Diabetes is one of the most prevalent chronic conditions that has a high association with death from cardiovascular disease(s). An impaired cardiac function independent of vascular disease suggests the existence of a primary myocardial defect in diabetes mellitus. We and others have documented that myocardial performance is impaired in the hearts of chronically diabetic rats and rabbits. Abnormalities in the contractile proteins and regulatory proteins could be responsible for the mechanical defects in streptozotocin (STZ)-diabetic hearts. The major focus of research on contractile proteins in the diabetic state has been on myosin ATPase and its isoenzymes. However, in the contractile protein system, this could be only one of the mechanisms that might be a controlling factor in myofilament contraction in diabetes. To define the role of cardiac contractile as well as regulatory proteins (troponin-tropomyosin) as a whole in the regulation of actomyosin system in diabetic cardiomyopathy, individual proteins of the cardiac system were reconstituted under controlled conditions. Enzymatic data confirmed a diminished calcium sensitivity in the regulation of the cardiac actomyosin system when regulatory protein(s) complex was recombined from diabetic hearts. This diminished calcium sensitivity along with shifts in cardiac myosin heavy chain (V1 → V3) could contribute to the impaired cardiac function in the hearts of chronic diabetic rats. It has also been reported that sarcomeric proteins such as myosin light chain-2 (MLC-2) and troponin I (TnI) could be involved in regulating muscle contraction and in calcium sensitivity. Since phosphorylation of cardiac TnI is associated with altered maximum enzymatic activity and calcium force relationship in isolated muscle preparations, TnI phosphorylation could contribute to depressed myocardial contractility in experimental diabetes. While we have yet to understand the exact function of each component in cardiac muscle and their behavior in concert where all of them act in tandem, we have focussed on the role of contractile proteins and their regulation in diabetes in this review. We have also included a brief discussion on other relevant intracellular components. In summary, there is substantial evidence to suggest that there are independent processes associated with diabetes which effect cardiac performance in experimental animals and in man. The focus of this review has been the explication of a biochemical defect which underlies cardiac contractile dysfunction in experimental models of diabetes.

**Keywords:** Diabetes; Contractile proteins; Myosin ATPase; Troponin; Contractile function; Calcium sensitivity

1. **Introduction**

Diabetes is a complex disorder resulting in large and small vessel disease and impaired organ function. Diabetes is characterized by hyperglycemia, a relative lack of insulin, an inclination to vascular disease and neuropathy. Several investigations in experimental animals and humans indicate that diabetes mellitus is associated with a specific cardiomyopathy [1] and further a depressed cardiac function independent of vascular disease suggests the existence of a primary myocardial defect in diabetes mellitus [2]. In the hearts of larger animals including man, it has been hard to identify systolic dysfunction in diabetics that is independent of the effects of the disease on other components of the heart and its vasculature, although clear impairment of diastolic relaxation has been demonstrated across a number of species [3,4]. Myocardial dysfunction in chronic diabetes in animals is associated with depression of the ATPase activities of contractile proteins and abnormalities in sarcoplasmic reticular (SR) and sarcolemmal (SL) calcium transport. All these abnormalities have been reported to be reversible by treatment of diabetic animals with...
insulin. Mechanical, ultrastructural, and biochemical [5–7] studies conducted in different diabetic animal models demonstrate that diabetes influences myocardial contractility, regulation and changes in cardiac energetics. Studies indicate that the key pathology relating to diabetes lies in part at the intracellular level of cardiomyocytes. The major component of cardiomyocytes, namely contractile proteins, could partly explain the clinical events in diabetic cardiomyopathy. In recent years, data suggest the importance of other aspects of the contractile machinery, its regulatory system and the various components of the intracellular compartment which could directly or indirectly influence the function of contractile proteins. While we have yet to understand the exact function of each component and their behavior in concert where all of them act in tandem, we have concentrated on the mechanism of regulation of contractile apparatus in diabetes. While this article reviews more extensively the regulatory and contractile proteins, we have also included a brief discussion on other relevant intracellular components.

