Mitochondrial NAD\(^+\)/NADH Redox State and Diabetic Cardiomyopathy

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Abstract

Significance: Diabetic cardiomyopathy (DCM) is a frequent complication occurring even in well-controlled asymptomatic diabetic patients, and it may advance to heart failure (HF).

Recent Advances: The diabetic heart is characterized by a state of “metabolic rigidity” involving enhanced rates of fatty acid uptake and mitochondrial oxidation as the predominant energy source, and it exhibits mitochondrial electron transport chain defects. These alterations promote redox state changes evidenced by a decreased NAD\(^+\)/NADH ratio associated with an increase in acetyl-CoA/CoA ratio. NAD\(^+\) is a co-substrate for deacetylases, sirtuins, and a critical molecule in metabolism and redox signaling; whereas acetyl-CoA promotes protein lysine acetylation, affecting mitochondrial integrity and causing epigenetic changes.

Critical Issues: DCM lacks specific therapies with treatment only in later disease stages using standard, palliative HF interventions. Traditional therapy targeting neurohormonal signaling and hemodynamics failed to improve mortality rates. Though mitochondrial redox state changes occur in the heart with obesity and diabetes, how the mitochondrial NAD\(^+\)/NADH redox couple connects the remodeled energy metabolism with mitochondrial and cytosolic antioxidant defense and nuclear epigenetic changes remains to be determined. Mitochondrial therapies targeting the mitochondrial NAD\(^+\)/NADH redox ratio may alleviate cardiac dysfunction.

Future Directions: Specific therapies must be supported by an optimal understanding of changes in mitochondrial redox state and how it influences other cellular compartments; this field has begun to surface as a therapeutic target for the diabetic heart. We propose an approach based on an alternate mitochondrial electron transport that normalizes the mitochondrial redox state and improves cardiac function in diabetes. Antioxid. Redox Signal. 30, 375–398.

Keywords: NAD, NADH, redox balance, mitochondria, diabetes, cardiomyopathy

Diabetes and the Heart

Clinical overview of diabetes mellitus incidence and etiology

Diabetes mellitus (DM) has been a rapidly growing health epidemic worldwide (47, 53). As a chronic metabolic disease resulting from either autoimmune destruction of the insulin-secreting pancreatic \(\beta\) cells (type 1 DM, T1D) or impaired insulin secretion secondary to systemic insulin resistance (type 2 DM, T2D), DM culminates in chronic hyperglycemia and dyslipidemia (8). Although historically considered separate conditions, contemporary evaluation of T1D and T2D suggests a significant overlap in presentation, complications, and health outcomes, particularly with respect to the heart (8).

The heart as a primary target of DM pathology

Cardiovascular disease is the leading cause of morbidity and mortality in DM patients. There is an appreciable contribution to DM cardiovascular pathology from atherosclerotic...
disease; however, this review will emphasize the effect of the diabetic milieu directly on the myocardium through processes that promote myocardial dysfunction. Disease pathology in the myocardium is indicated by the presence of a unique clinical entity, diabetic cardiomyopathy (DCM), which was first documented in the 1970's in a small DM patient cohort for which no attributable origin could be defined (159). This pathological condition garnered support as clinically important by epidemiological data in the Framingham study establishing a link between DM and heart failure (HF), which demonstrated DM as an independent risk factor for cardiac events when correcting for comorbidities such as hypertension, coronary artery disease, and dyslipidemia (99). Indeed, DCM develops in a large proportion of well-controlled DM patients, emphasizing the need for investigation into the underlying molecular events driving disease and development of targeted therapies.

Since its initial observation, DCM has been further characterized, and it is typically indicated by a number of morphological and molecular changes (summarized in Fig. 1). Gross structural aspects of DCM include an increase in left ventricle (LV) mass and wall thickness. Histological examination reveals interstitial fibrosis, and cardiomyocyte rarefaction and hypertrophy (66). Current clinical diagnosis of DCM relies on non-invasive imaging, that is, echocardiography or MRI. An early decline in diastolic function (ventricular filling/relaxation) often occurs without detectable changes in ejection fraction (diastolic HF with preserved ejection fraction) in both human subjects (95) and animal models (55). This subclinical diastolic dysfunction in DM patients is also evident by analysis of blood flow velocities at the LV valves using Doppler echocardiography, demonstrating prolonged filling times and rates (7, 166). As diastolic function persists, systolic dysfunction may develop and evolve into ultimately HF with reduced ejection fraction, an end-stage condition without specific therapeutic approaches.

The underlying mechanism that initiates the pathology and leads to compromised cardiac function has yet to be fully understood. In addition to the derangement of circulating substrates, metabolic changes in the myocardium are apparent very early in DM, preceding compromised pump function and thus pointing toward the crucial pathogenic role of energy metabolism.

**Energy Metabolism in the Diabetic Heart**

Optimal contractile function depends on continuous mitochondrial oxidative metabolism to form the ATP needed for cardiac function. Unfortunately, in DM, mitochondrial function is compromised, leading to decreased ATP production and increased ROS generation.

FIG. 1. Morphological and metabolic features of diabetic cardiomyopathy. Diabetic cardiomyopathy is defined by the presence of an initial decreased myocardial diastolic dysfunction [heart failure with preserved ejection fraction (55)] in the absence of diabetes-induced standard cardiac risk factors, and may evolve to systolic dysfunction (heart failure with reduced ejection fraction) and congestive heart failure. These functional abnormalities are progressively induced by thickness and stiffness of the ventricular wall (defect in cardiomyocyte relaxation, interstitial fibrosis, and cardiomyocyte hypertrophy), cardiomyocyte death, and eventually contractile dysfunction. The heart relies on a large and constant energy supplied by oxidative metabolism. The normal heart is free to switch fuel substrates for oxidation and ATP synthesis to respond to energy demands, whereas the diabetic heart is insulin resistant and metabolically inflexible experiencing a decrease in glucose oxidation, and an increase in FA oxidation.

Diabetic cardiomyopathy is considered a disease of the myocardial energetic metabolism characterized by a decreased efficiency of oxygen utilization induced by mitochondrial dysfunction. Healthy mitochondria release minimal ROS levels that are compatible with the physiological signaling, whereas the ROS flux released by diabetic cardiac mitochondria is reported to be increased by most studies. Other critical processes that are interrelated with mitochondrial failure are changes in the redox environment (described in this review) and Ca²⁺ handling (not shown in the picture). FA, fatty acids; LV, left ventricle; ROS, reactive oxygen species.
daily to sustain the cardiac output. To accomplish this energetic mission, cardiac mitochondria transform the chemical energy stored in fuel substrates (fatty acids [FA], glucose, lactate, ketone bodies, and amino acids) into ATP through oxidative phosphorylation (Fig. 2). The normal adult heart obtains 60% of energy from FA oxidation with the remaining 40% originating from the oxidation of other fuel substrates, and it has the flexibility to switch substrates as energy sources depending on physiological conditions (55). This characteristic is vital for the ability of the normal heart to respond to the energy demand as energetic substrates have different energy efficiency, which is defined as the amount of ATP produced for the amount of oxygen consumed, and assessed by the P/O ratio. FA oxidation generates the greatest ATP yield by using the highest amount of oxygen (P/O ~ 2.3). Glucose is the most efficient energy substrate with a P/O ratio of 2.58. (55). The oxidation of β-hydroxybutyrate (βHB) has an intermediate efficiency with a P/O ~ 2.5. βHB is oxidized by the normal heart in proportion to its availability at the expense of FA and glucose (147); the diabetic heart acquires the ability to shift the acetyl-CoA toward ketone body synthesis, a characteristic of the fetal heart (48). Glucose oxidation is required for normal cardiac metabolism as a decrease induces diastolic dysfunction (1, 181). A reversal back to a fetal metabolic state with overreliance on glucose oxidation and decreased FA oxidation occurs in the failing heart, and is associated with a state of “energy starvation” as glucose, although a low oxygen-consuming substrate, is also a low ATP-yield fuel (per mole). Conversely, an excessive dependence on FA oxidation occurs in the diabetic heart, which supports the central postulate to explain the negative impact of metabolic inflexibility on cardiac function in diabetes.

**Alterations in the cardiac metabolic profile: changes in substrate utilization**

There is an increased FA utilization in the hearts of asymptomatic diabetic human subjects, suggesting that changes in mitochondrial metabolism precede cardiac pathology and are detrimental to the heart (82). Insulin resistance is not the primary mechanism for this metabolic switch in models of insulin resistance and obesity (32). Cardiomyocyte-specific deletion of the insulin receptor leads to decreased insulin-stimulated glucose uptake/oxidation and decreased FA oxidation but only modest cardiac dysfunction in the absence of systemic insulin resistance (19). This work suggests that although cardiac insulin resistance is an important event over the course of obesity-T2D, increased FA availability to the myocardium induced by systemic insulin resistance is required to trigger cardiac dysfunction in DM.

