Mitochondrial Dynamics in the Regulation of Nutrient Utilization and Energy Expenditure

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Introduction

This review investigates the role nutrition (over and under nutrition) affect mitochondrial behavior.

Conclusions

Mitochondria fragment under conditions of over nutrition; and, when capable, will increase their energy wasting capacity by reducing the coupling of the electron transport chain via the production of uncoupling protein.

Mitochondria elongate, protecting against mitophagy (autophagy of mitochondria), and will couple the electron transport chain under conditions of under nutrition.

Amendments

Cell Metabolism
Review

Mitochondrial Dynamics in the Regulation of Nutrient Utilization and Energy Expenditure

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Mitochondrial fusion, fission, and mitophagy form an essential axis of mitochondrial quality control. However, quality control might not be the only task carried out by mitochondrial dynamics. Recent studies link mitochondrial dynamics to the balance between energy demand and nutrient supply, suggesting changes in mitochondrial architecture as a mechanism for bioenergetic adaptation to metabolic demands. By favoring either connected or fragmented architectures, mitochondrial dynamics regulates bioenergetic efficiency and energy expenditure. Placement of bioenergetic adaptation and quality control as competing tasks of mitochondrial dynamics might provide a new mechanism, linking excess nutrient environment to progressive mitochondrial dysfunction, common to age-related diseases.

Intro

Introduction

As our relationship with mitochondria evolves, we remain fascinated with the impact of this organelle in two seemingly unrelated conditions: aging and metabolic diseases. While aging involves insufficiency of mitochondrial quality control and turnover mechanisms (such as autophagy), type 2 diabetes and obesity are influenced by the ability of the organism to deal with excess nutrient environment. The observation that both conditions are impacted by the duration of exposure to excess nutrient environment raises the question, are the tasks of handling nutrients in excess and maintaining quality control ever in conflict? In this review, we discuss evidence to support a hypothesis that adaptation to excess nutrient environment interferes with quality control functions and, as a result, affects mitochondrial function in a magnitude that reflects the duration to which the organism was exposed to excess nutrient environment.

In response to changes in energy demand and supply, the organism adapts by adjusting both its capacity and/or efficiency of ATP production. *Mitochondrial bioenergetic efficiency* is defined as the ATP produced in the mitochondria per molecule of nutrient (Figure 1), and *mitochondrial ATP synthesis capacity* is defined as the rate at which ATP is produced per unit of time.

As an adaptation to excess nutrients, the organism recruits mechanisms to utilize nutrients first by storage and then by waste (heat generation). While spending time at the gym may be the appropriate way to waste energy and keep healthy, reducing bioenergetic efficiency might enable energy waste in tissues other than muscle and in individuals that are less compatible with the gym.

Studies in the field of mitochondrial dynamics have identified an intriguing link between energy demand and supply balance and mitochondrial architecture. Cells exposed to a rich-nutrient environment tend to keep their mitochondria in a separated (fragmented) state, and mitochondria in cells under starvation tend to remain for a longer duration in the connected (elongated) state (Molina et al., 2009; Gomes et al., 2011). Thus, it appears that bioenergetic adaptation that involves changes to bioenergetic efficiency and mitochondrial ATP synthesis capacity also implies remodeling of mitochondrial architecture.

However, bioenergetic adaptation is not the only mitochondrial task that involves changes to mitochondrial architecture. A vital task that engages the fusion and fission machinery is the mitochondrial life cycle (Twig et al., 2008a). The mitochondrial life cycle represents continuous changes to mitochondrial architecture through fusion and fission events. These brief transitions between connected and separated mitochondria enable the reorganization of mitochondrial components and the elimination of damaged material, thereby maintaining a healthy mitochondrial population. One can appreciate that the life cycle of mitochondria would be compromised if mitochondrial fusion or fission were disabled to allow for bioenergetic adaptation. Therefore, under certain nutrient environments, bioenergetic adaptation and quality control might represent conflicting tasks.

That mitochondrial quality control has evolved within the same mechanism that controls for bioenergetic efficiency is not surprising, given the understanding that a low-nutrient environment (caloric restriction) may support increased longevity.

Adaptation of bioenergetic efficiency and ATP synthesis capacity to nutrient availability differs among tissues and is intimately linked to their specific physiology. Thus, we will focus on three paradigmatic tissues that show different bioenergetic efficiencies and mechanisms of adaptation to nutrient availability:

(1) Brown adipose tissue: When stimulated, brown adipocytes can go through an acute and robust transition from high to low bioenergetic efficiency. Under these stimulatory conditions, energy obtained from mitochondrial nutrient oxidation is almost entirely directed toward heat production rather than ATP synthesis (reviewed in Cannon and Nedergaard, 2004).
(2) Muscle: Muscle cells harbor higher bioenergetic efficiency as compared to either beta cells (Affourtit and Brand, 2006) or stimulated brown fat. In the contracting red muscle, nutrient oxidation is primarily directed

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Intro: Aging involves insufficient mitochondrial quality controls, like autophagy. This review covers the effect of nutrient overload and mitochondrial function.

In response to nutrient overload, the cell adapts by changing the capacity (amount of mitochondria) and efficiency (ability to produce) of cell energy production. Bioenergetic efficiency is defined as the amount of ATP produced per molecule of nutrient. The cells respond to nutrient overload by creating added nutrient storage, then further adapt by wasting energy.

Cells exposed to nutrient rich environments experience changes in mitochondrial shape and structure - they tend to have fragmented mitochondria, while cells under starvation conditions tend to stay elongated.

Bioenergetic (cell energy) ability is not the only driver of changes in mitochondria architecture. Mitochondria are constantly undergoing fusion (combining two mitochondria) and fission (one mitochondrion splitting apart). They undergo these processes to separate out damaged sections of themselves, so sometimes the bioenergetic need for fusion or fission is at odds with the need to separate out or dilute damaged section of mitochondria

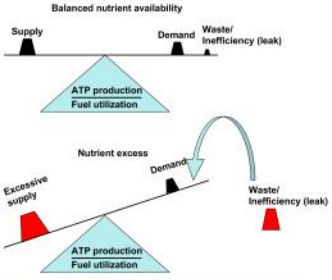

Figure 1. Regulation of Cellular Bioenergetic Efficiency under Conditions of Nutrient Excess

In the balanced state fuel/nutrient "supply" is sufficient to sustain energy (ATP) "demand." Under this condition, "waste" or inefficiency in the form of heat is minor. Nutrient excess, characterized by "excessive supply" in the absence of a parallel increase in "demand," represents a situation in which the energy required to satisfy ATP demand is lower than the available energy. This is compensated for by addition of an energy sink that does not involve ATP synthesis. This component is inefficiency/waste in the form of heat. The major mechanism for inefficiency/waste in the form of heat is mitochondrial proton "leak." This mechanism can slow down nutrient accumulation and prevent the development of reductive stress (accumulation of NADH) and ROS production.

towards production of ATP in the mitochondria (Chappell and Perry, 1954) to support contraction. Thus, the oxidative muscle is a good example of high mitochondrial ATP synthesis capacity and likely high bioenergetic efficiency (Marcinek et al., 2004).

(3) Beta cells: Mitochondria in pancreatic beta cells serve as nutrient sensors and signal generators for insulin secretion. Nutrients are "sensed" through their metabolism, which involves nutrient oxidation mediated by beta cell mitochondria (Ashcroft et al., 1984; reviewed in Deeney et al., 2000). Therefore, bioenergetic efficiency is expected to be highly regulated to allow proper insulin secretion.

Although the mechanisms for tissue-specific differences in bioenergetic efficiency are understood to a certain extent, less is known about the contribution of mitochondrial dynamics to tissue and diet-dependent bioenergetic efficiency and mitochondrial ATP synthesis capacity. Mitochondrial dynamics is a concept that comprises mitochondrial architecture resulting from movement, tethering, fusion, and fission events. Multiple evidences demonstrate that mitochondrial dynamics are important for cell viability, senescence, mitochondria health, bioenergetic function, quality control, and intracellular signaling (reviewed in Liesa et al., 2009; reviewed in Twig et al., 2008b). On the other hand, we are now beginning to understand how nutrients and the cellular metabolic state are regulating mitochondrial dynamics in different tissues and vice versa, particularly in the beta cell, brown adipose tissue, and muscle (Molina et al., 2009; Quirós et al., 2012; Sebastián et al., 2012). Along with this, the relevance of mitochondrial dynamics in the specific physiology of different tissues has only been revealed recently, mostly thanks to different mouse models harboring tissue-specific deletions of core components regulating mitochondrial dynamics (Chen et al., 2007, 2010; Chen et al., 2011; Ishihara et al., 2009; Sebastián et al., 2012; Wakabayashi et al., 2009; Zhang et al., 2011).

In this context, the aim of this review is to summarize the current understanding of mitochondrial bioenergetic function and efficiency regulation by nutrient availability and energy demand in health and disease. We will discuss how mitochondrial dynamics may be required for proper adaptation to the diverse bioenergetic requirements. In the last section, we will provide a model in which adaptation to sustained exposure to nutrient excess results in prolonged changes to mitochondrial dynamics. These changes can impact mitochondrial quality control and thereby contribute to the mitochondrial dysfunction characteristic of metabolic and other age-related diseases.

Regulation of Cellular Bioenergetics by Nutrients

How Can Bioenergetic Efficiency Affect Cellular Functionality and Viability?

Intuitively, it is expected that conditions of limited nutrient availability will increase the ratio of ATP produced per nutrient consumed, thereby reducing and optimizing the consumption of nutrients. Mechanisms to increase energy efficiency are expected to diverse between tissues that are primarily relying on "anaerobic" glycolysis and those that are relying primarily on oxidative metabolism for the production of ATP.

In this regard, recent studies performed in transformed cell lines demonstrate that starvation increases mitochondrial ATP synthesis capacity (ATP production per unit of time). This increase involves the formation of ATP synthase dimers at the cristae curvatures, which show higher activity (Gomes et al., 2011). This result may represent a shift from "anaerobic" glycolysis (to lactate) toward mitochondrial respiration under starvation, as respiration can produce more ATP per molecule of glucose. In oxidative cell types, one would also expect the activation of mechanisms that increase mitochondrial bioenergetic efficiency to ensure survival under limited availability of nutrients. Mechanisms enhancing mitochondrial bioenergetic efficiency have not been described in detail under these conditions. On the other hand, increased mitochondrial ATP synthesis capacity reported in transformed cell lines (Gomes et al., 2011) was associated with and dependent on changes in mitochondrial dynamics, which were presented as decreased fission rates and mitochondrial elongation. This change in dynamics suggests that elongation could be an active mechanism contributing to increased mitochondrial bioenergetic efficiency.

Decreased bioenergetic efficiency refers to the diversion of the energy obtained from nutrient oxidation toward heat production, most commonly by increased uncoupled respiration. Decreased bioenergetic efficiency may serve as a protective mechanism from the detrimental effects associated with nutrient overload. This is achieved through the reduction of reactive oxygen species (ROS) production and by the enhanced removal of excess nutrients and their potentially cytotoxic metabolites (Figure 1).

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Bioenergetic (cell energy) ability is not the only driver of changes in mitochondria architecture. Mitochondria are constantly undergoing fusion (combining two mitochondria) and fission (one mitochondrion splitting apart). They undergo these processes to separate out damaged sections of themselves, so sometimes the bioenergetic need for fusion or fission is at odds with the need to separate out or dilute damaged section of mitochondria.

Three tissues that respond differently to nutrient excess:

1. Brown Fat Tissue: These cells undergo significant changes in their bioenergetic efficiency (from high to low, with increased nutrient availability). In a low efficiency state, nutrient oxidation (utilization) is used for heat generation (wasting energy), not ATP synthesis.

2. Muscle Tissue: These cells have high bioenergetic efficiency (producing substantial energy with each nutrient molecule) - presumably to support contraction of the muscle cells.

3. Pancreatic Tissue (specifically, insulin producing beta cells): Mitochondrial oxidation of nutrients plays a critical role in the tight control of insulin secretion, so it is not going to swing heavily - rather, it is going to be defined to a narrow range of efficiency.

More is being learned on the topic thanks to animal models with specific gene deletions for fusion and fission proteins in mitochondria.

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The flow of electron-mediated proton translocation in the respiratory chain can be compared to a flow of water in a garden hose (see the "Understanding Mechanisms of Bioenergetic Efficiency and Changes in ATP Synthesis Capacity by Respiration Studies" section for a more detailed bioenergetics description). NADH, resulting from nutrient oxidation, feeds the hose inlet with water, while ATP synthase controls the hose final outlet. The pressure that the flow of water generates in the hose is the mitochondrial membrane potential ($\Delta\psi_m$). The flow of water and pressure in the hose are determined by the rates of NADH production and ATP synthesis. The minimum and maximum values of pressure that the hose can hold are determined by the material and integrity of the hose, not by the flow of water or the inlets and outlets (i.e., the range of $\Delta\psi_m$ in mV is determined by thermodynamics and the integrity of the organelle). ATP synthesis is determined by ATP demand, meaning that the hose outlet is controlled by ATP demand. If the hose could hold unlimited pressure, we would not have to be concerned with any parameter beyond ATP demand. However, this is not the case. The hose, as it turns out, has some cuts through which water can escape, when pressure builds up. A pressure valve that can divert excess water through a safe conduit can reduce the pressure in the hose and prevent water leakage through the "cuts" in the hose (increasing or maintaining the flow of water). In our analogy, the escape of water through the cuts represents the escape of electrons to produce ROS. The pressure valve represents the combination of inducible and inherent uncoupled respiration, the latter being caused by the inherent proton leak of the inner membrane. Inducible uncoupling can include uncoupling protein 1 (UCP1) activation in brown fat and the permeability transition pore opening. Inherent, nonactivated, proton leak is directly (but nonlinearly) correlated to the membrane potential and is mediated, in part, by inner membrane proteins (such as adenine nucleotide translocator or nonactivated UCP1 in brown fat) (Parker et al., 2009). The balance between ATP demand and nutrient supply determines both the rate of ATP synthesis and the level of ROS produced by mitochondria.

