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Protective Mechanisms of Mitochondria and Heart Function in Diabetes

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Abstract

Significance: The heart depends on continuous mitochondrial ATP supply and maintained redox balance to properly develop force, particularly under increased workload. During diabetes, however, myocardial energetic-redox balance is perturbed, contributing to the systolic and diastolic dysfunction known as diabetic cardiomyopathy (DC). ***Critical Issues:*** How these energetic and redox alterations intertwine to influence the DC progression is still poorly understood. Excessive bioavailability of both glucose and fatty acids (FAs) play a central role, leading, among other effects, to mitochondrial dysfunction. However, where and how this nutrient excess affects mitochondrial and cytoplasmic energetic/redox crossroads remains to be defined in greater detail. ***Recent Advances:*** We review how high glucose alters cellular redox balance and affects mitochondrial DNA. Next, we address how lipid excess, either stored in lipid droplets or utilized by mitochondria, affects performance in diabetic hearts by influencing cardiac energetic and redox assets. Finally, we examine how the reciprocal energetic/redox influence between mitochondrial and cytoplasmic compartments shapes myocardial mechanical activity during the course of DC, focusing especially on the glutathione and thioredoxin systems. ***Future Directions:*** Protecting mitochondria from losing their ability to generate energy, and to control their own reactive oxygen species emission is essential to prevent the onset and/or to slow down DC progression. We highlight mechanisms enforced by the diabetic heart to counteract glucose/FAs surplus-induced damage, such as lipid storage, enhanced mitochondria-lipid droplet interaction, and upregulation of key antioxidant enzymes. Learning more on the nature and location of mechanisms sheltering mitochondrial functions would certainly help in further optimizing therapies for human DC. *Antioxid. Redox Signal*. 22, 1563–1586.

Mitochondria and Heart Function

Redox and energetics of heart function

THE HEART DEPENDS on continuous oxidative metabolism to maintain ATP supply and redox balance for optimal contractile function. More than 90% of heart metabolism is aerobic (166, 180). A 70 kg human male at rest consumes 430 L of O_2 per day (177), which can increase 5- to 10-fold depending on physical activity (207). About 90% of this O_2 will be channeled to mitochondrial respiration (166); about 10% of O_2 usage is nonmitochondrial (79). Consequently, mitochondria are central to aerobic life, and their energetic and redox functions are pivotal for health, disease, and aging (36, 69, 107).

Cardiac output measures heart effectiveness as a pump; it can be calculated by multiplying the heart rate (beats/min) by stroke volume (ml/beat). For an average resting heart rate of 72 beats/min and a stroke volume of 70 ml/beat, the cardiac output is $\sim$5 L/min. In humans, the average total blood volume is about 5 L; therefore, at rest, one side of the heart pumps all the blood in the body in 1 min (177). The *in vivo* rate of cardiac basal metabolism is 5–10 times higher than skeletal or smooth muscle (79).

To carry out its mechanical work, the heart transduces the chemical energy stored in fatty acids (FAs), glucose, and other substrates into mechanical and electrical energy (124). About 20–30% of the O_2 is used to maintain the mitochondrial membrane potential. The remaining 60–70% of O_2 is

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used to supply mitochondrial ATP for protein synthesis, maintenance of transmembrane Na^+ and Ca^{2+} gradients (55–65%), and resting acto-myosin ATPase ($\sim$5%) (79).

At rest, the heart cycles about 6 kg of ATP every day while beating about 100,000 times (142). Mitochondria provide the bulk of the ATP needed for cardiac muscle contraction (180). Oxidative phosphorylation (OxPhos) represents the dominant source of energy for matching metabolic/contractile demand. Indeed, mitochondrial dysfunction contributes to heart mechanical failure (142, 145); therefore, the control and regulation of OxPhos is of paramount importance in this organ (53, 54).

The respiratory chain reduces O_2 efficiently through cytochrome oxidase, but the electron flux can be diverted to produce the free radical superoxide, O_2^{-}. As a result of this, mitochondria generate reactive oxygen species (ROS) in the respiratory chain mainly through complex I and III (138, 183, 191). Muscle mitochondria produce $\sim$85–90% of the O_2^{-} generated (10, 38), predominantly from complex I when mitochondria are in state 4 (high proton motive force and ATP) with a reduced coenzyme Q pool and at high matrix $NADH/NAD^+$ ratio (138).

The reported extent of electron diversion from the respiratory chain to produce $O_2^{\bullet -}$ varies widely, ranging from 0.15% to 2% (8, 24, 38, 96, 181). Recently, a study with heart mitochondria from mouse, rat, and guinea pig re-examined these values. They were found to be higher than those previously reported, because this time the contribution of ROS scavenging was taken into account (8). These results underscore the role played by the antioxidant defenses, especially under state 3 respiration, that is, when O_2 consumption is maximal.

Redox signaling

Originally considered an unavoidable byproduct of OxPhos, there is now a general awareness that ROS are important signaling molecules. When emission is controlled, H_2O_2 can act as a rather specific signaling molecule but it can readily become damaging when reaching a supra-physiological level (155). Cellular and mitochondrial physiological levels of ROS are attained when production and scavenging are balanced. Pathophysiological levels of ROS can occur due to alterations in production, overwhelming of antioxidant defenses, or both (5, 176). Oxidative stress plays an important role in mediating mitochondrial dysfunction and cell death during aging and heart failure (85).

The signaling role of H_2O_2 is based on its lifetime and ability of permeating membranes, because it freely diffuses throughout cellular compartments. Consequently, H_2O_2 can propagate intracellular physiological and pathophysiological signals (6, 219). As a mild oxidant, H_2O_2 reacts with cysteine (Cys) residues in proteins, a reaction modulated by the local environment (*e.g.*, pH) (59). Multiple biochemical networks that control cell cycle, stress response, cell migration and adhesion, energy metabolism, redox balance, cell contraction, and ion channels can be influenced by H_2O_2 (59, 62, 66, 105).

The H_2O_2 generated after SOD catalyzed $O_2^{\bullet -}$ dismutation has several possible fates. An extensive array of antioxidant defenses present in mitochondrial and cytoplasmic compartments act as H_2O_2 gatekeepers (Fig. 1). These scavenging systems continuously offset ROS generation from the respiratory chain and other sites (105). The glutathione peroxidase/glutathione reductase system removes H_2O_2 using GSH as an electron source, and the thioredoxin peroxidase/thioredoxin reductase system uses electrons from thioredoxin [$Trx(SH)_2$]. The oxidized (disulfide) forms of glutathione (GSSG) and thioredoxin (TrxSS) are converted back into the reduced state from electrons provided by NADPH (80, 111).

Redox biology

Every substrate degraded in the cell generates electrons, phosphate, and carbon fluxes (47, 48) building up into redox and phosphorylation potentials that are used to drive transport, catabolic, and anabolic reactions (Fig. 1). Redox and phosphorylation potentials are inextricably linked as exemplified by mitochondrial NADH, a redox hinge directing electrons to OxPhos and to the antioxidant systems through transhydrogenase *via* NADPH (5, 95, 169) (Fig. 1). Directing electrons from NADH to NADPH is important for mitochondria to control ROS emission, even under normal conditions, especially under state 3 respiration (8).

We use the term ''redox'' to refer to the four main redox couples, $NADH/NAD^+$, $NADPH/NADP^+$, GSH/GSSG, and $TrxSH_2$/TrxSS (105, 107, 165). These couples are, to a certain extent, inter-convertible (105). The redox environment (RE) is a metric defined by the contribution of the different redox couples as a function of both their redox potential and the concentration of the reduced species (102, 105, 171) (see ''Protecting mitochondrial function in the diabetic heart: an overview'').

Figure 2 illustrates an example of the thermodynamic feasibility of electron transfer ($n=2$) from NADPH to oxidized thioredoxin, TrxSS, in the mitochondrial matrix at pH = 7.2. This can be determined by calculating ΔG according to Eq. 2 (107). Considering a ratio $NADPH:NADP^+$ = 0.7/0.3 ($NADP^+$ = 30%; black square symbol in Fig. 2), and of $Trx(SH)_2$:TrxSS = 0.8/0.2 (TrxSS = 20%; red square symbol in Fig. 2), we can write:

$$\Delta E = E_2 - E_1 = E_{TrxSS} - E_{NADP} = -322.8\ mV - (-343.6\ mV) = 20.8\ mV \quad \text{(Eq. 1)}$$

$$\Delta G = -n\ F\ \Delta E = -2 \times (0.0964\ kJ/mV) \times (20.8\ mV) = -4.01\ kJ \quad \text{(Eq. 2)}$$

A negative ΔG indicates that the transfer of electrons from NADPH to TrxSS is thermodynamically favorable under these conditions. According to Figure 2, it becomes clear that $NADPH:NADP^+$ is the redox couple with the highest reductive potential, thus the main electron donor in control of oxidative stress. From NADPH, the reductive potential decreases as follows: $NADH/NAD^+$ > Trx(SH)2/TrxSS > GSH/GSSG (105). NADPH is a cofactor for glutathione reductase and thioredoxin reductase, generating reduced glutathione and reduced thioredoxin (Fig. 1) (5, 8, 105, 182). NADPH serves as an electron donor in a number of other essential cellular functions, including *de novo* FA and cholesterol biosynthesis (194).

