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Mitochondrial quality control in insulin resistance and diabetes

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Abstract

Diabetes is increasingly prevalent and a primary contributor to the major causes of disability and death. Despite the central role of mitochondria in metabolism, the relationship between mitochondrial quality and insulin action remains unclear. An increasing number of genetically-engineered and aging rodent models are shedding additional light on the mitochondrion's role in regulating glucose metabolism and insulin sensitivity by modulating mitochondrial morphology, function and quality control pathways. Clarification of the role of mitochondria in regulating key cellular processes including metabolic flux, autophagy, and apoptosis will drive the development of novel therapeutic strategies for maintaining mitochondrial quality and improving human health.

Diabetes currently affects nearly 10% of the US population with an additional 30% of the population categorized as pre-diabetic because of marked metabolic dysfunction. Interventions to address this epidemic will require understanding the full spectrum of diverse etiologies that contribute to the pathogenesis insulin resistance and type 2 diabetes. As a central player in metabolism and the "powerhouse" of the cell, the mitochondrion has long been implicated in the regulation of metabolic flux and insulin action although, to date, this remains an active and contentious area of research.[1,2] A number of recent reviews have examined the role of mitochondrial quality control and dysfunction in the regulation of insulin sensitivity and pathobiology of diabetes.[3,4] Here we present additional findings from rodent models that are contributing to our understanding of the mitochondrion's complex role in regulating insulin sensitivity. The integration of this information has allowed us to postulate potential pathways of therapeutic intervention.

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The specific type of mitochondrial damage impacts insulin sensitivity

The mitochondrion is the primary site of cellular oxidative respiration, where ~90% of cellular oxygen contributes to metabolism. During oxidative respiration, ~1–5% of this is converted to reactive oxygen species as byproducts of the electron transport complexes (ETCs).[5] Therefore mitochondria are the primary cellular source and target of endogenous reactive oxygen species (ROS). Although the production of ROS is shown to be critical for the maintenance of insulin action, [6] chronic elevation of ROS is detrimental to the cell by promoting lipid peroxidation, DNA damage, and organelle bilayer damage. Oxidative stress of mitochondrial origin has long been implicated in aging and the pathobiology of select chronic diseases. Mouse models have been generated to modulate oxidative damage by modulating ROS production or scavenging to test the free radical hypothesis of aging and chronic disease. Models of increased oxidative damage include knockouts of superoxide dismutase (SOD)1 and SOD2, and glutathione peroxidase.[7–10] By comparison, over-expression of these antioxidant enzymes reduced oxidative damage.[11–13] Variability in outcomes including lifespan and endpoints associated with age-related diseases have challenged a causal role of oxidative damage in aging; alternatively, these findings may indicate that the specific type and location of oxidative defense is critical. Several oxidative stress sensitive or resistant mouse models (e.g., knockout or transgenic models of superoxide dismutases, catalases, glutathione peroxidases, etc.) have been examined to understand the effects of oxidant status on metabolism and insulin sensitivity and the results of these studies are described in greater detail in a recent review.[14]

One model that does support the hypothesized role of mitochondrial oxidative stress in select settings of aging and insulin resistance is the mitochondrial catalase (mCAT) mouse in which the antioxidant enzyme catalase is targeted to the mitochondrial matrix.[13] In the mitochondrial matrix, catalase removes hydrogen peroxide, a by-product of mitochondrial metabolism (Figure 1A). Mitochondrially-targeted catalase expression was detected in brain, skeletal muscle and in the heart, and this mouse model was shown to have increased mean and median lifespan (i.e., by 5 and 5.5 months respectively), reduced cardiac pathology, delayed cataract formation, and reduced cancer incidence compared to wildtype littermates. In addition to the beneficial effects of increased oxidative defense on lifespan [15], the mitochondrial expression of catalase protected against insulin resistance induced with aging and high fat feeding.[16,17] These phenotypic outcomes in the mCAT mouse were associated with reduced hydrogen peroxide production and oxidative damage, and diminished mtDNA mutation in cardiac and skeletal muscle.

Of interest, mCAT reduced mitochondrial hydrogen peroxide production in mouse striated muscle by ~45% independent of mouse age. The reduction in hydrogen peroxide production by mCAT protected against age-related mitochondrial protein and DNA damage that translated into preserved rates of oxygen consumption and mitochondrial ATP synthesis.[17] The maintenance of muscle metabolism during aging mirrored the preservation of whole body energy expenditure. Moreover, the intrinsic protection of mitochondrial function in mCAT mice protected animals from age-associated decline in insulin sensitivity. Similar to the protection from mitochondrial dysfunction and insulin resistance observed in mCAT mice, animals treated with a pharmacological mCAT mimetic, SS-31, were protected from

age and high fat diet-induced metabolic dysfunction.[16] These findings showing reversal of the age-associated decline in energy expenditure and muscle insulin sensitivity by mCAT associated with the maintenance of mitochondrial quality and function suggests an important link between mitochondrial health, insulin action, and metabolic homeostasis. Collectively, studies in mitochondrially-targeted mouse models, including the mCAT model, indicate that the specific type of mitochondrial stress or damage may dictate the resulting metabolic phenotype.

The degree of mitochondrial damage impacts insulin sensitivity

To test the impact of mtDNA health on metabolic homeostasis and lifespan, several laboratories have studied the polymerase gamma mutator mouse, a knock-in line generated by mutating the proofreading domain of DNA polymerase gamma (i.e., the mitochondrial DNA polymerase, Figure 1B).[18,19] Mutation of the proof-reading domain of Polg1 results in mtDNA point and deletion mutations that accumulate to high levels in all tissues. The homozygous polymerase gamma mutant mice show a severely decreased lifespan (maximum survival of 460 days) and a variety of phenotypes suggestive of accelerated aging. If mitochondrial dysfunction leads to insulin resistance, then the high degree of mitochondrial dysfunction including reduced oxidative capacity in the polymerase gamma mutator mice might be predicted to promote hyperglycemia, however recent studies of the homozygous mutator mice found resting hypoglycemia, higher respiratory exchange ratios, and enhanced glucose tolerance.[20] Thus, because of the marked impairment in oxidative function and the inability of the cell to utilize fatty acids as a fuel source, the mutator mouse is forced to rely more heavily on glucose derived from hepatic gluconeogenesis and muscle glycolysis to drive ATP production.

