1. INTRODUCTION

1.1. Cardiovascular disease

Cardiovascular disease (CVD) remains the leading cause of mortality and morbidity in individuals with type 2 diabetes (T2D). CVD accounts for at least 80% of deaths in T2D patients (Hayat et al., 2004). As the global incidence of obesity and diabetes increases, it can be anticipated that the socioeconomic burden of CVD and health care costs will continue to rise. CVD mortality is increased in subjects with the metabolic syndrome (MS), a cluster of dyslipidemia, hyperglycemia, and hypertension, insulin resistance, obesity and impaired glucose tolerance. The presence of the MS is a strong predictor of CVD and death (Sundstrom et al., 2006). A recent study showed that the overweight and obese are at higher cardiovascular risk regardless of metabolic status (Ärnlöv et al., 2010) and the presence of one MS component is sufficient to increase the risk of CVD (Ho et al., 2008).

Insulin resistance is the major underlying abnormality in patients with T2D and is considered to be the main link between diabetes and CVD. The pathophysiology of cardiac disease in insulin-resistant states such as diabetes and obesity is complex and involves an increased incidence of coronary atherosclerosis, hypercoagulability and hemodynamic abnormalities including elevated cardiac output, altered vascular reactivity, hypertension, diastolic dysfunction and contractile dysfunction (Abel, 2008).

1.2. The diabetic heart

Accumulating data from experimental, pathologic and clinical studies have documented functional and structural alterations of the heart in diabetic patients and animal models. The most frequent and the earliest functional abnormality observed by echocardiography of
type 2 diabetic hearts is decreased diastolic compliance (Semeniuk et al., 2002). Reduced compliance co-exists with systolic dysfunction which may be evident as reduced ejection fraction. Diabetes is also a significant risk factor for left ventricular hypertrophy (LVH) and this has been demonstrated in type 2 diabetic populations. Higher left ventricular (LV) wall thickness and mass are the common structural alterations in diabetic hearts (Devereux et al., 2000).

Diabetes and prolonged effect of hyperglycemia can have metabolic and vascular consequences that lead to changes in cellular function and structure of the diabetic heart. Das et al. (1987) have reported thickening of the capillary basement membrane, myocellular atrophy and hypertrophy, interstitial and myocardial fibrosis and collagen deposition in endomyocardial biopsies from diabetic patients, all of which can result in compromised myocardial function. Cardiac fibrosis is another common structural problem seen in the diabetic heart.

Novel insights into underlying molecular and pathophysiological mechanisms that increase the vulnerability of the diabetic heart to failure have been proposed. Advanced glycosylation end products (AGEs) are formed non-enzymatically when glucose reacts with the free amino groups of proteins (Singh et al., 2014). Elevated levels of AGEs cause cross-linking between myocardial collagen fibres leading to a decrease in compliance and heart failure (HF) (Susac, 2007). AGEs upregulate the genetic expression of nuclear factor kappa B (NFkB), endothelin and fibronectin which have been linked with myocardial fibrosis, inflammatory changes and increased apoptosis (Singh et al., 2014). In addition, increased levels of interleukin-6 (IL-6) (Bahrami et al., 2008) and leptin (Xu et al., 2004) in obese, T2D subjects stimulate growth factors that increase LV mass and LVH.
1.3. Cardiac metabolism

The heart is one of the most metabolically active organs of the body and its metabolic requirements are the largest of any organ in the body. Normal cardiac metabolism is essential for contractile function and maintenance of cellular homeostasis. Generally, the heart is an energy-consuming organ that requires a constant supply of fuel and oxygen in order to maintain its intracellular adenosine triphosphate (ATP) level which in turn is essential for the uninterrupted myocardial contraction/relaxation cycle. The human heart produces and consumes between 3.5 and 5 kg of ATP every day to sustain pumping (Opie and Lopaschuk, 2004). The generation of this energy depends on the cardiac environment including coronary flow, substrate supply, hormones, and nutritional status (Stanley et al., 2005; Lopaschuk et al., 2007). The heart predominantly metabolizes three fuels- fatty acids (FAs), glucose and lactate and to a lesser extent other oxidizable substrates. There is a retrocontrol of FA and glucose utilization but the heart functions best when it oxidizes both substrates simultaneously (Taegtmeyer, 2000). Under physiological conditions, more amount of ATP (60-80%) is produced from the mitochondrial oxidation of long chain FAs (60–70%) and remainder is provided by glucose metabolism. However, in the stressed state (e.g., ischemia, pressure load and injury), the heart switches to the more efficient fuel, glucose since the amount of ATP generated per molecule of oxygen consumed is greater when glucose is oxidized. A large body of work has suggested that altered myocardial substrate metabolism may contribute to contractile dysfunction in the hearts of diabetics (Boudina and Abel, 2007).

Free fatty acids (FFAs) bound to albumin, FA esters present in chylomicrons and very-low-density lipoproteins (VLDL) are the major lipids for cardiac metabolism. FFAs are transported into the cardiomyocyte by either passive diffusion or by transport proteins
(FA translocase [FAT] or FA binding proteins [FABP]). The level of circulating FFAs largely determines FFA uptake in the heart (Abel et al., 2012).

1.4. Cardiac insulin signaling

Insulin plays a key role in the regulation of several aspects of cardiovascular physiology, including cardiac contractility, vascular tonicity and metabolism of lipids, glucose and proteins (Bertrand et al., 2008; Muniyappa et al., 2007). The metabolic role of insulin in the heart under physiological conditions is the regulation of substrate (glucose) utilization. The vascular actions of insulin are mediated through stimulation of the production of nitric oxide (NO), a potent vasodilator in the endothelium through endothelial nitric oxide synthetase (eNOS). NO action leads to capillary recruitment, vasodilatation, increased blood flow and subsequent augmentation of glucose disposal in insulin target tissues (Bertrand et al., 2008).

Insulin, synthesized and released from pancreatic β-cells, induces glucose uptake into cardiomyocytes upon binding to the cell surface insulin receptor (IR). Binding activates the tyrosine kinase activity of the receptor’s β-subunit. This leads to autophosphorylation as well as tyrosine phosphorylation of several insulin receptor substrates (IRS) 1 to 4. These substrates, in turn, interact with phosphatidylinositol 3-kinase (PI3K) which activates a serine/threonine (ser/thr) phosphorylation cascade of pleckstrin homology (PH)-domain containing protein-phosphoinositide-dependent protein kinase 1(PDK1) and the ser/thr protein kinase B (PKB)/Akt. Activated Akt regulates glucose uptake by phosphorylating Akt substrate of 160 kDa (AS160) (Kim et al., 2011). AS160 normally inhibits translocation of glucose transporter-4 (GLUT-4) through its interaction with Rab GTPase protein. The inhibitory phosphorylation of AS160 favors the GTP-loaded state of Rab and
relieves the inhibitory effect on GLUT-4, stimulating its translocation to the plasma membrane. In this way, insulin promotes the docking and fusion of GLUT-4 containing vesicles to the plasma membrane and finally stimulates glucose uptake. Diagrammatic representation of insulin signaling pathway is given in Fig.1.

**Fig.1 Insulin signal transduction pathway.** Binding of insulin to its receptor, initiates autophosphorylation of IRS-1, which in turn triggers a cascade of downstream phosphorylation events that activate PI3K and Akt. Thus, insulin activated Akt inhibits AS160 (inhibits GLUT-4 translocation through its interaction with Rab GTPase protein) thereby favours GLUT-4 translocation to the plasma membrane. (Adopted and modified from Thorn et al., 2013).

