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Mitochondrial Biogenesis: Regulation By Endogenous Gases during Inflammation and Organ Stress

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Abstract

The influence of mitochondrial dysfunction on pathological states involving inflammatory and/or oxidative stress in tissues that do not show frank cellular apoptosis or necrosis has been rather difficult to unravel, and the literature is replete with contradictory information. Although such discrepancies have many potential causes related to the type of injurious agent, the severity and duration of the injury, and the particular cells and tissues and the functions involved, it is the successful induction of cellular adaptive responses that ultimately governs the resolution of mitochondrial dysfunction and survival of the cell. Much recent attention has been devoted to unraveling the signaling pathways that activate mitochondrial biogenesis and other processes involved in mitochondrial quality control (QC) during inflammatory and oxidative stress with an eye towards the development of novel targets for therapeutic mitigation of the resultant tissue damage. This review provides a brief overview of this emerging field with an emphasis on the role of signaling through the endogenous gases (NO, CO and H₂S) and a redox-based approach that brings transparency to key factors that contribute to the resolution of mitochondrial dysfunction and the maintenance of cell vitality. We make the case that targeted stimulation of mitochondrial biogenesis could be a potentially valuable approach for the development of new therapies for the treatment of diseases for which mitochondrial damage is a major consideration.

INTRODUCTION

Mitochondria function in eukaryotic cells not only as the major site of ATP production, but as coordinators of numerous metabolic reactions through the Krebs cycle, fatty acid metabolism, and oxidative phosphorylation (OXPHOS) [1]. And these tiny, double-membrane enclosed organelles are involved in the regulation of heme metabolism, cell proliferation, inflammation and counter-inflammation, and cell viability. During processes involving electron transport, they generate small amounts of reactive oxygen species (ROS) that may degrade the performance of the organelles, but also provide redox signals for mitochondrial maintenance (**Figure 1**).

The preservation of the cell's mitochondrial pool at steady state is accomplished by the process of mitochondrial biogenesis, which entails the activation of a transcriptional network for mitochondrial rejuvenation that is linked to the degradation and elimination of senescent and damaged mitochondria via mitochondrial autophagy or mitophagy. Classically, mitochondrial biogenesis has been deemed a response to increased work, as in exercising muscle, or to changes in substrate availability, as in starvation. Recent studies have linked cell respiratory defects and ROS production to the activation of mitochondrial biogenesis as a strategy used by the cell to respond to diverse mitochondrial disturbances [2-6].

Data on the deterioration of mitochondrial function during pathological states such as ischemia, metabolic stress, inflammation, and oxidative stress are sometimes contradictory, as various studies have reported impaired [7, 8], unchanged [9, 10], or even improved [11, 12] respiratory function. Such discrepancies have many potential causes, such as the type of injury, the severity and duration of the injury, the cell type and its function, and the experimental conditions. Other events are superimposed, like the variables involved in the activation of cellular adaptive responses that promote the resolution of mitochondrial dysfunction [13]. These complexities have made it difficult to devise rational mitochondrial-based therapies and confirm their modes of action on mitochondria and their impact on the resolution of cell damage. This review provides a brief overview of this area and a redox-based approach to the problem.

The activation of mitochondrial biogenesis is known to occur after disturbances in oxygen delivery to tissues for both hypoxia and hyperoxia. For instance, after hypoxia, mitochondrial biogenesis is activated in the heart and the brain by mechanisms discussed later that involve energy limitation, but which have not been entirely mapped out [14, 15]. During periods of oxidative or electrophilic stress, the transcription factor nuclear factor (erythroid-derived2)-like2 (NFE2L2 or Nrf2), which is involved in increasing the levels of endogenous antioxidants and attenuating apoptosis, has been shown to activate mitochondrial biogenesis through the inducible heme oxygenase-1 (HO-1) system [5, 16]. In a model of chemical oxidative damage simulating renal ischemia-reperfusion, for instance, it has been shown that increased mitochondrial biogenesis accelerates the recovery of mitochondrial function through p38 and epidermal growth factor receptor activation of PGC-1 α [17]. In another study, these investigators found that mitochondrial biogenesis can be mediated via 5-HT receptors and suggested that 5-HT-agonists may be effective for the treatment of mitochondrial and cell injury [18].