Different tissues employ different mechanisms in their response to nutrient overload. The selection of specific compensatory mechanisms allows each tissue to maintain its unique primary function, while minimizing side effects related to ROS production. In certain cell types, compensatory mechanisms are placed upstream of the mitochondria, preventing their exposure to high levels of fuel. However, in beta cells, brown adipose tissue, and muscle, mounting evidence suggests that conditions of nutrient excess that increase fuel availability to the mitochondria might modulate bioenergetic efficiency and mitochondrial ATP synthesis capacity (Koves et al., 2008; Bonnard et al., 2008; Rothwell and Stock, 1979; Wikstrom et al., 2007).

Understanding Mechanisms of Bioenergetic Efficiency and Changes in ATP Synthesis Capacity by Respiration Studies

Mitochondria from any tissue can provide energy in the form of ATP as a result of nutrient oxidation (Chance and Williams, 1955; Mitchell, 1961). Oxidation of nutrients will provide electrons to the mitochondrial electron transport chain (constituted by four complexes) in the form of NADH and $FADH_2$. The sequential transport of electrons from complex I or II to III and IV extrudes protons from the matrix to the intermembrane space, generating an electrochemical gradient ($\Delta\mu_H^+$) resulting in a difference in charge ($\Delta\psi$) and in proton concentration (ΔpH). The mitochondrial membrane potential ($\Delta\psi_m$) is the main contributor to $\Delta\mu_H^+$ (reviewed in Nicholls and Ferguson, 2002). In intact mitochondria, maximal and minimal $\Delta\psi_m$ values are around 225 and 90 mV, respectively. This range in mV is dictated by the thermodynamic stability of functional mitochondria and represents the balance between proton extrusion and re-entry. Energy from proton re-entry through complex V is used for the synthesis of ATP from ADP. The state at which isolated mitochondria are synthesizing ATP at maximal rates is named state 3 (Chance and Williams, 1955), and it occurs at intermediate $\Delta\psi_m$ values (~140 mV). As such, this state is characterized by a high rate of both proton extrusion and re-entry (reviewed in Nicholls and Ferguson, 2002).

Proton re-entry through mechanisms that do not involve complex V and ATP synthesis are referred to as uncoupled respiration. Uncoupled respiration results in the generation of heat and is not controlled by ATP turnover (reviewed in Nicholls and Ferguson, 2002). It is important to distinguish between two different types of respiratory states resulting from uncoupling. These two respiratory states determined in isolated mitochondria show major functional differences and might mimic respiratory states under different physiological conditions in vivo:

(1) Respiration controlled by inherent proton leak. This is typically measured in vitro, in isolated mitochondria in which ATP synthesis has been inhibited either by ADP exhaustion (state 4) or by the use of complex V inhibitor oligomycin. It is also referred to as respiration controlled by basal proton conductance (Parker et al., 2009) and can mimic physiological conditions of decreased mitochondrial ATP demand and high nutrient availability.

(2) Respiration controlled by inducible uncouplers. This type of uncoupled respiration can be experimentally mimicked by the addition of chemical compounds, such as FCCP, or by activation of endogenous uncoupling proteins/molecules located in the inner mitochondrial membrane, such as UCP1. The activation of these endogenous uncouplers takes the control of respiration from ATP synthesis. Under these conditions, respiration is controlled by the capacity of the respiratory chain and by the availability of mitochondrial fuels. This type of respiration is also characterized by decreases $\Delta\psi_m$ values, due to increased proton re-entry. It is also referred to as inducible proton conductance (Parker et al., 2009).

A key difference between these two types of uncoupled respiration is the membrane potential at which they are conducted. Mitochondrial respiration controlled by inherent proton leak, which occurs in coupled mitochondria under conditions of low ATP synthesis and high nutrient availability, is associated with higher $\Delta\psi_m$ values and maintains low oxygen consumption rates. The high $\Delta\psi_m$ values result from a combination of decreased rates of proton re-entry through ATP synthase and low values of proton conductance contributed by the inherent proton leak. The combination of these effects maintains $\Delta\psi_m$ values within the range dictated by thermodynamic stability of intact mitochondria. This state is associated with relatively higher ROS generation, as a consequence of the increase in $\Delta\psi_m$.

1. Starvation conditions for the cells leads to increases in ATP (cell energy) production per second/minute, etc. (mitochondria generate energy more rapidly). This also leads to more ATP synthase complexes grouping together and increased activity of these ATP synthases. This might be a result of a switch from anaerobic glycolysis (oxygen independent energy generation) to oxidative phosphorylation (oxygen dependent energy generation). The mitochondria also would experience an increase in efficiency (getting the most energy per molecule of nutrient), which is dependent on if the mitochondria can fuse (fusion).

   Decreased mitochondrial efficiency is present when there is nutrient overload and mitochondria switch to producing more heat instead of energy (ATP) - this is accomplished through uncoupling the mitochondria through the production of more Uncoupling Protein (UCP).

In marked contrast, mitochondria treated with uncouplers (such as FCCP) have decreased $\Delta\psi$ value, which causes an increase in respiration rates to values higher or close to state 3. The concomitant increase in respiration maintains $\Delta\psi_m$ values within the range of thermodynamic stability (~90–120 mV). In this case, absolute values of calories from nutrients used for heat generation will be higher in uncoupler-induced respiration compared to inherent proton leak controlled respiration. Therefore, respiration that is activated by uncouplers is characterized by decreased bioenergetic efficiency and lower mitochondrial ATP synthesis capacity, as it drives nutrient oxidation toward heat generation. Furthermore, it is associated with lower ROS production, as $\Delta\psi_m$ values are reduced. The description of these basic differences between the two types of uncoupled respiration is relevant to understanding the physiological consequences of nutrient-mediated changes in respiration rates, $\Delta\psi_m$, and mitochondrial dynamics described in the "Relationship between Bioenergetic Efficiency and Mitochondrial Dynamics" section.

Nutrient Availability Control of Mitochondrial Respiration

Mitochondrial respiration is controlled by three different processes: (1) ATP turnover, determined by cellular ATP consumption and matrix ADP levels; (2) substrate utilization, determined by fuel availability inside the mitochondrial matrix and its oxidation to generate NADH, $FADH_2$; and (3) proton leak, determined by the inherent permeability of the inner membrane to protons. Understanding the contribution of each of these processes is essential to predict under which physiological and mitochondrial respiratory states, nutrient availability will be determining mitochondrial respiration and $\Delta\psi_m$.

In isolated mitochondria under state 3, where maximal ATP synthesis rates are induced, both nutrient utilization and ATP turnover exert a similar control over respiration and thus over $\Delta\psi_m$. This control can be quantified as the control coefficient over the mitochondrial respiratory flux. A value of 1 for this coefficient represents an absolute control of a process over respiration. Under state 3, ATP turnover was found to have a control coefficient value of >0.5, while nutrient utilization has a control coefficient of <0.4. (see Hafner et al., 1990). This finding in isolated mitochondria supports the idea that ATP demand has the main control over the rate of mitochondrial respiration in intact cells under physiological conditions, while mitochondrial nutrient availability and the inherent proton leak have relative lower control over respiration rates.

However, in an intact cell, the metabolic processes providing $NADH/FADH_2$ to the mitochondrial matrix, including glycolysis, fatty acid oxidation, and TCA cycle, can control respiration with a flux control coefficient over respiration between 0.15–0.3 under resting conditions (reviewed in Nicholls and Ferguson, 2002; Hafner et al., 1990). Therefore, although ATP turnover has a major influence controlling respiration and membrane potential (control coefficient value 0.5), under conditions of high ATP demand, nutrient utilization and its availability can still have a significant control over respiration and the exact mitochondrial $\Delta\psi$ values in intact cells. Furthermore, nutrient availability will have even a greater control over respiration after the induction of uncoupling with either pharmacological uncouplers or by stimulation of uncoupling mechanisms such as UCP1 in intact cells and in isolated mitochondria, as ATP turnover will have a reduced control over mitochondrial respiration under these conditions. Overall, the fact that the control coefficient of each process over mitochondrial respiratory flux can vary suggests that under certain physiological scenarios mitochondrial nutrient availability may control the mitochondrial $\Delta\psi$ (within the range dictated by thermodynamics; around 90–225 mV).

Of particular relevance for this review, in certain cell types, including nutrient sensors such as the beta cell, nutrient availability has a higher flux control coefficient and greater control over mitochondrial respiration and membrane potential than in other cell types (i.e., muscle cells). Consistent with this, recent evidence confirmed previous findings that mitochondrial hyperpolarization is proportional to the increase in extracellular nutrient concentration (glucose and pyruvate) in beta cell line (Goehring et al., 2012; Wikstrom et al., 2007; Danial et al., 2008; Heart et al., 2006).

Furthermore, uncoupling protein 1 in the brown adipocyte demonstrates a system where proteins determining basal proton conductance and thereby mitochondrial respiration can be activated by nutrients per se (Rial et al., 1983; Parker et al., 2009; Shabalina et al., 2008). That brown adipose tissue evolved to utilize fatty acids as a signal for nutrient wasting brings up a potential general concept that nutrients with high caloric content can activate thermogenesis and exert important control over respiration per se, or even increase their own oxidation. This mechanism could promote "nutrient wasting" in the form of heat generation under conditions of increased nutrient supply (Figure 1). Such regulatory pathways decreasing bioenergetic efficiency could exist in other tissues, but likely through other mediators and/or regulators. These regulatory pathways are expected to be relevant in nutrient sensors, which harbor high nutrient permeability. These mechanisms could involve and/or require changes in mitochondrial dynamics, as discussed in the "Relationship between Bioenergetic Efficiency and Mitochondrial Dynamics" section.

In this regard, obesity and diabetes research have put forward mitochondrial "nutrient wasting" in the form of heat as an important concept in metabolic adaptation. This concept is based on the rational that inducing thermogenesis through increased mitochondrial nutrient oxidation in certain tissues, including muscle, brown adipose tissue, or beige adipocytes, could potentially compensate for the deregulated energetic balance associated with nutrient excess (Levine et al., 1999; Schutz et al., 1984; Wu et al., 2012). Consequently, understanding how this mitochondrial "nutrient wasting" process is regulated in all cell types and in a tissue-specific manner might prove useful for the treatment of conditions associated with excess nutrients.

Cells that should be particularly susceptible to nutrient supply and demand imbalance are those allowing nutrient permeability regardless of their energy demand. Such cells are the nutrient sensors, the regulators and the storage organs: the beta cells, the hepatocytes, and the adipocytes. In the case of white adipocytes, high nutrient permeability allows for storage of nutrients in the form of triacylglicerides. However, in the nutrient sensors (e.g., beta cells), nutrient oxidation and ATP/ADP ratio serve as a sensing mechanism and a signal generator for insulin secretion. This ability of the beta cell to

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2. Mitochondrial respiration (use of oxygen as a necessary component of energy consumption) is controlled by the consumption of ATP (cellular ATP consumption), the amount of NADH and FADH available (coming from the TCA cycle), and the proton leak (the permeability of the inner mitochondrial membrane to allow protons from the intermembrane space to the matrix). ATP turnover thereby also plays a huge role in mitochondrial membrane potential.

control and modulate its mitochondrial bioenergetics according to nutrient supply is essential to maintain its function in nutrient stimulated insulin secretion. It might also play a role in maintaining beta cell viability through the removal of excess nutrients that if left to accumulate may have a toxic effect (reviewed in Prentki et al., 2002; reviewed in Muoio and Newgard, 2006).

In the "Relationship between Bioenergetic Efficiency and Mitochondrial Dynamics" section, we will discuss evidence for the role of mitochondrial dynamics and morphology in regulating energy efficiency and nutrient wasting.

Effects of Nutrient Excess on Mitochondrial Bioenergetics in Brown Adipose Tissue, Muscle, and the Beta Cell

Brown Adipose Tissue. Mitochondria from brown adipose tissue harbor UCP1, activation of which generates heat through dissipation of mitochondrial membrane potential and increased respiratory rates (Aquila et al., 1985; Heaton et al., 1978; Nicholls, 1974; Nicholls et al., 1978). UCP1 is used as a specific marker to detect brown adipocytes within other tissues. The brown adipocyte represents a model in which a large shift in bioenergetic efficiency can be acutely induced through hormonal stimulation. Activation of nonshivering thermogenesis in human brown adipocytes by cold is achieved by the increase in fatty acid availability to the mitochondria and their oxidation, which is the result of norepinephrine (NE)- induced lipolysis (reviewed in Cannon and Nedergaard, 2004; Ouellet et al., 2012). In the case of rodents, high fat diet (a form of nutrient excess) increases brown adipose tissue (BAT) mass. This is mainly thanks to the increase in brown fat proliferation and differentiation, which result in the increase in UCP1 expression and the expansion of mitochondrial mass per cell in rodent models (Himms-Hagen et al., 1981; Rothwell and Stock, 1979). Whether an increase in the activity of this diet-induced expanded BAT in rodents contributes to what was defined as diet-induced thermogenesis is controversial (reviewed in Kozak, 2010).

