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Review

Mitochondrial dysfunction and lipotoxicity

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ABSTRACT

Mitochondrial dysfunction in skeletal muscle has been suggested to underlie the development of insulin resistance and type 2 diabetes mellitus. Reduced mitochondrial capacity will contribute to the accumulation of lipid intermediates, desensitizing insulin signaling and leading to insulin resistance. Why mitochondrial function is reduced in the (pre-)diabetic state is, however, so far unknown. Although it is tempting to suggest that skeletal muscle insulin resistance may result from an inherited or acquired reduction in mitochondrial function in the pre-diabetic state, it cannot be excluded that mitochondrial dysfunction may in fact be the consequence of the insulin-resistant/diabetic state. Lipotoxicity, the deleterious effects of accumulating fatty acids in skeletal muscle cells, may lie at the basis of mitochondrial dysfunction: next to producing energy, mitochondria are also the major source of reactive oxygen species (ROS). Fatty acids accumulating in the vicinity of mitochondria are vulnerable to ROS-induced lipid peroxidation. Subsequently, these lipid peroxides could have lipotoxic effects on mtDNA, RNA and proteins of the mitochondrial machinery, leading to mitochondrial dysfunction. Indeed, increased lipid peroxidation has been reported in insulin resistant skeletal muscle and the mitochondrial uncoupling protein-3, which has been suggested to prevent lipid-induced mitochondrial damage, is reduced in subjects with an impaired glucose tolerance and in type 2 diabetic patients. These findings support the hypothesis that fat accumulation in skeletal muscle may precede the reduction in mitochondrial function that is observed in type 2 diabetes mellitus.

1. Introduction

The prevalence of type 2 diabetes mellitus is increasing dramatically and reaches epidemic proportions worldwide. The WHO predicts that 350–400 million people will suffer from type 2 diabetes in the year 2030 [1]. The increase in the prevalence of type 2 diabetes mellitus is a reflection of the alarming (and still increasing) number of people that are overweight. Environmental factors like consumption of high-fat and/or energy-rich diets and low levels of physical activity are likely to underlie this high prevalence of obesity.

In obesity, the accumulation of excessive fat in non-adipose tissues such as liver, heart and muscle (=ectopic fat accumulation) negatively impacts health. In sedentary humans, fat accumulation in skeletal muscle (and liver) strongly associates with insulin resistance, predisposing to the development of type 2 diabetes mellitus [2]. Indeed, both type 2 diabetic patients [3] and their diabetes-prone first-degree relatives [4,5] are characterized by high levels of intramyocellular lipids (IMCL) and muscular insulin resistance. On the contrary, endurance-trained athletes, who are among the most insulin-sensitive subjects, are also characterized by high IMCL levels [3,6], indicating that high IMCL levels *per se* do not necessarily lead to insulin resistance. We (and others) have previously suggested that the increase in IMCL following endurance training serves to match the training-induced increase in oxidative capacity and reliance on fat as a substrate during exercise [6]. In contrast, the increase in IMCL under obesogenic/diabetogenic conditions is due to a surplus of fat availability (high plasma FFA levels, high-fat diets) and is NOT matched by improved oxidative capacity. Under the latter conditions the intermediates of IMCL metabolism such as fatty acyl-CoA, diacylglycerol and ceramides will also accumulate and especially these intermediates impede cellular insulin signalling (for review see: [7,8]). The above concept predicts that a low oxidative capacity–combined with high IMCL levels–predisposes to the development of insulin resistance. Indeed, (pre-)diabetic subjects are characterized by low oxidative capacity. In fact, in the recent 5–7 years, skeletal muscle mitochondrial dysfunction has been implicated in the aetiology of insulin resistance and type 2 diabetes mellitus.

2. Mitochondrial dysfunction and type 2 diabetes

In 2002, Kelley et al. [9] were one of the first to raise the issue of mitochondrial dysfunction in type 2 diabetes mellitus. Thus, the

