FORUM REVIEW ARTICLE

Metabolic Alterations Induce Oxidative Stress in Diabetic and Failing Hearts: Different Pathways, Same Outcome

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Abstract

Significance: Several authors have proposed a link between altered cardiac energy substrate metabolism and reactive oxygen species (ROS) generation. A cogent evidence of this association has been found in diabetic cardiomyopathy (dCM); however, experimental findings in animal models of heart failure (HF) and in human myocardium also seem to support the coexistence of the two alterations in HF. **Critical Issues:** Two important questions remain open: whether pathological changes in metabolism play an important role in enhancing oxidative stress and whether there is a common pathway linking altered substrate utilization and activation of ROS-generating enzymes, independently of the underlying cardiac pathology. In this regard, the comparison between dCM and HF is intriguing, in that these pathological conditions display very different cardiac metabolic phenotypes. **Recent Advances:** Our literature review on this topic indicates that a vast body of knowledge is now available documenting the relationship between the metabolism of energy substrates and ROS generation in dCM. In some cases, biochemical mechanisms have been identified. On the other hand, only a few and relatively recent studies have explored this phenomenon in HF and their conclusions are not consistent. **Future Directions:** Better methods of investigation, especially *in vivo*, will be necessary to test whether the metabolic fate of certain substrates is causally linked to ROS production. If successful, these studies will place a new emphasis on the potential clinical relevance of metabolic modulators, which might indirectly mitigate cardiac oxidative stress in dCM, HF, and, possibly, in other pathological conditions. *Antioxid. Redox Signal.* 22, 1502–1514.

Introduction

THE PATHOPHYSIOLOGY OF cardiovascular diseases involves, almost invariably, the occurrence of oxidative stress (23), a major cause of progressive cellular sufferance and death. Oxidative stress can be initiated by various pathological processes, some of which are well characterized—for instance inflammation—while the role of others is only partially understood. Diabetic cardiomyopathy (dCM) and heart failure (HF) are two conditions in which oxidative stress seems to play a major pathogenic role (12, 32, 87, 89, 90). The consequences of cardiac oxidative damage are well characterized and result in abnormal calcium handling, apoptosis, fibrosis, and cardiac hypertrophy. Unfortunately, clinical and preclinical studies testing antioxidant strategies

have not led to positive results. Hafstad *et al.* stated that “the failure of these therapies might be due to lack of knowledge about the sources, spatial distribution, local concentration and time-course of reactive oxygen species (ROS) production and removal” in such pathologies (32) (see also Hafstad *et al.* in this Forum). One of the aspects that deserve particular consideration is the causative link between altered cardiac energy substrate metabolism and oxidative stress. Modulating cardiac substrate utilization might, indeed, prove an interesting therapeutic strategy for the attenuation of ROS-related biochemical and functional damage. The present review will propose a parallelism between dCM and HF, both of which are characterized by marked myocardial metabolic alterations as well as by high oxidative stress. In both cases, the scientific literature supports more than a pure association,

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but rather a possible cause-effect relationship between the two phenomena. Intriguingly, however, the metabolic changes follow opposite directions: In dCM, the heart relies mainly on fatty acids for its metabolism (25), whereas the failing heart loses part of its capacity to oxidize fat and relies more on carbohydrates (83). We will describe how different metabolic alterations can lead to the same effect, namely high oxidative stress.

Myocardial Energy Substrate Metabolism Under Physiological Conditions

Under resting conditions and in inter-prandial phases, the healthy heart obtains 70%–80% of the energy from the oxidation of free fatty acids (FFA) and the remainder from

carbohydrates. These metabolites are defined as cardiac energy substrates. The reader is referred to extensive reviews on this topic (53, 82–84); however, a synthetic description of cardiac substrate metabolism is necessary to better understand how its derangement can favor oxidative stress in dCM and HF.

Fatty acid metabolism

Circulating FFA enter cardiomyocytes either by passive diffusion or by facilitated transport due to cell membrane free fatty acid translocase (FAT), also known as “cluster of differentiation 36” (CD36). Deletion (45) or inhibition (55) of FAT/CD36 causes a 50%–60% decrease in cardiac FFA uptake. In the cytosolic compartment, long-chain FFA are