Mitochondrial expansion induced by high-fat-diet in rodent brown fat shows that when ATP demand is not the main drive for oxygen consumption (i.e., conditions characterized by increased uncoupling such as in the activated brown fat), nutrient excess and increased fuel availability to the mitochondria does not impair bioenergetic function. This lack of toxicity could be explained by the association between mitochondrial membrane potential and escape of electrons from the electron transport chain to generate ROS (Brand et al., 2004). Coupled respiration normally occurs at higher values of membrane potential as compared to uncoupled respiration, which generates heat through UCP1 activation or other uncouplers. This means that uncoupled mitochondria will potentially generate less ROS when compared to coupled mitochondria under conditions of nutrient excess. Following the metaphor of the hose, mitochondria from brown fat would have a second valve, constituted by UCP1, which would allow increasing water flow, while avoiding high pressure and any damage to the hose. The lack of this second valve with high capacity in muscle mitochondria might explain why diets similar to the ones inducing mitochondrial expansion in brown fat cause mitochondrial oxidative damage and dysfunction in muscle (decreased citrate synthase activity and decreased expression of complex IV subunits) (Bonnard et al., 2008) (see the next section). Thus, nutrient excess in the form of high-fat diet can expand mitochondrial capacity in some tissues, whereas mitochondria from other tissues might be damaged by the same diet.

Muscle. Current data suggest potential mechanisms by which nutrient supply and demand imbalance might affect muscle mitochondrial function. Nutrient excess in the form of long-term high-fat diet results in the accumulation of toxic levels of intermediates of fatty acid metabolism. Some of these intermediates were shown to be a result of incomplete mitochondrial fatty acid oxidation and to contribute to impaired insulin signaling and to decreased glucose oxidation (Koves et al., 2008). Furthermore, this accumulation could potentially contribute to the failure of mitochondrial electron transport chain function reported in skeletal muscle from type-2-diabetic patients (Kelley et al., 2002). Other studies show that increased ROS generation, caused by nutrient excess through long-term feeding of a high-sucrose and high-fat diet, is likely to cause self-inflicted oxidative damage to the mitochondria and their dysfunction, the latter taking place after the onset of insulin resistance (Bonnard et al., 2008). Thus, excessive ROS production would be a major contributor to insulin resistance. These mechanisms would suggest that decreased mitochondrial function is not a regulated process but rather caused by damaging effects caused by nutrient excess.

Other studies suggest that decreased mitochondrial electron transport chain (ETC) function reported in diabetic muscle might be a compensatory and a regulated mechanism that may be preventing insulin resistance, although sometimes not successfully. These studies characterized two mouse models of a "primary" reduction in ETC complexes activity, which are muscle-specific knockouts of the apoptosis-inducing factor (AIF) and the transcription factor A mitochondrial (TFAM), respectively (Pospisilik et al., 2007; Wredenberg et al., 2006). These knockout mice showed improved insulin sensitivity (Wredenberg et al., 2006; Pospisilik et al., 2007) and protection from high fat diet-induced obesity (Pospisilik et al., 2007). These findings suggest that the observed decrease in mitochondrial bioenergetic function in type 2 diabetics could be preventing mitochondrial-mediated toxicity associated with nutrient excess. This would favor the hypothesis that inherited or induced transcriptional downregulation of mitochondrial transcripts (Mootha et al., 2003; Patti et al., 2003, Petersen et al., 2004) is a protective mechanism which counteracts insulin resistance, rather than a pathogenic mechanism contributing to insulin resistance.

A potential explanation for the beneficial effect of reduced ETC activity is that reduction in the mass of coupled mitochondria in the muscle exposed to nutrient excess and low ATP demand might serve as a mechanism for avoiding ROS-mediated insulin resistance.

Another mechanism that could cope with toxicity associated with nutrient excess is muscle uncoupled respiration. Increase of proton conductance can decrease mitochondrial ROS production and can enhance the removal of toxic intermediates by completing their oxidation (see the previous section). However, nutrient-overload-induced uncoupling and its relationship to ROS production in muscle is still controversial, and the conclusions are different depending on the study, diets, mouse models, and even the mitochondrial population analyzed

(subsarcolemal versus intermyofibrillar mitochondria) (Asami et al., 2008; Mollica et al., 2006; Almind et al., 2007; Fink et al., 2007; Nabben et al., 2011a, 2011b).

These inconsistent findings might reflect the inability of oxidative muscle to promote a large shift in bioenergetic efficiency. A large increase in uncoupling capacity by nutrient excess, as in brown fat, could severely compromise ATP synthesis and thus oxidative muscle contractile function and calcium homeostasis. Furthermore, muscle is a "nutrient-consuming organ," and it has a steady supply of nutrients in vivo. In addition to fatty acids, these include glucose during the fed state, glycogen during the initial phase of starvation, and ketone bodies during intermediate starvation. Therefore, it makes physiological sense that high-caloric nutrients, such as fatty acids, do not by and large increase uncoupling capacity in oxidative muscle (inducible proton conductance) as in brown fat. On the other hand, it is of relevance to study the regulation of the basal proton conductance or the inherent proton leak in muscle, as this tissue accounts for the major part of nutrient oxidation and thus for the overall organism metabolic efficiency. Thus, the study of mechanisms controlling inherent proton leak in muscle might reveal mechanisms coping with nutrient excess.

The Beta Cell. The beta cell gauges glucose, free fatty acid, and amino acid availability in the bloodstream and secretes insulin accordingly (reviewed in Deeney et al., 2000; reviewed in Rutter, 2001). This gauging is performed through nutrient oxidation and mitochondrial respiration. Mechanistically, the main signal stimulating insulin secretion is increased cytosolic ATP/ADP ratio, through glucose oxidation and likely increased mitochondrial ATP synthesis. In addition, various studies show that byproducts of nutrient oxidation in the mitochondria, including Malonyl-CoA, ROS, and GTP, serve as mediators of insulin secretion, also termed as "secretagogues" (Pi et al., 2007; reviewed in Prentki et al., 1997; Kibbey et al., 2007; reviewed in Rutter, 2001). Some amino acids can stimulate insulin secretion by providing Acetyl-CoA to the Krebs cycle and increasing mitochondrial ATP synthesis (Floyd et al., 1966; reviewed in Poitout and Robertson, 2008). Along with this, there are also additive effects on insulin secretion by simultaneous

"How Can Bioenergetic Efficiency Affect Cellular Functionality and Viability?" section).

Perhaps, since the ability to adapt to excess supply has rarely if ever been selected for, beta cells are designed to be sensitive to ROS as a mechanism for nutrient sensing. As such, the beta cells have low antioxidant activity. ROS production mediated by high nutrients is utilized in the beta cell to couple nutrient oxidation to insulin secretion independently of changes in mitochondrial ATP synthesis (Pi et al., 2007). Therefore, insulin secretion could occur under conditions in which the ATP demand in the beta cell is low. However, an abnormal situation of permanent nutrient excess or continuous exposure to fat (such as type 2 diabetes) would cause mitochondrial damage or decreased function by sustained overproduction of ROS combined with reduced antioxidant activity.

Given the importance of ATP production, ROS, and mitochondrial-derived coupling factors in insulin secretion, one would expect that respiration would be very efficiently coupled to ATP synthesis in beta cells. However, the case is exactly the opposite. Beta cell mitochondria show higher levels of inherent proton leak than do mitochondria from other tissues (e.g., muscle-derived cells) (Affourtit and Brand, 2006). Although it might seem counterintuitive, uncoupled respiration allows limiting ROS-mediated toxicity caused by nutrient excess. This is consistent with the fact that beta cells require other mechanisms to control ROS production, as they harbor low antioxidant activity. Thus, mitochondrial uncoupling is one of the few antioxidant mechanisms described so far that maintains proportionality between nutrient oxidation and insulin secretion through ROS production. At the same time, uncoupling should be tightly regulated in a relatively short period of time, as ATP/ADP ratio is a signal for insulin secretion, which requires efficient and coupled ATP synthesis.

We can conclude that mitochondria in the beta cell have some bioenergetic properties that fall in between mitochondria from muscle and brown fat, which permit executing their specific physiological function related to nutrient sensing.

3. Mitochondria in brown fat cells contain far more UCP (uncoupling protein), which activates heat generation, through increased respiratory rates. This increase in UCP in brown fat cells is due to increased circulating free fatty acids, which are stimulated by epinephrine. Nutrient excess (like a high fat diet for rodents) increases the levels of brown fat cell number and UCP expression and increasing mitochondrial mass.

   In brown fat, when cell energy demand is not the primary driver of oxygen consumption, nutrient excess does not lead to bioenergetic/metabolic dysfunction. This is through greater UCP expression, thereby lowering the mitochondrial membrane potential (more positive mitochondrial membrane), because more protons are passing by without generating energy, thereby increasing the need to use substrate to pump protons back into the intermembrane space. This leads to less electron slippage and less ROS production, because it acts as a "blow valve" to release pressure building up from the intermembrane space.

   This "blow off valve" may be only possible in certain tissues, thereby having nutrient excess can be handled effectively in brown fat cells, for example, but poorly handled by other tissues (like muscle), because they do not have the capacity to express UCP as readily (if at all), leading to greater oxidative stress and damage to the cells not capable of ramping up their UCP levels. Also, long term nutrient excess leads to a toxic level of fat intermediates as they are incompletely cleaved due to a backup of oxidation (utilization for energy) - these fat intermediates can then lead to reduced insulin signaling (maybe they're talking about ceramides here?). Excess ROS (reactive oxygen species, oxidative stress) also leads to decreased insulin sensitivity.

   It is possible that the reduction in mitochondrial energy production may be due to a reduction in transcription of mitochondrial proteins to protect against the toxic effects of excess nutrients (namely, increased fat intermediates and increased oxidative stress).

   In beta cells of the pancreas, the role of these cells is different from muscle and brown fat cells, so the effect of excess nutrients may be different, as well. Beta cells have to be extremely responsive to cell ATP levels, because if there is an increase in ATP, the beta cell is activated to release insulin - this is why an increase in blood sugar is picked up by the beta cell, thereby releasing insulin through the increase in substrate for the mitochondria to generate more ATP. Notably, beta cells can also be sensitized to release insulin independent of change in cell energy; rather, it would release insulin with an increase in reactive oxygen species. This might increase in an excess nutrient situation, because the energy demand in the beta cells may be low, but the nutrients are exceeding that needed, so the drive to push electrons through the complexes would be high, leading to more electrons slipping and creating ROS. This is exacerbated by the fact that beta cells should be sensitive to ROS and therefor have low antioxidant capabilities. Beta cells allow nutrients into the cells based on availability, not based on demand, so this may be a key point differentiating them from something like muscle cells. This leads the beta cells, presumably, to be more vulnerable to nutrient excess.

insulin secretion, also termed as "secretagogues" (Pi et al., 2007; reviewed in Prentki et al., 1997; Kibbey et al., 2007; reviewed in Rutter, 2001). Some amino acids can stimulate insulin secretion by providing Acetyl-CoA to the Krebs cycle and increasing mitochondrial ATP synthesis (Floyd et al., 1966; reviewed in Poitout and Robertson, 2008). Along with this, there are also additive effects on insulin secretion by simultaneous presence of different nutrients. Fatty acids can modulate glucose-stimulated insulin secretion, through their beta oxidation, through the generation of monoglycerides and acyl-CoA, or by direct interaction with plasma membrane receptors (reviewed in Poitout and Robertson, 2008). Since beta cells import and metabolize nutrients based on availability, and not on demand, mechanisms that handle excess nutrient availability are of particular value.

How do beta cell mitochondria respond to nutrient excess? Long-term exposure of beta cells to high levels of glucose, lipids, or their combination has deleterious effects on beta cell mitochondrial function, physiology, and viability. The observation that glucose synergizes with free fatty acids in producing the toxic effects of nutrient excess suggests that the two converge onto a common product (reviewed in Poitout and Robertson, 2008; reviewed in Prentki et al., 2002; reviewed in Deeney et al., 2000). The usual suspect would be a situation of reductive stress characterized by increase in NADH, which, in the absence of increased ATP demand, generates mitochondrial hyperpolarization and produces excess ROS (see the hose metaphor in the coupled ATP synthesis.

We can conclude that mitochondria in the beta cell have some bioenergetic properties that fall in between mitochondria from muscle and brown fat, which permit executing their specific physiological function related to nutrient sensing.

Relationship between Bioenergetic Efficiency and Mitochondrial Dynamics

In this section, we will summarize the changes observed in mitochondrial dynamics associated with conditions requiring a bioenergetic adaptation. This association raises different questions that are essential to answer in order to understand the relevance of this association:

(1) What comes first, changes in mitochondrial dynamics or changes in bioenergetic efficiency? Which factor serves the other? In this section, we will discuss evidence showing that changes in dynamics modulate bioenergetic efficiency and vice versa. It is likely that the cell type and the metabolic state are major determinants in this relationship.

(2) If bioenergetic adaptation requires changes in mitochondrial dynamics, what are the consequences for mitochondrial quality control?

Regarding the first question, specific modulation of mitochondrial bioenergetics has been shown to cause profound changes

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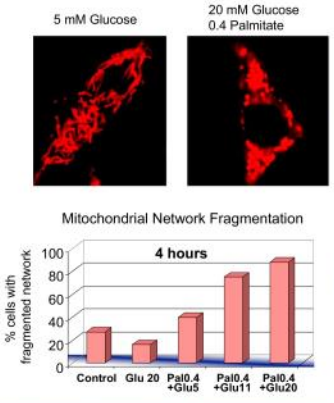

Figure 2. Nutrient Excess Induces Mitochondrial Fragmentation in the Beta Cell

INS-1 cells treated for 4 hr with different concentrations of glucose and fatty acids (palmitate conjugated to BSA). The upper panel shows representative images of INS-1 cells cultured with physiological glucose concentrations (5 mM glucose) and with high glucose and high fatty acid concentrations (20 mM glucose + 0.4 mM palmitate BSA at 4:1 ratio) for 4 hr. Mitochondria are shown in red and were labeled with DsRed targeted to the mitochondria. Cells exposed to high levels of nutrients (20 mM glucose+ 0.4 mM palmitate) show fragmentation and the formation of spherical mitochondria (ball shape), whereas mitochondria with 5 mM glucose appear tubular. The bar graph shows the percentage of cells with fragmented mitochondria after 4 hr incubation with different concentrations of glucose and palmitate (in mM). Note the additive effect of glucose and fatty acids causing fragmentation. See Molina et al. (2009) for more details.

to mitochondrial dynamics. These changes were to a large extent, interpreted in the context of quality control activation (Twig et al., 2008a; reviewed in Twig et al., 2008b). However, new evidence suggests that changes in mitochondrial structure mediated by nutrients and their metabolites might represent an adaptation to the changes in ATP demand and supply.

