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Critical Review

Mitochondrial Energy Metabolism and Redox State in Dyslipidemias

Anibal E. Vercesi[1], Roger F. Castilho[1], Alicia J. Kowaltowski[2] and Helena C. F. Oliveira[3]
[1]*Departamento de Patologia Clínica, Faculdade de Ciências Médicas, Universidade Estadual de Campinas, Campinas, Brazil*
[2]*Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo, Brazil*
[3]*Departamento de Fisiologia e Biofísica, Instituto de Biologia, Universidade Estadual de Campinas, Brazil*

Summary

Changes in mitochondrial function are intimately associated with metabolic diseases. Here, we review recent evidence relating alterations in mitochondrial energy metabolism, ion transport and redox state in hypercholesterolemia and hypertriglyceridemia. We focus mainly on changes in mitochondrial respiration, K^+ and Ca^{2+} transport, reactive oxygen species generation and susceptibility to mitochondrial permeability transition.

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Keywords Mitochondria; metabolic syndrome; hyperlipidemia; free radicals; K^+ channel; oxidative stress.

INTRODUCTION

Until recently, the recognized roles of mitochondria included being the site of some metabolic pathways (citric acid cycle, fatty acid beta oxidation, and amino acid oxidation), oxidative phosphorylation, and, exclusively in brown adipose tissue, non-shivering thermogenesis. With more recent work in the area, mitochondria have emerged as a center of attention in many new functions including cell signaling, injury, and death. Evidence has been provided that mitochondria comprise one of the main pathways that lead to cell death by apoptosis in vertebrate cells, since they contain many proteins involved in the regulation of this process (for reviews, see *1*, *2*). Furthermore, cell death occurring secondarily to oxidative stress is often the consequence of increased rates of reactive oxygen species (ROS) production by these organelles (for reviews, see *3*, *4*).

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Address correspondence to: Anibal E. Vercesi, Departamento de Patologia Clínica, Faculdade de Ciências Médicas, Universidade Estadual de Campinas, Campinas, SP 13083-877, Brazil.
E-mail: anibal@unicamp.br

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ROS release by mitochondria is altered by changes in metabolic rates, mitochondrial redox potential, Ca^{2+} levels and the electrochemical membrane potential (*4*, *5*). One of the most effective mechanisms that control mitochondrial ROS production is the uncoupling of respiration from oxidative phosphorylation (*6*). Mild decreases in the inner membrane potential resulting from this uncoupling very significantly reduce mitochondrial ROS production. On the other hand, high levels of Ca^{2+} increase mitochondrial generation of ROS, which seems to be the result of alterations in lipid packing and domain formation in the inner mitochondrial membrane mediated by Ca^{2+} binding to cardiolipin (*7*), leading to mitochondrial oxidative stress (*8*). Oxidative changes in inner membrane proteins lead to a form of non-selective permeabilization of this membrane (*9*) known as the mitochondrial permeability transition (MPT) (*10*). MPT allows the entrance of molecules up to 1.5 kDa and water, causing large amplitude mitochondrial swelling, elimination of the proton electrochemical potential and oxidative phosphorylation (*4*, *8*). Ca^{2+}-induced MPT is enhanced by a variety of conditions or compounds called inducers (for a list see *11*). Most of these inducers enhance Ca^{2+}-induced mitochondrial oxidative stress, such as oxidants of pyridine nucleotides (*12*). Indeed, we observed that mitochondrial Ca^{2+} overload in association with an oxidized state of NADPH causes membrane protein polymerization through thiol crosslinking and MPT (*9*). We proposed (*8*) that these oxidative modifications of membrane proteins are the consequence of a deficient supply of reducing equivalents from NADPH to the antioxidant systems glutathione peroxidase/reductase and thioredoxin peroxidase/reductase that normally remove locally-generated ROS (*4*, *5*). When MPT occurs in a large number of mitochondria, necrotic cell death follows. On the other hand, MPT in a smaller fraction of mitochondria within a cell can lead to apoptosis due to the release of pro-apoptotic factors from this organelle (*13*).