Glucose uptake in the heart is mediated by two isoforms of transporters- GLUT-1 and GLUT-4 (Luiken et al., 2004). GLUT-4 translocation represents the major mechanism that regulates glucose entry in the beating heart whereas insulin mediated activation of GLUT-1 plays a lesser role (Abel, 2004). Further, glucose metabolism is about 4-fold greater in heart than in skeletal muscle and adipose tissue, due to a greater expression of GLUT-4 proteins in the heart than in other organs (Fischer et al., 1997).
1.4.1. Regulation of insulin signaling

Given the importance of insulin in the regulation of metabolic and growth-promoting functions, its actions are highly regulated by auto-regulation (homologous desensitization), whereby downstream enzymes inhibit crucial upstream components, mainly the IR and IRS proteins. The IR-β and the IRS proteins undergo ser/thr phosphorylation, which may attenuate signaling by decreasing insulin-stimulated tyr phosphorylation of both proteins (Boura-Halfon and Zick, 2009). This mechanism represents a key step in the feedback control process of insulin signaling. Interestingly, many of the ser/thr kinases involved in the negative modulation of IRS are downstream effectors of PI3K, such as atypical protein kinase C (aPKC), mammalian target of rapamycin (mTOR) and S6kinase (S6K1) (Hiratani et al., 2005). Regulation of IR activity is mostly associated with ser/thr phosphorylation by PKC, receptor internalization and receptor dephosphorylation by specific tyr-phosphatases (Youngren, 2007).

1.5. Cardiac insulin resistance

Cardiac insulin resistance is a key factor in the development of hypertension, T2D and CVD. The insulin resistant heart fails to adjust to the changing energy demands due to increased delivery of FA and reduced ability to use glucose. This results in FAs being predominantly utilized instead of glucose causing cellular stress. Disturbances in glucose and lipid metabolism as result of insulin resistance can potentially worsen metabolic capacity and efficiency of the working heart which may favor structural and functional changes in the heart that may lead to the development of heart disease and HF (Gray and Kim, 2011).
Studies have strongly suggested that the major negative regulation of insulin action is the increased ser/thr phosphorylation of IRS proteins (principally IRS-1) (Zick, 2005; Draznin, 2006; Muoio and Newgard, 2008). Ser/thr phosphorylation in specific residues of IRS-1 can induce the dissociation of IRS proteins from the IR, block tyr phosphorylation sites of IRS proteins, release the IRS proteins from intracellular complexes that maintain them in close proximity to the receptor, induce degradation of IRS proteins, or turn IRS proteins into inhibitors of the IR kinase (IRK) (Zick, 2005). Thus, in contrast to a signal promoting tyr phosphorylation, excessive ser/thr phosphorylation of IRS proteins could become detrimental for normal metabolic insulin signaling, causing insulin resistance.

IRS proteins contain more than 70 ser/thr residues that are potential targets for phosphorylation. A number of serine kinases that phosphorylate IRS and weaken insulin signaling have been identified: c-Jun N terminal kinase (JNK) (ser307), protein kinase C (PKC)\( \theta \) (ser1101), PKC\( \zeta \) (ser323), PKCa (ser307), salt inducible kinase (SIK) (ser794), mitogen-activated protein kinase (MAPK) (ser616), mTor/S6K-1 (ser616/ser636) among others (Arkan, 2005; Draznin, 2006; Zick, 2005).

Interestingly, it is becoming apparent that inducers of insulin resistance such as tumor necrosis factor-\( \alpha \) (TNF-\( \alpha \)), FFAs, angiotensin-II (Ang-II) and cellular stress make use of similar mechanisms by activating a set of IRS ser/thr kinases that phosphorylate the IRS proteins and inhibit their functions (Carvalheira et al., 2003; Herschkovitz et al., 2007).

Mitochondrial dysfunction-induced insulin resistance in heart has also been reported (Lowell and Shulman, 2005). Decrease in mitochondrial FA oxidation, caused by mitochondrial dysfunction and/or reduced mitochondrial content, produces increased levels of
intracellular fatty acyl CoA and diacylglycerol (DAG). These molecules activate novel PKC, which in turn activates a serine kinase cascade leading to increased ser phosphorylation of IRS-1 thereby disrupt insulin signaling pathway.

1.6. Altered substrate metabolism

Glucose and lipids are the major substrates that supply energy to the heart upon oxidation. The mammalian heart possesses the capacity of oxidizing the available substrate to maintain a steady level of ATP required for contraction. Although, oxidation of FA is predominant in the adult heart, the use of glucose, lactate and ketones can be enhanced in certain pathological conditions. This flexibility in substrate use is important for normal cardiac function. Obesity, diabetes and/or insulin resistance independently or in combination affect this flexibility due to alteration in substrate (glucose) availability or due to impairment in transcriptional regulation of oxidation pathways. For example, obesity in mice enhances cardiac FA oxidation and reduces glucose oxidation independently of diabetes (Buchanan et al., 2005).

In the diabetic heart, myocardial glucose utilization is diminished at least in part because of insulin resistance, impaired pyruvate dehydrogenase activity, and reduced GLUT-4 content. Cardiac expression of GLUT-4 is compromised likely due to elevated FA (Armoni et al., 2005). A growing body of evidence indicates that perturbations in cardiac energy metabolism and insulin resistance are the earliest diabetes-induced alterations in in the myocardium (Lopaschuk et al., 2002). In obese or T2D animals, although there is hyperglycemia and hyperinsulinemia, cardiac glucose uptake is reduced as a consequence of reduced GLUT-4 protein and impaired insulin signaling (Carroll et al., 2005). A study by Buchanan et al. (2005) revealed that cardiac glucose oxidation is reduced at 4 weeks of age and
was associated with increased FA oxidation in ob/ob and db/db mice. Impaired myocardial glucose uptake was observed in T2D patients with hypertriglyceridemia, suggesting that myocardial insulin resistance in these patients is associated with hypertriglyceridemia and augmented plasma FA levels (Monti et al., 2004). A rise in intracellular FFA in the cardiac cell also leads to reduced insulin-mediated GLUT-4 translocation to the plasma membrane.

Under normal conditions, 70–90% of the esterified FA that enters cardiomyocytes are oxidized for ATP generation, whereas 10–30% is converted to triglycerides (TG) (Stanley et al., 2005). In the normal heart, intracellular TG level is constant, indicating a balance of lipogenesis and lipolysis (Lindsey et al., 2008). During obesity or diabetes conditions, FA supply supercedes the cellular oxidative capacity, resulting in intracellular TG accumulation which is associated with lipotoxicity (Unger, 2002). Although TG is unlikely to be a direct mediator of cell apoptosis, augmented lipolysis expands fatty acyl-CoA levels, which may be a key factor mediating cell apoptosis (Unger, 2002; Jagasia and McNulty, 2003). In addition FFA oxidation generates reactive oxygen species (ROS) which induces mitochondrial dysfunction and apoptosis (Barouch et al., 2006).

The mechanisms for obesity-related alteration in cardiac substrate utilization involve enhanced FA supply and reduced glucose availability and leptin resistance (Sloan et al., 2011). The mechanisms for increased cardiac FA uptake and oxidation include impaired glucose transport, enhanced long-chain FA uptake through relocation of the FA transporter cluster of differentiation (CD36) in the sarcolema (Ouwens et al., 2007) and decreased mitochondrial carnitine palmitoyltransferase-1 (CPT-1) activity, reduction in the expression of genes involved in FA oxidation, inactivation of transcriptional pathways such as the peroxisome proliferator–activated receptor-α (PPAR-α)/ PPAR gamma
coactivator-1α (PGC-1α) signaling network and impaired mitochondrial oxidative capacity (Huss et al., 2005; Boudina et al., 2009; Su et al., 2014).

1.7. Myocardial metabolic remodeling

Myocardial metabolic remodeling is the process in which the heart loses its ability to utilize different substrates, becoming dependent primarily on the metabolism of a single substrate such as glucose or FAs for energy production. Myocardial metabolic remodeling is central to the pathogenesis of diabetic heart. Altered myocardial substrate metabolism has emerged as an important contributor to the development of MS-associated heart disease (Lopaschuk, 2002). Insulin resistance and T2D are generally characterized by reduced glucose uptake and metabolism and enhanced FA metabolism. The shift in oxidation towards FA, at the cost of glucose causes metabolic disturbances especially lipid overload in the myocardium.

1.8. Lipid metabolism

Lipid accumulation, reduced FA uptake but inefficient oxidation and increased lipogenesis are encountered in insulin resistant states.

