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Diabetes-Induced Alterations in the Extracellular Matrix and Their Impact on Myocardial Function

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Abstract

Diabetes is an increasing public health problem that is expected to escalate in the future due to the growing incidence of obesity in the western world.While this disease is well known for its devastating effects on the kidneys and vascular system, diabetic individuals can develop cardiac dysfunction, termed diabetic cardiomyopathy, in the absence of other cardiovascular risk factors such as hypertension or atherosclerosis. While much effort has gone into understanding the effects of elevated glucose or altered insulin sensitivity on cellular components within the heart, significant changes in the cardiac extracellular matrix (ECM) have also been noted. In this review article we highlight what is currently known regarding the effects diabetes has on both the expression and chemical modification of proteins within the ECM and how the fibrotic response often observed as a consequence of this disease can contribute to reduced cardiac function.

Keywords

diabetes; extracellular matrix; heart; advanced glycation end products (AGE); fibrosis; myofibroblast

Introduction

According to the National Institute of Diabetes and Digestive and Kidney Diseases, as of 2010 approximately 8.3% of the U.S. population, or about 25 million individuals, are affected by diabetes mellitus (DM). There are two types of diabetes: type 1 and type 2. Type 1, sometimes referred to as juvenile-onset, typically affects children or young adults and accounts for a small percentage (~5%) of all diabetes cases. In contrast, type 2 diabetes, often associated with obesity, accounts for greater than 90% of diagnosed diabetes cases. The cardiovascular complications of diabetes are well known and remain a leading cause of morbidity and mortality in individuals with this disease (Jaffe et al., 1984; Lehto et al., 1994; Shehadeh & Regan, 1995). Patients with diabetes have a higher risk for hypertension, myocardial infarction, vascular dysfunction, and heart failure (Nichols et al., 2004). In fact,

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over 65% of diabetic patients die due to cardiovascular complications (Ares-Carrasco et al., 2009).

The effects of diabetes on the cardiovascular system have been extensively described; however, the mechanisms of these effects have not been fully elucidated. This is in part due to the complex and multifactoral nature of diabetes. Among other things, diabetes has profound effects on the expression, organization, and modification of extracellular matrix (ECM) components in many organs (Fig. 1). As discussed in this review article, tissue remodeling and fibrosis appear to be a direct consequence of diabetes in several organs including the heart. Nonenzymatic alterations in ECM proteins due to elevated glucose levels are also a consequence of this disease. Changes in the accumulation and modification of ECM proteins impact the mechanical properties of the ECM and have deleterious consequences on cardiac function. Elevated glucose levels have also been shown to result in direct activation of several cell types transforming these cells into a contractile, myofibroblast phenotype, which can further exacerbate myocardial remodeling and fibrosis. This review article describes the alterations in the ECM resulting from diabetes and the effects these alterations can have on myocardial function.

Diabetes and the Extracellular Matrix

The ECM is a dynamic network comprised of structural proteins, proteoglycans, growth factors/cytokines, and enzymes that provides essential cues and scaffolding for tissue organization and structure. Alterations in ECM accumulation, composition, and organization can adversely affect organ function. Regulation of ECM homeostasis is a complex balance between biochemical factors, mechanical forces transduced via cell-ECM interactions, and cell-cell communication. During the onset and progression of DM, changes in ECM protein expression occur, leading to structural modifications in the ECM network and altered cell-ECM and cell-cell interactions (Siperstein et al., 1968). Subsequently, these changes lead to diabetes-induced organdependent diseases such as nephropathy, retinopathy, and diabetic cardiomyopathy. While organ-specific changes in ECM are summarized in Table 1, in this section we will review DM-induced structural alterations to the cardiac ECM and discuss how DM-dependent changes to the ECM of other organs (i.e., kidneys, liver, and pancreas) affect myocardial function.

Diabetes Mellitus and the Cardiac ECM

The cardiac ECM consists of a well-defined network of collagen (Caulfield & Borg, 1979), with fibrillar collagen types I and III localized within the myocardial interstitium and nonfibrillar collagen types IV and VI, and the glycoproteins fibronectin and laminin predominating in the myocyte basement membrane (Eghbali &Weber, 1990; Bishop & Laurent, 1995). A number of studies have examined alterations in cardiac ECM expression associated with diabetes. Previous human studies comparing diabetic and nondiabetic human patients revealed an increased presence of type I, III, and VI collagen in the hearts of both groups; however, only collagen type III expression was significantly different between the two groups. No increase in collagen IV and V was detected (Shimizu et al., 1993). As DM progresses, the left ventricle may hypertrophy and hypertension-induced diastolic dysfunction can occur (Regan et al., 1981; Shapiro et al., 1981*a*, 1981*b*). Diabetes-induced

ECM remodeling may also influence the development of cardiomyopathy, atherosclerosis, carotid artery disease, and interstitial fibrosis (Seneviratne, 1977; Regan et al., 1981). For example, alloxan-induced diabetes in rats demonstrated a significant increase in collagen type VI when compared to collagen types I and IV and other ECM proteins (Spiro & Crowley, 1993). In diabetic cardiomyopathy, characterized as cardiac dysfunction in diabetic patients in the absence of other cardiovascular risk factors such as hypertension or coronary artery disease, excess collagen deposition, particularly collagens I, III, and IV, contributes to impaired LV function (Aneja et al., 2008).