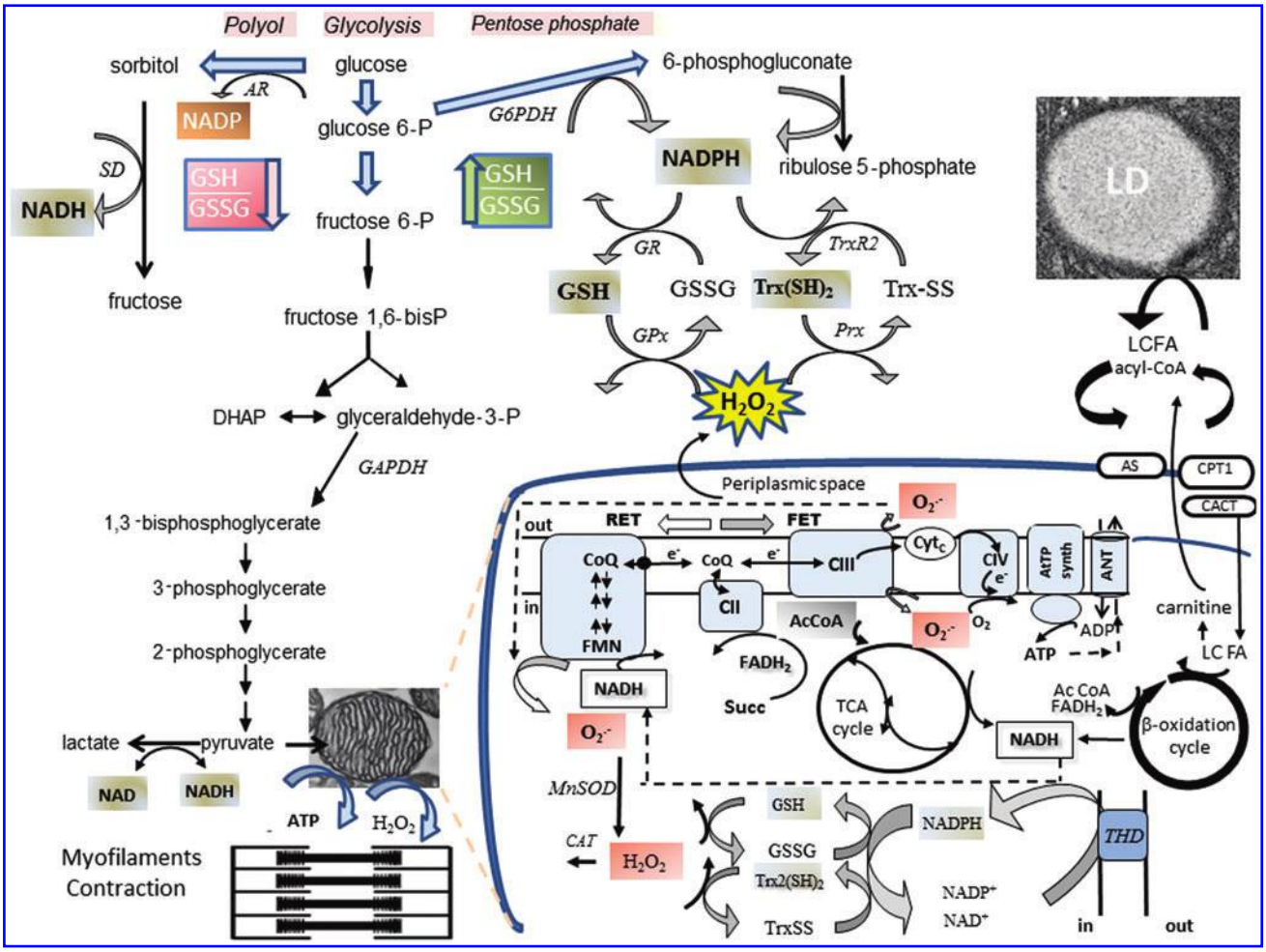

FIG. 1. Redox systems, glucose, and lipid degradation pathways in the heart. Depicted are mitochondrial reactive oxygen species (ROS)-driven cytoplasmic-redox balance, major energetic, and redox pathways in mitochondria and the antagonistic decreasing or increasing effects on GSH and NADPH pools produced by the polyol and Pentose Phosphate (PP) pathways, respectively. ROS can inactivate GAPDH *via* oxidation (68). Downstream glycolysis alternates the fates of pyruvate toward oxidation in mitochondria or excretion as lactate, resulting in both cases in NADH oxidation, affecting redox as well as cellular carbon balance. All these pathways are possible and may be relevant in a catabolic organ such as the heart. The cytoplasmic GSH/Trx scavenging systems are mirrored by a similar antioxidant array in the mitochondrial matrix. The scheme shows the respiratory complexes (I, II, III, IV), the ATPsynthase (ATPsynth), and the adenine nucleotide translocator (ANT) in the inner mitochondrial membrane. Also displayed are the major sites of superoxide ($O_2^{\bullet -}$) production and its conversion to H_2O_2 by manganese superoxide dismutase (MnSOD), the tricarboxylic acid cycle, and the H_2O_2-scavenging pathways in the matrix. $O_2^{\bullet -}$ can be generated by complex I and III of the electron transport chain through forward- or reverse-electron transport (FET or RET) that depends on NADH- or FADH-linked substrates such as glutamate/malate (G/M) or succinate (Succ), donating electrons to complex I or II, respectively. The two main modes of electron transport, FET and RET, produce markedly different ROS overflow (RET > > FET). In RET, electrons flow from CII to CI instead of CIII, and this occurs in mitochondria oxidizing high amounts of Succ, but FET is considered the physiological mode. The NADPH/$NADP^+$ couple with the highest (negative) redox potential (~ -360 to -400 mV) is the main electron donor of the large-capacity glutathione (GSH, GSSG) and the thioredoxin ($Trx[SH]_2$, TrxSS) systems that are responsible for scavenging H_2O_2 *via* glutathione peroxidase (GPx) and peroxiredoxin (Prx) enzymes, respectively. NADH is converted to NADPH *via* transhydrogenase (THD). On entry into the cell, long chain fatty acid (LCFA) first gets activated to LCFA-CoA and is either oxidized in the mitochondria *via* β-oxidation or forms triacylglycerol (TAG) by esterification that can accumulate in lipid droplets (LD). TAG catabolism starts with adipose triglyceride lipase (ATGL) followed by hormone-sensitive lipase (HSL) and monoacylglycerol lipase (MGL) that completes the lipolytic cascade by hydrolyzing monoacylglycerol (MAG) to glycerol and FAs. The LCFA thus produced is first activated to its Co-A form by acyl-CoA synthetase (AS) before entering the mitochondria *via* carnitine palmitoyl transferase 1 (CPT1) that catalyzes the conversion of LCFA-CoA to long-chain acylcarnitine, subsequently translocated across the inner mitochondrial membrane by carnitine acyl carnitine transferase (CACT) that exchanges carnitine for acylcarnitine. In the matrix, acylcarnitine is converted back to acyl CoA and catabolized *via* β-oxidation. The end product of each cycle of β-oxidation is acetyl-CoA (Ac CoA), shortening the LCFA by 2 carbons. Ac CoA then enters the TCA cycle for complete oxidation, rendering reducing equivalents in the form of the electron donors NADH and $FADH_2$ and leading to ATP synthesis *via* OxPhos in the respiratory chain. Ultimately, ATP is utilized by the contractile machinery to transduce chemical energy into mechanical work (myofilament contraction). ROS may also affect contractile performance *via* signaling or redox modification of sensitive Cys from, for example, myosin heavy chain, ryanodine receptor. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

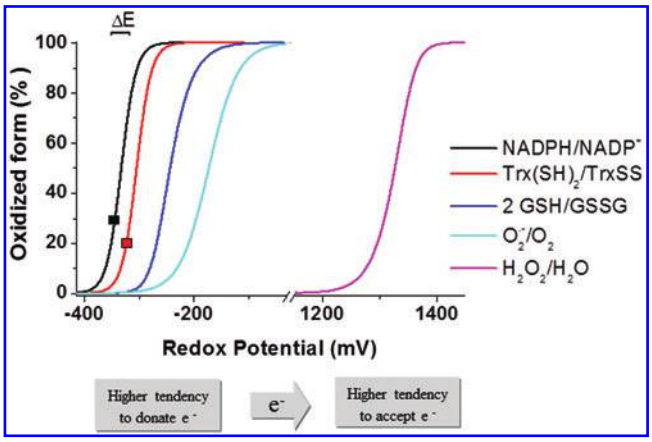

FIG. 2. Nernst representation of main mitochondrial redox couples and the direction of electron flow. Values plotted were obtained with Eq. 2 (see ''Redox biology''). The $NADP^+$/NADPH couple exhibits the highest reductive potential (*i.e.*, more negative redox potential), thus providing the reducing equivalents (electrons) needed for the other redox couples. Due to their ROS (H_2O_2)-scavenging capacity, the thioredoxin (Trx) and glutathione (GSH) systems become oxidized; hence, they need to be continuously provided with electrons from NADPH. Both Trx and GSH systems can reduce H_2O_2 to water (through peroxiredoxin and glutathione peroxidase, respectively), in a reaction that is highly favorable thermodynamically. Colored *squares* correspond to physiologically relevant redox potentials. The standard redox potentials used in this graph are as follows: −320 mV for NADH and NADPH, −292 mV for $Trx(SH)_2$, −240 mV for GSH, −160 mV for superoxide, and 1.349 V for H_2O_2. In the *y*-axis,% oxidized form = ([oxidized]/total)*100. The analysis was performed at 37°C, pH = 7.2. Reproduced from Kembro *et al.* (107). To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

The flow of electrons from substrates to NAD^+ is regulated at many levels to balance utilization of carbohydrate, fat, and amino acid-derived precursors (Fig. 1) (33, 43, 88, 165). NAD^+ is used as a substrate for several enzymes, including poly(ADP-ribose) polymerases-1 (PARP-1) and sirtuins, whose targets and end products critically regulate growth and death of cardiovascular cell types (148).

Protein Cys thiols are sensitive redox sensors because of their low pKa, that is, deprotonated as thiolate anions at physiological pH. This ionization markedly enhances reactivity with oxidants such as H_2O_2, providing a basis for selective redox signaling because only select thiols exist in this state (3, 59, 150). Protein sensors respond to alterations in local redox state with a change in conformation, stability, molecular interactions, and activity, thereby serving as signal transducers. Numerous proteins with diverse functions such as ion transporters, receptors, kinases, phosphatases, transcription factors, and structural proteins can serve as signal transducers in response to alterations in local redox state (33, 43, 197). A paradigmatic target for redox alterations is the cardiac ryanodine receptor (RyR2) with 90 Cys residues per subunit, of which approximately 20 are in a reduced state, thus sensitive to modifications, including S-nitrosylation, S-glutathionylation, and disulfide crosslinking (170). In general, the RyR2 open probability and therefore sarcoplasmic reticulum (SR) Ca release is increased by oxidizing conditions, whereas reducing reagents such as dithiothreitol reverses these effects (67, 170). Irreversible and detrimental RyR2 activation may be caused by prolonged exposure to a high dose of oxidizing agents (67, 129).

The mechanisms involved in the regulation of redox alterations at cellular and mitochondrial levels are relevant for diabetes, because preservation of mitochondrial and contractile functions depend on the RE (see ''Protecting mitochondrial function in the diabetic heart: an overview'').

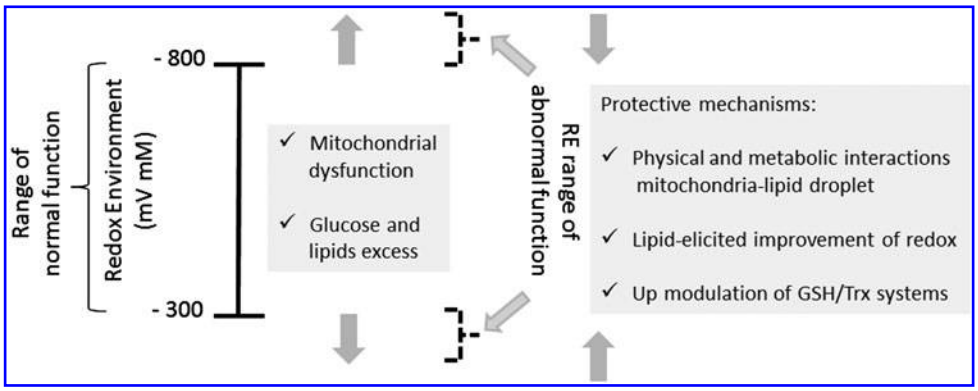

FIG. 3. Role of protective mechanisms in redox modulation of heart function in diabetes. The scheme postulates that protective mechanisms are relevant for the diabetic heart because of its vulnerability to oxidative damage. The redox environment (RE) values −300 to −800 mV m*M* depicted in the scale at the *left* of the scheme represent the extremes of the physiological range and were obtained both in experimental (52) and in computational modeling studies (105). The more oxidizing conditions under which the heart functions in diabetes impair contractility and Ca^{2+} handling. As a result of mitochondrial redox impairment, energetic and heart contraction become dysfunctional; contractility and Ca^{2+} transients are lower and relaxation is slower (16, 187). Compartmentalization plays a significant role in the control of ROS levels, the RE, and dynamic behavior, as suggested by experimentally validated computational modeling (105). Although each compartment exhibits its own dynamics, the interdependence of their REs is mediated by the exchange of redox species (*e.g.*, GSH, ROS).

Mitochondrial Dysfunction in Diabetes

Hyperglycemia, oxidative stress, and cardiac dysfunction

Both T1DM and T2DM result in hyperglycemia that contributes to oxidative stress, a major risk factor in the onset and progression of diabetes. Hyperglycemia is also a major driver of mitochondrial dysfunction in the diabetic myocardium affecting its morphodynamics (see Galloway and Yoon in this Forum) as well as energy/redox functions (135) (see Table 1 and ''Defining mitochondrial energetic and redox dysfunction in diabetes''). Obesity, aging, and unhealthy nutrition all contribute to oxidizing conditions that alter insulin sensitivity (156). A large body of clinical evidence correlates length of exposure and level of hyperglycemia to diabetic complications (115). Epidemiological and cohort studies compellingly showed that hyperglycemia is associated with worse cardiovascular outcome (160). Good, but not intensive (162, 200) glycemic control is effective in reducing cardiovascular morbidity.

As an initiating cause of tissue damage in diabetes (140), acute hyperglycemia is associated with enhanced oxidative stress biomarkers such as F2-isosprostanes. This relationship is supported by the decrease in oxidative stress elicited by anti-diabetic drugs (*e.g.*, biguanides, thiazolidinediones) (78). Long-term hyperglycemia leads to accumulation of glycated biomolecules and advanced glycation end products (AGEs). Glycation corresponds to chemical (nonenzymatic) reactions of reducing sugars with amino groups from, for example, proteins, lipids, nucleic acids, and impairing their function [for a review, Giacco and Brownlee (77)]

Major mechanisms mediating hyperglycemia-elicited tissue and mitochondrial damage are as follows (77): (i) increased flux of glucose and other sugars through the polyol pathway (Fig. 1) and intracellular formation of AGEs; (ii) enhanced expression of the receptor for AGEs and its activating ligands; (iii) activation of protein kinase C isoforms; and iv) over-activity of the hexosamine pathway. These mechanisms can be activated by mitochondrial ROS overflow (Fig. 1), and the idea was advanced that this may be due to hyperglycemia, leading to tissue damage in the diabetic microvasculature (77).

