17 Mitochondrial Function, Dysfunction, and Adaptation in the Liver during the Development of Diabetes

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CONTENTS

ABSTRACT

Due to its epidemiological dimensions, there are tremendous efforts to understand the ultimate pathways that lead from modern Western lifestyles to the development of insulin resistance and, finally, overt type 2 diabetes (T2DM), which is often accompanied by nonalcoholic fatty liver disease (NAFLD). The insulin-resistant liver is intimately involved in T2DM, since it importantly contributes to high circulating blood glucose levels due to the unsuppressed release of glucose, even in the fasted state. There is a large body of literature on the "involvement" of mitochondrial dysfunction in the liver in the development of T2DM. However, it is unclear if mitochondrial dysfunction causes hepatic insulin resistance, thereby truly contributing to the development of T2DM and NAFLD, or if it is just a consequence. Also, the term *mitochondrial dysfunction* has been used in a very uncritical way. Finally, there seems to be a continuum of mitochondrial changes during the development of NAFLD, from the initial benign steatosis to nonalcoholic steatohepatitis (NASH). In this chapter, we summarize the current knowledge on mitochondrial functions and their failure and critically review the existing literature on these processes in the liver during the development of T2DM and NASH.

Keywords: type 2 diabetes mellitus, type 1 diabetes mellitus, mitochondrial function, insulin resistance, mitochondrial metabolism

INTRODUCTION

Due to its epidemiological dimensions, there are tremendous efforts to understand the ultimate pathways which lead from modern western lifestyle to the development of insulin resistance and, finally, overt type 2 diabetes (T2DM). The insulin-resistant liver is intimately involved in this process, since it importantly contributes to high circulating blood glucose, even in the fasted state, as a consequence of unsuppressed release of glucose derived from gluconeogenesis (DeFronzo et al. 1982). There is a

large body of literature on the "involvement" of mitochondrial dysfunction in the liver in the development of T2DM. However, in our opinion, it is still unclear if mitochondrial dysfunction causes hepatic insulin resistance, thereby truly contributing to the development of T2DM, or if it is just a consequence, if it exists at all. Even if the former were the case, it is unclear what would be the initial cause for the mitochondrial problem. As an alternative, it may be the metabolic environment of developing T2DM leading to mitochondrial dysfunction, which in turn may, or may not, be involved in further aggravation of disease progression. Again, it would be unclear which of the circulating factors or altered intracellular pathways would be to blame ultimately for causing the mitochondrial problem.

Also, in our opinion, the term "mitochondrial dysfunction" has been used in a very uncritical way. Finally, there seems to be a continuum of events during the development of nonalcoholic fatty liver disease (NAFLD), which is very often found in patients with T2DM, from the initial benign steatosis to nonalcoholic steatohepatitis (NASH).

In this book chapter, we summarize and critically review the existing literature on mitochondrial function during the development of T2DM and NASH, which may explain why so many controversial data have been reported. An extensive review on the same topic, however with a major focus on the role of reactive oxygen and nitrogen species (ROS and RNS) generated by the mitochondria in NASH, has been published very recently (Begriche et al. 2013). Thus, we discuss in this chapter mostly those studies in which the state of the patient or the experimental animal regarding insulin resistance and diabetic state has been reported.

Mitochondrial Functions

Another important problem is that mitochondrial function is restricted by many authors to the organelle's role in ATP production and production of ROS, while many other and probably even more vital functions are generally overlooked (Figure 17.1).

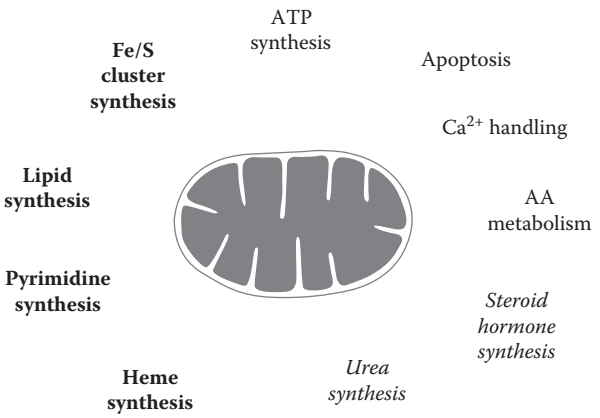

FIGURE 17.1 Essential functions that cannot be replaced by cytosolic activities (bold: left); Special functions typical for a special cell type (italics; lower right).

ATP Synthesis

The best-known function of mitochondria is the production of ATP by converting the redox potential of NADH and $FADH_2$ into an electrochemical potential across the inner mitochondrial membrane, which is then used by the ATP synthase to produce high-energy phosphate (Figure 17.1). In order to perform this task, the inner mitochondrial membrane is organized into membranous compartments called "cristae," which are packed with the four large membrane complexes of the electron transport chain and the ATP synthase, which is located at the tips of these cristae, thus contributing to or even causing their curvature (Davies et al. 2012). Since 13 essential subunits of these complexes are encoded by the small mitochondrial genome, any changes of the mtDNA sequence or its expression may cause a severe disturbance of this whole system of oxidative phosphorylation (OXPHOS). NADH and $FADH_2$ are derived from the oxidative degradation of carbohydrate or amino acid–derived pyruvate or fatty acids to CO_2.

Ca^{2+} Handling

Probably the second most well-known function is the involvement of mitochondria in cellular Ca^{2+} handling. It has long been known that isolated organelles are able to take up this divalent cation, driven by the electrochemical potential (negatively charged inside). However, only recently the Ca^{2+} channel responsible for this process was cloned, and was found to be surprisingly complex, being composed of the true channel, the mitochondrial Ca^{2+} uniporter (MCU) (Baughman et al. 2011, De Stefani et al. 2011), and several associated regulatory proteins (MICUs) (Mallilankaraman et al. 2012, Kovacs-Bogdan et al. 2014, De Stefani and Rizzuto 2014). With the detailed characterization of the properties of the channel, it is now clear that it only operates at very high Ca^{2+} concentrations, which would immediately lead to cell death, if they would be present all over the cytosol. Therefore, it is now well accepted that mitochondria are taking up Ca^{2+} only at cellular microdomains within the cytosol, where Ca^{2+} does indeed reach micromolar concentrations, that is, at contact sites with the endoplasmic reticulum and maybe at the subsarcolemmal region of muscle cells and cardiomyocytes (Pendin et al. 2014). One of the purposes of this mechanism may be the activation of TCA enzymes in order to match oxidative metabolism with high Ca^{2+} turnover, for example, during muscle contraction (Denton and McCormack 1990). Another mechanism may be shaping of Ca^{2+} transients in time and space (Pendin et al. 2014).

However, ATP synthesis and Ca^{2+} handling seem to be dispensable for many cell types, at least for a considerable time (see below), and other functions of the organelle, which are less well known, are probably the true essential mitochondrial functions which are needed to maintain cellular homeostasis.

Synthesis of Membrane Lipids

It is now clear that mitochondria are necessary for the synthesis of all cellular membrane lipids. Precursors are imported from parts of the endoplasmic reticulum called mitochondria-associated membranes (MAMs), converted into products in the inner mitochondrial membrane and either retained or exported again. This is best described

for the import of phosphatidylserine from MAMs via the outer to the inner membrane, where some of it is converted to phosphatidylethanolamine, which remains in both mitochondria or is transferred to all other cellular membranes, that is, the endoplasmatic reticulum, the nuclear envelope, and the plasma membrane (Tatsuta et al. 2014). Thus, mitochondria are needed to synthetize the lipid building blocks for all cellular compartments during regular cellular turnover, but even more during enhanced cell proliferation, which may occur in the liver under pathological conditions.