Our laboratories have focused recently on alterations in mitochondrial energy metabolism, redox state and ion

transport in genetic models of dyslipidemias. This paper will describe our main findings and present a brief critical review of potential roles for this organelle in disorders of lipid metabolism.

MITOCHONDRIAL ALTERATIONS IN HYPERCHOLESTEROLEMIA

Low density lipoprotein (LDL) receptor mutations cause familial hypercholesterolemia, a major autossomal dominant disorder associated with increased risk of premature coronary heart disease due to the development of severe atherosclerosis (*14*, *15*), a leading cause of death in the western world. One of the most consistent hypotheses for atherogenesis (*16*) postulates that this disease is triggered by LDL oxidation (*17*) caused by ROS from circulating and vascular wall cells (*18*, *19*). However, it is unclear where and how conditions of oxidative stress are established.

Since mitochondria are the main intracellular sites of ROS generation and are also targets for oxidative damage (*8*), we analyzed oxidative metabolism of these organelles in atherosclerosis-prone, hypercholesterolemic LDL receptor knockout mice (*20*). We observed that liver, heart and brain mitochondria and intact spleen mononuclear cells isolated from the knockout mice presented much higher rates of ROS production than mitochondria from control mice. In contrast to the controls, the knockout mouse mitochondria were not able to sustain a reduced state of matrix NADPH and, in accordance with our earlier findings (*12*), had a higher susceptibility to develop MPT (*20*).

We hypothesized that the lower content of reduced nucleotides in the knockout hepatocytes could be the result of higher rates of lipogenesis, since these cells are deficient in taking up exogenous cholesterol. The lipogenesis/steroidogenesis processes consume large amounts of reducing equivalents from NADPH. In fact, hepatic secretion rates of triglycerides and cholesterol *in vivo* were about twofold higher in the knockout than in control mice. Also, *de novo* synthesis of cholesterol and other lipids measured by the ^3H_2O incorporation techniques were significantly increased in the livers of knockout as compared to control mice (*20*). The biosynthesis of 1 mole of cholesterol oxidizes 24 moles of NADPH (*21*). Therefore, the lower content of reduced nucleotides in knockout mitochondria probably reflected the higher output and/or lower input of reducing equivalents between mitochondria and the cytosol via substrate shuttling mechanisms. Although this may not be the exclusive causative mechanism, it certainly contributes to reduce the NADPH pool in the knockout mitochondria.

Thus, the LDL receptor defect leads to two important proatherogenic effects: increased extracellular levels of oxidizable substrate (LDL) and an imbalance in cell redox processes. The latter phenomenon is responsible for local oxidative stress, triggering lipoprotein oxidation, which in its turn induces mitochondrial damage (*22*, *23*). The resulting vicious cycle leads to cell death and progress of atherogenesis in hypercholesterolemia caused by the lack of LDL receptor (see proposed model in Fig. 1). This mitochondrially-mediated redox imbalance (NADPH oxidation) may be an important step in the pathogenesis of several other diseases that also have increased lipogenesis and hyperlipidemia, such as diabetes, nephrotic syndrome, obesity, and metabolic syndrome.

The main sources of mitochondrial NADPH in animals are isocitrate dehydrogenase (*24*, *25*) and the transmembrane nicotinamide nucleotide transhydrogenase (*26*), which transfers electrons from NADH to $NADP^+$ using the electrochemical gradient as a source of energy. Interestingly, endogenous mitochondrial levels of isocitrate and other citric acid cycle intermediates are significantly lower in LDL receptor knockout mice (Paim, Velho, Castilho, Oliveira and Vercesi, unpublished observations). This is compatible with a chronic enhanced use of isocitrate as a source of NADPH in these mitochondria. Indeed, supplementing mitochondria from LDL receptor knockout mice with isocitrate effectively reverses the lack of NADPH, enhanced occurrence of MPT and augmented ROS release observed (*20*). Given the beneficial effects of isocitrate supplementation *in vitro*, we tested an *in vivo* supplementation of knockout mice with citrate. In fact, we observed a significant improvement in the capacity of the knockout mitochondria to sustain the reduced state of NADPH (Paim, Velho, Castilho, Oliveira and Vercesi, unpublished observations), partially reversing mitochondrial dysfunction in LDL receptor knockout cells.

