Prevention of Mitochondrial Oxidative Damage as a Therapeutic Strategy in Diabetes

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Hyperglycemia causes many of the pathological consequences of both type 1 and type 2 diabetes. Much of this damage is suggested to be a consequence of elevated production of reactive oxygen species by the mitochondrial respiratory chain during hyperglycemia. Mitochondrial radical production associated with hyperglycemia will also disrupt glucose-stimulated insulin secretion by pancreatic β-cells, because pancreatic β-cells are particularly susceptible to oxidative damage. Therefore, mitochondrial radical production in response to hyperglycemia contributes to both the progression and pathological complications of diabetes. Consequently, strategies to decrease mitochondrial radical production and oxidative damage may have therapeutic potential. This could be achieved by the use of antioxidants or by decreasing the mitochondrial membrane potential. Here, we outline the background to these strategies and discuss how antioxidants targeted to mitochondria, or selective mitochondrial uncoupling, may be potential therapies for diabetes. Diabetes 53 (Suppl. 1):S110–S118, 2004

Oxidative damage due to hyperglycemia contributes to the microvascular pathology of diabetes that occurs particularly in the retina, renal glomerulus, and peripheral nerves, causing blindness, renal failure, and peripheral neuropathy (1–4). Although the death of β-cells that underlies type 1 diabetes is probably due to an autoimmune response, the particular susceptibility of β-cells to oxidative damage from reactive oxygen species (ROS) produced during inflammation may be a predisposing factor (5,6). Supporting this, the streptozotocin and alloxan models of diabetes in rodents use ROS production to kill β-cells, and oxidative damage to β-cells during hyperglycemia may contribute to the progression of the disorder (7,8). The association between hyperglycemia and oxidative damage has been noted for some time with various sources proposed for the underlying ROS (1,2). Recently, it has been suggested that increased mitochondrial ROS production during hyperglycemia may be central to much of the pathology of diabetes (3,9). Furthermore, because β-cell mitochondria play a central role in glucose-stimulated insulin secretion (GSIS), damage to β-cell mitochondria will attenuate this response (7). Therefore, mitochondrial ROS production and oxidative damage may contribute to the onset, progression, and pathological consequences of both type 1 and type 2 diabetes. Here, we outline how mitochondrial oxidative damage occurs, consider the mechanisms by which it may contribute to the pathophysiology of diabetes, and discuss potential therapeutic strategies to prevent it.

MITOCHONDRIAL OXIDATIVE DAMAGE
Metabolism strips electrons from fatty acids, sugars, and amino acids and accumulates them on the soluble electron carrier NADH and on protein-bound FADH2 (Fig. 1). The electrons are then passed down the mitochondrial respiratory chain to drive ATP synthesis by oxidative phosphorylation. As the electrons move down the potential energy gradient from NADH/FADH2 to oxygen, the redox energy is conserved by pumping protons across the inner membrane to build up a proton electrochemical potential gradient (ΔΨm+). This gradient, composed of a substantial membrane potential and a smaller pH gradient, is used by the ATP synthase to make ATP, which is then mostly exported to the cytoplasm to carry out work. Protons can also reenter the mitochondrial matrix through nonspecific leak pathways and via proteins such as uncoupling proteins (UCPs), which may catalyze an inducible proton transport activity in the inner membrane. In both cases, redox energy is dissipated as heat rather than being used to make ATP.

