Alterations in sarcoplasmic reticulum and mitochondrial functions in diabetic cardiomyopathy

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NS Dhalla, S Rangi, S Zieroth, Y-J Xu. Alterations in sarcoplasmic reticulum and mitochondrial functions in diabetic cardiomyopathy. Exp Clin Cardiol 2012;17(3):115-120.

Although diabetes due to insulin deficiency or insulin resistance is a major cause of heart disease, the pathogenesis of cardiac dysfunction during the development of diabetic cardiomyopathy is not fully understood. Varying degrees of defects in subcellular organelles, such as sarcolemma, mitochondria, sarcoplasmic reticulum, myofibrils and extracellular matrix have been observed in the diabetic heart. These subcellular abnormalities in chronic diabetes become evident with the occurrence of hormonal imbalance, metabolic defects, oxidative stress and intracellular Ca^{2+} overload. During the initial stages of diabetes, hormonal imbalances, including elevated plasma levels of catecholamines and angiotensin II, as well as metabolic defects, appear to favour the development of oxidative stress; these changes lead to subcellular defects in the myocardium. Reductions in sarcoplasmic reticular Ca^{2+} pump and Ca^{2+} release channel function are associated with cardiac dysfunction, whereas alterations in sarcolemmal Na^+/Ca^{2+} exchanger and Na^+/K^+ ATPase activities contribute to intracellular Ca^{2+} overload at late stages of diabetes. The continued accumulation of Ca^{2+} in mitochondria produces Ca^{2+} overload in these organelles, and this change induces impairment of energy production and depletion of energy stores as well as further promotion of oxidative stress in chronic diabetes. Generation of oxyradicals due to impaired electron transport results in the opening of mitochondrial pores, leakage of toxic proteins and myocardial cell damage in diabetes. These observations support the view that alterations in sarcoplasmic reticular and mitochondrial functions produce intracellular Ca^{2+} overload and depletion of energy stores and, thus, play an important role in the development of cardiac dysfunction in diabetic cardiomyopathy.

Key Words: *Diabetic cardiomyopathy; Mitochondria; Sarcoplasmic reticulum; Subcellular remodelling*

Diabetes is one of the major metabolic diseases and affects a large number of people. It has been estimated that the prevalence of diabetes will increase from 2.8% in 2000 to 4.4% in 2030 (1). Diabetes is caused either by insulin deficiency, due to a lack of insulin production, or insulin resistance, in which the target cells no longer respond to insulin. These insulin-related abnormalities cause membrane defects that are associated with changes in cation permeability, especially for Ca^{2+}. It is now well accepted that altered Ca^{2+} permeability results in defects in smooth muscle, endothelial cells, cardiac muscle and neuronal cells, which can lead to microangiopathy, atherosclerosis, cardiomyopathy and neuropathy, respectively (2). Ultimately, these processes induce local and generalized ischemia, contractile dysfunction, loss of sympathetic influences and, eventually, impaired cardiac performance (2-4). In epidemiological studies, diabetes is associated with an increased incidence of heart failure even after adjusting for the presence of hypertension and coronary artery disease (5). Although the exact reasons for cardiac dysfunction in diabetic cardiomyopathy are not clear, metabolic derangements with respect to excessive use of free fatty acids (FFAs) and the reduced use of glucose, as well as hormonal imbalances and subcellular defects due to insulin deficiency or insulin resistance have been suggested to account for structural defects in the diabetic heart (2,3). In addition, marked alterations in biochemical and functional activities of different subcellular organelles such as extracellular matrix, sarcolemma (SL), sarcoplasmic reticulum (SR), mitochondria and myofibrils have been identified in the heart under chronic diabetic conditions (2). Because of the role of the SR in Ca^{2+} handling and the role of mitochondria in energy production (3,6), the present review intends to focus on the mechanisms, as well as the significance, of SR and mitochondrial defects in diabetic cardiomyopathy. Because Ca^{2+} handling defects in cardiac membranes are invariably associated with the occurrence of intracellular Ca^{2+} overload (3,4,6), the involvement of this mechanism in the pathogenesis of diabetic cardiomyopathy will also be discussed briefly. The contribution of changes in SL, which maintains Ca^{2+} homeostasis, myofibrils, which serve as contractile machinery, and extracellular matrix, which controls the permeability of cations, during the development of diabetic cardiomyopathy have been described previously (3,7) and, thus, it is not our intention to de-emphasize their role in diabetes-induced cardiac dysfunction.