Within the vascular cell wall, we propose that an enhanced mitochondrially-originated oxidative state may contribute toward lipoprotein oxidation, which, together with the enhanced susceptibility to MPT-mediated cell death, may be a causal effect in the development of atherosclerotic lesions. Furthermore, we believe that enhanced mitochondrial oxidative stress and susceptibility to MPT may be a contributing factor toward ischemia-related tissue lesions occurring in stroke and heart attack in familiar hypercholesterolemia.

Treatment of hypercholesterolemia with statins could reverse these mitochondrial effects, since it decreases intracellular cholesterol synthesis and preserves NADPH levels. However, we found that isolated liver mitochondria from LDL receptor knockout mice treated with lovastatin presented a higher susceptibility to Ca^{2+}-induced MPT than untreated animals (*27*). Indeed, statins induced MPT even in wild-type animals, suggesting this is an effect of the drugs themselves. *In vitro* experiments showed that lovastatin induces MPT in isolated liver and muscle mitochondria, indicating that this effect is unrelated to the ability of statins to reduced cholesterol synthesis (*27*). The ability of statins to induce MPT may explain statin-induced apoptosis observed in cultured cells (*28*, *29*). Statin-induced MPT also may be related to the deleterious side effects of these drugs, including myotoxicity, rhabdomyolysis and liver toxicity (*30*).

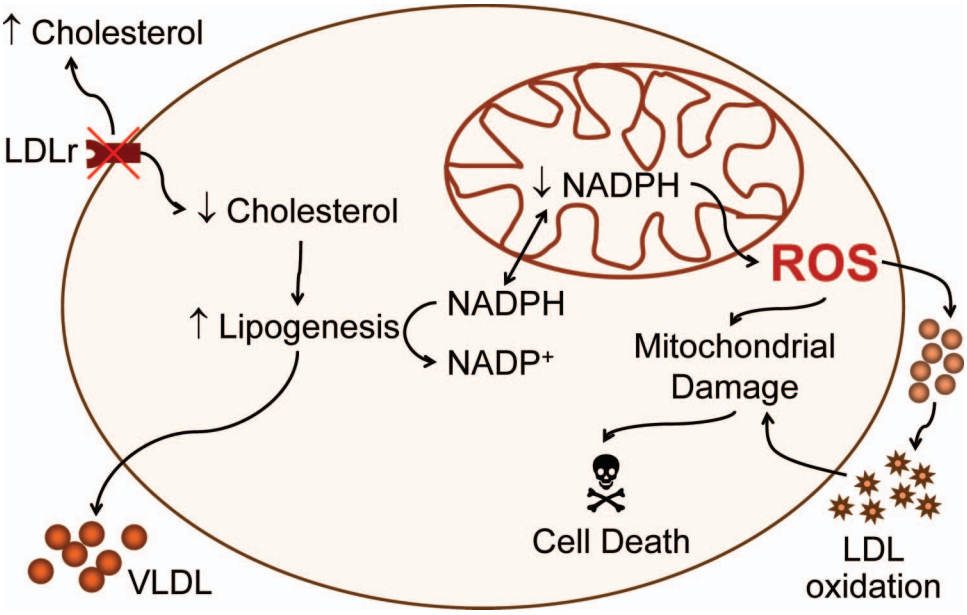

Figure 1. Mitochondrial ROS release is increased in LDL receptor knockout mice. The lack of LDL receptors (LDLr) results in a lack of cholesterol transport into the cell, stimulating intracellular lipogenesis and VLDL secretion, augmenting plasma lipid levels. NADPH is used for lipogenesis, resulting in decreased cytoplasmic and mitochondrial NADPH/$NADP^+$ ratios. Since many ROS removal systems depend on NADPH as a redox source, mitochondrial ROS accumulate and are released at higher levels. Higher ROS release under these conditions may contribute toward tissue oxidative damage, LDL oxidation and atherosclerosis.