FIG. 1. FFA and carbohydrate metabolisms in the cardiomyocyte. FFA enter cardiomyocyte *via* FAT/CD36. In the cytosol, FFA are converted into TG for storage or into FA-CoA esters by FACS and successively conjugated with carnitine by CPT-1 to form fatty acylcarnitine. CPT-1 is the key-limiting step determining FFA entry in mitochondria and its activity is regulated by the inhibitor malonyl-CoA. The concentration of malonyl-CoA depends on the balance between its synthesis catalyzed by ACC and its degradation catalyzed by MCD. Fatty acylcarnitine enters mitochondria *via* CT and is converted into FA-CoA by CPT-2. In mitochondria, FA-CoA undergo complete degradation in the β -oxidation cycle, leading to the production of acetyl-CoA, which is then catabolized in the tricarboxylic acid cycle to generate FADH_2 and NADH , that is, the reducing equivalents feeding the electron (e^-) transport chain. Glucose enters cardiomyocytes *via* the GLUT1 and GLUT4 (GLUT1/4). Once in the cardiomyocyte, glucose is phosphorylated by HK. G-6P derived from exogenous glucose and the intracellular glycogen pool undergo progressive breakdown through the glycolytic pathway or, to a lesser extent, the oxPPP and the polyol pathway. Most of the pyruvate generated from glycolysis or lactate dehydrogenation is decarboxylated in mitochondria to form acetyl-CoA. This reaction is catalyzed by PDH. ACC, acetyl-CoA carboxylase; CPT, carnitine palmitoyltransferase; CT, carnitine:acylcarnitine translocase; F-1,6-P, fructose-1,6-biphosphate; F-6-P, fructose-6-phosphate; FA, fatty acids; FA-CoA, fatty acyl-coenzyme-A; FACS, fatty acyl-CoA synthase; FAT/CD36, free fatty acid translocase/cluster of differentiation 36; FATP, fatty acid transport protein; FFA, free fatty acids; G-6P, glucose-6-phosphate; GLUT, glucose transporter; HK, hexokinases; MCD, malonyl-CoA decarboxylase; oxPPP, oxidative pentose phosphate pathway; PDH, pyruvate dehydrogenase; PFK-1, phosphofructokinase 1; Q, ubiquinone; TG, triglycerides; UCP, uncoupling protein.

converted into long-chain fatty acyl-coenzyme-A (CoA) esters by fatty acyl-CoA synthase and successively conjugated with carnitine by carnitine palmitoyltransferase-1 (CPT-1) to form long-chain fatty acylcarnitine (99) (Fig. 1). CPT-1 is the key-limiting step determining the rate of FFA entry in mitochondria, and its activity is regulated allosterically by the inhibitor malonyl-CoA (42, 53, 58, 68). The concentration of malonyl-CoA depends on the balance between its synthesis catalyzed by acetyl-CoA carboxylase (ACC) (52, 74), and its degradation is catalyzed by malonyl-CoA decarboxylase (MCD) (21, 75) (Fig. 1), the activity of which is regulated by the so-called “fuel sensor” AMP-activated protein kinase (AMPK) (22, 33). In response to low levels of chemical energy, AMPK is activated and exerts an inhibitory action on ACC with consequent reduction of malonyl-CoA levels and an increase in CPT-1 activity. In mitochondria, long-chain fatty acyl-CoA undergo complete degradation in the β -oxidation cycle, leading to the production of acetyl-CoA, which is then catabolized in the tricarboxylic acid cycle (TCA) to generate FADH_2 and NADH , that is, the reducing equivalents that feed the electron transport chain (ETC) (Fig. 1).

Carbohydrate metabolism

As mentioned earlier, while the heart relies mainly on FFA, ~10%–40% of the acetyl-CoA used for ATP production is derived from the oxidation of pyruvate, the final product of lactate dehydrogenation and glycolysis (29, 82, 98) (Fig. 1).

Glucose transport into cardiomyocytes is facilitated by the glucose transporters (GLUTs) of sarcolemma membrane. The main cardiac GLUT isoforms are GLUT1 and GLUT4 (38), with the first constitutively active, while the second translocates to the sarcolemma in response to insulin, increased workload, and ischemia (61, 102). AMPK activation is also involved in this process (62). Once in the cardiomyocyte, glucose is phosphorylated by hexokinases (20). Glucose-6-phosphate derived from exogenous glucose and intracellular glycogen pool (14) undergoes progressive breakdown through the glycolytic pathway or, to a lesser extent, the oxidative pentose phosphate pathway (oxPPP) and the hexosamine biosynthetic pathway (43) (Fig. 1).

Most of the pyruvate generated from glycolysis or lactate dehydrogenation is decarboxylated in mitochondria to form acetyl-CoA (70). This reaction is a key-limiting step for carbohydrate oxidation and is catalyzed by pyruvate dehydrogenase (PDH), located in the mitochondrial matrix. PDH is finely regulated by specific PDH kinase (PDK) and phosphatase and functions as a sensor of fuel availability. For instance, high rates of FFA β -oxidation lead to increased levels of acetyl-CoA/free CoA and NADH/NAD^+ ratios and subsequent activation of PDK, which, in turn, inhibits PDH. The mutual inhibition between FFA and carbohydrate oxidation was first described by Randle in the 1960s and generally referred to as the “Randle cycle” (64, 71).

TCA and ETC

TCA is responsible for the oxidation of acetyl-CoA generated from energy substrate breakdown. The chemical energy is accumulated in reducing equivalents and utilized for the synthesis of ATP by the ETC. Each cycle of the TCA generates three NADH and one FADH_2 , in addition to one molecule of GTP.

The oxidation by the ETC of all reducing equivalents available to mitochondria leads to an electrochemical gradient that, while essential for ATP synthesis, can also promote ROS production under pathological conditions such as diabetes.