DM-induced alterations to the vascular ECM are associated with several cardiovascular complications. In some diabetic models, changes in proteoglycans, notably decreases in heparan sulfate and increased levels of chondroitin and dermatan sulfate (Heickendorff et al., 1994; Tabas et al., 2007), have been observed. Tabas and colleagues (2007) suggested that changes in proteoglycan expression may lead to increased low density lipoprotein retention in the arterial wall, thus promoting atherosclerosis. In rat and pig diabetic atherosclerotic models, there is a loss of elastin in the intima associated with an increase in elastin fragmentation, yet the cause for elastin breakdown and turnover is unclear (Kwan et al., 1988; McDonald et al., 2007). Previous work has also shown an increase in collagen deposition by vascular smooth muscle cells (SMCs) during DM. The newly synthesized collagen can undergo advanced nonenzymatic glycation, which can increase low density lipoprotein binding and retention to the arterial wall, thus promoting atherosclerosis (Brownlee et al., 1985; McDonald et al., 2007). In addition, gylcation of collagen within the vascular wall contributes to decreased vascular compliance observed in diabetic models (Aronson, 2003), which can result in an increased load on the heart. Aortic SMCs isolated from type II diabetic rat models showed an increase in collagen type I mRNA levels and a decrease in transcription of fibronectin mRNA, respectively (Song & Ergul, 2006). Furthermore, aortic SMCs cultured in high glucose media showed an increase in collagen types I and III protein expression when compared to control cells cultured in normal glucose media (Bouguerra et al., 2004).

Involvement of Other Organs in Diabetes-Induced Myocardial Dysfunction

As can be seen in Table 1, diabetes is a multifactoral disease, affecting multiple organ systems whose own functional changes can impact cardiac performance. For example, during the early stages of diabetic nephropathy, acute kidney disease is associated with increased excretion of protein in the urine, a phenomenon called proteinuria (Abbate et al., 2006; Jefferson et al., 2008). Recent epidemiological studies have shown that during DM, hypertension and heart failure (HF) have a linear correlation with proteinuria, 93% and 26%, respectively (Tarnow et al., 1994;Wang et al., 1996). As diabetic nephropathy progresses, renal fibrosis and hypertrophy increase leading to a decline in glomerular function and filtration (Dalla Vestra et al., 2000; Osterby et al., 2001). During DM, the expression of normal ECM proteins in the glomerular basement membrane (GBM) and mesangium are increased, altering the structure of the diabetic kidney. The GBM and mesangium are thickened by increased synthesis of collagen types IV and V, fibronectin and laminin (Kiryu et al., 1994; Zhu et al., 1994; Osterby et al., 2001; Mason & Wahab, 2003). As the disease progresses, laminin and heparan sulfate proteoglycan expression decreases in the GBM and

heparan sulfate proteoglycan is reduced in the mesangium (Falk et al., 1983; Ikeda et al., 1991; Vernier et al., 1992). Renal decline and ultimately failure does not occur until the mesangium has expanded, impeding normal kidney filtration (Steffes et al., 1989).

Renal failure and inhibition of filtration contribute to several cardiac pathologies such as hypertension, fibrosis, cardiomyopathy, hypertrophy, and atherosclerosis (Rubler et al., 1972; Anavekar et al., 2004). Rubler and colleagues (1972) reported an association between cardiac hypertrophy and fibrosis in deceased individuals diagnosed with diabetic nephropathy, while another postmortem study looked at the relationship between hypertension and diabetes and found an increase in cardiac fibrosis in hypertensive diabetic individuals compared to hypertensive or diabetic only individuals (van Hoeven & Factor, 1990). A similar study showed that cardiac fibrosis was exacerbated in hypertensive diabetic patients compared to diabetic and nondiabetic control patients (Frustaci et al., 2000). Besides the pathological alterations that occur in the myocardium during diabetic nephropathy, physiological characteristics indicative of cardiac hypertrophy and cardiomyopathy have been reported (Poirier et al., 2001). For example, echocardiograph studies showed an increase in diastolic and/or systolic dysfunction during the progression of diabetic nephropathy (Miyazato et al., 2005).

The most prevalent form of liver disease induced during DM associated with cardiovascular dysfunction is nonalcoholic fatty liver disease (NAFLD), characterized by spontaneous lipid accumulation independent of alcohol consumption (Levinthal & Tavill, 1999). Traditionally, NAFLD can promote atherosclerosic events independent of DM; however, recent studies have shown that DM further exacerbates vascular damage (Targher et al., 2005). A cohort study showed that patients diagnosed with NAFLD had decreased blood flow and increased intima thickness (Targher, 2004; Villanova et al., 2005) that positively correlated with NAFLD progression. Another population based study showed that a strong correlation existed between hepatic steatosis and atherosclerotic plaque development (Volzke et al., 2005).

Cardiometabolic syndrome (CMS) is a grouping of several cardiovascular risk factors, such as hyperglycemia, obesity, hypertension, dyslipidemia, and albumineria (Lastra & Manrique, 2007), that is often used as an indicator for cardiovascular disease, stroke, type II diabetes, and chronic kidney disease. In type II DM, there is partial loss of pancreatic β-cell function, such as glucose stimulated insulin secretion, which affects the body's ability to remove glucose from the bloodstream, while in type I DM, the β-cell is attacked and destroyed by the host's immune system, eliminating all insulin secretion. Therefore, β-cell function and activity is the center of DM development and progression. After DM develops, it can further damage the pancreas by inducing alterations in pancreatic ECM proteins. Fibronectin, along with collagens type I, III, and IV, have been shown to be increased and secreted into the interstitium and islet exocrine interface by pancreatic stellate cells or islet cells during diabetes (Ko et al., 2006; Hayden et al., 2008). Specifically during type II DM, fibrosis has been shown to be the main cause of pancreatic failure and CMS prevalence. Previous literature has shown that activation of pancreatic stellate and islet cells is promoted by the renin-angiotensin system (RAS) and transforming growth factor beta 1 (TGF-β1), a profibrotic cytokine (Yoshikawa et al., 2002; Ko et al., 2006). RAS inhibition, either by

angiotensin converting enzyme inhibitors or angiotensin receptor blockers, has improved CMS frequency and had positive effects on preventing type II diabetes and balancing glucose metabolism (Lastra & Manrique, 2007).