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authors observed that mitochondrial morphology was altered in type 2 diabetic patients, including a reduction in mitochondrial area size and an elevated number of damaged mitochondria. Mitochondrial surface area was found to correlate positively with insulin stimulated glucose disposal [9]. In addition, they reported that the activity of rotenone sensitive NADH:O2 oxidoreductase, reflecting the overall activity of the respiratory chain, and citrate synthase were reduced in type 2 diabetic patients [9]. One year later, the interest for mitochondrial aberrations in the development of type 2 diabetes mellitus exploded when two independent studies were simultaneously published in different high-impact journals. These studies showed a coordinated reduction in the expression of genes encoding key enzymes in oxidative mitochondrial metabolism in (first-degree relatives of) diabetic patients [10,11]. Using micro-arrays it was shown that the mRNA expression of genes that encode for proteins involved in mitochondrial metabolism were mildly, but consistently lower expressed in muscle of type 2 diabetic patients and/or first-degree relatives of type 2 diabetic patients. Many of these genes are under the control of the transcription co-activator PGC1α, which was also found to be reduced in type 2 diabetic patients [10,11] and in family history-positive non-diabetic subjects [10]. This reduction in PGC1α gene expression has been confirmed by others [12,13]. However, it should be noted that changes in PGC1α mRNA expression should be interpreted with caution, as PGC1α has been shown to be regulated by post-translational modification and thus mRNA levels do not necessarily reflect activity of the protein [14].

Following these indications for aberrations in mitochondrial function in the diabetic state, several authors have applied non-invasive magnetic resonance spectroscopy (MRS) for the *in vivo* determination of skeletal muscle mitochondrial function. One way in doing so is by determining the rate of unidirectional flux through ATP synthase (ATP synthetic flux, fATPase) in the non-exercising state. This can be done by using the magnetization (saturation) transfer method in which the signal derived from ATP is temporarily suppressed and subsequent changes in the steady state MR signal of free phosphate are quantified to calculate unidirectional ATP synthesis rate. Simultaneously, flux through the tricarboxylic acid (TCA or Krebs) cycle can be determined non-invasively by carbon-13 (^{13}C) MRS. Using this ^{13}C-and ^{31}P-MRS method, Petersen et al. [15] reported that muscular fATPase and oxidative metabolism (TCA cycle flux) were decreased by ~40% in elderly subjects when compared to BMI-matched young, healthy subjects without a family history of type 2 diabetes subjects. The older volunteers were also characterized by elevated levels of muscular fat and by muscular insulin resistance, suggesting that an age-associated decline in mitochondrial function might contribute to the development of insulin resistance. Using similar methodology, the same research group later found that IMCL and fATPase were, respectively, 80% higher and 30% lower in lean, but insulin-resistant offspring of type 2 diabetic patients who were matched for age, BMI and habitual physical activity with insulin sensitive healthy subjects [16]. Consistent with this finding, Szendroedi et al. [17] recently reported that *in vivo* ATP synthesis rate was decreased by 27% in non-obese metabolically well-controlled type 2 diabetic patients compared to young, healthy control subjects.

We have applied an alternative ^{31}P-MRS method to investigate *in vivo* mitochondrial function by measuring the kinetics of phosphocreatine (PCr) resynthesis during recovery from submaximal exercise [18,19]. Importantly, and in contrast to the magnetization saturation transfer method, the PCR-recovery methodology assesses mitochondrial function under conditions of increased metabolic demand. When we then compared *in vivo* mitochondrial function in type 2 diabetic patients versus normoglycemic, obese control subjects matched for BMI [20], we showed that *in vivo* mitochondrial function was compromised by ~45% in type 2 diabetic patients [20]. Interestingly however, IMCL content was similar between the groups, suggesting that impaired mitochondrial function may be a more important determinant of diabetes than IMCL levels *per se*. Recently, we have confirmed this observation of reduced *in vivo* mitochondrial function in type 2 diabetic patients and in addition showed that also first-degree relatives of type 2 diabetic patients (FDR) had a similar reduction in mitochondrial function [21]. Again, in these subjects that were all matched for BMI, no differences in IMCL content were observed between healthy obese, FDR and type 2 diabetic subjects [21]. Taken together, these studies are consistent in showing a reduction in *in vivo* mitochondrial function in insulin resistant subjects and/or type 2 diabetic patients.

