Perspectives in Diabetes

Oxidative Capacity, Lipotoxicity, and Mitochondrial Damage in Type 2 Diabetes

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Recent evidence points toward decreased oxidative capacity and mitochondrial aberrations as a major contributor to the development of insulin resistance and type 2 diabetes. In this article we will provide an integrative view on the interrelation between decreased oxidative capacity, lipotoxicity, and mitochondrial aberrations in type 2 diabetes. Type 2 diabetes is characterized by disturbances in fatty acid metabolism and is accompanied by accumulation of fatty acids in nonadipose tissues. In metabolically active tissues, such as skeletal muscle, fatty acids are prone to so-called oxidative damage. In addition to producing energy, mitochondria are also a major source of reactive oxygen species, which can lead to lipid peroxidation. In particular, the mitochondrial matrix, which contains DNA, RNA, and numerous enzymes necessary for substrate oxidation, is sensitive to peroxide-induced oxidative damage and needs to be protected against the formation and accumulation of lipids and lipid peroxides. Recent evidence reports that mitochondrial uncoupling is involved in the protection of the mitochondrial matrix against lipid-induced mitochondrial damage. Disturbances in this protection mechanism can contribute to the development of type 2 diabetes. *Diabetes* 53: 1412–1417, 2004

S keletal muscle is responsible for a major part of postprandial glucose uptake, and resistance of the muscle toward insulin action is a common characteristic of type 2 diabetes (1). It has been suggested that defects in the capacity to metabolize glucose and fatty acids lead to insulin resistance of skeletal muscle (2). Indeed, a reduced fat oxidative capacity has been reported in type 2 diabetic patients (3). In addition, plasma free fatty acid (FFA) levels are increased in obese and diabetic patients, and in the long term, increased FFA levels together with diminished fat oxidative capacity will lead to the accumulation of fatty acids and triacylglycerol in nonadipose tissues, such as the β-cell, heart, liver, and skeletal muscle. Importantly, the accumulation of triacylglycerol inside muscle cells (intramyocellular lipids [IMCLs]) has been shown to correlate very strongly with insulin resistance (4,5). In elegant studies by Boden and colleagues (6,7), it was shown that elevation of plasma FFA levels during a hyperinsulinemic-euglycemic clamp induces skeletal muscle insulin resistance, but only after 2–4 h of fat infusion. The delay in effect of plasma fatty acids on insulin action suggests an indirect effect of FFAs. This notion is supported by the finding that fatty acid–induced insulin resistance is accompanied by accumulation of diacylglycerol (DAG) and triacylglycerol in human skeletal muscle (8), suggesting that muscular fat accumulation induces insulin resistance. DAG in particular has been suggested to be responsible for the induction of insulin resistance by activating distinct isoforms of protein kinase C (PKC). Subsequently, activated PKC could directly interact with insulin signaling by phosphorylating and inhibiting tyrosine kinase activity of the insulin receptor and tyrosine phosphorylation of insulin receptor substrate-1 (rev. in 2,9). Alternatively, PKC could activate the IKK-β/NF-κB pathway (8), which has been suggested to be involved in the fatty acid–induced impairment of insulin action (rev. in 10). However, regardless of the exact mechanism, there is compelling evidence that a diminished skeletal muscle oxidative capacity, through fat accumulation inside muscle cells, could induce skeletal muscle insulin resistance and contribute to the development of type 2 diabetes. The question of what causes the decreased oxidative capacity still remains.

Mitochondrial dysfunction as a cause of type 2 diabetes? In the search for causal factors that explain the decreased capacity to oxidize fatty acids by skeletal muscle, most laboratories have focused on deviations in one or more enzymes and/or proteins. For example, carnitine palmitoyltransferase-1 (CPT-1) acts to convert long-chain acyl-CoA into long-chain acyl-carnitine, which is necessary to allow mitochondrial fatty acid uptake, and CPT-1 is therefore the rate-limiting enzyme in the oxidation of fatty acids. Indeed, a reduced CPT-1 activity has been found in skeletal muscle of obese insulin-resistant subjects (11). CPT-1 activity can be inhibited by malonyl-CoA, which is synthesized from acetyl-CoA through the enzyme acetyl-CoA carboxylase-2 (ACC2). Mice lacking ACC2 in skeletal muscle have increased fat oxidative capacity and are protected against high-fat diet–induced

