Mitochondrial Uncoupling: A Key Contributor to Reduced Cardiac Efficiency in Diabetes

Cardiovascular disease is the primary cause of death in individuals with obesity and diabetes. However, the underlying mechanisms for cardiac dysfunction are partially understood. Studies have suggested that altered cardiac metabolism may play a role. The diabetic heart is characterized by increased fatty acid oxidation, increased myocardial oxygen consumption, and reduced cardiac efficiency. Here, we review possible mechanisms for reduced cardiac efficiency in obesity and diabetes by focusing on the potential role of mitochondrial uncoupling.

Diabetes is a worldwide epidemic (112). Cardiovascular disease remains the major cause of morbidity and mortality in people with diabetes. Epidemiological studies suggest that the prevalence of heart failure exceeds that which can be accounted for by other risk factors such as hypertension and vascular disease (76). Despite these observations, the mechanisms for cardiac dysfunction in diabetes remain relatively obscure. Therefore, attention has focused on the role of altered substrate metabolism in the development of cardiac dysfunction in diabetes (10, 56). In diabetes and obesity, the proportion of cardiac energy that is derived from the oxidation of fatty acids (FAs) is increased and energy derived from glucose oxidation and glycolysis is reduced (77, 101). This results in increased myocardial oxygen consumption (MVO₂). However, cardiac work is either unchanged or reduced, thus cardiac efficiency (CE) (which is the ratio between cardiac work and MVO₂) is reduced in diabetic animals (10, 18) and in obese insulin-resistant humans (72). Therefore, it is important to understand the cellular mechanisms responsible for metabolic alterations and reduced CE in diabetes. This may allow the identification of potential targets for the prevention of these abnormalities. In this review, recent advances in our understanding of metabolic disturbances in animal models of obesity and Type 2 diabetes will be discussed.

Mechanisms for Cardiac Dysfunction in Diabetic Hearts

Cardiomyopathy develops in obesity and diabetes independently of additional risk factors such as coronary heart disease or hypertension (8, 36). Thus systolic and diastolic dysfunction can be detected in obese subjects and animals (3, 10, 89). Several mechanisms have been proposed to explain contractile dysfunction in obesity and diabetes. One mechanism involves increased myocardial apoptosis due to the lipotoxic injury (80). An imbalance between FA uptake and oxidation may result in the accumulation of excess lipid in the cardiomyocyte that leads to the production of toxic lipid intermediates that may precipitate apoptosis (55, 95, 111) and contribute to systolic dysfunction (23, 105). Studies in animal models with increased lipid accumulation in the heart have provided insight into the link between lipid deposition, cell death, and the development of cardiac dysfunction. For example, obese Zucker diabetic fatty (ZDF) rats exhibit increased levels of myocardial triglyceride (TG) that are associated with accumulation of lipid intermediates such as ceramide, activation of inducible NO synthase (iNOS), and increased apoptosis. Therapies that reduce intramyocardial lipid accumulation are associated with reversal of these changes and amelioration of cardiac dysfunction (111). More severe myocyte lipotoxic injury develops in mice overexpressing the long-chain acyl-CoA synthase (MHC-ACS). These mice also exhibit increased myocardial TG content and increased apoptosis and develop a progressive cardiomyopathy (23). It has been suggested that saturated FA (such as palmitic acid) might be a more potent inducer of apoptosis than unsaturated FA because they generate more toxic intermediates and are less efficiently incorporated into TG (55). In contrast, heart-specific overexpression of peroxisome proliferator-activated receptor (PPAR) α is associated with increased FA oxidation rates and upregulation of FA oxidation genes (38). Despite this, these mice exhibit myocardial TG accumulation that is exacerbated by a diet rich in long-chain FA (LCFA) (37). Although not proven, it is likely that lipid accumulation occurs because FA uptake exceeds mitochondrial FA oxidative capacity. It is also possible that some of the increased FAs following uptake could be preferentially shunted toward TG synthesis after interacting with specific isoforms of acyl CoA synthase. In diabetes, FA uptake is increased as a consequence of increased delivery of free FA (FFA) and increased FA transport...
Despite increased rates of FA oxidation, it is also likely that FA uptake may exceed oxidation, thereby leading to the accumulation of lipid in the heart, which will lead to lipotoxicity and cell death. Moreover, lipid accumulation may contribute to myocardial insulin resistance (69).