Transcriptional control of cardiac metabolism

Enzymes involved in FFA and carbohydrate metabolisms are finely regulated at transcriptional level. Important transcription factors for FFA metabolism are the three isoforms of peroxisome proliferator-activated receptors (PPARs): $\text{PPAR}\alpha$, $\text{PPAR}\beta/\delta$, and $\text{PPAR}\gamma$ (35, 100). They form heterodimers with the retinoid X receptor- α ($\text{RXR}\alpha$) and the $\text{PPAR}\gamma$ coactivator-1 (PGC-1) and bind to the PPAR response element located in the promoter regions of many genes encoding for proteins involved in metabolism. $\text{PPAR}\alpha$ is highly expressed in the heart, activated by long-chain FFA, and regulates the expression of numerous enzymes involved in different steps of FFA metabolism, from uptake (FAT/CD36, FATP1) to mitochondrial entry (CPT-1, MCD) and β -oxidation (acyl-CoA dehydrogenase, 3-ketoacyl-CoA thiolase) (35, 100) (Fig. 2).

Metabolic Alterations Promoting Cardiac Oxidative Stress in dCM

Type I and II diabetes are characterized by hyperglycemia and hyperlipidemia. This excess of circulating substrate concentration, along with the lack of insulin or insulin resistance in type I and II diabetes, respectively, leads to changes in myocardial metabolism characterized by enhanced FFA oxidation and markedly impaired glucose oxidation (11, 95). This cardiac metabolic phenotype favors oxidative stress, that is, the imbalance between mitochondrial and/or cytosolic ROS generation and antioxidant defenses. Most of the studies on cardiac metabolism in dCM have been

FIG. 2. Transcriptional control of cardiac fatty acid metabolism. The PGC-1 forms a heterodimer with PPAR and the $\text{RXR}\alpha$. The heterodimer binds the PPRE located in the promoter regions of many genes encoding for proteins/enzymes involved in FFA metabolism. PPAR, peroxisome proliferator-activated receptors; PPRE, PPAR responsive element; PGC-1, $\text{PPAR}\gamma$ coactivator-1; $\text{RXR}\alpha$, retinoid X receptor- α .

performed in experimental models and only a few have been performed in patients. Oxidative stress was documented in atrial tissue from patients with type II diabetes (2). The authors of this study found an increased mitochondrial hydrogen peroxide (H_2O_2) generation and high levels of hydroxynonenal-modified and 3-nitrotyrosine-modified proteins, two hallmarks of oxidative stress. Interestingly, H_2O_2 emission, reflecting the net balance between H_2O_2 generation in the ETC and scavenging by the matrix antioxidant system, was increased regardless of the type of oxidized substrate or the respiratory state. Moreover, the atrial tissue displayed impaired mitochondrial capacity to oxidize palmitoyl-carnitine and glutamate. A strength of this study was the recruitment of an appropriate control group, in that both nondiabetic and diabetic patients had a body mass index of $\sim 32 \text{ kg/m}^2$. However, the contribution of obesity in the development of mitochondrial alterations in atria of diabetic patients could not be determined. A more recent study in a similar model of atrial tissue from type II diabetic patients elucidated this particular aspect (60). Interestingly, the authors showed that both diabetes and obesity impair atrial muscle contractility, but only diabetes also causes mitochondrial dysfunction and oxidative stress. They concluded that hyperglycemia is the major driver of oxidative stress in the diabetic heart. In the next few paragraphs, we will try to

separate the relative contribution of glucose and FFA from the pathogenesis of cardiac oxidative stress in dCM.

Glucose metabolism and ROS generation

Nonmitochondrial ROS generation. Hyperglycemia triggers nonmitochondrial ROS generation through several interconnected pathways, forming a complex network (Fig. 3). A recently identified mechanism is of particular interest, since it involves the uptake of glucose by cardiomyocytes through a less known transporter, namely the sodium/glucose co-transporter (SGLT1) (6). Rat cardiomyocytes exposed to a high glucose concentration displayed the activation of the small GTPase Ras-related C3 botulinum toxin substrate 1, followed by activation of the NADPH oxidase Nox2 and therefore superoxide production. Interestingly, Nox2 can also be activated by 3-O-methyl-D-glucopyranoside and α -methyl-D-glucopyranoside, nonmetabolizable glucose analogues; this process is inhibited by the SGLT1 inhibitor phlorizin but not by the GLUT1 and GLUT4 inhibitor phloretin. Under these experimental conditions, superoxide production by Nox2 is also inhibited by 6-aminonicotinamide, a blocker of the oxPPP. Therefore, even if activated by excessive glucose uptake, NADPH oxidase cannot generate superoxide if not provided with the substrate NADPH

