Published in final edited form as:

Trends Endocrinol Metab. 2009 May ; 20(4): 186–193. doi:10.1016/j.tem.2009.01.004.

Mitochondrial protein phosphorylation: instigator or target of lipotoxicity?

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Abstract

Lipotoxicity occurs as a consequence of chronic exposure of non-adipose tissue and cells to elevated concentrations of fatty acids, triglycerides and/or cholesterol. The contribution of mitochondria to lipotoxic cell dysfunction, damage and death is associated with elevated production of reactive oxygen species and initiation of apoptosis. Although there is a broad consensus on the involvement of these phenomena with lipotoxicity, the molecular mechanisms that initiate, mediate and trigger mitochondrial dysfunction in response to substrate overload remain unclear. Here, we focus on protein phosphorylation as an important phenomenon in lipotoxicity that harms mitochondria-related signal transduction and integration in cellular metabolism. Moreover, the degradation of mitochondria by mitophagy is discussed as an important landmark that leads to cellular apoptosis in lipotoxicity.

Mitochondrial relay function

The term ‘lipotoxicity’ describes the dysfunction of non-adipose tissue and cells that face chronic exposure to elevated fatty acids, triglycerides and/or cholesterol [1–3]. When plasma levels of prandial and postprandial fatty acids, triglycerides or cholesterol exceed the uptake capacity of adipose tissue, non-adipose tissue becomes overloaded with lipids, resulting in several metabolic imbalances and/or diseases. Once the oxidative capacity of these cells is exhausted, lipotoxic pathways are initiated that yield cellular dysfunction and, at worst, result in cell death. Although multiple lipotoxic events have been described [1–3] (e.g. induction of endoplasmic reticulum stress [4,5] by disruption of its structural integrity [6]) recent findings point to mitochondrial dysfunction as a key phenomenon in lipotoxicity leading to ominous signals [7–11].

The mitochondria not only serve as the power plant of the cell but also act as a cellular relay, sensing multiple cellular and environmental parameters, including energy status, oxygen availability and activation patterns of cellular signal-transduction pathways. Mitochondria then integrate these inputs, adapting their activity and functions to, ultimately, tune cellular responsiveness with respect to the current demand of the cell [12] (Figure 1). In spite of the important tasks of the mitochondrion and in contrast to its functions in energy metabolism, its contribution to signal transduction has not been thoroughly investigated, and the

connection between its role as a powerhouse and that of a metabolic sensor under conditions of balanced and imbalanced substrate supply remains mostly unclear. Nevertheless, excessive nutrition of cells by either lipids (i.e. free fatty acids, triglycerides and phospholipids) or D-glucose not only represents a tremendous metabolic challenge but also affects the functionality of this organelle [13]. Thus, in most metabolic disorders, mitochondrial dysfunction occurs regardless of substrate type and contributes to cell and tissue dysfunction [12]. However, most reports focus on excessive mitochondrial reactive oxygen species (ROS) production and mitochondrial-dependent apoptosis as the common endpoint of such a calamitous development [14], and little is known about the molecular events that are responsible for initial changes in the mitochondrial relay function to substrate overload and the consequences for the cell signal-transduction machinery. Here, we highlight the recent literature that describes protein phosphorylation as crucial for the contribution of mitochondria to cellular signal transduction and the organelle's adaptation and dysfunction following substrate overflow. Accordingly, we enforce the concept of mitochondria as a signaling platform that integrates the cell's metabolic status with signal transduction and discuss protein phosphorylation in the mitochondria and mitophagy as early putative players in lipotoxicity, preceding mitochondrial-triggered cell death.

Controlling mitochondrial signaling with phosphorylation and dephosphorylation

So far, more than 60 mitochondrial proteins, including many proteins of the inner mitochondrial membranes (such as subunits of the respiratory-chain complexes or the adenine nucleotide transporter), have been identified as phosphoproteins [15], thus indicating an existing mitochondrial phosphoproteome. In addition to mitochondrial protein phosphorylation by matrix kinases, the existence of specific mitochondrial phosphatases represents an exciting new area of research [15–17]. In fact, reversible protein phosphorylation represents a potentially important but so far largely unnoticed principle in regulating mitochondrial function(s). Cytosolic phosphatases, for example, have been localized in the mitochondrial matrix (e.g. the regulatory and catalytic subunits of protein phosphatase 2A [PP2A], mitogen-activated protein kinase phosphatase 1 and dual-specificity phosphatase 3) [18]; therefore, there is growing evidence for mitochondria-specific phosphatases. In the inner membrane and matrix of mitochondria, a serine (threonine) phosphatase acting on cyclic adenosine monophosphate (cAMP)-dependent phosphoproteins, such as complex I, was shown to play an important part in regulating mitochondria respiratory function [19]. Furthermore, Pagliarini and co-workers [20] identified a specific mitochondrial-localized phosphotyrosine phosphatase, protein tyrosine phosphatase localized to the mitochondrion 1, that is crucially involved in ATP synthesis and insulin secretion. Thus, beside altered kinase activity, modulation of matrix protein phosphatase activity represents an intriguing mechanism for controlling mitochondrial signaling function and warrants further investigation.