Another important mechanism of hyperglycemic damage involves alterations in mitochondrial DNA (mtDNA) that encodes 37 genes, among them 13 essential OxPhos components (198), thus potentially causing energetic failure and contributing to atherosclerosis (215). Recent work postulates that the metabolic status determined by the balance of substrate supply and demand (*e.g.*, influenced by physical activity or overnutrition) affects mitochondria fission/fusion dynamics, redox/energetics, and the integrity of their genome (152). Substrate excess would promote mitochondrial fragmentation favoring mtDNA modification, whereas higher demand than supply (*e.g.*, by caloric restriction, exercise) would trigger mitochondrial fusion bolstering cell survival while maintaining mtDNA integrity (152). The importance of this topic has been further underscored by latest work showing that relatively subtle changes in heteroplasmic levels can have dramatic effects on a patient's phenotype (153).

Deleterious mtDNA mutations can create an intracellular mixture of mutant and normal mtDNAs, a state known as ''heteroplasmy'' (147). Using one of the most common human pathogenic mtDNA point mutation, the 3243A > G that perturbs mitochondrial protein synthesis, Picard and collaborators created cybrids with a different heteroplasmy dose (153). Increasing mtDNA heteroplasmy caused changes in mitochondrial energetics, ultrastructure, cristae and matrix electron density, and cytoplasmic ribosomal content. Distinct heteroplasmic levels may explain perturbations in mitochondrial energetics (see: Defining mitochondrial energetic and redox dysfunction in diabetes), and *via* mitochondrially mediated retrograde signaling (involving Ca^{2+}, redox, and metabolic intermediates) (153), determine distinct patterns of nuclear gene expression associated with different disease phenotypes with diabetes being among them. Mechanisms mediated by mtDNA damage may be relevant for DC, because they connect basic mitochondrial biology such as fusion/fission (see Galloway and Yoon, in this Forum) that impinge on apoptosis (130) and are closely integrated with the mitophagy quality control pathway (147, 192) (see Kubli and Gustafsson, in this Forum). This is a crucial research area with potentially very important therapeutic implications.

Hyperglycemia not only adversely affects the microvasculature but also alters myocardial redox stress, in both experimental and human diabetes. In the presence of high glucose, the activity of the Trx system was attenuated in response to an increase in abundance of its inhibitor TxnIP, leading to oxidative stress. The increase in TxnIP occurred in experimental models of T1DM (streptozotocin [STZ]-treated rats) and T2DM (Goto–Kakizaki rats), as well as in human biopsy specimens from patients with T2DM and coronary artery disease (46). In two animal models of T2DM, the Zucker Diabetic Fatty (ZDF) rat and the *db/db* mouse (16, 55, 187), the increase in polyols mediates, at least in part, the redox imbalance exhibited by the diabetic heart (Fig. 1) (55). Different mechanisms may explain how hyperglycemia-driven polyol activation can lead to tissue injury, including consumption of NAPDH (27, 208) and AGEs generation (157). This evidence motivated clinical studies of aldose reductase inhibitors that show beneficial effects on diabetic neuropathy (82, 97) and cardiac performance (168), the latter thought to be due to improved cardiac autonomic function. Another potential mechanism involves glucose shunting from glycolysis to the polyol pathway driven by inactivation of the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH). GAPDH activity can be inhibited by increased NADH:NAD ratio from NADH generated by sorbitol dehydrogenase in the polyol pathway (27), and/or H_2O_2-mediated oxidation of critical Cys-149 and −153 from its catalytic domain (68).

Protecting mitochondrial function in the diabetic heart: an overview

Here, we will refer to protective mechanisms as those leading to a differential reaction of the cell/organism based on a distinct gene expression-physiological make up that result in an advantageous functional response to short- and long-term demand or stress. A detailed understanding on *what*, *when*, and *how* the heart activates these protective mechanisms may contribute novel therapeutic approaches by means of learning how to stimulate them.

Alterations in the mitochondrial metabolic state affect the energy/redox status of cardiomyocytes, thus negatively reverberating on heart mechanical and electrical functions (43,

Table 1. Energetic-Redox Impairments of Heart Mitochondria in Diabetes

Source (experimental model)	*Energetic*	*Redox*	*Ref.*
Human atrium (T2DM)	↓ St.3 (G/M)[a]	↑ H_2O_2 emission (Pcarnit/M-/Glut-/Succ; St. 4/3)[b] ↓ GSH/GSSG[c]	(1)
Human atrium (T2DM)	↓ St.3 SSM(G/M; Pcarnit/M)[b] ↔ IFM (G/M; Pcarnit/M)[b] ↓ Complex I, IV activ.&WB (SSM) ↔ Complex I, III, IV activ.&WB (IFM)	n.d.[d]	(58)
Human atrium (overweight, obese, diabetic)	↓ St3 (Pcarnit; Pyr/M)(N, OW, O/D)[a] ↓ RCR (Pcarnit; Pyr/M)(N, OW, O/D)[a] ↓ Complex I (OW, O/D) ↓ Complex II+III (N, OW, O/D) ↔ Complex IV (N, OW, O/D)	↑ ROS (N, OW, O/D)[a] ↑ MnSOD, Cat (N, OW, O/D) ↔ Cu,Zn SOD	(135)
ob/ob mice (T2DM)	↓ St.3, St.4 (G/M; Pyr/M)[a] ↓ St.3 (Pcarnit/M)[a] ↓ ATP synthesis ↓ Complex I, III, V (WB)	n.d.[d]	(22)
db/db mice (T2DM)	↓ St.3 (G/M; Pyr/M)[a] ↓ St.3 (Pcarnit/M)[a] ↓ ATP synthesis (G/M; Pyr/M)[a] ↓ Complex V, ANT (WB)	↑ H_2O_2 emission (Succ/Oligo/Rot; St. 4)[b] ↑ lipid and protein peroxidation (MDA, 4-HNE)[e]	(23)
db/db mice (T2DM)	↓ St.3 SSM (G/M)[b] ↓ St.3, St.4 SSM (Pcarnit)[b] ↓ Complex I, III, IV, V (SSM) ↔ Complex I, III, IV, V (IFM) ↓ $\Delta\Psi_m$ SSM ↓ ANT SSM (WB)	↑ MDA+4-HAE (SSM)[b] ↔ MDA+4-HAE (IFM)[b]	(60)
db/db mice (T2DM)	↓ RCR (G/M; Succ; TMPD)[b] ↓ Complex I	↑ H_2O_2 emission (G/M; Succ; St.4 & St.3)[b] ↓ TrxR2/Trx2/Prx3[b] ↔ Cat, MnSOD, GPx4, Prx3, GR, TrxR2, Trx2 (WB)[b,e]	(187)
db/db mice (T2DM)	↓ St.3 (Pyr/M; Pcarnit/M)[b]	↓ NADH, NAD+NADH[b]	(112)
Akita mice (T1DM, insulin deficient)	↓ St.3 (G/M; Pyr/M)[b] ↓ ATP synthesis (G/M; Pyr/M)[b] ↓ Complex V, ANT (WB)[e]	↓ H_2O_2 emission ↔ Aconitase activity; SOD2, Prx3 (WB)	(32)
STZ-treated Wistar rat (T1DM)	↓ St.3 (Pyr/M; αKG)[b,f] ↓ PDH[b] + Ins: ↑ St.3	n.d.[d]	(72)
STZ-treated Sprague-Dawley rat (T1DM)	↓ St.3 (G/M; Succ/Rot)[b,g] ↑ St.4 (G/M; Succ/Rot)[b] + Ins: ↑ St.3&4 (G/M; Succ/Rot)[b,] ↓ Activity complex I and II (SDH)[b]	↑ 4-HNE (70 kDa protein FDA subunit SDH)[b] + Ins: ↓ 4-HNE	(114)
STZ-treated Sprague–Dawley rat (T1DM)	↓ St.4 (Succ)[b,h]	↓ H_2O_2 emission (G/M)[b] ↑ GSH ↔ $O_2^{\bullet-}$	(92)
OVE26 mouse (T1DM)	↓ RCR (Pyr/M)[b] ↓ St.3	↓ GSH[b]	(175)
STZ-treated Swiss-Webster mice (T1DM)	↓ St.4 SSM(Succ)[b,i] ↓ St.4 IFM (G/M; Succ; Cyt C/DUBQ [complex III])[b] ↔ IFM (G/M; Pcarnit/M)[b]	↑ MDA+4-HAE SSM[b] ↑ NT, MDA+4-HAE IFM[b]	(61)

[a]Measured in permeabilized atrial myofibers.
[b]Measured in isolated mitochondria.
[c]Measured in atrial tissue.
[d]Not determined.
[e]Measured in tissue homogenate.
[f]From Flarsheim *et al*. (72), 2–4 weeks after STZ-treatment.
[g]Data from Lashin *et al.* (114) correspond to 4–6 weeks' diabetic rats after STZ treatment (single injection), and 2 weeks of insulin treatment of 6 weeks' diabetic animals.
[h]Data informed from Herlein *et al.* (92) correspond to 2 months' diabetic rats (changes were not evident after 2 weeks of STZ treatment).
[i]From Dabkowski *et al.* (61), 5 weeks after STZ treatment.

↑, increase; ↓, decrease; ↔, unchanged; activ.&WB, activity and Western blot; αKG, alpha ketoglutarate; αKGDH, alpha ketoglutarate dehydrogenase; Cat, catalase; Cyt C/decylubiq, cytochrome C/decylubiquinol; $\Delta\Psi_m$, mitochondrial membrane potential; G/M, glutamate/malate; GPx4, glutathione peroxidase 4; GR, glutathione reductase; 4-HNE, 4-hydroxy-2-nonenal; 4-HAE, hydroxyalkenal; IFM, interfibrilar mitochondria; +Ins, insulin-treated diabetic animal; MDA, malondialdehyde; MnSOD, manganese superoxide dismutase; Cu,Zn SOD, copper, zinc superoxide dismutase; N, OW, O/D, normal, overweight, obese/diabetic; NT, nitrotyrosine; Oligo, oligomycin; PDH, pyruvate dehydrogenase; Pcarnit, palmitoyl carnitine; Prx3, peroxiredoxin 3; Pyr/M, pyruvate/malate; Rot, rotenone; RCR, respiratory coupling ratio (St.3/St.4); St. 3 or 4, states 3 or 4 respiration; SSM, subsarcolemmal mitochondria; Succ, succinate; TrxR2, thioredoxin reductase 2; Trx2, thioredoxin2; SDH, succinate dehydrogenase; WB, Western blot.

187). The heart from diabetic subjects is constantly challenged by elevated circulating levels of glucose and FAs. Mitochondria are both source and target of oxidative stress, thus OxPhos should be somehow sheltered from marked variations in cardiac redox conditions to maintain proper myocardial function. A balanced cellular/mitochondrial RE is vital for optimal energy supply and excitation-contraction (EC) coupling (Fig. 3) (see ''Redox-optimized ROS balance''). Thus, key mitochondrial functions that keep reliable energy supply and control of ROS emission to levels compatible with signaling can be the target of protection.

Protective mechanisms are relevant for the human diabetic heart because of its vulnerability to oxidative damage, according to the following basic physiological facts:

(i) The human heart handles the highest amount of O_2. This organ represents 0.4% of the total body weight and processes 11% of the total O_2 consumed (166); thus, the specific ratio of the relative amount of O_2 consumed per relative organ weight is 27.5 (=11%/0.4%), higher than the relative O_2 consumption by other organs. Comparatively, the skeletal muscle represents 42%, the brain 2%, and the lungs 0.9% of the total body weight and consumes 20%, 10%, and 4%, respectively, of the total O_2; thus, the specific ratios are only 0.48, 5, and 4.5. According to these numbers, the heart handles 57-, 5.5-, and 6-fold higher amounts of O_2 on a specific basis than skeletal muscle, the brain, and the lungs, respectively. If we consider that cardiomyocytes are postmitotic cells, and that they process these high amounts of O_2 throughout an individual's lifetime, then it can be easily understood that the heart is especially exposed to possible oxidative damage.

(ii) More than 90% of heart metabolism is aerobic, and OxPhos represents the dominant source of energy for matching metabolic/contractile demand. Thus, mitochondria provide the bulk of the ATP needed for cardiac muscle contraction and sarcolemmal and sarcoplasmic ion transport (166, 180).