Acute tissue damage is often attended by periods of active inflammation, and mitochondria are frequently damaged not only by the original insult, but by the oxidative and nitrosative stress accompanying inflammation. For instance, in severe infections such as sepsis, the overproduction of ROS and NO leads to mitochondrial DNA (mtDNA) damage, and the elevated levels of oxidants and NO act to stimulate mitochondrial biogenesis through a complex crosstalk between mitochondria and nucleus that yields a net increase in the synthesis of new organelles [19] [14].

Some years ago, Suliman et al. demonstrated in the rat liver and heart that lipopolysaccharide stimulates mitochondrial biogenesis in response to inflammatory cell damage [20, 21]. In a later study, this group showed the simultaneous occurrence of mtDNA damage and compensatory mitochondrial biogenesis after exposure to *E. coli* that results from the activation of toll-like receptor 4 (TLR4) signaling pathways and nuclear factor- κ B (NF- κ B)-dependent cytokine production [22]. More recently, mitochondrial biogenesis has been shown capable of restoring oxidative metabolism in experimental murine peritonitis, thus providing a potential mechanism to alter sepsis outcome [23]. Indeed, the combined laboratory and clinical data in sepsis clearly suggest that mitochondrial dysfunction is closely linked to the onset of multiple organ failure and that the capacity to resolve this condition may depend on the ability to rebuild mitochondrial number and function [24]. Simply put, failure to maintain adequate an mitochondrial number and function inhibits the apposite resolution of inflammation. Indeed, recent research provides support for idea that activation of mitochondrial biogenesis in muscle influences survival in critical illness [25]. Accordingly, the search for strategies to maintain and protect mitochondrial biogenesis has been proposed as an innovative direction for providing new ways for preventing end-organ failure in sepsis and other severe inflammatory states [19].

Although mitochondria are constantly renewed through the normal quality control (QC) processes of mitochondrial biogenesis, fission, fusion, and mitophagy [26], homeostatic mitochondrial biogenesis proceeds at a low rate. As an adaptive response however, mitochondrial biogenesis can be greatly accelerated, allowing the cell to meet new energy demands created by injury or genetic, metabolic, or dietary events, thereby lessening the impact of changes in environment or disease on its survival [27]. This requires coordination of both nuclear and mitochondrial genes that encode for mitochondrial proteins.

A primary regulatory assembly for mitochondrial biogenesis involves the nuclear transcription factors (NRF-1 and NRF-2) and the co-activators peroxisome proliferator-activated receptor γ co-activator family (PGC-1 α and 1 β and PPRC1). PGC-1 α interacts with cAMP response element-binding protein and NRF-1 and 2 to regulate the transcription of multiple genes involved in mitochondrial biogenesis and energy metabolism [28]. These proteins provide a direct link between external physiological stimuli and the regulation of mitochondrial biogenesis that can be induced physiologically as well as manipulated pharmacologically.

Today, only a small number of chemical compounds are known that explicitly induce mitochondrial biogenesis without first causing mitochondrial damage [29]. Moreover, after an episode of sub-lethal cell damage, the activation of mitochondrial biogenesis accelerates cell repair and regeneration. Therefore, targeted stimulation of mitochondrial biogenesis would seem to be a potentially valuable approach for the development of new therapies for the treatment of injuries and diseases characterized by mitochondrial damage.

NO and Mitochondrial Biogenesis

Endogenous nitric oxide (NO) production serves as a potent paracrine agent with numerous effects on mitochondrial physiology. The ability of endothelial-derived NO to activate the transcriptional machinery driving mitochondrial biogenesis is well established [30].