2. Contractile proteins in diabetes

Insulin-deficient diabetes elicits alterations in contractile protein synthesis and marked changes in cardiac function which are hallmarks of the diabetic heart [8,9]. Regan et al. [10] reported an abnormal diastolic pressure–volume relationship which accompanied depressed ventricular function in the hearts of diabetic dogs. Numerous other studies [11–15] indicated either decreased contractility or incomplete relaxation of the myocardium, suggesting a primary abnormality in the contractile apparatus. Pengkulkul et al. [16] explored the effects of streptozotocin-induced diabetes on cardiac performance and metabolism, indicating abnormal myocardial function in rats. Complete reversal of streptozotocin-induced cardiomyopathy by chronic insulin treatment suggested that this condition was due to insulin deficiency and not due to a primary cardiotoxic effect of streptozotocin [17]. Chronic treatment of diabetic rats with a calcium channel blocker (verapamil) resulted in an improvement of cardiac contractile ATPase and sarcoplasmic reticular Ca$^{2+}$ pump activities [18,19]. We also demonstrated altered papillary muscle mechanics and changes in contractile proteins in the alloxan-induced diabetic rabbit model [2,20]. Diminished velocity of shortening, an increased duration of isometric contraction–relaxation and contractile proteins were the prominent abnormalities observed that could be reversed by insulin therapy [3]. Pierce and Dhalla [21] described a depressed cardiac myofibrillar adenosine triphosphatase (ATPase) activity in diabetic rats, which correlated well with the changes in contractile dysfunction. The β-myosin heavy chain, the isoform of cardiac myosin in chronic diabetes, correlated well with decreased ATPase activity and shortening velocity. Myosin ATPase activity along with a shift in myosin isoenzyme distribution is only one of the mechanisms in the contractile protein system that might be a controlling factor. Kinetic schemes suggest that Ca$^{2+}$ ATPase of myosin measured in vitro is only remotely related to physiologic enzymatic activity which occurs in the cell where actin and other regulatory proteins (viz. troponin-tropomyosin) are also present. It is well known that the human heart is primarily V₁ even in the absence of disease. Therefore, it would be important not only to focus on myosin ATPase but also on the regulatory integrated system containing the troponin-tropomyosin system. In a study on myocardial mechanical and myosin enzymology in streptozotocin (STZ) diabetic rats, Takeda et al. [22] indicated that diabetes influences myocardial contractility, shifts V₁ → V₃ and changes cardiac energetics. Based on these observations, they further suggest that post-receptor processes may play a role in myocardial mechanical responses to catecholamines in STZ diabetic animals. The same group [23] investigated the effects of endurance swimming training on myocardial contractility and myosin isoenzymes. Physical training improved abnormal glucose metabolism and also influenced myocardial catecholamine responsiveness and energetics in myocardial contraction. In an earlier study, Dillman [24] reported that fructose feeding increases calcium-activated myosin ATPase activity and changes myosin isoenzyme distribution in the diabetic rat heart. Schaffer et al. [25] explored the basis for myocardial mechanical defects associated with non-insulin-dependent diabetes (NIDDM). They have demonstrated that the two types of diabetic cardiomyopathy (Type I and Type II) share some common characteristics (e.g., myosin ATPase and isoenzyme distribution). It has been suggested that other contractile proteins besides the key protein, myosin, may also be altered in the diabetic heart [26].

2.1. Role of myosin isoenzymes

The major focus of research on contractile proteins in abnormal states including diabetes have focussed on myosin ATPase and isoenzymes [27–30]. The relationship between myosin ATPase activity and speed of cardiac muscle shortening was confirmed by Schwartz et al. [31] and they noted a relationship between the maximum velocity of shortening and myosin isoenzyme composition in rat heart. The myosin heavy chain exists in two genetically controlled molecular forms in mammalian ventricular myocardium, resulting in three isoenzymes, [32] namely V₁ (αα), V₂ (αβ) and V₃ (ββ). Cardiac muscle in which myosin is predominantly made up of an alpha heavy chain exhibits increased velocity of contraction, high ATPase activity and enhanced energy costs of contraction as compared to cardiac myosin with predominantly the beta form. The myosin isoenzymes demonstrate marked shifts in rodent hearts during pathologic states such as hypertensive hypertrophy, diabetes, myocardial infarction, and increas-
ing age. Our laboratory and others have demonstrated that isomyosin distribution shifts from \( V_1 \) to \( V_2 \) in parallel with the contractile protein ATPase data in diabetic rat hearts [5,6,21,27,33]. Dimlan's group [34] has shown that changes in cardiac substrate consumption could influence myosin isoenzyme predominance. In another study, Rupp et al. [35] reported that intermittent fasting for 6 weeks (rats) was sufficient to induce changes in the pattern of myosin isoenzymes and in the activity of SR Ca\(^{2+}\) pump ATPase similar to those seen in the diabetic heart. The decreased velocity of contraction in diabetes could be explained wholly or in part by these changes in myosin isoforms in rat models. Human ventricular myosin is predominantly in the \( V_2 \) (\( \beta \beta \)) isoform. This probably explains the failure to observe changes in myosin ATPase activities in human hearts with profound disease, yet as noted previously the same type of heart shows depressed myofibrillar ATPase curves [36]. Subtle differences could exist in human myosin heavy chain that are not detectable by one-dimensional pyrophosphate gels. It is plausible that very small isoenzyme shifts, in association with other major alterations of the contractile proteins, could result in more marked changes in myofibrillar activity.