Summary of Proteins Regulating Mitochondrial Dynamics

Mitochondrial architecture is determined by motility, fusion, and fission events. Mitochondrial fusion in mammals is mediated by mitofusins (Mfn1 and Mfn2, located in the outer mitochondrial membrane) and optic atrophy gene 1 (Opa1, located in the inner membrane) (reviewed in Liesa et al., 2009). These three proteins require GTPase activity to mediate mitochondrial fusion. Proteolytic processing of Opa1 controls its fusion activity but also an Opa1 fusion-independent role, controlling cristae structure remodeling (reviewed in Liesa et al., 2009; Ishihara et al., 2006; Frezza et al., 2006). On the other hand, mitochondrial fission is mediated by fission 1 protein (Fis1, located in the outer mitochondrial membrane), mitochondrial fission factor (Mff, located in the outer mitochondrial membrane), and dynamin-related protein 1 (Drp1, which is mostly cytosolic and translocates to the outer mitochondrial membrane during fission). Drp1 recruitment to the outer mitochondrial membrane and GTP hydrolysis are required for Drp1-mediated fission (reviewed in Liesa et al., 2009). Mff and Fis1 do not harbor GTPase activity, and different studies show that they mediate fission by recruiting Drp1 (or other factors) to the mitochondria to a different extent. MiD49 and MiD51 have been recently described to recruit Drp1 to the mitochondria, although the role of MiD49- and MiD51-mediated recruitment in mitochondrial fission is still under investigation (Palmer et al., 2011; Losón et al., 2013). Of note, Drp1, Fis1, and Mff also control peroxisomal fission (Schrader, 2006; Waterham et al., 2007; Gandre-Babbe and van der Bliek, 2008).

Mitochondrial Fragmentation, Proton Leak, and Maximal Respiratory Capacity: Effects of Chemical Uncouplers and Nutrient Excess

The addition of chemical uncouplers (i.e., FCCP or CCCP) causes complete mitochondrial network fragmentation, Drp1 recruitment to the outer membrane, and OPA1 degradation (Duvezin-Caubet et al., 2006; Griparic et al., 2007; Ishihara et al., 2006; Song et al., 2007; Legros et al., 2002; Cereghetti et al., 2008). In addition, more-recent studies show that depolarization by CCCP also triggers the proteasome-dependent degradation of additional mitochondrial fusion proteins (Mfn1 and Mfn2) and other outer-membrane proteins. However, this proteasome-dependent degradation of Mfns requires the overexpression of the E3-ubiquitin-ligase Parkin (Tanaka et al., 2010; Ziviani et al., 2010; Chan et al., 2011). These studies demonstrated that mitochondrial fission is stimulated and fusion is inhibited in depolarized and uncoupled mitochondria through Drp1 recruitment and OPA1/Mfn degradation, respectively. This suggests the possibility that fragmentation is advantageous for a system working at maximal respiratory capacity or for effective uncoupled respiration and depolarization.

Depolarization, decreased mitochondrial ATP synthesis efficiency, or inhibition of fusion is not equivalent to mitochondrial dysfunction. Consistent with this, the use of uncouplers can mimic physiological conditions of nutrient excess and thus increase nutrient oxidation and electron transport chain activity, such as in the activated brown fat or in the beta cell.

Consistent with this idea, studies exposing beta cells to nutrient excess (Molina et al., 2009) or to conditions that uncouple mitochondria with a physiological stimulus show increased respiration and robust fragmentation of the mitochondrial network (see Figure 2). Thus, it is likely that fragmentation is also associated with both maximal respiratory rates and increased proton conductance.

In this regard, there are some differences between the fragmentation observed under FCCP and the fragmentation observed under a rich-nutrient environment or oxidative stress. Treatment with uncouplers results in fragmentation and the generation of doughnut (bagel)-shaped mitochondria (Liu and Hajnóczky, 2011). Nutrient-induced fragmentation in the beta cell is accompanied by increase in mitochondrial diameter to form ball-shaped instead of doughnut (bagel-shaped)

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4. Changing mitochondrial bioenergetics leads to dramatic changes in mitochondrial morphology. Adding mitochondrial uncouplers, drug induced versions of what UCP does, leads to mitochondrial fragmentation through DRP1 recruitment and OPA1 degradation. Mitochondria will also depolarize as they receive too many protons entering the matrix, which also stimulates the proteasome (a protein degradation pathway) to be activated to destroy mitochondrial fusion proteins (MFNs). Fragmentation of mitochondria is also thought to be linked with maximal respiration.

   There are differences between fragmentation through nutrient excess and fragmentation from uncoupling drugs (CCCP) as uncoupling drugs lead to fragmented mitochondria with a donut-like shape compared to a fuller, ball shape from nutrient excess.

mitochondria (Molina et al., 2009) (see Figure 2). The difference between the two conditions might hint to the potential different roles of the fragmentation and the increase in diameter. Fragmentation might support increased respiration, and the increase in diameter might support increased inherent proton leak. Indeed, these different morphologies can be explained by mitochondrial membrane potential values. FCCP causes massive mitochondrial depolarization (see the "Regulation of Cellular Bioenergetics by Nutrients" section; it can reach 90 mV), whereas nutrient excess increases mitochondrial membrane potential (Goehring et al., 2012; Wikstrom et al., 2007; Danial et al., 2008; Heart et al., 2006). Indeed, oligomycin, which markedly increases membrane potential (up to 220 mV in isolated mitochondria; see the "Regulation of Cellular Bioenergetics by Nutrients" section) was shown to cause fragmentation (Legros et al., 2002). Therefore, the increase in mitochondrial diameter with high nutrients could be a consequence of the increase in inherent proton leak associated with high membrane potential values. On the other hand, FCCP would artificially increase proton conductance *by itself* (induced) and would not activate the inherent proton leak.

The common denominator between uncoupler-induced respiration and basal proton conductance increased by high nutrients is higher respiration and a decrease in ATP synthesis efficiency (less ATP per molecule of nutrient oxidized). The most conspicuous difference lies in the values of the mitochondrial membrane potential.

The mechanism by which fragmentation may benefit a condition of maximal respiration under uncoupler is not yet understood. Among other possibilities, fragmentation might represent a change in cristae structure that allows increased nutrient import. This would also be consistent with the dual role of OPA1 in mitochondrial fusion and cristae remodeling (Frezza et al., 2006). Thus, OPA1 processing/degradation could be one of the molecular mechanisms behind changes in cristae structure induced by uncouplers, facilitating nutrient import and/or inhibiting mitochondrial ATP synthase dimerization.

Since fragmentation is associated with increased proton conductance, one might consider the possibility that mitochondrial fission proteins, such as Drp1, might facilitate it. At least in some systems, there is evidence that Drp1-mediated fragmentation might promote proton conductance through the permeability transition pore due to increased recruitment of Bax (Montessuit et al., 2010). In other systems, Drp1 recruitment to the outer mitochondrial membrane triggered cristae remodeling (Germain et al., 2005) and FCCP promoted Drp1 recruitment (Cereghetti et al., 2008). However, this does not mean that all forms of fragmentation facilitate proton conductance. Nevertheless, it raises the potential role of fragmentation as a first step in the conversion of a cell into a high proton conductance and high respiration state.

Mitochondrial Elongation and Bioenergetic Function: Changes in Dynamics Associated with Situations Requiring Increased ATP Synthesis Capacity

The opposite condition to nutrient excess, starvation, causes an acute inhibition of mitochondrial fission, by inhibiting Drp1 recruitment to the mitochondria, and mitochondrial elongation due to unopposed fusion (Gomes et al., 2011; Rambold et al., 2011). These studies show that elongation prevented the removal of mitochondria by the starvation-induced autophagy. In addition, it causes an increase in mitochondrial cristae number, which is associated with the dimerization of the ATP synthase and thus higher ATP synthesis activity (Gomes et al., 2011). Therefore, starvation would elongate mitochondria in order to increase ATP synthesis capacity and thus sustain the ATP demand required during periods of limited nutrient availability. Furthermore, one could expect mitochondria from oxidative cell types under starvation to be more coupled and to produce ATP more efficiently, as increased ATP synthesis capacity alone would deplete the limited amount of nutrients faster.

In a similar manner, mitochondrial elongation occurs during G1/S phase of the cell cycle, which is characterized by a large increase in ATP demand to support biogenic processes. Consequently, mitochondrial elongation during G1/S phase could permit high ATP synthesis rates that can sustain cell duplication (Mitra et al., 2009). These observations are consistent with respirometry studies, in which it was demonstrated that cells at G1 phase have increased levels of coupled respiration and membrane potential (Schieke et al., 2008).

Consistent with the notion that mitochondrial elongation promotes increased mitochondrial ATP synthesis capacity is the association of elongation with cell senescence (Lee et al., 2007; Yoon et al., 2006). Senescence involves a decreased capacity of proliferation, homeostatic imbalance, and thus decreased capacity of mitochondrial biogenesis. Under this condition, increased ATP synthesis capacity and/or bioenergetic efficiency serves as an adaptation to reduced mitochondrial biogenesis. Mitochondrial fusion provides additional benefit, as it allows for sustaining functional mitochondria with higher number of mutated mitochondrial DNA (mtDNA) copies per senescent cell by complementation. Indeed, senescent cells show increased inherent proton leak that might be caused by damage to the inner mitochondrial membrane (Hutter et al., 2004). This leak is compensated by increasing absolute values of basal respiration (compared to nonsenescent cells) and thus maintaining the fraction of respiration coupled to ATP synthesis (Hutter et al., 2004). It will be interesting to determine whether senescent cells can maintain the same degree of mitochondrial ATP synthesis capacity when mitochondrial fragmentation is induced and when mitochondrial elongation is prevented.

The senescent cell represents a situation of decreased bioenergetic capacity and decreased work load, while the starved cell has both capacity and workload increased. These different needs may explain the difference in the molecular mechanism under each condition: senescent cells show reduced Fis1 and Drp1 expression and slightly increased Mfn protein levels, whereas starved cells show no changes in total proteins levels, only in Drp1 recruitment to the mitochondria (Mai et al., 2010; Lee et al., 2007; Yoon et al., 2006; Gomes et al., 2011).

Other acute stresses, such as apoptosis activation (early stages) and oxidative stress (hydrogen peroxide treatment), have been shown to induce mitochondrial elongation. These changes were shown to facilitate ATP synthesis (Jendrach et al., 2008; Tondera et al., 2009).

The examples reviewed here illustrate that respiration under uncoupling as found in nutrient excess (or treatment with uncouplers) is associated with fragmentation and inhibition of

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5. Starvation (low nutrient presence) leads to reduced fission, reduced DRP (the main fission protein) activation (phosphorylation). This also leads to unopposed fusion, thereby elongating mitochondria. This increased size of mitochondria protects against autophagy; it also serves to increase the cristae of mitochondria, allowing more ATP synthases (the enzyme that produces ATP, cell energy) to be closer together, or to interact, even. Mitochondrial elongation also occurs when cell energy demand is increased, like that of cell division (G/S Phase, where the cell is generating new proteins and new DNA). In cell senescence, the cell still has mitochondria that produce large amounts of energy, but they produce fewer mitochondria, so they rely more on the mitochondria that are present.

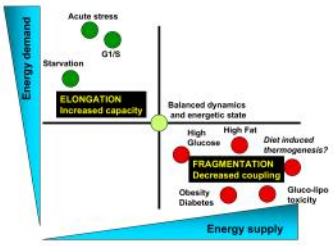

Figure 3. The Balance of Energy Supply and Demand Is Associated with Corresponding Changes to Mitochondrial Architecture and to Bioenergetic Efficiency

Physiological processes associated with increased energy demand and decreased energy supply, such as acute stress, starvation, and G1/S phase, are characterized by mitochondrial elongation and by respiration coupled to ATP synthesis. On the other hand, physiological processes associated with decreased energy demand and increased supply (high levels of nutrients, obesity, and type 2 diabetes) are associated with mitochondrial fragmentation and decreased coupling (associated with heat generation or decreased mitochondrial function).

fusion, whereas the opposite situation, starvation, is associated with inhibition of fission and increased ATP synthesis (and potentially with more coupled mitochondria under starvation). This comparison strengthens the hypothesis that mitochondrial dynamics plays an active role in changes in mitochondrial bioenergetic efficiency and capacity (see the summary in Figure 3).

The Link between Bioenergetic Efficiency and Mitochondrial Architecture: The Pancreatic Beta Cell

As discussed above, the beta cell exquisitely adapts nutrient oxidation to nutrient availability, thereby coupling the latter to insulin secretion. This makes the beta cell an attractive model for the study of the relationship between mitochondrial dynamics and cellular bioenergetic efficiency.

Beta cell mitochondria respond to nutrient excess by profound changes to mitochondrial architecture and dynamics. Exposure of beta cell line INS1 to high fat alone or in combination with glucose leads to mitochondrial fragmentation, which is detected after 4 and 24 hr of addition of high glucose and high fat (Molina et al., 2009) (Figure 2). Remarkably, the two nutrients show an additive effect in terms of inducing fragmentation. This suggests that the two are likely to activate the same fragmentation mechanism.