Mitochondrial ROS production. The mitochondrial respiratory chain is the major site of ROS production within the cell. Superoxide is thought to be produced continually as a byproduct of normal respiration through the one-electron reduction of molecular oxygen (Fig. 1) (10,11). Superoxide itself damages iron sulfur center–containing enzymes such as aconitate (12) and can also react with nitric oxide to form the damaging oxidant peroxynitrite, which is more reactive than either precursor (13). Nitric oxide diffuses easily into mitochondria and may also be produced there (14). The mitochondrial enzyme manganese superoxide dismutase (MnSOD) converts superoxide to hydrogen peroxide, which, in the presence of ferrous or cuprous ions, forms the highly reactive hydroxyl radical, which damages all classes of biomolecules. The availability of free iron and copper within mitochondria is uncer-
tain, although the reaction of superoxide with the iron sulfur center in aconitase releases ferrous iron (12). Consequently, mitochondrial superoxide production initiates a range of damaging reactions through the production of superoxide, hydrogen peroxide, ferrous iron, hydroxyl radical, and peroxynitrite, which can damage lipids, proteins, and nucleic acids (15). Mitochondrial function is particularly susceptible to oxidative damage, leading to decreased mitochondrial ATP synthesis, cellular calcium dyshomeostasis, and induction of the mitochondrial permeability transition, all of which predispose cells to necrosis or apoptosis (15).

Mitochondrial antioxidant defenses. Mitochondria have a range of defenses against oxidative damage (Fig. 1). The antioxidant enzyme MnSOD converts superoxide to hydrogen peroxide (16). The mitochondrial isoform of glutathione peroxidase and the thioredoxin-dependent enzyme peroxiredoxin III both detoxify hydrogen peroxide (17); alternatively, hydrogen peroxide can diffuse from the mitochondria into the cytoplasm. The mitochondrial glutathione pool is distinct from that in the cytosol and is maintained in its reduced state by a mitochondrial isoform of glutathione reductase (17). This enzyme requires NADPH, which is produced within mitochondria by the

![Mitochondrial oxidative damage](image)

**FIG. 1.** Mitochondrial oxidative damage. The mitochondrial respiratory chain (top) passes electrons from the electron carriers NADH and FADH$_2$ through the respiratory chain to oxygen. This leads to the pumping of protons across the mitochondrial inner membrane to establish a proton electrochemical potential gradient ($\Delta$\(\mu_m\), negative inside: only the membrane potential ($\Delta$\(\mu_m\)) component of $\Delta$\(\mu_m\) is shown. The $\Delta$\(\mu_m\) is used to drive ATP synthesis by the $F_0F_1$ ATP synthase. The exchange of ATP and ADP across the inner membrane is catalyzed by the adenine nucleotide transporter (ANT) and the movement of inorganic phosphate ($P_i$) is catalyzed by the phosphate carrier (PC) (top left). There are also proton leak pathways that dissipate $\Delta$\(\mu_m\) without formation of ATP (top right). The respiratory chain also produces superoxide ($O_2^-$), which can react with and damage iron sulfur proteins such as aconitase, thereby ejecting ferrous iron. Superoxide also reacts with nitric oxide (NO) to form peroxynitrite (ONOO$^-$). In the presence of ferrous iron, hydrogen peroxide forms the very reactive hydroxyl radical (OH). Both peroxynitrite and hydroxyl radical can cause extensive oxidative damage (bottom right). The defenses against oxidative damage (bottom left) include MnSOD, and the hydrogen peroxide it produces is degraded by glutathione peroxidase (GPX) and peroxiredoxin III (PRX III). Glutathione (GSH) is regenerated from glutathione disulfide (GSSG) by the action of glutathione reductase (GR), and the NADPH for this is in part supplied by a transhydrogenase (TH).
NADP-dependent isocitrate dehydrogenase and through a \( \Delta \mu_{H^+} \)-dependent transhydrogenase (17). Within the mitochondrial phospholipid bilayer, the fat-soluble antioxidants vitamin E and Coenzyme Q both prevent lipid peroxidation, while Coenzyme Q also recycles vitamin E and is itself regenerated by the respiratory chain (18). The mitochondrial isoform of phospholipid hydroperoxide glutathione peroxidase degrades lipid peroxides within the mitochondrial inner membrane (17). There are also a range of mechanisms to repair or degrade oxidatively damaged lipid, protein, and DNA (19).