The allosteric activation of FA uptake and mitochondrial oxidation precedes the transcriptional regulation of metabolic remodeling. Hyperlipidemia, common in obesity and DM, arises from both diet and adipose lipolysis that increases circulating FA. Excessive FA cardiac uptake is observed in rodent models of T1D (122), obesity (138), and T2D (40) indicating that cardiac FA utilization is largely driven by circulating lipid availability. Once inside cardiomyocytes, FA are esterified to fatty acyl-CoA esters and transported into mitochondria to be oxidized as energetic fuel (Fig. 2), translocate to the nucleus, or undergo esterification to triglycerides (TG) for cytosolic storage (105). Within the nucleus, FA activate transcription factors such as the nuclear peroxisome proliferator-activated receptor-alpha (PPARα) to further promote mitochondrial FA oxidation, and PPARγ coactivator 1α (PGC-1α) to improve mitochondrial oxidative capacity through a coordinated transcriptional program that drives metabolic remodeling (59, 112). PPARα increases the expression of genes that promote FA utilization and inhibit glucose oxidation (59, 73); its expression is increased in several rodent models of DCM (65), and its cardiac-specific overexpression phenocopies the diabetic heart (65). Despite increased FA utilization, there remains an excessive intramyocardial TG accumulation leading to incompletely oxidized and potentially detrimental lipid metabolites (29, 110) that contributes to cardiac damage. Which one of these two mechanisms of “lipotoxicity” is prevalent is not known. Strong evidence in favor of the detrimental effect of overreliance on mitochondrial FA oxidation for ATP originates from studies showing that increased FA oxidation comes with a higher oxygen cost, thus reducing cardiac efficiency (cardiac contractile force as a function of oxygen consumed) in obese (28), insulin-resistant (32), T1D (90), and T2D diabetic rodents (90).

Despite the detrimental role of excessive FA in cardiac function in DM, there is intriguing evidence of an acute beneficial effect of palmitate on contractile function in hearts from diabetic rodents exposed to high glucose (24, 63) and adrenergic stimulation to mimic the diabetic heart during stress (188). Diabetic hearts efficiently oxidized the excess of FA and developed a higher contractile force whereas palmitate was detrimental on normal hearts, consistent with the notion that increased FA availability to the normal heart may trigger cardiac pathology. The short-term benefit of FA on the diabetic heart is further addressed in subsequent sections.

**Mitochondrial electron transport chain defects in the diabetic heart**

Measuring the cardiac high-energy phosphate metabolites such as phosphocreatine/ATP ratio (PCr/ATP) as an indirect assessment of oxidative phosphorylation shows that DM patients with normal cardiac function have a decreased PCr/ATP that is negatively correlated with circulating FA (168). Oxidative phosphorylation rates of mitochondria from atrial tissue of T2D patients are decreased (9). More recently, an impaired mitochondrial function with cardiac contractile dysfunction was reported in diabetic patients but not in “metabolically healthy” obese patients (125), suggesting that mitochondrial dysfunction is central to developing cardiac dysfunction in the transition from obesity to DM.

Despite the lack of consensus regarding the specific mitochondrial electron transport chain (ETC) site defective in the DM heart in both human subjects (Table 1) and animal models (Table 2), heart mitochondria are very susceptible to DM-induced ETC dysfunction in comparison with kidney and liver mitochondria (34). The impact of ETC defect on limiting FA oxidation in the DM heart is also unclear. Mitochondrial palmitoylcarnitine oxidation was decreased in cardiac samples from T2D subjects (9), suggesting that the ETC defects limit FA oxidation. In contrast, in rodent models of DM, increased FA oxidation is not limited by defects in complexes I (192) and III (27). Intriguingly, complex I inhibition with rotenone led to a metabolic shift to increase FA oxidation and glutamate utilization to compensate for the impaired energy production (199).
FIG. 2. Metabolism of fuel substrates drives the levels of reduced equivalents (NADH and NADPH) in normal cardiomyocytes. Normal adult heart obtains 40% of energy from metabolism of glucose, lactate, and ketones (mainly βHB), with the remaining 60% delivered from FA oxidation (55). Glucose uptake into cardiomyocytes is insulin dependent, whereas FA and βHB uptake is not hormonally regulated, and is driven by their bloodstream availability (FAcirc, βHBcirc) (17, 147). Glucose enters cardiomyocytes predominantly via the insulin-dependent glucose transporter 4 (GLUT4), and it follows multiple metabolic pathways including glycolysis, glycogen synthesis, and polyol pathway (with sorbitol and fructose formation), or is shuttled into the hexosamine biosynthetic or pentose phosphate pathways (with 6-Phosphogluconate, 6-PG, and ribulose 5-phosphate formation). Pyruvate is either converted to lactate or transported into mitochondria via the MPC, and converted by PDH to acetyl-CoA (AcCoA) for the TCA cycle. PDH is inhibited by pyruvate kinase (PDK4) that is activated by an excess of AcCoA and NADH. For simplicity, fluxes through glycogen synthesis and hexosamine pathways are not shown. After entry into cardiomyocytes (via CD36 and FA translocase, FAT), long chain FA are activated to FA-CoA that is either esterified as triacylglycerol (stored in the cytosol, not shown) or enters the mitochondria via carnitine palmitoyltransferases (CPT1 and 2), and they are oxidized via FA β-oxidation. The end products of each FA β-oxidation cycle are NADH, FADH2, and acetyl-CoA, which are further oxidized by ETC complexes or TCA, respectively, ultimately leading to ATP synthesis via mitochondrial oxidative phosphorylation. FA β-oxidation is controlled at different steps, including the inhibitory effect of malonylCoA (formed from AcCoA via AcCoA carboxylase, ACC), FADH2/FAD+ and NADH/NAD+ redox ratios, and acetyl-CoA/CoA ratio, all of which are unfavorable to FA oxidation. MalonylCoA is degraded by MCD, thus releasing its inhibitory effect on CPT1. The control of FA β-oxidation provided by post-translational modifications of β-oxidation enzymes and their transcriptional regulation is not depicted in the figure. GDH converts glutamate to α-ketoglutarate that is coupled to either NAD+ or NADPH reduction. Mitochondrial isoforms of MEs also can reduce NADP+ to NADPH. Mitochondrial oxidative phosphorylation provides more than 95% of the cardiac ATP (55), with the remainder derived from glycolysis. Electrons are transferred from the reducing equivalents, NADH and FADH2, to oxygen by the ETC complexes, whereas an electrochemical gradient is built across the mitochondrial IM, which is used by the ATP synthase (complex V) to form ATP. Mitochondria-generated ATP is transferred to the cytosol by the mitochondrial and cytosolic creatine kinases (CK) for contractile apparatus, sarcoplasmic reticulum Ca2+-ATPase, and other ion pumps. Components of the contractile apparatus and calcium handling are affected by oxidative damage (9, 10), supporting a close link between mitochondrial bioenergetics, redox state, and myocardial contraction. The inset represents an electron micrograph of cardiac muscle showing mouse interfibrillar mitochondria. For simplicity, the nicotinamide nucleotide transhydrogenase, a mitochondrial IM enzyme that reduces NADPH by oxidizing NADH and using the mitochondrial proton motive force, is not shown in this figure. The reduced NADH and NADPH are shown in red. βHB, β-hydroxybutyrate; CPT1, carnitine palmitoyltransferase 1; ETC, electron transport chain; GDH, glutamate dehydrogenase; IM, inner membrane; MCD, malonylCoA decarboxylase; ME, malic enzyme; MPC, mitochondrial pyruvate carrier; PDH, pyruvate dehydrogenase; TCA, tricarboxylic acid cycle.
Will the increase in mitochondrial FA oxidation efficiently maintain a positive cardiac energy balance? Part of the electrochemical proton gradient generated by FA oxidation, usually used to generate ATP, is reported to be consumed in T2D (27) and some (111, 192) but not all (33, 81) T1D models to generate heat as a result of mitochondrial uncoupling, and did not match a parallel increase in cardiac contractile force leading to cardiac inefficiency. The data suggest that in some models of diabetes the heart may become energy starved.

### Table 1. Bioenergetic Impairment in the Hearts of Human Subjects with Obesity and T2D

| Tissue/sample analyzed | Type of bioenergetic impairment | Ref. |
|------------------------|---------------------------------|------|
| Atrial fibers          | ↓ Respiratory state 3 rate (with glutamate and palmitoylcarnitine, but not with pyruvate and succinate) in permeabilized atrial fibers from T2D patients with coronary artery bypass graft surgery | (9)  |
| Atrial fibers          | ↓ Respiratory state 3 rate (with glutamate/malate and succinate) in permeabilized atrial fibers from T2D patients with coronary artery bypass graft surgery | (57) |
| Atrial fibers          | ↓ Respiratory state 3 rate and RCR (with palmitoylcarnitine and pyruvate/malate) in permeabilized fibers from obese and T2D patients | (125) |
| Atrial fibers          | Complex I activity in obese patients |  |
| Atrial fibers          | Complex I and III activities in obese T2D patients |  |
| Atrial mitochondria    | Respiratory state 3 rate (with glutamate/malate and palmitoylcarnitine) | (52) |
| Atrial mitochondria    | Complex I and IV activities in subsarcolemmal mitochondria from atrial appendages in T2D patients |  |

↓, decreased; RCR, respiratory control ratio (state 3, ADP induced/state 4, ADP limited); T2D, type 2 diabetes mellitus.