Synthesis of Nucleic Acids

Mitochondria are essential to synthesize uridine, the common precursor for all pyrimidine derivatives during their de novo synthesis from carbamoyl-phosphate and aspartate. They catalyze the conversion of dihydroorotate to orotate by an electron transfer step by dihydroorotate dehydrogenase in the inner mitochondrial membrane (Berg et al. 2002). The final product of this pathway, uridine, is used to produce UTP needed for RNA synthesis, and also for the generation of UTP glucose, which is needed for glycogen synthesis as well as the synthesis of complex carbohydrates (Berg et al. 2002). UTP is converted to other pyrimidine-triphosphates CTP and TTP, needed for RNA and, as deoxynucleotides, for DNA synthesis, respectively. Although the nucleotides needed for RNA synthesis in a nondividing liver cell are mostly recycled from the preexisting pool by salvage pathways, during proliferation or enhanced transcription of genes, for example, during the acute phase response, mitochondria are necessary to provide essential nucleic acid building blocks.

Amino Acid Metabolism

Mitochondria are deeply involved via deamination and transamination reactions in amino acid metabolism (Berg et al. 2002). Therefore, especially organs like the liver, which not only uses amino acids to cover synthesis of its own proteins during regular turnover, but which produces and excretes large amounts of proteins, are highly dependent on this mitochondrial function.

Heme Synthesis

Mitochondria synthetize 5-aminolevulinate from succinyl-CoA and glycine and export it to the cytosol as the precursor for all kinds of heme groups (porphyrines), which are not only essential parts for hemoglobin and mitochondrial RC complexes, but also for the wide variety of cytochromes present in the endoplasmic reticulum, especially in the liver (Berg et al. 2002).

Synthesis of FeS Clusters

While all of the above-mentioned processes can be transferred to other parts of the cell, as can be seen in unicellular organisms without mitochondria, the one and only truly essential mitochondrial function seems to be the synthesis of FeS clusters (Stehling et al. 2014). These organisms, which are unable to respire, live in anaerobic environments, however they contain double-membrane compartments called mitosomes, which produce these highly complex protein machineries (Shiflett and Johnson 2010). FeS clusters are intricate parts of the mitochondrial electron

transport chain; however, they are also needed in other cellular compartments like the nucleus, where they are parts of DNA repair complexes, for example.

Apoptosis

Apoptosis is often considered as a mitochondrial dysfunction, since it is a mechanism destroying the cell. However, we rather consider this process being an extremely important *function*, since it is highly controlled and only occurs when the decision has been made that a cell is no longer important or even dangerous for the whole organism. When a cellular defect has been detected by various sophisticated surveillance mechanisms, either caused by intrinsic problems (most importantly irreparable DNA damage) or by extrinsic causes like infection with a pathogen, mitochondria finally execute apoptosis by releasing cytochrome c and other factors from the tightly closed intra-cristae space through highly regulated cristae junctions to activate the death cascade (Martinou and Youle 2011).

In addition to these ubiquitous functions, there are tissue-specific functions of the organelles, with urea synthesis in the liver being the probably best-known example, where the initial steps, the synthesis of carbamoylphosphate and citrulline, take place in the matrix. Also the synthesis of steroid hormones in the adrenal cortex from cholesterol is performed within mitochondria (Berg et al. 2002). However, when their proteome was compared, it was found that, quite surprisingly, only about one-third of the ca. 1.100 reliably annotated mitochondrial proteins encoded in the nucleus are shared by 14 different mouse tissues, while the remaining proteins are cell type specific and tissue pairs share only about 75% of their protein equipment (Pagliarini et al. 2008).

Mitochondrial Dysfunction

Mitochondrial dysfunction is, in the first place, considered as the inability to produce sufficient ATP to maintain cellular homeostasis. However, low levels of ATP are not necessarily a sign of mitochondrial problems. In the second place, the overproduction of ROS is thought to be one of the most common consequences of mitochondrial dysfunction, leading to cellular damage, and third, the inability to handle cytosolic Ca^{2+}, independent of ATP levels, is also believed to be one of the main problems when mitochondria do not work properly. As already mentioned above, it is the *inability* to properly execute apoptosis which should be considered as mitochondrial dysfunction and not the process of apoptosis itself. This inability is a hallmark of almost any cancer cell thereby escaping destruction although having accumulated the necessary chromosomal rearrangements which lead to tumor formation (Hanahan and Weinberg 2011).

ATP Synthesis

Surprisingly, the synthesis of high-energy phosphate by the OXPHOS system is dispensable in mammalian cells. Several cell lines have been generated lacking completely the mitochondrial genome, therefore containing a nonfunctional RC (called ρ0 cells; King and Attardi 1996, Bayona-Bafaluy et al. 2003). These cells

need uridine for nucleic acid synthesis, since they cannot oxidize dihydroorotate (see above). They also need high concentrations of pyruvate in the medium, which simply serves as an electron acceptor, being reduced to lactate, thereby allowing the ATP generating flux from glucose to lactate through glycolysis running at maximum speed. Only few such cell lines have been established from single clones after treating cells with mitochondrial replication inhibitors, indicating that a considerable amount of rearrangements in the nuclear genome has to occur to enable cells to maintain that state forever. Such cell lines as well as cell lines containing severe mutations of mtDNA very often proliferate as fast as control cells, although they contain low levels of ATP. However, also the content of ADP and AMP is low, so that the energy charge, the ratio of high-to-low energy phosphate, remains maintained. We concluded that low ATP levels may be an adaption to enable maximal glycolytic flux under such conditions, which is then the only source of ATP, since glycolysis is inhibited by high ATP concentrations (von Kleist-Retzow et al. 2007). In these cells, ATP produced by glycolysis is even imported into the mitochondria and hydrolyzed to ADP and phosphate by reverse action of the ATP synthase. This process generates a net negative charge inside, thus maintaining the electrochemical gradient needed to import all the enzymes and substrates needed to perform the essential synthesis reactions discussed above (Appleby et al. 1999).

However, completely unexpected, cells can even survive in vivo for a surprisingly long time without a functional RC. This state was achieved by cell-type specific depletion of the mitochondrial transcription factor A (TFAM), which is essential for the replication and maintenance of mtDNA. For example, skeletal muscle–specific TFAM KO mice contain no detectable TFAM at 1 month, followed by no detectable RC subunits at 4 months due to slow mitochondria turnover (Wredenberg et al. 2006). However, animals were still alive and had to be sacrificed only a few weeks later due to general weakness and respiratory failure (Wredenberg, personal communication). An even more impressive gap of at least 1 month between the total absence of RC subunits and death of the animals was observed in the brain cortex after TFAM knockout in cortical neurons (Sorensen et al. 2001). Finally, we have shown previously that epidermal stem cells and their descendants can even hyperproliferate and form a well-functioning epidermis without any mtDNA and consequently without RC (Baris et al. 2011). In conclusion, these results show that cells can survive without ATP-producing mitochondria for a surprisingly long time even in vivo, emphasizing again that ATP generation is obviously not the most essential function of the organelles.

Ca^{2+} Handling

A Ca^{2+} handling defect can be seen in model cells with mitochondrial impairment due to mtDNA mutations (Brini et al. 1999, von Kleist-Retzow et al. 2007). Surprisingly, mice without the mitochondrial Ca^{2+} uniporter MCU have a very moderate skeletal muscle phenotype, showing that also this function is dispensable in vivo, at least in most tissues under basal conditions (Pan et al. 2013). However, in cells which experience high fluctuations of Ca^{2+} like the heart or pacemaking dopaminergic neurons, the inability to remove from the cytosol a sufficient amount of Ca^{2+} from locations of high concentrations may indeed impede cell function under conditions of high Ca^{2+} load.