Increased availability of lipid (energy substrate) and increased delivery of fat to the heart muscle (Murdolo and Smith, 2006) have been reported in both obesity and obesity-induced T2D. Lipid accumulation is believed to be the consequence of a mismatch between the rate of FA uptake and the oxidative capacity of cardiomyocytes.

Studies suggest that alterations in cardiac FA metabolism may lead to the accumulation of products such as TG, DAG and ceramide (Sharma et al., 2004; Wilson et al., 2007). This may lead to dysfunction and/or myocyte death and eventually, cardiac function depression, a process known as lipotoxicity. Accumulation of TG is believed to be a major element in the pathogenesis of myocardial dysfunction in
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diabetes. Lipid overload in the heart induced by a transgenic approach in normal rodents resulted in cardiac dysfunction (Christoffersen et al., 2003; Yagyu et al., 2003) while prevention of TG accumulation ameliorate cardiac dysfunction in diabetic mice (Yokoyama et al., 2004). It has been reported that the hearts of Zucker diabetic fatty (ZDF) rats display a specific systolic dysfunction as a proposed consequence of excess lipid accumulation in cardiomyocytes (Sharma et al., 2004). In obese diabetic rats, lipid accumulation is associated with reduced FA oxidation with consequent accumulation of ceramide and induction of apoptosis (Dyntar et al., 2001).

The underlying molecular mechanisms in the regulation of cardiac FA metabolism at the gene level are given below.

1.8.1 SREBP-1c in lipogenesis

De novo lipogenesis (DNL) is the metabolic pathway which converts excess glucose into FA and ultimately TG. Regulation of the lipogenic flux involves a complex network of nuclear receptors. For example, sterol regulatory element binding proteins (SREBPs) are a family of transcription factors which belong to the basic helix-loop-helix leucine zipper family, co-ordinately regulate lipid homeostasis by controlling the expression of enzymes of lipid metabolism. The three SREBP isoforms, SREBP-1a, SREBP-1c and SREBP-2, have different roles in endogenous cholesterol, FA, TG and phospholipid synthesis. In vivo studies using transgenic and knockout mice suggest that SREBP-1c regulates genes involved in FA synthesis and TG metabolism, whereas SREBP-2 regulates the genes of cholesterol synthesis. The SREBP-1a isoform seems to be implicated in both pathways.

SREBPs become active upon proteolytic processing of the inactivate precursor in the endoplasmic reticulum, which releases a transcriptionally active N-terminal basic helix-loop-helix zip domain. The active mature form translocates to the nucleus and promotes a
lipogenic program in the liver. SREBP activates itself and its target genes by binding to sterol regulatory elements. Dysregulation of SREBPs was shown to be involved in T2D, dyslipidemia and hepatic steatosis (Raghow et al., 2008).

SREBP-1c is transcriptionally regulated by liver X receptor (LXR) and AMP-activated protein kinase (AMPK). SREBP-1c transcription is greatly stimulated by insulin (Yellaturu et al., 2005). Insulin activates SREBP-1c promoter primarily by increasing the activity of LXR (Chen et al., 2004). A synthetic LXR ligand, T0901317, increases hepatic SREBP-1c mRNA levels and mRNAs for lipogenic enzymes when administered to mice resulting in fatty liver and hypertriglyceridemia (Repa et al., 2000). Gene knockout mice lacking SREBP-1c show a blunted increase in lipogenic mRNAs in response to T0901317, confirming the role of SREBP-1c in this response (Liang et al., 2002).

SREBP-1c is activated during conditions of over-nutrition (Gallagher et al., 2010; Vallim et al., 2010). SREBP-1c expression is depressed during fasting but increases markedly when animals are fed a high carbohydrate diet. Miyazaki et al. (2004) reported an induction of the hepatic SREBP-1 isoform in mice following 7 days on a 60% fructose diet.

SREBP-1c has been implicated in the development of human metabolic physiopathology such as obesity, T2D, dyslipidaemia, atherosclerosis, MS and lipodystrophy (Friedman, 2006). Both insulin resistance and glucose can induce this lipogenic transcription factor (Wong et al., 2010). There is a correlation between reduced ejection fraction and lipid accumulation within cardiomyocytes of patients with the MS and increased levels of SREBP-1c and PPARγ in the heart (Marfella et al., 2009). This suggests that overactivation of SREBP-1c may promote lipid deposition in cardiomyocytes by upregulating PPARγ, in turn causing lipotoxicity and subsequent contractile dysfunction.
1.8.2. Transcriptional regulation of FA oxidation

The transcription factor PPAR-α (Barger and Kelly, 2000) and its coactivator PGC-1α (Liang and Ward, 2006) regulate genes involved in both FA uptake and metabolism. Dysregulation of PPAR-α/PGC-1α signaling causes imbalance in FA uptake and oxidation which in turn results in lipid accumulation, lipotoxicity, and cardiac dysfunction. PPAR-α is a nuclear receptor, which functions as a transcriptional regulator in tissues with high metabolic activity including liver, heart, kidney and skeletal muscle (Huss and Kelly, 2004). In the myocardium PPAR provides energy by regulating genes involved in cellular FA uptake, intracellular FA transport, mitochondrial and peroxisomal FA uptake and β-oxidation (Rakhshandehroo et al., 2010). Long-chain FAs are the most studied among the various endogenous ligands for PPAR-α. Activation of PPAR-α would increase the oxidation of FAs, thus decreasing tissue content of lipids and minimizing lipotoxicity (Fig.2).

![Fig. 2 Regulation of long-chain FA uptake and oxidation in cardiomyocytes.](image)

FA are imported from the blood facilitated by FAT, CD36. FAs then are activated by acetyl coA synthetase (ACS) to acyl-CoA. Acyl-coA is converted into acyl-carnitine by CPT-1. This is then translocated in to the mitochondria by CAT. In the matrix, CPT-II regenerates acyl-CoA, which enters β-oxidation and is further processed to ATP. Besides oxidation, cytoplasmic FA can be stored or can interact with PPARs to stimulate the expression of genes coding for lipid metabolic enzymes and transporters. (Adopted from Drikx et al., 2011).
Multiple studies have shown an inverse relationship between reduced PPAR-α activity and abnormalities in FA metabolism leading to the development of insulin resistance and alterations in glucose metabolism (Lee et al., 2003; Ferre, 2004). Animal studies suggest that the activation of PPAR-α improved the insulin resistance that was triggered by the excessive production and accumulation of lipids (Nagai et al., 2001; Ye et al., 2001). Zhou et al. (2000) showed that the hearts of an animal model of T2D (ZDF rat) possess elevated intracellular lipid deposition and contractile dysfunction, potentially because of impairment of the PPAR system. Treatment of cultured cardiac myocytes with PPAR-α agonists induces expression of many genes involved in FA catabolic pathways (Gilde et al., 2003), including FA transport, esterification, binding and β-oxidation. PPAR-α agonists also exhibit anti-inflammatory effects both in the vascular wall and the liver (Zandbergen and Plutzky, 2007). Overall, these studies suggest that one of the mechanisms whereby cellular FA oxidation rates are increased in the diabetic state occurs via activation of the PPAR-α gene regulatory pathway.

1.8.3. AMPK in lipid metabolism

AMPK is an important mediator of energy balance in various tissues, including the heart (Wong et al., 2010). The heterotrimeric protein AMPK is constituted by a catalytic subunit (α) and 2 regulatory subunits (β and γ). Each of these three subunits takes on a specific role in both the stability and activity of AMPK (Stapleton et al., 1996). The α-subunit contains the catalytic domain as well as a phosphorylation site for upstream kinases that regulate its activity (Crute et al., 1998). The β subunit interacts with both α and γ subunits and plays an obligatory role in AMPK complex formation (Dasgupta, and Milbrandt, 2009). Specifically, the γ subunit of AMPK has ability to sensitively detect shifts in the AMP:ATP ratio (Adams et al., 2004). Under
conditions of inhibited ATP production or elevated ATP consumption, such as ischemia or exercise, the AMP/ATP ratio is elevated and AMPK is stimulated. AMP binds to γ subunit of AMPK and activates the α catalytic subunit in an allosteric manner by upstream kinase liver kinase B1 (LKB1).

