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Minireview

Liver mitochondria and insulin resistance*

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With a steadily increasing prevalence, insulin resistance (IR) is a major public health issue. This syndrome is defined as a set of metabolic dysfunctions associated with, or contributing to, a range of serious health problems. These disorders include type 2 diabetes, metabolic syndrome, obesity, and non-alcoholic steatohepatitis (NASH). According to the literature in the field, several cell types like β-cell, myocyte, hepatocyte and/or adipocyte, as well as related complex signaling environment involved in peripheral insulin sensitivity are believed to be central in this pathology. Because of the central role of the liver in the whole-body energy homeostasis, liver insulin sensitivity and its potential relationship with mitochondrial oxidative phosphorylation appear to be crucial. The following short review highlights how liver mitochondria could be implicated in IR and should therefore be considered as a specific therapeutic target in the future.

Keywords: mitochondria, insulin resistance, oxidative phosphorylation, membrane potential

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INTRODUCTION

Mitochondria play a key role in energy metabolism by generating most of the energy used by cells. The redox power from substrate oxidation is provided to the respiratory chain by reduced equivalents ($NADH,H^+$; $FADH_2$) or directly by specific dehydrogenases *via* electron transferring proteins to the quinone pool. Electron flow is conveyed along the mitochondrial respiratory chain and part of its energy is converted to an electrochemical force by pumping out protons across the inner mitochondrial membrane. This generates the so-called proton motive force (Δp) that can be used to synthesize ATP or exchange proteins or ions (Ca^{2+}) across the inner mitochondrial membrane. The efficiency with which reduced equivalents are used to generate ATP by mitochondrial oxidative phosphorylation is dependent on mitochondrion coupling. Uncoupling the proton transport across the membrane participates in the regulation of energy homeostasis and defaults in electron transfer can enhance reactive oxygen species (ROS) production. Under conditions of excess in energy intake and tight mitochondrial coupling, Δp can rise to a maximum. Thus mitochondrial respiratory complexes are highly reduced and may release electrons directly to oxygen resulting in a higher ROS production that could alter cell functioning and lead to several pathologies. In this context, we study the links between mitochondria and insulin resistance (IR) with a focus on the role of mitochondrial membrane potential. Three major players are implicated in IR: β-cells, hepatocytes and other insulin-dependent cells (i.e., myocytes and adipocytes). Each of them contributes to the development of an abnormal glucose homeostasis and represents a valuable therapeutic target. Because of our interest in liver metabolism, we focus on the relationship between IR and alterations of oxidative phosphorylation in liver cells.

HEPATIC MITOCHONDRIAL ALTERATIONS IN INSULIN-RESISTANT PATIENTS AND ANIMAL MODELS

For more than ten years evidence has accumulated which reveals the importance of liver mitochondria in the physiopathology of IR. The first evidence was observed on biopsies from diabetic patients or from IR animal models using electron microscopy that revealed modifications in mitochondria morphology. In rats fed *ad libitum* with a high-fat diet for 8 weeks, mitochondrion degenerative changes such as rarefied matrix and loss of cristae have been shown (Lieber *et al.*, 2004; Kim *et al.*, 2008). Furthermore, rat models of IR and hypertension (Ren2) also present swollen mitochondria and decreased matrix density (Kim *et al.*, 2008). These observations strengthen not only the relationship between altered mitochondrial functioning and IR but also the importance of mitochondrial structure and organization in mitochondrial function. A decrease peroxisome proliferator-activated receptor gamma coactivator 1-α (PGC1-α) expression, a transcription factor controlling mitochondrial biogenesis, linked to an inactivation of AMPK expression, could be responsible for a decreased number of mitochondria (Patti *et al.*, 2003). However, a decrease in mitochondria biogenesis could not explain all the mitochondrial alterations reported in this pathology (Morino *et al.*, 2005). We also have to consider intrinsic mitochondrial dysfunctions leading to decrease in respiration and ATP production (Perez-Carreras *et al.*, 2003). Surprisingly, a large number of subunits of OXPHOS have been found to be overexpressed in diabetic patients (Takamura *et al.*, 2008). Unexpectedly, OXPHOS alterations are also frequently reported but the resulting picture of mitochondrial activity and efficiency remains unclear. The OXPHOS activity assessed in isolated liver mitochondria from either rats exposed to a high-fat