In diabetes, cardiac function occurs under more oxidizing conditions, in part due to the aforementioned effects of hyperglycemia (see ''Hyperglycemia, oxidative stress and cardiac dysfunction''). In response to oxidative stress, the ROS-scavenging systems in the mitochondrial matrix can be up-modulated. The key role played by redox balance in diabetic muscle was demonstrated by preincubating cardiomyocytes or heart trabeculae from T2DM mice and rat with the cell permeable GSH ethyl ester (GSHee) to rescue contractility in high glucose (16, 187).

Oxidizing conditions in diabetes readily affect contractility and Ca^{2+} handling (1, 187). As a result of mitochondrial redox impairment, energetics and heart contraction become dysfunctional; contraction and Ca^{2+} transients are lower and relaxation is slower. In T2DM diabetic mice as compared with control animals, energy/redox stressful conditions produced evident contractile impairment (15, 16, 51, 187). From an energetic standpoint, three main deficits were observed (Table 1): (i) lower mitochondrial respiration; (ii) reduced activity of individual respiratory complexes; and (iii) impaired OxPhos coupling. The energetic deficit was concomitant with mitochondrial redox alterations that affected the organelle itself as well as the cytoplasmic redox status. Interventions targeted to improving cytoplasmic redox propagated to mitochondria, thereby improving the cellular redox status and the contractile response (187). Mitochondrial thioredoxin reductase (TRxR2), together with the reductase and peroxidase of the GSH system, are major ROS emission controllers from mitochondria, especially during state 3 respiration (8, 105). Improved redox status rescued cardiomyocyte contraction in T2DM mice (187).

These results demonstrate that myocardial redox and contraction are causally related, and that reciprocal influence exists between the REs of cytoplasmic and mitochondrial compartments (95, 105, 106). Overall, the data strongly suggest that mitochondrial energetic/redox dysfunctions underlie defective EC coupling in the diabetic heart.

Defining mitochondrial energetic and redox dysfunction in diabetes

Impairment of mitochondrial respiration, alterations in structure, and respiratory chain complexes have been described in both T1DM and T2DM animal models (Table 1). Diabetes leads to defects in the electron transport chain that promote OxPhos uncoupling, defective electron transport, and excess ROS (see ''Hyperglycemia, oxidative stress and cardiac dysfunction''). Earlier (72, 112, 154) and more recent studies (32,114,175) demonstrated impaired state 3 respiration in heart mitochondria isolated from STZ-treated diabetic rats and T2DM *db/db* and obese *ob/ob* mice models.

The state $4 \rightarrow 3$ transition is fundamental in mitochondrial physiology, representing the shift from highly energized (*i.e.*, high $\Delta\Psi_m$) and reduced redox status, with low respiration and ATP synthesis fluxes, to high respiration and ATP synthesis in response to elevated ATP demand (52). Impaired energetic transition concomitant with decreased OxPhos coupling due to higher state 4 respiration and low respiratory reserve have been observed in heart mitochondria from *db/db* mice (60, 187) and STZ-treated Sprague–Dawley rats (114) (Fig. 4). Impairments in the state $4 \rightarrow 3$ transition both at the level of electron transport in the respiratory chain and of the ANT and/or ATP synthase are likely liable, at least in part, for the sustained rise in mitochondrial ROS levels from *db/db* hearts (187) (see ''Mitochondrial H_2O_2 emission and the antioxidant systems: critical targets of protective mechanisms''). Another mechanism of mitochondrial uncoupling in hearts from *db/db* mice involves increased uncoupling protein (UCP) activity (31). The increased proton leak in *db/db* mice mitochondria was almost completely restored to control levels after addition of GDP, acting as an inhibitor of UCPs. Superoxide and FAs can activate UCPs, for example, UCP3 in skeletal muscle. Based on the premise of UCP activation by superoxide, a qualitative model of ROS-induced mitochondrial uncoupling and reduced cardiac efficiency in T2DM was proposed (31). According to this model, the T2DM heart increased reliance on FAs and their higher delivery of reducing equivalents to the electron transport chain enhanced ROS generation, with lipid peroxidation, in turn, augmenting UCP activity. Increased mitochondrial uncoupling, without concomitant ATP synthesis, will increase cardiac oxygen consumption and FA oxidation further. In *ob/ob* mice, despite reduced expression of complexes I, III, and V of the respiratory chain, FA-induced increase in heart O_2 consumption occurred along with decreased ATP/O ratios

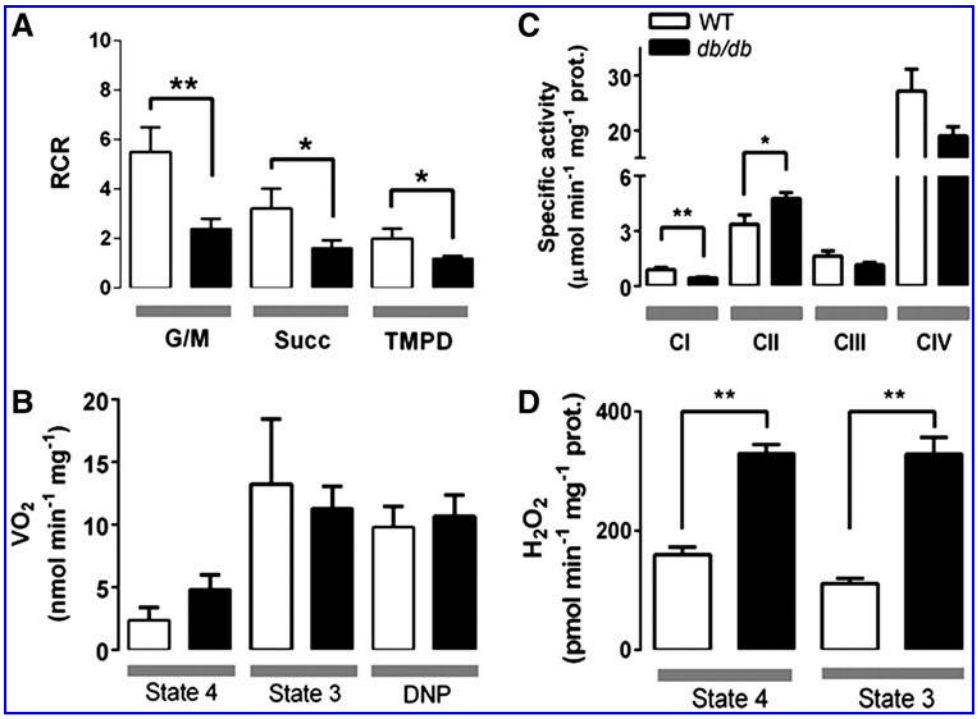

FIG. 4. Energetic and redox behavior of WT and *db/db* mouse mitochondria. Respiration in freshly isolated heart mitochondria from WT and *db/db* mouse was analyzed with a SeaHorse Bioscience XF96 analyzer (187). **(A)** Mitochondria were assayed under state 4, that is, substrate but no ADP, and state 3, that is, substrate and ADP present, respiration (VO_2) with substrates from complex I (5 m*M* glutamate/malate, G/M), complex II (5 m*M* Succinate, Succ), or complex IV (0.5 m*M* TMPD and 3 m*M* Na-ascorbate). State 3 was induced with 1 m*M* ADP in all cases. The Respiratory Coupling Ratio (RCR) was determined as the ratio of state 3/state 4. The bars plotted correspond to $n = 8$ replicates from two experiments. **(B)** Displayed are the state 4, state 3 and uncoupled respiration with dinitrophenol (50 μM DNP) of mitochondria under FET (5 m*M* G/M). **(C)** Specific activity of the individual respiratory complexes from *db/db* and WT heart mitochondria. The activity of individual complexes from the respiratory chain was determined in freshly isolated mitochondria monitored by spectrophotometry after the reduction or oxidation of a natural or artificial substrate (19). Complex I was determined through the oxidation of NADH at 340 nm. Complex II was assessed by monitoring the reduction of dichlorophenol indophenol at 600 nm. Complex III and complex IV activities were monitored after the reduction or oxidation of cytochrome c at 540 nm, respectively. **(D)** Shown are the H_2O_2-specific emission rates in state 4 or state 3 respiration under FET (5 m*M* G/M). H_2O_2 was monitored with the Amplex Red assay. The data presented in this figure are redrawn from Tocchetti *et al.* (187).

and increased mitochondrial state 4 respiration, suggesting mitochondrial uncoupling (22).

Impaired cardiac efficiency and mitochondrial uncoupling have been observed in some but not all animal models of T1DM. For example, heart mitochondria from Sprague–Dawley rats after 2–4 weeks of STZ treatment exhibited significantly decreased state 3 and increased state 4 respiration with substrates of complex I and II (Table 1); these defects could be recovered by insulin (114). However, insulin-deficient and severely hyperglycemic Akita mice $Ins2^{+/-}$ that exhibit improper folding of pro-insulin due to a mutation in the *Ins2* gene (167) displayed diminished state 3 respiration and ATP synthesis with complex I substrates but not reduced cardiac efficiency despite increased FA utilization (32). Similar to Akita mice, 2 months' STZ-induced T1DM rats showed increased UCP3 expression, but decreased mitochondrial state 4 respiration at unchanged ADP/O ratios (92) (Table 1).

Using an *in situ* approach, Anderson *et al.* showed that mitochondria from human diabetic hearts have specific impairments that limit their maximal capacity to oxidize palmitoyl-carnitine and glutamate (1). These authors also showed that the propensity to release ROS was elevated in the diabetic mitochondria and correlated with GSH depletion and other markers of oxidative stress. Thus, mitochondria contribute toward increasing the oxidative burden and toward a marked decline in the electro-mechanical function in diabetic hearts over time, ultimately leading to heart failure (1). Recent studies performed in diabetic human subjects with body mass index (BMI in kg/m^2) corresponding to obese (>30), overweight (25–30), and normal (<25) provided direct evidence of the link between mitochondrial dysfunction and myocardial contractile impairment at the onset of clinical cardiomyopathy (135). State 3 respiration and OxPhos coupling measured as respiratory coupling ratio (RCR) were significantly decreased in atrial tissue exposed to palmitoyl carnitine or pyruvate/malate from diabetic patients, independently from BMI. The reduced respiratory activity was underlain by decreased activities of complex I and complex II + III, whereas complex IV was not affected (135). Importantly, ROS levels were increased along with up-modulation of MnSOD and catalase at unchanged Cu, Zn SOD (Table 1).

Association between increased free radical generation and progression of diabetic complications in various tissues

(including the heart) has been demonstrated in numerous studies [see Bugger and Abel and Styskal *et al.* (31, 184) for review]. Abundant and compelling experimental and clinical evidence indicate that ROS levels are increased in multiple tissues in T1DM and T2DM (31, 179, 184). Several reports have shown ROS-mediated post-translational modifications in complex II in STZ-T1DM rats (114) or in enzymes from energy metabolism in alloxan-T1DM rats (189). During the heart adaptation to diabetes, a rapid and highly coordinated upregulation of protein expression from β-oxidation occurred while TCA cycle and electron transport proteins remained unchanged or were slightly decreased (190).

Cardiomyocyte contractile deficiency due to oxidative stress could be prevented in T1DM or T2DM (*db/db*) mice by overexpressing catalase (211), the mitochondrial form of superoxide dismutase, MnSOD (174) or increasing the GSH pool (187). In the latter case, increased cellular GSH decreased overall oxidative stress and rescued TRxR2 activity (182, 187). In the OVE26 T1DM mouse, state 3 and OxPhos coupling were significantly decreased along with the mitochondrial GSH pool (175). Crossing a transgenic mouse overexpressing heart-specific MnSOD with OVE26 normalized mitochondrial energetic/redox and rescued impaired cardiomyocyte contractility (174) (Table 1).

Other antioxidant components than MnSOD, such as glutathione peroxidase 4 (GPx4) and thioredoxin 2 (Trx2), play a crucial role in avoiding oxidative stress and preserving from oxidative damage. Unlike homozygous knockout mice for MnSOD that die postnatally (118), GPx4 or Trx2 knockout results in embryonic lethality (151, 210). Heterozygous knockout mice for MnSOD (*Sod2*$^{+/-}$, GPx4 (*Gpx4*$^{+/-}$) or Trx2 (*Trx2*$^{+/-}$) display $\sim$50% reduced expression of these proteins in several tissues, becoming less resistant to oxidative stress while accumulating oxidative damage (101, 151, 184, 210). Importantly, mitochondria isolated from *Trx2*$^{+/-}$ mice exhibit significantly lower ATP generation and higher levels of H_2O_2 emission ($\sim$30%) than wild type, with brain and heart mitochondria being the most affected (151). On the contrary, transgenic mice overexpressing SOD2 (100) or GPX4 (158) show decreased lipid peroxidation and maintained mitochondrial ATP synthesis, concomitant with less vulnerability to paraquat- or diquat-elicited oxidative stress and cell death.