AMPK has effects on glucose transport, lipid and protein synthesis and fuel metabolism (Fig. 3). The phosphorylation at thr172 of α subunit of the enzyme is responsible for metabolic changes via phosphorylation of various downstream substrates. Activated AMPK directly impacts FA metabolism through inhibition of acetyl-CoA carboxylase (ACC), which is responsible for the synthesis of malonyl CoA, a potent inhibitor of CPT-1. AMPK has also been implicated in FA delivery to cardiomyocytes through its regulation of the FA transporter CD36. As a result, AMPK acts to maintain ATP production and contractile function by increasing glucose and FA uptake and oxidation in the heart (Luiken et al., 2003). AMPK enhances insulin signaling by stimulating glucose uptake and by modulating insulin secretion by pancreatic beta-cells. Additionally, AMPK regulates a wide array of other physiological events like cellular growth and proliferation and mitochondrial function and biogenesis. Reduced activities of AMPK in tissues of diabetic animals have been observed (Ruderman et al., 2004; Bonnard et al., 2008). Given the wide range of metabolic functions, reduced activity of AMPK might contribute to dysfunction in the heart in obesity and insulin resistant states.

The molecular mechanisms by which AMPK inhibits cleavage and transcriptional activation of SREBP via direct phosphorylation have been well explained. SREBP-1c and-2, but not SREBP-1α, is characterized as conserved substrate of AMPK. It is demonstrated that AMPK interacts with and directly phosphorylates SREBP-1c and -2. ser372 phosphorylation of SREBP-1c by AMPK is sufficient and
necessary for the inhibition of proteolytic processing and transcriptional activity of SREBP-1c in response to polyphenols and metformin. AMPK stimulates ser372 phosphorylation, suppresses SREBP-1c maturation and represses SREBP-1c target gene expression in hepatocytes exposed to high glucose, leading to reduced lipogenesis and lipid accumulation. Hepatic activation of AMPK by the synthetic polyphenol S17834 protects against hepatic steatosis, hyperlipidemia, and accelerated atherosclerosis in a diet-induced insulin resistant model (Li et al., 2011).

Fig. 3 Schematic representation of the subunit structure and activation of AMPK. Increase of AMP/ATP ratio and intracellular calcium activate respectively LKB1 and Ca\textsuperscript{2+}/calmodulin-dependent protein kinase kinase (CAMKK) which in turn phosphorylate AMPK on thr172. AMPK activation leads to decreased lipid and cholesterol synthesis and increased FA oxidation, glucose uptake and mitochondrial biogenesis (Adopted and modified form Ruderman et al., 2010).

1.8.4. FA uptake transport proteins

FA uptake in the myocardium is facilitated by three transport proteins, CD36/FAT, FA transport protein-1 (FATP-1), and FABP of which CD36 appears to play a major role. CD36 translocates from intracellular endosomes to the sarcolemmal membrane and aids in the transport of FA into the cardiomyocytes (Luiken et al., 2004).
During obesity and diabetes, the high rate of FA uptake facilitated by these FA transporters, leads to augmented FA oxidation and TG storage. In STZ-induced diabetes, the increase in plasmalemmal CD36 and FABP amplifies FA uptake, an effect resulting from increased CD36 and FABP protein expression (Luiken et al., 2002). It is reported that the basal level of CD36 appears low and it is increased in animal models of obesity and after high calorie diet feeding (Koonen et al. 2007).

CPT-1 a downstream target of PPAR-α, serves a key regulatory role in controlling the rate of FA uptake by the mitochondria (Kerner and Hoppel, 2000). It catalyzes the formation of long-chain acylcarnitine from long-chain acyl-CoA in the compartment between the inner and outer mitochondrial membranes. There are two isoforms of CPT-1: CPT-1 alpha predominates in the liver and CPT-1 beta is the main isoform in the heart (McGarry et al., 1983). CPT-1 beta is 30-fold more sensitive to malonyl-CoA inhibition than is CPT-1 alpha (Weis et al., 1994). Studies suggest that removal of malonyl CoA inhibition of CPT-1 facilitates the increase in FA oxidation in perfused rat hearts (Goodwin and Taegtmeyer 2000; Reszko et al., 2004).

1.9. Oxidative stress

1.9.1. Alteration in FA metabolism leads to reactive oxygen species (ROS) generation

Oxidative stress is a state of mismatch between the formation of ROS and oxidative byproducts and their clearance by antioxidants. In healthy tissue, appropriate ROS levels are maintained by endogenous antioxidant systems by neutralizing or scavenging the ROS in order to minimize oxidative damage (Venardos et al., 2007). When production of ROS is elevated and/or the level of antioxidants is decreased, this balance is disturbed which results in oxidative stress. Increased oxidative stress or excessive ROS directly induce cellular injury by reacting with major biomolecules DNA, lipids and proteins resulting in oxidative damage to these molecules (Seddon et al., 2007).
Recent studies suggest that in addition to causing toxicity and cellular damage, ROS also participate in cell signaling through activation of redox-sensitive signaling cascades. Thus, oxidative stress can cause disruption in normal mechanisms of cellular signaling by oxidative modification of proteins. Many studies evidenced that oxidative stress alters the function of transcription factors (NFκB) and proteins of the signaling pathways including insulin signaling in the heart (Ceriello and Motz, 2004; Rasmussen et al., 2010). Oxidative stress causes insulin resistance and reduces glucose uptake through activation of serine kinases like p38 MAPK, JNK and PKC (Tiganis, 2011).

Many studies show that antioxidant treatment or inhibition of ROS-generating oxidases improves insulin sensitivity. Several clinical and animal studies have demonstrated that fruit and vegetable consumption, those particularly rich in vitamins C, E and other antioxidants, improves insulin sensitivity (Stanner et al., 2004).

The role of oxidative stress in the pathophysiology of CVD is well established (Griendling and FitzGerald, 2003). Oxidative stress is associated with obesity and insulin resistance, plays a causal role in the cardiac complications (Ritchie, 2009) and is implicated in diabetic cardiomyopathy (Bugger and Abel, 2010), congestive cardiomyopathy (Pankuweit et al., 2004), hypertensive heart disease and in other diabetic complications (Shahbaz et al., 2010). Excessive ROS and insulin resistance together play an active role as mediators of myocardial remodeling and cardiac dysfunction.

1.9.2. Sources of ROS in cardiac tissue

There are multiple sources of ROS in the cardiomyocyte including reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX), xanthine oxidase (XO) and mitochondrial electron transport
chain (ETC) (Muller et al., 2005). The primary factor governing mitochondrial ROS generation is the redox state of the ETC. Electrons mainly leaks from complexes I and III of the ETC under disease conditions cannot be dissipated by oxidative phosphorylation. This increases mitochondrial proton gradient resulting in the generation of incompletely reduced forms of oxygen particularly superoxide anion ($\text{O}_2^-$) (Brand et al., 2004).

The uncoupling proteins 1, 2 and 3 (UCP-1, UCP-2, and UCP-3) are members of the anion carrier proteins located in the inner membrane of mitochondria. UCPs play an important role in minimizing ROS production from the ETC. Both UCPs-2 and -3 are proposed to mildly uncouple respiration, allowing a more rapid electron flux, thus reducing membrane potential and ROS production.

