

Nutrient availability links mitochondria, apoptosis, and obesity

Francesca Pintus^{1,2}, Giovanni Floris², and Alessandro Rufini¹

¹ Medical Research Council, Toxicology Unit/University of Leicester, LE1 1QH, Leicester UK

² Dipartimento di Scienze della Vita e dell'Ambiente, Università di Cagliari, 09042 Monserrato, Italy

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Correspondence to: Alessandro Rufini, PhD; **E-mail:** ar230@le.ac.uk

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Abstract: Mitochondria are the dominant source of the cellular energy requirements through oxidative phosphorylation, but they are also central players in apoptosis. Nutrient availability may have been the main evolutionary driving force behind these opposite mitochondrial functions: production of energy to sustain life and release of apoptotic proteins to trigger cell death. Here, we explore the link between nutrients, mitochondria and apoptosis with known and potential implications for age-related decline and metabolic syndromes.

INTRODUCTION

The endosymbiotic theory proposes that mitochondria evolved from an atypical encounter of two prokaryotes that ended up in a symbiotic relationship. One bacterium was phagocytosed but not killed, and became progressively specialized in producing energy through oxidative phosphorylation - it became a mitochondrion. This pivotal event shaped the course of evolution, and was fundamental to the development of eukaryotic cells and metazoa [1, 2]. Progressively mitochondria turned into plastic organelles, specialized in energy production, but they also developed into an efficient cell-killing machine at the core of the apoptotic process. Growing evidence suggests that this mitochondrial apoptotic function is tightly linked to nutrient availability and respiratory efficiency, with important implications for health and aging.

Mitochondria structure

Millions of years of evolution have substantially modified the initial symbiotic relationship and, while mitochondria specialized in energy production, many regulatory functions have been shifted to the hosting cell. The actual human mitochondrial DNA is a circular molecule of 16Kb and encodes a handful of proteins, tRNAs and rRNAs. Only 13 proteins are encoded by

mitochondrial DNA, forming only a small proportion of the components of the electron transport chain (ETC). All the remaining mitochondrial proteins are nuclear encoded [3].

Mitochondria are composed of two highly specialized membranes, the **mitochondrial outer membrane** (MOM) and the **inner membrane** (MIM), which possess different structural and functional characteristics. These membranes define two separate mitochondrial compartments: the **mitochondrial matrix** (MM) and the **intermembrane space** (IMS) [4] (Figure 1).

The MOM is smooth and widely permeable to ions and molecules of up to 10KDa. Proteins localized in the MOM are mainly involved in solute exchange, protein import and fatty acid uptake for β -oxidation.

Conversely, the MIM is impermeable to most solutes and ions, a property that allows complexes of the ETC to build up the proton gradient required for oxidative phosphorylation. Consequently, specific carriers exist to transport individual molecules across this barrier. Common to all mitochondria are the ADP/ATP carrier, which exports ATP into the cytoplasm while ADP is imported into the matrix, the phosphate transporter and the pyruvate carrier. Other systems transport citric acid cycle intermediates that are used by cells in cytosolic

pathways such as fatty acid synthesis and gluconeogenesis. For example, di- and tricarboxylate carriers exchange malate or succinate for HPO_4^{2-} and citrate and isocitrate for malate, respectively. Since fatty acid β -oxidation occurs in the mitochondrial matrix, long-chain fatty acids are actively transported from the cytoplasm into the mitochondria by the carnitine palmitoyl transferase system of the MIM [5, 6]. Moreover, the MIM contains several transport shuttles used to transport the reducing agents NAD(P)(H) and $\text{FAD(H}_2)$ across the membrane. An example is the malate-aspartate shuttle in which the electrons of NADH are given to oxaloacetate forming malate after which malate is transported across the inner membrane and reoxidized back to oxaloacetate by malate dehydrogenase converting NAD^+ to NADH in the mitochondrial matrix (Figure 1).