Diabetes and Myofibroblast Activation

Accumulation of collagen within the ECM, or fibrosis, in a number of organs is associated with the appearance of myofibroblasts. Also referred to as contractile fibroblasts, the myofibroblast is most simply described as an “activated” fibroblast that expresses proteins and qualities characteristic of a smooth muscle cell (Fig. 2; Brown et al., 2005). Namely, they express the contractile protein α-smooth muscle actin (α-SMA) and the intermediate filament protein desmin typical of smooth muscle cells (Gabbiani, 2003; Brown et al., 2005). In this heightened state of activity, the myofibroblast exhibits increased motility, contractility, and secretion of ECM components such as collagen and fibronectin (Porter & Turner, 2009). Myofibroblasts are crucial to remodeling injured tissue, such as the wound healing response; however, the persistence of myofibroblasts has been correlated with a number of pathological fibrotic conditions (Naugle et al., 2006; Hinz, 2007). While the actions and fibrotic consequences resulting from the presence of this specific cell type may be universal throughout the body, myofibroblasts are heterogeneous in their origins. The factors that stimulate their transdifferentiation also vary and are dependent on the microenvironment, tissue, or organ system in which the cells are found (Hinz et al., 2007).

Cardiac Myofibroblast Origins and Activation

In the heart, the most widely accepted dogma suggests that cardiac myofibroblasts are derived from resident fibroblasts within the myocardium (Krenning et al., 2010). This argument has been disputed on the basis that myofibroblasts, both within the same and different organ systems, display significant differences in gene expression, and it has been suggested that they be considered distinct cell types (Chang et al., 2002; Krenning et al., 2010). In addition, myofibroblasts in other organs have been shown to originate from different cellular precursors. For example, in the liver and pancreas, myofibroblasts are derived from stellate cells. In the kidney, they arise, at least in part, from tubular epithelial cells via an epithelial-mesenchymal transformation (Gress et al., 1998; Yang & Liu, 2001; Cassiman et al., 2002). Myofibroblasts may also arise from other undifferentiated connective tissue cells such as pericytes or mesenchymal stem cells. Currently, there is substantial debate about the exact origin(s) of the cardiac myofibroblast (Leask, 2010). If, as is commonly proposed, myofibroblasts arise from resident cardiac fibroblasts, this could complicate comparison between model systems. Recent work has shown that the number of fibroblasts in the heart varies temporally during development and with species (Banerjee et al., 2007). This work would suggest that the rat heart may have the potential to produce more myofibroblasts compared to the mouse heart under pathological conditions and that the already elevated level of collagen within the rat heart would provide increased substrates for nonenzymatic modification associated with elevated glucose during diabetes.

Studies examining fibrosis have shown myofibroblasts to be the largest contributors of ECM in the heart and other organs (Naugle et al., 2006). Myofibroblasts have been associated with a multitude of cardiac diseases in which normally quiescent fibroblasts transdifferentiate

into myofibroblasts in response to diverse signals including cardiomyocyte distress and cell death (Brown et al., 2005; Porter & Turner, 2009). Of the many factors and hormones associated with cardiac fibrosis, a subset has been shown to drive myofibroblast activation in the heart. Transforming growth factor-β has long been recognized as a profibrotic cytokine and is among the most potent mediator in the transition of fibroblasts to the myofibroblast phenotype (Gabbiani, 2003; Desmoulière et al., 2005; Leask, 2008). When TGF-β is neutralized via antibody treatment, cardiac fibroblast activation is inhibited (Kuwahara et al., 2002). Angiotensin II (Ang II), also known to stimulate profibrotic remodeling in the heart, may indirectly induce myofibroblast transdifferentiation by activating TGF-β (Gao et al., 2009; Yang et al., 2009). Downstream of TGF-β, Endothelin-1 (ET-1), a vaso-constrictive protein found in many cases of heart failure, has been shown to induce lung and cardiac fibroblasts to transition into myofibroblasts (Shi-Wen et al., 2004; Nishida et al., 2007). Cardiac myofibroblasts respond to a variety of factors and stimuli that direct their production of ECM proteins such as proinflammatory cytokines (Interleukins 1 and 6, tumor necrosis factor-a, TGF-β), vasoactive proteins (Ang II, ET-1, natriuretic peptides A and B), noradrenaline, ischemia and reperfusion, and mechanical stimuli (Porter & Turner, 2009).

Myofibroblasts and Diabetes

Since fibrosis disrupts the normal function of a number of organs in diabetic individuals, myofibroblasts, as one of the primary modulators of ECM production and turnover, are a potential therapeutic target for preventing pathological matrix remodeling. In tubulointerstitial fibrosis observed in diabetic nephropathy, increased numbers of myofibroblasts have been associated with excessive deposition of ECM (Gilbert & Cooper, 1999; Li et al., 2004). Myofibroblasts of the kidney are derived from tubular epithelial cells via the process of epithelial-mesenchymal transformation involving several mediators including TGF-β (Li et al., 2004). Interestingly, the work by Li et al. (2004) showed that myofibroblasts can be activated in the presence of advanced glycation end products (AGEs) with or without TGF-β. This response was demonstrated to involve the AGE receptor (RAGE) and ERK1/2 MAP kinase activation *in vitro*. Others have also shown that myofibroblast formation can occur via activation of RAGE, but not independent of TGF-β (Oldfield et al., 2001). As AGEs are a consequence of prolonged hyperglycemia, this finding provides a unique paradigm when considering therapeutic targets of fibrosis in diabetes compared to other fibrotic disease states (Goldin et al., 2006).