ROS Production

It is still found in many reviews that "5% of the oxygen consumed by mitochondria" is first converted to potentially damaging superoxide, which is only then detoxified by scavenger systems. These numbers might be true for isolated mitochondrial preparations, but certainly not in vivo. The actual production of superoxide is extremely difficult to measure, however assays using newly developed in vivo indicators have shown that the true rate is probably very low (Cocheme et al. 2011). Only after poisoning with RC blockers, in the absence of proper free radical scavenging systems or in certain situations of high inner membrane potential, but low ATP consumption rate, free radicals like superoxide, H_2O_2, and RNS will truly accumulate and may then damage mitochondrial nucleic acids, proteins, and membrane lipids (Murphy 2009). However, we have shown that such free radicals generated by the RC do not cause mutations in the nuclear genome (Hoffmann et al. 2004), clearly showing that mitochondrial radicals are probably not causally involved in chromosome aberrations, be it in cancer or in the progress of aging.

Also, one of the most popular theories of aging, the free radical theory, postulating a vicious cycle of mutations of mtDNA causing RC dysfunction, leading to the generation of ROS causing more mutations of mtDNA, etc., has been elegantly disproven recently. One of the strongest arguments was provided when it was shown that a mouse model of premature aging, which accumulates a large number of mutations in their mtDNA (mutator mouse), does not show any signs of enhanced ROS production or downstream modifications of proteins or lipids (Trifunovic et al. 2004, 2005). The second argument is that such a vicious cycle should lead to random accumulation of mtDNA mutations during aging, while single cell analysis has shown that clonal expansion of single founder mtDNA mutations occurs during a lifetime (Khrapko et al. 1999, Wiesner et al. 2006).

Finally, it is also a widespread misconception that a nonfunctioning RC would inevitably lead to increased ROS production. On the contrary, it has been shown many times that the absence of the RC results in lowered intracellular ROS levels (Trifunovic et al. 2005, Schauen et al. 2006). Finally, there is some evidence that a certain ROS "tone" coming from the mitochondrial RC is a necessary prerequisite maintaining cellular homeostasis, since these ROS may be important signaling molecules (Schauen et al. 2006, Owusu-Ansah and Banerjee 2009).

DIABETES

From Obesity via Insulin Resistance to Type 2 Diabetes

The prevalence of diabetes is still increasing and 10 years ago the number of people who will suffer from diabetes in 2030 was estimated to be 366 million (Wild et al. 2004). Unfortunately, these days there are already more than 387 million people who live with diabetes and according to the new estimations for 2035, 592 million people are supposed to have diabetes, reaching then a 10% prevalence in adults (updated sixth diabetes atlas 2014 of the international diabetes federation (Cho et al. 2014). What is the ultimate cause for T2DM? At the moment, there is no obvious answer for this question, but insulin resistance and β-cell dysfunction are two major contributors.

The β cells are responsible to sense blood glucose levels and after a meal they secrete insulin, which tells the muscle and fat tissue to take up glucose, while it signals to the liver to stop glucose production from precursors (gluconeogenesis). If these peripheral tissues fail to work properly, insulin resistance evolves. An impairment of insulin secretion and peripherial glucose disposal lead to diabetes. A polygenetic predisposition definitely impacts the development of diabetes and there are about 70 established genetic loci identified, with numbers still increasing (Mahajan et al. 2014).

However, exogenous or environmental factors, like diet and exercise, are also the main contributors to develop T2DM. It was shown that about 90% of the new diabetes cases could be assigned to an adverse lifestyle (Krebs and Roden 2004), with the major lifestyle contributor being food. Excessive and prolonged high-calorie intake, in the form of highly palatable food which contains high fat as well as high sucrose, worsens or even directly leads to insulin resistance and NAFLD (Johnson et al. 2013, Kahle et al. 2013, Vos and Lavine 2013). In animal studies, rodents are usually treated with diets containing high fat and high sucrose to evoke insulin resistance and T2DM (Franko et al. 2014, Verbeek et al. 2014). It is not quite clear why it is hard to stop overeating, in rodents as well as in man, but high fat and sucrose diets were shown to lead to addiction like behaviors in rats and it is discussed whether highly palatable food could also be addictive for humans (Benton 2010, Garber and Lustig 2011, Gearhardt et al. 2011).

A second important factor is the sedentary nature of modern lifestyle which has probably an even higher impact. In the Australian diabetes, obesity, and lifestyle study (AusDiab), more than 4800 individuals without diagnosed diabetes were analyzed for self-reported sitting and TV viewing time. Sitting time was associated with high BMI, fasting insulin, and 2 h post-load plasma glucose (Thorp et al. 2010). A meta-analysis, summarizing data from more than 175,000 individuals reported by four studies, demonstrated that prolonged TV viewing is associated with an increased risk of T2DM. The risk score for T2DM with greater TV viewing remained significant even when obesity-associated parameters were also adjusted. Vice versa, the reduction of TV viewing time was shown to be beneficial in children in terms of BMI and energy intake even when physical activity remained unchanged (Grontved and Hu 2011). Furthermore, weight loss *per se* is also a powerful tool to reduce diabetes incidence and lifestyle interventions were demonstrated to be more effective than antidiabetic drugs (Krebs and Roden 2004).

Obesity

Obesity, which is usually originated from an imbalance between food intake and energy expenditure, is postulated to be a risk factor independent of insulin resistance and is associated with a 70%–80% risk to develop NAFLD and a 15%–20% risk to develop NASH (Choudhury and Sanyal 2005). Excess nutrient intake and obesity *per se* may also impact mitochondrial performance. Obese subjects showed a downregulated expression of energy metabolism genes in the liver compared to lean controls (Pihlajamaki et al. 2009). Morbidly obese, nondiabetic subjects exhibited lower skeletal muscle mitochondrial respiration compared to obese and lean controls and laparoscopic adjustable gastric banding bariatric surgery on these very obese patients led to a pronounced weight loss as well as elevated mitochondrial respiration

in muscle (Vijgen et al. 2013). Roux-en-y gastric bypass was also applied to high fat, high sucrose (HF-HSD)-fed mice and resulted in weight loss, but also insulin resistance, high fat mass and low complex I activity as well as ATP content in the liver were normalized (Verbeek et al. 2014). These results suggest that obesity may disturb mitochondrial performance and that pronounced weight loss is able to revert the suppressed mitochondrial capacity. Since most NAFLD/NASH patients are obese, it is not possible at this moment to distinguish between alterations in mitochondrial function due to obesity, NAFLD or, finally, NASH, respectively.

