through the microcirculation in fat and skeletal muscles. Facilitation of blood flow to insulin-sensitive tissues, such as skeletal muscle, would lead to an increase in glucose delivery to these tissues. Improvement of coronary blood flow may also be beneficial for microvascular disease-related diabetic cardiomyopathy. Captopril has been demonstrated to increase the number of perfused capillaries and epicardial perfusion rate, and to prevent the increase of coronary perfusion pressure and end-diastolic pressure in diabetic rats (251). ACE inhibitors can also improve insulin action at the cellular level (252, 253). ACE inhibition independently increases the basal and insulin-stimulated rate of glucose uptake in skeletal muscle in insulin-resistant obese Zucker rats by improving postreceptor insulin signaling and enhancing GLUT-4 translocation to the cell membrane (254). The action of ACE inhibitors on angiotensin II may improve fibrosis in myocardium and functional and structural changes of small vessels in diabetes. ACE inhibitors have been demonstrated to reduce cardiovascular disease in diabetic patients, particularly diabetic patients with hypertension (255). Angiotensin II receptor blockers and aldosterone inhibitors may also have similar effects on myocardial fibrosis in diabetic patients. Because of local effects of angiotensin II in diabetic myocardium, control of myocardial fibrosis should be started at an early stage rather than only in patients who have hypertension or vascular complications.

Steps to reduce atherogenesis consist of the basic treatment for vascular disease and should be started at the early stage of diabetic cardiomyopathy because abnormalities in lipid metabolism are already present. Exercise may improve glucose homeostasis by reducing the glucose/insulin ratio and increasing insulin sensitivity. Studies have shown that exercise training increases whole body insulin sensitivity and glucose oxidation by skeletal and cardiac muscle. The improvement may be associated with both attenuation of reduction in myocardial GLUT-4 transporters (256) and increase in myocardial sarcolemmal GLUT-4 protein in di-

abetic hearts (257). Changes in myocardial metabolism involving a shift from glucose to fat metabolism in diabetes mellitus increase plasma levels of triglycerides and cholesterol, and these may be lowered by exercise training, resulting in improved myocardial sarcoplasmic reticulum function and vascular function. In addition, exercise training improves cardiac output (258) and reverses the changes in contractile properties of the heart in streptozotocin-diabetic rats (259). Improvements in cardiac function are also mediated by decreasing the severity of the diabetic state (260). Improving vascular endothelial dysfunction by exercise training may also play an important role. However, whereas low-intensity exercise training seems to improve cardiovascular function, some types of endurance training may further decrease the reduced myocardial  $\text{Ca}^{2+}$ -activated ATPase and  $\beta$ -adrenergic receptor number in diabetes (261).

Sympathetic cardiac hyperinnervation can occur concurrently with denervation in diabetic neuropathy and could potentially cause arrhythmia and sudden death. Direct evaluation of myocardial sympathetic innervation has shown the correlation of autonomic dysfunction with diabetic control. In a prospective study over a mean of 4 yr, myocardial sympathetic innervation was investigated in 12 type I diabetic patients using myocardial  $^{123}\text{I}$ -MIBG scintigraphy in conjunction with cardiovascular autonomic function tests using QTc interval and QT dispersion. Global MIBG uptake increased from baseline to follow-up in patients with good glycemic control and decreased in the poor control group, suggesting that long-term poor glycemic control is associated with the progression of LV adrenergic denervation. The standard autonomic function tests were no different in each group (262). However, even in the early stage of diabetes, cardiac sympathetic denervation is only partially reversed with improved metabolic control (263, 264). The role of oxidative stress in the development of CAN suggests that antioxidants may have beneficial effects on the cardiac autonomic nervous system through a decline in oxidative stress. This was demonstrated in a double-blind randomized controlled trial, with 50 type II diabetic patients with CAN assigned to treatment with vitamin E or placebo for 4 months. The results show that chronic vitamin E administration improves the ratio of cardiac sympathetic to parasympathetic tone in patients with type II diabetes, which might be mediated by a decline in oxidative stress (265). This is supported by other antioxidant studies in diabetic animals using vitamin E (266) and acetyl-L-carnitine treatment (267) as well as in diabetic patients using lipoic acid (268–271). In addition, ACE inhibitors such as quinapril have also been shown to significantly improve CAN in diabetic patients (272). Similarly, aldose reductase inhibitors have demonstrated clinical improvement not only in CAN but also in cardiac performance. The effect on cardiovascular performance of sorbinil was studied in patients with diabetic autonomic neuropathy who were free of atherosclerotic coronary artery disease. After 1 yr of treatment, significant improvement was demonstrated in both the resting cardiac output and the maximal cardiac output, suggesting that the use of an aldose reductase inhibitor may be useful in treating suboptimal cardiovascular performance in patients with diabetic CAN (273). Furthermore, decreased MIBG uptake and increased norepi-



nephrene content in diabetic myocardium were completely prevented by insulin therapy started immediately after streptozotocin injection and partially, but significantly, by aldose reductase inhibitor administered immediately after streptozotocin injection. Heterogeneous MIBG distribution also disappeared with the aldose reductase inhibitor therapy. In contrast, diabetic rats treated with insulin or aldose reductase inhibitor therapy that was started 4 wk after streptozotocin injection showed no improvement in MIBG uptake, suggesting the importance of early intervention (274).