CARDIAC SR DYSFUNCTION IN DIABETES

By virtue of its ability to release and accumulate Ca^{2+}, SR are considered to play a major role in the process of excitation-contraction coupling in the myocardium. Voltage-gated L-type Ca^{2+} channels in the SL membrane are activated on depolarization of cardiomyocytes and permit the entry of Ca^{2+}, which releases more Ca^{2+} from the SR Ca^{2+} stores through the ryanodine receptor (6). This Ca^{2+} reaches myofibrils, binds to troponin C, releases the inhibition of actomyosin by troponin I and triggers the sliding of thick and thin filaments resulting in cardiac contraction. The increased level of cytosolic Ca^{2+} is then lowered by the combined actions of the SR Ca^{2+} pump ATPase (SERCA2a), the SL Na^+/Ca^{2+} exchanger and the SL Ca^{2+}-stimulated ATPase, as well as the mitochondrial uniporter (6). It has been shown that SERCA2a is the main mediator for lowering the cytoplasmic concentration of Ca^{2+} and is regulated by a SR protein, phospholamban. Although Ca^{2+} is bound to calsequestrin in the lumen of SR, the formation of a quaternary complex of SR proteins (junction, triadin, calsequestrin and the ryanodine receptor) is believed to release Ca^{2+} from the SR (8). Thus, different SR proteins are involved in the accumulation, binding and release of Ca^{2+} from the SR tubules, and changes in their contents and activities appear to disturb Ca^{2+} homeostasis. In fact, a depression in the SR Ca^{2+}-release activity would depress cardiac contraction, whereas a decrease in the SR Ca^{2+}- uptake activity would impair cardiac relaxation (6).

A wide variety of alterations in the SR Ca^{2+}-transport activities have been observed in hearts during the development of chronic diabetes induced by streptozotocin (2,3). Ganguly et al (9) reported that a decrease in Ca^{2+}-uptake activity in SR vesicles was associated with a

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depression in SERCA2a activity; these changes in diabetic animals were prevented by insulin administration. Similar defects in the cardiac SR Ca^{2+}-transport activity due to diabetes were shown by other investigators (10,11). Abnormalities in SR Ca^{2+} release were also identified in the diabetic heart (12,13). By using an experimental model of alloxan-induced diabetes in rats, Golfman et al (14) showed that SR Ca^{2+}-uptake and Ca^{2+}-release activities were depressed in the diabetic heart. Furthermore, impaired Ca^{2+}-uptake and Ca^{2+}-release activities were seen in noninsulin-dependent diabetic hearts (15,16). A depression of SR Ca^{2+} release channels in diabetes was also evident from experiments using the rapid cooling myocardial contracture technique as well as ryanodine receptor binding studies (17). These observations suggest that SR function in the diabetic heart may be defective as well as contributory to depressed cardiac performance in chronic diabetes. Depression of the SR Ca^{2+}-uptake activity for a prolonged period in chronic diabetes can also produce intracellular Ca^{2+} overload.

MECHANISMS OF SR DEFECTS

The decreased SR-transport activities in the diabetic heart may be related to alterations in protein content as well as regulatory mechanisms in the SR membrane. In this regard, decreases in Ca^{2+} uptake and Ca^{2+} release were associated with depressions in the levels of SERCA2a, ryanodine receptor and phospholamban proteins in diabetic hearts (12,18,19), except in one study where phospholamban protien content was increased (12). While some investigators have also reported reductions in SR ryanodine receptor (20,21) and phospholamban protein content (20), others have shown no change in SERCA2a protein content (20-22) in the diabetic heart. The SERCA2a messenger RNA levels were either unaltered (21,22) or decreased (20), and messenger RNA levels for the ryanodine receptor were depressed (20,21), whereas those for phospholamban were either unaltered (20) or increased (21). The conflicting results with respect to gene and protein expressions for SERCA2a and phospholamban may be due to differences in the duration and severity of diabetes in these various studies. Differential changes in SR activities and protein content during the development of diabetes have also been described previously (14,23,24). Nonetheless, these observations support the view that alterations in SR function and SR remodelling occur in the diabetic heart (3). Remodelling of other subcellular organelles, including SL and myofibrils, has also been reported during the development of diabetic cardiomyopathy (3,4).