In the case of the homozygous mutator mice, the extensive mitochondrial damage starting early in life and subsequent adaptation to the overwhelming metabolic defects may confound a clear view of the mitochondrion's role. This perspective is supported by the finding that young, pre-progeroid polgamma mutator mice (prior to the development of extensive mitochondrial dysfunction), appear to exhibit starvation as indicated by reduced body fat, lower glucose levels, increased ghrelin, decreased leptin, and dramatically increased fibroblast growth factor 21 levels.[21] The heterozygous polymerase gamma mutator mice are an interesting counterpoint to the homozygous mutator mice, as heterozygosity fails to promote the high levels of mtDNA deletion mutations observed in muscles from homozygous animals.[22] The absence of increased mtDNA deletion mutations in the heterozygous mutator mice may underlie the improvement in lifespan compared to homozygous mice. Moreover, in contrast to the homozygous animals, emerging unpublished data from the Hevener laboratory shows that heterozygosity confers glucose intolerance and an obesity phenotype. Thus, the heterozygous mutator mice may provide, perhaps in combination with other genetic mouse models of altered mtDNA quality control, a model that better recapitulates the degree of mitochondrial damage and dysfunction observed in aging and with chronic disease.

The tissue localization of mitochondrial damage impacts insulin sensitivity

The mouse models discussed above center on global alterations in mitochondrial quality and function. Another possible link between mitochondrial DNA quality and insulin sensitivity lies in the focal accumulation of mtDNA deletion mutations in aging muscle and subsequent muscle mass loss. Sarcopenia, the loss of skeletal muscle mass and function[23], has been implicated in the pathogenesis of insulin resistance and diabetes.[24–26] In humans, sarcopenia begins at ~25 years of age and contributes to a 10% loss of muscle mass by age 50, and a 50% by age 80 [27–29]. The etiology of sarcopenia is multifactorial and currently the proposed mechanisms contributing to the decline in muscle mass with aging include increased contraction-induced injury[30], altered satellite cell regulation[31–33], motor unit decline[34], and endocrine dysfunction (e.g., insulin-like growth factor signaling)[35].

Declining muscle mass with aging in humans has been attributed to both fiber loss and fiber atrophy [36] that is mediated by apoptosis and necrosis [37,38]. Down-regulation of apoptosis signaling preserves muscle mass and enhances muscle function in aged animals. Conversely, stimulation of apoptotic signaling accelerates muscle aging and sarcopenia, for example, in mice lacking the antioxidant enzyme copper/zinc-dependent superoxide dismutase,[39] or animals with an interleukin 10-deficiency.[40] In aging human muscle biopsies, increased levels of apoptosis have been detected by terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL).[41–43] Concomitant to apoptosis, cellular necrosis is another mechanism of muscle fiber loss and is readily observed in aging rat muscle.[44] Despite the clear roles of apoptosis and necrosis in sarcopenia, the initiating factors triggering activation of these cell death pathways have remained elusive.

Recently, the focal accumulation of mtDNA deletion mutations and their associated ETC deficiencies were identified as the predominant cause of muscle fiber apoptosis and necrosis in aging skeletal muscle.[45] Cheema *et al.* used five diverse markers of apoptosis and necrosis to localize cell death events in individual quadriceps muscle fibers of 36-month old rats.[46] Fibers positive for markers of apoptosis or necrosis were identified and these fibers were characterized for a loss of ETC activity by sequential and serial staining for cytochrome c oxidase (COX) and succinate dehydrogenase (SDH). Seventy percent of myofibers positive for activated caspase-3 (an apoptosis marker) lacked COX activity (and are therefore ETC deficient), while 86% of myofibers positive for C5b-9 and 78% myofibers positive for CD68 (both necrosis markers) were ETC deficient. Importantly, apoptosis and necrosis were observed in the longest and most atrophic ETC-deficient fibers, which argues against cell death causing the ETC deficiency. These data indicate the significance of ETC deficient fiber segments as the primary driver of muscle cell apoptosis and necrosis associated with fiber loss in sarcopenia.

Age-induced ETC-deficient muscle fibers arise from the intracellular, clonal accumulation of mitochondrial DNA deletion mutations.[47–49] Mitochondria contain their own unique 16 kb circular DNA genome that encodes 13 proteins of the ETC. The mitochondrial genome is abundant in most nucleated cells including 2 to 10 DNA copies per mitochondrion [50] and 10–100 mitochondria per cell [51]. During aging, deletion mutations occur in mtDNA where a portion of the genome is eliminated by unknown mechanisms.

When mtDNA deletion mutation containing genomes accumulate to >90% of the total mtDNA within a cell, the defective genomes interfere with the normal transcription and translation of the mitochondrially-encoded ETC components and promote segmental ETC deficiency. Mitochondrial DNA deletion mutations have been liked with segmental ETC deficiencies in muscle from aged rats [52], monkeys [53] and humans [54]. Although the specific deletion mutation varies from fiber to fiber, within a single fiber, ETC-deficient segments contain identical mtDNA deletion mutations. These findings suggest that once a mitochondrial deletion mutation occurs, it expands clonally from its point of origin throughout the fiber[52]. Because multiple copies of the mtDNA exist in the mitochondrial reticulum, both wild-type and mutant mtDNA coexist in a state of heteroplasmy. The cellular impact of heteroplasmy is largely dependent on the mutant to wild-type mtDNA ratio. The expression level at which specific mutations cause a segmental ETC deficit is termed the phenotypic threshold effect.[55,56] Herbst et al. quantified absolute levels of mutant and wild-type mtDNA along the length of skeletal muscle fibers. The mtDNA deletion mutations approached 100% of the mitochondrial genomes within the ETC deficient fiber segment and the threshold level of mutation for expression of the ETC deficient phenotype was 90% in both rats [49] and humans [54]. Mutation levels below 90% of total mtDNA do not express an ETC deficit or fiber apoptosis or necrosis.

In aggregate, these studies provide a molecular basis for the muscle fiber loss that contributes to sarcopenia, and this muscle mass loss, in combination with other muscle intrinsic and extrinsic factors, is likely to underlie metabolic dysfunction and insulin resistance as well as the drive the development of type 2 diabetes. (Figure 2).[45] In this theoretical model, focal mtDNA deletion mutation accumulation is the critical process that appears to exceed mitophagy in aging muscle fibers and culminates in fiber loss. This model highlights features of this hypothesis that cannot be replicated currently in cell culture or by studying other experimental mouse models including: requirements for aged cells, somatically derived mtDNA deletion mutations, mutation accumulation in adult, differentiated muscle fibers and fiber breakage and loss. If mechanisms of mtDNA deletion mutation initiation and accumulation could be identified, then therapeutic approaches to control mtDNA deletion mutation induced fiber loss and the ensuing metabolic dysfunction could be devised.