Correspondingly, during inflammation, the genomic induction of inducible NOS (iNOS or NOS2) augments mitochondrial biogenesis [13, 31]. Physiological levels of NO stimulate mitochondrial biogenesis via cGMP and PGC-1 α [32, 33]. NOS3 (eNOS) activates cGMP generation and up-regulates the expression of PGC-1 α and NRF-1, thus activating mitochondrial biogenesis [33]. NO also causes vasodilation, which improves the availability of oxygen and carbon substrate for cell respiration and metabolism. In contrast, under inflammatory or certain other pathological conditions, excessive NO production directly inhibits respiration mostly through blockade of mitochondrial Complexes I (NADH dehydrogenase) and IV (cytochrome *c* oxidase, COX), markedly reducing OXPHOS [34, 35]. This may also enhance the incomplete reduction of oxygen, increasing ROS production and stimulating AMP kinase (AMPK). Moreover, NO reacts chemically with superoxide to produce peroxynitrite (ONOO⁻), leading to the direct oxidative damage to and nitration of mitochondrial macromolecules [36, 37].

NO plays an important role in skeletal muscle mitochondrial biogenesis [38]. Several studies have shown that treatment of cells with NO donors increases mitochondrial biogenesis, demonstrating the biosynthesis of functional mitochondria [39, 40]. Treatment of rat primary skeletal muscle cultures with the NO donor S-nitroso-N-acetylpenicillamine (SNAP) has led to a significant increase in myocyte mitochondrial content [41], an effect also observed in other tissues. In other studies, treatment of primary cultures of brown adipocytes (mitochondria-rich cells) with SNAP increases mitochondrial size and mtDNA content [33]. This stimulatory effect of SNAP was completely abolished by supplementation of the medium with oxyhemoglobin, a potent NO scavenger, implying that the induction depends on NO generation. Nisoli et al. first reported that eNOS ^{-/-} mice had reduced skeletal muscle mitochondrial content [40]. The NO-dependent signaling mechanisms involved in mitochondrial proliferation are complex and not entirely understood, but in general, increased CREB1, NRF-1, and PGC-1 α expression levels are required (**Figure 2**) [39, 40].

Several drugs with NO-releasing properties currently used in clinical practice, mainly for cardiovascular diseases, may also affect NOS mRNA, protein or activity levels with special modulation of eNOS [42]. NO-directed therapeutic approaches to improve skeletal muscle mitochondrial performance include diet, exercise, and pharmacological interventions targeted at increasing mitochondrial content and improving OXPHOS efficiency [43]. Furthermore, exercise training in patients with mitochondrial myopathy improves muscle strength and oxidative capacity [44], in part through NO-dependent mechanisms, but further studies are needed to determine the safety, effectiveness, and the most appropriate exercise training regimen.

Supplementation with arginine or citrulline has been proposed for the treatment of mitochondrial disorders associated with NO deficiency [45]. L-arginine was first used as an NO precursor in the treatment of a mitochondrial encephalopathy with stroke-like syndrome (MELAS) [46, 47]. This therapeutic approach is supported by the finding of low levels of serum arginine [46] and citrulline [48] and endothelial dysfunction [49] in patients with MELAS. Based on these findings and the NO deficiency observed in muscle fibers of patients with mitochondrial myopathy and cytochrome *c* oxidase deficiency, it was proposed that arginine and citrulline supplementation could have a broader application in

mitochondrial diseases, especially in those with muscle symptoms [45]. However, the rationale for simple NO-based therapy depends on the capacity of the patient to increase the rate of proliferation of mitochondria with intact OXPHOS systems, a limitation in patients with hereditary mtDNA mutations and a low background heteroplasmy.

Of further interest, HCT 1026, a non-steroidal anti-inflammatory derivative of flurbiprofen that releases NO, has been tested in Duchenne's muscular dystrophy and shown to decelerate disease progression [50]. In the same study, arterially-delivered donor stem cells in association with HCT 1026 offered enhanced therapeutic efficacy. This treatment demonstrated better results than the combination of L-arginine and deflazacort [51]. In another studies, Buono et al. [52] showed that an NO-releasing drug, molsidomine had therapeutic efficacy in dystrophic mice. The drug increased regenerating muscle fibers and enhanced muscle function due to increased proliferation of satellite cells. These studies open up novel possibilities for treatment of mitochondrial dysfunction, since the drugs have already been tested for safety in humans, and could be used in longer term treatments.