diabetes (12). In addition, activation of the enzyme AMP-activated protein kinase (AMPK), which inactivates ACC2 and reduces malonyl-CoA levels, by exercise or AICAR (5-aminoimidazole-4-carboxamide ribonucleoside) results in an improvement in insulin-stimulated glucose uptake (13,14). Although these studies have provided clues toward possible defects in skeletal muscle, there is no uniform answer to the question of what causes the decreased skeletal muscle oxidative capacity. Some light into this comes from two recent and independent studies showing a coordinated reduction of oxidative genes in human type 2 diabetes (15,16). Both studies used DNA microarrays to identify genes that were downregulated in skeletal muscle from type 2 diabetic patients. Both studies reported that a cluster of oxidative genes, under the control of the peroxisome proliferator–activated receptor (PPAR)-γ coactivator (PGC1), was reduced in diabetes. In one of these studies, the reduction in this cluster of oxidative genes was well correlated with whole-body aerobic capacity (16). This finding is compatible with the early finding of a decreased whole-body aerobic capacity in patients with type 2 diabetes and their first-degree relatives (17,18), as well as with the general accepted concept that regular physical exercise is beneficial in the prevention of type 2 diabetes. Most important, however, these studies show that it is not a defect in one single gene or protein involved in substrate oxidation that is responsible for the decreased oxidative capacity of skeletal muscle. In fact, these findings point toward a phenotype of general mitochondrial dysfunction in type 2 diabetes. Interestingly, Kelley et al. (19) recently reported an impaired functional capacity of mitochondria in skeletal muscle of diabetic patients. Mitochondria of type 2 diabetic patients had reduced capacity of the electron transport chain (as measured by NADH:O2 oxidoreductase activity) and reduced citrate synthase activity. To investigate a potential explanation for the reduced oxidative capacity, Kelley et al. also examined mitochondrial morphology. They found that mitochondria from type 2 diabetic patients were smaller and that mitochondrial area correlated with insulin sensitivity (19). They suggested that muscular lipid accumulation might have been responsible for the alterations in mitochondrial functioning, although they did not investigate this topic.

Mitochondrial damage: a role for lipid peroxides? Apart from producing energy, mitochondria are also a major source of reactive oxygen species (ROS) (20). These ROS products have a very short half-life and react rapidly with DNA, protein, and lipids, thereby leading to so-called oxidative damage. Fatty acids are particularly prone to oxidative damage, resulting in the formation of lipid peroxides. These peroxide products are cytotoxic and highly reactive, leading to free-radical damage to proteins and DNA. Therefore, accumulation of fatty acids in the vicinity of the mitochondrial matrix, where oxidative processes take place, makes them prone to lipid peroxidation, which eventually may result in damaged mitochondrial proteins and reduced oxidative capacity as a consequence. Such a situation might be the case in type 2 diabetes. As mentioned above, patients with type 2 diabetes are characterized by high plasma FFA levels (21) and a reduced fat oxidative capacity (3). Under such conditions,

fatty acids that cannot be oxidized will accumulate in the muscle cell (4,5), and the increased load of fatty acids on the mitochondrial membrane will lead to the entrance of neutral fatty acids into the mitochondrial matrix (22), where they are prone to peroxidation (see "A role for UCP3 in preventing lipid-induced mitochondrial damage?" for further explanation). Consistent with this idea is the recent finding that skeletal muscle of obese insulin-resistant subjects contained a higher amount of intramyocellular lipids and, more importantly, these lipids showed a higher degree of lipid peroxidation (23). Also, in the elderly it was recently shown that muscular lipid accumulation is related to mitochondrial dysfunction and insulin resistance (24), and aging is associated with accumulation of ROS-induced mutations in control sites of mitochondrial DNA replication (25). Together, these results suggest that lipid accumulation in muscle cells, as observed in type 2 diabetes (4,5), could impair mitochondrial oxidative capacity due to lipid peroxidation–induced damage to mitochondria. In turn, the reduced mitochondrial oxidative capacity would further exacerbate the storage of lipids inside the muscle cell. Together, with a suggested reduced PGC1 activity, which would limit mitochondrial biogenesis (26), a positive feedback loop would exist in which mitochondrial functioning would rapidly deteriorate. The question then arises, are we equipped with a system to interrupt this positive feedback loop?