In vivo mitochondrial function is not only determined by intrinsic mitochondrial function, but also by mitochondrial content and even substrate supply to the skeletal muscle. To address if the mitochondria *per se* have a reduced capacity, *ex vivo* studies are needed. Three studies have been published that examined *ex vivo* mitochondrial function (to determine "intrinsic mitochondrial function") using high-resolution respirometry in muscle of type 2 diabetic patients. Boushel et al. [22] found that ADP-stimulated state 3 respiration, both with substrates for complex I and/or complex II, was normal in permeabilized skinned muscle fibers from type 2 diabetic patients, when corrected for mitochondrial density. In contrast, Mogensen et al. [23] reported that maximal ADP-stimulated respiration (state 3) with pyruvate as a substrate and respiration through the electron transport chain (ETC) was reduced in mitochondria isolated from diabetic patients. However, both studies did not determine *in vivo* mitochondrial function. In our study outlined above, in which we showed reduced *in vivo* mitochondrial function in type 2 diabetic patients and first-degree relatives [21], we also determined *ex vivo* mitochondrial function. We showed that a lower *ex vivo* (ADP-stimulated and maximally uncoupled) mitochondrial respiration underlies the reduction in *in vivo* mitochondrial function. Interestingly, we found similar tendencies of reduced intrinsic mitochondrial function in first-degree relatives of type 2 diabetic patients. These findings were not explained by reduced mitochondrial density, as both CS and mtDNA copy number were similar in all groups [21].

Taken together, there is indeed evidence for abnormalities in *in vivo* and intrinsic mitochondrial function in the (pre-)diabetic state. This led others to formulate the hypothesis that this (acquired or inherited) reduced mitochondrial function in the pre-diabetic state leads to accumulation of fat in muscle, thereby contributing to the development of insulin resistance [15,16].

3. Mitochondrial lipotoxicity

Although the above-mentioned hypothesis sounds firm, there are also data suggesting that mitochondrial dysfunction is not required for the development of type 2 diabetes mellitus or even the accumulation of muscular fat. First of all, several studies with genetically manipulated mouse models have suggested that a reduction in mitochondrial oxidative capacity in fact improves insulin sensitivity. Thus, Pospisilik et al. [24] generated mice with a deletion of the mitochondrial flavoprotein apoptosis-inducing factor (AIF) resulting in dysfunction of the oxidative phosphorylation. In contrast to what was expected, these mice had improved glucose tolerance and were protected from high-fat diet induced insulin resistance. Similarly, Wredenberg et al. [25] reported that mice with a skeletal muscle-specific deletion of mitochondrial transcription factor A (Tfam), resulting in marked reduction in oxidative phosphorylation capacity, were normal insulin sensitive and had an increased peripheral glucose uptake upon a glucose tolerance test. Also, PPARα ablation in muscle–thereby decreasing fat oxidative capacity–resulted in improvements in glucose homeostasis, whereas PPARα overexpression–improving oxidative capacity–had the opposite effects [26]. Together, these studies in genetically modified animals have suggested that in fact an ablation of mitochondrial oxidative capacity improves instead of impairs glucose homeostasis. Although

these studies could be interpreted as evidence against a causal role for mitochondrial dysfunction in the development of muscular insulin resistance, such an interpretation would be an oversimplification. It has been known for decades that in muscle (and other tissues) substrate competition between glucose and fatty acids determines the ultimate mix of fuel used for ATP production, as for example elegantly postulated in the Randle hypothesis [27]. A forced reduction/induction of the capacity to burn one substrate, will lead to a compensatory increase in the other substrate. The results of the above-mentioned mouse models can be explained by such substrate competition, as was also discussed by Finck et al. [26]. In fact, blockers of fat oxidation have previously been used for the treatment of type 2 diabetes. For example, we have previously shown that etomoxir, which reduces fat oxidation via inhibition of CPT1, indeed results in a reduction in fat oxidation but also an improvement in glucose metabolism, including enhanced basal GLUT4 translocation and lowering of plasma glucose levels [28]. In addition, part of the results in the animal studies is most likely explained by activation of the enzyme AMPK, a sensor of the energy status of the cell. Most likely, ablation of components of the oxidative phosphorylation system resulting in reduced mitochondrial function, will activate AMPK and thereby improve GLUT4 translocation and glucose uptake. For these reasons, the value of studies genetically disturbing oxidative phosphorylation for understanding the role of mitochondrial dysfunction in the development of type 2 diabetes mellitus is limited and interpretation of these studies should be done with more caution.