**Mitochondrial ROS production in vivo.** Many diverse pro-oxidant and antioxidant processes are ongoing in mitochondria. Oxidative damage occurs whenever the ROS produced by mitochondria evade detoxification, and the steady-state level of oxidative damage depends on the relative rates of damage accumulation, repair, and degradation (15,20). That mitochondrial ROS production occurs at all times is suggested by mice lacking MnSOD, which die within a few days of birth (21), while those lacking the cytosolic isofrom Cu,ZnSOD survive (22). Further evidence of mitochondrial ROS production under normal conditions is the efflux of hydrogen peroxide from intact mitochondria and from perfused organs, suggesting that mitochondria produce superoxide, which is then converted to hydrogen peroxide in vivo (11). There is also evidence that, under certain conditions, mitochondrial DNA and protein accumulate greater oxidative damage in vivo than the rest of the cell (19).

Complex III produces large amounts of superoxide when inhibited by antimycin, which stabilizes a ubisemiquinone radical at ubiquinol binding site \( o \) (10). This ubisemiquinone radical transfers a single electron to oxygen to form superoxide on the outside of the mitochondrial inner membrane (10,23). Complex I produces superoxide from NADH when it is inhibited by rotenone by a \( \Delta \mu_{H^+} \)-independent mechanism (24,25). Complex I also generates superoxide from ubiquinol when there is a sufficiently large \( \Delta \mu_{H^+} \), to drive reverse electron transport through complex I in intact mitochondria (23,26). In this case, superoxide is produced on the matrix side of the inner membrane, and its generation is inhibited by rotenone or an uncoupler (23). The maximum rate of superoxide production by antimycin-inhibited complex III is generally greater than that of complex I, which has led some to assume that the situation in vivo is similar. However, in the absence of antimycin, superoxide production by complex III is minimal (23), and it seems probable that in vivo complex I is the major source of superoxide through reversed electron transport (23,24,26) and possibly also from forward electron transport (25).

Many other enzymes associated with mitochondria can also produce superoxide or hydrogen peroxide, but even though their contribution to ROS formation in vivo is unclear, the current tacit assumption that only complexes I and III produce ROS may have to be reassessed. Even so, some conclusions about ROS formation by the respiratory chain are possible (23). Older ideas that mitochondrial ROS production is a simple function of the rate of oxygen consumption, with faster respiration linked to a greater rate of ROS production, have been abandoned. Instead, we now recognize that mitochondrial ROS production is favored by high levels of reduction of the respiratory electron carriers, particularly the Coenzyme Q pool, and by a large \( \Delta \mu_{H^+} \). These conditions favor superoxide production from complex I by enhancing reverse electron transport and may also act by increasing the lifetime of the semiquinone radical at the \( o \) site in complex III. Furthermore, because the rates of the nonenzymatic reactions of oxygen with radical intermediates to form superoxide are proportional to the local oxygen concentration, a high local oxygen concentration will also favor superoxide production (27). All of the conditions that favor superoxide production occur when mitochondria are respiring but not making ATP (state 4). In contrast, when mitochondria are actively making ATP (state 3), the lower \( \Delta \mu_{H^+} \), increased oxidation of electron carrier pools, and lower local oxygen concentration will decrease superoxide production.

**Uncoupling and mitochondrial ROS production.** As an elevated \( \Delta \mu_{H^+} \) favors superoxide production, limiting the magnitude of this gradient under state 4 conditions should decrease superoxide production (28). Artificial uncouplers such as 2,4-dinitrophenol (DNP) make the inner membrane permeable to protons, thereby lowering the \( \Delta \mu_{H^+} \), which accelerates respiration and consequently oxidizes the electron carrier pools (28,29). Uncouplers are typically lipophilic weak acids for which both the protonated and unprotonated forms are lipid soluble, thus enabling them to catalyze proton movement through the phospholipid bilayer by lowering the activation energy for proton leak. Even low concentrations of artificial uncouplers have been shown to lower the rate of superoxide production by mitochondria (28).