### 2.2. Role of troponin subunits

Myosin ATPase activity along with a shift in myosin isoenzyme distribution is only one of the mechanisms in the contractile protein system in various pathologic states that might be a controlling factor in myofilament contraction. It is more likely that changes in the other components of the contractile system, besides myosin, have a greater influence. Thus, it would be important to focus not only on myosin ATPase but also on the integrated actomyosin system containing the regulatory complex (troponin-tropomyosin; TnTm). In vertebrate striated muscle, regulatory components of the thin filaments (troponin-tropomyosin) are responsible for transducing the effect of free calcium in contractile protein activation and for inhibiting this activity when calcium is absent [37]. The thin filament, a key protein complex for the control of muscular contraction, displays several molecular and calcium binding variations in cardiac and skeletal muscle. These regulatory proteins undergo genetic changes with development and in overloaded myocardium. Diminished Ca\(^{2+}\) sensitivity of skinned cardiac muscle contractility coincident with troponin T-band shifts in the chronically diabetic rat has been demonstrated [38,39].

In humans, earlier work of Alpert and Gordon [40] and later Pagani et al. [36] showed that myofibrillar ATPase is downregulated in the diseased human heart without any obvious changes in the myosin enzymology. Changes in the troponin-tropomyosin (regulatory proteins) complex could explain different myofibrillar or actomyosin response, resulting in altered contractile function in different animal models. To define the role of cardiac regulatory proteins in diabetic cardiomyopathy, the experiments were conducted to compare the regulated actomyosin system by isolating and purifying the different proteins, recombining them under controlled conditions and studying their regulated ATPase activity. In this way the role of cardiac myosin and/or TnTm (troponin-tropomyosin) complex in diabetic cardiomyopathy could be analyzed independently as well as in a reconstituted form.

Earlier investigations from our laboratory and other groups have reported cardiac myofibrillar and myosin abnormalities in the contractile apparatus in experimental (Fig. 1) and genetic models of chronic diabetes. The figure demonstrates the Ca\(^{2+}\)-dependent activities of myofibrillar Mg\(^{2+}\) ATPase in the hearts of control and diabetic rats with increasing free calcium concentrations. Myofibrillar ATPase activity is depressed in diabetics as compared to controls across the spectrum of calcium concentrations. In another study, we examined the role of troponin-tropomyosin (TnTm) in the regulation of the cardiac actomyosin system in the diabetic animal model. Enzymatic data suggested a diminished calcium sensitivity in the regulation of cardiac actomyosin system when regulatory protein(s) complex was recombined from diabetic hearts. The composite data of the study are shown in Fig. 2. Ca\(^{2+}\)-dependent cardiac actomyosin ATPase activity using control or diabetic myosin in the presence of control or diabetic regulatory complex (TnTm) was examined. Actomyosin ATPase in the hearts of diabetic animals was partially reversed when myosin from diabetic rats was regulated with the regulatory protein complex isolated from control hearts (see DC in Fig. 2), suggesting that the regulatory proteins can partially upregulate cardiac myosin in a pathologic rat model of diabetes.

Regulatory proteins from the cardiac muscle of chronic diabetic rats and control animals displayed differences in TnI and TnT on SDS slab gels. Thus reversal in the regulated actomyosin ATPase in diabetic hearts with the addition of TnTm from control animals may be explained.