However, next to these mice studies, other observations have been reported that question the hypothesis that mitochondrial dysfunction underlies the development of type 2 diabetes mellitus or muscular fat accumulation. Thus, the acute elevation of circulating plasma fatty acids results in marked muscular fat accumulation and insulin resistance [29]—and importantly, these effects can also be induced in healthy or even endurance trained subjects, indicating independence of mitochondrial dysfunction. Furthermore, short-term consumption of high-fat diets (7 days) increased IMCL levels by 50% in lean, young and healthy subjects [30], whereas it has been shown that human subjects are capable of adjusting their fat oxidation to high-fat intake within this time-frame [31]. Moreover, it is important to realize that the suggestion that mitochondrial dysfunction precedes the development of insulin resistance originates from cross-sectional human studies in which a reduced mitochondrial function was always accompanied by increased/high muscular lipid content. For example, in offspring of type 2 diabetic patients a 30% reduction in mitochondrial function was already accompanied by a ~80% increase in IMCL content [16]. In our study, IMCL levels were comparable between diabetic patients, FDR and controls [21], despite marked differences in mitochondrial function. Finally, as recently addressed by Holloszy [32] it can be questioned if a 20–40% reduction in *in vivo* mitochondrial function or mitochondrial content as has been reported, would affect substrate oxidation and thereby fat accumulation in the resting state, given that mitochondria work at a very low fraction of their maximal capacity at rest.

Therefore, the question "cause or consequence" remains unanswered, and it is equally well possible that mitochondrial dysfunction in these studies is in fact the consequence of high concentrations of fat in the muscle cells: mitochondrial lipotoxicity (Fig. 1). Recent studies support this view: In a study where Szendroedi et al. [17] reported a 27% decrease in basal ATP synthesis rates in type 2 diabetic patients, they also found that plasma FFA levels correlated negatively with mitochondrial function, suggesting that lipid oversupply to the muscle may deteriorate mitochondrial function. Furthermore, the reduced PGC1α gene expression that has been reported in insulin resistant subjects might also be the consequence of elevated plasma and/or muscular fatty acid levels. Thus, we and others recently showed that the acute elevation of plasma fatty acids, leading to accumulation of intramuscular lipids, was accompanied by down-

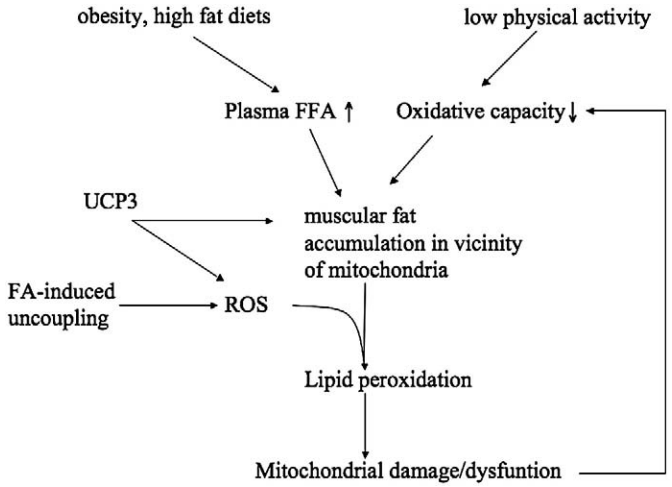

Fig. 1. Simplified schematic presentation of the "lipotoxicity hypothesis." In diabetogenic conditions (obesity, high-fat diets, low physical activity), fatty acids will accumulate in muscle cells in the vicinity of mitochondria. These fatty acids can exert lipotoxic effects on mitochondrial function, thereby further aggregating oxidative capacity and muscle fat accumulation.

regulation of the transcriptional co-activator PGC1α, as well as other genes involved in mitochondrial metabolism [33,34]. Also, the observed aberrations in mitochondrial morphology, i.e. smaller but most of all damaged mitochondria that were reported in type 2 diabetic patients [9] fit with the concept of lipotoxicity in skeletal muscle leading to mitochondrial dysfunction.