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Abbreviations: DNP, 2,4-dinitrophenol; IR, insulin resistance; mG-3PdH, mitochondrial glyceraldehyde-3-phosphate dehydrogenase; NASH, non-alcoholic steatohepatitis; Δp, proton motive force; PGC1-α, peroxisome proliferator-activated receptor gamma coactivator 1-α; ROS, reactive oxygen species; TH, thyroid hormone

diet, animal models of NASH or obesity is reported to be either decreased (Raffaella *et al.*, 2008; Mollica *et al.*, 2009), unchanged (Serviddio *et al.*, 2008) or even increased (Brady *et al.*, 1985; Chavin *et al.*, 1999; Raffaella *et al.*, 2008) when compared with controls. Respiratory chain complex activities, independently of membrane environment, also exhibit conflicting results (Perez-Carreras *et al.*, 2003). Hepatic ATP content is decreased but the intrinsic activity of complex V is unchanged (Chavin *et al.*, 1999; Serviddio *et al.*, 2008; Vial *et al.*, 2010). In a recent study on rats fed a high-fat diet during 8 weeks and presenting insulin-resistant state, we found impairments of mitochondrial respiration in intact liver cells as well as in isolated mitochondria (Vial *et al.*, 2010). Mitochondrial production of ROS was higher in our study and is also frequently reported to be increased in the physiopathology of IR. Thus, inhibition of respiration could be caused by this sort of excessive mitochondrial ROS production. With unchanged mitochondrial coupling (Vial *et al.*, 2010), inhibition of oxidative phosphorylation would lead to a reduced matrix redox potential. Mitochondrial redox state and cytosolic redox state are linked together, at a given Δp *via* the malate-aspartate shuttle (at the glutamate carrier). Surprisingly, in our IR model, cytosolic redox state was more oxidized while it was reduced in the matrix. Such a finding could be explained by an increased mitochondrial membrane potential. Taken together, the high membrane potential and inhibition of OXPHOS could explain the increase in ROS production. Despite intracellular antioxidant mechanisms, ROS overproduction could damage all cellular compounds: DNA, proteins and lipids potentially causing cell damage and death. With aging, accumulation of mitochondrial impairments would lead to a decreased oxidative capacity that could in turn promote intracellular lipid storage. The later is considered by some authors as a major player in the development of IR. Indeed, several studies show that an inhibition of β-oxidation induces DAG accumulation that activates PKC pathway and eventually inhibits insulin signaling (Zhang *et al.*, 2007).

Contrary to these arguments, other authors argue in favor of an elevated β-oxidation in hepatocytes being at the origin of IR and steatosis (Pessayre *et al.*, 2004; Pessayre, 2007). These authors suggest that an adaptive mechanism limits excessive intracellular storage of fatty acids and triglycerides intracellular storage. Indeed, higher fatty acid availability could activate β-oxidation on