The response of mitochondrial quality control pathways impacts insulin sensitivity

The role of mitochondrial turnover, or mitophagy, in the maintenance of mitochondrial quality and insulin sensitivity is an emerging area of investigation. Cellular stress resistance against metabolic insult is critical for disease prevention and longevity [57–59]. The heat shock response is an evolutionarily conserved defense system engaged during stress (e.g., nutrient oversupply) so as to maintain cellular homeostasis. Heat shock proteins are a conserved family of chaperones responsible for protein folding and are identified by molecular mass. HSP72 is the isoform most highly induced during cellular stress and with chronic endurance exercise [60]. Interestingly, basal HSP72 levels and induction response to cellular stress are diminished in muscle from obese and type 2 diabetic patients [61,62].

Conversely, HSP72 expression in skeletal muscle prevents obesity and insulin resistance in mice, but the underlying mechanisms of this observation are largely unresolved.[63]

To better understand the role of HSP72 in regulating insulin sensitivity genetic and pharmacological interventions were performed to study the impact of loss- and gain-of-HSP72-function on muscle metabolism and insulin action. Gain of function approaches protected mice from genetic- and high fat diet-induced obesity and insulin resistance while enhancing oxidative metabolism via increased mitochondrial mass.[63] As would be expected, these animals, overexpressing HSP72 in skeletal muscle, showed an enhanced running capacity including a 3-fold increase in running time to exhaustion.

In contrast, loss of HSP72 function conferred an obesity/insulin resistance phenotype marked by muscle lipid accumulation and reduced oxidative capacity. Transmission electron microscopy showed that HSP72 deletion produced an enlarged, hyper-fused muscle mitochondrial network. The aberrant mitochondrial morphology in muscle of KO mice prompted the interrogation of mitochondrial fission-fusion-mitophagy dynamics. Using molecular approaches to perturb mitochondrial membrane potential and redox status, it was determined that HSP72 is a critical regulator of stress-induced mitochondrial triage signaling. In the context of HSP72 deletion, it was shown that Parkin, an E3 ubiquitin ligase known to regulate mitochondrial turnover, mitophagy, was unable to ubiquitinate and control its own protein expression or that of its central target the mitochondrial fusion proteins, mitofusins (Mfn). Degradation of the outer mitochondrial membrane fusion proteins allows for the separation and isolation of damaged mitochondrial contents for autophagosome incorporation and lysosomal degradation.[64] In wild-type cells, it was shown that HSP72 rapidly translocates to depolarized mitochondria prior to Parkin recruitment and immunoprecipitates with both Parkin and Mfn2 only after specific mitochondrial insult, i.e., carbonyl cyanide *m*-chlorophenyl hydrazone, CCCP. At the same time as this work, the Youle laboratory, using high throughput proteomics, confirmed that HSP72 is a Parkin binding partner.[65] In addition to an impairment in Parkin action, feedback inhibition of macroautophagy (p62 and LC3B processing) and mitochondrial fission signaling of Drp1 was observed.

To determine whether the impaired Parkin action could account for the reduction in oxygen consumption and insulin action of the HSP72 KO mouse line, primary myotubes from Parkin null animals were generated. Real-time respirometry showed a significant reduction in oxygen consumption as well as basal and maximally stimulated ATP synthesis. Insulin-stimulated glucose disposal was reduced by 85% in myotubes lacking Parkin.[66] Although the Parkin null mouse is protected against HFD-induced obesity and insulin resistance due to malabsorption and compensatory adaptations in adipose tissue, we speculate that muscle-specific deletion of Parkin using the Lox-Cre approach should phenocopy findings observed in the Parkin null myotubes. In support of this notion, studies by David Walker and colleagues have shown that Parkin overexpression in *Drosophila* promoted smaller mitochondria, a reduction in aged-induced protein aggregates in flight muscle, and improved longevity in both males and females.[67] Similar studies in mammalian models have yet to be explored however these gain-of-function studies in flies are internally consistent with the loss-of-function studies in murine cells. These data suggest that Parkin may be a critical

mediator of mitochondrial function and insulin sensitivity at least in skeletal muscle, and that pharmaceutical strategies to bolster Parkin action with aging may improve metabolic health (Figure 3). Although HSP72 chaperones a host of regulatory proteins and transcription factors, its role in controlling mitochondrial function and health appears central for its beneficial effects on insulin sensitivity. Collectively findings suggest that strategies to maintain HSP72 may provide therapeutic benefit to enhance mitochondrial quality and insulin sensitivity to combat complications associated with metabolic diseases including type 2 diabetes. Since BGP-15, an HSP72 co-inducer, is currently in human clinical trials for the treatment of type 2 diabetes, data will be emerging in short order confirming or refuting the notion that HSP72 improves insulin sensitivity by modulating mitochondrial function. Regardless of the clinical outcome, additional molecular studies focused on interrogating the mechanistic link between mitochondrial quality and insulin action are warranted.

Conclusion

The ongoing societal impact of diabetes necessitates improved preventive measures and therapeutic interventions. Considerable evidence points to mitochondria as both an etiology and likely therapeutic target in the fight against diseases associated with metabolic dysfunction including type 2 diabetes. However as we have attempted to illustrate in this review, if we want to succeed at developing novel strategies to combat metabolic dysfunction by targeting the mitochondrion we must first answer the remaining questions regarding the type, degree and localization of mitochondrial damage and dysfunction contribute to impaired metabolism, as well as elucidate the cellular pathways responsible for maintaining mitochondrial quality (Figure 4). The mitochondrion’s centrality in cellular processes predicates its role in complex diseases associated with metabolic dysfunction.

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Bibliography

1. Goodpaster BH. Mitochondrial deficiency is associated with insulin resistance. Diabetes. 2013; 62(4):1032–1035. [PubMed: 23520282]
2. Holloszy JO. "Deficiency" of mitochondria in muscle does not cause insulin resistance. Diabetes. 2013; 62(4):1036–1040. [PubMed: 23520283]
3. Fischer F, Hamann A, Osiewacz HD. Mitochondrial quality control: An integrated network of pathways. Trends in biochemical sciences. 2012; 37(7):284–292. [PubMed: 22410198]
4. Montgomery MK, Turner N. Mitochondrial dysfunction and insulin resistance: An update. Endocr Connect. 2015; 4(1):R1–R15. [PubMed: 25385852]
5. Boveris A, Chance B. The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. The Biochemical journal. 1973; 134(3):707–716. [PubMed: 4749271]
6. Loh K, Deng H, Fukushima A, Cai X, Boivin B, Galic S, Bruce C, Shields BJ, Skiba B, Ooms LM, Stepto N, et al. Reactive oxygen species enhance insulin sensitivity. Cell metabolism. 2009; 10(4): 260–272. [PubMed: 19808019]