4. High-fat diet induced mitochondrial dysfunction

Several studies have tested the concept that in fact muscular fat accumulation may precede the development of mitochondrial dysfunction. Most of these studies have used high-fat diets to manipulate the IMCL content and assess the impact on mitochondrial function. Sparks et al. [35] were the first to show that feeding C57Bl/6J mice a high-fat diet for 3 weeks resulted in the down regulation of genes involved in mitochondrial oxidative phosphorylation [35]. In addition, these authors showed that in humans consumption of a high-fat diet (50 En% fat) for 3 days resulted in down regulation of PGC1α and its target genes involved in oxidative phosphorylation as compared to an isocaloric normal fat diet (37 En% fat) [35]. In contrast, Turner et al. [36] reported that consumption of a high-fat diet for 5–20 weeks in C57Bl/6J mice induced insulin resistance despite an improved mitochondrial oxidative capacity. Similarly, we found that an 8-week high-fat dietary intervention in rats did not reduce *ex vivo* mitochondrial function, as assessed by high-resolution respirometry in isolated mitochondria. Moreover, the high-fat diet increased the protein content of PGC1α [37], and the increase in PGC1α protein content correlated with the protein content of several proteins of the OXPHOS system, suggesting that in fact mitochondrial density was increased after high-fat feeding. These improvements in mitochondrial metabolism were accompanied by marked accumulation of fat in skeletal muscle [37]. In concordance, Hancock et al. [38] showed that high-fat diets for 4–5 weeks increased mitochondrial density via a stimulatory effect of fatty acid levels on PPARδ and PGC1α protein content. Together, these data tend to suggest that high-fat diets–although leading to insulin resistance in rodents–are not accompanied by mitochondrial dysfunction. Rather they lead to improved mitochondrial oxidative capacity, arguing against a role for lipotoxicity in the development of diabetes-related mitochondrial dysfunction. However, the matter may be more complicated than can initially be deduced from these data. For example, two

studies have examined the time-course of changes in mitochondrial function upon high-fat diets. Thus, Chanseaume et al. [39] fed rats high-fat diets for 0, 3, 6, 9, 14, 20, or 40 days and determined *ex vivo* mitochondrial activity in permeabilized muscle fibers. They showed a transiently enhanced activity of the oxidative phosphorylation after 14 days, but a significant decrease at day 40. Similarly, Laurent et al. [40] showed in rats–using non-invasive imaging–that ATP synthesis rates decreased by 50% within 24 h of raising the fat content in the diet, but ATP synthesis returned to normal values after 2–3 weeks on the HF diet. However, prolonged high-fat feeding for longer than one month resulted in consistently lower ATP synthesis rates by 30–50% accompanied by steadily augmenting IMCL levels [40]. In ZDF rats, we recently showed that *in vivo* mitochondrial function (determined using non-invasive magnetic resonance spectroscopy) declines similarly with age (from 6 to 18 weeks of age) in diabetic ZDF rats and insulin-sensitive fa/+rats [41]. However, examination of intrinsic mitochondrial function–ADP-stimulated and maximally uncoupled respiration in isolated mitochondria–revealed that mitochondrial oxidative capacity also gradually decreased with age in the insulin-sensitive fa/+rats. In contrast, however, mitochondrial respiration initially improved in ZDF rats (from week 6 to week 12), when IMCL accumulates and type 2 diabetes develops, but subsequently showed a very rapid decline at an age of 18 weeks when overt diabetes develops (Lenears et al., Obesity, in press). Finally, Bonnard et al. [42] showed that 1 month of high-fat, high-sucrose diet feeding was sufficient to induce glucose intolerance in mice, without any evidence of mitochondrial dysfunction. However, when the diet intervention was extended up to 16 weeks, they observed altered mitochondrial biogenesis, structure, and function in mouse muscle tissue, accompanied by the induction of a diabetic state [42]. Interestingly, they showed that ROS production in skeletal muscle of diet-induced diabetic mice was increased after 16 weeks of high-fat diet, but not after 4 weeks. Finally, in muscle cells, they showed that glucose- or lipid-induced ROS production resulted in mitochondrial alterations *in vitro*, and these effects were blocked by antioxidant treatment. Interestingly, recent reports have provided strong experimental evidence for ROS production in the development of insulin resistance [43,44], suggesting that ROS production may link muscular fat accumulation, mitochondrial dysfunction and insulin resistance.

Taken together, these studies are overall consistent in showing that: (1) rodents have the capacity to (initially) adapt mitochondrial function to a high fat intake, (2) mitochondrial dysfunction only occurs when high-fat diets are continued for a longer period and (3) muscular fat accumulation and insulin resistance develop before mitochondrial function deteriorates. Therefore, these data are consistent with the hypothesis that mitochondrial dysfunction may be a consequence rather than cause of muscular fat accumulation, and that it develops when the protective mechanisms can–on the longer term–no longer cope with high levels of muscular fatty acids, and as a consequence lipotoxicity occurs. It should be noted however that such lipid-induced mitochondrial dysfunction may lead to progressive deterioration of muscular oxidative capacity and accumulation of lipid intermediates in skeletal muscle, therefore not excluding that mitochondrial dysfunction in fact does play a role in the etiology of type 2 diabetes mellitus. Unfortunately, so far human studies that manipulated muscular fat content and examined the effect on mitochondrial function are lacking.