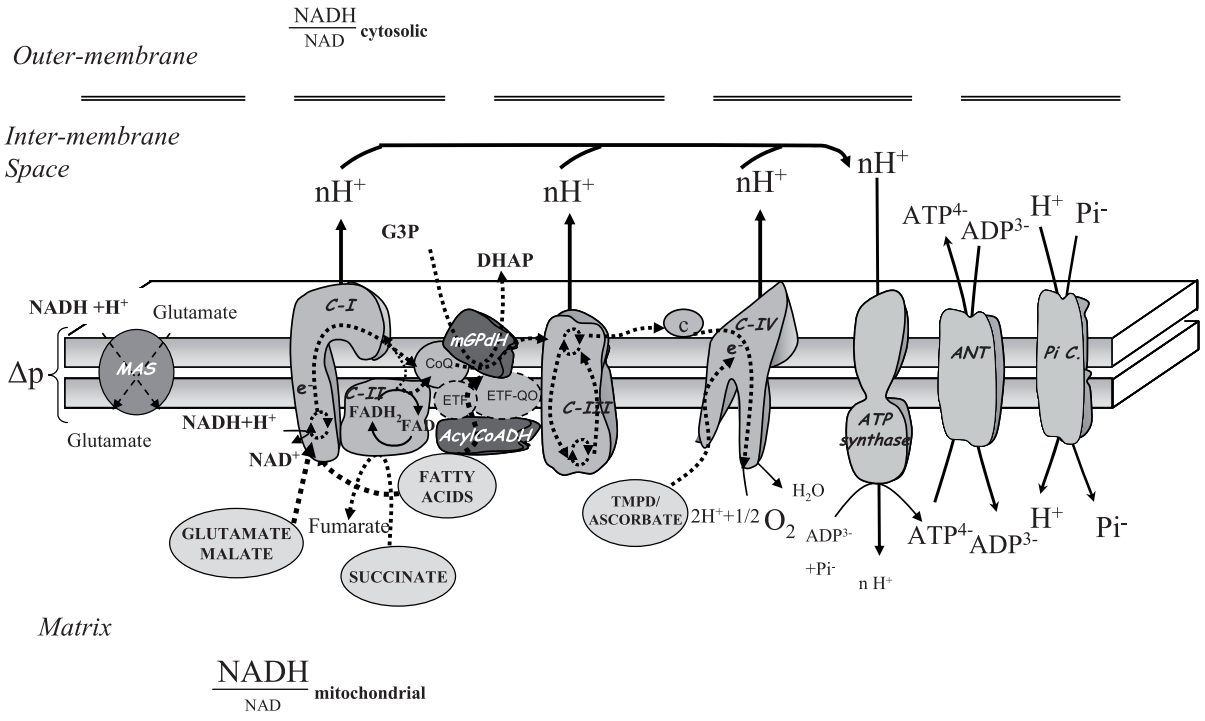

Figure 1. Mitochondrial reducing power translocation and oxidative phosphorylation, the story of membrane potential (Δp)
Oxidative phosphorylation pathway is a serie of successive transduction of potentials from nutrient chemical energy to phosphate potential (ATP/ADP.Pi), which is the energy source for different biological activities. Nutrient oxidation in cytosol and in mitochondria produces reducing equivalents ($NADH,H^+FADH_2$). Specific dehydrogenases (AcylCoAdH, mG3PdH) can transfer their electrons directly to quinone pool, without producing these reducing equivalents. Chemical energy is also converted to membrane potential (Δp) by the respiratory chain, which links redox reaction to proton extrusion from the matrix to the inter-membrane space. The high electrochemical gradient (–180 mV) generated then allows ATP synthesis from ADP and Pi as well as other functions like ion and substrates transport. Because the inner mitochondrial membrane is impermeable to reducing equivalents and adenylic nucleotides, these compounds must be translocated by carrier systems like malate-aspartate shuttle (MAS) and adenine nucleotide translocase (ANT). Due to the electrogenic properties, these exchanges across the mitochondrial membrane depend on electrochemical gradient. The gradient's energy allows the entry of reducing equivalents to the matrix and the exit of ATP to cytosol. Hence, membrane potential controls in part the $NADH/NAD^+$ and ATP/ADP ratios in cytosol and in mitochondrial matrix, managing then redox and phosphate potential, two important factors in cell metabolism. MAS: malate-aspartate shuttle (allowing transfer of $NADH,H^+$ linked to glutamate/asparte and malate/α-ketoglutarate transport), C-I to C-IV: complex 1 to 4. mGPdH: mitochondrial glycerol 3-phosphate dehydrogenase, AcylCoAdH: acylCoA dehydrogenase, ETF/ETF-QO : electron transfer flavoprotein-ubiquinon/oxidoreductase, c: cytochrome *c*, ANT: adenine nucleotide translocator, PiC: phosphate carrier.

the one hand by lowering CPT1 inhibition by malonyl-CoA (Cook & Gamble, 1987) and on the other hand *via* PPAR-α activation. This step would be unstable and a vicious circle could begin due to a high rate of β-oxidation providing large amounts of reduced equivalents ($NADH,H^+$ and $FADH_2$) and electrons to the respiratory chain regardless of the ATP demand. Thus, oxidative phosphorylation would be unbalanced, promoting successively increasing ROS production and mitochondrial and cellular damages. Yet, excessive free radical production would activate stress proteins such as serine-threonine-kinase (Jun kinase and IKKβ) that are able to reduce insulin signal transduction with phosphorylation of serine 307 and serine 312 on IRS proteins. Finally, some authors suggest that IR could be explained only by excess in ROS production (Mollica *et al.*, 2009): it could initiate mitochondrial degeneration and insulin signaling alteration.

However, in a recent study, we demonstrated that changes of matrix and cytosolic redox potentials, associated with an elevated mitochondrial membrane potential, could regulate metabolic pathways control in the IR state (Vial *et al.*, 2010). This phenomenon could be prior to other alterations such as hyperglycaemia that could subsequently damage liver cells. Changes in mitochondrial membrane potential could then affect the nature of oxidative substrate supply and the control *via* the malate-aspartate shuttle. At a high potential, because complex 1 works near equilibrium, mitochondrial NADH is high and the respiration is based on NADH oxidation. When the potential drops, such as in the presence of the uncoupling agent 2,4-dinitrophenol (DNP), the transfer of the NADH reduced equivalents would no longer be possible, and respiration will be slowed down if no other substrate supplies reduced equivalents independently of the malate-aspartate shuttle. If substrates can supply $FADH_2$ or reduced equivalents directly to the quinone pool, the potential would remain high, allowing the transfer of NADH and its subsequent oxidation to be maintained (Leverve & Fontaine, 2001). According to this theory, the mitochondrial membrane potential could then actively regulate the type of substrates oxidized and thus the level of IR.