In the absence of uncouplers, the mitochondrial inner membrane is still slightly permeable to protons, but intriguingly this permeability increases dramatically at high \( \Delta \mu_{H^+} \) (state 4) compared with when the mitochondria are making ATP (state 3) (30–32). The increased leakiness of the mitochondrial inner membrane to protons may be to minimize superoxide production in state 4 by limiting the maximum \( \Delta \mu_{H^+} \) (28). Although the mechanism of this proton leak is not certain, it is quite distinct from the thermogenic inducible proton leak catalyzed by UCP1 (33). This protein is confined to brown adipose tissue mitochondria, and its function is primarily thermogenic (33). There is considerable interest in two close homologues of UCP1 called (perhaps misleadingly) UCP2 and UCP3, which are also found in the mitochondrial inner membrane (33). UCP2 mRNA is widely expressed, but the expression of these UCPs increases in response to 2,4-dinitrophenol (DNP) make the inner membrane permeable to protons, thereby lowering the \( \Delta \mu_{H^+} \), which accelerates respiration and consequently oxidizes the electron carrier pools (28,29). Uncouplers are typically lipophilic weak acids for which both the protonated and unprotonated forms are lipid soluble, thus enabling them to catalyze proton movement through the phospholipid bilayer by lowering the activation energy for proton leak. Even low concentrations of artificial uncouplers have been shown to lower the rate of superoxide production by mitochondria (28).

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Mitochondrial membrane potential when superoxide is generated under state 4 conditions. Echtay et al. (35) have proposed a simple feedback cycle in which mitochondrial oxidative stress acutely and chronically upregulates the proton translocating activity of UCPs to lower the $\Delta \mu_{\text{H}^+}$ and thus decrease superoxide production (Fig. 2). However, until the physiological role(s) of UCP2 and UCP3 are fully clarified, the significance of the interaction of ROS with UCPs in vivo will remain uncertain (33).

A further issue to consider is the role of UCP2 within pancreatic $\beta$-cells. Glucose sensing by pancreatic $\beta$-cells uses ATP as a coupling factor between glucose metabolism and insulin secretion (outlined in Fig. 3). Mitochondria in $\beta$-cells contain UCP2, and because uncoupling protein activity lowers the $\Delta \mu_{\text{H}^+}$, it is also a means of attenuating GSIS (Fig. 2). The islets of UCP2$^{(-/-)}$ mice exhibit markedly higher ATP levels and improved GSIS, demonstrating that endogenous $\beta$-cell UCP2 is activated and that this impairs GSIS (36). Furthermore, the hyperglycemia of ob/ob mice (a model of type 2 diabetes) is significantly reduced by ablation of UCP2. This supports the principle that reducing UCP2 activity in $\beta$-cells is a valid means of treating diabetes (36), but also indicates that while uncoupling and activation of UCP can reduce ROS production in peripheral tissue, it may disrupt GSIS in $\beta$-cells.

**MITOCHONDRIAL OXIDATIVE DAMAGE IN DIABETES**

Much of the long-term pathology of diabetes occurs as a consequence of persistent hyperglycemia. Four consequences of hyperglycemia of particular pathological relevance (1) are the formation, auto-oxidation, and interaction with cell receptors of advanced glycation end products (AGEs); activation of various isoforms of protein kinase C; induction of the polyol pathway; and increased hexosamine pathway flux. Many of these pathways have long been associated with elevated oxidative stress (1), for example,