Excessive concentration of ROS causes an inequity between mitochondrial uncoupling and lipid oxidation resulting in oxidative stress (Kim et al., 2008). UCPs -2 and -3 have been reported to be activated by ROS or ROS by-products to induce proton leak, thus providing a negative feedback loop for control of mitochondrial ROS production. UCP-2 knockout mice have elevated ROS production in macrophages (Arsenijevic et al., 2000) further supporting the role of UCPs in protecting against ROS production and oxidative damage. Strategies that provide cell protection from oxidative stress by enhancing UCP-2 expression have been successful. For example, overexpression of UCP-2 in cultured neonatal cardiomyocytes made these cells more resistant to oxidative stress and mitigated the detrimental loss of mitochondrial membrane potential upon exposure to hydrogen peroxide (Teshima et al., 2003).
1.10. Cardiac remodeling

Remodeling of the heart is derived from intra- and extracellular structural changes of the myocardium and elicits structural changes of the LV wall. Further, mechanical load on the LV wall causes LV dilation. Thus, the extent of LV remodeling could be a predictive factor for mortality and morbidity of a patient with myocardial infarction (MI) (Verma et al., 2008).

The underlying mechanism of LV remodeling is multifactorial and many biological reactions are involved in the time course of remodeling including 1) oxidative stress and inflammatory reactions in injured myocardium (Neri et al., 2013), 2) cardiodepressive reactions due to the production of reactive oxygen species (ROS) and inflammatory cytokines (Suematsu et al., 2003), 3) changes in extracellular matrix following activation of matrix metalloproteinases (MMPs) (Fan et al., 2012), 4) structural changes of myocardium in response to mechanical stress, and 5) synthesis of collagens and myocardial fibrosis (Fan et al., 2012). These reactive processes are related each other and proceed from acute reactions to chronic changes. LV remodeling ultimately elicits LV dilatation usually with LV dysfunction.

1.10.1. Cardiac hypertrophy

Myocardial hypertrophy is described as the increase of the cardiomyocyte size and number in response to stress conditions like increase of pressure and mechanical stretch. Myocardial hypertrophy has two subtypes: concentric hypertrophy and eccentric hypertrophy. Concentric hypertrophy is usually induced by pressure-overload and is characterized by significant increase of LV wall thickness, force generation, and impaired filling state due to stiffness (diastolic dysfunction). Eccentric hypertrophy is mainly caused by volume-overload and is characterized by moderate increase of wall thickness with impressive chamber dilation (systolic dysfunction) (Fig.4).
In the compensated state, an increase in diameter of cardiomyocytes and number of sarcomeres in parallel elevates the contractile force generated per cell to meet the required cardiac output. However, the compensatory increase of cardiac output cannot last indefinitely and will gradually proceed to life-threatening cardiac episodes, such as MI, arrhythmia, and HF.

 Decompensation could be due to (1) inadequate oxygen and nutrition supply (2) reduced myocardial capillary density, (3) reduced space for coronary artery vasodilatation and (4) capillary compression, which together make the hypertrophied cardiomyocytes more vulnerable to HF (Kleiner et al., 2006). Decompensation contributes to chamber dilation, reduced myocyte contractility and cardiac output.

Cardiac hypertrophy can be physiological or pathological. Physiological hypertrophy is reversible and occurs during maturation, pregnancy and exercise, without morbid effect on cardiac function (Nadal-Ginard et al., 2003). Pathological hypertrophy occurs following membrane bound receptor activation in response to pathological stress signals (e.g. neurohormonal activation, aortic stenosis, oxidative stress, in ÁDPPDWLRQ or cardiac injury). Initially, it may be adaptive but can proceed to decompensation and HF (Maillet et al., 2013). This is often associated with impaired myocardial vascularization, unfavorable changes in the extracellular matrix composition and fibrosis.

![Fig. 4 Physiological and pathological hypertrophic response to stimuli](image)
GLUT-4 deficiency has a causative role in the induction of cardiac hypertrophy. There is evidence that demand-driven and substrate-limited reduced GLUT-4 expression plays a pathological role in hypertrophic myocardium. When cardiac GLUT-4 expression levels were reduced below a ‘threshold’ level (<5% of that seen in wild type animals), a dramatic hypertrophy (85% increase in cardiac weight index) and fibrosis were evident (Kaczmarczyk et al., 2003). Furthermore, impaired Ca\textsuperscript{2+} homeostasis with sustained elevation of Ca\textsuperscript{2+} levels is known to constitute a growth induction signal in the cardiomyocyte (Barry et al., 2008).

1.10.2. Cardiac fibrosis

Cardiac fibrosis refers to the inappropriate proliferation of interstitial fibroblasts and associated deposition of matrix proteins. Under normal conditions, cardiac fibroblasts secrete the major protein components of the extracellular matrix (ECM), such as collagen, elastin, fibronectin and others which together provide structural support for the heart. When collagen secretion is in excess, it makes the ventricular wall stiffer which eventually leads to valvular heart disease (Walker et al., 2004). The fibrotic process is characterized by increased deposition of connective tissue and excessive deposition of collagen which results in the replacement of the normal tissue and functional decrement.

Cardiac fibroblasts are multifunctional cells that contribute to cardiac homeostasis by maintaining the matrix network. Moreover, fibroblasts are critically involved in remodeling through formation of replacement scar tissue with high collagen content in the injured myocardium (Maekawa et al., 2004). Extensive interstitial fibrosis formation leads to increase of ventricular stiffness, which impairs normal LV diastolic function and results in inadequate filling. Fibrosis is considered as the prominent myocardial structural change in hypertensive heart patients (Kuwahara et al., 2004).
ROS and cytokines like transforming growth factor-beta (TGF-β1) and IL-6 stimulate cardiac fibroblasts to proliferate, differentiate and synthesize ECM proteins (Yue et al., 2011). Buildup of matrix leaves the organ stiff and inflexible, unable to properly relax and function and hence leads to myocyte hypertrophy and diastolic and systolic ventricular dysfunction (Kuwahara et al., 2002).

1.10.2.1. TGF-β1

TGF-β1, a profibrotic cytokine involved in tissue repair and its continued production underlies the progression of fibrosis (Khan and Sheppard, 2006). TGF-β1 is synthesized by the myocytes and fibroblasts at the site of myocardial tissue injury. TGF-β1 increases matrix deposition especially collagen in order to repair tissue damage. However, in abnormal circumstances excessive production of collagen results in pathological scarring or fibrosis.

1.10.2.2. Collagen

Collagen is one of the most important structural proteins of the ECM that exhibit typical triple helical shape due to the spatial orientation of their 3 α-polypeptide chains. Fibroblasts are responsible for the biosynthesis of collagens-type I and type III in the heart (Kanekar et al., 1988). Fibrillar collagen serves as a structural framework for cardiomyocytes and the intramyocardial vasculature, imparts cardiac tissue with physical properties that include stiffness and resistance to deformation (Burlew and Weber, 2000). Furthermore, fibrillar collagen connects the contractile elements of adjacent cardiomyocytes and acts as a transducer of cardiac muscle contraction.

1.10.2.3. Alpha smooth muscle actin (α-SMA)

Myofibroblasts are metabolically and morphologically distinctive cells expressing alpha smooth muscle actin (α-SMA), an isoform of actin. α-SMA that predominates within vascular smooth cells is a highly preserved protein that provides mechanical support and shape for the
cell. It is one of the major components of contractile apparatus in the muscle cell that enable cell movements (Cherng et al., 2008).

In the activated state, myofibroblasts cease to proliferate and start to synthesize large amounts of extracellular component proteins. The expression of α-SMA correlates with the activation of myofibroblasts. Therefore, α-SMA is commonly used as a marker of myofibroblast transformation (Singh and Hall, 2008). Increased α-SMA expression reveals transition to fibrotic phenotype and is seen in fibrotic hearts subjected to pressure or volume overload (Hinz et al., 2001). Fig. 5 represents the fibrogenic process in the myocardium.