7. Elchuri S, Oberley TD, Qi W, Eisenstein RS, Jackson Roberts L, Van Remmen H, Epstein CJ, Huang TT. Cuznsod deficiency leads to persistent and widespread oxidative damage and hepatocarcinogenesis later in life. Oncogene. 2005; 24(3):367–380. [PubMed: 15531919]

8. Muller FL, Song W, Liu Y, Chaudhuri A, Pieke-Dahl S, Strong R, Huang TT, Epstein CJ, Roberts LJ 2nd, Csete M, Faulkner JA, et al. Absence of cuzn superoxide dismutase leads to elevated oxidative stress and acceleration of age-dependent skeletal muscle atrophy. Free radical biology & medicine. 2006; 40(11):1993–2004. [PubMed: 16716900]

9. Van Remmen H, Ikeno Y, Hamilton M, Pahlavani M, Wolf N, Thorpe SR, Alderson NL, Baynes JW, Epstein CJ, Huang TT, Nelson J, et al. Life-long reduction in mnsod activity results in increased DNA damage and higher incidence of cancer but does not accelerate aging. Physiological genomics. 2003; 16(1):29–37. [PubMed: 14679299]

10. Van Remmen H, Williams MD, Guo Z, Estlack L, Yang H, Carlson EJ, Epstein CJ, Huang TT, Richardson A. Knockout mice heterozygous for sod2 show alterations in cardiac mitochondrial function and apoptosis. Am J Physiol Heart Circ Physiol. 2001; 281(3):H1422–H1432. [PubMed: 11514315]

11. Ran Q, Liang H, Gu M, Qi W, Walter CA, Roberts LJ 2nd, Herman B, Richardson A, Van Remmen H. Transgenic mice overexpressing glutathione peroxidase 4 are protected against oxidative stress-induced apoptosis. The Journal of biological chemistry. 2004; 279(53):55137–55146. [PubMed: 15496407]

12. Ran Q, Gu M, Van Remmen H, Strong R, Roberts JL, Richardson A. Glutathione peroxidase 4 protects cortical neurons from oxidative injury and amyloid toxicity. Journal of neuroscience research. 2006; 84(1):202–208. [PubMed: 16673405]

13. Schriner SE, Linford NJ, Martin GM, Treuting P, Ogburn CE, Emond M, Coskun PE, Ladiges W, Wolf N, Van Remmen H, Wallace DC, et al. Extension of murine life span by overexpression of catalase targeted to mitochondria. Science. 2005; 308(5730):1909–1911. [PubMed: 15879174] ** This paper demonstrates one of the first examples of genetically enginered mouse with an extended lifespan and a direct test of the free radical hypothesis of aging.

14. Styskal J, Van Remmen H, Richardson A, Salmon AB. Oxidative stress and diabetes: What can we learn about insulin resistance from antioxidant mutant mouse models? Free radical biology & medicine. 2012; 52(1):46–58. [PubMed: 22056908]

15. Treuting PM, Linford NJ, Knoblaugh SE, Emond MJ, Morton JF, Martin GM, Rabinovitch PS, Ladiges WC. Reduction of age-associated pathology in old mice by overexpression of catalase in mitochondria. The journals of gerontology. 2008; 63(8):813–822. [PubMed: 18772469]

16. Anderson EJ, Lustig ME, Boyle KE, Woodlief TL, Kane DA, Lin CT, Price JW 3rd, Kang L, Rabinovitch PS, Szeto HH, Houmard JA, et al. Mitochondrial h2o2 emission and cellular redox state link excess fat intake to insulin resistance in both rodents and humans. The Journal of clinical investigation. 2009; 119(3):573–581. [PubMed: 19188683]

17. Lee HY, Choi CS, Birkenfeld AL, Alves TC, Jornayvaz FR, Jurczak MJ, Zhang D, Woo DK, Shadel GS, Ladiges W, Rabinovitch PS, et al. Targeted expression of catalase to mitochondria prevents age-associated reductions in mitochondrial function and insulin resistance. Cell metabolism. 2010; 12(6):668–674. [PubMed: 21109199] **This paper shows direct evidence of the protective effect of mCAT on mitochondrial quality and subsequent prevention of age-associated insulin resistance.

18. Trifunovic A, Wredenberg A, Falkenberg M, Spelbrink JN, Rovio AT, Bruder CE, Bohlooly YM, Gidlof S, Oldfors A, Wibom R, Tornell J, et al. Premature ageing in mice expressing defective mitochondrial DNA polymerase. Nature. 2004; 429(6990):417–423. [PubMed: 15164064] **This paper describes the generation and testing of the role of mitochondrial DNA mutations in aging and disease.

19. Kujoth GC, Hiona A, Pugh TD, Someya S, Panzer K, Wohlgemuth SE, Hofer T, Seo AY, Sullivan R, Jobling WA, Morrow JD, et al. Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging. Science. 2005; 309(5733):481–484. [PubMed: 16020738] **This paper describes the generation and testing of the role of mitochondrial DNA mutations in aging and disease.