Impaired SR function in the diabetic heart is not only associated with depressions in the contents of Ca^{2+}-cycling proteins but may also be due to a decrease in the phosphorylation of phospholamban (18,19,25). Although the activities of cyclic AMP-dependent protein kinase and Ca^{2+} dependent protein kinase were increased, the reduction in phospholamban phosphorylation was attributed to an increase in SR-associated protein phosphotase activity (25). The decrease in SR function in the diabetic heart has also been suggested to be due to an increase in the formation of advanced glycation end products (26). Because NADPH oxidase activation (27) and increased phosphotidyl inositol turnover (28) have been observed in diabetic hearts, it is likely that the depression in SR function may be due to oxidative modification of SR proteins and/or alterations in the phospholipid composition of the SR membrane, respectively. Furthermore, in view of the increase in the activities of proteolytic enzymes due to hyperglycemia and diabetes (7,29), alterations in SR function may be a consequence of proteolysis of membrane proteins. The functional significance of changes in SR proteins is suggested from observations indicating that depressed levels of SERCA2a are associated with reduced cardiac performance in the diabetic heart (30,31). In addition, overexpression of SERCA2a in transgenic mice was found to improve cardiac function in diabetic cardiomyopathy (32).

DEVELOPMENT OF INTRACELLULAR Ca^{2+} OVERLOAD

Several investigators have examined diabetes-induced changes in the intracellular concentration of free Ca^{2+} ($[Ca^{2+}]_i$) in cardiomyocytes as well as total Ca^{2+} content in the myocardium. The basal $(Ca^{2+})_i$ was observed to be either unaltered (33), increased (34) or decreased (35-37) in cardiomyocytes from diabetic animals. Elevated levels of $(Ca^{2+})_i$ in the diabetic heart appear to be due to depression of the SR Ca^{2+}-uptake activity (9-11). On the other hand, the inability to detect the elevated levels of $(Ca^{2+})_i$ in diabetic cardiomyocytes may be due to accumulation of Ca^{2+} in mitochondria because these organelles are known to serve as Ca^{2+} sinks in pathological conditions (6,38). Because $(Ca^{2+})_i$ in cardiomyocytes is maintained by Ca^{2+} influx and Ca^{2+} efflux at the SL level as well as Ca^{2+} release and Ca^{2+} uptake by both SR and mitochondria (38), it is possible that the observed differences in the results for $(Ca^{2+})_i$ in the diabetic heart from different laboratories may be due to differential changes in Ca^{2+}-transport activities in these subcellular organelles. Such differences in the observed changes in $(Ca^{2+})_i$ may also be attributable to differences in the stage and severity of diabetes (3). It should be noted that depressed Ca^{2+} efflux (32,39) and SL Ca^{2+} pump ATPase activity (40,41) as well as increased Ca^{2+} influx (28,42) and increased SL Ca^{2+}/Mg^{2+} ecto-ATPase activity (3,43) in the diabetic heart would also raise $(Ca^{2+})_i$ and, thus, contribute to the occurrence of intracellular Ca^{2+} overload.