### Table 2. Bioenergetic Impairment in the Hearts of T1D and T2D Animal Models

| Type of diabetes   | Type of bioenergetic impairment | Ref. |
|--------------------|---------------------------------|------|
| Heart tissue, LV fibers, and isolated mitochondria T1D | ↓ Respiratory state 3 rate (with succinate and TMPD + ascorbate, but not with glutamate/malate) in cardiac mitochondria isolated from guinea pigs with STZ-induced T1D | (189) |
|                   | ↓ Respiratory state 3 rate (with pyruvate/malate) and RCR in cardiac mitochondria isolated from OVE26 mice with T1D | (173) |
|                   | ↓ Respiratory state 3 rate (with pyruvate/malate) in cardiac mitochondria isolated from rats with STZ-induced T1D; rates were improved with insulin treatment | (67)  |
|                   | ↓ Respiratory state 3 rate (with glutamate and succinate) | (192) |
|                   | ↓ Complex I and II activities |  |
|                   | FA oxidation is not limited |  |
|                   | ↓ Respiratory state 4 rate (with succinate), ↔ ATP in cardiac mitochondria isolated from rats with STZ-induced T1D | (81)  |
|                   | ↓ Respiratory state 3 rate (with glutamate/malate and succinate), improved after insulin treatment | (111) |
|                   | ↓ Complex I and II activities in cardiac mitochondria isolated from rats with STZ-induced T1DM | (33)  |
|                   | ↓ Respiratory state 3 rate (with glutamate/malate and pyruvate/malate, but not with palmitoylcarnitine) in LV fibers from Akita mice with T1D | (111) |
|                   | ↓ Respiratory state 3 rate (with glutamate/malate and succinate) that was improved after insulin treatment |  |
|                   | ↓ Complex I and II activities in cardiac mitochondria isolated from rats with STZ-induced T1D |  |
|                   | ↓ Respiratory state 3 rate (with glutamate/malate and palmitoylcarnitine) in SSM (but not in IFM) | (54)  |
|                   | ↓ Complexes I, III, IV, and V in SSM (but not in IFM) in cardiac mitochondria isolated from db/db mice |  |
|                   | ↓ Respiratory state 3 rate (with glutamate/malate, pyruvate/malate, palmitoylcarnitine) | (28)  |
|                   | ↓ Respiratory state 4 rate (with palmitoylcarnitine), ↓ ATP due to FA-induced uncoupling in LV fibers from db/db mice | (188) |
|                   | RCR (with glutamate/malate, succinate, TMPD) in cardiac mitochondria isolated from db/db mice with T2D |  |
|                   | ↓ Respiratory state 3 rate (with glutamate/malate, pyruvate/malate, and palmitoylcarnitine), ↓ ATP in LV fibers from ob/ob mice | (27)  |
|                   | ↔ Respiratory state 3 rate, ↑ respiratory state 4 rate (with palmitoylcarnitine), ↓ ATP due to mitochondrial uncoupling in LV fibers from db/db mice | (93)  |
|                   | ↓ Complex I and IV activities in LV tissue from ZDF rats | (151) |

↓, decreased; ↔, unchanged; LV, left ventricle; IFM, interfibrillar mitochondria (between the myofibrilles); TMPD, N,N,N’,N’-tetramethyl-p-phenylenediamine; RCR, respiratory control ratio (state 3, ADP induced/state 4 ADP limited); STZ, streptozotocin; SSM, subsarcolemmal mitochondria (beneath the sarcolemma); T1D, type 1 diabetes mellitus.
Consequences of Mitochondrial Metabolic Changes on Critical Pathogenic Mechanisms of DCM

Oxidative stress

Two major features of DCM are particularly sensitive to antioxidant therapy, namely cardiac hypertrophy (130) and fibrosis (205), highlighting the role of oxidative stress as a critical pathogenic mechanism for DCM (174, 201). Multiple sources contribute to reactive oxygen species (ROS) in the obese and DM heart, including NADPH oxidases (92, 171), uncoupled nitric oxide synthase (62), monoamine oxidase in both humans (57) and animal models (180), and ETC. The classical concept is that the univalent reduction of oxygen to form superoxide occurs with increased electron pressure at specific ETC sites caused by interruption of the electron flow in ETC defects. A novel mechanism of increased oxidative stress in the diabetic heart was reported by Nakamura et al. (129), demonstrating oxidative stress-induced activation of p53 signaling leading to increased cytochrome c oxidase assembly protein and subsequent increases in mitochondrial respiration, FA oxidation, and ROS generation in T1D and T2D mouse models. These data support a novel concept about the nuclear-mitochondrial interaction, and they indicate that early genomic instability induced by the diabetic milieu activates p53 pathway in the heart to induce mitochondrial oxidative stress (183). In addition, they bring forth an interesting hypothesis that an increase, rather than a decrease, in mitochondrial electron flow may increase oxidative stress in the diabetic heart.

Is the oxidation of excessive energetic sources responsible for the increased oxidative stress? Mitochondrial oxidation of excessive glucose and subsequent ROS generation has been put forth as the unifying pathogenic mechanism for chronic diabetic complications based on work in endothelial cells that are readily permeable to glucose in the absence of insulin (136). During the development of overfeeding-induced obesity and DM, the heart oxidizes an excessive amount of FA, increasing the reducing equivalent pool (NADH and FADH2, Fig. 2), leading to increased ETC electron flow, and generating a greater mitochondrial inner membrane electrochemical gradient that is usually used to drive ATP synthase. In the normal heart, mitochondrial oxygen consumption increases when the proton gradient is collapsed by an uncoupler (a compound that dissociates ADP phosphorylation), indicating a physiologic limitation of oxidative phosphorylation by the phosphorylation apparatus (ATP synthase, adenine nucleotide translocase, and mitochondrial phosphate carrier) (157). Therefore, the larger electrochemical gradient produced during substrate oversupply that is not consumed for ADP phosphorylation may increase electron pressure at ETC sites that are known to leak electrons and form superoxide such as the Qo in complex III (42). In addition, sites in the mitochondrial FA oxidation pathway also leak electrons during increased electron flux in normal heart mitochondria, independent of the proton gradient magnitude (177). We reported similar findings from a T1D rodent model examining mitochondria from kidney proximal tubules, a structure that also over-relies on mitochondrial FA oxidation in DM (158). ETC defects that interrupt electron flow create additional sites for increased electron pressure and leak as recently reported for complex II (192). Interestingly, mice bearing a genetic complex I defect in the heart do not exhibit increased oxidative stress (100), implying that mitochondrial sites other than ETC are also responsible for superoxide formation in the diabetic heart. Alternatively, complex III may be the principal site of ROS generation during excessive FA oxidation by diabetic cardiac mitochondria, and a defect in complex I is protective by limiting electron flow into complex III.

Oxidative stress triggers a vicious cycle whereupon mitochondria are both the site and targets of oxidative damage. The induction of the mitochondrial transition pore opening is sensitive to oxidative stress, adding to mitochondrial dysfunction (175) and inducing apoptosis (184). In addition, ROS lead to cardiac fibrosis and damage the contractile apparatus (174). Mitochondrial uncoupling proteins dissipate the inner membrane potential and are inducible by oxidative stress, suggesting that they protect mitochondria by reducing the electron pressure created by excessive substrate oxidation. Indeed, human gene polymorphisms that decrease uncoupling protein 2 expression lead to enhanced oxidative stress (162).

Mitochondrial biogenesis

Mitochondrial biogenesis depends on a coordinated program to regulate the formation of all mitochondrial components. This process is orchestrated by PGC-1α that activates the nuclear respiratory factors 1 and 2 (NRF-1 and NRF-2) and enhances expression of the nuclear-encoded mitochondrial transcription factor A (TFAM) (200). TFAM binds to mtDNA, and it initiates mitochondrial transcription and genome replication (36). PGC-1α is considered the master regulator of mitochondrial biogenesis (102), and is beneficial for cardiac function during development and adaptation, but leads to cardiomyopathy with overexpression (160). Although PGC-1 coactivator is required for high-capacity mitochondrial respiratory function (13, 113), it is dispensable for maintenance of mitochondrial density and cardiac function under basal conditions in the adult heart.

Decreased cardiac PGC-1α is noted early in genetically induced DM (58); whereas during high fat diet (HFD)-induced insulin resistance, PGC-1α is initially increased, but then progressively declines (124). Cardiac cells cultured with various FA concentrations exhibit an increase in PGC-1α-mediated mitochondrial biogenic response with high FA levels (124). Increased PGC-1α, mtDNA content, and mitochondrial mass have been reported in some models of T1D and T2D (27, 173) though this biogenic response was associated with decreased mitochondrial function, suggesting an accumulation of defective mitochondria. As in animal studies, cardiac mitochondrial biogenesis is reduced in patients with advanced stages of DM (152, 154).