Excessive fibrotic scarring is also a problem in the manifestation of proliferative diabetic retinopathy and other proliferative retinal diseases where fibroblast to myofibroblast differentiation has been described (Walshe et al., 1992; Bochaton-Piallat et al., 2000). An increased presence of myofibroblasts in the diabetic eye contributes to the formation of scar-like epiretinal membranes contributing to retinal detachment. One study has shown that this myofibroblast transition is linked to TGF-β, TGF-β receptor II, and the EDA splice variant of fibronectin in proliferative diabetic retinopathy (Bochaton-Piallat et al., 2000). Another clinical study discovered reduced expression of bone morphogenetic protein 6 (BMP6) in myofibroblast progenitor cells in type 1 diabetic patients compared to healthy controls. Since BMP6 has been shown to have anti-TGF-β effects, it is hypothesized that cells lacking

BMP6 may be potential contributors to disease-related tissue remodeling (Nguyen et al., 2006). Interestingly, the opposite seems to be true in dermal wounds where impairment in wound healing is observed in both diabetic humans and genetically diabetic mice (Greenhalgh et al., 1990; Brem & Tomic-Canic, 2007). In a wound healing study, Darby et al. (1997) demonstrated that nonobese diabetic mice showed reduced cell proliferation, myofibroblast transdifferentiation, procollagen I mRNA, and the presence of abnormal cell death in dermal wounds of diabetic mice compared to wild-type controls. Just as myofibroblasts are diverse in their origins, studies suggest that differences in diabetes-induced myofibroblast differentiation and mediators depend on the microenvironment and organ system.

Cardiac Myofibroblasts and Diabetes

Though patients with diabetic cardiomyopathy develop pathological cardiac fibrosis, there have been few studies investigating the role of the cardiac myofibroblast in this disease. In a study comparing the fibrotic properties of aldosterone and high levels of glucose (to simulate hyperglycemia observed in diabetes), a similar increase in cardiac myofibroblast proliferation was seen with both treatments *in vitro* (Neumann et al., 2002). This suggests that high glucose levels may be a stimulus of cardiac myofibroblast action and subsequent remodeling of the ECM by these cells. Conversely, in a rat streptozotocin-induced type 1 diabetic model, a reduction in α-SMA-positive cardiac myofibroblasts was reported; however, an increase in proliferation of cardiac fibroblasts in animals diabetic for 6 weeks was observed (Shamhart et al., 2009). Another *in vitro* study investigating human cardiac fibroblasts was carried out using methylglyoxal-modified collagen where methlglyoxal has been shown to be a component of the diabetic environment involved in carbonyl stress and impacts cell adhesion by modifying integrin binding sites on collagen (Dobler et al., 2006; Yuen et al., 2010). Findings showed increases in α-SMA and EDA-fibronectin positive myofibroblasts that was dependent upon TGF-β but not Rho-kinase. The formation of myofibroblasts by methylglyoxal-modified collagen resulted in enhanced collagen gel contraction and migration compared to cardiac fibroblasts on nontreated collagen (Yuen et al., 2010). Additionally, a decrease in adherence was observed in cells exposed to methylglyoxal treated collagen (Yuen et al., 2010). These findings support the suggestion that glycated collagen as seen in the diabetic is able to promote differentiation of fibroblasts into myofibroblasts, activating these cells independent of other biochemical factors. In agreement with this, epithelial cells have been shown to transdifferentiate into myofibroblasts under the influences of AGEs working through the RAGE receptor (Schäfer et al., 2006). While the present studies report interesting information regarding the role of myofibroblasts in the manifestation of diabetic cardiomyopathy, there is still much to be investigated to therapeutically target these cells.

Diabetes, Ages, and the Ecm

It is well established that proteins in the ECM undergo not only enzyme-mediated modification, but also modification through direct chemical reaction with other biomolecules. Of central importance to conditions related to both type I and II diabetes are the class of modification products referred to as advanced glycation end products, or AGEs.

Broad in scope, the AGEs represent proteins or lipids that have been glycated via a nonenzymatic process long known to food chemists as the Maillard reaction. Found in a variety of tissues, AGEs have been correlated to normal aging (Dunn et al., 1989; Sell et al., 1996; Frey et al., 1998; Ulrich & Cerami, 2001), elevated glucose associated with diabetes (Goh & Cooper, 2008), vascular complications (Goldin et al., 2006), and cardiac disease (Tikellis et al., 2008; Boudina & Abel, 2010) among other conditions. In all of the syndromes where AGEs are now implicated, the proteins undergoing glycation are long-lived, with collagen in its different forms featured prominently.

Initially investigated in terms of varying glucose concentrations and exposure times to proteins, a more complete picture has emerged showing that metabolic pathways can, and do, also provide the starting materials necessary for formation of AGEs (Hamada et al., 1996).A variety of carbohydrates (i.e., glucose, fructose, triose phosphates, or methylglyoxal) supplies the reactive intermediates (α-hydroxy aldehydes and ketones, α, β-unsaturated aldehydes and ketones, hydroxyalkenals, and enediols), which form the final AGE adduct via a variety of mechanisms (dehydration, rearrangement, Cannizarro reaction, and Michael addition) (Baynes, 2001).When reactive carbonyl compounds, derived from carbohydrates are combined with high levels of reactive oxygen species (as would be present in tissues under oxidative stress), the result is also the formation of AGEs (Smit & Garrits, 2010). Formation of AGEs is made more complex by the fact that in many cases a single reactive intermediate can be derived from multiple carbohydrate sources, and so a focus on a single precursor, such as glucose in relation to diabetes, is unlikely to fully account for the formation of AGEs (because levels of triose phosphates and methylglyoxal will also vary). As a result, development of therapeutic agents has turned to blocking endogenous AGE receptors (Bierhaus et al., 2005, 2006) or development of agents capable of breaking the AGE inter- and intramolecular cross-links that have formed (Candido et al., 2003).