Insulin resistance, hyperinsulinemia, atherogenic dyslipidemia, hypertension, abdominal obesity, and impaired hemostasis are risk factors for cardiovascular disease. Insulin resistance results from a combination of genetic and environmental factors and contributes to type II diabetes mellitus. Obesity is associated with insulin resistance, and weight loss has been shown to correct insulin resistance in diabetes (275, 276), implying that weight reduction through lifestyle modifications (low-calorie diet and exercise) and antiobesity drugs (orlistat, sibutramine, etc.) is the first step of treatment of insulin resistance. Metformin and the thiazolidinediones are used to treat insulin resistance and work through different mechanisms. Metformin reduces FFA efflux from fat cells, thereby suppressing hepatic glucose production, and indirectly improving peripheral insulin sensitivity and endothelial function. In contrast, thiazolidinediones improve peripheral insulin sensitivity by reducing circulating FFAs but also by increasing production of adiponectin, which improves insulin sensitivity. Thiazolidinediones also improve endothelial function and may prevent or delay the onset of diabetes (277). Combination oral hypoglycemic therapy may be ideal for maintaining adequate glycemic control and improving insulin resistance in patients with type II diabetes. In obese type II diabetic patients inadequately controlled on metformin alone, addition of the insulin-sensitizing agent rosiglitazone improves glycemic control, insulin sensitivity, and  $\beta$ -cell function to a clinically important extent (278). Combination of pioglitazone and metformin has also shown to significantly improve insulin sensitivity as compared with metformin monotherapy in patients recently diagnosed with type II diabetes (279). Troglitazone has been shown to reduce plasma insulin levels and restore coronary circulation by improving insulin resistance in type II diabetic patients (280). In addition, weekly iv IGF-I bolus therapy has been demonstrated to be effective in inducing sustained insulin sensitivity in a patient with type I diabetes mellitus and massive insulin resistance (281). Finally, both ACE inhibitors and exercise may be beneficial for improving insulin resistance. In skeletal muscle, exercise has been demonstrated to recruit a separate pool of GLUT4 to that activated by insulin (282). This leads to an additive effect of insulin and exercise on glucose uptake. Furthermore, exercise increases skeletal muscle GLUT4 gene and protein expression (283). Should similar changes occur in cardiac muscle, it is conceivable that exercise may have a direct beneficial effect on myocardial function also. The improvement of insulin resistance may result in improvement of cardiac function. Glipizide has been shown to reduce the degree of insulin resistance in the myocardium and improves cardiac function in diabetes. In a neonatal streptozotocin-induced rat model of type II dia-

betes, animals treated with glipizide for 1 yr exhibited improved myocardial contractile function relative to the vehicle-fed or *ad lib*-fed diabetic animals. Heart rate was significantly elevated, and there was a tendency for both the rate of relaxation and contractility to be elevated in the glipizide-treated group (284). Finally, improvement in insulin sensitivity of cardiac muscle may have benefits other than improved energy utilization. Insulin and IGF-I share multiple intracellular signaling pathways, and both receptors mediate antiapoptotic effects. Improvements in the signaling of these molecules may have an effect to preserve cardiomyocyte number.

## V. Summary and Conclusions

In this review we present evidence that strongly supports the existence of diabetic cardiomyopathy as a distinct clinical entity. The pathophysiology of the condition remains to be fully elucidated, but includes interstitial fibrosis, cardiomyocyte loss, impaired energy utilization, small vessel disease, and neuropathy. Prominent functional consequences include diastolic and systolic dysfunction, which may manifest as dyspnea and exercise intolerance. Traditional cardiac risks such as hypertension, atherosclerosis, and dyslipidemia are common in diabetic patients and further compromise cardiac status. Currently, no specific therapeutic strategies can be recommended for diabetic cardiomyopathy, but management of traditional risk factors and lifestyle modification programs already established in the management of cardiac disease should be instituted. Further research is urgently required into the molecular basis of diabetic cardiomyopathy such that more appropriate therapies may be formulated and tested.

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Address all correspondence and requests for reprints to: Prof. T. Marwick, University of Queensland Department of Medicine, Princess Alexandra Hospital, Ipswich Road, Brisbane, Queensland 4012, Australia. E-mail: tmarwick@soms.uq.edu.au

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