Calcium handling

Cardiomyocyte contraction depends on intracellular calcium concentration and mitochondrial-generated ATP. Ca$^{2+}$ influx via the L-type Ca$^{2+}$ channels triggers sarcoplasmic reticulum Ca$^{2+}$ release, which dramatically increases the cytosolic-free Ca$^{2+}$ to trigger contraction. Ventricular relaxation and filling is induced by Ca$^{2+}$ removal from cytosol...
primarily by the ATP-dependent sarcoplasmic reticulum Ca-
ATPase 2a (SERCA2a). A decreased excitation-contraction
coupling induced by decreased SERCA2a was reported in
the hearts of both T1D (133) and T2D (20) rodents. Recent
research has focused on mitochondrial Ca\(^{2+}\) handling in
DM. Normal mitochondria act not only as Ca\(^{2+}\) buffers to
prevent cytosolic Ca\(^{2+}\) overload but also as sensors with
Ca\(^{2+}\)-sensitive mitochondrial dehydrogenases that are ac-
vated to improve mitochondrial oxidative capacity (74).
Decreased mitochondrial Ca\(^{2+}\) uptake precedes the contrac-
tile decline in a model of T1D (67), suggesting that Ca\(^{2+}\)
handling is compromised in the DM heart.

Overall, alterations in mitochondrial biogenesis and me-
tabolism are linked to contractile dysfunction via oxidative
stress and Ca\(^{2+}\) mishandling, likely contributing to reduced
diastolic function. However, earlier events driving the meta-
abolic inflexibility that precede these critical pathogenic
loops have yet to be uncovered.

**Mitochondria as Mediators of Redox Status and
Signaling in DCM**

**The concept of redox status and signaling**

Redox reactions involve electron transfer between reduced and
oxidized compounds. Classic examples are the transfer of electrons in
the mitochondrial ETC from reduced to oxidized subunits according to their redox potential until the ultimate tetra-
vental addition of electrons to oxygen. There are four major redox couples (redox players) (97) that reflect the cellular
redox status, and they are involved in redox signaling:
NAD\(^{+}\) (oxidized)/NADH (reduced), NADP\(^{+}\)/NADPH,
GSSG (glutathione disulfide)/GSH (glutathione), as well as
TrxSS (oxidized disulfide thioredoxin)/TrxSH2 (reduced
thioredoxin) (Fig. 3).

Redox state is compartmentalized within the cell. En-
ergized mitochondria have a high NADH concentration to
provide electrons for oxidative phosphorylation (100). In the
cytosol, NAD\(^{+}\) exceeds NADH, reflecting a relatively ox-
idized state with NAD\(^{+}\) available as a cofactor for oxidative
reactions (i.e., glyceraldehyde-3-phosphate dehydrogenase
reaction in glycolysis). In contrast, the cytosolic NADPH/
NADP\(^{+}\) ratio is maintained in a reduced state via several
enzymatic reactions (Fig. 3) to drive reductive biosynthesis
and maintain antioxidant defense. The cytosolic GSH/GSSG
couple is also maintained in a reduced state for ROS detox-
ification. These redox couples are interconnected (Fig. 3).

In mitochondria, the nicotinamide nucleotide transhy-
drogenase (NNT) reduces NADP\(^{+}\) to NADPH to maintain the
mitochondrial NADPH/NADH redox couple and to supply
NADPH for the NAD-consuming enzymes to replenish NAD.
NNT maintains the NADPH/NAD\(^{+}\) ratio several fold higher than the NADH/NAD\(^{+}\) in
mitochondria (161).

In conclusion, redox signaling regulates metabolism whereas
metabolic state influences redox signaling. The NAD\(^{+}\)/NADH
redox couple is a critical node integrating metabolic and sig-
naling events. Our discussion will emphasize changes in the
NAD\(^{+}\)/NADH redox couple in the heart over the course of
obesity, insulin resistance, and DM.

**Total NAD pool**

The redox signaling network linked to the NAD\(^{+}\)/NADH
couple depends on the total extramitochondrial and mito-
thondrial NAD pools (Fig. 4). NAD is a substrate for en-
zymes, including the family of sirtuins (SIRTs) and poly
ADP ribose polymerases (PARPs), which continuously
converts NAD\(^{+}\) to nicotinamide. As NAD is continuously
reduced, cardiomyocytes must maintain a constant pool by
*de novo* synthesis from diet-derived tryptophan and conver-
sion of nicotinamide mononucleotide (NMN), nicotinamide
(NAM), or nicotinamide riboside (NR) to NAD. However,
mammalian cells, including cardiomyocytes, rely mainly on
the NAD salvage pathways that recycle NAM generated by
NAD-consuming enzymes to replenish NAD. Transformation
of NAM to NMN is catalyzed by nicotinamide phospho-
ribosyltransferases (NAMPTs), rate-limiting enzymes in
the salvage pathway. Conversion of NMN to NAD is then
catalyzed by NAD nucleoside diphosphokinases (NMNATs)
with different isoforms (NMNAT1 is nuclear, NMNAT2 is in
the Golgi apparatus, and NMNAT3 is mitochondrial) supporting
organellar cellular NAD pools. In cardiomyocytes, the mito-
thondrial NAD pool is relatively high, matching its critical
role in mitochondrial function (4). It is unclear whether NAD
pools are completely segregated or exchanged between
subcellular compartments. Recently, it was shown that nu-
clear and mitochondrial NAD concentrations match the Mi-
chaeils constants of the respective SIRTs that are also
compartmentalized (Fig. 4). The rate of NAD depletion is
similar in the nucleus and cytosol on PARP activation,
whereas mitochondrial NAD is minimally affected by the
cytosolic NAD-consuming enzymes, further indicating that
the mitochondrial NAD pool is finely balanced. Mitochon-
drial NAD pool is dependent on the conversion of NMN via
NMNAT3 and the potential import of cytosolic NAD through
transport mechanisms that are not fully elucidated in mam-
mals (35), although its existence is supported by the fact that
exogenously added NAD leads to greater accumulation in
mitochondria than the cytosol (145).

Compared with exercise-induced cardiac hypertrophy,
pathological cardiac hypertrophy is associated with a de-
crease in the cardiomyocyte NAD pool whereas NAD re-
pletion inhibits the cardiac hypertrophic response (144). Why
is the cardiomyocyte NAD pool decreased in cardiac path-
ology? One explanation is that chronic oxidative stress with
secondary DNA alterations stimulates PARP to consume NAD for DNA repair (143). Oxidative stress may also cause NAD loss via connexins that are sensitive to oxidative stress (144). A reduced NAMPT and NAD synthetic pathways may be also responsible for NAD depletion (144). NAMPT inhibition with either an NAD analog (139) or a genetic approach (91) leads to a decreased NAD pool in cardiomyocytes associated with mitochondrial dysfunction, insulin resistance, and decreased cardiomyocyte ability to respond to stress. Is the diabetic or obese heart NAD deficient? The myocardial FIG. 3. The four main redox couples governing the redox balance in cardiac mitochondria (NAD+/NADH, NADP+/NADPH, GSH/GSSG, and TrxSH2/TrxSS) are hinged by NNT. Normal cardiomyocytes maintain a constant NAD pool mostly by converting biosynthetic precursors via the salvage pathway rather than de novo synthesis [not shown in the figure, revised in (83)]. Mitochondrial NAD content is increased by the import of cytosolic NAD through hypothetical transport mechanisms (35) that were identified in many species but not in mammals. However, it is believed that a bi-directional mitochondrial-cytosol transport system exists for both NAD and its precursors because exogenously added NAD leads to a greater accumulation in mitochondria than the cytosol (145). NAD+ is both a coenzyme for redox enzymes and an enzyme substrate for nonredox reactions in which the adenine diphosphate ribose moiety of NAD+ is cleaved, leading to the depletion of the NAD pool (not shown). Nicotinamide adenine nucleotide (NAD+) is a phosphate acceptor being converted to the phosphorylated form, NADP+, via the enzyme NAD kinase (120). Only the cytosolic isomer of the NAD kinase is depicted in the figure. Therefore, NAD+ is a precursor for NADP+. Both NAD+ and NADP+ are hybrid acceptors, and are converted to the reduced forms, NADH and NADPH. NADH transfers reducing equivalents that are derived from fuel oxidation, and, therefore, the NAD+/NADH couple is critical for energy generation. The NADPH/NADP+ redox couple is central to anabolism and antioxidant defense by donating electrons to glutathione (GSH/GSSG) and thioredoxin (TrxSH2/TrxSS) systems, both of which are critical in scavenging H2O2 (generated from superoxide, O2-, by dismutation) via the enzymes GR, GPx, and thioredoxin reductase-Prx, respectively. Mitochondrial antioxidant system is mirrored by a similar scavenging mechanism in the cytosol. In these reactions, the reduced and oxidized members of the redox couples interconvert but are not consumed. Unlike these antioxidant mechanisms, catalase, which also scavenges H2O2, does not require reducing equivalents from NADPH for its function. Mitochondrial NADH/NAD+ and NADPH/NADP+ redox couples are linked by the enzyme NNT that reduces NADP+ at the expense of NADH oxidation and utilizing the mitochondrial IM proton motive force to drive this process. NNT maintains mitochondrial matrix NADPH/NADP+ pool in a reduced form, and it is a physiologically relevant source of NADPH to drive the enzymatic degradation of H2O2. The figure shows that the mitochondrial redox state of the NADH/ NAD+ and NADPH/NADP+ redox couples are maintained different as the nucleotides have different metabolic roles. The NADH/ NAD+ pool supports the divergent transfer of electrons from fuel substrates to both the ETC and antioxidant system via NNT, and thus is only partially reduced in comparison to NADPH/NADP+. NNT maintains the NADPH/NADP+ ratio several fold higher than the NADH/NAD+ (161). Cytosolic NADPH is regenerated via the pentose phosphate pathway, and redox reactions are catalyzed by isocitrate dehydrogenase, malic enzyme, aldehyde dehydrogenase, and methylene tetrahydrofolate dehydrogenase (115). The cytosolic NADH is imported in mitochondria by redox shuttles, most commonly the M-A and glycerol 3 phosphate shuttles (G3P). GPx, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione; GSSG, glutathione disulfide; H2O2, hydrogen peroxide; NNT, nicotinamide nucleotide transhydrogenase; M-A, malate-aspartate; Prx, peroxiredoxin. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars.
NAD pool was decreased in a model of DM, and NAD replenishment was protective against streptozotocin (STZ)-induced destruction of pancreatic β-cells (104). A more specific question then emerges: Are subcellular NAD pools altered in the obese and DM heart? This is yet to be determined. Whole body increases in NAD availability appear to benefit metabolic health, especially with conditions of nutrient excess such as obesity and metabolic syndrome. Boosting NAD with NR increases the NAD⁺/NADH ratio (41). However, skeletal muscle-specific overexpression of NAMPT increased the NAD pool in parallel with NADH without altering the NAD⁺/NADH ratio in any cellular compartment (69). As the mitochondrial membrane is impermeable for NADH, the reducing equivalents generated in the cytosol are transferred to the mitochondria via redox shuttles. Although the NMMAT3 isoform is present in mitochondria, the mitochondrial NAD salvage pathway has not been fully elucidated. Exogenous NAD increases mitochondrial function, but a mammalian NAD transporter has yet to be found. NNT reduces NADP⁺ to NADPH at the expense of NADH oxidation. NA, nicotinic acid; NAM, nicotinamide; NMN, nicotinamide mononucleotide; NR, nicotinamide riboside; PARP, poly(ADP-ribose) polymerase. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