Mitochondrial uncoupling as a mechanism to reduce lipid peroxidation? In living cells, adenosine triphosphate (ATP) is resynthesized continuously in the mitochondria. To resynthesize ATP from ADP, substrates such as fat, carbohydrate, and proteins are metabolized, resulting in the production of NADH and FADH2. Subsequently, NADH and FADH2 can be oxidized to NAD+, FAD, and H+ in the respiratory chain. According to the chemiosmotic hypothesis of Mitchell (27), the protons are transported to the cytosolic side of the inner mitochondrial membrane by a series of reactions. This eventually generates a proton gradient across the membrane, which causes protons to flow back across the inner mitochondrial membrane through a so-called F_0F_1 complex. The energy thus generated is used by ATPase to transform ADP into ATP. However, mitochondrial respiration also results in the formation of ROS such as superoxide and hydrogen peroxide. Moreover, when the proton gradient becomes too high, the electron transport is slowed and the formation of superoxide and ROS is increased. One way to prevent excessive ROS production is by lowering the mitochondrial proton gradient by, for example, uncoupling substrate oxidation from ATP production. Indeed, mild mitochondrial uncoupling, thereby lowering the proton gradient, results in a pronounced decrease in mitochondrial production of ROS (28). Recently, Echtay et al. (29) have shown a signaling role for 4-hydroxy-2-nonenal, an important byproduct of lipid peroxidation, in the regulation of mitochondrial uncoupling, suggesting a negative feedback loop (Fig. 1). When mitochondrial ROS production becomes excessive, and thus lipid peroxides are formed, mitochondrial uncoupling would reduce ROS production, thereby reducing the formation of lipid peroxides. In skeletal muscle, 4-hydroxy-2-nonenal–induced uncoupling was shown to be accomplished by the mitochondrial
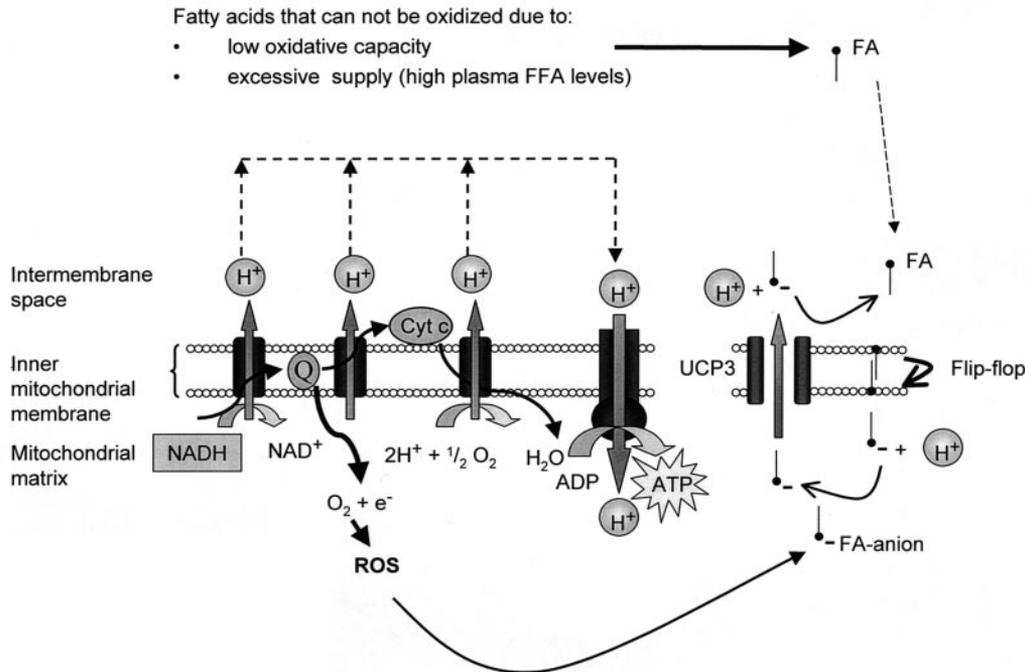
FIG. 1. A role for UCP3 in the protection of mitochondria against lipid peroxidation. Fatty acids that cannot be oxidized (i.e., cannot be converted to fatty acyl-CoA) can enter the mitochondrial matrix as neutral fatty acids by a so-called flip-flop mechanism (44). Here, these fatty acids will be deprotonated and become fatty acid anions, thereby lowering the proton gradient. The mitochondrial matrix is also the site where substrate oxidation and oxidative phosphorylation takes place and ROS are formed. These ROS can attack fatty acid anions and produce lipid peroxides, which are highly reactive and can lead to oxidative damage to DNA, RNA, and enzymes inside the matrix. UCP3 exports the fatty acid anions and/or peroxides to prevent damage to the mitochondrial matrix and lowers the proton gradient, thereby reducing ROS formation. Activation of UCP3 by lipid peroxides facilitates the export of fatty acid anions/peroxides, resulting in a negative feedback loop to prevent mitochondrial damage. In the insulin-resistant state, UCP3 levels are decreased and levels of lipid peroxides are increased. This might ultimately lead to mitochondrial aberrations.