![Diagram showing mitochondrial ROS production and oxidative damage](image-url)
through auto-oxidation of AGEs or through the action of the polyol pathway depleting cytosolic NADPH and thereby decreasing the cellular glutathione/glutathione disulfide ratio. Recently, a hypothesis was proposed that suggests that all these processes are a consequence of overproduction of superoxide by the mitochondrial respiratory chain during hyperglycemia (3,9,39). This is thought to occur because hyperglycemia increases the flow of electrons to the respiratory chain by maintaining large mitochondrial NADH/NAD and FADH2/FAD ratios under conditions of high glucose/H9004/H9262/H11001 (Fig. 3). Thus, mitochondria in many tissues would spend more time under state 4-like conditions of low respiration rate, high glucose/H9004/H9262/H11001, and reduced electron carriers, all of which favor superoxide formation. However, it should be noted that the validity of this link between hyperglycemia and increased mitochondrial superoxide production has not yet been demonstrated. Indeed, in isolated hepatocytes, simply increasing the glucose concentration does not increase glucose/H9004/H9262/H11001, mitochondrial respiration rate, or cytosolic NADH/NAD ratio; instead, most of the excess glucose is converted to glycogen (40). Consequently, the link between hyperglycemia and increased mitochondrial superoxide production may turn out to be due to some other interaction with mitochondria, not mediated directly by the redox state of electron carriers.

The evidence for increased mitochondrial ROS production during hyperglycemia comes from experiments on cultured endothelial cells, where raising the glucose concentration from 5 to 30 mmol/l increased cytosolic ROS production, as measured by the rate of oxidation of dichlorodihydrofluorescein to dichlorofluorescein (DCF) (9). The reaction between hydrogen peroxide and dichlorodihydrofluorescein is catalyzed by intracellular peroxidases; thus, an increase in cellular DCF oxidation is consistent with elevated mitochondrial superoxide production forming hydrogen peroxide, which then diffuses to the cytoplasm. DCF oxidation was disrupted by overexpression of mitochondrial MnSOD, suggesting that the proximal ROS produced was superoxide within the mitochondrial matrix. However, it is unclear why overexpression of MnSOD abolished the DCF oxidation signal, because MnSOD should convert the superoxide to hydrogen peroxide, which should then efflux from the mitochondria and enhance the DCF signal. This finding suggests that the nature of the ROS being measured in these experiments remains uncertain. DCF oxidation was also blocked by inhibitors of mitochondrial pyruvate uptake and of succinate dehydrogenase, but not by rotenone, suggesting that reverse electron transport was not involved. ROS production was also blocked by overexpression of UCP1, indicating dependence. The increase in mitochondrial ROS production during hyperglycemia comes from experiments on cultured endothelial cells, where raising the glucose concentration from 5 to 30 mmol/l increased cytosolic ROS production, as measured by the rate of oxidation of dichlorodihydrofluorescein to dichlorofluorescein (DCF) (9). The reaction between hydrogen peroxide and dichlorodihydrofluorescein is catalyzed by intracellular peroxidases; thus, an increase in cellular DCF oxidation is consistent with elevated mitochondrial superoxide production forming hydrogen peroxide, which then diffuses to the cytoplasm. DCF oxidation was disrupted by overexpression of mitochondrial MnSOD, suggesting that the proximal ROS produced was superoxide within the mitochondrial matrix. However, it is unclear why overexpression of MnSOD abolished the DCF oxidation signal, because MnSOD should convert the superoxide to hydrogen peroxide, which should then efflux from the mitochondria and enhance the DCF signal. This finding suggests that the nature of the ROS being measured in these experiments remains uncertain. DCF oxidation was also blocked by inhibitors of mitochondrial pyruvate uptake and of succinate dehydrogenase, but not by rotenone, suggesting that reverse electron transport was not involved. ROS production was also blocked by overexpression of UCP1, indicating dependence. The increase in mitochondrial ROS production during hyperglycemia comes from experiments on cultured endothelial cells, where raising the glucose concentration from 5 to 30 mmol/l increased cytosolic ROS production, as measured by the rate of oxidation of dichlorodihydrofluorescein to dichlorofluorescein (DCF) (9). The reaction between hydrogen peroxide and dichlorodihydrofluorescein is catalyzed by intracellular peroxidases; thus, an increase in cellular DCF oxidation is consistent with elevated mitochondrial superoxide production forming hydrogen peroxide, which then diffuses to the cytoplasm. DCF oxidation was disrupted by overexpression of mitochondrial MnSOD, suggesting that the proximal ROS produced was superoxide within the mitochondrial matrix. However, it is unclear why overexpression of MnSOD abolished the DCF oxidation signal, because MnSOD should convert the superoxide to hydrogen peroxide, which should then efflux from the mitochondria and enhance the DCF signal. This finding suggests that the nature of the ROS being measured in these experiments remains uncertain. DCF oxidation was also blocked by inhibitors of mitochondrial pyruvate uptake and of succinate dehydrogenase, but not by rotenone, suggesting that reverse electron transport was not involved. ROS production was also blocked by overexpression of UCP1, indicating dependence. The increase in mitochondrial ROS...
production associated with hyperglycemia also led to the activation of the redox-sensitive cytosolic transcription factor Nfκb, the formation of AGEs, and the activation of protein kinase C, all of which were prevented by blocking mitochondrial ROS production with MnSOD, respiratory inhibitors, or UCP1 (9).