Thus, mitochondrial fragmentation in the beta cell is an early event that could be directly associated with increased nutrient oxidation. Mechanistically, the observed nutrient-induced fragmentation is mediated by inhibition of mitochondrial fusion (as shown by decreased sharing in mitochondrial matrix protein content; see Figure 4). Similar studies revealed a marked decrease in mitochondrial fusion in primary mouse islets exposed to high glucose and high fatty acids for 48 hr (Molina et al., 2009).

Is nutrient-induced fragmentation unique to the beta cell? A model in which this question can be addressed is the brown adipocyte. The brown adipocyte allows for hormonal mediated induction of uncoupled respiration in less than 5 min. This system is an example of a sharp increase in nutrient availability and in proton conductance, moving from efficient respiration to the most inefficient respiration in terms of ATP synthesis.

Activated brown fat preferably oxidizes fatty acids, which would be a similar situation to high fat exposure in the beta cell. Brown adipocytes go through complete mitochondrial network fragmentation upon induction of uncoupled respiration, supporting the observed correlation between the two (unpublished data). Consequently, determination of the importance of mitochondrial fragmentation to brown fat activation can be a strong evidence that fragmentation is required to stimulate and/or enhance uncoupled respiration.

Increase in uncoupled respiration in the beta cell may serve as a mechanism to remove excess nutrient and set bioenergetic efficiency to balance beta cell nutrient supply and demand (see Figure 1). High glucose and particularly high fatty acids have multiple toxic effects in the beta cell, not only related to excessive ROS production (Las et al., 2011; reviewed in Poitout and Robertson, 2008). The increase in uncoupled respiration could be a mechanism to decrease bioenergetic efficiency in the beta cell, and thus to getting rid of the excess nutrients within the beta cell, by oxidizing them to generate heat. In this regard, Barbara Corkey and Marc Prentki suggested that increased nutrient oxidation and metabolic cycling in response to nutrient excess were mechanisms acting to permit beta cell detoxification. Consistent with this, increased uncoupled respiration and heat generation would be a mechanism to permit beta cell detoxification from the excess of nutrients through their oxidation without overproduction of ROS. Interestingly, fatty acid excess is more toxic for beta cells than is high glucose (reviewed in Poitout and Robertson, 2008). This might explain why fragmentation is higher in the presence of fatty acids excess than in the presence of high glucose in the beta cell. Fatty acids might require extra detoxification capacity within the beta cell because of their higher caloric content and their potential cytotoxic intermediates as a result of their incomplete oxidation (as in muscle) (Koves et al., 2008).

The difference between fatty acids and glucose in mediating fragmentation can be explained by the following hypotheses.

Respiration with fatty acids as substrates is associated with increased mitochondrial proton leak and concomitantly with lower values of membrane potential, whereas glucose oxidation (feeding pyruvate to the mitochondria) occurs with relatively higher membrane potential and thus lower proton leak. Therefore, one could hypothesize that fatty acids are more efficient at inducing fragmentation because their oxidation and other additional effects mediated by fatty acids per se are associated with a higher proton leak.

Fatty acid oxidation has been associated with higher ROS production by the electron transport chain. This is in part due to an additional site for superoxide formation (ETF-Qo, an exclusive site for electron entry into the ETC through fatty acid beta oxidation) (Seifert et al., 2010). Fragmentation and uncoupling might therefore be a protective mechanism that prevents oxidative damage. This would suggest that ROS, and not the fatty

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6. Beta cells exposed to high fat or high fat and glucose experience mitochondrial fragmentation, with the combination of the two causing an additive effect - this could mean they work through the same mechanism. This would likely mean that mitochondrial fragmentation at early stages might be due to increased nutrient utilization.

   Fragmentation can also be manifested, in brown fat cells, through hormonal mechanisms - hormones binding to the cell, inducing cellular signaling within the cell, causing a promotion of the fragmentation of mitochondria.

   High glucose and high fat (especially), have toxic effects in beta cells, significantly increasing ROS, thereby damaging the cells (potentially). (This could be why chronic exposure leads to high levels of insulin secretion, but eventually leads to beta cell "exhaustion" - death). Mitochondrial fragmentation is a way for the beta cells to "detox" from the elevated nutrient availability.

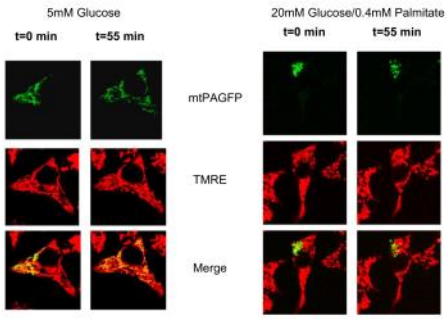

Figure 4. Mitochondrial Fragmentation Induced by Nutrient Excess in the Beta Cell Is Caused by Decreased Mitochondrial Fusion

Mitochondrial fusion activity was quantified with mitochondrial matrix-targeted photoactivatable GFP (mtPAGFP, green). A portion of the mitochondrial population within a cell is labeled by laser photoconversion, and the sharing of the photoconverted molecules across the mitochondrial population through fusion events is monitored. Over 55 min, the majority of mitochondria acquire photoconverted PA-GFP molecules. As a result of the dilution of the signal across the cell, the intensity is diminished. TMRE (red) labeling was used to visualize the entire mitochondrial population. Right panels: INS-1 cells expressing mtPAGFP exposed to nutrient overload (20 mM glucose + 0.4 mM palmitate-BSA) for 4 hr show a dramatic reduction in fusion rates, as shown by the lack of mtPAGFP sharing with other mitochondria. Note that due to the lack of fusion, the mtPAGFP intensity in the labeled mitochondria remains unchanged. Left panels: INS-1 control cells (5 mM glucose) present almost all mitochondria labeled with mtPAGFP 55 min after photoactivation. The images are adapted from Molina et al. (2009) with permission.

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acids or their beta oxidation, are the main activators of fragmentation and uncoupling. In this regard, mitochondrial superoxide has been shown to activate uncoupled respiration (Echtay et al., 2002). Therefore, one would expect antioxidants to decrease fragmentation and proton leak induced by high fatty acids.

An alternative mechanism would be that the fatty acids per se could be causing fragmentation by directly interfering with the fusion and fission machinery. Indeed, fatty acids were shown to activate uncoupled respiration in brown fat through UCP1 (Nicholls and Locke, 1984; Williamson, 1970). The mechanism for this activation would be more likely related to their chemical structure, rather than to fatty acid metabolism or an intrinsic protonophoric activity (Shabalina et al., 2008). In this context, a proportion of this activation related to fatty acid chemical structure was UCP1-independent (Shabalina et al., 2008). Therefore, one could expect that certain fatty acids could be simultaneously signaling mitochondria fragmentation and consequently uncoupled respiration in a UCP1-independent manner, in addition to being the fuels oxidized by mitochondria. Consistent with this, phospholipase activity in the mitochondria is required for mitochondrial fusion mediated by mitofusins (Choi et al., 2006). This study shows a direct connection between acidic lipids generated in the mitochondria by phospholipase activity and fusion (Choi et al., 2006). Thus, fatty acid excess or acidic lipid moieties could be modulating fusion by interfering in these phospholipase-dependent processes or others currently unknown (Huang et al., 2011). However, this pathway has not been described in beta cells or brown adipocytes so far.

Ultimately, reductive stress and increased ROS generation are associated with mitochondrial fragmentation. In some cases, this fragmentation could relieve from reductive stress and ROS generation by decreasing mitochondrial membrane potential through cristae remodeling and OPA1 processing (i.e., nutrient excess). At the same time, mitochondrial fragmentation could be recruited by mechanisms or physiological processes depolarizing the mitochondria, to amplify or enhance the capacity of these processes lowering the mitochondrial membrane potential.

The Primary Role of Mitochondrial Dynamics in Bioenergetic Efficiency: Lessons from Genetic Models

Thus far, we have described the association of mitochondrial network fragmentation and elongation with bioenergetic efficiency. Examination of genetic models in which alteration of mitochondrial dynamics proteins is the primary change may allow us to better understand the cause and effect relationship between the two.

Effects of Specific Changes in Mitochondrial Dynamics on Mitochondrial Bioenergetic Efficiency

Early studies showed that shifting the balance toward *fusion* protected from cell death and shifting it toward *fission* increased susceptibility to apoptosis (Frank et al., 2001; Lee et al., 2004). Consistent with this observation, apoptosis has been associated with complete mitochondrial fragmentation (Frank et al., 2001). Furthermore, smaller and fragmented mitochondria were found in skeletal muscle from type-2-diabetic and obese subjects, conditions that are associated with decreased electron transport chain activity and decreased Mfn2 expression (Bach et al., 2003; Kelley et al., 2002). Together, these findings led to the initial impression that mitochondrial fragmentation impairs mitochondrial respiratory function and is deleterious to cell viability.

However, these generalizations were found to be inaccurate. For example, inhibition of mitochondrial *fission* through Drp1 modulation impairs mitochondrial function. HeLa cells with reduced Drp1 expression showed decreased complex IV activity and a decrease in both state 3 respiration (maximal ATP synthesis rates) and state 4 respiration (proton leak or uncoupling) (Benard et al., 2007). Drp1 knockdown induced a decrease in mtDNA copy number in cell culture (Parone et al., 2008), and complete Drp1 abrogation in mice and humans caused

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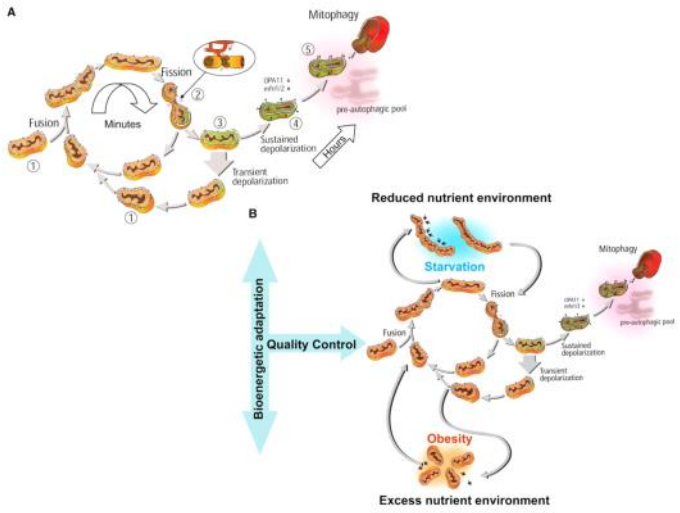

Figure 5. The Life Cycle of Mitochondria and Its Regulation by Nutrient Availability

(A) The life cycle of the mitochondria. The cycle is characterized by fusion and fission events. Fusion generates a network in which components of the two mitochondria are mixed and reorganized (1). Fission that follows within minutes splits fused mitochondria into two daughter mitochondria with disparate membrane potential (2). The daughter with the higher membrane potential is the first to return to the cycle of fusion and fission, while the daughter with more depolarized membrane potential will remain solitary until its membrane potential recovers (3). If membrane potential remains depolarized, this mitochondrion will lose its ability to fuse and become part of the preautophagic pool characterized by solitary, depolarized mitochondria (4). With a delay of 1–3 hr, these mitochondria are eliminated by autophagy (5).

(B) Changes to nutrient availability and energy demand can divert mitochondria from the life cycle and extend their stay in the post fusion state (elongation) or the post fission state (fragmentation). Elongation of mitochondria is a result of increased fusion or decreased fission activity (top section). This is typical for states of increased energy efficiency (starvation, acute stress, and senescence). Shortening of mitochondria is a result of decreased fusion activity or increased fission activity (bottom section). This is typical for states of reduced bioenergetic efficiency (increased uncoupled respiration). Since bioenergetic adaptation to high energy supply requires the arrest of the mitochondria life cycle, extended exposure to excess nutrient environment is expected to impact quality control, a condition that will contribute to reduced longevity.

lethality with brain developmental defects and severe neurodegeneration (Ishihara et al., 2009; Wakabayashi et al., 2009; Waterham et al., 2007). Therefore, Drp1-mediated fission is important to maintain proper quality control (Twig et al., 2008a), electron transport chain function, mtDNA integrity, and cell viability. Drp1 also mediates peroxisomal fission and some of the physiological changes induced by Drp1 modulation can be attributed to effects on peroxisome function (Schrader, 2006; Waterham et al., 2007).

Mitochondrial fusion and fission occur sequentially in a repeating cycle (see Figure 5). The direct implication of this realization is that inhibition of either fusion or fission arrest the cycle. Indeed, similar bioenergetic defects are observed in cells in which *fusion* is inhibited. As an example, skeletal muscle harboring simultaneous deletions in Mfn1 and Mfn2 expression (Mfn double knockout) show decreased number of mtDNA copies, increased mutation and deletion load, and decreased mitochondrial respiration (Chen et al., 2010). On the other hand, an ineffective compensatory increase in mitochondrial mass and complex II activity has been observed in Mfn double-knockout muscles (Chen et al., 2010). The bioenergetic defect and the accompanying expansion of mitochondrial mass resemble the histopathology of patients harboring mutations in mtDNA causing MERRF (myoclonic epilepsy with red ragged fibers). Thus, absence of fusion alters mtDNA homeostasis and electron transport chain function in a similar manner to the

inhibition of fission. The mechanisms by which lack of fusion or fission would decrease mtDNA levels are not clear. While mitochondrial fusion is the main mechanism proposed to allow complementation of functional components in mitochondria harboring mutated mtDNA copies, lack of complementation per se cannot explain why decreased fusion should decrease mtDNA levels (along with a compensatory increase in mass and transcription of nuclear-encoded mitochondrial components).