Additional mechanisms for cardiac dysfunction in diabetes exist. Increased FA oxidation that characterizes hearts of Type 1 and Type 2 diabetes (15, 45) is associated with increased production of reactive oxygen species (ROS) (107). In addition to impairing mitochondrial coupling (33), ROS can also oxidize lipids and proteins and other intracellular molecules such as NO to produce highly reactive products, which are damaging to the cell (20, 83, 106). Additional mechanisms implicated in the development of cardiac dysfunction in diabetes include increased activation of the renin-angiotensin system (40), stimulation of pro-inflammatory pathways (92), activation of protein kinases (103), and impaired calcium homeostasis (11). Changes in cardiac metabolism have also been proposed to play a potential role in the development of cardiac dysfunction in diabetes (91). Early studies on cardiac metabolism were conducted in Type 1 diabetes and showed that FA oxidation was increased and that carbohydrate use was decreased (56, 74, 88). In this review, we will focus on more recent observations regarding metabolic alterations in mouse models of Type 2 diabetes, obesity, and insulin resistance.

Substrate Metabolism in Type 2 Diabetic Hearts

Obesity and Type 2 diabetes often coexist and share many underlying pathophysiological mechanisms such as insulin resistance. Recent studies indicate that obesity, insulin resistance, and Type 2 diabetes are associated with disturbances in myocardial insulin signaling (29, 59, 68, 70) as well as glucose and FA utilization (10, 15, 59).

Decreased glucose utilization

Glucose enters the cardiomyocytes via facilitative glucose transporters, with GLUT1 and GLUT4 being quantitatively most important. Myocardial glucose transport is impaired in the diabetic heart (7, 66). It has long been known that the expression of GLUT1 and GLUT4 is reduced in the hearts of diabetics (2, 41). However, in some models, defective glucose utilization cannot be accounted for by reduced transporter expression as little or no reduction in heart GLUT4 content is observed in obese insulin-resistant db/db and ob/ob mice (51, 59). GLUT4 translocation has not been directly examined in ob/ob and db/db mouse hearts. However, insulin-mediated activation of Akt and insulin-mediated glucose uptake is markedly impaired in the cardiomyocytes of ob/ob mice (59). Insulin-mediated glucose uptake is also impaired in db/db mouse cardiomyocytes, although the degree of impairment in insulin-mediated Akt activation was minor (19). Impaired insulin-mediated GLUT4 translocation was also observed in cardiomyocytes of obese Zucker rats (96). Taken together, impaired insulin-mediated GLUT4 translocation is likely to contribute to reduced insulin-mediated glucose utilization in the heart. Insulin resistance may play additional roles in reducing glucose utilization in the heart. For example, mice with cardiomyocyte-restricted deletion of insulin receptors (CIRKO) have impaired glucose oxidation despite increased expression of GLUT4 (9). Myocardial insulin resistance is also associated with reduced glucose oxidation and glycolysis rates in ob/ob and db/db mice (15). Although reduced insulin-stimulated glucose uptake may contribute to decreased glucose utilization in these hearts, the existence of a fixed defect in basal rates of glucose oxidation in the absence of insulin signaling in CIRKO hearts and in association with severe insulin resistance in ob/ob hearts suggests that additional mechanisms for reduced myocardial glucose utilization may exist in the obese and insulin-resistant state. One mechanism could be reduced flux through pyruvate dehydrogenase (PDH), which has been described in the hearts of ZDF rats (22) and ob/ob mice (13). A second mechanism for reduced glucose oxidation in diabetes is reduced mitochondrial oxidative capacity, as suggested by the observations of generalized mitochondrial defects in the hearts of ob/ob mice (13).

Increased FA utilization

The sources of FA for the heart are FFA bound to albumin and TGs that are present in chylomicrons and very-low-density lipoproteins, both of which are elevated in the diabetic condition (1, 57, 65). FFAs are known ligands for PPARs. PPARα is an important transcriptional regulator of genes, whose products regulate FA metabolism in tissues with high FA oxidation rates, such as the heart. Studies using gain and loss of function strategies have shown that PPARα regulates genes involved in cardiac FA utilization including 1) FA uptake, 2) FA esterification, 3) FA transport into mitochondria, and 4) mitochondrial β-oxidation. Indeed, cardiac expression of genes involved in FA uptake (CD36/FAT and FATP), mitochondrial FA transport (CPT1 and CPT2), and mitochondrial β-oxidation (MCAD, LCAD, and VLCAD) are reduced in PPARα knockout mice (PPARα−/−) (6, 54). Conversely, transgenic mice overexpressing PPARα in the heart results in a metabolic phenotype of enhanced FA utilization that mimics the altered FA metabolism observed in Type 2 diabetic mice (38). In these animals, glucose oxidation is reduced as a result of increased expression of pyruvate dehydrogenase kinase (PDK), which phosphorylates PDH and inhibits its activity. Increased FA oxidation in diabetic hearts cannot be entirely accounted for by the activation of
Increased MVO$_2$ and reduced CE