Insulin Resistance

Insulin resistance and diabetes were reported to be associated with steatosis and NASH, however there are NASH patients who show normal glucose metabolism. In human studies, the HOMA (homeostasis model assessment) index is usually used to assess insulin resistance, which is calculated from fasting insulin and fasting glucose values. Most of the patients with NAFLD exhibit insulin resistance or diabetes (Cortez-Pinto et al. 1999), however about 10%–20% of them do not (Larter et al. 2010). Chitturi and colleagues reported that 98% of the NASH patients analyzed exhibited insulin resistance, revealed by high HOMA-IR (Chitturi et al. 2002). Furthermore, 87% and 50% of the NASH patients, respectively, were characterized by impaired glucose metabolism and overt T2DM (Chitturi et al. 2002). Therefore, insulin resistance may play an important role in the development of NAFLD. Prolonged fat and sucrose intake leads to ectopic lipid accumulation in the liver and this was postulated to cause liver insulin resistance by changing the phosphorylation state of insulin receptor machinery (Lowell and Shulman 2005, Szendroedi and Roden 2009). Insulin binds to its tyrosine kinase receptor (IR), which undergoes a conformational change, so that the tyrosine kinase domain is activated via autophosphorylation. This phosphorylation step recruits the insulin receptor substrate proteins (IRS) and the IR phosphorylates IRS on tyrosine residues. The phosphorylated IRS serves as a scaffold recruiting several effector kinases such as phospho-inositol-3-kinase (PI3K). These kinases activate other target proteins (like protein kinase B; PKB/AKT) which modulate transcription factors like FOXO1 and in turn modify gene regulation and control metabolic pathways, as well as cell proliferation and cell survival/death processes (for a current review see Boucher 2014). This highly complex system could be impaired by many different ways. Here we only point out the role of lipid intermediates like diacylglycerols, which can activate classical and novel PKC family members such as α, β and δ, ε, and these kinases are able to phosphorylate IR and IRS on serine residues. Serine phosphorylation inhibits tyrosine phosphorylation and in turn the recruitment of downstream substrates, which could result in diminished insulin signaling efficiency (Lowell and Shulman 2005, Szendroedi and Roden 2009, Boucher et al. 2014).

However, insulin resistance itself could also contribute to fat accumulation (Biddinger et al. 2008, Semple et al. 2009), therefore, it is hard to conclude whether steatosis causes insulin resistance or vice versa, since both states can induce the other (Larter et al. 2010). Furthermore, the role of insulin resistance in the development of NASH is not well understood, since liver steatosis further develops to NASH in some patients with insulin resistance, however in others it does not (Choudhury and Sanyal 2005).

Diabetes

Pancreatic β cells are flexible cells, they can expand their mass and number, can increase insulin production and secretion, and they can even form new β cells upon demand (Chang-Chen et al. 2008). Thus, the insulin-resistant state can be preserved for decades due to increased insulin secretion, but eventually β-cell failure and in turn diabetes develop. At the time of diagnosis, about 70% or 25%–50% reduction in β-cell mass were found in T1DM or T2DM patients, respectively (Chang-Chen et al. 2008). There are many reasons why β cells cannot infinitely compensate for insulin resistance. First, the function of β cells is especially dependent on mitochondrial performance, since insulin secretion is tightly coupled to mitochondrial ATP production. If ATP production in the mitochondria is suppressed, ATP-dependent potassium channels are not properly closed, membrane depolarization as well as calcium influx are diminished, resulting in disturbed insulin secretion (Lowell and Shulman 2005). High lipid levels and their intermediates were shown to directly disturb β-cell function, a phenomenon called lipotoxicity. Pancreatic lipid levels were demonstrated to be doubled in patients with T2DM and negatively correlated with β-cell performance (Szendroedi and Roden 2009). A physiological rise of glucose concentration is known to stimulate insulin secretion, however permanently high glucose levels were demonstrated to be detrimental for β-cell function, known as glucotoxicity. Chronic hyperglycemia caused diminished β-cell mass and function and ROS are one of the postulated major mediators of this process (Chang-Chen et al. 2008). Patients with T2DM are usually characterized by high lipid and glucose levels, therefore they are even facing "glucolipotoxicity." The effect of hyperglycemia and hyperlipidemia are assumed to synergize, since the detrimental effects of lipotoxicity on β-cell death is enhanced by high glucose levels. A proposed mechanism of glucolipotoxicity includes a suppressed fatty acid oxidation by high malonyl-CoA levels, which inhibits the mitochondrial fatty acid transporter CPT1 (Chang-Chen et al. 2008). Endoplasmic reticulum stress was also found to play a crucial role in β-cell dysfunction due to obesity and diabetes (Chang-Chen et al. 2008).

Possible Role of Mitochondrial Alterations in the Development of Diabetes

Since mitochondria play a central role in nutrient metabolism, it is not surprising that mitochondrial alterations in the liver have been "associated" with steatosis, NASH, and diabetes (Pessayre and Fromenty 2005). However, it is still debated whether mitochondrial changes, if they exist at all, are the cause or the consequence of, or alternatively, an adaptation to steatosis or diabetes (Larter et al. 2010, Koliaki and Roden 2014). There are dozens of reports pointing to the "changes in mitochondrial function" when patients with NAFLD or mouse models with steatosis or NASH were analyzed (for an excellent summary, see Begriche et al. 2013). However, the vast majority of these studies unfortunately do not report on circulating insulin or glucose levels or other important measures of glucose metabolism, like tolerance tests (Brady et al. 1985, Chavin et al. 1999, Serviddio et al. 2008a,b), so final conclusions regarding insulin resistance and mitochondrial function are impossible to be drawn from these studies at this point.

In this review, we are mainly summarizing data about liver mitochondrial alterations in NAFLD, but our main focus will be on studies with reported insulin resistance and/or diabetes. We will also shortly discuss mitochondrial alterations in skeletal muscle and heart, since they may also influence the development and progression of disease. One should again note that it is not an easy task to distinguish between hyperlipidemia in the circulation, ectopic lipid accumulation in the liver, liver insulin resistance, diminished whole body glucose tolerance, and manifested diabetes. All these single factors can preexist alone or in combination for years or decades without turning into more serious metabolic problems.

ROS, A Possible Contributor to Mitochondrial Dysfunction

What is the mechanism for mitochondrial impairment in NAFLD? Reactive oxygen species are one of the postulated key molecules, which possibly lead to mitochondrial dysfunction upon steatosis (for detailed information see Chapter 1). According to the "ROS theory," increased supply of energy, especially fatty acids, cannot be compensated completely by increased fatty acid transport and β-oxidation (Pessayre and Fromenty 2005). Fatty acids are accumulating and may additionally disturb the insulin signaling pathways, as discussed above (Lowell and Shulman 2005). Elevated fatty acid oxidation results in higher levels of the reducing equivalents NADH and $FADH_2$, which are donating electrons to the mitochondrial complexes I and II. Electron transport runs at high speed and reactive superoxide molecules are created by complexes I and III. These superoxide molecules are reduced by the mitochondrial Mn-SOD to hydrogen peroxide, which in turn is converted by glutathione peroxidase or catalase to water (Patti and Corvera 2010, Begriche et al. 2013). On the other hand, superoxide and hydrogen peroxide are known to activate the uncoupling proteins (UCPs) (Echtay et al. 2002), which are proton transporters and dissipate the electron flow from creating a proton gradient, which is used to drive the ATP synthase. Thus, UCP activation by ROS may result in diminished ATP production.

What Makes Liver Mitochondria Special?

Liver mitochondria have a smaller size, less cristae and total cellular area covered by mitochondria is much lower in the liver when compared to, for example, the heart (Veltri et al. 1990). Liver mitochondria show less maximal activities for CIII and CIV, however state 3 respiration and respiratory control ratio are similar in liver and muscle mitochondria (Benard et al. 2006). Furthermore, in liver mitochondria, the maximal capacity for the entry of electrons is primarily carried out by CII rather than CI (Holmstrom et al. 2012, Lapuente-Brun et al. 2013, Franko et al. 2014), while muscle shows a similar contribution of both complexes (Holmstrom et al. 2012) (Franko and Wiesner unpublished data). On the other hand, the maximal possible rate of ATP synthesis was higher in liver than in muscle mitochondria (Schmid et al. 2008).

What Makes Liver Mitochondria Special in Diabetic Situations?