Peroxiredoxins (Prxs) are important pieces of the cellular antioxidant array system. Six Prxs, 1–6, are known in mammals, of which Prx 1, 2, and 6 are cytoplasmic; Prx4 is located in the endoplasmic reticulum and Prx3 is located in the mitochondria, while Prx5 is distributed in various compartments, among them being peroxisomes and mitochondria (56). The key roles played by Prxs in maintaining proper cellular redox balance and signaling have been highlighted by many studies dealing with transgenic mice. Knockout mice for Prx3 (*Prdx3*$^{-/-}$) (117), Prx 4 (*Prdx4*$^{-/-}$) (64), and Prx6 (*Prdx6*$^{-/-}$) (206) exhibit higher sensitivity to oxidant stressors, for example, paraquat or t-butylhydroperoxide, and lower survival rates accompanied by tissue damage and enhanced protein oxidation levels. In contrast, mitochondria from transgenic mice overexpressing Prx3 (PRDX3) (40, 131) have reduced levels of H_2O_2 emission, and cells from the same mice exhibit increased resistance to cell death and apoptosis along with enhanced glucose sensitivity as manifested by reduced blood glucose levels and increased glucose clearance. Similar findings were reported for transgenic mice overexpressing human PRDX4 when subjected to STZ-induced T1DM (64). *Prdx6*$^{-/-}$ knockout mice develop a phenotype similar to early-stage T2DM as can be judged by reduced glucose-dependent insulin secretion, increased insulin resistance, and altered plasma lipid and inflammation profiles (149). The recent work of Pacifici *et al.* (149) is relevant, because it demonstrates that redox balance can be an upstream event leading to the onset of diabetes, since Prx6 activity is sensitive to oxidation and inhibited by H_2O_2 excess (161). The fact that unlike *Prdx6*$^{-/-}$ (149), *Sod1*$^{-/-}$ knockout mice showed glucose intolerance without changes in peripheral and hepatic insulin sensitivity (139) underscores the pivotal impact of Prx6 alteration on redox balance/signaling, and the role that this modification may have on the physiopathology of diabetes and its related complications.

The cytoplasmic GSH/Trx systems from both T2DM mice and rat hearts can be challenged by oxidant stress triggered by high glucose. In *db/db* mice, the additional burden of enhanced ROS emission from mitochondria is also present (23, 187) and in the ZDF rat there is an apparent deficit in the cytoplasmic GSH pool (16). Since the redox status of cytoplasmic and mitochondrial compartments is inter-dependent (105), a more oxidative cytoplasmic environment can potentially influence mitochondrial redox assets (5, 103, 112, 187) and cause lipid and protein oxidative damage as well in both T2DM (23, 60) and T1DM (61, 114) (Table 1).

T1DM and T2DM can exert differential effects on mitochondrial subpopulations, subsarcolemmal (SSM), and interfibrillar (IFM) in human and rodents (58, 60, 61). In T2DM mitochondria from human atrium (58) and *db/db* mice hearts (60), the SSM (not the IFM) subpopulation was affected. Decreased complex I and IV activities and protein levels likely underlie the state 3 respiration impairment exhibited by SSM in the presence of substrates of complex I and palmitoyl carnitine/malate in humans (58) (Table 1). In T2DM mice, the SSM subpopulation exhibited increased levels of markers of oxidative damage (MDA + 4-HAE) accompanied by defective state 3 respiration and decline in the activities of complex I, III, IV, and V, with the latter concomitant with decreased $\Delta\Psi_m$ and protein levels of the adenine nucleotide translocator (60) (Table 1). In T1DM STZ-treated mice (Table 1), mitochondria from the IFM subpopulation were the most affected, exhibiting decreased state 4 respiration with substrates from complex I, II, and III (61). Increased $O_2^{\bullet -}$ generation along with markers of oxidative damage of protein (nitrotyrosine) and lipids (MDA and 4-HNE) were also present in the IFM subpopulation from T1DM mice (61). Although present knowledge indicates that T1DM primarily influences the IFM whereas T2DM affects the SSM, the underlying reason for this is unclear.

Overall, the available evidence shows that several components of the mitochondrial respiratory chain and the antioxidant defenses are affected in diabetic animals, especially T2DM. Indeed, mitochondria represent a source of oxidative stress in diabetes and, as a result, their function can be impaired. A basic understanding of the differential response exhibited by IFM and SSM mitochondrial subpopulations inside the cardiomyocyte is a subject of great potential interest for tuning therapeutic strategies.

Complex I, a key target of energetic dysfunction. Complex I is a proton pump that is redox driven by electron

transfer from NADH to ubiquinone (CoQ). Complex I constitutes the largest respiratory protein complex containing a covalently bound, redox-active flavin, as well as multiple iron–sulfur centers.

Mitochondrial respiration from substrates of complex I appears to be one of the most sensitive to the diabetic condition (Table 1 and Fig. 4C). In T2DM, the RCR (state3/state4) was significantly decreased in *db/db* mice (Fig. 4A). Post-translational modification of mitochondrial subunits from complex I can either promote or attenuate the generation of $O_2^{\bullet -}$ (88). Thiol oxidation of complex I promotes $O_2^{\bullet -}$ generation, whereas S-nitrosation suppresses activity and reduces $O_2^{\bullet -}$ leak (44). Recent data show that reversible S-nitrosation of Cys39 on the ND3 subunit of complex I is cardioprotective during reperfusion after ischemic injury. This selective modification slows the reactivation of mitochondria during the crucial early minutes of reperfusion, thereby decreasing $O_2^{\bullet -}$ production (42). S-glutathiolation, which may be enhanced under oxidant stress, was shown to preserve complex I activity, decrease $O_2^{\bullet -}$ production under some conditions, and inhibit complex I activity to enhance $O_2^{\bullet -}$ production under others (186).

Although impairment of complex I appears as a relevant and constant defect across chronic T1DM and T2DM conditions, mitochondrial energetic dysfunction in diabetes is a complex failure, involving the other electron carriers. The *in vivo* scenario can be even more complicated. Indeed, it is generally accepted that electron transfer in mitochondrial membranes depends on random collisions between small diffusing molecules (coenzyme Q and cytochrome *c*) and complexes (I–IV), independently embedded in the bilayer (86, 93). However, an emerging concept is that there are preferential associations between specific complexes. In fact, the biogenesis and function of the mitochondrial respiratory chain involve organization in super-complexes or respirasomes of the electron transport carriers (17). In human cells, respirasome biogenesis involves a complex I assembly intermediate acting as a scaffold for the combined incorporation of complexes III and IV subunits. The process is completed with the incorporation of complex I NADH dehydrogenase catalytic module, which leads to the respirasome activation (136).

Metabolic control analysis was utilized to discriminate between the *random collision* and *super-complex* models of electron carrier organization in the mitochondrial membrane (17). According to the former model, each enzyme will be rate controlling to a different extent; whereas in the latter, the whole metabolic pathway would behave as a single super-complex and inhibition of any one of its components would elicit the same flux control. Using bovine heart mitochondria and sub-mitochondrial particles devoid of substrate permeability barriers, Bianchi and collaborators investigated the flux control coefficients of the complexes involved in aerobic NADH oxidation (I, III, IV) and in succinate oxidation (II, III, IV) (17). Complex I and III were found to be highly rate controlling compared with NADH oxidation, representing strong kinetic evidence of the existence of functionally relevant association between these two complexes, whereas Complex IV appeared to be randomly distributed (17).

The control exerted by complex I on respiration was found to be higher when analyzed in working heart trabeculae (53) than in isolated mitochondria (116). As respiration increases control of the respiratory flux, there is a shift from demand or ''pull'' to supply or ''push,'' implying from downstream NADH (*e.g.*, respiration, ANT, ATPase) to upstream (*e.g.*, TCA cycle) processes, respectively (49, 74).

Overall, complex I represents a relevant rate-controlling step of respiration in cardiac muscle subjected to workload. In chronic diabetes, the high control exerted by complex I on mitochondrial respiration depends on its low activity, likely due to low abundance, as compared with the other respiratory complexes, at least in mice (Fig. 4C). From control analysis, we expect a higher flux control from steps catalyzed by less abundant enzymes or electron carriers because of smaller V_{max}. Moreover, complex I is a target of chemical modifications, for example, Cys oxidation, nitrosylation, which negatively affect its activity under ischemic injury. Consequently, we conclude that complex I is a main target that is responsible for mitochondrial energetic dysfunction in T1DM and T2DM.

Mitochondrial H_2O_2 emission and the antioxidant systems: critical targets of protective mechanisms. In aging and heart failure, mitochondria-derived oxidative stress plays an important role in mediating dysfunction of these organelles and cell death. Modulating the release of H_2O_2 from mitochondria is crucial for overall cellular redox conditions. In turn, redox-dependent signaling pathways are vital for normal cell function (102, 184), for example, the well-known effect of ROS to trigger increased expression of antioxidant enzymes *via* the Nrf2 antioxidant response element (144).

The increase in oxidative stress with obesity has been linked to alterations in mitochondrial function and insulin resistance (184). Mechanistically, it was postulated that obesity causes insulin resistance through modulation of oxidative stress. The causal link between oxidative stress and insulin resistance was shown in insulin-responsive cell lines (3T3L1, L6 myotubes) that significantly decreased their insulin sensitivity in response to H_2O_2 (65, 75, 184).

In vivo studies performed with lines of knockout mice lacking SOD1 ($Sod1^{-/-}$) or transgenic mice overexpressing SOD1 show increased sensitivity to oxidative stress/damage causing agents (217) or ischemia/reperfusion (212) in the former case; conversely, augmented resistance to toxic/oxidant agents was present in the latter group (41, 134). Mitochondrially targeted overexpression of Cu,Zn SOD or expression of catalase conferred protection to cardiac oxidative stress and left ventricle (LV) dysfunction provoked by zidovudine (AZT), an antiretroviral used to treat HIV/AIDS (109). Conversely, Cu,Zn SOD depletion targeted to mitochondria worsened LV function. These studies indicate that the decreased propensity for or susceptibility to AZT-elicited oxidative stress confers protection to cardiomyocytes from lytic events that correlate with improved function of hearts overexpressing Cu,Zn SOD or catalase (109, 184).

Perturbation of signaling pathways, pro-inflammatory cytokines, and redox-mediated protein modifications are among the underlying mechanisms proposed to lead from oxidative stress to insulin resistance (184). The detrimental effects of obesity on glucose homeostasis can be diminished by reduction of oxidative stress associated with high-fat feeding. Under metabolic stress due to obesity or elevated consumption of high-caloric diets, antioxidants play a significant role in the regulation of glucose homeostasis (184). Superoxide dismutase (SOD) is a main player of the antioxidant systems;

two of the three main isoforms of SOD in mammals are CuZn SOD (SOD1) and Mn SOD (SOD2). SOD2 homozygous knockout mice do not survive (195), but heterozygous ones are viable and show a general trend to oxidative damage (184). For example, when glucose tolerance was examined in *in vivo* SOD2 overexpressing mice fed with a high-fat diet, they exhibited normal tolerance as opposed to the lower threshold found in wild-type animals under the same conditions (94). Thus, data from mice overexpressing SOD2 (94), catalase (2), or mitochondria-localized peroxiredoxin 3 (40) highlight the relevant role played by mitochondria in controlling both oxidative stress and glucose regulation under high-fat feeding conditions.

Where ROS emission is concerned, T2DM mitochondria exhibited remarkable differences as well as similarities. In mice, mitochondria from diabetic hearts displayed significantly greater H_2O_2 emission than wild type under forward electron transport (Fig. 4D), the physiological mode of electron transport. In stark contrast, mitochondria from diabetic rats showed lower ROS emission than their lean controls (16, 51). Another important difference was that in mitochondria from diabetic mice, ROS emission did not decrease during the state 4→3 transition (Fig. 4D). Unlike mice, mitochondria from diabetic rats lowered ROS emission in state 3 respiration to a similar extent than lean controls. These differences may originate from species-specific protective mechanisms. Indeed, heart mitochondria from mice divert a much higher portion of the respiratory flux to ROS than rats and guinea pigs (8).