![Fig. 1. Ca\(^{2+}\)-dependent Mg\(^{2+}\) ATPase activity of cardiac myofibrils versus increasing free calcium concentrations in control (C) and diabetic (D) animals. *P < 0.05 when compared against D.](image-url)
by either the different content of TnTm subunits present or the different isoenzymatic makeup of the regulatory protein subunits in diabetic cardiomyopathy. Immunoelectrophoretic data demonstrated a downregulation of cardiac TnI in the diabetic hearts. Since molecular expression and protein analysis data demonstrate that the cardiac TnI isoform is the only one present in the adult heart, it is suggested that the downregulation of actomyosin could be attributed to reduction in TnI content or modification of the TnI molecule which could not be recognized by immunoelectrophoresis by the specific monoclonal antibody. TnI-1 (adult cardiac). In a recent report, Liu et al. [43] suggest that increased TnI phosphorylation measured under in vitro conditions may contribute to the depression in cardiac myofibrillar ATPase activity in chronic diabetes. The abnormality in the troponin-tropomyosin system observed in diabetes could be causally related in pathophysiologic states in other experimental animals and humans. Shifts in contractile regulatory protein subunits troponin T and troponin I in cardiac hypertrophy have been documented [44]. The same group demonstrated diminished Ca-sensitivity of skinned cardiac muscle contractility coincident with troponin T-band shifts in the diabetic rat [38].

2.3. Role of phosphorylation in cardiac myofibrillar proteins

The regulatory components of the thin filaments, TnI and TnT, affect the Ca$^{2+}$ sensitivity of isometric tension [45]. A number of studies have documented the effect of PKC and PKA phosphorylation of TnI, TnT, MLC-2 and C protein on Ca$^{2+}$-stimulated MgATPase activity in the normal heart [46–49]. In contrast to PKC phosphorylation of TnI, both direct phosphorylation of MLC-2 with PKC or receptor-mediated stimulation of PKC results in increased Ca$^{2+}$ sensitivity and ATPase activity in skinned cardiac myocytes and myofibrils, respectively [50]. Venema and Kuo [49] investigated the phosphorylation of cardiac myofibrillar proteins by PKC in isolated adult rat cardiomyocytes. PKC-induced phosphorylation of cTnI resulted in a reduced maximal activity of myofibrillar MgATPase with no significant change in Ca$^{2+}$ sensitivity. Besides TnI, MLC-2 and C protein were also phosphorylated. MLC-2 is phosphorylated to a small extent and has never been shown to be an effective substrate for PKC either in vitro or in situ [48]. C-protein phosphorylation as stimulated by phorbol esters does not have a dominant role in mediating the differential functional effects on the MgATPase activity. Damron et al. [51] have reported that arachidonic acid enhances the contractility of individual muscle cells mediated through the phosphorylation of myofibrillar proteins, particularly TnI and MLC-2. TnI phosphorylation decreases myofilament responsiveness to Ca$^{2+}$ when the NH$_2$-terminal extension is involved [45]. In another study on the effect of PKC activation in intact and skinned muscles from normal and diseased human myocardium, Gwatmey and Hajjar [52] suggest that the altered Ca$^{2+}$ sensitivity of the myofilaments and contractile activation could be due to phosphorylation of TnI and TnT. Studies show that the preferred PKC phosphorylation site, Thr-144, in bovine troponin I might be important for the observed decrease in Ca$^{2+}$-stimulated actomyosin MgATPase. It has been proposed that the increased rate of relaxation in cardiac muscle due to adrenergic stimulation could be attributed to TnI phosphorylation [54].

2.4. Myofilament calcium sensitivity alterations in diabetic cardiomyopathy

An important observation relating to Ca-sensitivity of tension development in diabetic cardiomyopathy was made by Akella et al. [38]. Previous evidence also suggested that regulatory properties may be impaired in cardiomyopathy [55,56,43,57–60]. Other data from rat skinned ventricular myocytes demonstrate depressed velocity of shortening when the cells are subjected to the $\alpha_1$-adrenergic agonist, phenylephrine, an agent known to stimulate PKC activity. In contrast, activation of the adenylate cyclase–PKA pathway in cardiac myocytes and skinned muscle preparations by $\beta$-adrenergic stimulation leads to decreased Ca$^{2+}$ sensitivity and enhanced muscle relaxation whereas shortening velocity is unchanged or altered [61–63]. These biochemical and physiologic data would indicate that the negative inotropic properties observed in cardiac muscle preparations incubated with phorbol ester and $\alpha$-adrenergic agonists may be PKC-mediated. They also suggest that a
possible mechanism for the decreased ATPase activity and depressed contractile performance observed in the pathologic state could involve upregulation of the PKC pathway as opposed to the PKA mechanism.