**Fig. 5** Fibrogenesis cascade in the myocardium. In response to a variety of stimuli, cardiac fibroblasts proliferate, differentiate and produce cytokines like TGF-β1 that in turn stimulate myofibroblast differentiation (Adopted and modified from Yue et al., 2011).
1.10.2.4. Matrix metalloproteinases (MMPs) in ECM remodeling

The MMPs are a family of Zn-dependent proteolytic enzymes that cleave structural proteins of the ECM (Spinale, 2007). The MMPs are regulated at both pre- and post-transcriptional levels. Post-transcriptional level includes regulation by substrate interaction, and endogenous physiological inhibitors called tissue inhibitors of metalloproteinases (TIMPs). TIMPs are a class of inhibitory proteins that regulate MMP activities. MMPs and TIMPs play an important role in cardiac repair and LV remodeling. A key process in LV enlargement is activation of MMPs in the ECM. Under normal conditions, the ratio of MMPs to TIMPs is tightly regulated to control myocardial proteolytic activity. In pathological situations, this balance is disturbed and the activated MMPs degrade the ECM, disrupting the fibrillar collagen network and cause alterations in collagen turnover and collagen content. It is reported that ROS mediate MMP induction or TIMP reduction causing collagen synthesis followed by changes in the structure of myocardial tissue (Siwki and Colucci, 2004).

1.11. Mitochondria – structure and function

Mitochondrion is a subcellular organelle that plays a critical role in cellular energy metabolism, contraction, Ca\(^{2+}\) homeostasis, ROS generation and apoptosis (McFalls et al., 2003; Crow and Mani et al., 2004). Mitochondria adopt different shapes depending on the cell type and the metabolic demands of the cell. It is composed of an outer and inner membrane, a narrow intermembrane space and a large inner cavity filled with fluid (matrix) (Sasaki, 2010). The outer membrane contains specialized transport proteins called porins that are relatively permeable to small molecules (< 5 kDa). The inner membrane is highly impermeable to small ions due to the high concentration of a phospholipid, cardiolipin. The inner membrane has inward folds called
cristae containing the respiratory chain complexes for the generation of ATP. ATP-production by oxidative phosphorylation involves the coordinated reactions of respiration chain complexes (Complex I, II, III, IV and V), which couple electron transfer between an electron donor (such as NADH) and an electron acceptor (such as oxygen) with the transfer of H^+ ions (protons) across the membrane (Sack, 2006). The cristae protrude into the central space of the mitochondrion, the matrix. The core of the mitochondria is the matrix that harbours the mitochondrial DNA (mtDNA). The matrix consists of numerous compounds such as proteins, lipid and nucleic acids.

Mitochondria have their own genome called mtDNA, which is a closed circular double-stranded DNA molecule of ~16.5 kb. There are two promoters in mtDNA (the light-strand and heavy-strand promoters) directing the production of 22 mitochondrial tRNAs, 2 mitochondrial rRNAs, and 13 proteins that constitute parts of the oxidative phosphorylation complex I, III, IV, and V.

1.12. Mitochondrial dysfunction

Mitochondria are the main source of energy in eukaryotic cells and are abundant in high-energy-requiring organs like the heart. Mitochondrial quality is precisely controlled by mitochondrial remodeling mechanisms including biogenesis and repair, dynamics, and mitophagy (Green, 2011). Disturbance in any of these mechanisms results in mitochondrial dysfunction which is widely seen in CVD. Considering the close relationship between workload and energy generation demand, cardiac hypertrophy will inevitably lead to alterations in mitochondrial function. Recent investigations suggest that mitochondrial dysfunction likely occurs in the hearts of humans with diabetes or insulin resistance and is tightly associated with the cardiac hypertrophy as well as hypertension (Puddu et al., 2007).
It has been documented that myocardial energetics attenuation and subsequent mitochondrial dysfunction mediates cardiac contractile dysfunction in obese subjects and makes them more susceptible to HF (Abel et al. 2008). Increased myocardial oxygen consumption, limiting ATP production, decreased mitochondrial oxidative capacity and reduced cardiac efficiency have been demonstrated in obese humans (Peterson et al., 2004) and animals (Mazumder et al., 2004).

1.13. Molecular mechanisms of mitochondrial dysfunction in diabetic myocardium

1.13.1. Impaired mitochondrial calcium homeostasis

Mitochondrial calcium is important for the activation of tricarboxylic acid (TCA) cycle and for increasing ATP production. \( \text{Ca}^{2+} \) coordinates ATP supply and demand for cardiomyocyte contraction. Impaired mitochondrial \( \text{Ca}^{2+} \) handling may compromise cardiac energy metabolism and contribute to the development of contractile dysfunction in diabetic hearts and is associated with impaired heart function (Belke et al., 2004). Altered \( \text{Ca}^{2+} \) homeostasis can result from decreased glycolysis, increased oxidative stress and decreased cardiac expression of sarcoplasmic reticulum calcium ATPase (SERCA2a) and \( \text{Na}^+/{\text{Ca}}^{2+} \) exchanger (NCX) as these are the main machineries responsible for removing \( \text{Ca}^{2+} \) from cytosol. Impaired sarcoplasmic-reticulum \( \text{Ca}^{2+} \) release or reduced re-uptake results in intramitochondrial \( \text{Ca}^{2+} \) accumulation during contraction. This reduces the attendant increase in dehydrogenase activation and ATP synthesis. Reduced ATP synthesis and lower cytosolic \( \text{Ca}^{2+} \) transients may both contribute to contractile dysfunction.

1.13.2. Impaired mitochondrial biogenesis

Mitochondrial biogenesis is a complex and dynamic process responsible for mitochondrial component synthesis and assembly. This process controls mitochondrial content/density and maintains energy
production and cardiac contraction. Mitochondrial biogenesis should be finely controlled to match cardiac growth and cardiac work. The process is driven through PGC-1α, a master regulator of energy metabolism. Transcription factors like nuclear respiratory factors (NRF)-1 and -2 and mitochondrial transcription factor A (TFAM) activate the expression of both nuclear and mitochondrial genes essential for mitochondrial biogenesis and are in turn regulated by PGC-1α (Wu et al., 1999).

1.13.2.1. Transcriptional control of mitochondrial biogenesis: the central role of PGC-1α

PGC-1α is the first discovered member of a family of transcriptional coactivators. The other two members are PGC-1β (also known as PERC) and PGC-1α related coactivator (PRC). Of these three proteins, PGC-1α exhibits a tissue-enriched expression pattern and is highly inducible by exercise, starvation or cold (Pugiserver et al., 2003). PGC-1α is now widely recognized as a master regulator/central mediator of both energy metabolism and mitochondrial biogenesis. Activated PGC-1α coactivates NRF1 and -2 and TFAM which in turn facilitate the production of new mitochondria in the heart and is involved in expression of nuclear-encoded subunits of the mitochondrial respiratory chain.

Changes in PGC-1α expression are likely to have a functional impact. Downregulation of PGC-1α and its target genes have been observed in a number of rodent models of HF raising the intriguing possibility that impaired mitochondrial biogenesis can be a causal mechanism for mitochondrial dysfunction in HF (Garnier et al., 2003; Arany et al., 2006) On the other hand, PGC-1α over expression causes uncontrolled mitochondrial proliferation, contractile dysfunction and dilated cardiomyopathy (Lehman et al., 2000), suggesting that a well-balanced expression of PGC-1α is necessary for optimal cardiac function.
PGC-1α activity is influenced by various posttranslational modifications. AMPK (Jager et al., 2007), p38 MAPK (Wright et al., 2007), and Akt (Li et al., 2007) phosphorylate PGC-1, modifying its stability and activity. Besides these phosphorylation sites, PGC-1 activity is also stimulated by deacetylation induced by the NAD⁺-dependent silence information regulator T1 (SIRT1) thereby induced mitochondrial biogenesis (Gerhart-Hines et al., 2007). Thus, the control of PGC-1α by AMPK and SIRT1 is suggestive of a link between mitochondrial biogenesis and metabolic signaling pathways.