Two distinct domains can be identified in the MIM: the inner boundary membrane (IBM), tightly attached to the MOM, and the cristae, which extend within the matrix space increasing the membranous surface and which enclose ETC complexes and ATP synthase for oxidative

phosphorylation. ETC complexes are organized into four large multi-subunits. Complex I (or NADH dehydrogenase) transfers two electrons from NADH , produced by the tri-carboxylic acid (TCA)/Krebs's cycle and β -oxidation, to the lipid-soluble carrier ubiquinone coenzyme-Q (CoQ). Complex II contains succinate dehydrogenase as the only membrane-bound enzyme of the TCA cycle; the electrons are driven from succinate to CoQ using FADH_2 as coenzyme. In complex III (CoQ - cytochrome c oxidoreductase) two electrons are removed from reduced CoQ and sequentially transferred to two molecules of cytochrome c. Reduced cytochrome c is then oxidized by complex IV (cytochrome c oxidase) that donates electrons to oxygen as the last step of the electron transfer. Complexes I, III and IV, are protons pumps which link electron transfer to the pumping of protons from the matrix into the intermembrane space in order to generate the proton gradient across the MIM. This gradient is then used backwards by ATP synthase, or F_1F_0 -ATPase to synthesize ATP. This is the major and most efficient source of energy in the cell and, as is well known, the main generator of reactive oxygen species (ROS) [7-9].

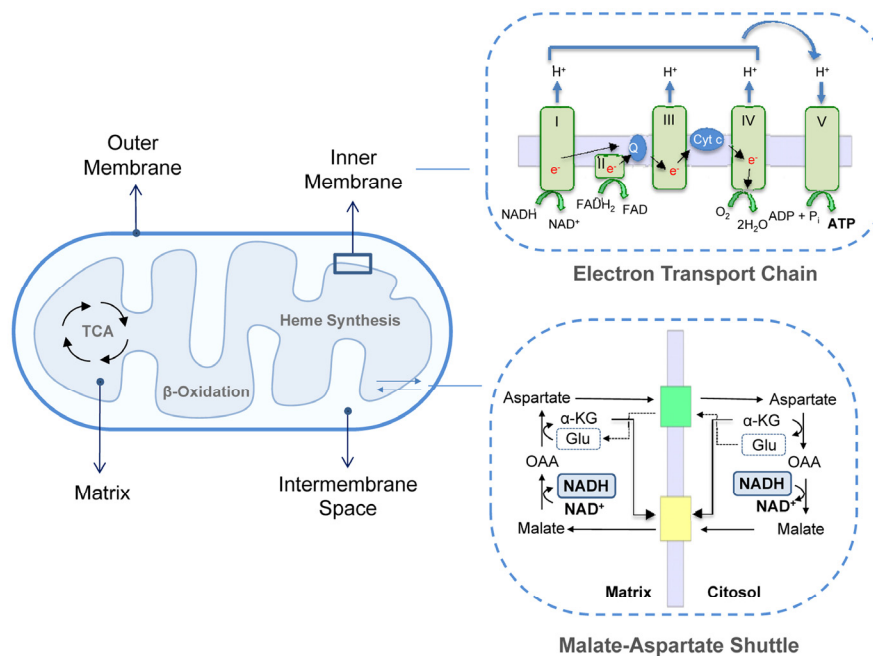


Figure 1. Mitochondrial structure. Mitochondrial membranes delimit the IMS and the matrix. This last compartment hosts the mitochondrial metabolic pathways, such as TCA cycle, β -oxidation and heme synthesis. MIM contains ETC complexes and ATP synthase. Complex I, III and IV extrude protons from the matrix in the IMS creating a proton gradient or mitochondrial membrane potential. The retrograde flux of ions promoted by complex V (ATP Synthase) liberates the energy necessary to phosphorylate ADP to ATP; upper inset. Fundamental for mitochondrial homeostasis and function are several exchange carries, such as the malate-aspartate shuttle in which cytosolic oxaloacetate is reduced to malate in a NADH -dependent reaction and malate is then imported in the mitochondrial matrix and oxidized back to oxaloacetate by malate dehydrogenase with the conversion of NAD^+ to NADH ; lower inset. α -KG, α -ketoglutarate; OAA, Oxaloacetate; Glu, Glutamate, cyt-c, cytochrome c.

The IMS is delimited by the outer and inner membranes and shows a protein composition different from the cytosol and matrix. Its most prominent member is cytochrome c, which has an essential role in the respiratory chain and in the apoptotic signaling pathway since it is released into the cytosol during the apoptotic process [10, 11]. In addition to cytochrome c, other potential apoptotic inducers are present, as well as enzymes such as adenylate kinase and creatine kinase.

The MM is the space enclosed by the inner membrane and includes a large range of enzymes that are part of the major metabolic pathways such as the TCA cycle, lipid and amino acid oxidation and urea and heme biosynthesis.