FIG. 3. Hyperglycemia- and hyperlipidemia-induced cardiomyocyte cytoplasmic ROS generation in dCM. Glucose enters cardiomyocytes via the SGLT1. The oxPPP provides NADPH for NADPH oxidase (Nox2), which generates superoxide ($\text{O}_2^{\bullet-}$). Nox2 needs the translocation of subunit P47phox to the membrane to be activated. The activation of the signaling pathway PKC β 2–small GTPase Ras-related C3 botulinum toxin substrate 1 (Rac1) by high glucose concentration induces this translocation. PKC β 2 is also activated by a positive feedback loop involving the RhoA/Rho kinase (ROCK) pathway and the overexpression of the iNOS. CaMKII hyper-phosphorylation caused by elevated intracellular calcium (Ca^{2+}) concentration is also probably involved in PKC β 2 activation. Finally, hyperglycemia also contributes to the generation of AGE that, through their receptor RAGE, induces oxidative stress. When present at high concentrations, LCFA can be reduced in peroxisomes before entering the mitochondria, thus generating H_2O_2 . AGE, advanced glycation end-products; CaMKII, calcium/calmodulin dependent kinase II; dCM, diabetic cardiomyopathy; G-6-P, glucose-6-phosphate; G6PD, G-6-P dehydrogenase; H_2O_2 , hydrogen peroxide; iNOS, inducible nitric oxide synthase; LCFA, long chain fatty acids; NCX, sodium-calcium exchanger; NHE, sodium-proton exchanger; NO, nitric oxide; ONOO^- , peroxynitrite; PKC β 2, protein kinase C β 2; ROS, reactive oxygen species; SGLT1, sodium/glucose co-transporter.

furnished by the oxPPP (6) (Fig. 3). This interesting finding is in agreement with a previous study in a rat model of type II diabetes (76). Boosting substrate metabolism by AMPK activation with glucagon-like peptide-1 can prevent NADPH oxidase activation, as recently shown in adult cardiomyocytes exposed to high glucose concentrations (7). These results might have important clinical implications; however, they have not yet been validated *in vivo*.

The studies mentioned earlier either demonstrated (76) or suggested (6) the involvement of PKC in the activation of Nox2 in response to high glucose concentrations, in accordance with other reports. In fact, it has been shown that Nox2 activation in cardiomyocytes exposed for 12 h to high glucose concentration was prevented by the PCK β 2 inhibitor LY333531 (96). Another study in cardiomyocytes exposed for 24 h to high glucose concentrations or isolated from type I diabetes rats found that PCK β 2 induction by hyperglycemia is involved in the production of ROS through a positive feedback loop involving the RhoA/Rho kinase pathway and the overexpression of the inducible nitric oxide synthase (78) (Fig. 3). These findings have been further supported by a more recent report (48). Of note, inducible nitric oxide synthase upregulation is known to be associated with nitrosative stress caused by peroxynitrite, the product of NO and superoxide reaction (36).

Another pathway linking hyperglycemia to ROS generation *via* NADPH oxidase involves the calcium/calmodulin-dependent kinase II (CaMKII) (66) (Fig. 3). The authors of this study found hyper-phosphorylation of CaMKII caused by elevated intracellular Ca²⁺ concentration in a rat model of type I diabetes, as well as in cultured neonatal rat cardiomyocytes exposed to high glucose concentrations. The proposed mechanism is the following: High glucose concentrations activate the Na⁺-H⁺ exchanger, leading the Na⁺-Ca²⁺ exchanger to work in a reverse mode to compensate for the increased intracellular concentration of Na⁺. The consequent cellular influx of Ca²⁺ causes CaMKII activation. Interestingly, Na⁺-H⁺ exchanger upregulation driven by PKC in response to high glucose has been shown in cultured vascular smooth muscle cells (97). Moreover, a link between PKC and CaMKII was already found in the hippocampus of type I diabetic rats (50).

Hyperglycemia also contributes to the generation of advanced glycation end-products (AGE) that, through their receptor RAGE, can induce oxidative stress (Fig. 3). While only a few groups explored the involvement of AGE and RAGE in cardiac oxidative stress, more solid evidence has been found in other cell types such as cardiac endothelial cells or pancreatic β -cells [for review, see Daffu *et al.* (19)]. Moreover, AGE can increase cellular oxidative stress through the inhibition of antioxidant defenses.

Mitochondrial ROS generation. Two hypotheses attempt to link mitochondrial respiration and ROS level: the “mild uncoupling” (9, 44) and the more recent “redox-optimized ROS balance” (4, 18). Mild uncoupling is related to the mitochondrial membrane potential, while redox-optimized ROS balance is related to the redox environment as the intermediate between mitochondrial respiration and ROS level. In mitochondria, superoxide is produced through complexes I and III of the ETC (37). High levels of ROS in cardiac mitochondria have been found in diabetic humans (2) and

experimental animals (76). Transgenic mice overexpressing manganese superoxide dismutase (SOD), that is, the mitochondrial isoform of this enzyme, are protected against oxidative damage caused by type I diabetes (77). Moreover, increased catalase expression or inhibition ETC complexes I and II with rotenone and thenoyltrifluoroacetone, respectively, attenuate ROS generation in cardiomyocytes from animals with type I and II diabetes, pointing to mitochondria as the primary source of ROS in these models (101) (see Aon *et al.* in this Forum). Other interesting results linking hyperglycemia to mitochondrial ROS generation came from a study demonstrating, in bovine aortic endothelial cells, a blockade of three pathways involved in hyperglycemia-induced alterations by the normalization of mitochondrial ROS (65). Unfortunately, this study did not investigate the signaling pathway linking hyperglycemia to mitochondrial ROS generation.