20. Saleem A, Safdar A, Kitaoka Y, Ma X, Marquez OS, Akhtar M, Nazli A, Suri R, Turnbull J, Tarnopolsky MA. Polymerase gamma mutator mice rely on increased glycolytic flux for energy production. Mitochondrion. 2015; 21:19–26. [PubMed: 25575636]
21. Wall CE, Whyte J, Suh JM, Fan W, Collins B, Liddle C, Yu RT, Atkins AR, Naviaux JC, Li K, Bright AT, et al. High-fat diet and fgf21 cooperatively promote aerobic thermogenesis in mtdna mutator mice. Proceedings of the National Academy of Sciences of the United States of America. 2015; 112(28):8714–8719. [PubMed: 26124126]
22. Vermulst M, Wanagat J, Kujoth GC, Bielas JH, Rabinovitch PS, Prolla TA, Loeb LA. DNA deletions and clonal mutations drive premature aging in mitochondrial mutator mice. Nature genetics. 2008; 40(4):392–394. [PubMed: 18311139]
23. Rosenberg IH. Sarcopenia: Origins and clinical relevance. The Journal of nutrition. 1997; 127(5 Suppl):990S–991S. [PubMed: 9164280]
24. Srikanthan P, Karlamangla AS. Relative muscle mass is inversely associated with insulin resistance and prediabetes. Findings from the third national health and nutrition examination survey. The Journal of clinical endocrinology and metabolism. 2011; 96(9):2898–2903. [PubMed: 21778224] *This paper utilized a national database with a novel method of muscle mass determination by bioimpedance to examine the relationship between muscle mass and insulin resistance.
25. Srikanthan P, Karlamangla AS. Muscle mass index as a predictor of longevity in older adults. Am J Med. 2014; 127(6):547–553. [PubMed: 24561114]
26. Kim TN, Choi KM. The implications of sarcopenia and sarcopenic obesity on cardiometabolic disease. J Cell Biochem. 2015; 116(7):1171–1178. [PubMed: 25545054]
27. Lexell J, Henriksson-Larsen K, Winblad B, Sjostrom M. Distribution of different fiber types in human skeletal muscles: Effects of aging studied in whole muscle cross sections. Muscle Nerve. 1983; 6:588–595. [PubMed: 6646161]
28. Lexell J, Taylor CC, Sjostrom M. What is the cause of the ageing atrophy? Total number, size and proportion of different fiber types studied in whole vastus lateralis muscle from 15- to 83-year-old men. Journal of the neurological sciences. 1988; 84(2-3):275–294. [PubMed: 3379447]
29. Mazzeo RS, Cavanagh P, Evans WJ, Fiatarone M, Hagberg J, et al. Exercise and physical activity for older adults. Med Sci Sports Exerc. 1998; 30(6):992–1008. [PubMed: 9624662]
30. Faulkner JA, Brooks SV, Zerba E. Muscle atrophy and weakness with aging: Contraction-induced injury as an underlying mechanism. The journals of gerontology. 1995; 50:124–129. [PubMed: 7493205]
31. Carlson BM. Factors influencing the repair and adaptation of muscles in aged individuals: Satellite cells and innervation. J Gerontol Series A. 1995; 50A:96–100.
32. Grounds MD. Age-associated changes in the response of skeletal muscle cells to exercise and regeneration. Ann N Y Acad Sci. 1998; 854:78–91. [PubMed: 9928422]
33. Gallegly JC, Turesky NA, Strotman BA, Gurley CM, Peterson CA, Dupont-Versteegden EE. Satellite cell regulation of muscle mass is altered at old age. J Appl Physiol (1985). 2004; 97(3): 1082–1090. [PubMed: 15121742]
34. Larsson L. Motor units: Remodeling in aged animals. J Gerontol Series A. 1995; 50A:91–95.
35. Hameed M, Orrell RW, Cobbold M, Goldspink G, Harridge SD. Expression of igf-i splice variants in young and old human skeletal muscle after high resistance exercise. J Physiol. 2003; 547(Pt 1): 247–254. [PubMed: 12562960]
36. Klein CS, Marsh GD, Petrella RJ, Rice CL. Muscle fiber number in the biceps brachii muscle of young and old men. Muscle & Nerve. 2003; 28(1):62–68. [PubMed: 12811774]
37. Dirks AJ, Leeuwenburgh C. Aging and lifelong calorie restriction result in adaptations of skeletal muscle apoptosis repressor, apoptosis-inducing factor, x-linked inhibitor of apoptosis, caspase-3, and caspase-12. Free radical biology & medicine. 2004; 36(1):27–39. [PubMed: 14732288]
38. Marzetti E, Hwang JC, Lees HA, Wohlgemuth SE, Dupont-Versteegden EE, Carter CS, Bernabei R, Leeuwenburgh C. Mitochondrial death effectors: Relevance to sarcopenia and disuse muscle atrophy. Biochimica et biophysica acta. 2010; 1800(3):235–244. [PubMed: 19450666]
39. Jang YC, Lustgarten MS, Liu Y, Muller FL, Bhattacharya A, Liang H, Salmon AB, Brooks SV, Larkin L, Hayworth CR, Richardson A, et al. Increased superoxide in vivo accelerates age-associated muscle atrophy through mitochondrial dysfunction and neuromuscular junction

degeneration. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2010; 24(5):1376–1390. [PubMed: 20040516]

40. Walston J, Fedarko N, Yang H, Leng S, Beamer B, Espinoza S, Lipton A, Zheng H, Becker K. The physical and biological characterization of a frail mouse model. The journals of gerontology Series A, Biological sciences and medical sciences. 2008; 63(4):391–398.
41. Strasser H, Tiefenthaler M, Steinlechner M, Bartsch G, Konwalinka G. Urinary incontinence in the elderly and age-dependent apoptosis of rhabdosphincter cells. Lancet. 1999; 354(9182):918–919. [PubMed: 10489956]
42. Malmgren LT, Jones CE, Bookman LM. Muscle fiber and satellite cell apoptosis in the aging human thyroarytenoid muscle: A stereological study with confocal laser scanning microscopy. Otolaryngol Head Neck Surg. 2001; 125(1):34–39. [PubMed: 11458211]
43. Whitman SA, Wacker MJ, Richmond SR, Godard MP. Contributions of the ubiquitin-proteasome pathway and apoptosis to human skeletal muscle wasting with age. Pflugers Archiv : European journal of physiology. 2005; 450(6):437–446. [PubMed: 15952031]
44. Fujisawa K. Some observations on the skeletal musculature of aged rats. I. Histological aspects. Journal of the neurological sciences. 1974; 22(3):353–366. [PubMed: 4134538]
45. Cheema N, Herbst A, McKenzie D, Aiken JM. Apoptosis and necrosis mediate skeletal muscle fiber loss in age-induced mitochondrial enzymatic abnormalities. Aging cell. 2015; 14(6):1085–1093. [PubMed: 26365892] **This paper was the first to identify focal accumulations of mtDNA mutations as the the etiology of the majority of apoptotic and necrotic muscle fibers in aging muscle.
47. Chung SS, Weindruch R, Schwarze SR, McKenzie DI, Aiken JM. Multiple age-associated mitochondrial DNA deletions in skeletal muscle of mice. Aging (Milano). 1994; 6(3):193–200. [PubMed: 7993927]
48. Wanagat J, Cao Z, Pathare P, Aiken JM. Mitochondrial DNA deletion mutations colocalize with segmental electron transport system abnormalities, muscle fiber atrophy, fiber splitting, and oxidative damage in sarcopenia. Faseb J. 2001; 15(2):322–332. [PubMed: 11156948] *This paper used the novel approach of laser microdissection to study individual ETC negative fibers harboring mtDNA mutations and described segmental atrophy in these fibers.
49. Herbst A, Pak JW, McKenzie D, Bua E, Bassiouni M, Aiken JM. Accumulation of mitochondrial DNA deletion mutations in aged muscle fibers: Evidence for a causal role in muscle fiber loss. The journals of gerontology. 2007; 62(3):235–245. [PubMed: 17389720]
50. Clayton DA. Replication of animal mitochondrial DNA. Cell. 1982; 28:693–705. [PubMed: 6178513]
51. Robin E, Wong R. Mitochondrial DNA molecules and virtual number of mitochondria per cell in mammalian cells. Journal of cellular physiology. 1988; 136:507–513. [PubMed: 3170646]
52. Cao Z, Wanagat J, McKiernan SH, Aiken JM. Mitochondrial DNA deletion mutations are concomitant with ragged red regions of individual, aged muscle fibers: Analysis by laser-capture microdissection. Nucleic acids research. 2001; 29(21):4502–4508. [PubMed: 11691938]
53. Gokey NG, Cao Z, Pak JW, Lee D, McKiernan SH, McKenzie D, Weindruch R, Aiken JM. Molecular analyses of mtdna deletion mutations in microdissected skeletal muscle fibers from aged rhesus monkeys. Aging cell. 2004; 3(5):319–326. [PubMed: 15379855]
54. Bua E, Johnson J, Herbst A, Delong B, McKenzie D, Salamat S, Aiken JM. Mitochondrial DNA deletion mutations accumulate intracellularly to detrimental levels in aged human skeletal muscle fibers. Am J Hum Genet. 2006; 79(3):469–480. [PubMed: 16909385]
55. Smeitink J, van den Heuvel L, DiMauro S. The genetics and pathology of oxidative phosphorylation. Nat Rev Genet. 2001; 2(5):342–352. [PubMed: 11331900]
56. Rossignol R, Faustin B, Rocher C, Malgat M, Mazat JP, Letellier T. Mitochondrial threshold effects. Biochemical Journal. 2003; 370(Pt 3):751–762. [PubMed: 12467494]
57. Balch WE, Morimoto RI, Dillin A, Kelly JW. Adapting proteostasis for disease intervention. Science. 2008; 319(5865):916–919. [PubMed: 18276881]
58. Morley JF, Morimoto RI. Regulation of longevity in caenorhabditis elegans by heat shock factor and molecular chaperones. Mol Biol Cell. 2004; 15(2):657–664. [PubMed: 14668486]