5. How can fatty acids lead to mitochondrial dysfunction in muscle?

Next to the production of ATP, mitochondria are also the major source of reactive oxygen species (ROS). Mitochondrial ROS can rapidly react with mtDNA, protein and lipids, thereby leading to oxidative damage. In muscle cells fatty acids accumulate in the vicinity of mitochondria and these fatty acids are very prone to ROS-induced oxidative damage, resulting in the formation of lipid peroxides. Especially accumulation of fatty acids in the inner mitochondrial membrane of mitochondria, at the site where ROS are formed, would be susceptible to peroxidation, subsequently inducing oxidative damage to the mitochondrial machinery. To prevent mitochondrial accumulation of fatty acids, their entry into mitochondria is regulated by the enzyme CPT1 that serves as an inward transporter of oxidizable fatty acids. However, this system cannot completely prevent the diffusion of non-metabolizable fatty acid into the mitochondria. Mitochondrial membranes consist of lipid bilayers and therefore fatty acids can still 'enter' the mitochondria matrix membrane via a so-called 'flip-flop' mechanism over the membrane [45]. However, these fatty acids cannot be oxidized due to the lack of acyl-CoA synthetase inside the matrix and likely are incorporated in the inner mitochondrial membrane, where they would be vulnerable to oxidative damage. This 'passive diffusion' is more likely to occur under conditions of high fatty acid availability and low demand, as is often the case in the pre-diabetic and diabetic state. This notion is supported by the observation that skeletal muscle of obese insulin resistant subjects not only contains a higher amount of intramyocellular lipid, but these lipids also showed a higher degree of lipid peroxidation [46]. It is tempting to suggest that these lipid peroxides lead to oxidative damage to mitochondrial structures and contribute to the reduced mitochondrial capacity observed in (pre-)diabetic patients [9]. Furthermore, this suggestion is also in accordance with the finding of Bonnard et al. [42] showing that high-fat diet induced mitochondrial dysfunction was associated with increased ROS production.

Mitochondrial ROS production depends highly on the proton gradient over the inner mitochondrial membrane (which drives ATP synthesis) and a high proton gradient will increase ROS production. One way to lower the proton gradient is by so-called mitochondrial uncoupling, a process in which a reduction of the proton gradient is not coupled to ATP production, but is due to proton leakage. Since the relationship between ROS production and the proton gradient is exponential, already a small decrease in membrane potential ("mild uncoupling") will reduce mitochondrial ROS production considerably [47]. Therefore, at the expense of a slight inefficiency in ATP production, mitochondrial uncoupling is a powerful tool to control ROS formation and hence to preserve mitochondrial functioning. Recently, this mitochondrial uncoupling was linked to the maintenance of mitochondrial function in human skeletal muscle during aging [48]. Thus, it has been observed that during aging mitochondrial function decreased in the well-coupled dorsal interosseus muscle while mitochondrial function was maintained in mildly uncoupled mitochondria from aging tibialis anterior muscle [48]. Although associative, these novel data suggest that mitochondrial uncoupling might protect against (age-induced) mitochondrial dysfunction.

As outlined above, especially fatty acids in the vicinity of mitochondria would be prone to oxidative damage, leading to the formation of lipid-peroxides. Interestingly, it is known for a long time that fatty acids are capable of mitochondrial uncoupling [49], but the reason why fatty acids do so is less clear. It can be hypothesized that fatty-acid induced uncoupling lowers mitochondrial ROS production and thereby prevents mitochondrial lipotoxicity. Although, so far no direct evidence for this hypothesis has been provided, we [50,51] and others [52,53] have previously suggested that the muscle specific mitochondrial uncoupling protein-3 (UCP3) may be involved in the protection of mitochondria against lipid-induced mitochondrial damage. UCP3 may do so by exporting non-metabolizable fatty acid anions or peroxides away from the mitochondrial inner membrane before they are attacked by ROS, or by directly lowering ROS production via mild mitochondrial uncoupling. We have recently shown that mitochondrial ROS