The dark side of mitochondrial protein phosphorylation

Although kinase activation under distinct, sometimes pathological, conditions represents a well-explored process in the regulation of cytosolic signal transduction, little is known about the contribution of protein dephosphorylation to mitochondrial signaling and signal transduction, metabolism and (dys)function under homeostatic conditions and conditions of substrate overload that lead to lipotoxicity. Because of their fundamental importance to cell homeostasis by governing signal-transduction processes, kinases – especially members of the Rous sarcoma virus oncogene protein kinases (Src) and protein kinase C (PKC) families – are commonly crucial in various pathologies. Intriguingly, homologous to the Src and/or PKC activation was found to govern multiple pathological processes in hyperglycemia [21–23] and lipotoxicity [24,25]. Because similar pathways were also described as leading to cellular differentiation [17], the actual physiological outcome might depend on the pattern of activated pathways rather than on a single phenomenon. Thus, it is feasible that changes in the mitochondrial phosphoproteome caused by alterations in kinase activities are fundamental for the decisive setting of signaling patterns for cell survival or the initiation of cellular dysfunction in lipotoxicity leading to cell death. Three different scenarios affecting mitochondrial protein phosphorylation can be postulated in lipotoxicity (Figure 2). First, increased substrate accumulation within the mitochondria in response to an excessive nutrition supply directly activates constitutive matrix kinases and leads to reversible protein phosphorylation that alters mitochondrial function. Second, lipotoxic conditions initiate cytosolic stress and trigger phosphorylation of distinct extramitochondrial mediator proteins that subsequently enter the mitochondria and yield mitochondrial dysfunction. Third, upon cytosolic stress, kinases associate at the inner mitochondrial membrane and affect mitochondrial homeostasis by phosphorylating crucial ion transporters that are essential for the proper functioning of the organelle. These three scenarios are discussed below.

Reversible protein phosphorylation by constitutive kinases as instigators for lipotoxicity

So far, evidence for the role of reversible protein phosphorylation as a key phenomenon for mitochondrial regulation comes from mitochondrial enzymes that are involved in metabolism and energy supply, such as mitochondrial pyruvate dehydrogenase [26,27], glycogen synthase kinase 3 β [28,29], and enzymes of fatty acid oxidation and the Krebs cycle [18]. However, little is known regarding how cytosolic signals are transferred into the mitochondria via protein phosphorylation.

It has been shown that mitochondrial kinases and phosphatases are under the control of intraluminal Ca²⁺ [18], an effector that is already known to adjust the activity of many metabolic and anti-oxidative enzymes within mitochondria [12,17]. This observation indicates that mitochondrial Ca²⁺ accumulation is a crucial phenomenon for the immediate regulation of mitochondrial protein phosphorylation.

p66Shc as a link between cytosolic protein phosphorylation and mitochondrial dysfunction in lipotoxicity

In addition to the classical modulation of mitochondrial proteins by phosphorylation, the shuttling of proteins from the cytosol or endoplasmic reticulum into the mitochondria upon

their phosphorylation by cytosolic kinases represents an important mechanism in pathology and aging. For example, the translocation of pro- and antiapoptotic Bcl-2 family members is frequently regulated by phosphorylation–dephosphorylation cycles [30]. In this respect, the Src-homology-2-domain-containing transforming protein 1 (p66Shc), which is predominantly localized in the cytosol [31] but also shown to interact with the mitochondrial heat-shock protein 70 [32], perhaps represents the most appealing factor. Under conditions of cytosolic oxidative stress, such as occurs under lipotoxic conditions or hyperglycemia, PKC β phosphorylates cytosolic p66Shc, which then binds to Pin1 and translocates into the intermembrane space, where it is activated, perhaps via phosphatase PP2A-mediated dephosphorylation [33,34]. In the intermembrane space, electron transfer between cytochrome c and activated p66Shc results in ROS generation that can initiate mitochondrial-induced apoptosis [35] (Figure 2). Although p66Shc-triggered ROS production is initiated by Ca²⁺ and accompanied by alterations in mitochondrial Ca²⁺ homeostasis [36], its effect on mitochondria does not necessarily depend on cytosolic Ca²⁺ elevation [37]. In fact, deletion of the p66Shc longevity gene has been associated with reduced systemic and organ oxidative stress and a delay in the development of atherosclerosis in high-fat-fed mice [38], indicating the potential involvement of p66Shc in lipid-associated vascular disease and lipotoxicity. In line with this assumption of an involvement of p66Shc in lipotoxicity, glucose-induced activation of PKC β has been reported [39]. Intriguingly, decreased methylation of the p66Shc promoter, which corresponded to elevated plasma levels of S-adenosylhomocysteine, has been reported in patients with end-stage renal disease [40]. These data indicate that p66Shc expression is subject to epigenetic modifications that might contribute to the susceptibility of patients to lipotoxicity and to the development of atherosclerosis (Figure 2).

Mitochondrial anchoring of kinases in promoting lipotoxicity

Although kinases that have been found in the mitochondria [17,18] might be *a priori* incorporated into the mitochondria as a consequence of continuous protein uptake, many kinases are specifically anchored to or into the mitochondria (or are recruited by the mitochondria) in response to a certain metabolic state [15] or distinct signaling events, such as Ca²⁺ elevation [18]. In view of the efficient protein-import machinery in mitochondria membranes that encompasses complexes of the translocases in the outer and inner membranes of mitochondria [41,42], one might expect that protein kinases utilize the existing machinery to enter the mitochondria. Although there is no evidence for separate pathway(s) and/or mechanism(s) to enter and/or associate with the mitochondria in response to specific signals and/or (pathological) conditions, the importance of anchoring proteins – such as A-kinase anchor protein 121 (AKAP121) [43–45], the downstream of kinase family member 4 [46] and protein tyrosine phosphatase D1 (PTPD1) – for the mitochondrial anchoring of cytosolic Src kinase (c-Src) might point to some specific features of kinase import into the mitochondria. At least four members of the Src family (Fgr, Fyn, Lyn and c-Src) and the C-terminal Src kinase have been found to be associated with mitochondria [17,44,47] and to represent the major kinases in reversible tyrosine phosphorylation of target proteins within mitochondria [48,49]. In the case of AKAP121 and PTPD1, c-Src has been shown to anchor the activated enzyme to the outer mitochondrial membrane [43,50]; however, recent evidence describing AKAP121 on the inner mitochondrial membrane [51]

also points to a possible association of Src family kinases with proteins of the inner mitochondrial membrane and supports the concept of reversible protein phosphorylation as an intriguing regulator of mitochondrial ion-carrier activity and, subsequently, their impact on the function of the organelle.