Cellular mitochondrial content can increase through growth and division of pre-existing organelles, a process known as biogenesis and promoted by the peroxisomal proliferative activated receptor- γ co-activator 1 α (PGC-1 α). PGC-1 α enhances transcription of nuclear encoded mitochondrial genes, causing an increase in mitochondrial number and function [12, 13]. In addition, mitochondria are subject to a plastic process of fusion and fission. Fusion generates complex tubular structures with high respiratory efficiency, whereas fission results in fragmented and less efficient mitochondria [12]. In mammals, but with orthologues in *Drosophila* and yeast, the dynamin-related GTPases mitofusin (Mfn) 1 and 2, and Opa1 are responsible for mitochondrial fusion. Mfn1 and 2 localize to the MOM, whereas Opa1 mediates fusion of the MIM allowing exchange of mitochondrial content. Conversely, dynamin-related protein 1 (Drp1) and fission protein 1 (Fis1) mediate fission: Fis1 localizes to the MOM and serves as an anchor for the recruitment of Drp1, the triggering event of mitochondrial fission [12]. Highly metabolic tissues (such as muscle) show increased fusion, stimulated by PGC-1 α -mediated increase of Mfn2 expression. Repression of Mfn2 decreases oxygen consumption and mitochondrial membrane potential [14-16].

Collectively, this chapter highlights mitochondrial specialization as energy hubs and effectors of cellular catabolism.

Mitochondrial apoptosis

Mitochondria are critical regulators of apoptotic cell death. Apoptosis is a highly conserved and regulated self-suicide mechanism in which there is DNA fragmentation, nuclear condensation and fragmentation into apoptotic bodies [17-23].

There are two main apoptotic pathways: the extrinsic pathway, triggered by cell surface receptor engagement and the intrinsic pathway triggered in response to cellular stress signals and dependent on mitochondria [24]. In the intrinsic pathway, proteins of the Bcl-2-family are crucial determinants of cellular fate. This family comprises antiapoptotic members such as Bcl-2, Bcl-w, Bcl-X_L and Mcl-1 and proapoptotic homologs, such as Bax and Bak or the BH3-only proteins Bid, Bim, Puma, Noxa, Bik and Bmf [25-28]. In response to death stimuli, Bax and Bak oligomerization triggers permeabilization of the MOM, followed by a drop in the membrane potential and release into the cytoplasm of apoptotic effector proteins. The Bax/Bak oligomerization is directly antagonized by antiapoptotic Bcl-2 members, which preserve integrity of the mitochondrial membranes [29, 30]. Finally, BH3-only proteins can displace Bcl-2 proteins from Bax and Bak allowing apoptosis to occur [31, 32]. Perhaps the most relevant protein released from mitochondria during apoptosis is cytochrome c, one of the proteins involved in ETC activity and energy production, which, in the presence of ATP, assembles Apaf-1 and procaspase 9 into the apoptosome. Cleavage and activation of procaspase 9 only occurs, however, when the inhibitory activity of another apoptosome component, IAP, is neutralised by another protein released from mitochondria, Smac/DIABLO [33]. Active caspase 9 is an initiator or apical caspase which in turn activates the executioner caspases 3 and 7 [34]. Briefly, caspases are a family of cysteine proteases that cleave their substrates specifically after aspartic acid residues. All caspases, like caspase 9, exist in cells as catalytically inactive pro-enzymes (zymogens). During apoptosis, initiator caspases activate effector caspases in a cascade of activation in which zymogens are converted into mature enzymes by proteolytic cleavage [35, 36]. Other proteins released during apoptosis include HTRA2, apoptosis inducing factor (AIF) and endonuclease G (endoG), which all play different roles in apoptotic cell death [10, 11, 37, 38].

Mitochondrial dynamics is also altered in apoptotic cells and inhibition of fusion by Bax-mediated repression of Mfn2 seems to be a prerequisite for loss of membrane potential and cytochrome c release. Mitochondria appear fragmented in apoptotic cells and ectopic expression of Mfn2 prevents Bax translocation to the MOM and protects cells from apoptosis [39-42]. Similarly, Drp1 activity is necessary for mitochondrial permeabilization and inhibition of Drp1 increases mitochondrial fusion and prevents release of cytochrome c [28, 40, 43].