uncoupling protein (UCP)-3 (29), which is in accordance with the earlier notion that UCP3 plays a role in the prevention of excessive ROS (30). Indeed, this negative feedback loop appears to be interrupted in the absence of UCP3, as UCP3-ablated mice have been shown to have increased skeletal muscle ROS production (30), increased lipid peroxidation, and damage to mitochondrial proteins (31). The question of whether deviations in this negative feedback loop could play a role in type 2 diabetes then remains.

A role for UCP3 in preventing lipid-induced mitochondrial damage? The recent finding of Echtay et al. (29), as outlined above, is well compatible with the suggested putative function of UCP3 in fatty acid metabolism. Based on a series of (human) experiments, we (32,33) and others (34,35) have previously suggested that the primary physiological function of muscle UCP3 is to export fatty acid anions from the mitochondrial matrix, rather than function as a UCP per se (36). Thus, UCP3 is upregulated under conditions of an abundance of fatty acid supply to the mitochondria, such as during fasting (37), high FFA levels (38), acute exercise (39), and high-fat feeding (36,38). On the other hand, downregulation of UCP3 occurs when fatty acid oxidation is increased or plasma FFA levels are lowered, such as with endurance training (40,41) and weight reduction (42). In addition, we recently reported that UCP3 protein content is inversely related to fat oxidative capacity of muscle, indicating an increased need for UCP3 in those muscles with a low-fat oxidative capacity (43).