Mitochondria in the liver are also different from the muscle in diabetic situations; here we summarize data reported from type 1 diabetic animal models. Heart and skeletal muscle mitochondria were found to have a defect in insulin-deficient diabetes in terms of respiration and proton conductance, whereas liver mitochondria did

not show any reduction in these parameters (Herlein et al. 2009). Heart mitochondria isolated from mice with a type 1-like diabetes state displayed less oxygen consumption compared to healthy controls, whereas kidney and liver mitochondria exhibited unchanged respiration (Bugger et al. 2009). In the nonobese diabetic (NOD) mouse, which is a genetic model for T1DM, mitochondrial respiration was reported to be decreased in skeletal muscle in the fasted state, however it remained unaltered in liver mitochondria, while in the fed state, liver mitochondria displayed a higher respiration, and muscle mitochondria showed an unchanged or decreased oxygen consumption, depending on complex I and II substrates (Jelenik et al. 2014). When we analyzed insulin-deficient, type 1 diabetic mice, we also noticed a striking difference in mitochondrial capacity comparing muscle and liver tissues. Mitochondrial performance was severely suppressed in the mitochondria of soleus muscle (Franko et al. 2012), however isolated liver mitochondria displayed normal complex II respiration or even elevated complex I oxygen consumption (Franko et al. 2014) (for details, see the section "Mitochondrial Function in the Liver in Type 1 Diabetes"). These results indicate that changes in mitochondrial capacity in type 1 diabetic states are tissue specific and conclusions drawn from one organ cannot be generalized. Moreover, heart and muscle mitochondrial function seems to be impaired in insulin-resistant and/or diabetic states, while liver rather can compensate for the disturbed metabolic state.

MITOCHONDRIAL FUNCTION IN THE LIVER DURING THE PROGRESSION OF STEATOSIS TO NASH

In the following chapters, we keep the terms steatosis, NAFLD, or NASH like they were used by the authors of the quoted references. To simplify the nomenclature, the term *fatty liver* is used equivalent to steatosis, defined by >5.5% intrahepatic fat content (Koliaki and Roden 2013). For a detailed review about NAFLD, see Chapters 18 and 12 or Begriche et al. (2013). In this section, we summarize the published studies on mitochondrial function in NAFLD, although insulin resistance and the diabetic state were not reported.

Human Studies

High levels of oxidized glutathione were found in isolated liver mitochondria of NASH patients, who did not show any other metabolic disorders. Furthermore, mRNA levels of the uncoupling protein UCP-2 were increased (Serviddio et al. 2008a), suggesting mitochondrial oxidative stress and mitochondrial uncoupling as a compensation for it in the liver of these NASH patients.

Rodent Studies

The leptin-deficient ob/ob mice serve as a mild model for NASH with hepatocellular necroinflammation and fibrosis (Begriche et al. 2013). In young, 5-week old ob/ob mice, lipid-driven respiration of liver mitochondria as well as activity of CPT-1, the main carrier for fatty acids in the inner mitochondrial membrane, was unaltered

compared to lean controls. In contrast, in old 6–9 months old ob/ob mice, liver mitochondrial respiration and CPT-1 activity were found to be increased (Brady et al. 1985). We also analyzed 7 months old ob/ob mice, which were normoglycemic, and liver mitochondria showed elevated respiration via CI or CII substrates as well as elevated CPT-1 activity (Franko and Wiesner, unpublished data). These results suggest that in the liver disease progression or ageing processes could affect mitochondrial function and the elevated mitochondrial capacity reported in older ob/ob mice could be an adaptation process, which is probably triggered by the oversupply of fatty acids. Chavin et al also studied young, 10–12-weeks old ob/ob mice and liver mitochondrial respiration was increased by CII substrate, but not with CI substrates compared to lean controls. Mitochondria exhibited a decreased membrane potential and upregulated UCP-2 levels, while liver ATP levels were reduced compared to lean controls (Chavin et al. 1999). These data suggest that UCP-2 upregulation and the reduced mitochondrial membrane potential could be a possible way to attenuate the redox pressure caused by the energy oversupply. The data also indicate that one should take care by choosing the appropriate substrate for respiration studies, since changes in the inner membrane RC complex stoichiometry may take place as an adaptation. Insulin receptor substrate (IRS) proteins are the key regulators for insulin signaling pathway connecting receptor activation to gene regulation. A diminished mitochondrial respiration was observed in 8–10-weeks old IRS-1/IRS-2 double KO mice as well as in 16–20-weeks old leptin-deficient ob/ob and leptin receptor mutant db/db mice compared to respective controls, in parallel with reduced mitochondrial number, respiratory control ratio, ATP level, and CI activity. In these animals, the level of heme, which is a cofactor needed for CIII and CIV, was also lower, in association with decreased CIII and CIV protein levels. FOXO1, a transcription factor activated via the insulin signaling pathway, as well as PGC-1α, a transcriptional coactivator responsible for mitochondrial biogenesis, were shown to control mitochondrial function in these insulin-resistant states (Cheng et al. 2009).

The methionine-choline-deficient (MCD) diet is proposed to cause more severe liver inflammation and fibrosis compared to ob/ob mice, however without causing insulin resistance. Three-week treatment resulted in normal CI-driven respiration, however CII respiration was increased in these animals, possibly again as an adaptation, but liver ATP level was decreased. After 7-and 11-weeks treatment, CII respiration was normal but liver ATP content still decreased (Serviddio et al. 2008a). Thus, the basal liver ATP level is probably not a reliable marker for mitochondrial performance (see below discussion of Cortez-Pinto et al. 1999), since liver ATP level was decreased at all time points, but mitochondrial respiration was not compromised at all.

MITOCHONDRIAL FUNCTION IN THE LIVER IN INSULIN-RESISTANT STATES

In this section, we summarize some of the published data on mitochondrial function in animals or patients, whose insulin-resistant state was reported. Subjects with fatty liver or NASH are also included in this section, since they are very probably

insulin resistant (see above). Our decision criteria to include a study in this paragraph were based on reported mild elevation in blood glucose levels (but not manifested diabetes with high fasted blood glucose), an elevated HOMA-IR, impaired glucose tolerance, and/or whole body or hepatic insulin resistance assessed by hyperinsulinemic–euglycemic clamp studies. Still one should bear in mind that the insulin-resistant state of the subjects determined by different methods will not necessarily lead to a homogenous group. HOMA-IR is an estimate for insulin resistance, calculated from fasting insulin and glucose values, while an euglycemic–hyperinsulinemic clamp study serves as the ultimate in vivo measurement. Also, the severity of insulin resistance probably impacts on the mitochondrial phenotype, as in extreme insulin-resistant states mitochondrial dysfunction was observed, at least in the muscle (Sleigh et al. 2011, Franko et al. 2012). However, according to our knowledge there is no common accepted one and only method to verify insulin resistance. Euglycemic–hyperinsulinemic clamp studies represent the gold standard for testing the efficacy of insulin in vivo, although because of its laborious, costly and time-consuming nature, only few laboratories perform this method routinely, especially in rodent studies (Fuchs et al. 2012). During the clamp procedure, a constant insulin dose is infused i.v., together with an i.v., glucose infusion. Glucose infusion is adjusted to maintain euglycemia in the presence of high insulin levels, so the infused dose will vary depending on the insulin sensitivity of the subject or animal. In an insulin-resistant subject, less glucose is needed to maintain euglycemia compared to a normal insulin-sensitive subject, since insulin is not able to decrease blood glucose levels properly due to hepatic and peripheral tissue insulin resistance. Furthermore, this technique is frequently combined with the use of radioactive isotopes, providing a unique possibility to dissect liver, muscle, or fat insulin sensitivity (Muniyappa et al. 2008).