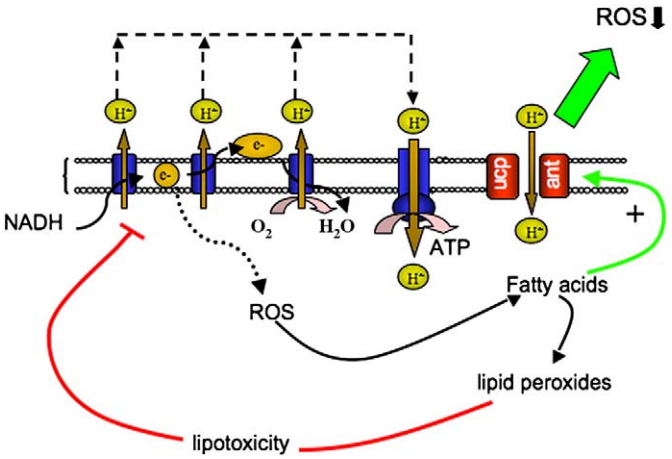

Fig. 2. Mitochondrial uncoupling as a proposed protective mechanisms against mitochondrial lipotoxicity: mitochondria produce reactive oxygen species (ROS) that can attack mitochondrial fatty acids, leading to lipid peroxides. These peroxides are highly reactive and could lead to damage to the mitochondrial machinery. However, fatty acids can activate processes leading to mitochondrial uncoupling, thereby reducing the proton gradient over the inner mitochondrial membrane. A lowering of the proton gradient leads to a reduction of ROS production, providing a feedback mechanism limiting mitochondrial lipotoxicity.

production, measured in a direct way by electron spin resonance of isolated mitochondria, increased with age in wild type mice, but this age-induced increase in mitochondrial ROS production was blunted in mice overexpressing UCP3 [54]. In addition, we have shown that the ablation of UCP3 was accompanied by higher levels of muscular lipid peroxidation [55]. We have shown that UCP3 is specifically upregulated in skeletal muscle when a dysbalance between fatty acid delivery and fatty acid oxidative capacity exists in muscle (for an extensive review, see: [51]). In short, UCP3 is upregulated with high-fat diets [56,57], fasting [58], etomoxir treatment (which inhibits the mitochondrial fatty acid oxidation) [28,59] and lipid infusion [33], all conditions that are associated by excessive lipid accumulation in skeletal muscle. On the other hand, interventions improving fat oxidative capacity, like endurance training [60,61] and weight loss [62], or lowering circulatory fatty acids ([63,64]) are associated with a decrease in UCP3. Interestingly, UCPs are activated by fatty acids and/or by peroxidation products of fatty acids [53], and when activated UCPs may reduce mitochondrial ROS production [53,54]. Therefore, it can be suggested that UCP3 is involved in the protection against mitochondrial lipotoxicity by lowering ROS production when activated by fatty acids (Fig. 2). In that respect, it is of interest to note that only type 2 diabetic patients and pre-diabetic subjects, who as outlined above are characterized by excessive fat accumulation in skeletal muscle and by mitochondrial dysfunction, have ~50% lower UCP3 protein levels [65,66] compared to obese control subjects who have similar IMCL content but normal mitochondrial function. This suggests that fat accumulation in skeletal muscle, combined with a defective protection mechanisms and probably elevated ROS levels, may lead to mitochondrial dysfunction.

6. Concluding remarks

In summary, although the evidence for mitochondrial dysfunction in type 2 diabetes mellitus is ample, there is so far no evidence that this reduced mitochondrial function is causal in the development of the disease. If anything, the results obtained so far tell us that the relation between mitochondrial function and the development of type 2 diabetes mellitus is not as simple as originally assumed. The initial suggestion that a reduced mitochondrial function precedes the accumulation of IMCL and insulin resistance is not supported by all studies, as several studies show no differences in IMCL despite reductions in mitochondrial function in insulin-resistant and/or diabetic subjects [17,20]. Therefore, it cannot be excluded that a reduced mitochondrial function may in fact be the consequence rather than cause of the diabetic state, for example caused by lipotoxic mechanisms [50]. The findings that plasma fatty acid levels reduce the expression of PGC1α [33,34] are negatively related to fasting muscular ATP synthesis [17], and impair insulin-stimulated ATP synthesis [67], as well as the observation of increased mitochondrial damage in type 2 diabetic patients [9] support this hypothesis. Whether mitochondrial dysfunction in type 2 diabetic patients and insulin-resistant subjects is due to reduced protective mechanisms against mitochondrial lipotoxicity deserves further study.

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