59. Hsu AL, Murphy CT, Kenyon C. Regulation of aging and age-related disease by daf-16 and heat-shock factor. Science. 2003; 300(5622):1142–1145. [PubMed: 12750521]

60. Febbraio MA, Koukoulas I. Hsp72 gene expression progressively increases in human skeletal muscle during prolonged, exhaustive exercise. J Appl Physiol. 2000; 89(3):1055–1060. [PubMed: 10956350]

61. Bruce CR, Carey AL, Hawley JA, Febbraio MA. Intramuscular heat shock protein 72 and heme oxygenase-1 mrna are reduced in patients with type 2 diabetes: Evidence that insulin resistance is associated with a disturbed antioxidant defense mechanism. Diabetes. 2003; 52(9):2338–2345. [PubMed: 12941774]

62. Chung J, Nguyen AK, Henstridge DC, Holmes AG, Chan MH, Mesa JL, Lancaster GI, Southgate RJ, Bruce CR, Duffy SJ, Horvath I, et al. Hsp72 protects against obesity-induced insulin resistance. Proc Natl Acad Sci U S A. 2008; 105(5):1739–1744. [PubMed: 18223156]

63. Henstridge DC, Bruce CR, Drew BG, Tory K, Kolonics A, Estevez E, Chung J, Watson N, Gardner T, Lee-Young RS, Connor T, et al. Activating hsp72 in rodent skeletal muscle increases mitochondrial number and oxidative capacity and decreases insulin resistance. Diabetes. 2014; 63(6):1881–1894. [PubMed: 24430435]

64. Yoshii SR, Kishi C, Ishihara N, Mizushima N. Parkin mediates proteasome-dependent protein degradation and rupture of the outer mitochondrial membrane. The Journal of biological chemistry. 2011; 286(22):19630–19640. [PubMed: 21454557]

65. Lazarou M, Sliter DA, Kane LA, Sarraf SA, Wang C, Burman JL, Sideris DP, Fogel AI, Youle RJ. The ubiquitin kinase pink1 recruits autophagy receptors to induce mitophagy. Nature. 2015; 524(7565):309–314. [PubMed: 26266977]

66. Drew BG, Ribas V, Le JA, Henstridge DC, Phun J, Zhou Z, Soleymani T, Daraei P, Sitz D, Vergnes L, Wanagat J, et al. Hsp72 is a mitochondrial stress sensor critical for parkin action, oxidative metabolism, and insulin sensitivity in skeletal muscle. Diabetes. 2014; 63(5):1488–1505. [PubMed: 24379352] **This paper is the first to link mitophagy and mitochondrial quality control with insulin resistance.

67. Rana A, Rera M, Walker DW. Parkin overexpression during aging reduces proteotoxicity, alters mitochondrial dynamics, and extends lifespan. Proceedings of the National Academy of Sciences of the United States of America. 2013; 110(21):8638–8643. [PubMed: 23650379]

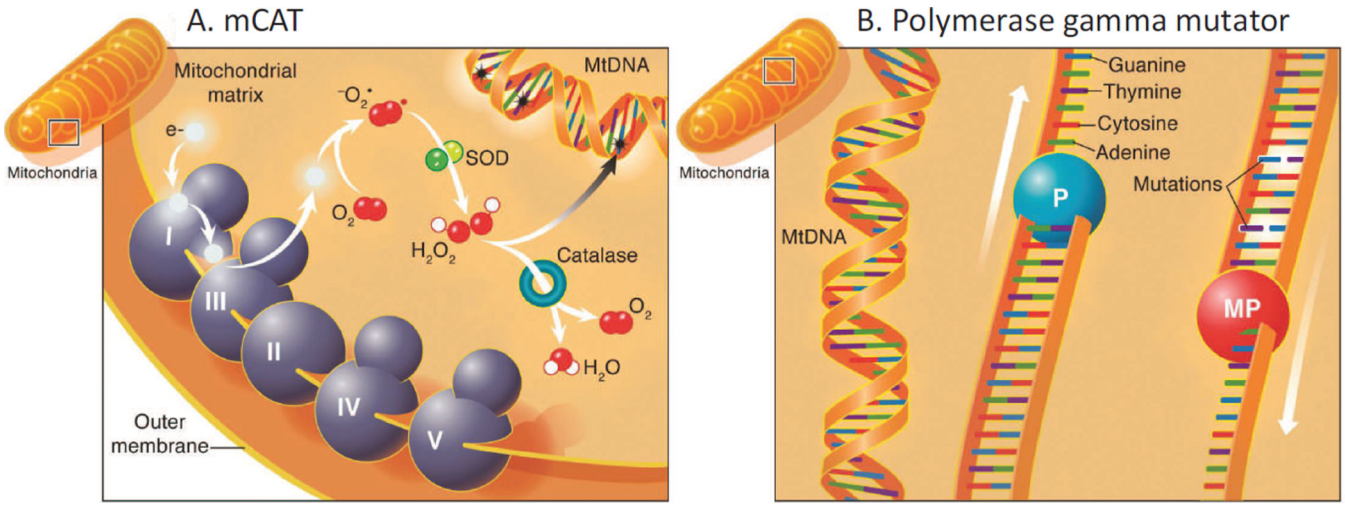

Figure 1. Genetically engineered mouse models of altered mtDNA quality

Panel A, the mCAT mouse has mitochondrially-targeted catalase that neutralizes endogenous hydrogen peroxide. Panel B, the polgamma mutator mouse expresses a mutant polymerase gamma (MP), which lacks proofreading function, resulting in both point and deletion mutations in the mtDNA.