Increased Ca^{2+} permeability due to changes in the composition of SL membranes has been suggested to raise $(Ca^{2+})_i$ in diabetic myocardium (44). Furthermore, marked depressions in SL Na^+/K^+ ATPase activity (45-47) and SL Na^+/Ca^{2+} exchanger activity (41,47-49) appear to play a major role in the development of intracellular Ca^{2+} overload in the diabetic heart. In this regard, a depression in SL Na^+/K^+ ATPase activity would increase the intracellular concentration of free Na^+($[Na^+]_i$) and this is exactly what was reported in diabetic cardiac muscle (50). Because SL Na^+/Ca^{2+} exchangers are considered to be involved in Ca^{2+} efflux under normal conditions (3,6), the observed depression of its activity in diabetic heart would decrease Ca^{2+} efflux and raise $(Ca^{2+})_i$ in cardiomyocytes. On the other hand, an increase in $(Na^+)_i$ in diabetes (50) can be seen to enhance the entry of Ca^{2+} in cardiomyocytes through the stimulation of SL Na^+/Ca^{2+} exchangers in reverse mode (3,6,38). In fact, a net gain of Ca^{2+} or increased level of total tissue Ca^{2+} has been demonstrated in the diabetic myocardium (15,16,51,52). It is noteworthy that interventions such as SL Ca^{2+} channel blockers and angiotensin receptor antagonists, which are known to attenuate the occurrence of intracellular Ca^{2+} overload, have been shown to partially prevent subcellular defects, cardiac dysfunction and ultrastructural damage in diabetic cardiomyopathy (13,53-55). These observations suggest that in addition to SR defects, alterations in SL Ca^{2+} cycling proteins may also participate in raising $(Ca^{2+})_i$ and inducing intracellular Ca^{2+} overload in the diabetic heart.

CARDIAC MITOCHONDRIAL DYSFUNCTION IN DIABETES

While defects in SR function may play a critical role in the development of impaired cardiac performance in chronic diabetes, abnormalities in other subcellular organelles, including mitochondria, have been identified to occur in the diabetic heart (2-4). It should be noted that the main function of mitochondria in the heart is to produce energy in the form of ATP, which is required for cardiac contractile activity. These organelles are known to be intimately involved in the process of glucose and FFA metabolism by cardiomyocytes, and their oxidative phosphorylation activity is generally impaired in different types of failing hearts (56-58). Defect in energy production are invariably associated with impairment of the electron transport system and the increased formation of oxyradicals in the mitochondria. Such events promote the development of oxidative stress, opening of mitochondrial pores, leakage of different mitochondrial proteins and the development of cardiomyocyte damage (2-4,16). In addition, mitochondria are known to serve as Ca^{2+} sinks in the cell and, when cardiomyocytes are faced with conditions of intracellular Ca^{2+} overload, it can result in Ca^{2+} overload in the mitochondria, defecencies in the process of oxidation of phosphorylation and enhanced production

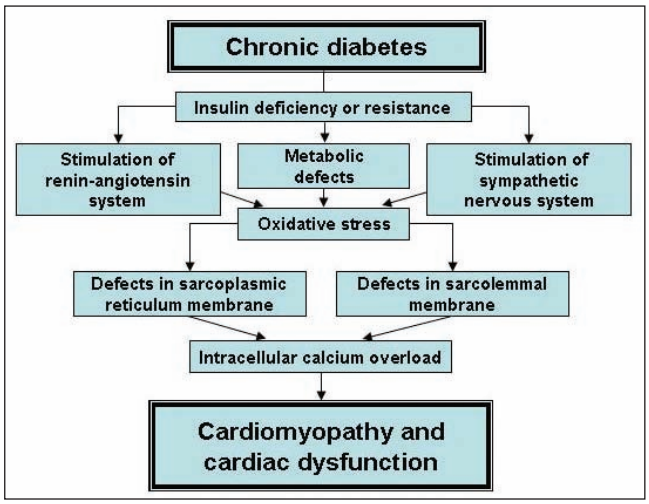

Figure 1) *Role of oxidative stress in sarcolemma and sarcoplasmic reticulum defects in the development of cardiomyopathy and cardiac dysfunction in chronic diabetes*