Human Studies

Intravenous fructose injection leads to a transient depletion of hepatic ATP pool due to a rapid conversion of fructose to glucose-6-phosphate, thus it was used to analyze liver mitochondrial function in obese NASH patients with probably mild insulin resistance. Basal and fructose-depleted ATP levels were similar in NASH patients compared to healthy controls, however ATP levels recovered to basal level after 1 h in control subjects, while recovery was impaired in NASH patients, suggesting a severe mitochondrial dysfunction (Cortez-Pinto et al. 1999). These data also clearly show that the basal ATP level is not an appropriate marker for mitochondrial performance, since NASH patient had normal values, and the diminished mitochondrial performance became only apparent after fructose depletion. In obese patients with steatosis or NASH, overall insulin sensitivity was assessed by euglycemic–hyperinsulinemic clamps and showed that the glucose infusion rate was lower compared to healthy controls, indicating whole body insulin resistance. However, these NASH patients exhibited similar hepatic insulin sensitivity compared to patients with steatosis. Transmission microscopy revealed swollen mitochondria with paracrystalline inclusion bodies in the liver biopsies of patients with NASH, but steatosis patients did not show such mitochondrial abnormalities (Sanyal et al. 2001). In accordance with

these findings, liver homogenates of overweight NASH patients with an elevated HOMA-IR index, suggested that patients were insulin resistant, exhibited reduced activities for all five RC complexes (Perez-Carreras et al. 2003). Furthermore, liver samples of morbidly obese but nondiabetic subjects with elevated HOMA-IR and increased liver fat content exhibited a decreased expression of OXPHOS genes compared to lean controls (Pihlajamaki et al. 2009). Sunny et al. studied obese NAFLD patients with high hepatic TG levels and showed that impaired hepatic insulin sensitivity compared to body fat, BMI, and age-matched subjects with low hepatic TG. Despite hepatic insulin resistance, oxidative and anaplerotic fluxes determined in liver mitochondria were elevated in NAFLD patients (Sunny et al. 2011). These data indicate that liver steatosis and insulin resistance do not necessarily lead to mitochondrial damage, and only severe forms of NASH are associated with mitochondrial abnormalities.

Rodent Studies

High fat diet in the combination with high sucrose (HF-HSD) supplementation is a powerful tool to evoke insulin resistance. Sucrose is a disaccharide composed of one glucose and one fructose molecule. Fructose is known to deplete the mitochondrial ATP pool in the liver (Cortez-Pinto et al. 1999) and is postulated to be a risk factor for the development of NAFLD (Ouyang et al. 2008). HF-HSD treatment of mice for 4 weeks evoked impaired glucose tolerance and a tendency for elevated HOMA-IR, but without diabetes. This short-term HF-HSD feeding neither caused symptoms of NASH nor did it reduce mitochondrial activity (Verbeek et al. 2014) (Verbeek, personal communication). These results support the data from humans, indicating that mitochondrial function remains preserved in most insulin-resistant states.

MITOCHONDRIAL FUNCTION IN THE LIVER IN OVERT TYPE 2 DIABETES

Diabetes is defined by fasting plasma glucose ≥7.0 mmol/L (126 mg/dL) for human subjects (WHO 2006, 2014). This definition was used to identify published data in this category. One should note that in rodent studies glucose is usually measured in whole blood taken from the tail vein, since plasma samples cannot conveniently be collected for routine and repeated measurements. Tail vein blood glucose levels are usually lower than plasma samples due to the presence of erythrocytes; however, fasting blood glucose levels in mice are usually higher than in humans.

Human Studies

Nuclear genes encoding OXPHOS proteins were shown to be upregulated in the liver of T2DM patients compared to age and BMI-matched nondiabetic controls (Misu et al. 2007). In a second study, this was also shown in the liver with steatosis of obese patients with T2DM compared to nonobese patients with T2DM (Takamura et al. 2008). On the other hand, morbidly obese patients with T2DM and steatosis were reported to exhibit reduced expression of OXPHOS genes compared to lean, healthy

controls (Pihlajamaki et al. 2009). Interestingly, this study also analyzed obese, nondiabetic subjects and gene expressions of this group showed very similar downregulation in terms of OXPHOS genes as the obese, diabetic group (Pihlajamaki et al. 2009). The apparent controversy in these studies is certainly due to the fact that they have used (1) different controls for their comparisons (lean healthy versus lean diabetics), (2) the studied subjects originated from different ethnicity (Japanese versus Caucasian Americans), and (3) the degree of obesity was different (morbidly obese vs. mildly obese subjects BMI 52 vs. 27) (Patti and Corvera 2010). Taken together, it remains still controversial whether and how mitochondrial gene expression is influenced by the diabetic situation in the human liver. Szendroedi et al. reported for the first time, using in vivo ^{31}P-NMR, that the liver of overweight patients with T2DM showed less energy-rich γ-ATP, however also less inorganic phosphate (Pi) levels than nondiabetic, age and BMI-matched or lean controls (Szendroedi et al. 2009). One possible explanation of these findings is "the energy deficit" hypothesis: excessive gluconeogenesis and/or lipogenesis, which are not properly suppressed due to insulin resistance, may evoke an ATP synthesis/demand dysbalance, leading to a drop of intracellular ATP if the hepatocytes are not able to compensate this situation. The lowered Pi levels, however, may indicate that the energy charge of the adenylate system is maintained. These T2DM patients were extensively characterized by euglycemic–hyperinsulinemic clamp studies, which revealed the whole body as well as hepatic insulin resistance (Szendroedi et al. 2009). To analyze in vivo ATP turnover in the liver, in contrast to steady-state levels, the ^{31}P-NMR method was further developed using saturation transfer (Petersen et al. 2004) by the same group. Overweight T2DM patients, who exhibited hepatic insulin resistance, were reported to show a reduced liver ATP synthetic flux rate (fATP) compared to nondiabetic age and BMI-matched controls. On the other hand, hepatic lipid content was not altered and the prevalence of steatosis was similar between both groups (Schmid et al. 2011). These results suggest that a lower mitochondrial ATP production rate is not necessarily due to ectopic lipid accumulation.

Rodent Studies

Liver mitochondrial function was compared in lean, diabetic Goto-Kakizaki rats to control Wistar rats and complex I- and complex II-driven mitochondrial respiration rate were found to be increased. The elevated mitochondrial respiration was associated with higher respiratory control ratios and less lipid peroxidation was found (Ferreira et al. 1999). In a later study of the same group, the higher mitochondrial respiration rate and increased membrane potential were associated with elevated complex activities in 6 months old, but not in 3-month-old Goto-Kakizaki rats (Ferreira et al. 2003). Buchner et al. compared two congenic mouse strains, called 6C1 and 6C2, derived from chromosome substitutions. The 6C1 strain fed with HF-HSD showed higher body weight, higher blood glucose levels, impaired glucose tolerance, and insulin resistance compared to 6C2. Genes coding for mitochondrial proteins were upregulated in 6C1 liver compared to 6C2 in parallel with elevated CI-driven mitochondrial respiration (Buchner et al. 2008, 2011). In our study, HF-HSD feeding of C57BL/6 mice for 6 months caused increased body

weight, high liver triglycerides, increased serum insulin, fasted glucose and triglyceride levels, and thus T2DM. The oxygen consumption of isolated liver mitochondria revealed normal CI- and CII-driven respiration, however an increased capacity to oxidize glycerol-3-phosphate, feeding electrons into the quinone pool before CIII, was found. Elevated mitochondrial respiration was not associated with higher mitochondrial RC protein levels, but TCA enzyme activities were increased in HF-HSD mice. Mitochondrial membrane potential measurements exhibited unchanged proton leak kinetics in HF-HSD mice (Franko et al. 2014). These results strongly support the concept that mitochondrial dysfunction is not a necessary prerequisite for steatosis, since massive lipid accumulation was observed in the presence of normal mitochondrial function. Our results and the former results rather indicate an adaptation process of the liver mitochondria under insulin-resistant and diabetic conditions.