Regarding the potential pathways involved in hyperglycemia-induced ROS generation, an elegant study recently described the increased expression and activity of the peptidyl-prolyl cis-trans isomerase (Pin1) and of the Ser-36 phosphorylation by the growth factor adapter protein (p66Shc), associated with an increased mitochondrial superoxide concentration in human aortic endothelial cells exposed to high glucose concentrations (67) (Fig. 4). Interestingly, the mitochondrial superoxide level was normalized when the cells exposed to high glucose concentrations were treated with an anti-Pin1 siRNA. Moreover, the same authors also showed Pin1 overexpression in aorta of type I diabetic mice. Using Pin1^{-/-} transgenic mice, the authors confirmed the involvement of this enzyme in the hyperglycemia-induced mitochondrial superoxide overproduction. Finally, an increased Pin1 expression and activity was also found in the blood of type II diabetic patients and correlates with HbA1c and fasting plasma glucose concentrations (67). Pin1 activity also correlates with the concentration of 8-iso-prostaglandin F2 α , a marker of oxidative stress. This study was focused on vascular dysfunction; however, it may be considered of interest in the cardiology field, since other studies demonstrated a beneficial effect of p66Shc deletion on cardiac function and oxidative state in models of diabetes (56, 59). For instance, isolated rat cardiomyocytes transduced with a dominant-negative p66Shc exhibit attenuated high glucose-induced ROS production and more stable mitochondrial energetics (56). Moreover, mice with type I diabetes display an increased phosphorylation and translocation of p66Shc in mitochondria (Fig. 4).

Other potential mitochondrial sources of ROS are monoamine oxidases (MAO) A and B. Although no available data support the involvement of MAO in dCM, a possible pathogenic role of these constitutive ROS generators has been recently found in mice with cardiac overload (39, 40) and it is plausible that these enzymes are involved in hyperglycemia-induced cardiac ROS generation in the setting of dCM. Interestingly, increased levels of MAO-A have been found in the heart of the streptozotocin-induced diabetic rat, which were normalized by multiple antioxidant administration (46).

Finally, the end products of lipid peroxidation (oxidation of unsaturated lipid by H₂O₂) such as malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE) (1) are known to be involved in mitochondrial ROS generation (47). Interestingly,

FIG. 4. Hyperglycemia- and hyperlipidemia-induced cardiomyocyte mitochondrial ROS generation in dCM. Hyperglycemia increases expression and activity of the peptidyl-prolyl cis-trans isomerase (Pin-1) and of the Ser-36 phosphorylation of the growth factor adapter protein (p66Shc) involved in superoxide generation $O_2^{\bullet-}$ in mitochondria. Hyperlipidemia induces increased cardiac FFA uptake and oxidation so that large amounts of the reducing equivalents NADH and $FADH_2$ are produced due to enhanced FA β -oxidation and acetyl-CoA oxidation in the tricarboxylic acid cycle. They cause an excessive flux of protons (H^+) across the electron (e^-) transport chain with consequent hyperpolarization of the inner mitochondrial membrane. The final effect is the inhibition of complex III and the excessive $O_2^{\bullet-}$ generation by complexes I and III. $O_2^{\bullet-}$ generation by ETC is counteracted by UCP and APOO. End-products of lipid peroxidation (oxidation of unsaturated lipid by H_2O_2) such as MDA and HNE (1) are also involved in mitochondrial ROS generation. Hyperglycemia-induced cytosolic ROS inhibits ALDH2, an enzyme involved in the oxidation and detoxification of aromatic and aliphatic aldehydes. ADP, adenosine diphosphate; ALDH2, aldehyde dehydrogenase 2; APOO, apolipoprotein O; ATP, adenosine triphosphate; ETC, electron transport chain; HNE, 4-hydroxy-2-nonenal; MDA, malondialdehyde; Pi, inorganic phosphate.

a recent study in type I diabetic rats found that hyperglycemia-induced ROS inhibits aldehyde dehydrogenase 2 (ALDH2), an enzyme involved in the oxidation and detoxification of aromatic and aliphatic aldehydes such as HNE (94). ALDH2 decreased activity is also correlated to the plasmatic concentration of glycosylated hemoglobin and cardiac alterations in another study using type I diabetic rats after an intraperitoneal injection of streptozotocin (93). Such findings revealed interactions between lipid- and glucose-induced ROS generation and between nonmitochondrial and mitochondrial ROS generation, thus adding a further level of complexity to the ROS generation network.