Increased FA oxidation in the diabetic heart is associated with increased MVO$_2$. Numerous reports have shown that increasing FA oxidation leads to higher MVO$_2$ relative to hearts that are predominantly oxidizing glucose (27, 71, 99). Indeed, there is a calculated 12—14% increase in efficiency of ATP production when shifting from 100% palmitate to 100% glucose. This difference in efficiency between FA and glucose is more pronounced when ATP-to-O ratios of glucose and FA oxidation are compared (21, 43). Thus the increase in cardiac FA oxidation that occurs in diabetes might be energetically detrimental because of the higher oxygen cost to produce ATP. Increased MVO$_2$ and decreased CE have been reported in obese insulin-resistant and diabetic mouse models (13, 15, 48, 59) and in obese humans (72). In contrast, studies in the hearts of ZDF rats perfused with a mixed substrate (glucose, lactate, pyruvate, and palmitate) did not reveal increased MVO$_2$, despite increased FA oxidation (102). In an independent study using oleate as a substrate, FA oxidation rates and MVO$_2$ were both reduced in ZDF rats (108). The mechanisms for these divergent results are unclear but may represent changes that are unique to the ZDF model.

The mechanisms for the diabetes-associated increase in MVO$_2$ and reduction in CE observed in most studies are incompletely understood. In addition to increased FA uptake and oxidation, which will both increase MVO$_2$, oxygen may be also consumed by FA esterification and ROS production (61, 99, 100). The existence of futile cycles that waste ATP in the cell may also contribute to decreased CE. This includes the mitochondrial and the cytosolic thioesterases (MTE and CTE) as well as long-chain acyl-CoA synthase (ACSL) reactions involved in cycling fatty acyl moieties (47). Durgan et al. (31) recently demonstrated that CTE 1 expression was induced by streptozotocin treatment and high-fat feeding, two conditions that are known to increase FA oxidation. Finally, the increased MVO$_2$ that characterizes the diabetic heart could also reflect increased mitochondrial uncoupling, which will be discussed in more detail later in this review. In summary, diabetes is associated with profound changes in myocardial substrate utilization, and multiple mechanisms are responsible for these changes. Glucose utilization is reduced, FA utilization is increased, and increased FA utilization is associated with increased MVO$_2$ and reduced CE.

Mitochondrial Dysfunction in Diabetes

Pyruvate (derived from glucose and lactate) and FA are oxidized in mitochondria and account for the bulk of intracellular ATP generation (87, 90). Mitochondrial function was first investigated in the hearts of experimental animal models of Type 1 diabetes and demonstrated a reduction in the maximal rates of respiration (73, 79). Using a proteomic approach, Turko et al. (93, 94) demonstrated an increase in the tyrosine nitration of certain mitochondrial proteins such as subunits of complex I, mitochondrial superoxide dismutase (SOD2), and succinyl-CoA:3-oxoacid CoA transferase (SCOT). In addition, upregulation of β-oxidation proteins and a selective downregulation of OXPHOS and other mitochondrial proteins, such as creatine kinase, voltage-dependent anion channel 1, HSP60, and Grp75, were observed. Altered mitochondrial respiration and increased mitochondrial biogenesis have also been reported in a separate study (84). In contrast to Type 1 diabetes, only a few studies have examined mitochondrial function in Type 2 diabetes and obesity. Early studies described reduced palmitoyl-carnitine supported state 3 respiration and reduced pyruvate dehydrogenase activity in db/db hearts (52, 53). We recently observed increased mitochondrial biogenesis as evidenced by increased mitochondrial DNA copy number relative to nuclear DNA copy number in ob/ob hearts (FIGURE 1). Despite this increase in mitochondrial mass, ob/ob mitochondria exhibit a global defect in oxidative phosphorylation and reduced expression of complex I, III, and V of the electron transport chain (ETC) (13). In the face of a clear reduction in mitochondrial OXPHOS capacity of ob/ob, mitochondria and FA oxidation rates are increased in working ob/ob hearts perfused with glucose and palmitate. These data, therefore, raise the possibility of increased mitochondrial uncoupling in these hearts. Indeed, examination of mitochondria from ob/ob hearts is consistent with this hypothesis (increased state 4 respiration and reduced ATP-to-O ratios). In our studies, mitochondrial uncoupling in ob/ob hearts was not associated with increased protein levels of the uncoupling protein (UCP) 3 (13). These observations suggest that mito-
Mitochondrial uncoupling in these hearts may reflect allosteric activation of uncoupling mechanisms that are independent of changes in the expression of mitochondrial UCPs.