The model that arose from these studies is that an increase in mitochondrial ROS in response to hyperglycemia is the proximal defect that leads to most of the other pathological consequences of hyperglycemia. There is no doubt this view will prove too simplistic and will have to be extended to accommodate other sites of ROS production (2). Nevertheless, this is a major new insight that suggests how preventing the production of superoxide by mitochondria, or increasing its rate of decomposition by antioxidants, may block many of the pathological consequences of hyperglycemia. In addition, β-cell mitochondria are essential for GSIS but are susceptible to oxidative damage during hyperglycemia that suppresses GSIS (7,8), thus contributing to the progression of the disease through a vicious cycle in which hyperglycemia causes oxidative damage, which in turn disrupts the ability of β-cells to respond to elevated blood glucose, leading to further hyperglycemia (2,7).

In summary, increased mitochondrial ROS production during hyperglycemia may be a major factor in the pathology of diabetes. This suggests that therapeutic strategies to limit mitochondrial radical production during hyperglycemia and to counteract their damaging effects may be useful complements to conventional therapies designed to normalize blood glucose.

ANTIOXIDANT THERAPIES

Because oxidative damage is part of the pathophysiology of diabetes, there is interest in determining whether antioxidants can decrease this damage (1). Too few large-scale double-blind trials on the use of antioxidants in diabetes have been carried out for conclusions (1). However, a few small-scale trials have suggested the efficacy of the natural antioxidants α-tocopherol (vitamin E), ascorbate (vitamin C), Coenzyme Q, and α-lipoic acid, although in other trials, the efficacy of ascorbate and α-tocopherol were ambiguous (1,2). Because these natural antioxidants can be given at high doses and have shown some efficacy in other degenerative diseases, there is a strong rationale for trialing them in diabetes (1,41), even though the uptake and distribution to tissues of hydrophobic natural antioxidants such as Coenzyme Q is often poor (42). In addition, many other artificial antioxidants are being developed, such as mimetics of SOD or peroxidase, that may be more potent than natural antioxidants and also have improved bioavailability, pharmacokinetics, and stability (43,44). Because these artificial antioxidants are novel drugs (vs. natural antioxidants), it will be more demanding to initiate clinical trials. Both the natural and artificial antioxidants distribute throughout the body, with only a small proportion reaching the mitochondria, where much of the oxidative damage associated with hyperglycemia may occur.