In summary, a very complex network has evolved to decide whether a cell should live or die, and mitochondria stand at the heart of this decision. Although perhaps better known for its pathogenic role in some human diseases, such as cancer and neurodegeneration [19, 34, 38, 44-52], apoptosis has important functional roles during development and in the immune response [19]. Strong evidence for this is the lymphopenia, cachexia and nervous system defects affecting mice with a mutation that abolishes the apoptotic function of cytochrome c [53]. Another developmental example is the formation of fingers, a process that involves apoptotic death of cells in the interdigital space.

Forms of apoptosis have been described even in unicellular organisms such as yeast [20, 54], which suggests that regulated cell “suicide” has been an ancient decision in evolution, and probably coevolved with the first endosymbiotic relationship [1, 2]. But while it is easy to understand the function of apoptosis in metazoans, it is more difficult to imagine its role in unicellular organisms: why would a single cell decide to die and evolve a machinery to achieve this? And, above all, why this pivotal role for mitochondria?

One reason may well be the availability of nutrients. When a colony of unicellular organisms is in good equilibrium with the available extracellular energy resources, proliferation and growth are possible. On the other hand, when resources are limiting, cell death has the effect of reducing colony size to a level where it is again in equilibrium with environmental nutrient supply. In this regard, the ability to die may confer an evolutionary advantage and provide a paradoxical mechanism of survival in conditions where nutrients are limiting. Thus, as mitochondria specialized in energy production, they also became the most obvious sensor of nutrient availability and the most obvious candidate to regulate cell death. It is therefore conceivable that nutrient availability may have been the main evolutionary driving force and ancient stressor that has selected mitochondria for the regulation of apoptosis, which would help explain why some apoptosis-related proteins influence mitochondrial respiration [55-57].

Mitochondria, apoptosis and nutrients

As mentioned above, in metazoa apoptosis has acquired additional functions important for body shaping during development and during immune responses [19, 58]. However, there remains a strong link between mitochondria, nutrient supply and apoptosis in multicellular organisms.

It is widely accepted that mitochondria play an important role during aging and, like apoptosis, this relationship is conserved from yeast to primates [59-61]. Many mouse models in which mitochondrial function is compromised show accelerated aging [62-68]. It has been disputed whether ROS, the harmful by-products of ETC activity, are important for this outcome [69]. Indeed, in some cases mitochondrial dysfunction correlates with increased ROS production and oxidative damage [63, 65, 66], although other studies failed to see a similar phenotype [70]. Nonetheless, these studies demonstrate that defects in mitochondrial activity aggravate aging. Caloric restriction (CR: reduced food intake without malnutrition), is regarded as one of the most successful approaches to prolong lifespan. From *C. elegans* to mice and primates, reduced food intake improves survival and delays age-associated decline, compared to ad libitum feeding [71]. CR also reduces accumulation of senescent cells in mice [72]. Several molecular pathways have been implicated in this process, such as dampened IGF/insulin signalling and mTOR kinase activity, increased activation of AMPK and reduced ROS production all of which act on or are consequent to mitochondrial function [73, 74]. CR promotes PGC-1 α activity and enhances expression of Mfn1 and 2, stimulating mitochondrial biogenesis and fusion [75]. Overall, CR potentiates mitochondrial activity, which is pivotal to its anti-aging outcome [76, 77] (Figure 2).

Although ad libitum feeding is standard laboratory practice, it is unlikely to reproduce animals' natural food intake, which is probably nearer to a regimen of CR. In this regard, since mitochondria evolved to coordinate energy production with food availability, their optimum performance coincides with CR, whereas excess of food intake will compromise mitochondrial energetic capacity [78, 79], probably through mTOR and PGC-1 α [74, 75, 77, 80].

Thus, we might envisage a scenario where mitochondria are susceptible to apoptosis as their efficiency of energy production, which is linked to nutritional status, declines. In other words, excess food intake will impair respiratory capacity and prime mitochondria for apoptosis, increasing cellular susceptibility to additional stress. This possibility could also be extended to unicellular organisms, such as yeast [81, 82] and might represent an alternative way to remove cells with inefficient mitochondria when other mechanisms, such as autophagy, are not in place. It is noteworthy that apoptotic proteins levels and cell death are increased in adipocytes of obese humans and rodents, and genetic depletion of the pro-apoptotic BH3-only protein Bid protects from liver steatosis and insulin resistance in

high fat diet regimes [83]. An opposite effect, an increase in anti-apoptotic proteins has been suggested to be neuroprotective in aged caloric-restricted mice [84]. In addition, obese individuals have reduced levels of PGC-1 α and Mfn2 and obesity results in mitochondrial fragmentation, which is known to reduce mitochondrial energetic efficiency and which has been observed in apoptotic cells [85-87].