Lipid metabolism and ROS generation

As described earlier, cardiac FFA uptake and oxidation is enhanced in patients (69) and in animal models of type I and II diabetes (11, 13, 95). During the early stages of the disease, insulin resistance mainly causes this metabolic alteration. In more advanced stages, high FFA levels activate PPAR α , which, in turn, upregulates the enzymes involved in FFA uptake and β -oxidation (26). Large amounts of the reducing equivalents NADH and $FADH_2$ are produced due to enhanced FFA β -oxidation and acetyl-CoA oxidation in the

TCA. They cause an excessive flux of protons across the ETC with consequent hyperpolarization of the inner mitochondrial membrane (Fig. 4). The final effect is the inhibition of complex III in the ETC and the excessive superoxide generation by complexes I and III (10, 80, 86). An increase in reverse electron flow has also been suggested as a potential mechanism (10). Other sources of superoxide have been identified in mitochondria (10), for instance, the electron transfer flavoprotein:Q oxidoreductase associated to FFA β -oxidation (80).

Interestingly, it has been recently shown that tumor suppressor p53 and synthesis of cytochrome c oxidase 2 are enhanced by hyperglycemia-induced ROS in both type I and II diabetic mice. This phenomenon leads to increased ETC complex IV activity and consequent enhancement of FFA oxidation and ROS production by the ETC (63), suggesting another possible interaction between hyperglycemia and hyperlipidemia-induced ROS. Of note, ROS generation by ETC is counteracted by uncoupling proteins (UCP), which allow the flux of protons back in the mitochondrial matrix, hence favoring oxygen consumption uncoupled from ATP production. In fact, the UCP isoform 3 has been found to be upregulated in type I diabetic hearts (13). Unfortunately, based on the mild uncoupling hypothesis, this potentially

protective mechanism necessarily lowers cardiac efficiency (15). Another protein recently identified as potentially involved in ETC uncoupling is the apolipoprotein O (88). On the one hand, this protein prevents ETC-derived ROS generation, while on the other hand it depletes mitochondrial-reducing equivalents, thus favoring the accumulation of FFA intermediate metabolites in cardiomyocytes. Among these, ceramides are known to directly interact with the ETC and induce ROS generation (28).

Finally, the FFA β -oxidation occurring in peroxisomes, although minor, can become a source of ROS in the diabetic heart, as recently shown in a mouse model of a high-fat diet (73) (Fig. 3).

Despite abundant data supporting the detrimental role of enhanced FFA oxidation in diabetic hearts, some experimental evidence suggests an apparently paradoxical acute beneficial effect of palmitate on redox homeostasis and cardiac function in type II diabetes mouse models (24, 85). When challenged with high glucose and the beta-adrenergic receptor agonist isoproterenol, cardiomyocytes isolated from db/db mice, a model of type II diabetes, display mitochondrial ROS overproduction and contractile dysfunction (85). These alterations could be prevented not only by the antioxidant molecule glutathione but also by exogenous palmitic acid. The mechanisms underlying this interesting phenomenon are not yet clear. The beneficial effects of palmitate on redox balance and cardiac contractility have been recently confirmed in the Zucker Diabetic fatty rat (8), a model of type II diabetes. The authors of this study demonstrate that palmitate is able to rescue contractile function of heart trabeculae harvested from diabetic (but not from normal) hearts exposed to high glucose. These beneficial effects are associated with an increased scavenging capacity of the antioxidant system (glutathione and thioredoxin) and reduced oxidative stress (8). In this regard, Aon *et al.* recently reviewed the cellular management of lipid excess focusing on the involvement of lipid droplets and hypothesized a "metabolic channeling of lipid utilization from close contacts between mitochondria and lipid droplets" (3).

Metabolic alterations and antioxidant defenses

Under physiological conditions, antioxidant defenses neutralize ROS to prevent oxidative stress. They include SOD, glutathione peroxidase, catalase, thioredoxin, vitamins C and E, and coenzyme Q₁₀ (35, 36, 57, 81, 100). Diabetes is characterized by an impairment of antioxidant defenses, which further amplifies oxidative stress due to increased ROS production. These biochemical alterations have been well characterized in the heart and recently reviewed (36). However, very little is known about the specific role played by hyperglycemia and hyperlipidemia. It has been recently shown that hyperlipidemia induces catalase overexpression in mice subjected to a high-fat diet (73). In this model, the increased concentration of H₂O₂ induces the activation of c-Jun N-terminal kinase, which promotes the phosphorylation of the transcription factor family FOXO, leading to catalase overexpression (73). The capacity of rat hearts to counteract the oxidative stress caused by a chronic high-fat/high-sugar diet by upregulating thioredoxin reductase-2 has been recently shown, although the signaling involved in this mechanism was not investigated (27). Conversely, other au-

thors found an increased expression of the thioredoxin endogenous inhibitor named thioredoxin interacting protein, and therefore reduced anti-oxidant defenses, after hyperglycemia in patients with diabetes and in rodents with type I and II diabetes (16). Lower pools of reduced glutathione and thioredoxin reductase-2 have been found in energetically/redox stressed mitochondria isolated from hearts of type II diabetic db/db mice (85). The authors of the study attribute a direct causative role to these alterations in determining cardiomyocyte contractile dysfunction.