1.13.2.2. Downstream targets of PGC-1α

NRF-1 and NRF-2 are the first identified nuclear transcription factors coactivated by PGC-1α and are implicated in the expression of multiple mitochondrial functions in vertebrates. NRF-1 and 2 have been linked to an increase in mitochondrial content indicating a role for these two in cardiac mitochondrial biogenesis (Finck and Kelly, 2007). Downregulated expression of NRF1/2 genes results in decreased expression of oxidative phosphorylation genes in prediabetic and diabetic muscle (Nisoli et al., 2007). NRFs induce expression of TFAM, an essential protein for the transcription and replication of mitochondrial genome. The TFAM promoter contains recognition sites for NRF-1 and/or NRF-2, thus allowing coordination between mitochondrial and nuclear activation during mitochondrial biogenesis. Cardiac-specific TFAM deletion results in decreased levels of mtDNA, impaired respiratory chain function, cardiac hypertrophy, and progressive cardiomyopathy (Hansson et al., 2004). Transcription and replication of mitochondrial DNA is thus driven by the nuclear-encoded TFAM induced by NRF-1 and -2.
1.13.3. Altered mitochondrial dynamics- fusion and fission imbalance

The number and morphology of mitochondria are regulated by two important processes namely mitochondrial fission and fusion. The change in mitochondrial number in diabetic hearts may reflect alterations in rates of mitochondrial fission and/or fusion events. In addition, mitochondrial fission and fusion have also been associated with mitochondrial fragmentation and apoptosis (Karbowski and Youle, 2003). Thus, an additional potential mechanism for mitochondrial impairment and contractile dysfunction may be altered mitochondrial fission/fusion machinery.

Fission and fusion processes are quality control mechanisms regulated by a series of GTPases: Mitochondria fusion is regulated by three large GTPase proteins: mitofusin (MFN1), mitofusin 2 (MFN2) and optic atrophy protein (OPA1) (Chen and Chan, 2005). MFN-1 and MFN-2 both localized in the outer mitochondrial membrane with the N-terminal (GTPase domain) and C-terminal (coiled-coil region) interacts with their homologous proteins in adjacent mitochondria to coordinate fusion (Chen et al., 2003). Fusion of the inner mitochondrial membrane is mediated by OPA1, a protein that faces the inter-membrane space (Meeusen et al., 2006). To complete mitochondrial fusion all the three proteins are required (Zorzano et al., 2010). For mitochondrial fission, two proteins are required. They are dynamin-related protein 1 (DRP1) and the mitochondrial outer membrane protein fission protein 1 (FIS1).

Fission/fusion imbalance can create small and fragmented mitochondria or fused mitochondria resulting in disorganization of the mitochondrial network leading to mitochondrial impairment, contractile dysfunction and apoptosis in diabetic heart (Yu et al., 2008) while defect in biogenesis results in reduced number of mitochondria (Morino et al., 2005). Therefore, a fine-tune regulation of mitochondrial biogenesis and dynamics is necessary to obtain and maintain functional mitochondria.
1.13.4. Mitochondria-mediated apoptosis

1.13.4.1. Role of cardiolipin

Cardiolipin, a highly specialized anionic phospholipid, representing 8–15% of the cardiac phospholipid mass is exclusively present in the inner mitochondrial membrane where it is synthesized (Paradies et al., 2009). It is an important player in the regulation of the mitochondrial phase of apoptotic process (Ostrander et al., 2001). Cardiolipin is bound to cytochrome c and changes in cardiolipin content or oxidative damage to the acyl chains of cardiolipin might alter the interaction between cytochrome c and cardiolipin. This leads to the detachment of cytochrome c from the mitochondrial inner membrane. Release of cytochrome c stimulates a series of events leading to caspases-mediated apoptosis (Iverson and Orrenius, 2004) Cardiolipin also serves as a mitochondrial target of BH3 interacting-domain death (Bid) agonist, a proapoptotic factor which promotes pore formation in the outer membrane. Cardiolipin, besides contributing to apoptosis, plays a key role in regulation of electron transport, mitochondrial bioenergetics and optimization of activities of inner membrane proteins engaged in oxidative phosphorylation and mitochondrial lipid and protein import (Houtkooper and Vaz, 2008).

1.13.4.2. Apoptosis

Apoptosis is a form of cell death leading to the elimination of cells without releasing harmful substances into the surrounding area. Apoptosis is a genetically determined process of cell self-destruction marked by shrinkage of the cell, condensation of chromatin and fragmentation into membrane-bound bodies that are eliminated by phagocytosis. The process contributes to both physiological and pathological processes (Elmore, 2007). The B-cell lymphoma 2 (BCL-2) family of proteins consisting of anti-apoptotic and pro-apoptotic
members in the mitochondria are the key regulators of apoptotic cell
death. The proapoptotic BCL-2 protein, BCL2-associated X protein
(BAX), resides in the cytosol and promotes apoptosis. The anti-apoptotic
protein BCL-2 inhibits apoptosis by several mechanisms-1) sequestering
death-driving cysteine proteases called caspases. 2) preventing the
release of mitochondrial apoptogenic factors such as cytochrome c and
apoptosis-inducing factor (AIF) into the cytoplasm and 3) reducing the
generation of reactive oxidants, which are requisite for the completion
of the apoptotic program (Cory and Adams, 2002). A shift in the
BAX/BCL-2 balance determines the susceptibility of a cell to apoptosis
(Yang et al., 2002).

Caspases are cysteine-dependent proteases that trigger the
executioning phase of apoptosis. Release of cytochrome c complexed
with apoptotic protease factor-1 (Apaf-1) activates caspase-9 which in
turn activates its downstream target caspase-3 to execute apoptosis.
Studies reported that the antiapoptotic factor BCL-2 is a downstream
death substrate for caspases and is inactivated by caspases (Yang et al.,
2002).

Emerging evidence suggest that high rate of apoptotic response is
associated with oxidative stress occurring in insulin resistance and T2D
(Yang and Peng, 2010). Williamson et al. (2010) reported evidence of
increased caspase activity in diabetic mice in association with changes
in mitochondrial membrane potential and mitochondrial membrane
transition pore (MPTP) opening. Studies have also noted changes in
oxidative protein damage and mitochondrial cardiolipin content, which
may alter mitochondrial membrane integrity and contribute to
mitochondrial-induced apoptosis (Dabkowski et al., 2010).
1.14. High fat, high fructose diet (HFFD)-an inducer of insulin resistance

Diet rich in fat and fructose represents the unhealthy human Western type diet and is used extensively by researchers to induce obesity, insulin resistance and the MS in experimental animals and humans. Furthermore, HFFD-induced model appears to be more relevant than the genetic and other nongenetic rodent models of the MS, since this diet can accelerate the development of cardiac dysfunction. Deng et al. (2007) demonstrated that feeding high-cholesterol/fructose for 15 weeks causes insulin resistance and impaired myocardial contractile performance. Rodents fed high carbohydrate and/or high fat diet for 8-16 weeks were observed to develop insulin resistance, hypertension, cardiac hypertrophy, increased cardiac stiffness, cardiac contractile dysfunction, cardiac inflammation and fibrosis, decreased cardiac function, cardiovascular remodeling, lipid accumulation, oxidative stress, depletion of antioxidants, and myocardial apoptosis (Ballal et al., 2010; Panchal et al., 2011; Huang et al., 2013). Thus, in this study, the HFFD-induced mice model of MS was employed to characterize the disease pathology and to investigate the disease-protective effect of TX.

1.15. Pharmacological interventions

Insulin resistance and MS are associated with metabolic risk and development of heart disease. Prevention and treatment are therefore of great importance. Preventive measures involving lifestyle recommendations (exercise, smoking cessation, weight loss) are the first line measures for MS-associated CVD management. Lifestyle changes that target dietary and exercise habits can reduce the progression of impaired glucose tolerance to fully developed T2D in subjects by up to 58% (Tuomilehto et al., 2001). However, the rapidly escalating number of patients with
T2D and MS strongly support the urgent need for pharmacological intervention, as well as the need to develop novel therapeutic approaches. In, particular, elderly patients with the MS require pharmacological treatment, usually for the remainder of their lives.

Clinical trials (Multiple Risk Factor Intervention Trial [MRFIT]) conducted ~350,000 men with and without diabetes for 12 years revealed that insulin resistance is an independent risk factor for CVD mortality. The presence of additional risk factors may confer a steeper rise in risk profile. This finding underlies the importance of addressing the multiple risk factors for CVD that are present in patients with MS. Thus, the American Diabetes Association (ADA) and the European Diabetes Policy Group strongly recommend that effective management of diabetes and CVD should address all the risk factors of MS in parallel.