In summary, the nutritional imbalance in western diets leads to mitochondrial dysfunction and higher susceptibility to apoptosis with dramatic consequences for metabolic syndromes such as insulin resistance and liver steatosis (Figure 2). It is already known that caloric

restriction protects from several stresses [74], and it would be interesting to investigate whether cells isolated from mice on different diets show different susceptibilities to apoptotic cell death via the intrinsic pathway and whether this correlates with the mitochondrial respiratory rate. In particular, adult stem cells could be intriguing candidates for further studies, as they show a particular sensitivity to nutrient availability, and their loss contributes to aging [88-90]. Increased nutrient-mediated susceptibility to cell death may cause stem cell repertoire exhaustion and accelerate aging in obese individuals in a mitochondria-dependent fashion, at least partially explaining the increased apoptotic rates in aged individuals [91-93].

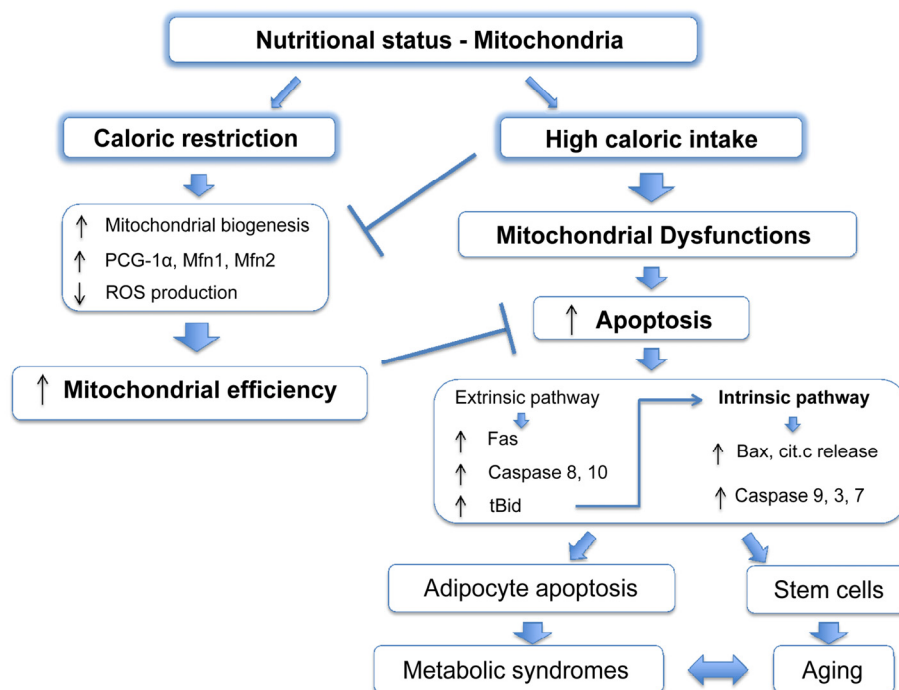


Figure 2. Mitochondrial apoptosis links nutritional status with metabolic syndromes. *Caloric restriction* leads to an improvement of the mitochondrial function stimulating mitochondrial biogenesis and fusion, and increasing ETC efficiency with a decreased production of ROS. *High caloric intake* decreases levels of PGC-1 α and Mfn2 leading to reduced mitochondrial fusion and compromising organelle functions. Moreover, in obesity apoptotic pathways proteins are upregulated and increased apoptosis has been reported in adipocytes. This cell death is strongly dependent on mitochondria as the extrinsic pathway can also activate the intrinsic pathway by caspase-8 mediated cleavage of the BH3-only protein Bid, resulting in the formation of the active truncated isoform tBid. Genetic depletion of Bid protects against obesity-induced metabolic syndrome. Other cell lines, such stem cells, might suffer a similar mitochondrial dysfunction during obesity and become more sensitive to apoptosis, which in turn, will worsen age and age-related metabolic syndromes.

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Conflict of Interest Statement

The authors of this manuscript have no conflict of interests to declare.

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