Studies aimed at determining the sites and levels of inter- versus intramolecular cross-linking have thus far focused on human serum albumin and lens tissue (Biemel et al., 2002) and ribonuclease A (Dai et al., 2008). In all three cases, glucosepane, a 7-membered ring formed from arginine and lysine residues, was the major cross-link observed. For diabetics, levels of glucosepane in collagen have been documented to be more than twice the levels found in nondiabetics (Sell et al., 2005), with results suggesting one cross-link for every two triple helical collagen molecules in diabetics (Dai et al., 2008). The rate of AGE accumulation has been correlated to not only carbohydrate concentration and protein turnover (Brownlee, 1995; Booth et al., 1997; Ferreira et al., 2003), but also to the simple process of tissue aging (Dunn et al., 1989, 1991). Under the hyperglycemic conditions common to diabetics, the rate of AGE formation is enhanced, and the impairment of cardiac function occurs at even earlier ages than found in the nondiabetic population (Nichols et al., 2004).

The impact of the formation and accumulation of AGEs has been documented in a variety of tissues. *In vitro* studies of cross-linking and articular collagen stiffness have correlated the decrease in instantaneous deformation that collagen experiences to increasing levels of AGEs present in the sample (Verzijl et al., 2002). *In vitro* results also suggest that glycation

yields products that are especially stable (Fathima et al., 2004) and retain a significant degree of their intrinsic three-dimensional structure (Yamada et al., 2004). Modification of lysine and arginine residues in collagen has been found to significantly reduce its *in vitro* rate of degradation (Gratzer et al., 2006), further supporting the hypothesis that collagen modification by AGEs is an essentially irreversible process (Hartog et al., 2007).

In addition to the chemical modification of collagen and other proteins, which may alter their intrinsic function, AGEs also exert effects via binding to a cell surface receptor of the immunoglobulin superfamily (Basta et al., 2002). These receptors for AGEs, or RAGE, also respond to other ligands that accumulate with age and degenerative diseases such as amyloid β-peptides (Du et al., 1997) and amyloid A (Yan et al., 2000). The S100/calgranulins, polypeptides that bind calcium and accumulate in locations of inflammation, will also bind to and trigger RAGE (Hofmann et al., 1999). Discussion of the promiscuous ligand binding of the RAGE receptor and the potential signaling pathways activated by these different interactions is beyond the scope of this review; however, the reader is referred to recent a review of RAGE-ligand interactions and signaling pathways (Bierhaus et al., 2005).

At this time it appears that a strong circumstantial case can be made for AGEs contributing to heart failure. The high degree of cross-linking that accompanies AGE accumulation decreases the compliance of cardiac tissue and induces diastolic dysfunction. In assessing the effect of AGE cross-links on cardiac mechanics in volume-overload hypertrophy, Herrmann et al. (2003) demonstrated that the use of aminoguanidine, a drug that prevents AGE formation, reduced myocardial longitudinal stiffness to that of control animals. Animals with volume overload hypertrophy that did not receive aminoguanidine had myocardial longitudinal stiffness coefficients 50–100% higher at the septum and left ventricle, respectively, than control animals. More direct evidence of the involvement of RAGE and its downstream signaling pathways in heart failure has been demonstrated by Nielsen et al. (2009), who used RAGE antibodies to block the receptor in type 2 diabetic mice. In the RAGE-blocked animals, systolic function was preserved and left ventricle chamber stiffness was markedly lower compared to diabetic animals in the control group that did not receive the RAGE-blocking antibody. These studies suggest that therapeutic targeting of the AGE-RAGE interaction may provide a new tool to manage cardiovascular complications associated with diabetes.

Diabetes and Cardiovascular Function

Diabetes has long been associated with increased incidence of cardiovascular diseases including hypertension, atherosclerosis, and coronary heart disease (Tahiliani & McNeill, 1986; Candido et al., 2003). Historically, it was thought that diabetes-associated changes in cardiac structure and function were dependent on accelerated atherosclerosis and hypertension seen in diabetic patients (Poornima et al., 2006). Though still somewhat controversial, the idea that diabetes itself can lead to dysfunction of the heart, a pathology termed diabetic cardiomyopathy (Rubler et al., 1972), in the absence of other cardiovascular complications is beginning to be widely accepted. Despite the appreciation for the significance of diabetes on cardiovascular health, the full ramifications of this disease as well as the mechanisms of its pathogenesis are not fully elucidated. In fact, a number of

discrepancies exist in the literature regarding the temporal progression of this disease. Some of the discrepancies noted in the pathological changes that are associated with diabetes are likely due to the different animal models utilized and the stages of diabetes examined. Indeed, there are a number of rodent models, including genetic (db/db leptin receptor deficient mice; ob/ob leptin deficient mice; Zucker diabetic rat) and toxin induced (streptozotoxin and alloxan), which recapitulate aspects of diabetes observed in the human population. The specific characteristics of these models and their phenotypes have been recently reviewed elsewhere (Poornima et al., 2006). It is also clear that diabetes-induced myocardial remodeling and pathogenesis are multifactorial and complex. Despite differences described in various studies, fibrosis, myocardial wall stiffness, and diastolic dysfunction have emerged as consistent and rather early characteristics of the diabetic heart.

Type I diabetes, often called juvenile onset diabetes, results from the autoimmune destruction of pancreatic β cells resulting in a lack of insulin production and elevated glucose levels. Type I diabetes is associated with up to a tenfold increase in microvascular disease (Retnakaran & Zinman, 2008). Despite this, a direct relationship between type I diabetes and disease of the heart and macrovasculature has been difficult to establish. The Wisconsin Epidemiological Study of Diabetic Retinopathy illustrated that elevated levels of protein glycosylation seen in diabetic individuals was only weakly correlated with increased susceptibility to myocardial infarction (Klein et al., 2004). In another epidemiological study, hyperglycemia was not significantly associated with coronary artery disease (Orchard et al., 2003). However, many of the epidemiological studies with type I diabetic patients are complicated by the fact that many of the patients are young in terms of typical cardiovascular disease onset. Several studies have indicated that atherosclerosis has an earlier onset in children with type I diabetes (Järvisalo et al., 2001; Dahl-Jørgensen et al., 2005). These individuals have increased tunica intima to tunica media ratios and decreased vascular flow velocities, indicators of early artery dysfunction. Long-term studies (Diabetes Control and Complication Trial) of type I diabetes patients illustrated that intensive insulin therapy yielded a 42% reduction in any cardiovascular events compared to patients on standard insulin therapy and a 52% reduction in nonfatal myocardial infarction, stroke or death due to a cardiovascular event (Nathan et al., 2005).