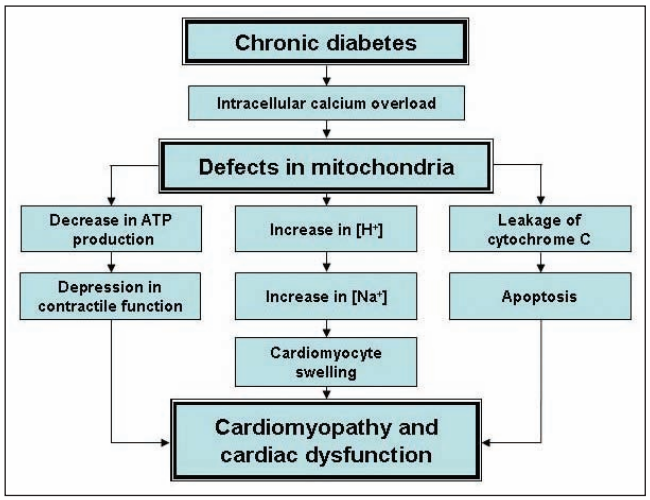

Figure 2) *Role of mitochondrial defects in the development of cardiomyopathy and cardiac dysfunction in chronic diabetes*

of oxyradicals (4,6,38). Thus, it is essential to discuss the role of mitochondria in the process of substrate utilization and energy production, regulation of intracellular Ca^{2+} and the development of oxidative stress in the diabetic heart.

ENERGY PRODUCTION AND OXIDATION OF FFA

Although plasma levels of both glucose and FFA are elevated in diabetic subjects, glucose metabolism is markedly reduced due to impaired glucose transport in cardiomyocytes (2,16). Glucose uptake in the diabetic myocardium is also reduced due to the elevated levels of FFA (57-59). On the other hand, FFA uptake is increased in the diabetic heart and FFAs are either incorporated into triglycerides or catabolized in the mitochondria (58). The increase in fatty acid oxidation in the diabetic heart (60-62) has been demonstrated to be due to the upregulation of perioxisome proliferator activated receptor α (PPARα) (63-65). Thus, it is evident that energy production in the diabetic myocardium is primarily dependent on the oxidation of FFA by mitochondria; however, increased oxidation of FFAs for a prolonged period can impair the electron transport chain and mitochondrial oxidative phosphorylation activity. In fact, the respiratory and oxidative phosphorylation activities of cardiac mitochondria have been observed to be depressed in chronic diabetes (66-68). These changes in mitochondrial function were found to be associated with the depletion of high energy phosphate stores as well as depression in the performance of diabetic hearts (67,68).

Excessive lipid uptake and increased mitochondrial fatty acid oxidation are now well known to be associated with the accumulation of lipid droplets in the myocardium and the development of diabetic cardiomyopathy (2,16). In this regard, the upregulation of PPARα has been reported to play a critical role in mediating diabetes-induced lipotoxicity and pathological alterations in the myocardium (69-72). High levels of FFAs in the diabetic myocardium have been suggested to be intimately involved in the pathogenesis of cardiac cell damage as well as cardiac dysfunction (2,73). Such observations are further supported by the fact that palmitate and long-chain saturated fatty acids were found to induce apoptosis and cell death in cardiomyocytes (74,75). Accordingly, a shift in myocardial metabolism with respect to increased uptake, utilization and oxidation of FFAs may play an important role in the development of diabetic cardiomyopathy.