NAD⁺ serves as a cofactor in fuel breakdown, whereas NADH is a substrate for complex I in the ETC and NNT. Therefore, the NAD⁺/NADH ratio reflects the overall status of mitochondrial metabolism. Although the NAD⁺/NADH redox ratio has been extensively studied in other organs, a description of the NAD⁺/NADH redox ratio in the obese or diabetic heart, and its involvement in diabetic cardiac pathology remains to be elucidated.

NADH is produced from NAD⁺ during energy substrate oxidation, serving as a key energy-transfer intermediate. In comparison with glucose oxidation that consumes 10 NAD⁺, palmitate (long-chain FA) consumes 31 NAD⁺ during mitochondrial oxidation. In addition to greater NAD⁺ consumption, the increased NADH from FA β-oxidation activates pyruvate dehydrogenase kinase to phosphorylate and inhibits pyruvate dehydrogenase, thus limiting glucose oxidation by...
the glucose-FA cycle (Randle cycle) (137, 150). It has not been determined whether increased circulating ketone bodies are associated with an increased cardiac uptake and oxidation. Therefore, mitochondrial FA oxidation is the major NADH producer in the diabetic heart.

NAD$^+$ adjusts cell metabolism to meet energetic challenges. During energy crises (caloric restriction, fasting, exercise), NAD$^+$ level rises (38); whereas during energy overload (i.e., increased fuel substrates in the form of FA), the NAD$^+$ decreases and its reduced form, NADH, increases. The amount of NADH production and NAD$^+$ consumption is tightly linked to energetic fuel oxidation, whereas mitochondrial NAD recycling (NADH consumption and NAD$^+$ production) is linked to the ETC and decreases with ETC defects. For example, Complex I (100, 183) defects lead to increased mitochondrial NADH content. The deficiency of frataxin, a mitochondrial protein integral to the assembly and function of iron-sulfur proteins in ETC complexes I, II, and III and aconitase (tricarboxylic acid cycle, TCA cycle), is associated with an 85-fold decrease in cardiomyocyte NAD$^+/$/NADH ratio and pathologic cardiac hypertrophy (194). Approaches to correct mitochondrial ETC defects increased NAD$^+$ content (3). In conclusion, the disruption of the electron flow to oxygen by ETC defects increases NADH, causing a highly reduced redox environment within mitochondria.

Cardiac NAD$^+$ is reduced in HF (89). Is there a change in the NAD$^+/$/NADH redox couple or its components in the obese and diabetic heart? Though a plethora of reasons suggest it is, direct measures of NAD$^+$ or NADH in the obese and diabetic heart are limited. In OLETF rats, a model of T2D, diastolic dysfunction, and stiffness correlated with ATP depletion; whereas the NAD$^+/$/NADH redox ratio was unchanged (109). In the ZDF rat T2D model, NADH was unchanged in cardiac tissue in the absence of dysfunction (24).

Although NADH/NAD$^+$ redox ratio determines the production of mitochondrial ROS, the NADPH/NADP$^+$ ratio is key to antioxidant defense. They are linked by the enzyme NNT that transfers electrons from NADH to NADP$^+$ (Fig. 3). Although they are engaged in distinct metabolic pathways, their reduced forms, NADH and NADPH, are spectrally identical, making specific quantification technically challenging, though fluorescence lifetime imaging has been promising in distinguishing the two reduced pyridine nucleotide cofactors in the heart (135). Most studies measure a mixture of NADH and NADPH (24, 188, 189). Using a protocol that does not allow NADPH interference (Cell Technology), we found an increase in NADH in isolated cardiac mitochondria energized with FA substrates (21); whereas Bhatt et al. report a decrease in the steady-state NAD(P)/H in diabetic cardiomyocytes incubated with high glucose and palmitate (24). These results suggest that the reducing power of NADH excess (induced by either excessive FA oxidation or defective complex I) may be split between ATP production and antioxidant defense (to NADPH, via NNT). The increase in the NADH/NAD$^+$ ratio and NAD$^+$ decrease is supported by our work showing decreased activity of mitochondrial NAD$^+$-dependent SIRT3 with secondary increases in protein lysine acetylation (21).

Two critical observations suggest that the NAD$^+/$/NADH ratio is decreased in the diabetic heart mitochondria: (i) The activity of NAD$^+$-dependent sirtuins is decreased, leading to increased lysine acetylation of mitochondrial proteins; (ii) NAD replenishment or interventions (i.e., caloric restriction) to normalize the NAD$^+/$/NADH are beneficial for DCM. Therefore, manipulating the NAD$^+/$/NADH redox couple with novel therapeutic strategies is a promising, yet unexplored target to improve cardiac mitochondrial metabolism and to alleviate DCM. The next sections will discuss these topics.

The NAD$^+/$/NADH Couple in Redox Signaling

The role of the NAD$^+$ as a cellular signaling regulator has only begun to be understood. NAD$^+$ modulates key cellular processes, including energetic metabolism, mitochondrial integrity, gene expression, cell death, and degeneration; all of these are altered in DCM.

NAD$^+$ is an electron acceptor and functions as a cofactor for enzymes that catalyze reduction-oxidation (redox) reactions. Redox reactions are readily reversible, and they do not contribute directly to changes in the total NAD pool in a specific subcellular compartment. NAD$^+$ and NADH interconvert but are not irreversibly consumed. NAD$^+$ participates in all major energetic pathways, including glycolysis, TCA, FA oxidation, ketone body metabolism, and ETC (Fig. 2).

Sirtuins

SIRTs remove an acetyl group from lysine residues in an NAD$^+$-dependent manner by cleaving NAD$^+$ to nicotinamide. SIRT2 was initially reported to mediate longevity in response to caloric restriction in Saccharomyces cerevisiae (94). Sirtuin orthologs also enhance lifespan in mammals, including mice (165). There are seven mammalian sirtuins that differ in their cellular localization, suggesting that sirtuin activities are compartmentalized in parallel with NAD$^+$ pools. Although all sirtuins are NAD$^+$ dependent, SIRT1 and 3 are well-known players in the heart, and they are the focus of this review.

Sirtuin1 has been observed in both the nucleus and the cytosol (185), where it serves several roles, including (i) epigenetic regulation by targeting specific lysine residues on histones to alter gene transcription, (ii) metabolic control by acting on transcription factors (p53, NF-κB, PGC1α, forkhead box O [FOXO]3a), and components of insulin signaling, and (iii) mediating the cardiomyocyte circadian clock. SIRT1 protein abundance is relatively stable, whereas its deacetylation of key lysine residues of histone 3 follows circadian variations (128). Fasting activates SIRT1 through adrenergic-dependent phosphorylation by cAMP/PKA to sensitize the enzyme to NAD$^+$ fluctuations and maintain energy homeostasis during stress (72). SIRT1 protects against pathologic cardiac hypertrophy as knockout mice exhibit developmental cardiac defects (43). Sustained SIRT1 overexpression causes cardiomyopathy whereas moderate SIRT1 expression ameliorates age-induced cardiac hypertrophy and dysfunction (5), suggesting that its effect is dose dependent. SIRT1 also protects mitochondrial function by...
activating PGC-1α (132) to increase mitochondrial FA oxidation (71). Deacetylation by SIRT1 facilitates insulin sensitivity. The effect of SIRT1 on mitochondrial FA oxidation is complex and varies according to the setting, a topic addressed later in this review. In a model of T1D, SIRT1 suppresses cardiomyocyte apoptosis (78). Overall, SIRT1 is critical to the heart in obesity and DM by regulating pathological hypertrophy, insulin sensitivity, and mitochondrial metabolism.