**Mitochondrial Uncoupling, Mediators, and Regulators**

Mitochondrial substrate oxidation results in the production of reducing equivalents (NADH and FADH$_2$) that enter the ETC and generate the electrochemical gradient across the mitochondrial membrane that is used to drive ATP synthesis. In perfectly coupled mitochondria, there would be no proton leak across the inner mitochondrial membrane, and the entire gradient generated by the respiratory chain would be used to generate ATP. However, it was observed that, in the absence of ADP, isolated mitochondria from several tissues continue to consume oxygen. Under these conditions, the potential energy of the proton gradient is not used to phosphorylate ADP to ATP. Thus the existence of proton transport across the mitochondrial inner membrane that is not coupled to ATP production (uncoupled respiration) clearly exists. UCPs represent a mechanism by which protons can re-enter the mitochondrial matrix bypassing ATP synthesis. In brown adipose tissue (BAT), this proton leak or uncoupled respiration is mediated by UCP1, which is abundantly expressed in this tissue and plays a role in thermogenesis in rodents (17, 50, 67). Apart from its role in nonshivering thermogenesis in BAT, the physiological significance of uncoupling is debated. In the last decade, other UCP homologs have been identified, based on sequence homology and mitochondrial location. UCP2 is expressed in most tissues (39, 42), UCP3 is mainly expressed in heart and skeletal muscle (12, 44, 97), and UCP4 and UCP5/BCMP1 are expressed in the brain (58, 78). Because of the 59 and 57% homology of UCP2 and UCP3 relative to UCP1, it has been proposed that they can also mediate proton leak. In support of this, overexpression of UCP2 in yeast leads to uncoupling of oxidative phosphorylation and decreased membrane potential (39, 42). Skeletal muscle induction of UCP3 also results in mitochondrial uncoupling (24). However, the possibility exist that these results could be artifacts resulting from overexpression (16). Deletion studies have provided additional insights. Thus skeletal muscle mitochondria lacking UCP3 were more coupled, suggesting an uncoupling activity for UCP3 in this tissue (98). Similarly, the rate of ATP synthesis was increased in UCP3KO mice (25), suggesting a role for UCP3 in the regulation of energy metabolism in muscle.

Uncoupling activity of UCP2 and UCP3 requires the presence of regulators, namely FA and purine nucleotides. Purine nucleotides (such as GDP) inhibit while FAs activate UCP2 and UCP3 when they are expressed in *E. coli* and reconstituted into liposomes.
important mechanism that seeks to reduce mitochondrial superoxide production, with the untoward consequence of reducing mitochondrial oxidative efficiency.

The heart expresses both UCP2 and UCP3 (13, 31, 35, 60, 64). However, their exact function in this organ is not well known. UCP3 is a PPARγ transcrip
tional target, and conditions associated with increased myocardial FA delivery are associated with increased UCP3 gene expression. Thus UCP2 and UCP3 protein expression is reduced in the hearts of PPARγ-null mice, whereas UCP3 protein expression is increased in the hearts of streptozotocin-treated mice, and both UCP2 and 3 protein content is elevated in db/db hearts (64). Conversely, UCP3 expression is reduced in the hypoxic heart, which predominantly uses glucose as substrate (35). Recently, UCP2 and UCP3 were shown to play an important role in antioxidant defenses after ischemia-reperfusion injury in the heart (60).

In addition to UCPs, the adenine nucleotide translocator (ANT) is a second candidate that may modulate mitochondrial energy efficiency. This protein was shown to mediate uncoupling by FA and to lower mitochondrial membrane potential when superoxide is generated. In contrast, in isolated mitochondria from BAT and skeletal muscle of SOD2 transgenic mice, neither UCP1 nor UCP3 activities, respectively, appear to be affected by reduced production of superoxide, despite increased oxidative capacity (85). Taken together, it might be possible to reconcile these observations by speculating that, under physiological conditions when superoxide generation is relatively low, there is little effect on UCPs. However, under pathophysiological conditions where FA flux and superoxide generation is increased, activation of mitochondrial uncoupling may represent an important mechanism that seeks to reduce mitochondrial superoxide production, with the untoward consequence of reducing mitochondrial oxidative efficiency.