3. Signal transduction and phosphorylation in diabetes

In a study on diabetes and tension in myocardium, Akella et al. [38] and Hoffmann et al. [39] observed altered calcium sensitivity in skinned ventricular specimens from diabetic rats. To substantiate the exact mechanism(s) of diabetes-induced changes, these data raise the possibility that myofilament proteins contribute to the contractile and other functional abnormalities. Factors that might modulate myocardial function include control parameters such as phosphorylation of troponin-I and MLC-2 (myosin light chain-2) in addition to adaptations in the structure and function of other sarcomeric proteins, many of which are potential protein kinase C (PKC) target functions. Although PKC isoform-specific modulation of the contractile protein system provides attractive hypotheses to explain altered contractile function, in diabetic cardiomyopathy, this has not been well understood and the explorations of the PKC effects could be very important.

An intracellular second messenger, protein kinase C (PKC), plays a crucial role in cell surface signal transduction [64–66]. PKC has been implicated in cellular growth and also reportedly phosphorylates myosin light chain-2 and troponin-I in cardiac myocytes, thereby modulating and also reportedly phosphorylates myosin light chain-2 in addition to adaptations in the structure and function of other sarcomeric proteins, many of which are potential protein kinase C (PKC) target functions. Although PKC isoform-specific modulation of the contractile protein system provides attractive hypotheses to explain altered contractile function, in diabetic cardiomyopathy, this has not been well understood and the explorations of the PKC effects could be very important.

Table 1

<table>
<thead>
<tr>
<th>Alterations in cardiac contractile regulatory proteins and sarcoplasmic reticular (SR) fraction in the experimental model(s) of diabetes</th>
<th>Control</th>
<th>Diabetic</th>
<th>Diabetic + insulin</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myofibrillar Ca(^{2+}).Mg(^{2+}) ATPase</td>
<td>–</td>
<td>↓</td>
<td>–</td>
<td>[21,26]</td>
</tr>
<tr>
<td>Myosin ATPase (Ca(^{2+}))</td>
<td>–</td>
<td>↓</td>
<td>–</td>
<td>[5,6,13,22,27]</td>
</tr>
<tr>
<td>Myosin enzyme</td>
<td>V(_1)</td>
<td>V(_1) → V(_3)</td>
<td>V(_1)</td>
<td>[5,6,22,27]</td>
</tr>
<tr>
<td>TnI phosphorylation</td>
<td>–</td>
<td>↑</td>
<td>–</td>
<td>[43]</td>
</tr>
<tr>
<td>TnT isoforms (no.)</td>
<td>2</td>
<td>3</td>
<td>N/A</td>
<td>[38,39]</td>
</tr>
<tr>
<td>SR ATPase</td>
<td>–</td>
<td>↓</td>
<td>–</td>
<td>[12,15,35]</td>
</tr>
<tr>
<td>Ca(^{2+})</td>
<td>–</td>
<td>↓</td>
<td>–</td>
<td>[11,12,19,35]</td>
</tr>
</tbody>
</table>

Data are compiled from different studies as referenced in the table. The arrows indicate marked changes (decrease or increase) in experimental diabetes. V\(_1\) = predominantly V\(_1\) isoenzyme; V\(_1\) → V\(_3\) = shifts to predominantly V3 isoenzyme in diabetes; N/A = not available. TnT isoforms show the number of isoforms in controls and diabetic state.

4. Summary

This article has reviewed the abnormalities seen in the contractile proteins and regulatory proteins that occur in the myocardium of chronically diabetic animals. In Table 1, we have summarized the major alterations in cardiac contractile proteins and regulatory proteins as well as changes in sarcoplasmic reticulum (SR) in the experimental model of diabetes. An overview of the role of the cardiac contractile apparatus including regulatory proteins (troponin-tropomyosin) in the regulation of the actomyosin system is presented. Specifically, the diminished calcium sensitivity along with shifts in cardiac myosin heavy chain, and changes in TnI, TnT have been described.
5. Overview

Despite dramatic advances in treatment strategies, diabetes remains one of the most common causes of cardiovascular morbidity and mortality. Associated coronary artery disease is a prominent feature of the cardiomyopathy seen in humans, but there is substantial evidence to suggest that there are independent processes associated with diabetes which effect cardiac performance in experimental animals and in man. The focus of this article has been the explication of a biochemical defect which could be correlated with cardiac contractile dysfunction in different experimental models of diabetes mellitus.

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References