Treatment of rats with toxins of pancreatic islet cells including streptozotocin (STZ) and alloxan have been widely used as models of type I diabetes. These models rapidly develop profound hyperglycemia and modest elevation of triglycerides. Due to the effects of the toxins, these animals have reduced plasma insulin levels and have been particularly useful for examining the effects of elevated glucose in the absence of hyperinsulinemia. Studies in these models generally report induction of myocyte atrophy and death as opposed to myocyte hypertrophy seen in some other models (Dhalla et al., 1985; Depre et al., 2000). The STZ rat model also has altered myocardial mechanical properties, which correlates to prolongation of ventricular contraction and relaxation (Depre et al., 2000; Scognamiglio et al., 2004). Other studies have illustrated a correlation between increased myocardial stiffness and impaired ventricular filling in the STZ model (Vadlamudi &McNeill, 1983; Jackson et al., 1985).

Most studies in diabetic patients or in animal models of type I diabetes have illustrated that diastolic dysfunction precedes systolic alterations. Within 7 days in the STZ model, evidence of diastolic dysfunction, including increased left ventricular end-diastolic pressure and chamber stiffness, is seen (Vadlamudi & McNeill, 1983; Jackson et al., 1985). Significant decline in left ventricular systolic pressure and left ventricular *dP*/*dt* max are not seen until several weeks later. Though the literature is contradictory, these changes in systolic function often appear to occur in the absence of myocardial hypertrophy or extensive fibrosis (Poornima et al., 2006). Administration of insulin partially attenuates the systolic abnormalities, including restoration of contractile protein function (Malhotra et al., 1981).

In type 2 diabetic patients, diastolic dysfunction again appears to precede systolic dysfunction and the development of heart failure (Poirier et al., 2001; Picano, 2003). The cardiac manifestations of type 2 diabetes vary considerably depending on the duration of the disease and the degree of hyperglycemia and hyperinsulinemia. Many of the animal models of type 2 diabetes are associated with the genetic perturbation of leptin and its receptors. The leptin receptor deficient mouse (db^{-}/db^{-}) has been widely used as a model of type 2 diabetes as it presents with a robust systolic and diastolic dysfunction (Aasum et al., 2003; van den Bergh et al., 2006; Nielsen et al., 2009). Characteristics of this genetic model include obesity, insulin resistance, and subsequently compensatory hyperinsulinemia, as well as elevated triglyceride and glucose levels. Elevated blood glucose levels are apparent as early as 8 weeks of age in the db^{-}/db^{-} mice (Nielsen et al., 2009). Measures of left ventricular contractility including ejection fraction are decreased by 12 weeks of age and progressively worsen in this model (Nielsen et al., 2009). Indices of diastolic function are reduced by 16 weeks of age as the mice begin to develop a dilated phenotype. Myocardial hypertrophy becomes apparent in these animals after 3 months of age (Belke et al., 2001; Barouch et al., 2003). Systolic function appears to be preserved in these mice through 6 months of age (Barouch et al., 2003), at least when examined *in vivo*. Some differences have been noted in these mice when isolated working heart preparations are used for the analyses (Belke et al., 2001), including earlier onset of systolic dysfunction. This is likely due to metabolic substrate limitations in the working heart models (Poornima et al., 2006) and not real physiological differences.

Cellular Mechanisms of Diabetes-Induced Myocardial Dysfunction

Many cellular changes have been described in the diabetic heart that contribute to depressed myocardial function. Most of the work to date has focused on the effects on cardiomyocytes as the contractile cells of the heart. Multiple diabetes-associated metabolic changes have been described including hyperlipidemia or the increase of triglycerides and nonessential fatty acids (NEFAs). Increased NEFAs can alter myocardial contractility through multiple molecular mechanisms (Shulman, 2000). Accumulation of NEFAs can lead to altered cellular insulin signaling and insulin resistance via activation of atypical Protein Kinase C isoforms (Kim et al., 2001). At least in skeletal muscle cells and adipocytes, NEFAs can also inactivate Akt signaling by up-regulation of the phosphatase PTEN (phosphatase and tensin homolog; Lawlor & Alessi, 2001). Accumulation of NEFAs also appears to activate caspase 3 leading to cardiomyocyte apoptosis (Halse et al., 2001).

It is clear that elevated glucose levels have direct effects on cardiac myocytes, including the generation of reactive oxygen species, glucose oxidation, and the production of superoxide, which lead to myocyte stress and death. As discussed above, diabetes also results in activation of ECM-producing cells and subsequently tissue fibrosis. Cardiac function is inherently dependent on the biophysical properties of the myocardium, which are at least partly due to the ECM. Cardiac fibrosis associated with a number of disorders including hypertension, aortic stenosis, and myocardial infarction results in increased myocardial stiffness. This in turn alters ventricular relaxation and contributes to diastolic dysfunction in a number of disorders. Accumulation of AGEs in ECM proteins such as collagen further exacerbates the mechanical changes of the myocardium and has been shown to decrease myocardial compliance (Norton et al., 1996; Aronson, 2003; van Heerebeek et al., 2008). In addition, accumulation of intracellular AGEs contributes to decreased calcium ion transport and reduced myocyte contractility (Bidasee et al., 2003). Treatment of STZ-treated rats with aminoguanidine, which prevents AGE formation, results in decreased AGE-modified collagen in the heart and diabetes-induced myocardial stiffness (Norton et al., 1996). Studies with ALT-711, which prevents glucose-derived crosslinks in collagen and other proteins, illustrated a significant reduction in myocardial collagen content and enhanced collagen solubility (Candido et al., 2003). These studies clearly illustrate that one mechanism of diabetes-induced diastolic dysfunction is through altered ECM deposition and cross-linking, which in turn alter myocardial compliance.