What happens when the supply of fatty acids mis-matches the oxidative capacity of the mitochondria? Under such conditions, fatty acids that cannot be oxidized will accumulate in the muscle cell. The increased load of fatty acids on the mitochondrial membrane will lead to the entrance of neutral fatty acids inside the mitochondrial matrix (44). However, here these fatty acids cannot be oxidized (only fatty acids in the acyl-CoA form can undergo β-oxidation) and will be deprotonated (due to the proton gradient across the inner mitochondrial membrane built up by the transport of electrons through the electron transfer chain), giving rise to fatty acid anions inside the mitochondrial matrix. Since mitochondrial membranes are impermeable to fatty acid anions (22), these fatty acid anions are trapped inside the mitochondrial matrix. At the site of the matrix, where ROS are formed, the fatty acid anions are prone to lipid peroxidation and could therefore induce mitochondrial damage. As UCP3 is able to export fatty acid anions (and/or fatty acid peroxides) across the mitochondrial membrane away from the matrix (45), we (32,33) and others (34,35) previously postulated that UCP3 is a fatty acid anion and/or peroxide exporter that protects the mitochondrial matrix against lipid peroxidation-induced mitochondrial damage (32,35). It is important to note that the mitochondrial matrix, in contrast to the intermembrane space, is much more vulnerable to oxidative damage because the matrix contains DNA, RNA, and numerous enzymes of (among others) the Krebs cycle, which are very sensitive to ROS (35). Please also note that by exporting fatty acid anions, UCP3 also lowers the proton gradient, thus possessing uncoupling activity and further reducing ROS formation. The activation of UCP3
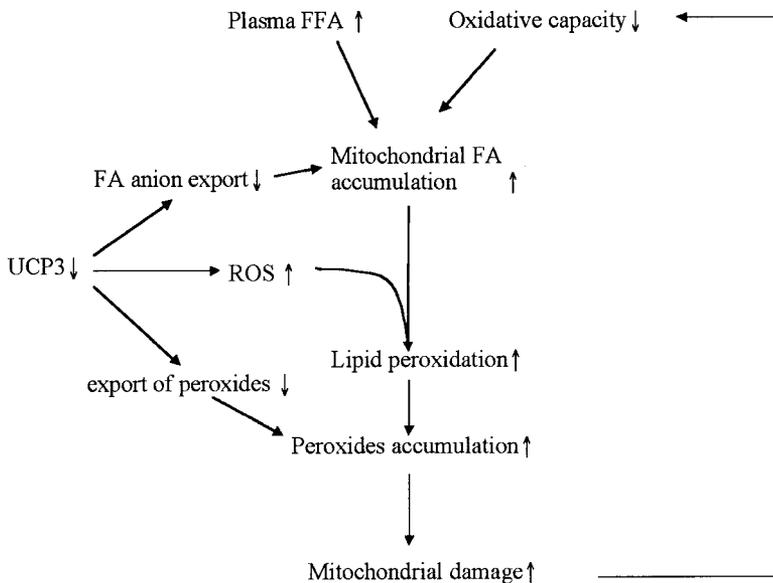
FIG. 2. Schematic representation of the distinct steps where the negative feedback mechanism is (or may be) defective in type 2 diabetes.

by 4-hydroxy-2-nonenal (29) is well compatible with this function of UCP3 and might reveal two potential functions for UCP3: *1*) lowering the proton gradient to reduce ROS production and therefore reduce peroxide formation, and *2*) exporting the formed lipid peroxides (or fatty acid anions before they become peroxidized) away from the mitochondrial matrix. In this way UCP3 might play an important role inside skeletal muscle in preventing mitochondrial damage induced by lipid peroxides (Fig. 1).

Involvement of UCP3 in the etiology of type 2 diabetes? Interestingly, it has been shown that humans with the exon 6 splice donor mutation in the UCP3 gene, resulting in decreased UCP3 levels, have diminished fat oxidative capacity (46). In addition, mice lacking UCP3 are characterized by disturbances in fat oxidation (47). Whether this diminished fat oxidative capacity is due to increased mitochondrial damage should be reinvestigated. Unfortunately, to our knowledge, no studies have been examined the longer-term effects of lack of UCP3. Intriguingly, however, type 2 diabetic patients, who are characterized by decreased oxidative phosphorylation activity, probably due to morphological changes to mitochondria (19) or mitochondrial DNA damage (48), have a 50% reduction in UCP3 levels (49). Also, aging, which is characterized by mitochondrial dysfunction (24) and accumulation of ROS-induced mutations in control sites of mitochondrial DNA replication (25), is accompanied by a reduction in UCP3 levels (50). These data suggest that in patients with type 2 diabetes and in the elderly, the reduction in UCP3 is a pathological condition in which low levels of UCP3 fail to sufficiently reduce ROS production and to export fatty acid anions and/or peroxides, ultimately leading to lipid-induced mitochondrial damage, indicating a defective feedback mechanism between lipid peroxides and mitochondrial uncoupling (Fig. 2). Therefore, more research is needed to better understand the mechanisms that lead to mitochondrial damage in the insulin-resistant state and the putative role of mitochondrial uncoupling, specifically that of UCP3.