Mitochondria-targeted antioxidants in vivo. Because mitochondrial oxidative damage is thought to be critical in the pathophysiology of diabetes, antioxidants that accumulate within mitochondria may offer more protection than untargeted antioxidants. As a first step toward testing this hypothesis, a strategy has been developed to deliver antioxidants to mitochondria by covalent attachment to the triphenylphosphonium cation through an alkyl chain (Fig. 4) (45,46). The delocalized positive charge of these lipophilic cations enables them to permeate lipid bilayers easily and to accumulate several hundred-fold within mitochondria, due to the large membrane potential (∆ψm: −150 to −170 mV, negative inside; Fig. 4). The plasma membrane potential (∆ψp, −30 to −60 mV, negative inside; Fig 4) also drives their accumulation from the extracellular fluid into cells, from where they are further concentrated within mitochondria. Because the natural antioxidants vitamin E and Coenzyme Q are thought to protect mitochondria from oxidative damage in vivo, mitochondria-targeted derivatives of these molecules were first developed (Fig. 4). Experiments in vitro showed that the mitochondria-targeted derivative of α-tocopherol (MitoVit E) and the mitochondria-targeted ubiquinone were rapidly and selectively accumulated by isolated mitochondria and by mitochondria within isolated cells (47–49). Importantly, the accumulation of these antioxidants by mitochondria protected them from oxidative damage far more effectively than untargeted antioxidants, suggesting that the accumulation of antioxidants within mitochondria does increase their efficacy. Most interestingly, these compounds were several hundred-fold more effective at preventing cell death in fibroblasts from Friedreich Ataxia patients (49a). Because cell death in this model is due to endogenous mitochondrial oxidative damage (50), it is suggested that the accumulation of antioxidants by mitochondria within cells blocks mitochondrial oxidative damage and that their uptake into mitochondria makes them far more effective than untargeted antioxidants.

Mitochondria-targeted antioxidants in vivo. If these mitochondria-targeted molecules are to have therapeutic potential in diabetes, then they must be taken up selectively by mitochondria in vivo. Because alkyltriphenylphosphonium cations pass easily through lipid bilayers by noncarrier-mediated transport, they should be taken up by the mitochondria of all tissues, in contrast to hydrophilic compounds, which rely on the tissue-specific expression of carriers for uptake (41). Mice were fed mitochondria-targeted antioxidants for several weeks, leading to stable steady-state concentrations within all tissues assessed, including the brain, heart, liver, and kidneys (51). Uptake was reversible, as shown by the rapid clearance of the simple lipophilic cation methyltriphenylphosphonium from all organs when oral administration stopped (51). That these compounds can enter the bloodstream and distribute to tissues in their intact active form was shown by solvent extraction of the brain, heart, and liver of mice fed MitoVit E, followed by mass spectrometry (51). These data are consistent with the following pharmacokinetic model: following absorption from the gut into the bloodstream, orally administered mitochondria-targeted antioxidants are taken up into all tissues by nonmediated movement through the lipid bilayer of the plasma membrane, assisted by the plasma membrane potential. From the cytosol, most of the lipophilic cations are taken up into mitochondria, driven by the large membrane potential. After several days of feeding, the cation concentration
within mitochondria comes to a steady-state distribution with circulating blood levels. At this point, the mitochondrial concentration will be several hundred-fold higher than that in the bloodstream. Because the mitochondrial pool of compound is in dynamic equilibrium, once feeding stops, the accumulated cations will re-equilibrate back into the bloodstream and be relatively rapidly excreted.

The levels of methyltriphenylphosphonium and MitoVit E that accumulated in mouse tissues in vivo after feeding were in the range of 5–20 nmol/g wet wt, or about 5–20 μmol/l in the tissue (51). Because these compounds accumulate within mitochondria, the intramitochondrial concentration will be about millimolar. These concentrations are likely to be in the therapeutically effective range, because mitochondria-targeted antioxidants prevented oxidative damage to isolated mitochondria at 1–2.5 μmol/l (48,49). Because these compounds will be further accumulated into cells, similar protective effects were found by incubating cultured cells with 500 nmol/l to 1 μmol/l mitochondria-targeted antioxidants (49,52). Therefore, oral delivery of well-tolerated doses of mitochondria-targeted antioxidants can deliver potentially therapeutic concentrations to mitochondria in vivo. Their efficacy at preventing oxidative damage to mitochondria in vivo is now being tested in mouse models of mitochondrial oxidative damage.