MITOCHONDRIAL ALTERATIONS IN HYPERTRIGLYCERIDEMIA

Hypertriglyceridemia and high free fatty acid concentrations are primary genetic disorders or features associated with metabolic syndrome, obesity, diabetes and other pathological states caused by alcoholism, drugs, hormone imbalances, infections, etc. (*31*, *32*). Although less clearly than for hypercholesterolemia, hypertriglyceridemia has been established as an independent risk factor for atherosclerotic heart disease by a number of recent studies, regardless of the presence of other risk factors (*33*). Several animal models are available to study secondary hypertriglyceridemia. However, few models of genetic hypertriglyceridemia have been created. A transgenic mouse that overexpresses human apolipoprotein CIII (*34*), which impairs adequate triglyceride removal from the plasma (*35*), is particularly useful since it does not exhibit altered glucose homeostasis (*36*) or obesity (Salerno, Patrício and Oliveira, unpublished observations).

In mice overexpressing apolipoprotein CIII, we found that mitochondria exhibit enhanced tendencies to undergo MPT (*37*). This effect may be related to enhanced levels of free fatty acids in these animals. Free fatty acids such as arachidonic acid may induce MPT in a manner dependent on their ability to oxidize mitochondrial pyridine nucleotides, promoting mitochondrial oxidative stress (*38*). In addition to presenting enhanced MPT, mitochondria from mice overexpressing apolipoprotein CIII present higher rates of respiration when in the resting (non-phosphorylating) state. This suggests that hypertriglyceridemia promotes mild mitochondrial uncoupling. Indeed, treatment of these animals with fibrates, which reduce lipid levels, reverses this effect (*37*).

Many metabolic diseases have been associated with changes in expression levels of mitochondrial uncoupling proteins (UCP), an endogenous mild uncoupling pathway (*39*). Interestingly, UCP expression is altered by obesity and diabetes (*40*, *41*) and in some conditions where circulating lipid levels are modified by hormones (*42*), dietary fat (*43*), and intravenous heparin plus lipid infusion (*44*). In an attempt to determine the mechanism through which hypertriglyceridemia enhances mitochondrial respiration, we measured the expression levels of uncoupling proteins in mitochondria from these mice, but found no changes. Furthermore, these higher respiratory rates were maintained in the presence of bovine serum albumin, carboxyatractyloside and GDP (*37*), indicating the uncoupling could not be attributable to anion transporters such as uncoupling proteins or the adenine nucleotide carrier.

An independent pathway that promotes mild mitochondrial uncoupling recently described in mitochondria is the concomitant activity of ATP-sensitive K^+ channels (mito-K_{ATP}, which allow K^+ to enter mitochondria) and K^+/H^+ exchangers (which transport K^+ out and H^+ into the matrix, Fig. 2) (*45*, *46*). Indeed, we found that enhanced mitochondrial