The energy-redox link in mitochondrial function

Redox-optimized ROS balance. Mitochondria need to ensure robust and reliable energy supply while maintaining physiological levels of ROS compatible with signaling functions. The Redox-Optimized ROS Balance (R-ORB) hypothesis provides a useful conceptual framework to understand how mitochondria can satisfy both energetic and redox requirements. According to R-ORB (7), the net ROS efflux from mitochondria depends on ROS production by the respiratory chain and ROS scavenging by the antioxidant systems. The balance between these processes is altered at both extremes of the RE (Fig. 5). The relationship between respiration and ROS emission shows that simultaneous optimal energy output and low ROS can be achieved at intermediate values of the RE (52). The constant, combined redox action of GSH and Trx2 systems prevents ROS overflow from mitochondria, in both states 4 and 3 respiration (8, 182). Taken together, the available data indicate that, when mitochondrial respiration is maximal, ROS efflux is strongly dependent on RE and tends toward a *minimum*. This behavior is evident both at pseudo steady state and during dynamic transitions in respiratory state (52).

Energetic and redox behavior of mitochondria under stress. Two key questions arise about the energy-redox link of mitochondrial function: How is it affected by the diabetic condition? And, how do protective mechanisms operate to keep it unharmed? To answer these questions, we first need to better understand the energetic and redox behavior of mitochondria under oxidative stress. This is a significant biological problem, because it is accepted that aging and chronic diseases such as diabetes and obesity contribute to the progressive deterioration of the redox status of cells and the organism (66). Available evidence shows that mitochondria can be a source of oxidative stress in hearts from T2DM animals. Reduced insulin signaling and increased oxidative stress can lead to increased autophagy (31, 146).

Regarding how oxidative stress influences the dependence of ROS emission on respiration, recent work sheds some light on this problem (52). When challenged with oxidants, mitochondria displayed H_2O_2 emission levels two-fold higher than controls. Oxidative stress shifts the RE toward more oxidized states, apparently due to a 50% decrease in GSH

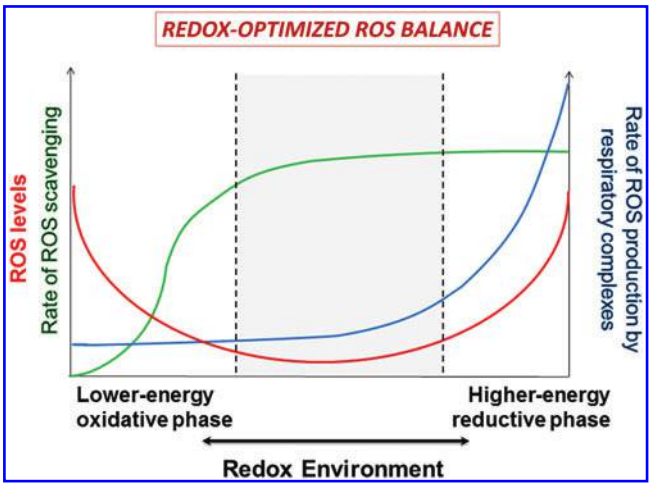

FIG. 5. The Redox-Optimized ROS Balance [R-ORB] hypothesis. According to R-ORB, the net flux of ROS released from mitochondria depends on ROS production by the respiratory chain and ROS scavenging. The concept of ROS balance accounts more explicitly for the role of the antioxidant defenses. Main postulates of the R-ORB hypothesis are as follows: (i) The extent of ROS imbalance is defined by the redox environment (RE); (ii) ROS levels attain a minimum, likely compatible with physiological signaling, at intermediate values of RE; and (iii) ROS overflow can occur at both extremes of redox environment, that is, highly reduced or highly oxidized, but the mechanisms involved are completely different. Under more reducing conditions, energized, tightly coupled, mitochondria will exhibit high redox potential and low respiratory flux. Thus, a higher probability of the respiratory carriers being reduced will happen, increasing ROS generation, in the presence of abundant O_2 (200 μM as compared with 3 μM in cells). Under these conditions, mild uncoupling will oxidize the RE, thus decreasing ROS in isolated mitochondria. In this way, the R-ORB hypothesis includes the mild-uncoupling mechanism, under the specific situation of a highly reduced RE. At more oxidized redox potentials, ROS overflow occurs as a consequence of overwhelmed ROS scavenging systems. This could be demonstrated in isolated mitochondria when additionally exposed to exogenous H_2O_2 and GSH depletion (7, 52). According to R-ORB, a decrease in ROS levels does not require compromising the efficiency of mitochondrial energy transduction (*e.g.* mild $\Delta\Psi$m uncoupling) but instead it postulates that under high energy demand, and despite large respiratory rates, ROS emission levels will be kept to a minimum by the ROS-scavenging systems. Reprinted by permission from Aon *et al.* (7). To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

(52). These redox changes were accompanied by lower respiration in stressed mitochondria. Likely, oxidative stress compromises the NADH availability for respiration due to augmented redirection of electrons to the ROS-scavenging systems, required to restore the antioxidant defenses. As a result, the mitochondrial energetic performance is lessened apart from ROS overflow.

Protective Mechanisms of Mitochondrial Function

Adaptive and maladaptive responses in diabetes

Adaptation is considered a beneficial set of mechanisms leading to a proper reaction of the organism toward metabolic disorder. In contrast, maladaptation implies mechanisms that result in disadvantageous effects on function. Specifically, within the altered glucose and FA metabolism commonly found in diabetes (185, 214), metabolic adaptation was deemed essential for maintaining proper heart contraction under stress.

Different kinds of mechanisms of metabolic and hormonal nature, including chemical modifications of signaling proteins, have been involved in either adaptive or maladaptive responses in diabetes. Impaired metabolism of energy-providing substrates and myocardial lipid accumulation are considered early events found in obese and insulin-resistant individuals. In humans and several animal models, obesity, insulin resistance, and T2DM are associated with an altered cardiac metabolism characterized by enhanced reliance on FAs and decreased glucose utilization despite hyperglycemia. These changes play a central role in the development of diabetic cardiomyopathy (DC) (172) (see ''Diabetic Cardiomyopathy,'' and Schilling; Sung *et al.*; and Schrauwen-Hinderling *et al.* in this Forum).

When the glycolytic flux exceeds the rate of glucose oxidation through OxPhos, glycolytic metabolites accumulate, for example, glucose, G6P, pyruvate, and lactate. In diabetes, the increased reliance of the heart on FAs results in pyruvate dehydrogenase complex (PDC) inhibition mediated by increased acetyl CoA levels and phosphorylation of pyruvate dehydrogenase kinase 4 (159). In addition, increased citrate levels also occur as a result of higher FA utilization, thus potentiating the phosphofructokinase (PFK) inhibition by ATP precluding glycolysis (159). Paradoxically, despite decreased glucose transporter expression, translocation, and insulin-mediated glucose transport, the rates of glucose uptake by the diabetic heart are comparable to the normal heart. With normal glucose influx into the cardiomyocyte, and the blocks at PFK and PDC, glucose metabolites accumulate (99, 159). This sequence is consistent with the Randle mechanism, according to which FAs inhibit glucose oxidation more than glycolysis and glycolysis more than glucose uptake (91).

The nuclear hormone receptor peroxisome proliferator-activated receptor α (PPARα) mediates the increase in gene expression involved in cardiac FA utilization (9, 11). Overexpression of cardiac PPARα in mice augmented the expression of FA utilization genes and, in parallel, decreased those related with glucose utilization, suggesting a role of this receptor in cardiac metabolism in diabetes (31).

A sequence of events for the initial adaptation and subsequent maladaptation of the heart to a diabetic environment was proposed (91):

- Elevated circulating FAs
- Activation of PPARα within the cardiomyocytes
- Induction of enzymes involved in FA oxidation
- Increased FA oxidation
- Inhibition of pyruvate dehydrogenase, limiting pyruvate oxidation that results in the accumulation of glycolytic intermediates as described earlier

With diabetes progression and/or the occurrence of additional stress on the heart (*e.g.*, hypertension), the following metabolic maladaptation may occur:

- Decreased PPARα expression
- Limit the FA oxidation capacity of the heart
- FA availability exceeds FA oxidation rates, provoking intramyocardial lipid accumulation
- Subsequent lipotoxicity plays a role in the development of contractile dysfunction

Energetic-redox adaptation in the diabetic heart subjected to hyperglycemia and workload

Hyperglycemia and hyperlipidemia associated with T2DM are thought to be responsible for decreasing mechanical efficiency of the heart (29, 205). Indeed, both factors pose a great challenge to mitochondrial function. However, it is still unclear whether FA utilization in the diabetic heart helps or hinders myocardial function in the presence of high glucose.

The current paradigm is that (i) lipid overload and the generation of lipid oxidation products contribute to myocardial damage (81, 193), and (ii) increased FA metabolism primarily inhibits glucose utilization through the classical negative feedback mechanism described by Randle (99, 159). The inhibition of glycolysis is then thought to contribute to dysfunction by redirection of sugar phosphates into detrimental side pathways (28).

In contrast to this prevailing wisdom, FAs such as palmitate (Palm) have been shown to help maintaining contractile function and speed of relaxation in isolated T2DM (*db/db*) myocytes or whole hearts treated with high glucose, but not in control hearts (Fig. 6) (187). High glucose (HG, 30 m*M*) had a strong negative impact on the isoproterenol (ISO) response in hearts and myocytes from *db/db* mice that could be reversed by the medium supplementation with Palm. HG+Palm restored the ISO effect on the Ca^{2+} transient and on sarcomere shortening in cells and improved LV hemodynamics in Langendorff-perfused *db/db* hearts (187). In contrast, in wild-type hearts, Palm addition to the HG perfusate inhibited LV function. A similar pattern was observed when contractile work was examined in cardiac trabeculae from control (Lean) and diabetic (ZDF) rats (16).

Mechanical efficiency for intact heart has been defined as the ratio of external cardiac power to cardiac energy expenditure (18). The latter was estimated from VO_2, given that the heart meets >95% of its energetic requirements under normal conditions *via* oxidation of carbohydrates and FAs (124). Previous work showed that the increase in FA oxidation augments myocardial VO_2, and cardiac efficiency is reduced in *ob/ob* and *db/db* mice, as well as in obese humans. On these premises, it was stated that cardiac efficiency is higher for a given VO_2, when the myocardium has relatively low rates of FA with respect to glucose and lactate oxidation (98, 124). One possible explanation for these apparently discrepant

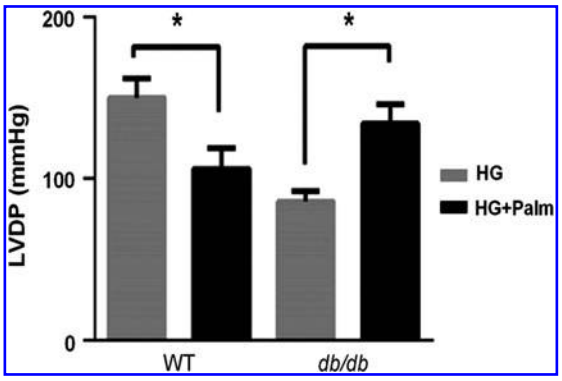

FIG. 6. Improvement of contractile function by palmitate in high glucose in *db/db* diabetic mouse. In the presence of high glucose (HG, 30 m*M*), palmitate (Palm) improved left ventricular developed pressure (LVDP) in perfused diabetic (*db/db*) mouse hearts but induced contractile dysfunction in wild-type (WT) hearts. Palm bound to albumin (4:1) concentration was 0.4 m*M* in WT and 0.8 m*M* in *db/db*. The data presented in this figure are redrawn from Tocchetti *et al.* (187). $*p < 0.05$.

results (15, 187) is that the vast majority of the previous studies was performed with low glucose (5–11 m*M*) (29, 98, 132, 213), including studies in ZDF hearts (39, 205). Work employing HG studied the heart susceptibility to ischemia-reperfusion injury in normal, nondiabetic rats (204). However, Boardman *et al.* (20) studied transitions from 5 to 26 m*M* glucose in T2DM (*db/db*) mouse hearts and observed decreased VO_2 concomitant with increased glucose *versus* FA oxidation, but the effects on contractility were not reported. The same group of investigators analyzed the effect of insulin on glucose, FA oxidation, and contractility in isolated working hearts from *db/db* mice. They found that with low or high glucose Palm decreased *db/db* heart performance (measured as cardiac output) as compared with wild type in both the absence and presence of insulin (87). Apparently, the increase in glucose oxidation and decrease in FA oxidation provoked by insulin under both substrate conditions did not influence cardiac output.