Mfn2 Deletion in Skeletal Muscle Exacerbates the Effects of Nutrient Excess on Bioenergetic Efficiency

Some of the first observations that associated fragmentation with excess nutrients were the decreased size of mitochondria in muscle from type-2-diabetic and obese humans or mouse models (Bach et al., 2003; Kelley et al., 2002). An accompanying decrease in Mfn2 expression provided a potential mechanism to the reduction in mitochondrial size (Bach et al., 2003; Bach et al., 2005). Consistent with this, specific deletion of Mfn2 in the muscle is associated with decreased mitochondrial ATP synthesis efficiency in permeabilized muscle fibers and with lower protein levels of different ETC subunits (Sebastián et al., 2012). This lower efficiency is explained by a mild decrease in ADP-stimulated respiration and by a mild increase in the leak, accompanied by an increase in ROS production (Sebastián et al., 2012). Therefore, as in the beta cell, fragmentation through inhibition of mitochondrial fusion is associated with increased proton leak in muscle. In addition, this specific deletion of Mfn2 in the muscle and mild repression in other tissues is sufficient to impair insulin signaling and exacerbates the deleterious effects of nutrient excess (high-fat diet) (Sebastián et al., 2012). These results suggest that Mfn2 expression in the muscle is required for a proper bioenergetic adaptation to nutrient excess in the form of high fat diet. Thus, Mfn2 deletion in skeletal muscle causes a prodiabetic effect that involves increased ROS generation and oxidative damage.

On the other hand, Mfn2 and other mitochondrial dynamics components are regulated by pathways activated during conditions of increased energy demand (i.e., exercise and cold exposure) and downregulated in type-2-diabetic patients (nutrient excess), involving the transcriptional coactivators PGC-1α and PGC-1β (Cartoni et al., 2005; Liesa et al., 2008; Soriano et al., 2006; Mootha et al., 2003; Patti et al., 2003). This regulation also supports, to a certain extent, the link between increased energy demand and mitochondrial elongation described before (see the illustration in Figure 3).

In conclusion, maintaining both fusion and fission events is the key parameter to the homeostasis of the bioenergetic function of the mitochondrial population within the cell. Specific defects in mitochondrial dynamics can generate cellular energetic states similar to conditions with altered nutrient supply and demand balance, as shown in muscle. In the specific case of long-term nutrient excess, it is possible that the extension in time of a short-term protective response to nutrient overload, such as fragmentation to reduce reductive stress, ROS, and membrane potential, has deleterious effects on mitochondrial quality control in the long term (Mouli et al., 2009; Twig et al., 2008a). These effects on mitochondrial quality control mediated by altered morphology can potentially explain the abnormal mitochondrial bioenergetic function and cumulative damage associated with metabolic diseases or aging.

Effects of Nutrient Availability on Mitochondrial Quality Control

Changes in mitochondrial dynamics affect quality control and can therefore influence bioenergetic capacity indirectly (Twig et al., 2008a). Moreover, recent evidence suggests that nutrients influence quality control function (Las et al., 2011; Singh et al., 2009). Therefore, for appropriate consideration of the relationship between mitochondrial dynamics and bioenergetics one has to consider how both interact with mitochondrial quality control mechanisms.

Mechanisms for Mitochondrial Quality Control and Its Regulation by Bioenergetics, Mitophagy, and Mitochondrial Dynamics

The use of confocal microscopy allows the visualization of single mitochondria units and specifically to track them over time (Twig et al., 2008a, 2010; reviewed in Liesa et al., 2009). A very relevant observation to our discussion is the finding that mitochondrial units with a cell are heterogeneous in terms of their bioenergetic activity (Wikstrom et al., 2007; reviewed in Wikstrom et al., 2009). This is reflected by the difference in mitochondrial membrane potential between the different units. Furthermore, this heterogeneity was modulated by nutrient excess and other metabolic changes (Wikstrom et al., 2007), which regulate mitochondrial dynamics (Molina et al., 2009). These data demonstrate that mitochondrial fusion and fission does not completely equilibrate the bioenergetic properties of the entire mitochondrial population. This stands in contrast to the mitochondrial complementation theory, which hypothesizes that mitochondrial fusion homogenizes the entire population, a conclusion drawn from the observation of shared matrix soluble components. Remarkably, however, decreasing mitochondrial fusion rate resulted in increased heterogeneity illustrating the contribution of mitochondrial dynamics to the maintenance of the mitochondrial bioenergetic function. The paradox could be settled by the understanding that fusion, fission and autophagy are all connected by one axis (Figure 5).

The quality control axis is centered on the fission event, which might generate two bioenergetically different mitochondria, one with a higher membrane potential and one with lower membrane potential. The single daughter mitochondrion with lower membrane potential has two options: (1) recover its membrane potential and regain the capacity to reconnect with the network or (2) remain in the solitary period, depolarized. If membrane potential is not restored during the solitary period, OPA1 will be degraded. Thus, the solitary mitochondria will not be able to re-engage with the network and will be degraded by mitophagy. One can conclude that fission is an important process isolating a potentially damaging organelle and that selective fusion governs the fate of the mitochondria to be autophagocytosed (Twig et al., 2008a). Within this context, long-term inhibition (days) of fission by Drp1 dominant negative overexpression can reduce the increase in respiration induced by uncouplers in intact cells (Twig et al., 2008a). These results should not be interpreted as evidence for the requirement of fragmentation to achieve maximal respiratory capacity (see the "Effects of Specific Changes in Mitochondrial Dynamics on Mitochondrial Bioenergetic Efficiency" section). The effects on bioenergetics caused by long-term inhibition of fission can be explained by accumulation of irreversibly damaged mitochondria that cannot be

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7. Shifting mitochondria toward fusion protects against cell death, and shifting mitochondria toward fission increases the chance of cell death. Cell death (apoptosis version) is linked to complete fragmentation of mitochondria - and, mitochondria in diabetic muscle has shown increased fragmentation. Impairing fission, however, impairs mitochondrial function.

   Loss of DRP (fission protein) reduces mitochondrial function, DNA health in mitochondria, and cell survival, which may also be due to DRP's effect on peroxisome function (because it also is involved in peroxisome function).

   Similar detrimental effects are also seen when fusion is inhibited, so it isn't just fission centric.

8. As stated before, reduced size of mitochondria is linked to diabetic tissues, as well as obese tissues. During exercise and other cell energy stress, there is an increase in MFN protein expression (this is a fusion protein) - this makes sense considering exercise reduces cell energy levels, thereby promoting mitochondrial elongation.

   Mitochondria morphology changes are centered around fission, creating two mitochondria - one with a high membrane potential and the other is a low membrane potential. The low membrane potential mitochondria can undergo two processes: 1. Recover its membrane potential (increasing membrane potential), or 2. remain with a low membrane potential and be tagged for mitophagy.

segregated (Twig et al., 2008a). This finding is supported by changes of membrane fluidity in isolated mitochondria from cells with downregulation of Drp1 (Benard et al., 2007), showing that the alteration is maintained when mitochondria are taken out of the cells and mitochondrial dynamics are absent.

Although it is widely accepted that fission events produce uneven daughters that are selected by autophagy, it might be appropriate to indicate that this was only shown in the beta cell and COS7 cells. Similarly, that mitochondrial autophagy is a housekeeping process that targets spontaneously depolarized mitochondria was thus far shown only in the beta cells.

Multiple studies have identified additional mechanisms for the inability of mitochondria in the solitary period to fuse and the signals that label them to be recognized and removed by the autophagic machinery. The U3-ubiquitin ligase Parkin (mutated in Parkinson's disease), through PINK1 serine-threonine kinase activity, is recruited to depolarized mitochondria to target them for mitophagy (Narendra et al., 2008; Vives-Bauza et al., 2010; Ziviani et al., 2010). In addition, Parkin ubiquitinates Mfn, promoting its degradation by the proteasome system and thus contributing to fusion inhibition of the solitary depolarized mitochondria (Chan et al., 2011; Tanaka et al., 2010; Ziviani et al., 2010). Therefore, we can define that these solitary and dysfunctional mitochondria, with no fusion capabilities, comprise the preautophagic pool of mitochondria.

A key component dictating the efficiency of mitochondrial quality control by fusion, fission, and autophagy is the ability of a full cycle to be completed and the number of cycles per day (Mouli et al., 2009). A mathematical model that runs multiple iterations of the cycle predicts that the rate of fusion and fission cycles determines the capacity of the pathway to restore quality upon damage. In this context, the effect of nutrient on the rate of fusion, fission, and the formation of the mitochondrial preautophagic pool may be considered as important in its effect on autophagy (Las et al., 2011; Singh et al., 2009).

Evidence of Mitochondrial Quality Control, Mitophagy, and Autophagy Modulation by Nutrients and Their Relationship to the Energetic State

Nutrient excess leads to the inhibition of fusion, resulting in fragmentation and an incomplete cycle of fusion, fission, and autophagy (Molina et al., 2009; Las et al., 2011). In addition, it does not allow for mitochondrial complementation and thus increases subcellular mitochondrial heterogeneity (Wikstrom et al., 2007). Given this lack of selective removal, one could expect that mitochondrial mass would decrease, as the population will be mostly comprised of small and depolarized mitochondria (Figure 5). Therefore, maintenance of mitochondrial health would only require stimulation of mitochondrial biogenesis. However, nutrient excess can impair autophagic flux by inhibiting lysosomes, which are required for autophagic degradation (Las et al., 2011). As a consequence, dysfunctional mitochondria will accumulate and will affect even mitochondria generated de novo (by unselective fusion and/or increased ROS production). These alterations can explain different reports demonstrating mitochondrial dysfunction in pathologies associated with an imbalance in nutrient supply and demand.

Turnover requires both fusion events and the segregation of damaged components by fission, which will not enter again into the network because fusion is bioenergetically selective (Figure 5). We suggest that the interaction between mitochondrial life cycle, dynamics, and bioenergetics evolved to adapt to changes in nutrient availability, which are physiologically comprised of feeding and fasting states. Any prolongation in the feeding or fasting state requires a bioenergetic adaptation that will shift the balance of mitochondrial dynamics. A prolonged shift will have deleterious effects on mitochondrial health and quality control. In the case of the fasting state, the shift in dynamics required for bioenergetic adaptation will homogenize the mitochondrial population, preventing the segregation, the formation of the preautophagic pool and the removal of damaged components by mitophagy. In the fed state and/or nutrient excess (particularly high fat), fragmentation and high respiratory rates can lead to damage, in addition to mechanisms affecting the autophagic machinery downstream of the preautophagic pool of mitochondria. This would cause the accumulation of dysfunctional units and the increase in ROS generation. In this context, it is likely that caloric restriction (or proper fed/fasting cycles) would promote a bioenergetic adaptation and a change in mitochondrial dynamics, permitting the most efficient mitochondrial quality control mechanisms. Thus, this interaction between bioenergetics adaptation, mitochondrial dynamics, and quality control could explain some of the beneficial effects associated with caloric restriction.

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REFERENCES

Affourtit, C., and Brand, M.D. (2006). Stronger control of ATP/ADP by proton leak in pancreatic beta-cells than skeletal muscle mitochondria. Biochem. J. *393*, 151–159.

Almind, K., Manieri, M., Sivitz, W.I., Cinti, S., and Kahn, C.R. (2007). Ectopic brown adipose tissue in muscle provides a mechanism for differences in risk of metabolic syndrome in mice. Proc. Natl. Acad. Sci. USA *104*, 2366–2371.

Aquila, H., Link, T.A., and Klingenberg, M. (1985). The uncoupling protein from brown fat mitochondria is related to the mitochondrial ADP/ATP carrier. Analysis of sequence homologies and of folding of the protein in the membrane. EMBO J. *4*, 2369–2376.

Asami, D.K., McDonald, R.B., Hagopian, K., Horwitz, B.A., Warman, D., Hsiao, A., Warden, C., and Ramsey, J.J. (2008). Effect of aging, caloric restriction, and uncoupling protein 3 (UCP3) on mitochondrial proton leak in mice. Exp. Gerontol. *43*, 1069–1076.

Ashcroft, F.M., Harrison, D.E., and Ashcroft, S.J. (1984). Glucose induces closure of single potassium channels in isolated rat pancreatic beta-cells. Nature *312*, 446–448.

Bach, D., Pich, S., Soriano, F.X., Vega, N., Baumgartner, B., Oriola, J., Daugaard, J.R., Lloberas, J., Camps, M., Zierath, J.R., et al. (2003). Mitofusin-2 determines mitochondrial network architecture and mitochondrial metabolism. A novel regulatory mechanism altered in obesity. J. Biol. Chem. *278*, 17190–17197.

Bach, D., Naon, D., Pich, S., Soriano, F.X., Vega, N., Rieusset, J., Laville, M., Guillet, C., Boirie, Y., Wallberg-Henriksson, H., et al. (2005). Expression of Mfn2, the Charcot-Marie-Tooth neuropathy type 2A gene, in human skeletal

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9. Nutrient excess leads to inhibition of fusion, increased fission, but also impaired autophagy, because lysosome activation (autophagy activation) is partly dictated by lacking nutrients (starvation) - creating an environment of fragmented mitochondria, but a heterogenous population of mitochondria, with some being primed for degradation through autophagy, because they are unhealthy (depolarized); however, they stick around, because autophagy is inhibited. This increase in the number of dysfunctional mitochondria increases the amount of ROS, hurting the cells.

muscle: effects of type 2 diabetes, obesity, weight loss, and the regulatory role of tumor necrosis factor alpha and interleukin-6. Diabetes *54*, 2685–2693.

Benard, G., Bellance, N., James, D., Parrone, P., Fernandez, H., Letellier, T., and Rossignol, R. (2007). Mitochondrial bioenergetics and structural network organization. J. Cell Sci. *120*, 838–848.