Uncoupling proteins as putative targets of kinases in lipotoxicity

Uncoupling protein (UCP)-2 and -3 contribute to fatty acid metabolism, counteract mitochondrial ROS production and are involved in the organelle's response in lipotoxicity [7,52–56]. In view of the obvious importance of mitochondrial Ca^{2+} uptake for regulating mitochondrial protein phosphorylation [18], it is notable that UCP2 and UCP3 facilitate mitochondrial Ca^{2+} sequestration [57,58], and this leads us to propose that UCP2 and UCP3 operate as an interface between mitochondrial energy metabolism and Ca^{2+} -dependent signal transduction for tuning the organelle's phosphorylation activity with respect to its metabolic obligation [59,60]. Moreover, UCPs might themselves be subject to reversible phosphorylation that modulates and/or controls their activity and/or functions [52,53,55,61]. Indeed, phosphorylation of serine residues on UCP1 was recently shown to be involved in cold acclimation [62], thus indicating phosphorylation of non-metabolic enzymes as a potential mechanism to modulate mitochondrial function. Further evidence for modulating UCP2 and UCP3 activity by phosphorylation comes from their involvement in mitochondrial Ca^{2+} uniporter activity [57,58]; recent findings have shown a modulatory role of PKC family members [63,64] and mitogen-activated protein kinase p38 (p38MAPK) [64,65] on mitochondrial Ca^{2+} sequestration. However, the role for kinases in targeting the uniporter and the role of UCP2 and UCP3 in Ca^{2+} conductance needs further investigation. It is to be noted that the effect of PKC and p38MAPK on mitochondrial Ca^{2+} sequestration might not be strong evidence that UCP2 and UCP3 activity can be modulated by phosphorylation. Interestingly, however, mitochondrial Ca^{2+} accumulation is bidirectionally modulated by PKC family members; the PKC β isoform reduces and the PKC ζ isoform increases mitochondrial Ca^{2+} uptake [63]. Based on motif scans (http://www.hprd.org/PhosphoMotif_finder), UCP2 has five (two threonine, two serine and one threonine/serine) and two (one serine and one threonine/serine) predicted phosphorylation sites for PKC and casein kinase II, respectively. Additionally, UCP3 has seven (four threonine and three serine), two (one serine and one threonine/serine) and one predicted phosphorylation site(s) for PKC, casein kinase II and cAMP-dependent protein kinase, respectively. Thus, it is possible that UCP is phosphorylated by these enzymes.

The only described interacting partners of UCP2 and UCP3 (but not UCP1), so far, are proteins of the 14–3–3 family [66]. These scaffold proteins preferentially bind to serine phosphorylation sites [67], and binding of 14–3–3 with its respective phosphopeptide is augmented by divalent cations, such as Ca^{2+} or Mg^{2+} . Remarkably, UCP2 (AA155–158) and UCP3 (AA157–160) but not UCP1 share an Arg-Xaa-Xaa-Ser serine phosphorylation motif for 14–3–3 binding at the N-terminal side of the second intermembrane loop, a mutation of which was found to result in functionally inactive UCP mutants [57,58]. Although the existence of such phosphorylation sites in UCP2 and UCP3 does not confer function, considering the existence of kinases and phosphatases in the mitochondrial matrix [16], the ability of UCP2 and UCP3 to specifically interact with 14–3–3 [66] and our own recent

findings using inactive mutants that might be defective in 14–3–3 binding [58] maintain such a possibility. However, whether reversible protein phosphorylation represents a regulatory mechanism for UCP2- and UCP3-dependent function remains unanswered. Such reversible protein phosphorylation might also affect Ca^{2+} -dependent signal transduction because of the mitochondria's elementary contribution to Ca^{2+} signaling [14,68–70] and, in addition, lipid metabolism owing to the protein's activity as a fatty acid carrier [71–73]. In line with this assumption, the involvement of UCPs as guardians against lipotoxicity has been proposed [9,10,74]. Accordingly, in view of the counteracting function of UCP2 and UCP3 against lipotoxicity [7–10,53,74–77] and their sensitivity to ROS [78–81], the involvement of ROS-mediated phosphorylation in the regulation and/or adaptation of UCP2 and UCP3 activity in lipotoxicity can be postulated and requires further investigation.

Besides UCPs, the voltage-dependent anion channels 1 and 2 that are located in the outer mitochondrial membrane have been found to be phosphorylated in a Ca^{2+} -dependent manner [18]. However, our present knowledge of whether additional ion transporters or key enzymes for mitochondrial regulation and function are reversibly phosphorylated is hindered by the still unrevealed identity of most of the mitochondrial ion carriers and channels [60]. Nevertheless, there is increasing evidence for a role of reversible protein phosphorylation of mitochondrial ion transporters by cytosolic or matrix kinases [62–65], thus pointing to a contribution of protein phosphorylation to the signaling function of the organelle (Figure 2).