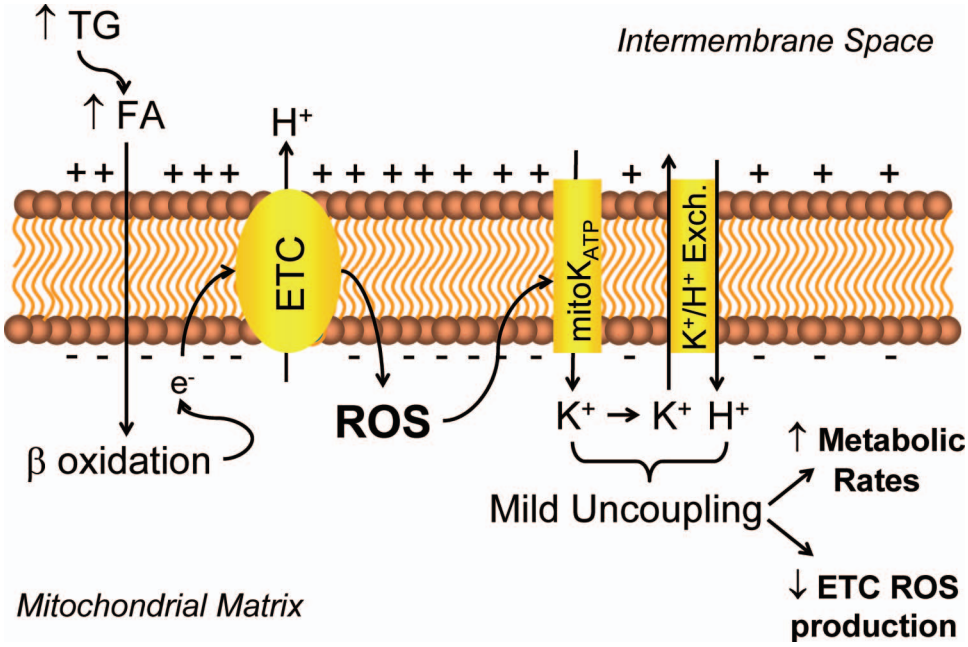

Figure 2. MitoK_{ATP} channels are activated in hypertriglyceridemia. Enhanced circulating triglycerides (TG) and fatty acids (FA) may lead to augmented mitochondrial β oxidation, which promotes high levels of ROS generation by the electron transport chain (ETC). Higher ROS levels activate mitoK_{ATP} channels, resulting in enhanced K^+ uptake into the matrix and exchange for H^+ by the K^+/H^+-exchanger. As a result, mild mitochondrial uncoupling occurs. The two main consequences of mild mitochondrial uncoupling are enhanced metabolic rates and the prevention of further ROS generation at the ETC.

K^+ cycling occurred in hypertriglyceridemic mice (*47*). Interestingly, the higher mitochondrial respiratory rates induced by mitoK_{ATP} activation in hypertriglyceridemia were reflected also at the tissue and animal levels (*47*). This suggests that the activity of mitoK_{ATP} induces a hypermetabolic state in hyperlipidemic animals, which may compensate at least partially for their higher levels of circulating lipids. This enhanced metabolic state promoted by mitoK_{ATP} may explain why these mice gain little weight and present low efficiency of ingested food conversion (*47*). Our results may also contribute to explain the higher energy expenditure and lower net efficiency for energy gain in rats submitted to diets rich in lipids (*48*, *49*).

The reasons for mitoK_{ATP} activation in hyperlipidemia are still undetermined. We hypothesize that the enhanced activity of this channel may be related to augmented levels of ROS in hypertriglyceridemia (see Fig. 2; *50*). MitoK_{ATP} is a redox-sensitive channel (*51*), and presents significant increases in activity when mitochondrial ROS release levels are enhanced (*52*). We are currently investigating if broad-spectrum antioxidants reverse the activation of mitoK_{ATP} in apolipoprotein CIII-overexpressing animals. Interestingly, because mitoK_{ATP} activity decreases ROS release by promoting mild mitochondrial uncoupling (*52*), this channel may contribute toward preventing oxidative tissue damage in this dyslipidemia.

CONCLUDING REMARKS

Given their central role in energy metabolism, it is not surprising that changes in mitochondrial function are associated with metabolic diseases such as dyslipidemias. Indeed, the role of mitochondria in these disorders adds to the numerous roles of these organelles in cell pathophysiology currently under investigation. We hope that a more detailed understanding of changes in mitochondrial energy metabolism, redox state and ion transport that occur in dyslipidemias will contribute toward a more thorough comprehension of these pathologies, pointing toward mechanisms in which they can be more effectively controlled.

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