MITOCHONDRIAL MEMBRANE POTENTIAL AS A SPECIFIC TARGET

With the aim of introducing energy loss and modifying mitochondrial membrane potential, Samuel *et al.* (2004) have demonstrated that administering DNP reduces hepatic steatosis linked to IR and inhibits hepatic glucose production using rats fed a high fat diet for 3 days which is a model known to induce hepatic IR with increases in liver triglycerides and total fatty acyl-CoA content (Kraegen *et al.*, 1991). Suppression of endogenous glucose production by insulin is diminished in these animals and could be attributed to impaired insulin-stimulated IRS-1 and IRS-2 tyrosine phosphorylation. Treatment with a low dose of the mitochondrial uncoupler DNP abrogated the development of fatty liver, hepatic insulin resistance and other associated disturbance.

Accordingly, Brand (2000) has proposed that elevated mitochondrial uncoupling helps to dissipate proton gradient and leads to decreased ROS production. Therefore, uncoupling could reduce oxidative stress and improve mitochondrial function essential to preserve cells. Supporting this theory, Speakman *et al.* (2004) showed that rodents with high muscle mitochondrial uncoupling survived the longest. Considering our second hypothesis based on modifying metabolic control with uncoupling, we have already tested DNP effect on hepatocyte metabolism. Surprisingly, the metabolic consequences of uncoupling oxidative phosphorylation were substrate dependent. Whereas in the presence of carbohydrates as substrate (i.e., ethanol as a cytosolic supplier of reduced equivalents), DNP induces a progressive decline in respiration after a transient burst associated with a collapse in Δp and a fall in ATP-to-ADP ratio. In the presence of octanoate or proline (matrix $FADH_2$ suppliers), the DNP effects on Δp and ATP-to-ADP ratio were minimized but a sustained increase in respiration was observed.

Finally, the use of DNP as a countermeasure of a high mitochondrial membrane potential could appear to be a good means to change substrates' oxidation but is far from being physiological. So the question is how to maintain NADH oxidation with such a high potential? One way to bypass the malate-asparte shuttle in order to maintain NADH oxidation is the G3P-DHAP shuttle. This shuttle enables oxidation of cytosolic NADH by transferring electrons directly to the quinone pool, independently of the membrane potential.

Ultimately, this would allow an increase in fatty-acid oxidation with the limitation that G3PdH is very weakly expressed in the liver. However, the Lou/C rat strain presents an increased liver activity of this enzyme. This is associated with a higher fatty-acid oxidation compared to control animals that could explain its relative resistance to obesity. Interestingly, the high rate of fatty-acid oxidation in Lou/C rat, related to a high mitochondrial glyceraldehyde-3-phosphate dehydrogenase (mG3PdH) activity, appears to be linked to a "metabolic hyperthyroid status" limited to liver, resulting from high thyroid hormone (TH) receptor transcription level (Taleux *et al.*, 2009). Overexpression of TH is effectively well-known to be associated with modified metabolism. At mitochondrial level, TH action is less than clear but recently Mollica *et al.* (2009) have reported spectacular effects of T2 administration on mitochondria isolated from rat fed a high-fat diet. It increases β-oxidation and CPT system activity with a high rate of respiration. This could be explained by an increase in $NADH,H^+$ oxidation to NAD^+ required for β-oxidation and tricarboxylic acid cycle, through a stimulation of proton leak and a decreased membrane potential, strengthening the importance of this latter parameter.

CONCLUSIONS

A series of experiments support the idea that mitochondria play a crucial role in IR. However, several important questions remain to be answered: What is the primary event in IR? Is it the reported inhibition of oxidative phosphorylation or ROS production? What are the upstream reasons? Is the ROS production involved not only through DNA mutation? Are there structural modifications such as reduced quinone pool and/or changes in mitochondrial membrane lipids contents? Does it increase mitochondrial membrane potential due to structural disorders? In other words: "which came first, the chicken or the egg?" In conclusion, it is clear that mitochondrial dysfunction is not so easy to explain. It involves interplay between substrate uptake, storage, and oxidation as well as their efficiency. Additionally, a

balanced interaction of various metabolic pathways within the mitochondrion itself is to be considered. In this scheme, a central point could be alterations of the mitochondrial membrane potential and their consequences. Although these conclusions are clearly relevant to the liver, it should be kept in mind that they do not necessarily apply to other tissues such as muscles. Thus, targeting mitochondria and especially the membrane potential with uncoupling agents or trying to bypass malate-aspartate shuttle dependent pathways by induction of G3PdH, may constitute new therapeutic approaches for treatment of IR.

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