Currently metabolic modulators and diabetic medications are the promising potential medical therapies for insulin resistance syndrome. Metabolic modulators like trimetazidine has been shown to increase glucose metabolism, decrease FFA metabolism, and potentially enhance myocardial contractile efficiency. Diabetic medications improve insulin sensitivity. For instance, metformin and thiazolidinediones serve as insulin sensitzers in patients with the insulin resistance. They reduce insulin resistance via activation of PPAR-γ, the transcription factor that promotes glucose uptake and insulin sensitivity but decreases circulating FFA (Hällsten et al., 2004). Glucagon-like peptide 1(GLP1), angiotensin converting enzyme (ACE) inhibitors, angiotensin-II type 1 receptor (AT1R) blockers, statins, lipid decreasing agents and recently Poly (ADP-ribose) polymerase (PARP) inhibitors are also recommended as therapeutics for diabetes associated CVD.
Chronic drug treatment may result in side effects like nausea and gastrointestinal disturbances which may affect the quality of life. For instance, metformin administration showed no improvement in myocardial glucose uptake and its use in HF patients is limited by its potential for lactic acidosis. Additionally, insulin secretagogues fail to address the underlying physiologic problem of insulin resistance and expose the patients to the potential negative effects of hyperinsulinemia. New and effective therapeutic interventions without or with minimal side effects are therefore required. This need can be accomplished by integrated approach of pharmacotherapy with natural substances.

The herbs themselves or their derivatives have been employed traditionally by native people in the treatment of diabetes, in the areas in which they grow. These include *Allium sativum, Gymnema sylvestre* and *Trigonella foenum-graecum*. Among others, all these herbal remedies are easily available and can provide a simpler, more natural way of controlling diabetes without any unpleasant side effects. Herbal products may contain several active constituents or compounds that can act by several modes of action to influence multiple biological pathways and to alleviate the diabetic symptoms, providing thereby multifaceted benefits (Kar *et al.*, 2003). Natural remedies are only alternative way that can save humans from harmful side effects of synthetic drugs.

Some of the natural products are found effective in treating metabolic diseases like insulin resistance and T2D and MS. One example is, resveratrol (trans-3,5,4'-trihydroxystibene), a naturally occurring polyphenol which is extracted from *Polygonum cuspidatum*. Resveratrol is present in blue berries, pea nuts, red grapes and wines. Studies have suggested that resveratrol prevented LVH, systolic dysfunction and hyperinsulinemia in high calorie diet-fed animals (Qin *et al.*, 2012). Quercetin, a flavonoid found in variety of plant based foods such as red onions, apples, tea, broccoli, capers, red grapes and berries which is
isolated from *Inflorescentia tiliae*. Similar to resveratrol, quercetin also reduced systolic blood pressure and improved cardiac function in rats fed high calorie diet (Panchal *et al.*, 2012). Rutin is a naturally occurring flavonol glycoside comprised of the flavonol quercetin and the disaccharide rutinose which is extracted from flower buds of the Chinese Scholar-tree, *Sophora japonica L.* (Fabaceae family). Rutin is present in tea, coffee, cereal grains and a variety of fruits and vegetables such as buckwheat, orange, grapefruit, lemon, berries, apples, broccoli and onion (Fan *et al.*, 2009). Rutin was found to improve metabolic profile and reduce changes in cardiovascular structure and function in high calorie diet fed animals (Panchal *et al.*, 2011).

### 1.16. Troxerutin

Troxerutin (TX) is a trihydroxyethylated derivative of the natural bioflavonoid rutin. Troxerutin is mainly constituted by monohydroxyethylrutosides (~5%), dihydroxyethylrutosides (~34%), trihydroxyethylrutosides (~46%) and tetrahydroxyethylrutosides (~5%). and are prepared by the hydroxyethylation of the phenolic groups of rutin Hydroxyethylrutosides (HR) refers to a mixture of semi-synthetic derivatives of the flavonoid rutin. Fig. 6 represents the structure of TX and rutin

![Molecular structures of TX (A) and rutin (B)](image)

**Fig. 6 Molecular structures of TX (A) and rutin (B)**
TX is available as a food supplement under many brand names such as troxevasin, troxeven, troxsal and venoruton in countries like Europe, Asia, New Zealand, Australia, and South America, both alone and in combination with other compounds. It is used as an anticoagulant, a radioprotective agent and for the treatment of chronic venous insufficiency (CVI), varicose veins, and hemorrhoids. Studies in humans and animals report that TX is safe and well tolerable. Consumption of 4 g/day for 6 months and 4 g/day by pregnant women showed no toxic side effects (Marhic, 1991; Wijayanegara et al., 1992).

About 10-15% of orally administered TX is absorbed and it is estimated that 30% is bound to plasma protein. The plasma concentration of TX and its metabolites (dihydroxy toluene and hydroxyl phenyl acetic acid) begin to increase from 4-8 hrs after administration, reaching the maximum level at 8-12 hrs. Half life of TX is reported to be 10-25 hrs (Wadworth et al., 1992). It is excreted in higher proportion in bile and lesser amount in urine (Wadworth et al., 1992).

1.16.1. Pharmacological actions of TX

Several studies have reported the pharmacological actions of TX pertaining mainly to its effect on blood vessels and hemodynamics. TX prevents thrombosis by inhibiting the coagulation of erythrocytes and thrombocytes, lowers blood viscosity and enhances the effective decoagulation rate of thrombocytes (Krupinski et al., 1996; Chen et al., 2011). TX increases the oxygen content in blood and the oxygen saturation and activates the breathing of cells (McEwan and McArdle, 1971). TX protects the endodermic cells of blood vessels by improving the resistance and density of capillary vessels, lowering their penetrating property and brittleness. By this it prevents dropsy caused by increase of penetrating of vessels, and alleviates the injuries caused to vessels by inflammatory substances (Belcaro et al., 1988; Boisseau et al., 1989; Glacet-Bernard et al., 1994; Gueguen-Duchesne et al., 1998).
TX improves microcirculation by the creation of new vessels and branch circulations and increase blood flow (Cesarone et al., 2003a; Cesarone et al., 2003b). TX is remarkably protective to excitotoxic brain injury in domoic acid-treated mice (Lu et al., 2013).

1.16.2. Biological effects of TX

Sufficient evidence has shown that TX has many biologic actions, such as antioxidative, anti-inflammatory, antierythrocytic, anti-fibrinolytic, anti-thrombotic, antineoplastic, and anti-radiation effects (Fan et al., 2009). TX is best suited for the treatment of the pre-varicose and varicose syndrome, varicose ulcers, chronic venous deficiency, and hemorrhoids. It exerts potent anti-varicose action by normalizing the permeability of vessels and by enhancing the capillary stability (Gueguen-Duchesne et al., 1998). TX can also be successfully applied for muscle pain and edemas due to traumatic vein and blood-flow disorders and hematomas.

1.16.3. Studies with TX

TX plays an important role in protection from D-galactose induced renal injury through its antioxidant and anti-inflammation properties (Fan et al., 2009). Studies report that TX has a protective role against high cholesterol-induced cognitive deficits in C57BL/6 strain mice and showed anti-diabetic activity (Lu et al., 2011). Supplementation of a high carbohydrate, high fat diet with rutin effectively attenuates or reverses the signs of MS, cardiovascular remodeling and non-alcoholic steatohepatitis in this rat model of diet-induced obesity and liver dysfunction (Panchal et al., 2011). Since TX has three more hydroxyl groups than rutin it can be envisaged that it has more beneficial effects than rutin.
Though there are a vast number of studies suggesting its pharmacological and disease-preventive properties, the mechanisms underlying the effects of TX on cardiac function has not been explored so far. This study was designed to evaluate the ability of TX to attenuate/reverse HFFD-induced adverse effects, in particular the components of MS, cardiac remodeling, mitochondrial dysfunction and apoptosis in HFFD-fed mice.