Summary

Despite having profound cardiovascular consequences, many questions remain regarding the mechanisms of diabetes-induced myocardial remodeling (Table 2). Addressing these questions is critical as the incidence of diabetes is rapidly on the rise. While diabetes and the biochemical changes in the body that occur during this disease (hyperglycemia, hyperinsulinemia, hyperlipidemia) have documented impacts on cardiac myocyte function, major functional consequences of diabetes in the heart are the result of changes that occur in the myocardial ECM including increased ECM deposition and glucose-induced modification of ECM components. Fibrosis is likely promoted by elevated glucose in several ways including: (1) the direct effects of elevated glucose on fibroblast gene expression and activation, (2) indirectly through the consequences of cardiomyocyte stress and death, and (3) the stimulation of the fibroblast AGE receptor (RAGE) by AGE-modified ECM. There are likely other mechanisms of diabetes-induced myocardial damage and remodeling as diabetes is a very complex process that affects many organs simultaneously. It is imperative that the mechanisms of diabetes-induced fibrosis be identified such that novel therapeutic approaches can be developed.

Key to developing new therapeutic strategies for controlling myocardial fibrosis observed in diabetes will be in-creased understanding of the role of the myofibroblast in diabetic cardiovascular disease. Identification of the mechanism(s) responsible for activation of fibroblasts and their transition into myofibroblasts would provide for an early intervention, likely to prevent much of the fibrosis associated with this cellular phenotype. Inhibiting the response of fibroblast/myofibroblast to AGE modifications within the matrix may also provide potential targets. Studies in diabetic mouse models have demonstrated improved

cardiac function following treatment with AGE-RAGE interaction blockers (either antibodies or soluble forms of the receptor), but as RAGE binds a number of different ligands, it is unclear what other effects such approaches may have. Recently, the RAGE crystal structure and identification of ligand binding sites were published; using this information it may be possible to design an inhibitor that specifically targets the AGE-RAGE interaction permitting further regulation of cellular response to diabetes associated changes within the ECM.

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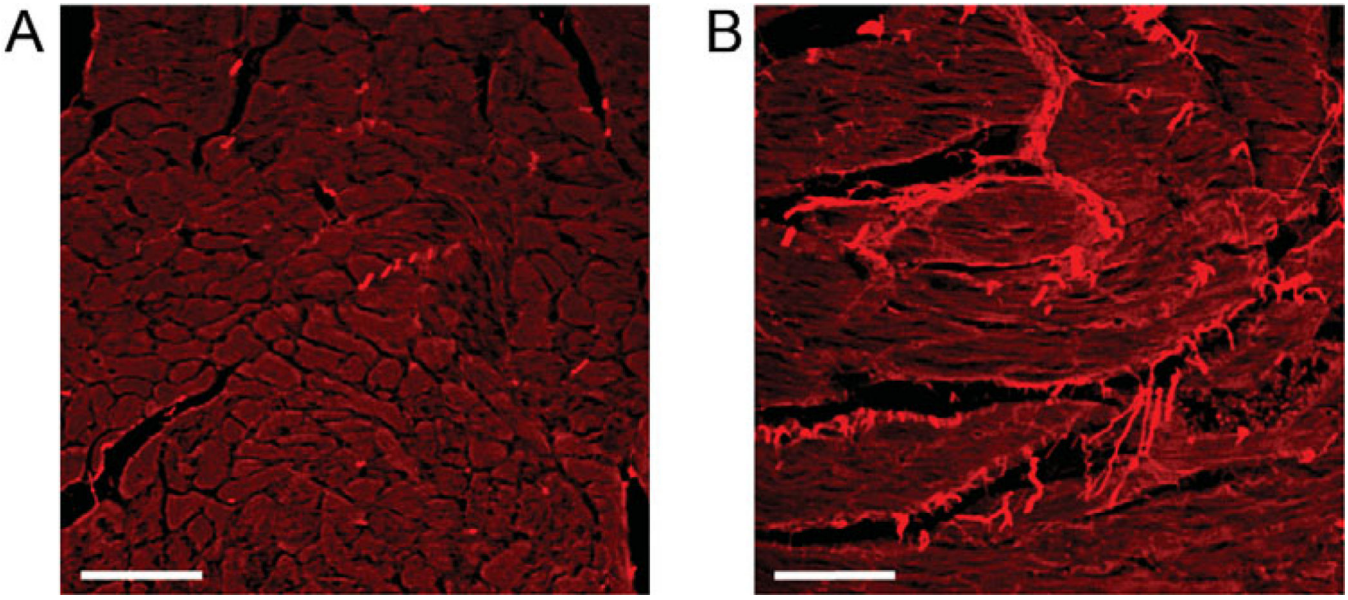

Figure 1.

Increased collagen expression in the diabetic heart. The db/db leptin receptor deficient mouse is a common model used to study the cardiovascular effects of type 2 diabetes. Picrosirus red staining of heart sections from control (**A**) and 12 week diabetic (**B**) mice illustrates the accumulation of collagen, as indicated by the bright red staining, which occurs in the diabetic heart. Scale bar = 50 μm.