Although no evidence is yet available in hearts, it should be mentioned that AGE have also been involved in the impairment of antioxidant defenses in human embryonic kidney cells in culture (15). Cells treated with two different AGE types presented an increase phosphorylation of FKHL1, which subsequently leads to a 70% downregulation of the MnSOD in mitochondria (15). Finally, the polyol pathway was found to be hyperactivated in the heart of BBZ rats, which develop spontaneous type II diabetes: The concentration of both sorbitol and fructose, products of this pathway, was increased in diabetic rat hearts at 12 and 48 weeks of age (49). Although this study did not investigate the involvement of this polyol pathway upregulation in oxidative stress, others hypothesized its causative role in the decreased availability of NADPH necessary for glutathione reductase (91).

Metabolic Alterations Promoting Cardiac Oxidative Stress in HF

Role of altered glucose metabolism

Very little is known about the involvement of metabolic alterations in the generation of ROS in HF. Moreover, some discrepancies are found among the few studies that investigated this phenomenon, due to complexity of the HF syndrome and its evolution. One of the first studies proposing a link between metabolic alterations and oxidative stress in HF was performed in dogs with pacing-induced HF (31). The authors provided evidence that NAD(P)H oxidase, fueled by NADPH deriving from an upregulated oxPPP, is an important source of superoxide in failing hearts. NADPH might also feed another superoxide generator, the uncoupled NO synthase. The inhibitor of NAD(P)H oxidase gp91^{ds-tat} abolished superoxide production in cardiac tissues homogenates, further confirming the involvement of this enzyme. While no changes in Nox-2 and Nox-4 expression were observed, the authors found an increased interaction between Nox-2 and its subunit p67^{phox}, leading to its activation. The same group then performed a similar study in left ventricular tissue samples collected from HF patients (30). Not only they confirmed the findings obtained in dogs, but also, by using chelerythrine and PP2, respectively PKC and Src kinase inhibitors, provided evidence that both PKC and Src kinase coordinate G6PD and NAD(P)H oxidase activation. Moreover, using the same HF dog model, other authors demonstrated decreased protein and transcript levels of aldose reductase in the myocardium (79), indicating polyol pathway downregulation. One possible interpretation of these findings in HF dogs and patients is that the co-existence of cardiac enhanced glucose uptake, oxPPP upregulation, and decreased polyol pathway might lead to an excessive flux through NADPH-generating enzymes that, in turn, might feed not only the anti-oxidant system (glutathione peroxidase) but

FIG. 5. Paradoxical effect of oxidative penthose pathway-derived NADPH as substrate for superoxide-generating enzymes in the failing heart. In the presence of NADPH oxidase upregulation and uncoupled NOS, NADPH generated by the oxidative penthose pathway may paradoxically fuel superoxide production. GP, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; NOS, nitric oxide synthase; SOD, superoxide dismutase.

also superoxide generators such as NADPH oxidase and uncoupled NO synthase (Fig. 5). To further explore, *in vivo*, the link between glucose metabolism and oxidative stress, the effects of raising glycemic levels from ~80 to ~170 mg/dL were tested in dogs with pacing-induced HF (92). In response to this acute glycemic peak, cardiac isoprostane output, an index of oxidative stress, increased by approximately two-fold, whereas oxPPP inhibition normalized oxidative stress and enhanced cardiac oxygen consumption, glucose oxidation, and stroke work. The clinical implications of these

findings are evident. For instance, they suggest that physiological postprandial glycemic peaks might induce a marked cardiac oxidative stress in HF patients. In theory, an attenuation of the oxPPP activity would mitigate this stress. But the balance between the anti-oxidant and the pro-oxidant effects of reducing equivalents provided by the oxPPP is very delicate. In fact, mice with 60% genetic reduction of G6PD activity were not more protected against cardiac remodeling and dysfunction after myocardial infarction or transverse aortic constriction. On the contrary, the outcome in these

FIG. 6. Different metabolic pathways lead to oxidative stress in dCM and HF. In dCM, hyperglycemia induces both oxPPP and polyol pathway activation while glycolysis is reduced due to completion by FFA. Increased reducing equivalents feed the overexpressed NAD(P)H oxidase (Nox) and uncoupled NOS, thus increasing ROS generation. In HF, glucose utilization by cardiomyocytes is increased due to concomitant downregulation of FFA catabolism. A pathologically higher fraction of glucose may then be utilized by the upregulated oxPPP, which provides NADPH for Nox and uncoupled NOS, thus increasing ROS generation. On the other hand, the polyol pathway might play a very minor role in this pathological condition. HF, heart failure.

mice was slightly worse compared with the control (34). In Dahl salt-sensitive rats fed a high-salt diet to induce progressive cardiac dysfunction, the oxPPP was found to be activated during the HF stage (41), consistent with findings in dogs (31). In these animals, administration of dichloroacetate, a PDH stimulator (Fig. 1), enhanced cardiac function and survival of the animals and activated the cardiac oxPPP. Although they could not provide direct evidence for it, the authors of this study attributed the beneficial effects of dichloroacetate to oxPPP activation. However, it is also possible that dichloroacetate redirected glucose from the oxPPP to the oxidative glycolytic pathway, thus subtracting substrate from NADPH-generating enzymes (Fig. 6). Only direct tracking of the entire glucose metabolic fate can help draw the right conclusions.