**Uncoupling to prevent oxidative damage.** In addition to inactivating ROS by antioxidants, another strategy is to decrease mitochondrial ROS production in diabetes by lowering the Δρ_{H+}. This could be done using low doses of artificial uncouplers to slightly lower the Δρ_{H+} and thus decrease mitochondrial ROS production (29). The uncoupler DNP has been used extensively in the past to treat obesity in humans, with surprisingly good results (29). However, unregulated administration, abuse, and the very narrow therapeutic window between efficacy and toxicity.

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**FIG. 4. Mitochondria-targeted antioxidants.** A generic mitochondria-targeted antioxidant is shown constructed by the covalent attachment of an antioxidant moiety (X) to the lipophilic triphenylphosphonium cation. This molecule is accumulated 5- to 10-fold into the cytoplasm driven by the plasma membrane potential (Δψ_{p}) and then further accumulated into the mitochondria 100- to 500-fold driven by the mitochondrial membrane potential (Δψ_{m}). The structures on the bottom are of two targeted antioxidants, MitoVit E and mitochondria-targeted Coenzyme Q (MitoQ).
led to its abandonment, although it is still used unofficially as a slimming agent (29). It should be possible to make a safe uncoupler for human use, although the challenge is to make one that is mild enough to decrease mitochondrial ROS production without significantly affecting mitochondrial ATP production, and to make these molecules tissue specific to reduce the possibility of compromising ATP production in nervous and cardiac tissue. A further complication is that decreasing the membrane potential of β-cell mitochondria would make insulin secretion less responsive to plasma glucose levels, which might be counterproductive. Indeed, it has been reported that DNP treatment of animals causes hyperglycemia (53).

An alternative way to decrease ΔΨm may be activating the inducible proton leak catalyzed by endogenous proteins such as the UCPs. However, whereas some molecules such as superoxide and retinoids do appear to activate UCPs (35, 38), the development of drugs designed to modulate this function will probably require better understanding of the physiological roles and molecular mechanisms of UCPs.

CONCLUSIONS

It seems probable that mitochondrial radical production and consequent oxidative damage contribute to the progression and pathophysiology of diabetes. Consequently, therapeutic strategies to decrease ROS production, or to intercept the ROS once formed, should be explored. Two approaches suggest themselves: selective uncoupling of mitochondria, and antioxidants. Whereas the development of uncoupling strategies is not imminent, the time is upon us to test antioxidant therapies in diabetes. As well as protecting peripheral tissues from hyperglycemia-induced oxidative damage, antioxidants may have the additional benefit of improving GSIS, both by preventing the damage to β-cells and possibly by blocking the proposed ROS activation of UCP2 in β-cells. Whereas suitable uncouplers should protect peripheral tissues, there is the danger that they would have counterproductive effects on β-cells, rendering insulin secretion less responsive to plasma glucose.

A first step in developing antioxidant therapies is to give large doses of natural antioxidants such as vitamin E, α-lipoic acid, or Coenzyme Q to see if this approach has potential. The advantage of natural antioxidants is their safety and that large oral doses are well tolerated. However, in other degenerative diseases, very large doses have been required to see beneficial effects, possibly because of their poor bioavailability and distribution within the body and the cell (42). If natural antioxidants show efficacy in large-scale patient trials, then it may be more effective to develop artificial antioxidants with improved potency, bioavailability, and pharmacokinetics. Among these, a case can be made for testing mitochondria-targeted antioxidants. To date, mitochondria-targeted versions of Coenzyme Q and vitamin E have been made and can be administered safely to mice (51). Experiments are now underway to see if these and other mitochondria-targeted antioxidants show efficacy against the pathologies associated with hyperglycemia in cell and rodent models of diabetes.

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