The differential response of the diabetic heart toward Palm in high glucose suggests the existence of at least short-term protective mechanisms directed to counteract the potential deleterious effects of substrate surplus.

Cardiac mechanisms for managing lipid affluence in diabetes

The omnivorous and flexible normal heart metabolism exploits the high energy yield potential of FA β-oxidation to generate ATP for its function (57). Under conditions of nutrient excess, the heart is able to increase lipid oxidation, but when this capacity is exceeded, FAs are diverted to neutral lipid pools. This response is believed to contribute to metabolic inflexibility, energetic inefficiency, insulin resistance of the myocardium, cellular damage, and contractile dysfunction (26, 124, 185). These metabolic abnormalities are thought to underlie heart failure and coronary artery disease, thus having a significant impact on morbidity and mortality in diabetes (73, 172).

The finding that, in hyperglycemia and hyperlipidemia, the diabetic heart responds to glucose and fat availability in a distinct manner from the nondiabetic heart (Fig. 6) prompted the idea that protective mechanisms may be at play in the former. In high glucose, the T2DM heart is able to perform more efficient lipid utilization, ensuring robust energy supply and control of the RE. Thus, protective mechanisms driven by increased efficiency of lipid degradation in hyperglycemia are seemingly active under high energy demand or workload and appear to be targeted to the mitochondria.

Lipid packaging in droplets. Lipids are main fuels for cellular energy and mitochondria their major oxidation site. Cells store FAs as triacylglycerol (TAG) and package them into cytoplasmic lipid droplets (LDs) (108, 178). New emerging data show the LD as a highly dynamic storage pool of FAs that can be used for energy reserve (199) (Fig. 1).

Cells protect themselves from lipotoxicity by either oxidizing FAs or sequestering them in LDs (83, 199). In electron micrographs, LDs can be easily detected in *db/db* (23) or *ob/ob* (76) but not in WT mice hearts. Under conditions of excess lipid supply, it is becoming clear that development of tissue lipotoxicity and dysfunction are linked to alterations in LD biogenesis and regulation of TAG hydrolysis (203). Since in response to lipid loading perilipins associate with LDs, the role of these proteins is currently under intense scrutiny (108, 113). Reported data indicate that reduced expression of perilipins may promote both lipolysis and fat oxidation, resulting in reduced intracellular TAG and adipose mass. On the other hand, excessive lypolysis and defective lipid storage may promote insulin resistance and impaired cardiac function through chronic mitochondrial FA overload [reviewed in Aon *et al.* (4)]. Consequently, lipid storage and utilization appears to be a tightly regulated cellular process.

Mitochondria-LD interactions. Recent evidence supports physical and metabolic interactions between LDs and mitochondria mediated by the scaffolding protein Plin5 (110, 201, 203). Plin5-expressing cells show decreased LD hydrolysis and palmitate β-oxidation when compared with controls. Instead, palmitate was increasingly incorporated into TAGs under basal conditions, whereas after stimulation, LD hydrolysis inhibition was lifted and FAs were released for β-oxidation (201). Together, this evidence suggests that Plin5 regulates LD hydrolysis and controls local FA flux to protect mitochondria against excessive exposure to FA (203). Acute exercise can trigger changes in the dynamics of LD assembly, morphology, localization, and mobilization in skeletal muscle, a process regulated by a broad genetic program affecting the spatial and metabolic interaction between mitochondria and LDs (110) (see Hafstad *et al.* and Schrauwen-Hinderling *et al.* in this Forum).

Noteworthy is that the occurrence of close contact between mitochondria and LD occurred in the T2DM heart, where the dependence on fat fueling is more prominent than in the normal heart (31, 123). In the T2DM scenario, packaging of TAGs in LDs may embody an adaptive response to avoid elevation of the intracellular concentration of free FAs (26, 119). Interestingly, Plin5 overexpression in heart tissue rendered tight mitochondrial clusters around LDs with mitochondria significantly larger but not higher in number (202).

Beyond physical interaction, direct contact between mitochondria and LDs along with their metabolic exchange may represent a key cellular mechanism for management of lipid

excess. By this means, mitochondria will ensure controlled delivery and utilization of lipids without compromising energy supply and redox balance. Based on the premise of metabolic links extending beyond physical contact between mitochondria-LDs, we proposed a hypothetical model of metabolic channeling for lipid utilization by mitochondria (4). According to this model, metabolic channeling represents a way by which mitochondria can manage lipid affluence in an energetically and redox-controlled fashion. Metabolic channeling of lipid utilization may also embody a mechanism directed to avoid excessive elevation of lipid concentration, while increasing the speed of β-oxidation (Fig. 1). The importance of these mechanisms cannot be overstated, because potentially, lipids can behave as both uncouplers and inhibitors of OxPhos (209). However, it is unknown as to *how* and to *what extent* the balance from these apparently contradictory behaviors influences mitochondrial energetics and the ROS emission.

Redox, lipid oxidation, and insulin resistance. Lipids have been implicated in the onset of insulin resistance in skeletal muscle, but the mechanisms accounting for this remain unclear. The debate centers around the role of mitochondrial lipid oxidation capacity and its impact on ROS emission and insulin signaling (71, 126, 137, 173). Overexpression of mitochondrial H_2O_2-consuming enzymes diminishes high-fat diet-induced loss of insulin sensitivity, suggesting that mitochondria may be a major contributor to insulin resistance associated with obesity (163, 184). This problem is relevant for the diabetic heart because of its heavy reliance on fats and its demand-led function (see Sung *et al.* and Schrauwen-Hinderling *et al.* in this Forum).

There are at least two leading opinions in this debate, and their postulates are quite different. One side attributes a main role to dysfunctional mitochondria with intrinsic deficiencies in OxPhos and deficits in fat oxidation. These deficits impinge on insulin signaling by diverting FAs away from oxidation toward production of diacylglycerols, ceramide, and other toxic lipid species (126, 164). The other side, without assuming inherent mitochondrial dysfunction, posits that the redox pressure placed on the respiratory system by persistent outpacing of energy supply with respect to demand is at the root of insulin resistance (137). In this view, even a small increase in energy demand will completely relieve the reducing pressure and ROS production in mitochondria created by a positive energy balance (see Hafstad *et al.* in this Forum).

An intriguing observation is that increased energy expenditure may represent the common underlying protective mechanism in many mouse models that resist diet-induced metabolic abnormalities (35, 137). Under this premise, it has been suggested that mitochondrial adaptations benefiting exercise performance are those favoring energy efficiency and glucose sparing, whereas therapeutic agents targeted against obesity and diabetes should promote energetic inefficiency and glucose utilization (137).

Upregulation of mitochondrial glutathione/thioredoxin systems

Maintaining unharmed antioxidant systems appears to be pivotal for preventing cardiac mitochondria from derailing to pathophysiological function. The Trx system is tightly regulated in skeletal muscle and critical as well for preserving the mitochondrial RE during exercise training (70). However, from studies performed in rat fed with a high-fat high-sucrose diet, it appears that the heart has an intrinsic ability to adapt its RE in response to nutrient overload, whereas the skeletal muscle does not (70).

The mitochondrial GSH/Trx antioxidant systems were affected in T2DM, *db/db* mice. In states 4 and 3 respiration mice, mitochondria diverted a much higher amount of the total O_2 consumed to ROS than the rat (8) and exhibited a deficit in the thioredoxin system (187). In *db/db* mice mitochondria, Trx2 was decreased and the activity of the thioredoxin reductase2 (TrxR2)/thioredoxin2 (Trx2)/peroxiredoxin3 (Prx3) system diminished. Preincubation of mitochondria with GSHee, a cell-permeable form of GSH, recovered Trx2 in mitochondria, decreased oxidative stress concomitantly with increased intracellular GSH, and enhanced contractility in cardiomyocytes. Likewise, palmitate improved intracellular redox status through higher GSH and decreased oxidative stress, while rescuing contractile performance of *db/db* cardiomyocytes (187) and T2DM ZDF heart trabeculae (16).

In diabetes, the hyperglycemia-triggered oxidative stress can be an additional burden to the cytoplasmic GSH/Trx systems (Fig. 1), and it may reverberate on mitochondria (5, 105). In the same line of reasoning, mounting evidence is pointing out the importance of compartmentalization in both H_2O_2 levels and antioxidant expression (102, 105, 163). As a matter of fact, antioxidant enzymes located in the mitochondria can prevent diet-induced loss in insulin sensitivity (2, 40). Conversely, mice lacking glutathione peroxidase 1 exhibit increased insulin sensitivity in response to a high-fat diet relative to wild-type controls (122).

The importance of protecting the antioxidant systems is further highlighted by recent data showing that under oxidative stress mitochondria exhibit lower respiration, higher ROS emission, and slower energetics, that is, lower rate of ADP consumption (52). This evidence is in agreement with studies indicating that reduced mitochondrial oxidative capacity coupled with increased ROS generation underlies the accumulation of intramuscular fat, insulin resistance, and muscle dysfunction in aging (143).

Diabetic Cardiomyopathy

DC is a life-threatening complication of T1DM and T2DM independent of coronary artery disease or hypertension (13, 45). Detected in 75% of asymptomatic diabetic patients (25), diastolic dysfunction is considered the earliest manifestation of contractile dysfunction in DC, and it is produced by even moderate decreases in ATP (30) (see Schilling in this Forum).

Three major interrelated processes underlie heart failure in DC: (i) substrate selection; (ii) redox status of contractile proteins; and (iii) Ca^{2+} handling.

Substrate selection-redox status: DC is impacted by alterations in metabolic substrate availability. FAs and glucose are the two major fuels driving heart contraction, and in T2DM and obesity, existing evidence indicates increased FA oxidation (12, 37, 124). While the healthy heart is flexible regarding fuel selection, the high circulating levels of glucose and lipids in T2DM lead to questions about which factors

contribute to dysfunction and which are beneficial as energy sources or redox donors (104).

The idea that FAs excess negatively regulates glucose oxidation in diabetes according to the Randle mechanism (91, 99, 124) remains a central postulate explaining some negative consequences of substrate shift in this metabolic disorder (see Sung *et al.* in this Forum). However, hyperglycemia *per se* can trigger cellular damage, independently from FA utilization (Fig. 1) (27). Hyperglycemia, a hallmark of diabetes, plays a central role in the pathogenesis of DC, acting as a trigger to several adaptive and maladaptive processes, involving, among others, the renin-angiotensin-aldosterone system, transporters of glucose, and AGEs (28, 77, 140, 157). How exactly hyperglycemia drives all these adverse effects, particularly in contracting myocardium, remains to be further elucidated (see Roul and Recchia in this Forum). However, the multiple routes followed by hyperglycemia to negatively affect cell function have been already well described in endothelial tissue (77). Reports have also explored the effects of high fat diets on the myocardium and skeletal muscle (71, 137). However, our grasp of the impact exerted by hyperglycemia and high FA on metabolism and redox/ROS balance remains incomplete (1, 51, 187) (Fig. 7).

Similarly, although metabolic remodeling in diabetes has been extensively studied (37, 39, 99, 124), the involvement of redox metabolism in T2DM heart contractile dysfunction remains only partially understood (194). Compelling evidence about the critical role played by perturbed cellular/mitochondrial redox, compromised mitochondrial energetics, and elevated ROS at the origin of contractile impairment in diabetes has been reported (2, 187). An important finding from these studies (187) is that the diabetic heart (Fig. 7) responds to glucose and fat availability in a different manner than the nondiabetic heart (Figs. 6 and 8).

Redox status-EC coupling: ROS-induced post-translational modifications of key components of the EC coupling machinery (34), and mitochondrial failure to provide enough ATP, underscore abnormal function of Ca^{2+} channels and pumps in DC. Perturbation of EC coupling in cardiac myocytes is a central impairment in heart failure (14, 145) and DC (21, 90, 128, 172). Reduced SR Ca^{2+} load is linked to decreased SR Ca^{2+} ATPase activity and increased Ca^{2+} leak from RyRs, resulting in lowered amplitude and velocity of cytosolic Ca^{2+} transients (14, 125, 141).