However, one could also argue that the reduction of UCP3 is not a pathological condition, but that UCP3 is reduced as part of the cluster of oxidative genes that are downregulated in type 2 diabetic patients (15,16) due to physical inactivity. However, there is evidence that this is not the case. Thus, the normal physiological response to physical inactivity and/or a low oxidative capacity is an upregulation, not a downregulation, of UCP3. Endurance-trained athletes with a high oxidative capacity are characterized by very low levels of UCP3 when compared with untrained subjects (41). In addition, UCP3 protein levels are highest in muscles with a low oxidative capacity (43). This inverse relation between oxidative capacity and UCP3 can be explained by its putative function. Thus, when oxidative capacity is high, such as in trained subjects or in type 1 muscle fibers and the heart, fatty acids can easily be oxidized and therefore are not likely to accumulate inside the mitochondria, and little UCP3 is needed to export fatty acid anions from the mitochondrial matrix. Under such conditions, low levels of UCP3 might even be beneficial in terms of energy efficiency, as the export of fatty acid anions by UCP3 leads to mitochondrial uncoupling and a reduction in the efficiency of mitochondrial energy production (32). Therefore, there is evidence that the decline in UCP3 levels in type 2 diabetic patients truly reflects a pathophysiological state. This idea is further supported by the finding that in pre-diabetic (impaired glucose tolerant) subjects, a 1-year lifestyle diabetes prevention program that included increased physical activity, which effectively resulted in improved metabolic control (51), resulted in a twofold increase in UCP3 (M. Mensink, P.S., M.K.C.H., E. Moonen-Kornips, G. Schaart, W. Saris, E. Blaak, unpublished observations), thereby restoring normal physiological UCP3 levels. Together, these results suggest that a reduced level of UCP3 in type 2 diabetic patients indicates a defective feedback mechanism between lipid peroxides and mitochondrial protection against fat accumulation and, therefore, could contribute to lipid-induced mitochondrial damage.

Targets for intervention. Considering the above, the question remains of how the defective feedback mechanism can be restored in type 2 diabetic patients. Most directly, agents that are able to induce UCP3 in a regulated manner should potentially be able to prevent lipid-induced mitochondrial damage. However, to our knowledge, no

agents that are able to specifically upregulate UCP3 are currently available. Alternatively, AMP-activated protein kinase (AMPK) might be a potential target to correct this defective feedback mechanism. Activation of AMPK leads to an improvement in insulin sensitivity, and it is tempting to speculate that this is due to an improvement of mitochondrial function. Interestingly, in human vascular endothelial cells, AMPK activation has indeed been shown to diminish the mitochondrial dysfunction that was caused by exposure to high glucose levels (52). Unfortunately, data on a similar effect in human skeletal muscle are lacking. However, acute activation of AMPK has been shown to acutely upregulate UCP3 (39), a response that could serve to protect mitochondria against lipid-induced mitochondrial damage. In addition, AMPK activation also induces mitochondrial biogenesis (53), probably through the induction of PGC1 (54). It could be reasoned that in type 2 diabetes, dysregulation of the AMPK signaling mechanism is responsible for the low UCP3 levels and hence contributes to mitochondrial dysfunction. In this context it is again noteworthy that physical activity, which is generally accepted to be beneficial in the treatment of type 2 diabetes, leads to the activation of AMPK (55). Future research is needed to test whether chronic AMPK activation in patients with type 2 diabetes restores UCP3 and thus contributes to the protection of mitochondria against lipid-induced mitochondrial damage.

Along the same lines of thinking, PGC1 is a potential target because it induces mitochondrial biogenesis and upregulates UCPs (56). Clearly, more research is needed to explore the exact role of these targets in mitochondrial damage in type 2 diabetes.

CONCLUSION

In this article, we provide, in an integrative view, a mechanistic explanation linking IMCL accumulation with mitochondrial dysfunction under conditions of insulin resistance. We propose that accumulation of fatty acids inside mitochondria might lead to increased production of lipid peroxides and damage to mitochondria. Mitochondrial uncoupling, by UCP3 or other unidentified mechanisms, might play an important role in the protection of mitochondria against these lipid peroxides by lowering the production of ROS. Therefore, in the search for the mechanisms underlying the reduced oxidative capacity observed in type 2 diabetes and aging, the putative role of mitochondrial uncoupling per se, and UCP3 in particular, in the prevention of peroxide-induced mitochondrial damage deserves in-depth investigation.

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