Mitophagy as a potential endpoint of lipotoxic changes after mitochondrial protein phosphorylation

There is accumulating evidence that mitochondrial membrane instability and altered ion flux triggered by numerous cellular phenomena (including ROS production, protein phosphorylation and modulation of UCP transcription) regulates macro-autophagy and cell death under a variety of processes in health and disease [82,83]. So far, mitophagy has been associated with a loss of mitochondrial membrane potential and a transient increase in UCP3 levels upon lipopolysaccharide exposure [84], the appearance of mitochondrial ROS [83,85–87], ionic imbalances [88] and high levels of mitochondrial fission protein 1 [89]. Thus, mitophagy has been causally associated with phenomena that are also reported as a consequence of lipotoxicity, indicating lipotoxicity as a feasible initiator of mitophagic processes. For more information on mitophagy, see Box 1. Moreover, recent evidence that points to mitochondrial localization of mitogen-activated protein kinase p42 as a key regulator for mitophagy convincingly demonstrates an important contribution of mitochondrial protein phosphorylation to mitophagy during cell stress [90]. In line with this assumption, parkinsonian-neurotoxin-induced mitophagy is dependent on MAPK signaling [91], and a yeast mitochondrial protein phosphatase homolog, Aup1p, that is similar to a family of protein phosphatase homologs in eukaryotic cells was described to be part of a signal-transduction mechanism that marks mitochondria for sequestration into autophagosomes [92]. Although autophagy is principally thought to be a crucial step in housekeeping and cellular adaptation to serious stress conditions, such as starvation [93] or infection [94], disturbed autophagy was also found to be associated with human diseases, including cancer [95], neurodegenerative diseases [96] and aging [93,94]. Accordingly, the

involvement of altered mitophagy in lipotoxicity that is due to phosphorylation events and promotes cellular dysfunction or even cell death seems to be a likely phenomenon and awaits further investigation.

Concluding remarks and future directions

The reversible phosphorylation of mitochondrial ion transporters represents an appealing mechanism for controlling mitochondrial activity by environment. Moreover, considering the involvement of kinases in pathological processes initiated by nutrition overload, mitochondrial ion carriers and enzymes might be the subjects of improper protein phosphorylation by pathologically activated kinases that, in turn, might transfer cellular stress to mitochondria; this might explain the strong connection between this organelle and cellular dysfunction in disease [14,97,98].

Altered protein phosphorylation in the course of lipotoxicity can have three lines of action. First, the enhanced substrate flow might exceed the oxidative capacity of the mitochondria and directly result in fatty acid oxidation and the generation of lipotoxic derivatives and ROS, leading to dysfunction of the mitochondrial phosphoproteome. Second, altered phosphorylation activity in the cytoplasm that yields activation of protein mediators such as p66Shc might cause their translocation to the mitochondria and trigger calamitous phenomena, such as ROS generation or opening of the permeability transition pore. Third, lipotoxicity-activated kinases might associate with the inner mitochondrial membrane and manipulate the activity of ion transporters, resulting in mitochondrial dysfunction because of ionic imbalance. These processes might trigger not only apoptosis but also mitophagy as an intriguing, yet underestimated, phenomenon of lipotoxic cell damage. After all, it is the interaction of the mitochondria with their cellular environment that reveals the organelle's fate and contribution to life or death. Future research will dissect the individual mechanisms of this interplay to provide not simply the basis of understanding but enough information to explore putative therapeutic intervention targets against lipotoxicity, which is certainly a noteworthy burden on today's human health.

Acknowledgements

The authors' research on mitochondrial ion homeostasis and the organelle's contribution to lipotoxicity is funded by the Austrian Science Funds (FWF, P20181-B05 and F3010-B05 to W.F.G.), the Franz-Lanyar-Stiftung (to W.F.G.) and the Austrian GenAu (GOLD II-C6 to G.M.K.).

References

1. Unger RH. Lipotoxic diseases. *Annu Rev Med.* 2002; 53:319–336. [PubMed: 11818477]
2. Schaffer JE. Lipotoxicity: when tissues overeat. *Curr Opin Lipidol.* 2003; 14:281–287. [PubMed: 12840659]
3. Unger RH. Minireview: weapons of lean body mass destruction: the role of ectopic lipids in the metabolic syndrome. *Endocrinology.* 2003; 144:5159–5165. [PubMed: 12960011]
4. Cunha DA, et al. Initiation and execution of lipotoxic ER stress in pancreatic β -cells. *J Cell Sci.* 2008; 121:2308–2318. [PubMed: 18559892]
5. Cnop M, et al. An update on lipotoxic endoplasmic reticulum stress in pancreatic beta-cells. *Biochem Soc Trans.* 2008; 36:909–915. [PubMed: 18793160]