OXIDATIVE STRESS AND MITOCHONDRIAL ALTERATIONS

It is becoming clear that oxidative stress generated by different sources, including mitochondria, is a major factor in the pathogenesis of diabetic cardiomyopathy (3,4,76-80). Elevated levels of plasma glucose and advanced protein glycation end products have been shown to be involved in contributing toward generating oxidative stress in the diabetic heart (81-85). Increased levels of plasma hormones, such as angiotensin II, catecholamines and endothelins, have also been considered to promote oxidative stress in diabetic cardiomyopathy (3,86). Cardiac mitochondria have been reported to generate reactive oxygen species including superoxide radicals, hydroxyl radicals and hydrogen peroxide due to microangiopathy and subsequent hypoxia in chronic diabetes (3,82,87). Impaired insulin signalling as well as auto-oxidation of glucose has also been demonstrated to affect mitochondria and to promote the development of oxidative stress in the diabetic heart (88,89). Hyperglycemia-induced apoptosis in the diabetic heart has been shown to involve mitochondrial cytochrome C-activated caspase-3, as well as depression in the mitochondrial reduced glutathione content (90,91). In fact, normalizing mitochondrial superoxide production as well as overexpression have been observed to prevent hyperglycemia-induced cell damage (88,92). Furthermore, diabetic cardiomyopathy and cardiac dysfunction have been prevented by different antioxidants such as vitamin E, catalase and metallothionein (3,93,94). Taken together, the oxidative stress generated through the participation of mitochondria, as a consequence of hyperglycemia, excessive utilization of FFAs, impaired electron transport and oxidative phosphorylation processes, seems to be a crucial factor for the genesis of diabetic cardiomyopathy.

Ca^{2+} HANDLING BY MITOCHONDRIA

In view of the ability of mitochondria to accumulate large amounts of Ca^{2+}, these organelles are known to prevent and/or delay the occurrence of intracellular Ca^{2+} overload in cardiomyocytes under different pathological conditions (6,38). During the development of cardiac dysfunction and intracellular Ca^{2+} overload in chronic diabetes, mitochondria are believed to continue accumulating Ca^{2+}, serving as a protective mechanism (3). However, these organelles become overloaded with Ca^{2+} with time and, thus, their respiratory and oxidative phosphorylation activities are impaired in the diabetic heart (3). Different investigators have reported a depression in the mitochondrial Ca^{2+} uptake activity in the diabetic myocardium under chronic conditions (14,67,68). Such a defect in mitochondrial Ca^{2+} uptake has been reported to occur following the loss of SR Ca^{2+}-pump activity in diabetic cardiomyopathy (24) and has been proposed to be due to oxidative stress (3,4). Mitochondrial abnormalities in the process of energy production in the diabetic heart have also been reported to increase the intracellular concentration of H^+, which is considered to promote the occurrence of $(Na^+)_i$ overload and cardiomyocyte swelling (2,16). Although mitochondria are being established as a source of

cellular Ca^{2+} signalling (95,96), the exact contribution of changes in these mechanisms in diabetic cardiomyopathy remains to be investigated. Nevertheless, these observations regarding the participation of mitochondria in the regulation of intracellular Ca^{2+} at early and late stages of diabetic cardiomyopathy are consistent with the view that mitochondria play an important role in health and disease (97).

CONCLUSIONS

From the foregoing discussion, it is clear that insulin deficiency or insulin resistance in diabetes is associated with a decrease in glucose utilization and an increase in the use of FFAs for the production of energy in cardiomyocytes. This metabolic shift, along with the elevated levels of different hormones, including angiotensin II and catecholamines, as well as hyperglycemia result in the occurrence of oxidative stress. Such mechanisms promote the development of intracellular Ca^{2+} overload due to alterations in the SR and SL Ca^{2+}-transport systems and, thus, lead to cardiac dysfunction. These events during the development of diabetic cardiomyopathy are depicted in Figure 1. It is known that excessive oxidation of FFAs for a prolonged period of time, as well as intracellular Ca^{2+} overload due to the loss of SR Ca^{2+}-pump activity impair the electron transport system, generate oxyradicals and contribute to further promoting oxidative stress in the diabetic heart. Defects in mitochondrial function in diabetes also result in the occurrence of $(Na^{+})_{i}$ overload and cardiomyocyte swelling. In addition, mitochondrial abnormalities lead to a depression in oxidative phosphorylation and depletion of high energy stress as well as release of mitochondrial cytochrome C and the induction of cellular death. A scheme depicting all of these mitochondrial alterations is shown in Figure 2. The present article has attempted to emphasize the role of changes in both SR and mitochondria in the pathogenesis of cardiac dysfunction in diabetic cardiomyopathy.

ACKNOWLEDGEMENTS: The research reported in this article was supported by a grant from the Canadian Institutes of Health Research. The infrastructure for this project was provided by the St Boniface Hospital Research Foundation (Winnipeg, Manitoba).

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