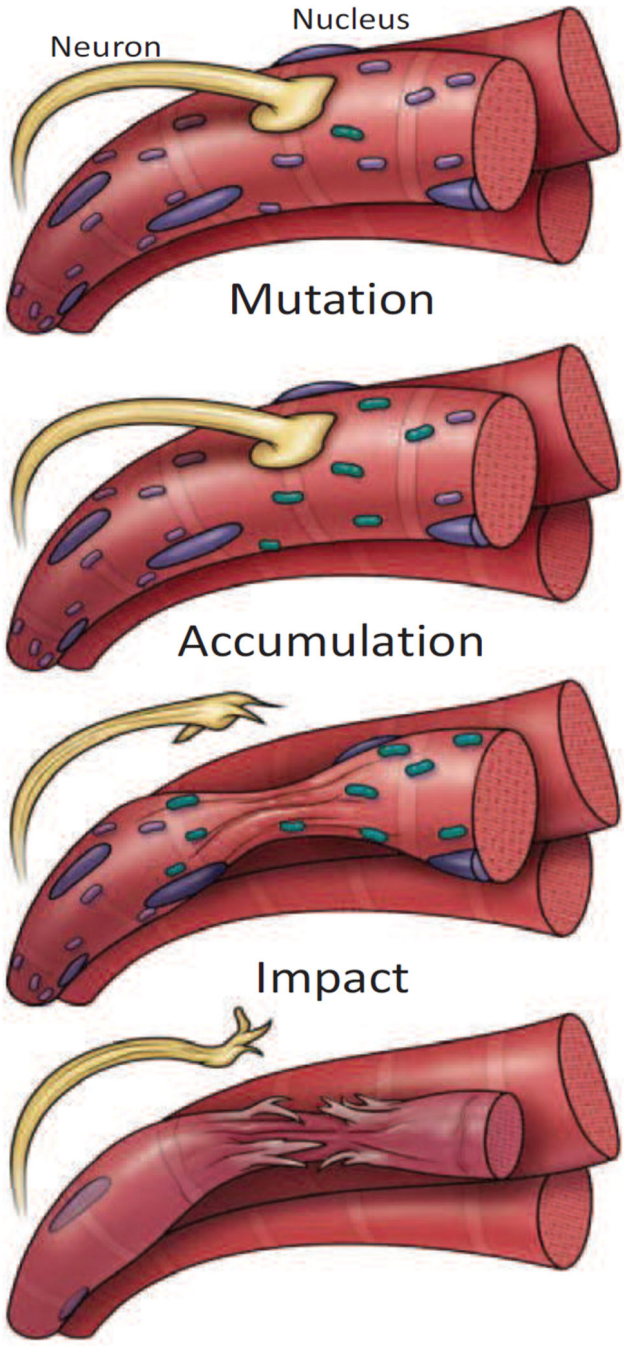

Figure 2. MtDNA Deletion Mutation Hypothesis of Muscle Fiber Loss

The top panel illustrates an aged healthy muscle fiber. Green mitochondrion represent somatically derived mtDNA deletion mutation event that occurs within a single mitochondrion in an individual muscle fiber. Over time, deletion mutations accumulate until they surpass the phenotypic threshold for disrupting the transcription and translation of mitochondrial ETC subunits. Accumulation of deletion mutations promotes the loss of cellular respiration, oxidative phosphorylation and the production of ATP. This metabolic insult contributes to muscle atrophy including fiber breakage and loss by apoptosis and

necrosis. This process is repeated in other muscle fibers and accelerates with advancing age cumulatively in sarcopenia.