6. Borradaile NM, et al. Disruption of endoplasmic reticulum structure and integrity in lipotoxic cell death. *J Lipid Res.* 2006; 47:2726–2737. [PubMed: 16960261]
7. Schrauwen P, Hesselink MK. The role of uncoupling protein 3 in fatty acid metabolism: protection against lipotoxicity? *Proc Nutr Soc.* 2004; 63:287–292. [PubMed: 15294045]
8. Schrauwen P, Hesselink MK. Oxidative capacity, lipotoxicity, and mitochondrial damage in type 2 diabetes. *Diabetes.* 2004; 53:1412–1417. [PubMed: 15161742]
9. Schrauwen P, et al. Effect of acute exercise on uncoupling protein 3 is a fat metabolism-mediated effect. *Am J Physiol Endocrinol Metab.* 2002; 282:E11–E17. [PubMed: 11739077]
10. Schrauwen P, et al. Uncoupling protein 3 as a mitochondrial fatty acid anion exporter. *FASEB J.* 2003; 17:2272–2274. [PubMed: 14525936]
11. Turner N, Heilbronn LK. Is mitochondrial dysfunction a cause of insulin resistance? *Trends Endocrinol Metab.* 2008; 19:324–330. [PubMed: 18804383]
12. Goldenthal MJ, Marin-Garcia J. Mitochondrial signaling pathways: a receiver/integrator organelle. *Mol Cell Biochem.* 2004; 262:1–16. [PubMed: 15532704]
13. Dulloo AG, et al. Substrate cycling between *de novo* lipogenesis and lipid oxidation: a thermogenic mechanism against skeletal muscle lipotoxicity and glucolipotoxicity. *Int J Obes Relat Metab Disord.* 2004; 28:S29–S37. [PubMed: 15592483]
14. Duchen MR. Mitochondria and Ca^{2+} in cell physiology and pathophysiology. *Cell Calcium.* 2000; 28:339–348. [PubMed: 11115373]
15. Pagliarini DJ, Dixon JE. Mitochondrial modulation: reversible phosphorylation takes center stage? *Trends Biochem Sci.* 2006; 31:26–34. [PubMed: 16337125]
16. Horbinski C, Chu CT. Kinase signaling cascades in the mitochondrion: a matter of life or death. *Free Radic Biol Med.* 2005; 38:2–11. [PubMed: 15589366]
17. McBride HM, et al. Mitochondria: more than just a powerhouse. *Curr Biol.* 2006; 16:R551–R560. [PubMed: 16860735]
18. Hopper RK, et al. Mitochondrial matrix phosphoproteome: effect of extra mitochondrial calcium. *Biochemistry.* 2006; 45:2524–2536. [PubMed: 16489745]
19. Signorile A, et al. Serine (threonine) phosphatase(s) acting on cAMP-dependent phosphoproteins in mammalian mitochondria. *FEBS Lett.* 2002; 512:91–94. [PubMed: 11852058]
20. Pagliarini DJ, et al. Involvement of a mitochondrial phosphatase in the regulation of ATP production and insulin secretion in pancreatic beta cells. *Mol Cell.* 2005; 19:197–207. [PubMed: 16039589]
21. Schaeffer G, et al. Intercellular signalling within vascular cells under high D-glucose involves free radical-triggered tyrosine kinase activation. *Diabetologia.* 2003; 46:773–783. [PubMed: 12811469]
22. Spitaler MM, Graier WF. Vascular targets of redox signalling in diabetes mellitus. *Diabetologia.* 2002; 45:476–494. [PubMed: 12032623]
23. Kominato R, et al. Src activation generates reactive oxygen species and impairs metabolism-secretion coupling in diabetic Goto-Kakizaki and ouabain-treated rat pancreatic islets. *Diabetologia.* 2008; 51:1226–1235. [PubMed: 18449527]
24. Russell AP. Lipotoxicity: the obese and endurance-trained paradox. *Int J Obes Relat Metab Disord.* 2004; 28(Suppl. 4):S66–S71. [PubMed: 15592489]
25. Giannarelli R, et al. Reducing insulin resistance with metformin: the evidence today. *Diabetes Metab.* 2003; 6S28–35. [PubMed: 14502098]
26. Linn TC, et al. Alpha-keto acid dehydrogenase complexes. X. Regulation of the activity of the pyruvate dehydrogenase complex from beef kidney mitochondria by phosphorylation and dephosphorylation. *Proc Natl Acad Sci U S A.* 1969; 62:234–241. [PubMed: 4306045]
27. Holness MJ, Sugden MC. Regulation of pyruvate dehydrogenase complex activity by reversible phosphorylation. *Biochem Soc Trans.* 2003; 31:1143–1151. [PubMed: 14641014]
28. Moule SK, et al. Regulation of protein kinase B and glycogen synthase kinase-3 by insulin and beta-adrenergic agonists in rat epididymal fat cells. Activation of protein kinase B by wortmannin-sensitive and -insensitive mechanisms. *J Biol Chem.* 1997; 272:7713–7719. [PubMed: 9065430]
29. Cross DA, et al. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature.* 1995; 378:785–789. [PubMed: 8524413]

30. Er E, et al. Mitochondria as the target of the pro-apoptotic protein Bax. *Biochim Biophys Acta*. 2006; 1757:1301–1311. [PubMed: 16836974]
31. Nemoto S, et al. The mammalian longevity-associated gene product p66shc regulates mitochondrial metabolism. *J Biol Chem*. 2006; 281:10555–10560. [PubMed: 16481327]
32. Orsini F, et al. The life span determinant p66Shc localizes to mitochondria where it associates with mitochondrial heat shock protein 70 and regulates trans-membrane potential. *J Biol Chem*. 2004; 279:25689–25695. [PubMed: 15078873]
33. Pinton P, et al. Protein kinase C beta and prolyl isomerase 1 regulate mitochondrial effects of the life-span determinant p66Shc. *Science*. 2007; 315:659–663. [PubMed: 17272725]
34. Hajnóczky G, Hoek JB. Cell signalling. Mitochondrial longevity pathways. *Science*. 2007; 315:607–609. [PubMed: 17272709]
35. Giorgio M, et al. Electron transfer between cytochrome c and p66Shc generates reactive oxygen species that trigger mitochondrial apoptosis. *Cell*. 2005; 122:221–233. [PubMed: 16051147]
36. Pinton P, Rizzuto R. p66Shc, oxidative stress and aging: Importing a lifespan determinant into mitochondria. *Cell Cycle*. 2008; 7:304–308. [PubMed: 18235239]
37. Pellegrini M, et al. p66SHC promotes T cell apoptosis by inducing mitochondrial dysfunction and impaired Ca²⁺ homeostasis. *Cell Death Differ*. 2007; 14:338–347. [PubMed: 16794602]
38. Napoli C, et al. Deletion of the p66Shc longevity gene reduces systemic and tissue oxidative stress, vascular cell apoptosis, and early atherogenesis in mice fed a high-fat diet. *Proc Natl Acad Sci U S A*. 2003; 100:2112–2116. [PubMed: 12571362]
39. Pinton P, et al. Dynamics of glucose-induced membrane recruitment of protein kinase C beta II in living pancreatic islet beta-cells. *J Biol Chem*. 2002; 277:37702–37710. [PubMed: 12149258]
40. Geisel J, et al. Decreased p66Shc promoter methylation in patients with end-stage renal disease. *Clin Chem Lab Med*. 2007; 45:1764–1770. [PubMed: 18067454]
41. Neupert W. Protein import into mitochondria. *Annu Rev Biochem*. 1997; 66:863–917. [PubMed: 9242927]
42. Pfanner N, Meijer M. The Tom and Tim machine. *Curr Biol*. 1997; 7:R100–R103. [PubMed: 9081657]
43. Cardone L, et al. Mitochondrial AKAP121 binds and targets protein tyrosine phosphatase D1, a novel positive regulator of src signaling. *Mol Cell Biol*. 2004; 24:4613–4626. [PubMed: 15143158]
44. Salvi M, et al. Tyrosine phosphorylation in mitochondria: a new frontier in mitochondrial signaling. *Free Radic Biol Med*. 2005; 38:1267–1277. [PubMed: 15855046]
45. Carlucci A, et al. Proteolysis of AKAP121 regulates mitochondrial activity during cellular hypoxia and brain ischaemia. *EMBO J*. 2008; 27:1073–1084. [PubMed: 18323779]
46. Itoh S, et al. Mitochondrial Dok-4 recruits Src kinase and regulates NF- κ B activation in endothelial cells. *J Biol Chem*. 2005; 280:26383–26396. [PubMed: 15855164]
47. Miyazaki T, et al. Regulation of cytochrome c oxidase activity by c-Src in osteoclasts. *J Cell Biol*. 2003; 160:709–718. [PubMed: 12615910]
48. Arachiche A, et al. Localization of PTP-1B, SHP-2, and Src exclusively in rat brain mitochondria and functional consequences. *J Biol Chem*. 2008; 283:24406–24411. [PubMed: 18583343]
49. Tibaldi E, et al. Src-Tyrosine kinases are major agents in mitochondrial tyrosine phosphorylation. *J Cell Biochem*. 2008; 104:840–849. [PubMed: 18247338]
50. Livigni A, et al. Mitochondrial AKAP121 links cAMP and src signaling to oxidative metabolism. *Mol Biol Cell*. 2006; 17:263–271. [PubMed: 16251349]
51. Sardanelli AM, et al. Occurrence of A-kinase anchor protein and associated cAMP-dependent protein kinase in the inner compartment of mammalian mitochondria. *FEBS Lett*. 2006; 580:5690–5696. [PubMed: 16996504]
52. Brand MD, et al. Mitochondrial superoxide: production, biological effects, and activation of uncoupling proteins. *Free Radic Biol Med*. 2004; 37:755–767. [PubMed: 15304252]
53. Brand MD, Esteves TC. Physiological functions of the mitochondrial uncoupling proteins UCP2 and UCP3. *Cell Metab*. 2005; 2:85–93. [PubMed: 16098826]