These impairments, ultimately, account for diastolic and systolic dysfunction in DC. Two important interrelated

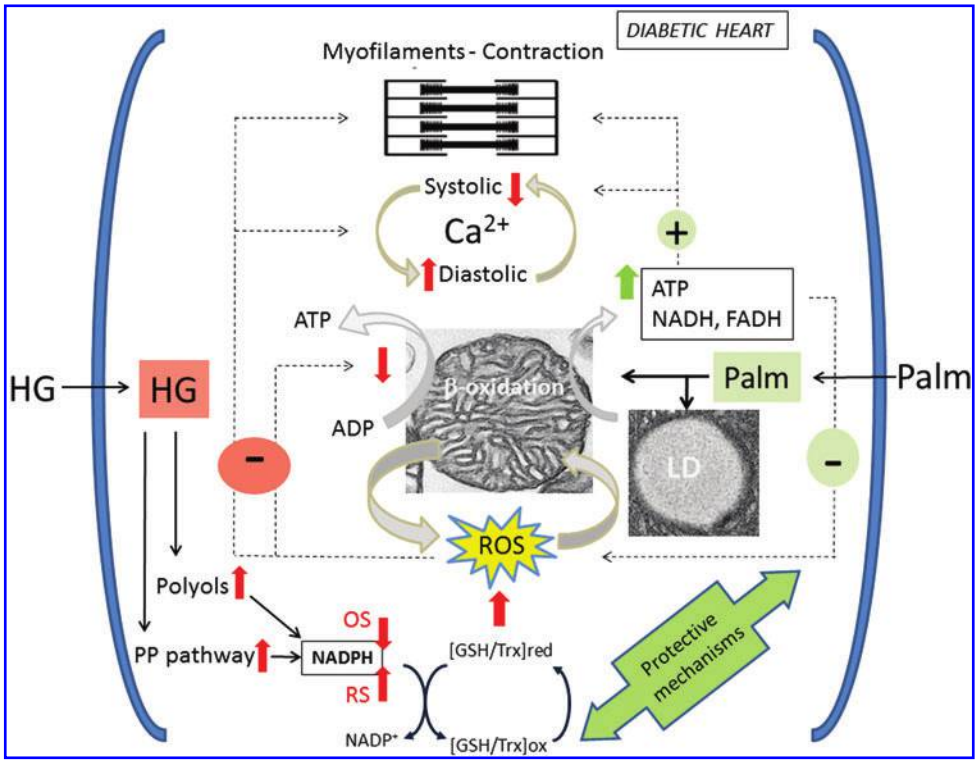

FIG. 7. Redox, energetic, and functional impact of hyperglycemia and palmitate in the diabetic heart. The scheme depicts the high-glucose (HG)-triggered activation of the polyol and pentose phosphate (PP) pathways leading to decreased or increased NADPH (denoted with a *down* or *up red arrow*, respectively) that could produce oxidative (OS) or, eventually, reductive (RS) stress. Palmitate (Palm) decreases ROS and increases both energy (ATP) and reducing equivalents (NADH, FADH) (both denoted with an *up green arrow*). Palm can be either metabolized in mitochondria *via* β-oxidation or stored as the triacylglycerol tripalmitin in LDs. The scheme shows acute Palm addition can produce a positive impact on excitation-contraction (EC) coupling and Ca^{2+} handling from both energy supply and reducing equivalents (187). In contrast, HG elicits oxidative stress, decreasing mitochondrial respiration and enhancing H_2O_2 emission (52) that exert a negative impact on Ca^{2+} handling and contractility (187). The *green box* denotes protective mechanisms that alleviate the adverse effects of glucose and lipids surplus. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

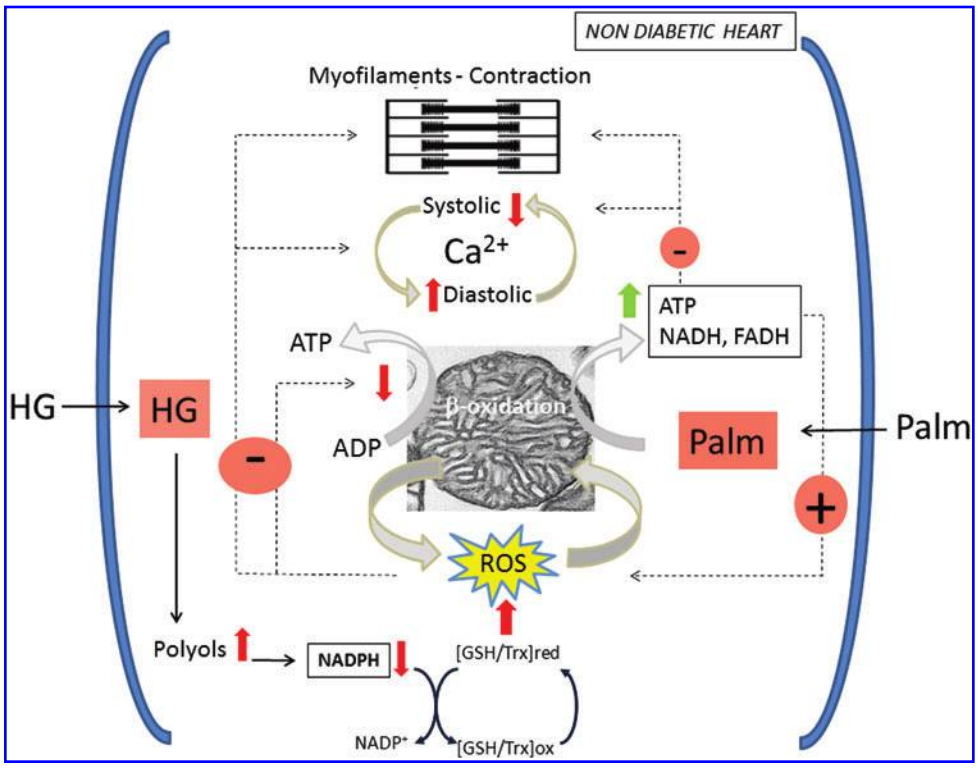

FIG. 8. Redox, energetic, and functional impact of hyperglycemia and palmitate in normal heart. The scheme depicts the negative effects on E-C coupling caused by both excess glucose and lipids. The key to symbols is the same as in Figure 7. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

aspects of Ca^{2+} handling participate in its onset, that is, the rate of Ca^{2+} decline after release, which regulates the speed of myocyte relaxation, and the levels of resting or end-diastolic Ca^{2+} that sets the extent of cell relaxation or diastolic tension (125). A previous work by Flarsheim *et al.* (72) already suggested that a defective cardiac relaxation unmasked by moderate workload may underlie the development of overt systolic and diastolic dysfunction in diabetes. In this study, the basal rates of heart contraction and relaxation in 4 week STZ-treated diabetic rats were not different from controls. However, ISO treatment unveiled a reduction in the peak of relaxation in diabetic hearts at similar rates of contraction in diabetic and controls (72). Importantly, mitochondrial Ca^{2+} uptake was also impaired, leading to the conclusion that diastolic dysfunction is likely the result of decreased ATP supply. Mitochondria may influence diastolic Ca^{2+} levels through diverse mechanisms, including the Ca^{2+} uniporter (31, 145). In heart failure, mitochondrial Ca^{2+} uptake is compromised (121), and this defect impacts both cytosolic Ca^{2+} and mitochondrial energetics. Because Ca^{2+} concentration is in the range where modulation of Ca^{2+}-sensitive dehydrogenase activation occurs (50), lower levels of this cation may also adversely affect TCA cycle function, thus contributing to impaired ATP generation in the heart. This underscores the fact that mitochondrial Ca^{2+} and energetics are bi-directionally linked phenomena (Figs. 7 and 8).

When ATP synthesis is reduced such as in heart failure (84), the Ca^{2+}ATPase SERCA2a and NCX functions are impaired (127), further perturbing Ca^{2+} cycling and leading to increased diastolic Ca^{2+} with adverse consequence on heart mechanics and increased risk of fatal arrhythmias (120). Similar events are likely to occur in DC. Several reports have indeed shown smaller systolic transients in cytosolic Ca^{2+} concentrations in T1DM myocytes (30, 141, 216). Moreover, when STZ myocytes are electrically stimulated, mitochondrial Ca^{2+} concentrations do not increase to the same extent as in normal cardiac cells (89). In STZ-induced diabetic animals, lower expression of SERCA2a is present (141, 218) and its forced expression improves or normalizes contractile function in these animals (188, 196).

Mitochondrial ROS excess in hearts from diabetic patients exposed to hyperglycemia and increased energy demand due to higher sympathetic activity appear to be directly involved in DC (187). Hearts from diabetic subjects are particularly prone to excess ROS, because sympathetic hyperactivation and hyperglycemia are present in a large cohort of these patients (63, 133). Importantly, sympathetic activation is a hallmark of acute and chronic heart failure. Recent reports suggest a possible link between elevated blood glucose and sympathetic over activity (63); this evidence will contribute to explaining how hyperglycemia may lead to progression of chronic heart failure and increase of morbidity and mortality in these patients (133).

Concluding Remarks

Glucose and lipids excess have the potential to render the cardiac RE more oxidized, compromising mitochondrial function while paving the way to the onset of DC and thus cardiac functional deficits. The distinct response of the diabetic heart when acutely infused with Palm in the presence of high glucose suggests the possibility that, under certain circumstances, FAs not only supply energy but also activate protective, redox-based mechanisms to temporarily preserve cardiac function under substrate excess. Very likely, these protective pathways converge on mitochondria to preserve their ability of supplying adequate energy, while maintaining proper redox balance and mtDNA integrity.

On the other hand, the potential of lipids to lead to energetic impairment and ROS overflow underscores the importance of keeping the free *in vivo* bulk concentration of these molecules under tight control. Given the relevant role played by mitochondria in the oxidation of FAs, these organelles must have *in vivo* mechanisms to protect them when operating in the presence of permanent lipid bioavailability. Here, we have summarized mechanisms of lipid utilization that, in the diabetic heart, depend on close spatial positioning between mitochondria and LD. Apparently, a cohort of proteins belonging to the perilipin family, and located in the LD periphery, guide or serve this process.

A better understanding of these processes may offer new therapeutic avenues for preventing or treating the dire consequences due to diabetes and other metabolic disorders on heart function as well as other organs such as the liver and skeletal muscle.

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Abbreviations Used

Ac CoA = acetyl CoA
AGEs = advanced glycation end products
ANT = adenine nucleotide translocator
AS = acyl-CoA synthetase
ATGL = adipose triglyceride lipase
CACT = carnitine acyl carnitine transferase
CPT1 = carnitine palmitoyl transferase 1
Cys = cysteine
DC = diabetic cardiomyopathy
DNP = dinitrophenol

Abbreviations Used (Cont.)

FA = fatty acid
FET = forward electron transport
G/M = glutamate/malate
GAPDH = glyceraldehyde 3 phosphate dehydrogenase
GPx = glutathione peroxidase
GSH = reduced glutathione
GSHee = GSH ethyl ester
GSSG = oxidized glutathione
HG = high glucose
HSL = hormone-sensitive lipase
ISO = isoproterenol
LCFA = long chain fatty acid
LD = lipid droplet
LV = left ventricle
LVDP = left ventricular developed pressure
MAG = monoacylglycerol
MGL = monoacylglycerol lipase
mtDNA = mitochondrial DNA
OxPhos = oxidative phosphorylation
Palm = palmitate
PDC = pyruvate dehydrogenase complex
PFK = phosphofructokinase
Plin5 = perilipin 5
PP = pentose phosphate
PPARα = nuclear hormone receptor peroxisome proliferator-activated receptor α
Prx = peroxiredoxin
Prx3 = peroxiredoxin 3
RCR = respiratory coupling ratio
RE = redox environment
RET = reverse electron transport
R-ORB = Redox-Optimized ROS Balance
ROS = reactive oxygen species
RyR = ryanodine receptor
SERCA = sarcoplasmic reticulum ATPase
SOD = superoxide dismutase
SR = sarcoplasmic reticulum
STZ = streptozotocin
T1DM = type 1 diabetes
T2DM = type 2 diabetes
TAG = triacylglycerol
THD = transhydrogenase
Trx = thioredoxin
$Trx(SH)_2$ = reduced thioredoxin
TRxR2 = thioredoxin reductase 2
TrxSS = oxidized thioredoxin
UCP = uncoupling protein
VO_2 = mitochondrial respiration
ZDF = Zucker Diabetic Fatty rat.

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