Bonnard, C., Durand, A., Peyrol, S., Chanseaume, E., Chauvin, M.A., Morio, B., Vidal, H., and Rieusset, J. (2008). Mitochondrial dysfunction results from oxidative stress in the skeletal muscle of diet-induced insulin-resistant mice. J. Clin. Invest. *118*, 789–800.

Brand, M.D., Affourtit, C., Esteves, T.C., Green, K., Lambert, A.J., Miwa, S., Pakay, J.L., and Parker, N. (2004). Mitochondrial superoxide: production, biological effects, and activation of uncoupling proteins. Free Radic. Biol. Med. *37*, 755–767.

Cannon, B., and Nedergaard, J. (2004). Brown adipose tissue: function and physiological significance. Physiol. Rev. *84*, 277–359.

Cartoni, R., Léger, B., Hock, M.B., Praz, M., Crettenand, A., Pich, S., Ziltener, J.L., Luthi, F., Dériaz, O., Zorzano, A., et al. (2005). Mitofusins 1/2 and ERRalpha expression are increased in human skeletal muscle after physical exercise. J. Physiol. *567*, 349–358.

Cereghetti, G.M., Stangherlin, A., Martins de Brito, O., Chang, C.R., Blackstone, C., Bernardi, P., and Scorrano, L. (2008). Dephosphorylation by calcineurin regulates translocation of Drp1 to mitochondria. Proc. Natl. Acad. Sci. USA *105*, 15803–15808.

Chan, N.C., Salazar, A.M., Pham, A.H., Sweredoski, M.J., Kolawa, N.J., Graham, R.L., Hess, S., and Chan, D.C. (2011). Broad activation of the ubiquitin-proteasome system by Parkin is critical for mitophagy. Hum. Mol. Genet. *20*, 1726–1737.

Chance, B., and Williams, G.R. (1955). Respiratory enzymes in oxidative phosphorylation. III. The steady state. J. Biol. Chem. *217*, 409–427.

Chappell, J.B., and Perry, S.V. (1954). The Respiratory and Adenosinetriphosphatase Activitiesof Skeletal-Muscle Mitochondria. Biochem. J. *55*, 586–595.

Chen, H., McCaffery, J.M., and Chan, D.C. (2007). Mitochondrial fusion protects against neurodegeneration in the cerebellum. Cell *130*, 548–562.

Chen, H., Vermulst, M., Wang, Y.E., Chomyn, A., Prolla, T.A., McCaffery, J.M., and Chan, D.C. (2010). Mitochondrial fusion is required for mtDNA stability in skeletal muscle and tolerance of mtDNA mutations. Cell *141*, 280–289.

Chen, Y., Liu, Y., and Dorn, G.W., 2nd. (2011). Mitochondrial fusion is essential for organelle function and cardiac homeostasis. Circ. Res. *109*, 1327–1331.

Choi, S.Y., Huang, P., Jenkins, G.M., Chan, D.C., Schiller, J., and Frohman, M.A. (2006). A common lipid links Mfn-mediated mitochondrial fusion and SNARE-regulated exocytosis. Nat. Cell Biol. *8*, 1255–1262.

Danial, N.N., Walensky, L.D., Zhang, C.Y., Choi, C.S., Fisher, J.K., Molina, A.J., Datta, S.R., Pitter, K.L., Bird, G.H., Wikstrom, J.D., et al. (2008). Dual role of proapoptotic BAD in insulin secretion and beta cell survival. Nat Med. *14*, 144–153.

Deeney, J.T., Prentki, M., and Corkey, B.E. (2000). Metabolic control of beta-cell function. Semin. Cell Dev. Biol. *11*, 267–275.

Duvezin-Caubet, S., Jagasia, R., Wagener, J., Hofmann, S., Trifunovic, A., Hansson, A., Chomyn, A., Bauer, M.F., Attardi, G., Larsson, N.G., et al. (2006). Proteolytic processing of OPA1 links mitochondrial dysfunction to alterations in mitochondrial morphology. J. Biol. Chem. *281*, 37972–37979.

Echtay, K.S., Roussel, D., St-Pierre, J., Jekabsons, M.B., Cadenas, S., Stuart, J.A., Harper, J.A., Roebuck, S.J., Morrison, A., Pickering, S., et al. (2002). Superoxide activates mitochondrial uncoupling proteins. Nature *415*, 96–99.

Fink, B.D., Herlein, J.A., Almind, K., Cinti, S., Kahn, C.R., and Sivitz, W.I. (2007). Mitochondrial proton leak in obesity-resistant and obesity-prone mice. Am. J. Physiol. Regul. Integr. Comp. Physiol. *293*, R1773–R1780.

Floyd, J.C., Jr., Fajans, S.S., Conn, J.W., Knopf, R.F., and Rull, J. (1966). Stimulation of insulin secretion by amino acids. J. Clin. Invest. *45*, 1487–1502.

Frank, S., Gaume, B., Bergmann-Leitner, E.S., Leitner, W.W., Robert, E.G., Catez, F., Smith, C.L., and Youle, R.J. (2001). The role of dynamin-related protein 1, a mediator of mitochondrial fission, in apoptosis. Dev. Cell *1*, 515–525.

Frezza, C., Cipolat, S., Martins de Brito, O., Micaroni, M., Beznoussenko, G.V., Rudka, T., Bartoli, D., Polishuck, R.S., Danial, N.N., De Strooper, B., and Scorrano, L. (2006). OPA1 controls apoptotic cristae remodeling independently from mitochondrial fusion. Cell *126*, 177–189.

Gandre-Babbe, S., and van der Bliek, A.M. (2008). The novel tail-anchored membrane protein Mff controls mitochondrial and peroxisomal fission in mammalian cells. Mol. Biol. Cell *19*, 2402–2412.

Germain, M., Mathai, J.P., McBride, H.M., and Shore, G.C. (2005). Endoplasmic reticulum BIK initiates DRP1-regulated remodelling of mitochondrial cristae during apoptosis. EMBO J. *24*, 1546–1556.

Goehring, I., Gerencser, A.A., Schmidt, S., Brand, M.D., Mulder, H., and Nicholls, D.G. (2012). Plasma membrane potential oscillations in insulin secreting Ins-1 832/13 cells do not require glycolysis and are not initiated by fluctuations in mitochondrial bioenergetics. J. Biol. Chem. *287*, 15706–15717.

Gomes, L.C., Di Benedetto, G., and Scorrano, L. (2011). During autophagy mitochondria elongate, are spared from degradation and sustain cell viability. Nat. Cell Biol. *13*, 589–598.

Griparic, L., Kanazawa, T., and van der Bliek, A.M. (2007). Regulation of the mitochondrial dynamin-like protein Opa1 by proteolytic cleavage. J. Cell Biol. *178*, 757–764.

Hafner, R.P., Brown, G.C., and Brand, M.D. (1990). Analysis of the control of respiration rate, phosphorylation rate, proton leak rate and protonmotive force in isolated mitochondria using the 'top-down' approach of metabolic control theory. Eur. J. Biochem. *188*, 313–319.

Heart, E., Corkey, R.F., Wikstrom, J.D., Shirihai, O.S., and Corkey, B.E. (2006). Glucose-dependent increase in mitochondrial membrane potential, but not cytoplasmic calcium, correlates with insulin secretion in single islet cells. Am J Physiol Endocrinol Metab. *290*, E143–E148.

Heaton, G.M., Wagenvoord, R.J., Kemp, A., Jr., and Nicholls, D.G. (1978). Brown-adipose-tissue mitochondria: photoaffinity labelling of the regulatory site of energy dissipation. Eur. J. Biochem. *82*, 515–521.

Himms-Hagen, J., Triandafillou, J., and Gwilliam, C. (1981). Brown adipose tissue of cafeteria-fed rats. Am. J. Physiol. *241*, E116–E120.

Huang, H., Gao, Q., Peng, X., Choi, S.Y., Sarma, K., Ren, H., Morris, A.J., and Frohman, M.A. (2011). piRNA-associated germline nuage formation and spermatogenesis require MitoPLD profusogenic mitochondrial-surface lipid signaling. Dev. Cell *20*, 376–387.

Hutter, E., Renner, K., Pfister, G., Stöckl, P., Jansen-Dürr, P., and Gnaiger, E. (2004). Senescence-associated changes in respiration and oxidative phosphorylation in primary human fibroblasts. Biochem. J. *380*, 919–928.

Ishihara, N., Fujita, Y., Oka, T., and Mihara, K. (2006). Regulation of mitochondrial morphology through proteolytic cleavage of OPA1. EMBO J. *25*, 2966–2977.

Ishihara, N., Nomura, M., Jofuku, A., Kato, H., Suzuki, S.O., Masuda, K., Otera, H., Nakanishi, Y., Nonaka, I., Goto, Y., et al. (2009). Mitochondrial fission factor Drp1 is essential for embryonic development and synapse formation in mice. Nat. Cell Biol. *11*, 958–966.

Jendrach, M., Mai, S., Pohl, S., Vöth, M., and Bereiter-Hahn, J. (2008). Short- and long-term alterations of mitochondrial morphology, dynamics and mtDNA after transient oxidative stress. Mitochondrion *8*, 293–304.

Kelley, D.E., He, J., Menshikova, E.V., and Ritov, V.B. (2002). Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. Diabetes *51*, 2944–2950.

Kibbey, R.G., Pongratz, R.L., Romanelli, A.J., Wollheim, C.B., Cline, G.W., and Shulman, G.I. (2007). Mitochondrial GTP regulates glucose-stimulated insulin secretion. Cell Metab. *5*, 253–264.

Koves, T.R., Ussher, J.R., Noland, R.C., Slentz, D., Mosedale, M., Ilkayeva, O., Bain, J., Stevens, R., Dyck, J.R., Newgard, C.B., et al. (2008). Mitochondrial overload and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance. Cell Metab. *7*, 45–56.

Kozak, L.P. (2010). Brown fat and the myth of diet-induced thermogenesis. Cell Metab. *11*, 263–267.

Las, G., Serada, S.B., Wikstrom, J.D., Twig, G., and Shirihai, O.S. (2011). Fatty acids suppress autophagic turnover in β-cells. J. Biol. Chem. *286*, 42534–42544.

Lee, Y.J., Jeong, S.Y., Karbowski, M., Smith, C.L., and Youle, R.J. (2004). Roles of the mammalian mitochondrial fission and fusion mediators Fis1, Drp1, and Opa1 in apoptosis. Mol. Biol. Cell *15*, 5001–5011.

Lee, S., Jeong, S.Y., Lim, W.C., Kim, S., Park, Y.Y., Sun, X., Youle, R.J., and Cho, H. (2007). Mitochondrial fission and fusion mediators, hFis1 and OPA1, modulate cellular senescence. J. Biol. Chem. *282*, 22977–22983.

Legros, F., Lombès, A., Frachon, P., and Rojo, M. (2002). Mitochondrial fusion in human cells is efficient, requires the inner membrane potential, and is mediated by mitofusins. Mol. Biol. Cell *13*, 4343–4354.

Levine, J.A., Eberhardt, N.L., and Jensen, M.D. (1999). Role of nonexercise activity thermogenesis in resistance to fat gain in humans. Science *283*, 212–214.

Liesa, M., Borda-d'Agua, B., Medina-Gómez, G., Lelliott, C.J., Paz, J.C., Rojo, M., Palacín, M., Vidal-Puig, A., and Zorzano, A. (2008). Mitochondrial fusion is increased by the nuclear coactivator PGC-1beta. PLoS ONE *3*, e3613.

Liesa, M., Palacín, M., and Zorzano, A. (2009). Mitochondrial dynamics in mammalian health and disease. Physiol. Rev. *89*, 799–845.

Liu, X., and Hajnóczky, G. (2011). Altered fusion dynamics underlie unique morphological changes in mitochondria during hypoxia-reoxygenation stress. Cell Death Differ. *18*, 1561–1572.

Losón, O.C., Song, Z., Chen, H., and Chan, D.C. (2013). Fis1, Mff, MiD49, and MiD51 mediate Drp1 recruitment in mitochondrial fission. Mol. Biol. Cell. *24*, 659–667.

Mai, S., Klinkenberg, M., Auburger, G., Bereiter-Hahn, J., and Jendrach, M. (2010). Decreased expression of Drp1 and Fis1 mediates mitochondrial elongation in senescent cells and enhances resistance to oxidative stress through PINK1. J. Cell Sci. *123*, 917–926.

Marcinek, D.J., Schenkman, K.A., Ciesielski, W.A., and Conley, K.E. (2004). Mitochondrial coupling in vivo in mouse skeletal muscle. Am. J. Physiol. Cell Physiol. *286*, C457–C463.

Mitchell, P. (1961). Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. Nature *191*, 144–148.

Mitra, K., Wunder, C., Roysam, B., Lin, G., and Lippincott-Schwartz, J. (2009). A hyperfused mitochondrial state achieved at G1-S regulates cyclin E buildup and entry into S phase. Proc. Natl. Acad. Sci. USA *106*, 11960–11965.

Molina, A.J., Wikstrom, J.D., Stiles, L., Las, G., Mohamed, H., Elorza, A., Walzer, G., Twig, G., Katz, S., Corkey, B.E., and Shirihai, O.S. (2009). Mitochondrial networking protects beta-cells from nutrient-induced apoptosis. Diabetes *58*, 2303–2315.

Mollica, M.P., Lionetti, L., Crescenzo, R., D'Andrea, E., Ferraro, M., Liverini, G., and Iossa, S. (2006). Heterogeneous bioenergetic behaviour of subsarcolemmal and intermyofibrillar mitochondria in fed and fasted rats. Cell. Mol. Life Sci. *63*, 358–366.