SIRT3 is the major mitochondrial NAD⁺-dependent deacetylase (118). Although the SIRT3-deficient mice do not exhibit baseline metabolic changes (118), an HFD regimen recapitulates the human metabolic syndrome characterized by obesity, glucose intolerance, dyslipidemia, liver steatosis, protein hyperacetylation, and chronic inflammation (86). A single nucleotide polymorphism in the human SIRT3 gene was identified that reduces enzymatic activity, suggesting a genetic component to the metabolic syndrome, and further underscored the role of sirtuins as master regulators of metabolism (86). Genetic manipulation of SIRT3 gene has revealed its role in deacetylating and increasing hepatic mitochondrial enzyme activity involved in oxidative phosphorylation, FA oxidation (84), antioxidant defense, and mediating the effect of caloric restriction on age-induced hearing loss (176). SIRT3 also mediates the circadian control of mitochondrial oxidative phosphorylation (142).

Sirtuin3 regulates cardiac changes in the setting of obesity and DM such as hypertrophy, insulin sensitivity, and fuel metabolism. SIRT3 knockout causes age-related cardiac hypertrophy and failure with pathologic challenges (106), whereas overexpression protects against pathological hypertrophy by activating FOXO3a and its downstream antioxidant genes, mitochondrial manganese Superoxide dismutase (MnSOD), and catalase (182). SIRT3 also targets cyclophilin D, a modulator of the mitochondrial permeability transition pore (mPTP), to suppress pore opening, apoptosis, and cardiac hypertrophy (79). Modulation of the mPTP and mitochondrial Ca⁺² by cyclophilin D maintains the mitochondrial metabolism and NAD⁺/NADH redox state, and it controls the mitochondrial acylome (134).

SIRT1 and SIRT3 act in concert to regulate energy metabolism during energetic crises. SIRT3 deacetylation activates enzymes involved in glycolysis (96, 140), FA oxidation (22, 84, 86), TCA cycle (176), and the ETC (2). During fasting, SIRT1 mediates a switch from glucose to FA oxidation in response to increased circulating FA (75) and increased NAD⁺ (37) in the muscle. By upregulating mitochondrial machinery during states of decreased fuel availability, SIRT3 appears to be a critical metabolic regulator of coupling substrate oxidation with the formation of reducing equivalents to ATP production, thus maximizing efficiency. However, most studies in this area have been performed in extracardiac tissues, comparing the fasting versus fed conditions and/or genetic manipulation of sirtuins. The effect of fasting on NAD⁺-dependent deacetylases has not been investigated in the heart.

SIRT1 and SIRT3 are classically defined as NAD⁺-dependent deacetylases, but more recent data suggest that SIRT3 depends on the NAD⁺/NADH ratio rather than the absolute [NAD⁺] (100). NADH exhibited a dose-dependent inhibition of SIRT3 activity despite constant NAD⁺ concentrations, suggesting that NADH competes with NAD at the binding site. In vitro experiments show that NADH inhibits SIRT3 at concentrations that are nonphysiologic, thus unlikely to regulate SIRT3 activity in vivo. This conundrum is complicated by the observation that mice bearing a genetic defect in complex I, the major NADH oxidation site, do not exhibit changes in mitochondrial NAD⁺ concentrations but rather a significant increase in NADH and a dramatic increase in lysine acetylation of cardiac proteins (100). We also showed that decreasing NADH consumption at complex I facilitates mitochondrial lysine acetylation in the diabetic heart (192), and that [NADH] rather than NAD⁺ correlates with increased SIRT3 activity (21).

NAD⁺-dependent sirtuins have been investigated as therapeutic targets in DCM. For example, resveratrol, a polyphenol and well-known SIRT1 activator, alleviated DCM in models of both T1D and T2D via activating sirt1, 2, 3, and 5 (14, 15), improved glucose metabolism in human subjects (23, 117), and decreased oxidative stress in cultured cardiomyocytes (186). In a rodent model of genetic obesity, resveratrol mitigated cardiac fibrosis and improved FA metabolism (18).

SIRT1 compete with PARPs for the NAD pool. PARP activity increases in chronic diseases, including obesity-induced DM due to increased DNA damage, and is reported to be a more rapid and efficient NAD⁺ consumer than SIRT1, suggesting that its activation may limit SIRT1 activity in these conditions. Competition for the NAD⁺ pool was validated in a mouse model of human accelerated aging where either PARP inhibition or NAD pool replenishment was protective (167). Increasing NAD availability by inhibiting PARP-1 increased SIRT1 activity, mitochondrial content, energy expenditure, oxidative metabolism, and protected against metabolic disease (16). PARP-1 inhibition in a mouse model of T1D alleviated DCM (148). However, therapeutic manipulation of PARP-1 activity with the purpose to correct the NAD⁺/NADH redox state, increase sirtuin activity, and protect against DCM should be considered with care due to the critical role of PARPs in the cellular response to stress and DNA repair.

### NAD⁺/NADH ratio, sirtuins, and mitochondrial health in DCM

NAD⁺ is a vital cofactor in the cardiac metabolic program and mitochondrial fitness (39) through regulating mitochondrial biogenesis, dynamics (fusion/fission processes), and mitochondrial quality control.

Mitochondrial mass depends on the formation of mitochondria (biogenesis) and degradation of defective mitochondria (mitophagy). Both SIRT1 and SIRT3 regulate PGC-1α, the master regulator of mitochondrial biogenesis. SIRT1 deacetylates and increases the transcriptional activity of PGC-1α (132), whereas SIRT3 is a PGC-1α target as its activation is required for PGC-1α-induced mitochondrial biogenesis (107). The decrease in nuclear NAD⁺ and SIRT1 activity caused a downregulation of TFAM, the major factor responsible for the replication and transcription of the mitochondrial genome, with decreased mitochondrial respiration and increased glycolysis, a pseudohypoxic phenomenon that is mitigated by NAD supplementation (76). SIRT1 and PGC-1α were recently found to be associated with mitochondrial DNA nucleoids and TFAM (26), suggesting that both SIRT1 and PGC-1α can directly affect mitochondrial transcription.
ETC protein subunits are encoded by both nuclear and mitochondrial genomes. Unbalanced gene expression results in accumulation of unassembled subunits and induces proteotoxic stress with activation of the mitochondrial unfolded protein response (UPR{\text{mit}}) (98) as part of mitochondrial quality control. The UPR{\text{mit}} induces a nuclear gene expression program, including FOXO3a, MnSOD, and catalase, along with stimulating autophagy. SIRT3 is a critical coordinator of the UPR{\text{mit}} by enhancing the antioxidant defense and mitophagy (141). SIRT3 exerts cardioprotection by activating the FOXO3a-Parkin-mediated mitophagy, thus preserving mitochondrial homeostasis and alleviating cardiomyopathy in a model of T1D (202). SIRT3 also regulates mitochondrial morphology by influencing factors that are responsible for fusion/fission processes (163).

Complex I is the major hybrid acceptor from mitochondrial NADH; its inhibition traps this redox couple in its reduced form with a decrease in NAD{\text{+}}, SIRT3 inhibition, and increased mitochondrial protein lysine acetylation with accelerated HF in the presence of stress (100). NR supplementation in mouse models of mitochondrial defects increased cellular NAD with an increase in mitochondrial biogenesis (41, 103). Known targets of the NAD-dependent deacetylase SIRT1 are the tumor suppressor p53, the myocyte-specific enhancer factor 2, the FOXO factor, and PGC-1{\text{a}}, all of which activate transcriptional programs and promote mitochondrial function (11). Gomes et al. (76) reported that the control of mitochondrial metabolism exerted by the nuclear NAD{\text{+}}-dependent SIRT1 is regulated by energy supply in the heart. With normal or increased energy supply, the acetyl-CoA-dependent acetyltransferase GCN5 acetylates and inhibits PGC-1{\text{a}} (64) to depress mitochondrial biogenesis and function. In contrast, caloric restriction increases nuclear NAD{\text{+}} and SIRT1 activity to improve the nuclear-mitochondrial communication and metabolism by alleviating the state of “pseudohypoxia” induced by the decreased NAD{\text{+}}, and it increases TFAM promoter activity to enhance transcription and mtDNA replication. This signaling pathway is believed to operate in insulin-resistant tissues, including the heart (146). In conclusion, NAD{\text{+}}-dependent sirtuins may be a key therapeutic target to maintain mitochondrial integrity in DCM.

**How Mitochondrial NAD{\text{+}}/NADH Redox Dysregulation Potentially Drives DCM**

The interplay between bioenergetics and NAD{\text{+}}/NADH redox state is a critical point of regulation in the heart. Understanding the nature of how this relationship fails in DM may reveal novel targets for treating DCM. We propose that an excess energy supply with a secondary shift toward a highly reduced NAD{\text{+}}/NADH redox couple is an early event in cardiac pathology of overfeeding-induced obesity and DM. In the setting of increased fuel availability, their full oxidation provides excessive NADH and acetyl-CoA.