Currently, an alternative tool to explore overall glucose metabolism and therefore elucidate these complex aspects might be provided by computational analysis of the metabolomic profile in myocardial extracts. Using metabolomics data in intact Langendorff-perfused hearts (glucose as the only substrate), Cortassa *et al.* (17) were able to calculate the control of the glucose fluxome, including glycolysis, oxPPP, glycogenolysis, and polyol pathways. They found that the higher the glycolytic flux, the lower the oxPPP and polyol pathways. Otherwise stated, the structure of control reveals that an overall increase in the glycolytic flux will decrease the flux redirection through oxPPP and polyol pathways. In dCM, due to hyperglycemia, the glycolytic enzymes are impaired by fatty acid metabolism and Randle cycle, probably leading to an overfeeding of oxPPP and polyol pathways and an exaggerate competition for NAD(P)H, limiting its availability for glutathione peroxidase. In the absence of hyperglycemia, the polyol pathway activation probably plays a very minor role in HF (Fig. 6). Glucose must necessarily enter the glycolytic or the oxPPP. In addition, as previously mentioned, the polyol pathway is downregulated in HF (79) while the oxPPP is upregulated (31). Therefore, if further studies confirm it, the different involvement of polyol pathway and oxPPP might be considered a hallmark difference between glucose metabolism-induced oxidative stress in dCM and HF.

Role of altered FFA metabolism

The failing heart metabolic phenotype is characterized by a decreased FFA oxidation, as described in the previous paragraphs. PPARs and PGC-1, transcription factors responsible for the regulation of enzymes involved in FFA metabolism (Fig. 2), are downregulated in HF (5). Interestingly, these same transcription factors might also regulate the expression of enzymes that play a role in the anti-oxidant defense. In PGC-1 α knockout mice, the decreased expression of anti-oxidant proteins such as SOD2 and thioredoxin reductase-2 likely contributed to decreased survival after transverse aortic constriction (54). In fact, myocardial oxidative stress markers 3'-nitrotyrosine and 4-hydroxynonenal were increased in association to more severe signs of HF compared with wild type. Similar results were found in PGC-1 β -deficient mice (48, 72).

Conclusions

Despite the opposite metabolic alterations found in dCM and HF, they seem to have at least one common denomi-

nator, namely the generation of ROS. However, simply by evaluating the current literature, it is difficult to identify alterations of the energy substrate metabolic pathways, shared by the two pathological conditions, which might be responsible for enhanced oxidative stress. One of these alterations is perhaps the oxPPP over-activation (Fig. 6). In both dCM and HF, an increased glucose channeling in the oxPPP leads to the generation of a large amount of NADPH feeding the NAD(P)H oxidase and, subsequently, increasing ROS generation. While not surprising in the failing heart, which relies mainly on glucose, the over-activation of this pathway is less intuitive in dCM, especially in insulin-resistant patients, and calls for the search of potential therapeutic targets, such as glucose transporter SGLT1 and the PKC, apparently playing a central role in ROS generation associated to carbohydrate metabolism. Cardiac metabolic modulation is still a potentially interesting option for the treatment of dCM and HF and might also prove efficacious to mitigate oxidative stress (51).

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Abbreviations Used

ACC = acetyl-CoA carboxylase
 AGE = advanced glycation end-products
 ALDH2 = aldehyde dehydrogenase 2
 AMPK = AMP-activated protein kinase
 CaMKII = calcium/calmodulin-dependent kinase II
 CD36 = cluster of differentiation 36
 CoA = coenzyme-A
 CPT-1 = carnitine palmitoyltransferase-1
 dCM = diabetic cardiomyopathy
 ETC = electron transport chain
 FAT = free fatty acids translocase
 FATP = fatty acid transport protein
 FFA = free fatty acids
 GLUTs = glucose transporters
 H₂O₂ = hydrogen peroxide
 HF = heart failure
 HNE = 4-hydroxy-2-nonenal
 MAO = monoamine oxidases
 MCD = malonyl-CoA decarboxylase
 MDA = malondialdehyde
 oxPPP = oxidative pentose phosphate pathway
 PDH = pyruvate dehydrogenase
 PDK = PDH kinase
 PGC-1 = PPAR γ coactivator-1
 PPARs = peroxisome proliferator-activated receptors
 ROS = reactive oxygen species
 RXR α = retinoid X receptor- α
 SGLT1 = sodium/glucose co-transporter
 SOD = superoxide dismutase
 TCA = tricarboxylic acid cycle
 UCP = uncoupling protein