Montessuit, S., Somasekharan, S.P., Terrones, O., Lucken-Ardjomande, S., Herzig, S., Schwarzenbacher, R., Manstein, D.J., Bossy-Wetzel, E., Basañez, G., Meda, P., and Martinou, J.C. (2010). Membrane remodeling induced by the dynamin-related protein Drp1 stimulates Bax oligomerization. Cell *142*, 889–901.

Mootha, V.K., Lindgren, C.M., Eriksson, K.F., Subramanian, A., Sihag, S., Lehar, J., Puigserver, P., Carlsson, E., Ridderstråle, M., Laurila, E., et al. (2003). PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat. Genet. *34*, 267–273.

Mouli, P.K., Twig, G., and Shirihai, O.S. (2009). Frequency and selectivity of mitochondrial fusion are key to its quality maintenance function. Biophys. J. *96*, 3509–3518.

Muoio, D.M., and Newgard, C.B. (2006). Obesity-related derangements in metabolic regulation. Annu. Rev. Biochem. *75*, 367–401.

Nabben, M., Hoeks, J., Moonen-Kornips, E., van Beurden, D., Briedé, J.J., Hesselink, M.K., Glatz, J.F., and Schrauwen, P. (2011a). Significance of uncoupling protein 3 in mitochondrial function upon mid- and long-term dietary high-fat exposure. FEBS Lett. *585*, 4010–4017.

Nabben, M., Shabalina, I.G., Moonen-Kornips, E., van Beurden, D., Cannon, B., Schrauwen, P., Nedergaard, J., and Hoeks, J. (2011b). Uncoupled respiration, ROS production, acute lipotoxicity and oxidative damage in isolated skeletal muscle mitochondria from UCP3-ablated mice. Biochim. Biophys. Acta *1807*, 1095–1105.

Narendra, D., Tanaka, A., Suen, D.F., and Youle, R.J. (2008). Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. J. Cell Biol. *183*, 795–803.

Nicholls, D.G. (1974). Hamster brown-adipose-tissue mitochondria. The control of respiration and the proton electrochemical potential gradient by possible physiological effectors of the proton conductance of the inner membrane. Eur. J. Biochem. *49*, 573–583.

Nicholls, D.G., and Ferguson, S.J. (2002). Bioenergetics 3 (London: Academic Press).

Nicholls, D.G., and Locke, R.M. (1984). Thermogenic mechanisms in brown fat. Physiol. Rev. *64*, 1–64.

Nicholls, D.G., Bernson, V.S., and Heaton, G.M. (1978). The identification of the component in the inner membrane of brown adipose tissue mitochondria responsible for regulating energy dissipation. Experientia Suppl. *32*, 89–93.

Ouellet, V., Labbé, S.M., Blondin, D.P., Phoenix, S., Guérin, B., Haman, F., Turcotte, E.E., Richard, D., and Carpentier, A.C. (2012). Brown adipose tissue oxidative metabolism contributes to energy expenditure during acute cold exposure in humans. J. Clin. Invest. *122*, 545–552.

Palmer, C.S., Osellame, L.D., Laine, D., Koutsopoulos, O.S., Frazier, A.E., and Ryan, M.T. (2011). MiD49 and MiD51, new components of the mitochondrial fission machinery. EMBO Rep. *12*, 565–573.

Parker, N.D., Crichton, P.G., Vidal-Puig, A.J., and Brand, M.D. (2009). Uncoupling protein-1 (UCP1) contributes to the basal proton conductance of brown adipose tissue mitochondria. J. Bioenerg. Biomembr. *41*, 335–342.

Parone, P.A., Da Cruz, S., Tondera, D., Mattenberger, Y., James, D.I., Maechler, P., Barja, F., and Martinou, J.C. (2008). Preventing mitochondrial fission impairs mitochondrial function and leads to loss of mitochondrial DNA. PLoS ONE *3*, e3257.

Patti, M.E., Butte, A.J., Crunkhorn, S., Cusi, K., Berria, R., Kashyap, S., Miyazaki, Y., Kohane, I., Costello, M., Saccone, R., et al. (2003). Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: Potential role of PGC1 and NRF1. Proc. Natl. Acad. Sci. USA *100*, 8466–8471.

Petersen, K.F., Dufour, S., Befroy, D., Garcia, R., and Shulman, G.I. (2004). Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. N. Engl. J. Med. *350*, 664–671.

Pi, J., Bai, Y., Zhang, Q., Wong, V., Floering, L.M., Daniel, K., Reece, J.M., Deeney, J.T., Andersen, M.E., Corkey, B.E., and Collins, S. (2007). Reactive oxygen species as a signal in glucose-stimulated insulin secretion. Diabetes *56*, 1783–1791.

Poitout, V., and Robertson, R.P. (2008). Glucolipotoxicity: fuel excess and beta-cell dysfunction. Endocr. Rev. *29*, 351–366.

Pospisilik, J.A., Knauf, C., Joza, N., Benit, P., Orthofer, M., Cani, P.D., Ebersberger, I., Nakashima, T., Sarao, R., Neely, G., et al. (2007). Targeted deletion of AIF decreases mitochondrial oxidative phosphorylation and protects from obesity and diabetes. Cell *131*, 476–491.

Prentki, M., Tornheim, K., and Corkey, B.E. (1997). Signal transduction mechanisms in nutrient-induced insulin secretion. Diabetologia *40(Suppl 2)*, S32–S41.

Prentki, M., Joly, E., El-Assaad, W., and Roduit, R. (2002). Malonyl-CoA signaling, lipid partitioning, and glucolipotoxicity: role in beta-cell adaptation and failure in the etiology of diabetes. Diabetes *51(Suppl 3)*, S405–S413.

Quirós, P.M., Ramsay, A.J., Sala, D., Fernández-Vizarra, E., Rodríguez, F., Peinado, J.R., Fernández-García, M.S., Vega, J.A., Enríquez, J.A., Zorzano, A., and López-Otín, C. (2012). Loss of mitochondrial protease OMA1 alters processing of the GTPase OPA1 and causes obesity and defective thermogenesis in mice. EMBO J. *31*, 2117–2133.

Rambold, A.S., Kostelecky, B., Elia, N., and Lippincott-Schwartz, J. (2011). Tubular network formation protects mitochondria from autophagosomal

degradation during nutrient starvation. Proc. Natl. Acad. Sci. USA *108*, 10190–10195.

Rial, E., Poustie, A., and Nicholls, D.G. (1983). Brown-adipose-tissue mitochondria: the regulation of the 32000-Mr uncoupling protein by fatty acids and purine nucleotides. Eur. J. Biochem. *137*, 197–203.

Rothwell, N.J., and Stock, M.J. (1979). A role for brown adipose tissue in diet-induced thermogenesis. Nature *281*, 31–35.

Rutter, G.A. (2001). Nutrient-secretion coupling in the pancreatic islet beta-cell: recent advances. Mol. Aspects Med. *22*, 247–284.

Schieke, S.M., McCoy, J.P., Jr., and Finkel, T. (2008). Coordination of mitochondrial bioenergetics with G1 phase cell cycle progression. Cell Cycle *7*, 1782–1787.

Schrader, M. (2006). Shared components of mitochondrial and peroxisomal division. Biochim. Biophys. Acta *1763*, 531–541.

Schutz, Y., Bessard, T., and Jéquier, E. (1984). Diet-induced thermogenesis measured over a whole day in obese and nonobese women. Am. J. Clin. Nutr. *40*, 542–552.

Sebastián, D., Hernández-Alvarez, M.I., Segalés, J., Sorianello, E., Muñoz, J.P., Sala, D., Waget, A., Liesa, M., Paz, J.C., Gopalacharyulu, P., et al. (2012). Mitofusin 2 (Mfn2) links mitochondrial and endoplasmic reticulum function with insulin signaling and is essential for normal glucose homeostasis. Proc. Natl. Acad. Sci. USA *109*, 5523–5528.

Seifert, E.L., Estey, C., Xuan, J.Y., and Harper, M.E. (2010). Electron transport chain-dependent and -independent mechanisms of mitochondrial H2O2 emission during long-chain fatty acid oxidation. J. Biol. Chem. *285*, 5748–5758.

Shabalina, I.G., Backlund, E.C., Bar-Tana, J., Cannon, B., and Nedergaard, J. (2008). Within brown-fat cells, UCP1-mediated fatty acid-induced uncoupling is independent of fatty acid metabolism. Biochim. Biophys. Acta *1777*, 642–650.

Singh, R., Kaushik, S., Wang, Y., Xiang, Y., Novak, I., Komatsu, M., Tanaka, K., Cuervo, A.M., and Czaja, M.J. (2009). Autophagy regulates lipid metabolism. Nature *458*, 1131–1135.

Song, Z., Chen, H., Fiket, M., Alexander, C., and Chan, D.C. (2007). OPA1 processing controls mitochondrial fusion and is regulated by mRNA splicing, membrane potential, and Yme1L. J. Cell Biol. *178*, 749–755.

Soriano, F.X., Liesa, M., Bach, D., Chan, D.C., Palacín, M., and Zorzano, A. (2006). Evidence for a mitochondrial regulatory pathway defined by peroxisome proliferator-activated receptor-gamma coactivator-1 alpha, estrogen-related receptor-alpha, and mitofusin 2. Diabetes *55*, 1783–1791.

Tanaka, A., Cleland, M.M., Xu, S., Narendra, D.P., Suen, D.F., Karbowski, M., and Youle, R.J. (2010). Proteasome and p97 mediate mitophagy and degradation of mitofusins induced by Parkin. J. Cell Biol. *191*, 1367–1380.

Tondera, D., Grandemange, S., Jourdain, A., Karbowski, M., Mattenberger, Y., Herzig, S., Da Cruz, S., Clerc, P., Raschke, I., Merkwirth, C., et al. (2009). SLP-2 is required for stress-induced mitochondrial hyperfusion. EMBO J. *28*, 1589–1600.

Twig, G., Elorza, A., Molina, A.J., Mohamed, H., Wikstrom, J.D., Walzer, G., Stiles, L., Haigh, S.E., Katz, S., Las, G., et al. (2008a). Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. EMBO J. *27*, 433–446.

Twig, G., Hyde, B., and Shirihai, O.S. (2008b). Mitochondrial fusion, fission and autophagy as a quality control axis: the bioenergetic view. Biochim. Biophys. Acta *1777*, 1092–1097.

Twig, G., Liu, X., Liesa, M., Wikstrom, J.D., Molina, A.J., Las, G., Yaniv, G., Hajnóczky, G., and Shirihai, O.S. (2010). Biophysical properties of mitochondrial fusion events in pancreatic beta-cells and cardiac cells unravel potential control mechanisms of its selectivity. Am. J. Physiol. Cell Physiol. *299*, C477–C487.

Vives-Bauza, C., Zhou, C., Huang, Y., Cui, M., de Vries, R.L., Kim, J., May, J., Tocilescu, M.A., Liu, W., Ko, H.S., et al. (2010). PINK1-dependent recruitment of Parkin to mitochondria in mitophagy. Proc. Natl. Acad. Sci. USA *107*, 378–383.

Wakabayashi, J., Zhang, Z., Wakabayashi, N., Tamura, Y., Fukaya, M., Kensler, T.W., Iijima, M., and Sesaki, H. (2009). The dynamin-related GTPase Drp1 is required for embryonic and brain development in mice. J. Cell Biol. *186*, 805–816.

Waterham, H.R., Koster, J., van Roermund, C.W., Mooyer, P.A., Wanders, R.J., and Leonard, J.V. (2007). A lethal defect of mitochondrial and peroxisomal fission. N. Engl. J. Med. *356*, 1736–1741.

Wikstrom, J.D., Katzman, S.M., Mohamed, H., Twig, G., Graf, S.A., Heart, E., Molina, A.J., Corkey, B.E., de Vargas, L.M., Danial, N.N., et al. (2007). beta-Cell mitochondria exhibit membrane potential heterogeneity that can be altered by stimulatory or toxic fuel levels. Diabetes *56*, 2569–2578.

Wikstrom, J.D., Twig, G., and Shirihai, O.S. (2009). What can mitochondrial heterogeneity tell us about mitochondrial dynamics and autophagy? Int. J. Biochem. Cell Biol. *41*, 1914–1927.

Williamson, J.R. (1970). Control of energy metabolism in hamster brown adipose tissue. J. Biol. Chem. *245*, 2043–2050.

Wu, J., Boström, P., Sparks, L.M., Ye, L., Choi, J.H., Giang, A.H., Khandekar, M., Virtanen, K.A., Nuutila, P., Schaart, G., et al. (2012). Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell *150*, 366–376.

Wredenberg, A., Freyer, C., Sandström, M.E., Katz, A., Wibom, R., Westerblad, H., and Larsson, N.G. (2006). Respiratory chain dysfunction in skeletal muscle does not cause insulin resistance. Biochem. Biophys. Res. Commun. *350*, 202–207.

Yoon, Y.S., Yoon, D.S., Lim, I.K., Yoon, S.H., Chung, H.Y., Rojo, M., Malka, F., Jou, M.J., Martinou, J.C., and Yoon, G. (2006). Formation of elongated giant mitochondria in DFO-induced cellular senescence: involvement of enhanced fusion process through modulation of Fis1. J. Cell. Physiol. *209*, 468–480.

Zhang, Z., Wakabayashi, N., Wakabayashi, J., Tamura, Y., Song, W.J., Sereda, S., Clerc, P., Polster, B.M., Aja, S.M., Pletnikov, M.V., et al. (2011). The dynamin-related GTPase Opa1 is required for glucose-stimulated ATP production in pancreatic beta cells. Mol. Biol. Cell *22*, 2235–2245.

Ziviani, E., Tao, R.N., and Whitworth, A.J. (2010). Drosophila parkin requires PINK1 for mitochondrial translocation and ubiquitinates mitofusin. Proc. Natl. Acad. Sci. USA *107*, 5018–5023.