**The role of high glucose**

Because hyperglycemia is the hallmark of diabetes, and carbohydrates are major parts of the Western diet, a reasonable question arises: Does glucose contribute to the increased mitochondrial Acetyl-CoA/CoA and NADH/NAD{\text{+}} ratios in the heart in these conditions? Optimal glycemic control in diabetes reduces cardiovascular morbidity (153), suggesting that hyperglycemia is an important pathogenic factor for cardiovascular complications. Glucose is taken up by cardiomyocytes via GLUT1 and GLUT4, with the latter being responsible for insulin-dependent glucose uptake. Absence of GLUT4 significantly decreased cardiac glucose uptake under insulin-stimulated conditions but did not fully abolish (61) the observed increase of basal cardiac glucose uptake and glycogen content (1). Because GLUT1 is responsible for the bulk of basal glucose uptake, these data suggest that circulating hyperglycemia increases GLUT1-mediated insulin-independent glucose uptake. However, glycolysis and pyruvate oxidation are decreased in the diabetic heart (164). This shift away from glucose oxidative metabolism is not restricted to diabetes as it precedes the diastolic dysfunction on angiotensin II infusion (126) and pressure-overload hypertrophy models (203). In addition, transgenic mice with a mutation that decreases pyruvate oxidation develop both diastolic (1) and systolic (181) dysfunction, supporting the concept that glucose oxidation is required for normal cardiac metabolism. Retaining the capacity to freely switch between energy substrates to optimally respond to energy demands is a characteristic of normal cardiomyocytes, skeletal and liver cells (172). The question that arises pertains to the role of hyperglycemia in DCM.

If cardiomyocyte glucose uptake is only moderately affected whereas glycolysis and pyruvate oxidation are inhibited, it is unlikely that hyperglycemia contributes to the increase in mitochondrial acetyl-CoA/CoA and decreased NAD{\text{+}}/NADH in the diabetic heart. Rather, the increased flux through alternative non-ATP-producing glucose pathways such as polyol pathway, activation of protein kinase C, formation of advanced glycation end products, and hexosamine pathways, recognized as central to diabetic chronic microvascular complications, are more likely to mediate cytosolic redox changes (Fig. 5). Do they affect the myocardium? Glucose conversion to sorbitol (via aldose reductase with NADPH oxidation) and then to fructose (via sorbitol dehydrogenase with NAD{\text{+}} reduction) has been extensively studied in microvascular complications, but its effect on myocardium in diabetes has not been established. High cytosolic glucose increases diacylglycerol, causing chronic activation of protein kinase C is forms and cardiomyopathy (193). Excessive glucose and glucose-derived dicarboxyls (i.e., methylglyoxal) react with lysine and arginine amino groups of proteins, forming advanced glycation end-products that damage cardiomyocytes. Intracellular targets of methylglyoxal have been reported, including mitochondrial proteins (156) and components of calcium cycling (187), indicating a direct effect on energetic metabolism and cardiac contraction (131). The hexosamine pathway provides the end product UDP-N-acetyl-glucosamine (UDP-GlcNAc) used by the enzyme O-GlcNAc transferase to modify proteins by O-GlcNAcylation, thus causing contractile dysfunction (149), epigenetic modifications, and changes in gene expression in the diabetic heart [reviewed in (101)]. Diversion of the glycolytic intermediates to pathways others than full oxidation and ATP production is favored by the inhibition of the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH).
by oxidation. In addition, chronic oxidative stress and ROS-mediated DNA damage activate DNA repair mechanisms, including PARP1 that polyADP-ribosylates and inhibits GAPDH, thus creating an amplification loop by depleting cytosolic NAD\(^+\) and inhibiting SIRT1 (Fig. 5).

The role of FA

In comparison with glucose, chronically excessive FA reach the mitochondria, and pose a great challenge to mitochondrial metabolism by increasing NADH, thus favoring ETC electron flow and mitochondrial inner membrane hyperpolarization that favors superoxide formation (108). In addition, the various ETC defects in the diabetic heart will further increase NADH. Therefore, in contrast to the general consensus that the diabetic heart functions under more oxidizing conditions that drive pathology (12), we propose that a reductive redox stress may precede and induce the observed oxidative stress.

A mainstream hypothesis in the field of DCM supports the dependency of the net mitochondrial ROS efflux on the mitochondrial redox environment, and it states that the ROS levels are minimal at intermediate redox environment values (redox optimized ROS balance hypothesis; for more details, readers are referred to an excellent review on this topic (12)). The mitochondrial redox environment is provided by the mitochondrial redox couples [NADH/NAD\(^+\), NADPH/NADP\(^+\), GSH/GSSG, and Trx(SH)\(_2\)/TrxSS], ranging from extremely reduced to oxidized values depending on mitochondrial energetic state. Although the mechanisms differ, both extremes are associated with increased mitochondrial ROS release. At a highly oxidized redox state, the mitochondrial antioxidant defense is overwhelmed. A highly reduced mitochondrial redox state is achieved when the levels of the reducing equivalents in the redox couples are
increased. As seen in Figure 3, primary changes in NADH may induce secondary alterations in other redox couples via the forward NNT reaction, and they may occur when NADH is either excessively produced or not oxidized. An excessive oxidation of energy substrates may cause increased mitochondrial NADH, whereas either mitochondrial ETC defects (i.e., complex I defect) or NNT deficiency may decrease NADH oxidation. However, NNT deficiency has been reported to protect the heart against overload-induced HF (135), and, therefore, this is unlikely to contribute to an overly reduced redox environment. Due to increased oxidation of energy substrates with high reducing power and mitochondrial ETC defects, diabetic heart mitochondria may achieve an extremely reduced redox environment. For example, we reported that the NADH/NAD⁺ ratio is increased in cardiac mitochondria oxidizing palmitoyl-CoA, and normalizing the NADH/NAD⁺ ratio is beneficial to the diabetic heart (21).

Our hypothesis does not exclude the redox-optimized ROS balance hypothesis, and it proposes that the effect of excessive FA oxidation by the metabolically inflexible diabetic heart is more complex, and exceeds the participation in oxidative stress. The early increase in the reactive metabolites, NADH and acetyl-CoA, may initiate cardiac damage through pathogenic mechanisms such as lysine acetylation of cardiac proteins. Increased circulating FA seems to be the primary event that changes the cardiac NAD⁺/NADH redox state during nutrient challenges. For example, long-term caloric restriction reduces circulating FA (68) and activates NAD⁺-dependent mitochondrial sirtuins to deacetylate ETC proteins and alleviate diastolic dysfunction and DCM in T2D (46). In contrast, a short-term (overnight) fasting triggers adipose lipolysis to increase circulating nonesterified FA (75, 80) and decrease cardiac NAD⁺.

In addition to NAD⁺/NADH redox couple regulating protein lysine acetylation and energetic metabolism (described in previous sections), the role of increased acetyl-CoA derived from oxidation of excessive fuel substrates is gaining recognition. The importance of acetyl-CoA as a sensor of caloric excess is supported by experiments in mice bearing a deficiency of carnitine acetyltransferase (transports mitochondrial acetyl-CoA to the cytosol), which exhibit increased mitochondrial lysine acetylation induced by excessive mitochondrial acetyl-CoA (127). In addition, an increase in nuclear acetyl-CoA caused histone lysine acetylation and induction of genes involved in utilizing the nutrient excess (197). Acetyl-CoA is used by acetyltransferases as a substrate to acetylate protein lysines. Histone acetyltransferases (p300, CBP, GCN5) regulate chromatin dynamics to activate gene transcription. General control of amino acid synthesis 5 (GCN5) is involved in lysine acetylation and inhibition of PGC-1α (114). A mitochondrial acetyltransferase, GCN5-1, was recently described to counter the effect of SIRT3 on ETC proteins (170). With low binding affinity for acetyl-CoA, acetyltransferases are controlled by acetyl-CoA availability within cellular compartments (196), and are inhibited by CoA, suggesting that the acetyl-CoA/(AcCoA) is equally important in regulating their activity (44). Acetyl-CoA can also promote nonenzymatic lysine acetylation (195), and is increased in skeletal muscle (110) and liver mitochondria (88) with high fat feeding.

Both acetyl-CoA excess and NAD⁺-dependent sirtuin deficiency favor lysine acetylation of cardiomyocyte proteins with a purportedly inhibitory effect on metabolic enzymes to limit the further generation of acetyl-CoA and NADH. This is illustrated in the context of SIRT3 deficiency where known targets, including mitochondrial FA oxidation enzymes (85, 169) and protein subunits of ETC complexes I and II (2, 45), are hyperacetylated and inhibited. However, this potentially sets in motion a vicious cycle that favors increased NADH and a mitochondrial reduced state, further facilitating protein lysine acetylation. In the setting of over-nutrition, a paradoxical association arises: increased cardiac FA oxidation enzymes and long chain acylCoA dehydrogenase, LCAD, activation, thus enhancing cardiac FA oxidation (6). We also reported that improving the NAD⁺/NADH redox state and decreasing lysine acetylation of FA oxidation enzymes limited mitochondrial FA oxidation and improved heart function in T1D (21) (Fig. 6). In conclusion, in the setting of high FA availability, mitochondrial FA oxidation enzymes are activated when hyperacetylated.