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skeletal muscle (75, 86). In rodents, two isoforms of ANT are expressed in the heart (ANT1 and ANT2), with ANT1 being the main isofrom of the adult heart (30). It has long been postulated that the uncoupling effect of LCFA (104) involves ANT. This protein is involved in the back transport of deprotonated LCFA from the matrix to the intermembrane space (4, 5). Oleate- and palmitate-induced uncoupling is inhibited by carboxyatractyloside (CAT) in rat hearts, and this CAT sensitivity correlated with ANT content (82). Indeed, in a recent study by Brand et al. (14), proton conductance of muscle mitochondria was decreased by 50% in ANT1-deficient mice. Interestingly, 4-hydroxynonenal-induced proton leak is inhibited by CAT in heart mitochondria but not by GDP, again implicating ANT as a major mediator of FA-induced uncoupling in the heart (32). In summary, UCPs (UCP2 and UCP3) are expressed in the heart and are activated by ROS, lipid peroxidation products, and FAs. Moreover, UCP expression is increased in some models of diabetes. ANTs also exhibit inducible uncoupling activity. Thus activation of uncoupling mechanisms would be expected to increase mitochondrial oxygen consumption in the face of increased FA utilization, thereby contributing importantly to altered substrate utilization and decreased myocardial efficiency in diabetes.

Mitochondrial Uncoupling and Cardiac Dysfunction in Diabetes

A study performed in heart failure patients demonstrated a positive correlation between plasma FFA concentrations and UCP2 and UCP3 expression in the heart and an inverse correlation with GLUT4 (63), leading to the hypothesis that mitochondrial uncoupling and reduced GLUT4 expression may contribute to altered energetics in the failing heart. Although UCP3 mRNA is increased in the hearts of streptozotocin-treated rats likely as a result of increased FFA concentrations and activation of PPARα, UCP3 mRNA expression is unchanged in the hearts of Zucker fatty fa/ra rats despite higher FFA concentration (46). In our studies, we observed that FA-induced uncoupling is increased in the absence of any changes in UCP3 protein content in ob/ob (13) and db/db mice (unpublished data). In both strains, perfusion of hearts with FA increases MVO₂ and decreases CE. In parallel, mitochondrial oxygen consumption rates are increased and ATP production is compromised following perfusion of hearts with FA (13, 15, 59). These observations provide strong evidence that FA-mediated activation of uncoupling mechanisms contributes to reducing CE in diabetic mouse hearts. These observations are also consistent with activation of UCPs and/or ANT leading to mitochondrial uncoupling despite the lack of any change in their expression. In addition to increased FA availability and utilization, other potential activators of uncoupling in the diabetic heart include increased superoxide generation. Indeed, we have observed increased hydrogen peroxide production and lipid peroxidation products in db/db mouse hearts (unpublished data). ROS or the lipid peroxidation products (increased in diabetes) activate UCP proteins and/or ANT to limit the production of ROS by reducing the mitochondrial membrane potential (32). These UCPs also rid the mitochondrial matrix of FA anions, which are also increased in the diabetic state. Thus our recent findings of increased MVO₂ and decreased CE in ob/ob and db/db mice and independent confirmation by How et al. (48) lead us to conclude that mitochondrial uncoupling likely plays a major role in the development of cardiac dysfunction in obesity and Type 2 diabetes. Recent human studies demonstrating reduced PCr-to-ATP ratios and circulating FFA concentrations are also consistent with this hypothesis (81).

Proposed Model

In this review, we have discussed potential mechanisms for reduced CE in the diabetic heart. We propose that activation of UCP3 and ANT by ROS and lipid peroxidation products (both of which are increased in obese Type 2 diabetic animals) induces an increase in FA-mediated proton leak. UCP- and/or ANT-mediated uncoupling contribute to reduced ATP synthesis and availability in the heart while increasing FA oxidation and O₂ consumption. The net effect is reduced efficiency of the diabetic heart (FIGURE 2). Impaired mitochondrial energetics may limit myocardial energetic reserves that we hypothesize will increase the susceptibility of diabetic hearts to failure.

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