54. Hoeks J, et al. Involvement of UCP3 in mild uncoupling and lipotoxicity. *Exp Gerontol.* 2006; 41:658–662. [PubMed: 16564663]
55. Jezek P, et al. Mitochondrial uncoupling proteins – facts and fantasies. *Physiol Res.* 2004; 53:S199–S211. [PubMed: 15119950]
56. Joseph JW, et al. Free fatty acid-induced beta-cell defects are dependent on uncoupling protein 2 expression. *J Biol Chem.* 2004; 279:51049–51056. [PubMed: 15448158]
57. Trenker M, et al. UCP2/3 – likely to be fundamental for mitochondrial Ca^{2+} uniport. *Nat Cell Biol.* 2008; 10:1237–1240.
58. Trenker M, et al. Uncoupling proteins 2 and 3 are fundamental for mitochondrial Ca^{2+} uniport. *Nat Cell Biol.* 2007; 9:445–452. [PubMed: 17351641]
59. Graier WF, et al. Mitochondrial Ca^{2+} , the secret behind the function of uncoupling proteins 2 and 3? *Cell Calcium.* 2008; 44:36–50. [PubMed: 18282596]
60. Graier WF, et al. Mitochondria and Ca^{2+} signaling: old guests, new functions. *Pflugers Arch.* 2007; 455:375–396. [PubMed: 17611770]
61. Jezek P, et al. Possible physiological roles of mitochondrial uncoupling proteins-UCPn: Mechanism of uncoupling protein action. *Int J Biochem Cell Biol.* 2002; 34:1190–1206. [PubMed: 12127570]
62. Carroll AM, et al. Identification of serine phosphorylation in mitochondrial uncoupling protein 1. *Biochim Biophys Acta.* 2008; 1777:1060–1065. [PubMed: 18486593]
63. Pinton P, et al. Long-term modulation of mitochondrial Ca^{2+} signals by protein kinase C isozymes. *J Cell Biol.* 2004; 165:223–232. [PubMed: 15096525]
64. Szanda G, et al. Participation of p38 MAPK and a novel-type protein kinase C in the control of mitochondrial Ca^{2+} uptake. *Cell Calcium.* 2008; 43:250–259. [PubMed: 17628663]
65. Montero M, et al. A novel regulatory mechanism of the mitochondrial Ca^{2+} uniporter revealed by the p38 mitogen-activated protein kinase inhibitor SB202190. *FASEB J.* 2002; 16:1955–1957. [PubMed: 12368236]
66. Pierrat B, et al. Uncoupling proteins 2 and 3 interact with members of the 14-3-3 family. *Eur J Biochem.* 2000; 267:2680–2687. [PubMed: 10785390]
67. Muslin AJ, et al. Interaction of 14-3-3 with signaling proteins is mediated by the recognition of phosphoserine. *Cell.* 1996; 84:889–897. [PubMed: 8601312]
68. Sorrentino V, Rizzuto R. Molecular genetics of Ca^{2+} stores and intracellular Ca^{2+} signalling. *Trends Pharmacol Sci.* 2001; 22:459–464. [PubMed: 11543873]
69. Nicholls DG. Mitochondria and calcium signaling. *Cell Calcium.* 2005; 38:311–317. [PubMed: 16087232]
70. Duchen MR. Mitochondria and calcium: from cell signalling to cell death. *J Physiol.* 2000; 529:57–68. [PubMed: 11080251]
71. Himms-Hagen J, Harper ME. Physiological role of UCP3 may be export of fatty acids from mitochondria when fatty acid oxidation predominates: an hypothesis. *Exp Biol Med.* 2001; 226:78–84.
72. Jaburek M, et al. Hydroperoxy fatty acid cycling mediated by mitochondrial uncoupling protein UCP2. *J Biol Chem.* 2004; 279:53097–53102. [PubMed: 15475368]
73. Seifert EL, et al. Essential role for UCP3 in mitochondrial adaptation to fasting but not in fatty acid oxidation or fatty acid anion export. *J Biol Chem.* 2008; 283:25124–25131. [PubMed: 18628202]
74. Echtay KS. Mitochondrial uncoupling proteins – what is their physiological role? *Free Radic Biol Med.* 2007; 43:1351–1371. [PubMed: 17936181]
75. Hagen T, Vidal-Puig A. Mitochondrial uncoupling proteins in human physiology and disease. *Minerva Med.* 2002; 93:41–57. [PubMed: 11850613]
76. Nedergaard J, et al. Uncoupling proteins: current status and therapeutic prospects. *EMBO Rep.* 2005; 6:917–921. [PubMed: 16179945]
77. Nicholls DG. The physiological regulation of uncoupling proteins. *Biochim Biophys Acta.* 2006; 1757:459–466. [PubMed: 16725104]
78. Echtay KS, Brand MD. 4-hydroxy-2-nonenal and uncoupling proteins: an approach for regulation of mitochondrial ROS production. *Redox Rep.* 2007; 12:26–29. [PubMed: 17263904]