This paradox suggests that the effect of sirtuins and lysine acetylation on mitochondrial FA oxidation depends on both the pre-existing biochemical environment and tissue type, and that additional regulatory mechanisms may operate during nutrient excess in the heart. The details underlying this unique mechanism have begun to unfold. One aspect of additional regulation occurs during HFD where SIRT3 is downregulated and the NAD⁺/NADH ratio is decreased; heat shock protein 10 (SIRT3 substrate) becomes acetylated and induces optimal folding of FA oxidation enzymes to enhance activity and, subsequently, mitochondrial FA oxidation (121). In addition, both enzymatic and nonenzymatic acetylation is operative during nutrient overload, leading to acetylation of lysine sites beyond those recognized as sirtuin targets. Future research will establish whether nonspecific lysine acetylation activates FA oxidation, overriding the inhibitory effect of SIRT3-targeted lysines. An interesting hypothesis was raised by Griffin et al. regarding the functional effect of the site-specific lysine acetylation; the authors noted that several proteomic studies have identified more than 2000 acetylated lysine residues in 400 mitochondrial proteins that function within metabolic pathways. In comparison to phosphorylation, the functional impact of the large number of lysine acetylation sites remains unknown (77). The authors suggest a similarity of acetylation with the oxidative changes of proteins that commonly occur with yet-to-be-known functional changes, but are likely determined by the intensity to which the protein is “decorated” with these post-translational modifications (77). This hypothesis emphasizes the need to understand the critical and specific role of the NADH/NAD⁺ and acetyl-CoA/CoA as energy sensors, between many others, and pathogenic factors in DCM.

Despite the mainstream postulate supporting the detrimental role of the chronic exposure to excessive FA on cardiac function in DM, there is evidence of an acute beneficial effect of palmitate on cardiac contractile function in models of T1D (189) and T2D (24, 188) during both basal and adrenergic stimulation. In the presence of high glucose, the diabetic hearts efficiently oxidized FA and developed a
Mitochondrial oxidation of high levels of glucose has been established as having a central mechanistic role in the development of chronic complications in tissues with predominantly insulin-independent glucose uptake, such as the endothelium during microvascular complications (31). In contrast, as pyruvate oxidation decreases in the DM heart, chronic hyperglycemia induces an excessive oxidative redox environment and exhausts mitochondrial antioxidant scavenging systems (24, 188, 189) via extra-mitochondrial mechanisms. As the glutathione and thioredoxin redox couples (major mitochondrial and cytosolic scavengers for the highly diffusible $\text{H}_2\text{O}_2$) become more oxidized during hyperglycemia, short-term mitochondrial palmitate oxidation provides reducing equivalents (NADH) that normalize the mitochondrial redox status, restore the antioxidant potential, and provide a higher energetic budget that rescues the diabetic heart. This emphasizes the short-term benefit of optimizing the high glucose-induced oxidative redox environment by using palmitate, a powerful reducing equivalent generator. A similar benefit was obtained with GSH, emphasizing the acute benefit of correcting the oxidized redox environment to where the net mitochondrial ROS flux is minimal. Chronic administration of palmitate has not been investigated; thus, the results remain in an unresolved contradiction to support the detrimental role of increased mitochondrial FA oxidation in cardiac function.

In light of metabolic changes, the existence of a difference in FA handling between the normal and diabetic heart is not surprising. Chronic exposure of the diabetic hearts to both hyperglycemia and excessive FA has multiple long-term metabolic effects. In addition to decreased insulin signaling, acetyl-CoA and NADH from excessive FA oxidation activate PDK4 (pyruvate dehydrogenase [PDH] inhibitor), thus inhibiting pyruvate oxidation and requiring enhanced FA oxidation to meet cellular demand. Moreover, metabolic gene expression reprogramming via PPARα facilitates FA oxidation.
and suppresses glucose oxidation. Therefore, in contrast to
the normal heart, the diabetic heart is conditioned to oxidize
the FA excess over glucose. Noteworthy is that ATP is not
always measured in DM myocardial samples, raising the
possibility that diabetic samples exposed to experimental
conditions of high glucose as the only energetic fuel may be
energetically deficient and this accounts for the observed
decline in cardiac function and palmitate benefit (188).

Acute palmitate has also been reported to rescue the dia-
abetic heart exposed to a β-adrenergic agonist mimicking a
physiological increase in workload (188). On physiologic
stress, the β-adrenergic-induced increase in cardiomyocyte
Ca$^{2+}$ levels stimulates TCA cycle enzymes (30) to produce
NADH to respond to increased NADH demand resulting
from increased consumption by the ETC to produce ATP for
contraction. FA oxidation fits this “parallel activation” (49),
whereas glucose oxidation by the diabetic heart does not [a
very informative review is provided in (198)]. On a non-
physiologic workload, such as an increase in preload or
postload, the primary mechanism to increase inotropism is
governed by the Frank Starling mechanism without an ap-
propriate adrenergic signaling and increase in Ca$^{2+}$ car-
diomyocyte level. The beneficial effect of palmitate has not been
studied when the diabetic heart is under these pathological
stresses.

Nevertheless, these experiments are in agreement with our
hypothesis that FA oxidation is detrimental to the normal
heart, and may trigger the cardiac pathology on substrate
oversupply. In the oxidized cytosolic redox environment
induced by hyperglycemia, mitochondrial FA oxidation offers
maximal reducing power and optimizes the redox environ-
ment to a level where the mitochondrial ROS release is
minimal. However, at higher concentrations, FA oxidation
induces an increase in ROS mitochondrial flux (50). We
propose that chronic, excessive FA oxidation by the meta-
bolically inflexible diabetic heart is more complex than
changing the level of oxidative stress, and includes lysine
decacylation and epigenetic changes as key mechanistic
factors in DCM.

**Future Directions**

Cardiac dysfunction induced by overfeeding-induced
obesity and diabetes has a multifactorial pathogenesis, and
the initiating mechanism is yet to be understood. These
conditions cause an increased energetic supply. The increase
in mitochondrial FA oxidation at the expense of glucose
oxidation indicates the onset of metabolic inflexibility. Ca-
loric restriction alleviates obesity and diabetes-induced dia-
stolic dysfunction in humans and mice; its health benefits are
partly mediated by sirtuins, enzymes that remove the acetyl
group from lysine residues on proteins, and regulate their
activity in an NAD$^+$-dependent manner. NADH, the reduced
form of NAD, is produced by fuel oxidation and consumed by
mitochondria to produce the oxidized form, NAD$^+$, in the
process of oxidative phosphorylation while ATP is formed.
In addition, NADH serves as an electron donor used by the
enzyme NNT in the forward reaction to form NADPH to
maintain optimal antioxidant response. Therefore, the NAD$^+$/
NADH redox ratio reflects the mitochondrial function, and it
is a cellular regulation node. Although the mitochondrial
NAD$^+$/NADH redox ratio has been studied in other organs,
its relationship with other cellular compartments and in-
volve in obesity and diabetes-induced cardiac pathology
remains to be elucidated. Exogenous administration of NAD
or its precursors increases mitochondrial NAD pool and is
beneficial to the metabolic health by maintaining mitochon-
drial integrity. Their use in the setting of the obese and dia-
betic heart needs further investigation. Normalizing the
mitochondrial redox by shifting electrons away from NADH
without changing the NAD pool proved a beneficial thera-
peutic approach for the diabetic heart (Fig. 6). The role of the
NNT to connect fuel oxidation with the antioxidant potential
also needs to be unfolded.

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**Abbreviations Used**

\( \beta HB = \beta -\text{hydroxybutyrate} \)

CPT1 = carnitine palmitoyltransferase 1

DM = diabetes mellitus

ETC = electron transport chain (mitochondrial)

FA = fatty acids

FOXO = forkhead box O

GAPDH = glyceraldehyde-3-phosphate dehydrogenase

GCN5 = general control of amino acid synthesis 5

GSH = glutathione

GSSG = glutathione disulfide

GPx = glutathione peroxidase

H2O2 = hydrogen peroxide

HF = heart failure

HFD = high fat diet

LCAD = long chain acylCoA dehydrogenase

LV = left ventricle

MCD = malonylCoA decarboxylase

MnSOD = manganese Superoxide dismutase

mPTP = mitochondrial permeability transition pore

NAM = nicotinamide

NAMPT = nicotinamide phosphoribosyltransferase

NMN = nicotinamide mononucleotide

NMNAT = NMN adenylyltransferase

NNT = nicotinamide nucleotide transhydrogenase

NRF = nuclear respiratory factor

PARP = poly(ADP ribose) polymerase

PDH = pyruvate dehydrogenase

PGC-1\( \alpha \) = Peroxisome proliferator-activated receptor gamma coactivator 1-alpha

PPAR\( \alpha \) = Peroxisome proliferator-activated receptor \( \alpha \)

Prx = peroxiredoxin

ROS = reactive oxygen species

SIRT = Sirtuin

STZ = streptozotocin

T1D = type 1 DM

T2D = type 2 DM

TCA = tricarboxylic Acid Cycle

TFAM = mitochondrial transcription factor A

TG = triglycerides

UPR\( \text{ mt } \) = mitochondrial unfolded protein response