On the other hand, there are other reports showing attenuated mitochondrial performance in rodents with diabetes. Sand rats (*Psammomys obesus*) were fed with a high caloric diet (HFD), which led to an increased body weight and insulin levels as well as manifested diabetes together with elevated liver triglyceride levels. Liver mitochondria isolated from HFD animals exhibited reduced mitochondrial respiration via CI substrates, whereas respiration via CII substrate remained unchanged (Bouderba et al. 2012). Holmstrom et al. compared obese, diabetic leptin receptor mutant db/db mice to lean controls and found reduced CI- and CII-driven respiration in liver homogenates. The protein levels of CI and CIV subunits were reduced in association with lower PGC-1α expression, however the mtDNA copy number was increased in db/db mice (Holmstrom et al. 2012). Very recently, Verbeek et al. reported an excellent longitudinal study on 4-, 12-, and 20-week HF-HSD-treated C57/BL6J mice. Interestingly, all three treatment groups showed elevated citrate synthase activity. The liver of 20-week HF-HSD mice exhibited obvious signs for NASH, reflected by hematoxylin and eosin stainings as well as collagen morphometry. Furthermore, these mice not only exhibit insulin resistance, assessed by glucose tolerance test and HOMA-IR, but also increased fasting blood glucose levels (Verbeek et al. 2014 and Verbeek, personal communication). Complex I and IV enzymes activities as well as ATP content were significantly decreased in the 20-week HF-HSD-treated group, however mtDNA content remained unchanged. Furthermore, ultrastructural analysis of the 20-week treated mice revealed mitochondrial membrane damage, rearranged cristae, and swollen mitochondria. HF-HSD treatment for 12 weeks evoked similar but less severe signs for NASH in the liver compared to 20-week treatment, but mitochondrial morphological and functional impairments were comparable to the 20-week group (Verbeek et al. 2014). Since 4-week HF-HSD treatment caused an impaired glucose tolerance, but neither NASH nor elevated blood glucose levels, we postulate from this and other studies (Szendroedi et al. 2009) that insulin resistance can precede manifested NASH.

MITOCHONDRIAL FUNCTION IN THE LIVER IN TYPE 1 DIABETES

To our knowledge, there are no human studies on liver mitochondrial performance in this state.

Rodent Studies

The Akita mice carrying a mutation in the insulin 2 gene are used as a model for T1DM with severe hyperglycemia. In the liver of these mice, an upregulation of mitochondrial genes and proteins were found, however neither mitochondrial respiration, analyzed via CI or CII substrates, nor mitochondrial mass or morphology were changed (Bugger et al. 2009). A chemical called streptozotocin (STZ) can be used to destroy pancreatic β cells in healthy animals and thus evokes an insulin-deficient state resembling T1DM. In the liver of STZ-treated mice, elevated levels of mRNAs for mitochondrial genes and for mitochondrial biogenesis regulating genes, as well as the cognate proteins were observed, together with higher mtDNA level and increased mitochondrial mass (Liu et al. 2009). These and results on insulin-treated hepatocytes (Liu et al. 2009) indicate that insulin may suppress mitochondrial biogenesis in the liver.

Importantly, one needs to take into account the time factor, namely the duration of diabetes, which does also impact the mitochondrial phenotype. Ferreira et al. investigated STZ-treated male Wistar rats 3 or 9 weeks after STZ injection: Although both STZ groups presented blood glucose levels higher than 400 mg/dL and reduced body weights, mitochondrial respiration stimulated by FCCP, respiratory control ratios, and the mitochondrial membrane potential were only elevated in the 9-week group, but were normal in the 3-week group. Both STZ groups showed elevated activities for CII and CIV at 3 and 9 weeks after injection (Ferreira et al. 2003). Furthermore, liver mitochondrial function was also studied in STZ-treated male Sprague–Dawley rats 2 or 8 weeks after injection. Mitochondrial respiration via CI or CII substrates, respiratory control ratios as well as mitochondrial membrane potential remained unaffected in both STZ groups presenting with severe hyperglycemia. Mitochondrial H_2O_2 production was elevated in the 2-week group but was reduced in the 8-week group (Herlein et al. 2009). We have also investigated the long-term effect of type 1 diabetes in male C57BL/6N mice and have not observed any sings for mitochondrial dysfunction. Euglycemic–hyperinsulinemic clamps proved hepatic insulin resistance in the treated mice, which was accompanied by very low insulin and very high blood glucose levels after 2 months. Mitochondrial protein levels were increased in liver mitochondria in parallel with elevated CI-driven respiration and with better coupling (Franko et al. 2014). Finally, the impact of the duration of diabetes (acute vs. chronic) on mitochondrial performance was also investigated in a recent study of Jelenik et al., where female NOD (nonobese diabetic) mice were examined, which represent an excellent genetic model for T1DM with autoimmune characteristics. Three different insulin-resistant NOD mice were investigated and compared to wild-type mice: (1) normoglycemic but insulin-resistant—NOD mice, (2) acute diabetic—NOD mice, and (3) chronic diabetic—NOD mice. The insulin-resistant and acute diabetic NOD mice displayed hepatic insulin resistance, verified by euglycemic–hyperinsulinemic clamps. In the fasted state normal mitochondrial respiration was observed in the liver of all mice (although no data were collected from chronic diabetic NOD mice). On the other hand, in the fed state an elevated mitochondrial respiration was found in the acute diabetic NOD compared to wild-type and insulin-resistant NOD mice. Mitochondria from the fed chronic diabetic

NOD mice also exhibited a higher respiration rate compared to wild-type controls, however it was lower than in acute diabetic mice. The expression of genes regulating mitochondrial biogenesis was upregulated in acute diabetic NOD mice compared to wild-type controls (Jelenik et al. 2014). These results indicate that the insulin-resistant state per se is not able to change mitochondrial function in the liver of T1DM mice, however acute diabetes evokes a prominent elevation of mitochondrial oxygen consumption, which later declines in the chronic diabetic state, but still remains higher than healthy controls. This study also points to the importance of the nutrition state, which clearly influences mitochondrial performance in the liver as well as in the muscle (Jelenik et al. 2014), maybe by influencing the assembly of RC supercomplexes in the inner membrane (Lapuente-Brun et al. 2013).

Taken together, these data suggest that in the T1DM state with high glucose but low insulin levels, in contrast to T2DM with usually elevation of both parameters, liver mitochondrial function is not impaired but rather compensates for the disturbed metabolic state.

DOES LIVER MITOCHONDRIAL DYSFUNCTION PER SE CAUSE LIVER INSULIN RESISTANCE?

An altered mitochondrial capacity could arise as a consequence of insulin resistance or the diabetes state. Thus, in the former sections, we collected data on mitochondrial function from studies which investigated liver ranging from steatosis to NASH. On the contrary, primary mitochondrial dysfunction was also postulated to play an important role in the development of insulin resistance. Therefore, in this section, we summarize some of the reports, in which mitochondrial proteins were deleted or in which drugs were used in vivo to manipulate mitochondrial function, followed by the analysis of the insulin-resistant status of the liver.