79. Echtay KS, et al. A signalling role for 4-hydroxy-2-nonenal in regulation of mitochondrial uncoupling. *EMBO J.* 2003; 22:4103–4110. [PubMed: 12912909]
80. Echtay KS, et al. Superoxide activates mitochondrial uncoupling protein 2 from the matrix side. Studies using targeted antioxidants. *J Biol Chem.* 2002; 277:47129–47135. [PubMed: 12372827]
81. Echtay KS, et al. Superoxide activates mitochondrial uncoupling proteins. *Nature.* 2002; 415:96–99. [PubMed: 11780125]
82. Kissová I, et al. Selective and non-selective autophagic degradation of mitochondria in yeast. *Autophagy.* 2007; 3:329–336. [PubMed: 17377488]
83. Vaquero EC, et al. Tocotrienols: balancing the mitochondrial crosstalk between apoptosis and autophagy. *Autophagy.* 2007; 3:652–654. [PubMed: 17932465]
84. Hickson-Bick DL, et al. Stimulation of mitochondrial biogenesis and autophagy by lipopolysaccharide in the neonatal rat cardiomyocyte protects against programmed cell death. *J Mol Cell Cardiol.* 2008; 44:411–418. [PubMed: 18062988]
85. Kim EH, et al. Sodium selenite induces superoxide-mediated mitochondrial damage and subsequent autophagic cell death in malignant glioma cells. *Cancer Res.* 2007; 67:6314–6324. [PubMed: 17616690]
86. Kim I, et al. Selective degradation of mitochondria by mitophagy. *Arch Biochem Biophys.* 2007; 462:245–253. [PubMed: 17475204]
87. Lemasters JJ. Selective mitochondrial autophagy, or mitophagy, as a targeted defense against oxidative stress, mitochondrial dysfunction, and aging. *Rejuvenation Res.* 2005; 8:3–5. [PubMed: 15798367]
88. Nowikovsky K, et al. Mdm38 protein depletion causes loss of mitochondrial K⁺/H⁺ exchange activity, osmotic swelling and mitophagy. *Cell Death Differ.* 2007; 14:1647–1656. [PubMed: 17541427]
89. Gomes LC, Scorrano L. High levels of Fis1, a pro-fission mitochondrial protein, trigger autophagy. *Biochim Biophys Acta.* 2008; 1777:860–866. [PubMed: 18515060]
90. Dagda RK, et al. Mitochondrially localized ERK2 regulates mitophagy and autophagic cell stress: implications for Parkinson's disease. *Autophagy.* 2008; 4:770–782. [PubMed: 18594198]
91. Chu CT, et al. Beclin 1-independent pathway of damage-induced mitophagy and autophagic stress: implications for neurodegeneration and cell death. *Autophagy.* 2007; 3:663–666. [PubMed: 17622797]
92. Tal R, et al. Aup1p, a yeast mitochondrial protein phosphatase homolog, is required for efficient stationary phase mitophagy and cell survival. *J Biol Chem.* 2007; 282:5617–5624. [PubMed: 17166847]
93. Todde V, et al. Autophagy: Principles and significance in health and disease. *Biochim Biophys Acta.* 2009; 1792:3–13. [PubMed: 19022377]
94. Levine B, Kroemer G. Autophagy in the pathogenesis of disease. *Cell.* 2008; 132:27–42. [PubMed: 18191218]
95. Grandemange S, et al. Mitochondrial dynamics and cancer. *Semin Cancer Biol.* 2009; 19:50–56. [PubMed: 19138741]
96. Komatsu M, et al. Loss of autophagy in the central nervous system causes neurodegeneration in mice. *Nature.* 2006; 441:880–884. [PubMed: 16625205]
97. Duchon MR. Roles of mitochondria in health and disease. *Diabetes.* 2004; 53:S96–S102. [PubMed: 14749273]
98. Duchon MR. Mitochondria in health and disease: perspectives on a new mitochondrial biology. *Mol Aspects Med.* 2004; 25:365–451. [PubMed: 15302203]
99. Giorgi C, et al. Ca²⁺ signaling, mitochondria and cell death. *Curr Mol Med.* 2008; 8:119–130. [PubMed: 18336292]
100. Jeong SY, Seol DW. The role of mitochondria in apoptosis. *BMB reports.* 2008; 41:11–22. [PubMed: 18304445]
101. Suen DF, et al. Mitochondrial dynamics and apoptosis. *Genes Dev.* 2008; 22:1577–1590. [PubMed: 18559474]