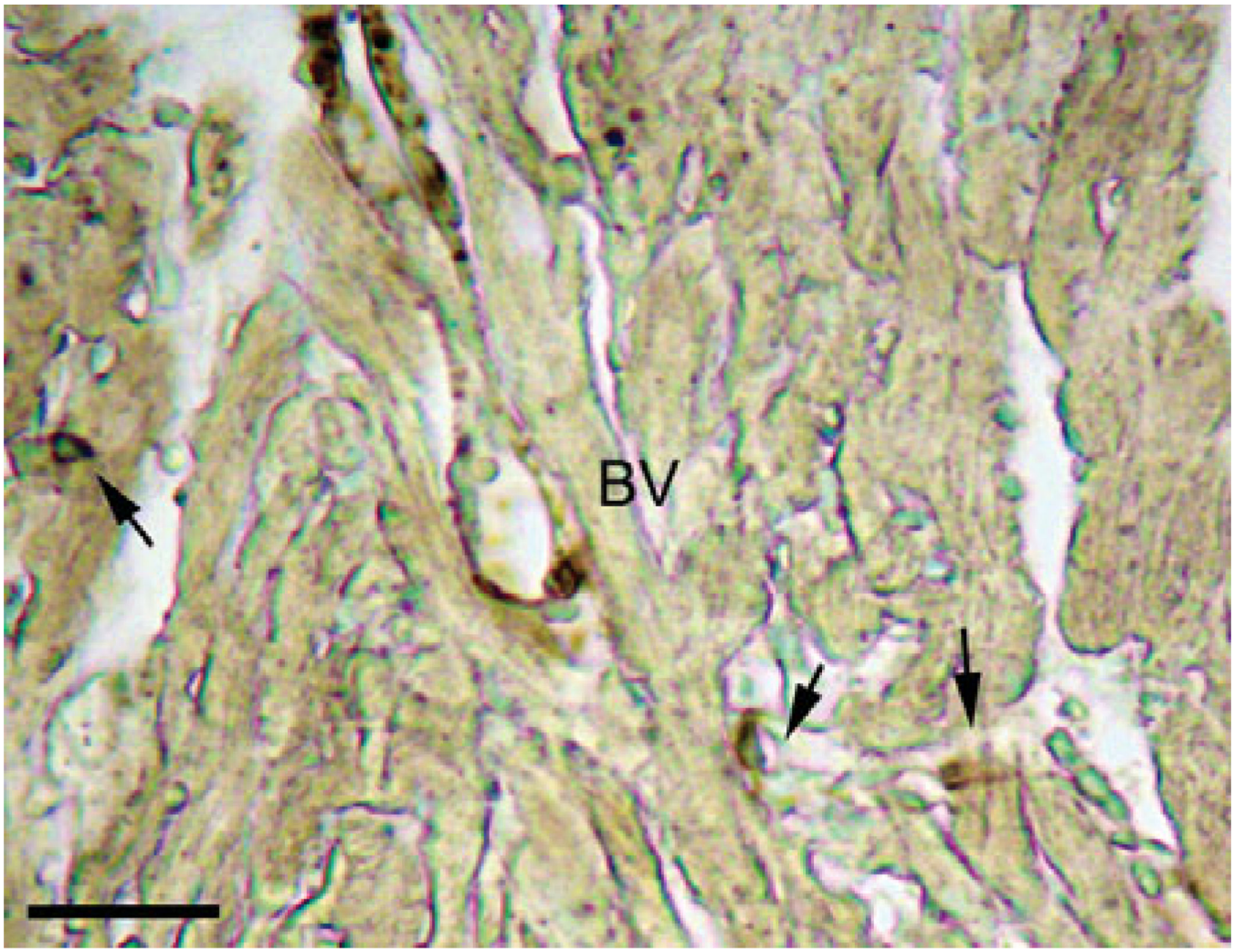

Figure 2.

Myofibroblasts in the heart. As myofibroblasts represent a contractile phenotype of the fibroblasts, staining with α-smooth muscle actin (dark brown color) reveals the location of several myofibroblasts (indicated by the arrows) in the mouse heart. Small in size, these cells reside in close proximity to neighboring cardiac myocytes and are clearly distinguishable from vascular smooth muscle cells localized around blood vessels (BV). Scale bar = 25 μm.

Table 1

Summary of Alterations in ECM Protein Expression During DM.*

	Cardiac	Renal	Hepatic	Pancreatic
Fibrillar collagen	↑Types I & III (myocardium and aorta)	↑Types I & III (late glumeruloscerosis)	↑Type I	↑Types I & III
Nonfibrillar collagen	Type VI (DC)	↑Types IV& V	↑Type IV	↑Type IV
Adhesive proteins	↓intimal elastin ↑elastin fragments (DA) ↑FN (aorta)	↑FN & LN (early DM) ↓LN (late DM)	↑LN	↑FN
Proteoglycans	↓HSP & ↑chondroitin & dermatan sulfate (DA)	↓HSP	↔	↔
Other changes associated with ECM proteins	↑ advanced glycated collagen (DA)	↑ macroalbuminuria	↑FN (ED-B), ↑IV 7s domain (serum) ↑VI (serum)	↔

* Arrows indicate increased (up arrow) or decreased (down arrow) presence of listed ECM proteins. (↔) denotes no reported changes to ECM protein expression.

FN, fibronectin; LN, laminin; HSP, heparin sulfate proteoglycan; ED-B, extra binding domain splicing variant; DC, diabetic cardiomyopathy; DA, diabetic atherosclerosis.

Table 2

Key Questions to Understanding the Role of the ECM in Diabetic Cardiomyopathy.

Does AGE modification of the cardiac ECM affect collagen turnover, and does this contribute to structural changes in myocardium?
What is the mechanism through which elevated glucose levels trigger an increase in collagen production by the cardiac fibroblasts?
Based on the crystal structure and known binding information for the AGE receptor (RAGE), can RAGE-AGE specific inhibitors be designed that block this interaction but not the binding of RAGE to other ligands?
How might intercellular communication between myocytes and fibroblasts result in increased fibrosis due to myocyte response to hyperglycemia?
Does AGE modification of collagen alter myocyte-ECM interactions contributing to increased myocyte apoptosis and necrosis observed in the diabetic heart?
What is the role of the myofibroblast in matrix remodeling in the diabetic heart?
What is the temporal course of AGE-collagen modification in the heart, and is there an ideal “therapeutic window” for administration of blocking agents that could prevent/delay onset of diabetic cardiomyopathy?