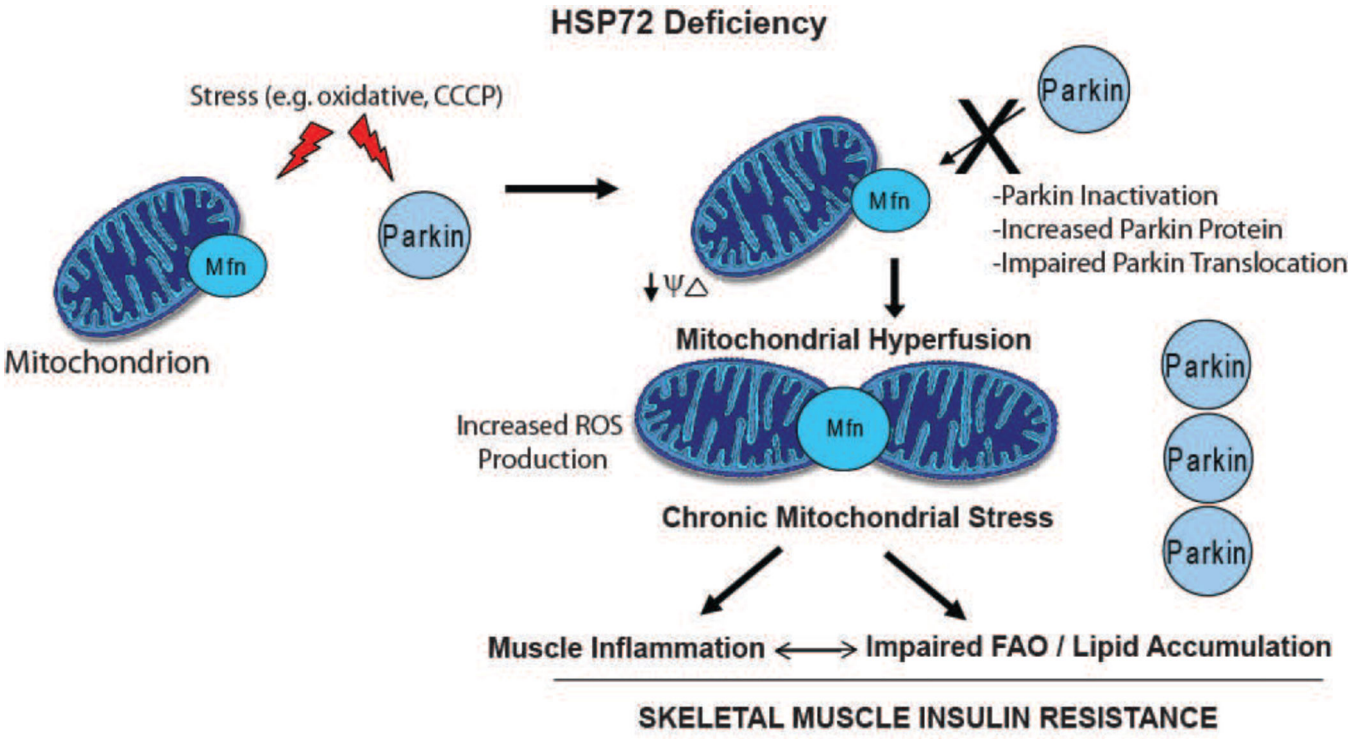

Figure 3. Proposed role of HSP72 in stress-induced mitophagy and insulin action

Mitochondrial stress induces rapid movement of HSP72 to the mitochondrion where it interacts with Mfn2 on the outer mitochondrial membrane. At a later time point, Parkin translocates to the mitochondrion and complexes with HSP72 and Mfn2. The interaction is specific to the mitochondrial stress, as CCCP (induced reduction in mitochondrial membrane potential), but not rotenone or antimycin, induces HSP72 mitochondrial translocation. In the context of HSP72 deficiency, mitochondrial stress fails to induce Parkin translocation and interaction with mitochondrial membrane proteins. Parkin, in the absence of HSP72, is unable to ubiquitinate itself or its targets, and in consequence, cytosolic Parkin and mitochondrial Mfn2 protein levels become elevated thus promoting the fusion and retention of unhealthy mitochondria to the network. We propose that this mechanism underlies the impairments in oxidative metabolism and the marked insulin resistance phenotype observed in HSP72-KO animals.[66]

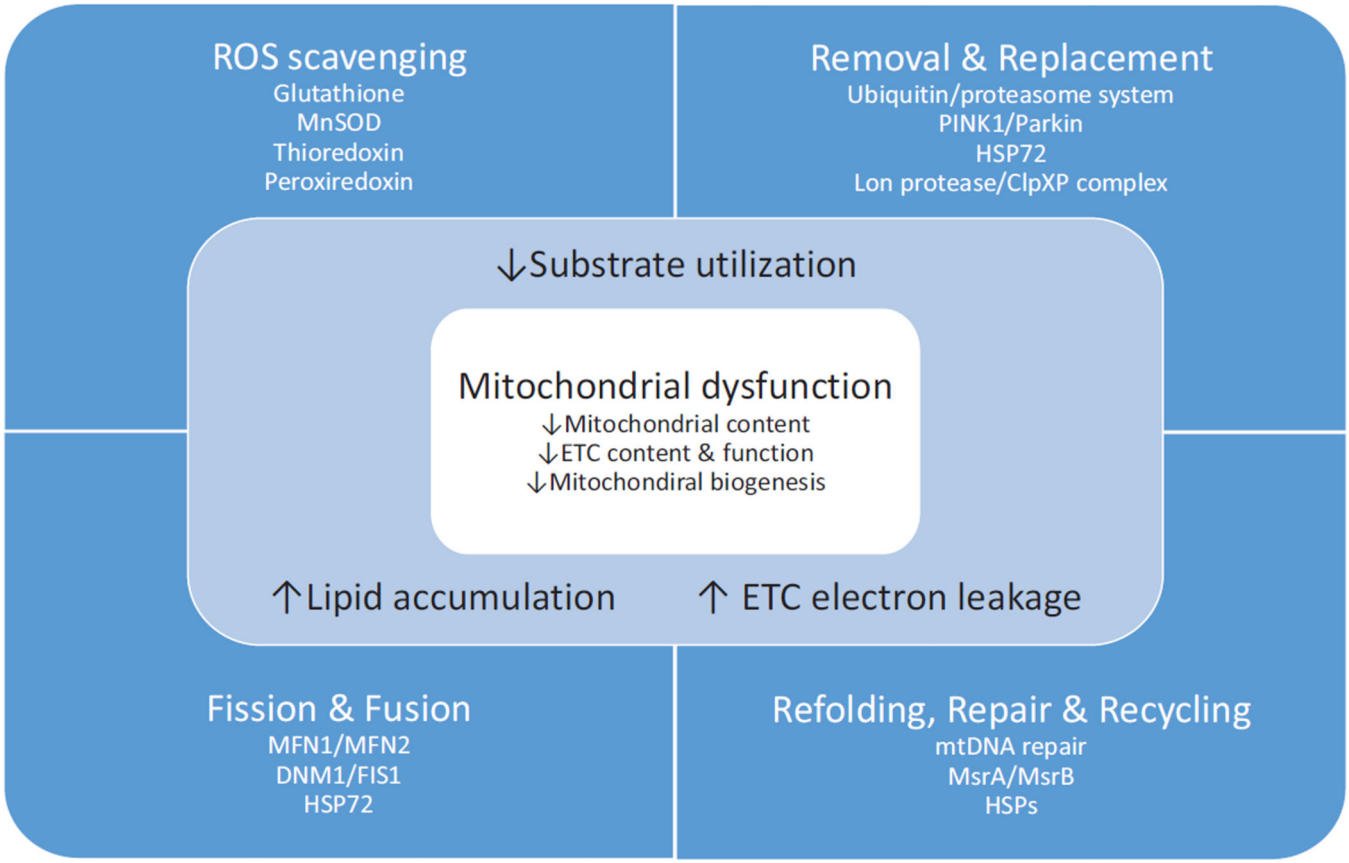

Figure 4. Interplay of mitochondrial quality control and mitochondrial dysfunction

Mitochondrial dysfunction encompasses reductions in mitochondrial content, ETC content and function and biogenesis. Subsequent decreases in substrate utilization trigger lipid accumulation and increased ROS production from the ETC and a vicious cycle of further mitochondrial dysfunction. Cellular mitochondrial quality control mechanisms including ROS scavenging, mitophagy, fission and fusion and biomolecular repair act to intervene. The quality control mechanisms offer therapeutic targets for maintaining mitochondrial function. Adapted from [3] and [4]. MnSOD, manganese superoxide dismutase. PINK1, PTEN-induced putative kinase 1. MFN1, mitofusin-1. MFN2, mitofusin-2. DNM1, dynamin 1. FIS1, mitochondrial fission 1 protein. MsrA, methionine sulfoxide reductase. MsrB, methionine sulfoxide reductase B.