102. Bras M, et al. Programmed cell death via mitochondria: different modes of dying. *Biochemistry (Mosc.)*. 2005; 70:231–239. [PubMed: 15807663]
103. Yu L, et al. The selectivity of autophagy and its role in cell death and survival. *Autophagy*. 2008; 4:567–573. [PubMed: 18362514]
104. Kanki T, Klionsky DJ. Mitophagy in yeast occurs through a selective mechanism. *J Biol Chem*. 2008; 283:32386–32393. [PubMed: 18818209]
105. Tolkovsky AM, et al. Mitochondrial disappearance from cells: a clue to the role of autophagy in programmed cell death and disease? *Biochimie*. 2002; 84:233–240. [PubMed: 12022954]
106. Tasdemir E, et al. Cell cycle-dependent induction of autophagy, mitophagy and reticulophagy. *Cell Cycle*. 2007; 6:2263–2267. [PubMed: 17890908]
107. Klionsky DJ. The molecular machinery of autophagy: unanswered questions. *J Cell Sci*. 2005; 118:7–18. [PubMed: 15615779]
108. Kundu M, Thompson CB. Autophagy: basic principles and relevance to disease. *Annu Rev Pathol*. 2008; 3:427–455. [PubMed: 18039129]
109. Meijer WH, et al. ATG genes involved in non-selective autophagy are conserved from yeast to man but the selective Cvt and pexophagy pathways also require organism-specific genes. *Autophagy*. 2007; 3:106–116. [PubMed: 17204848]

Box 1.**Mitophagy as a potential endpoint of lipotoxic changes after mitochondrial protein phosphorylation**

Besides apoptosis as a predominant endpoint of mitochondrial dysfunction, which has not been addressed herein because there are numerous excellent, recent reviews available [99–101], mitophagy represents another, perhaps underestimated, consequence of lipotoxicity [102,103]. Mitophagy, the controlled degradation of mitochondria, involves an autophagic sequestration of the organelle and its hydrolytic degradation in the lysosomes [86] and represents the second major protein-degradation pathway in eukaryotes beside the ubiquitin-proteasome pathway [93]. Mitophagy is not a rare event *per se* [104] and is not necessarily associated with cell death. In fact, this process occurs frequently during cell housekeeping in a variety of organisms [93,105] and is cyclically involved during the cell cycle [106] and aging [86]. In analogy to autophagy, two major mitophagic pathways can be postulated: macro-mitophagy and micro-mitophagy. During macro-mitophagy, the organelle is captured in a double-membrane vesicle that fuses with the lysosomal and/or vacuole membrane for further degradation. In the process of micro-mitophagy, mitochondria are directly assimilated by lysosomes and/or vacuoles [93]. The mechanism or mechanisms by which the phagocytotic machinery discriminates between healthy and damaged mitochondria remains unclear and awaits further investigation. It is to be noted, however, that the molecular mechanisms controlling autophagy crucially involve protein phosphorylation [107–109].

Figure 1.

Mitochondrial relay function. Mitochondria are extensively involved in many pivotal signaling processes of the cell and represent a major integration point between metabolic processes and signal transduction. Individual environmental stimuli (input signals) trigger distinct signal-transduction pathways that result in the generation of a vast amount of signaling molecules (mediators). Simultaneously, nutrition and substrates are metabolized and result in mediator production. Thus, mitochondria face multiple input signals that are integrated to release mediators to optimize the cellular homeostasis and responsiveness (cellular effect). Abbreviations: 4HNE, 4-hydroxynonenal; Bax, Bcl-2-associated X protein; Bcl2, B-cell lymphoma 2; CTP-1, mitochondrial citrate transporter 1; FA, fatty acids; FA-CoA, acyl Coenzyme A; NO, nitric oxide; $\text{HO}_2^{\bullet}$, perhydroxyl radical; ROS, reactive oxygen species; $\text{O}_2^{\bullet-}$, superoxide anion; Src, Rous sarcoma virus oncogene protein kinase.

Figure 2.

Lipotoxicity evokes mitochondrial dysfunction by affecting organelle-associated protein phosphorylation. Illustration of the proposed consequences of lipotoxicity on mitochondrial protein phosphorylation: (i) fatty acid overload exceeds the oxidative capacity of the mitochondria, resulting in enhanced radical production that is accompanied by generation of lipotoxic derivatives. ROS can affect constitutive matrix kinases and/or phosphatases, yielding deleterious changes in the mitochondrial phosphoproteome. (ii) Upon excess substrate flow, various enzymes and/or kinases are activated (e.g. Nox, JNK and/or PKC β), leading to phosphorylation of protein mediators, such as p66Shc, that translocate to the mitochondria and trigger organelle dysfunction. (iii) During the onset of lipotoxic responses, cytoplasmic kinases are activated and associate with the inner mitochondrial membrane to affect ion-transporter activity by reversible phosphorylation, causing mitochondrial dysfunction because of an ionic imbalance. Abbreviations: I, II, III, IV, complexes of the respiratory chain; Cyt c, cytochrome C; acyl-CoA, acyl-Coenzyme A; GPDH, glycerol-3-phosphate dehydrogenase; IMM, inner mitochondrial membrane; JNK, c-Jun N-terminal kinase; mtHsp70, mitochondrial heat-shock protein 70; NADH/H⁺, reduced nicotinamide adenine dinucleotide; Nox, (plasma) membrane-bound NADPH oxidases; OMM, outer

mitochondrial membrane; p66Shc, Src-homology-2-domain-containing transforming protein 1; PKC β , protein kinase C- β ; PTP, permeability transition pore; Q, ubiquinone; ROS, reactive oxygen species.