In mice, ablation of the mitochondrial long-chain acyl-CoA dehydrogenase, responsible for fatty acid oxidation, led to hepatic insulin resistance, diminished IRS-2 phosphorylation, and PI3K as well as AKT2 activities, which are the key enzymes transmitting the insulin signal to the cell, in association with decreased energy consumption and higher body fat mass (Zhang et al. 2007). These results suggest that primary mitochondrial dysfunction, in this case failure of β-oxidation, is possibly involved in the development of hepatic insulin resistance. Apoptosis-inducing factor (AIF) is probably involved in apoptosis and necessary for normal respiratory chain function. The deletion of this gene in mouse liver (LAIFKO mice) caused a coordinated downregulation of OXPHOS genes, diminished CI and CIV activities, ATP levels and CII-driven respiration, however respiratory control ratio was elevated. On the other hand, LAIFKO mice exhibited a better glucose and insulin tolerance as well as whole body insulin sensitivity and were protected against HFD-induced obesity (Pospisilik et al. 2007). These results indicate that a primary OXPHOS defect alone does not cause insulin resistance but, rather, can result in even higher insulin sensitivity.

Treatment with 2,4-dinitrophenol (DNP), which is a mitochondrial uncoupler, showed beneficial effects on HFD-fed rats in terms of hepatic insulin resistance in association with higher IRS-1 and IRS-2 tyrosin phosphorylation as well as PI3K

and AKT2 activities (Samuel et al. 2004). These results suggest that mild mitochondrial uncoupling could be favorable, since it increases fatty acid oxidation to cover increased energy expenditure and thus slows down the development of T2DM.

CONCLUSION

In conclusion, we do not have a clear picture yet about the relation between mitochondrial function in the liver and the development of insulin resistance and T2DM. Even the term mitochondrial dysfunction is not well defined, since there is not a single well accepted method easily used ex vivo representing mitochondrial performance in vivo. According to our experience, respiration measurements of freshly isolated mitochondria are a very reliable method to investigate maximal mitochondrial functional capacity. On the other hand, other methods give also valuable information about mitochondrial function, although from a different angle, and the more methods are used, the clearer the picture becomes. In some studies, upregulated levels of mitochondrial transcripts were observed (Misu et al. 2007, Takamura et al. 2008), and when mitochondrial oxygen consumption was measured, gene expression positively correlated with respiration (Buchner et al. 2011), while other studies did not report a correlation between these two parameters (Bugger et al. 2009, Holmstrom et al. 2012). Mitochondrial RC levels were reported to correlate with mitochondrial respiration in some animal models (Holmstrom et al. 2012, Franko et al. 2014), however the correlation was missing in others (Bugger et al. 2009, Franko et al. 2014). mtDNA copy number is claimed to be a good marker for mitochondrial mass in some (Liu et al. 2009), however other studies disagree (Wiesner 1997, Franko et al. 2008, Kim et al. 2008). In insulin-resistant/diabetic animals, mtDNA levels changed in parallel with mitochondrial respiration (Jelenik et al. 2014), however no correlation was also found (Holmstrom et al. 2012, Jelenik et al. 2014). In order to assess mitochondrial capacity, RC activities are also routinely measured. Mitochondrial oxygen consumption was shown to be only significantly impaired when RC activities are severely suppressed (Begriche et al. 2013) and respiration and activity measurements could change in the same direction, as expected (Serviddio et al. 2008b, Bouderba et al. 2012), but also behave differently (Serviddio et al. 2008b, Bouderba et al. 2012). These results indicate that mitochondrial transcript and protein levels as well as RC complex activities could, but not always do complement mitochondrial respiration measurements and conclusions drawn from the former methods generalized as mitochondrial function should be interpreted carefully. One should also appreciate that oxygen consumption protocols are different, since some laboratories investigate complex I and II respiration separately (Bugger et al. 2009, Herlein et al. 2009, Franko et al. 2014), while others analyze both in combination (Holmstrom et al. 2012, Jelenik et al. 2014). Most importantly, experiments studying liver mitochondrial capacity using complex I or complex II substrates separately could reveal significant differences between the two pathways, however the difference could disappear when both complex I and II substrates are applied together (Lapuente-Brun et al. 2013). Furthermore, the respiration rates for CI and CII substrates in liver mitochondria in insulin-resistant/diabetic states do not necessarily supplement each other: some studies reported higher CII and normal CI-driven respiration (Chavin et al. 1999,

Serviddio et al. 2008b), while others found reduced CI but normal CII-driven oxygen consumption (Bouderba et al. 2012) or both respiration rates were observed to be elevated (Ferreira et al. 1999) or even remained unchanged (Franko et al. 2014). Liver ATP levels in basal states were reported to be reduced (Szendroedi et al. 2009) or shown to be normal (Cortez-Pinto et al. 1999) in insulin-resistant patients. Moreover, ATP recovery after fructose injection and ATP synthesis flux were diminished in insulin-resistant subjects (Cortez-Pinto et al. 1999, Schmid et al. 2011). However, a low ATP level does not necessarily result from disturbed oxygen consumption, since in some obese animals a higher mitochondrial respiration capacity was observed although ATP level was reduced (Chavin et al. 1999, Serviddio et al. 2008b). In conclusion, the chosen protocol for measuring mitochondrial performance may strongly determine the outcome of the experiments and conclusions based on only one or two methods should be drawn carefully.

Another important issue is the diverse background of patients and animals. In this review, we summarized the data from patients and rodents displaying steatosis only, but some developed NASH with inflammation and fibrosis. Moreover, the overweight or obese state could also influence mitochondrial function (Patti and Corvera 2010, Verbeek et al. 2014) and some studies compared the analyzed patients with BMI-matched controls, some did not. When insulin produced by the pancreatic β cells cannot compensate anymore for insulin resistance, the blood glucose level rises and T2DM develops. If this stage is prolonged for a longer period, glucotoxicity will impair the function of organs (Krebs and Roden 2004, Roseman 2005). Furthermore, the results generated by Jelenik et al. importantly point to the nutrition state of the studied rodents, since in the fasting state no significant changes were found for diabetic, insulin-resistant rodents, however in the fed state a pronounced elevation in mitochondrial respiration was reported (Jelenik et al. 2014).

Finally, longitudinal studies provide an excellent opportunity for investigating mitochondrial capacity at different time points and they have called attention to the

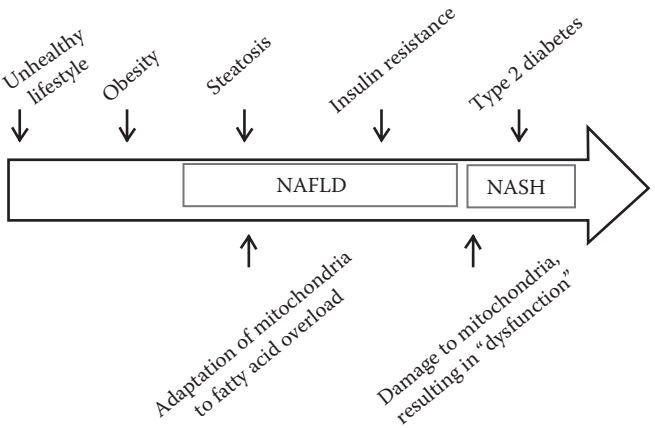

FIGURE 17.2 Possible changes of mitochondrial function during the "typical" development of T2DM.

disease duration, which was shown to significantly influence the mitochondrial performance. Most studies found that short-term duration of the disease evoked a compensatory rise in mitochondrial oxygen consumption, which usually vanished upon disease progression (Ferreira et al. 2003, Serviddio et al. 2008b, Jelenik et al. 2014); however, other authors did not observe significant changes among different time points (Ferreira et al. 2003, Herlein et al. 2009). Therefore, the found discrepancies on mitochondrial performance could probably be explained by the distinct disease states of the studied patients or animals.

Altogether, according to our own results (Franko et al. 2014) and other studies (Schiff et al. 2009, Turner 2013), mitochondria in the liver seem to be capable to adapt to the metabolic environment of NAFLD and insulin-resistant and diabetic states, however, seem to be damaged as soon as NASH develops (Figure 17.2).

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