In ischemia/reperfusion injuries (I/R) of the heart and brain, injury progression is associated with mitochondrial dysfunction, characterized by ATP depletion, calcium-induced opening of the mitochondrial permeability transition pore, and exacerbation of mitochondrial ROS release [53]. Recently, nitrite, recognized as an NO donor specifically in hypoxic/acidic conditions, has been proposed to mediate cytoprotection without substantially altering normal tissue. Many studies have investigated the effects of acute treatments with nitrite on ischemia, and one recent paper showed that long-term nitrite therapy, when initiated 24 hours after I/R, improved tissue and vascular regeneration, and led to better functional recovery [54]. Thus, the authors proposed that early and long-term nitrite therapy may be effective for the management of ischemic conditions such as stroke.

The CO/HO-1 System

The dogma that carbon monoxide (CO) is poisonous is certainly well-founded, particularly to the brain and heart, but emerging studies also suggest that low doses of CO can provide cytoprotection through its anti-inflammatory and anti-apoptotic properties [55, 56]. Basically, endogenous CO acts as a messenger gas that regulates mitochondrial function through its important impact on the control of mitochondrial bioenergetics and the regulation of biogenesis.

The latter is triggered mainly through increased mitochondrial ROS production that leads to induction of HO-1 (reviewed in [57]). Under such circumstances CO gas or the administration of CO-releasing molecules (CORMs) generate a positive feedback loop that contributes to the beneficial effects of the up-regulation of the HO-1/CO pathway (**Figure 2**).

CO is a non-reactive, inexpensive, and easy-to-produce gas, and is thus easily administered as an inhaled gas similar to NO, which is already in clinical use [58]. With pulses of inhaled CO, tissue levels of CO rise transiently and follow PO₂ dependent elimination kinetics. The targets of CO binding in tissue are also restricted mainly to reduced transition metals, such

as the iron centers in hemoglobin, myoglobin, cytochrome P450s, and cytochrome *c* oxidase [57, 58].

There is substantial preclinical evidence demonstrating beneficial effects of CO, administered as authentic CO gas or as a CORM in cardiovascular disease, stroke, sepsis, transplanted organ rejection, and acute organ injury [58-60]. CO was originally shown to modulate immune response and stimulate production of anti-inflammatory cytokines [59] and many such effects can be linked to the activation of mitochondrial biogenesis. Indeed, the transcriptional network of mitochondrial biogenesis is directly linked to the production of several counter-inflammatory cytokines including interleukin-10 (IL-10), IL-1 receptor antagonist, and suppressor of cytokine synthesis 3 (SOCS3) [16].

In human volunteers under normoxia, CO has a half-life of 4–5 hours, and CO elimination is primarily through exhalation by the lungs [58] with nominal further metabolism to CO₂ by mitochondria. Moreover, clinical investigators must consider that the critical safe upper limit of carboxyhemoglobin [HbCO] in human blood in normal individuals is unknown, and it is certainly lower in patient with cardiovascular and cerebral disease [61]. Ongoing clinical trials focused on the anti-inflammatory effects of CO have set an upper limit of exposure at 8% HbCO, the equivalent of smoking about two packs of cigarettes per day.

The first single, blinded, randomized, placebo-controlled Phase I trial to evaluate the clinical safety and pharmacokinetics of authentic inhaled CO in healthy humans has recently been completed [58]. CO gas has been evaluated for safety in healthy humans with rigorous dose-escalation studies and was acceptable for Phase II testing in lung disease patients, which are currently ongoing. In addition, inhaled CO is currently undergoing evaluation for safety and potential efficacy of CO in subjects with severe pulmonary arterial hypertension.

The application of CO releasing molecules (CORMs) are valuable alternative to inhaled CO, because they can be administered to biological systems via systemic routes and potentially in a tissue-specific manner thus avoiding unreliable inhaled application and allowing safe targeting organs such as the central nervous system. CORMs suppress the inflammatory response in glial cells [62] and might therefore exhibit neuroprotection. However, other findings have elicited some confusion as to the consistency of the protective effects, which may reflect the choice of CORM.

CORMs all possess a backbone carrier moiety, most commonly embodied as organometallic carbonyl complexes that must be stringently characterized from a metabolic and toxicological standpoint. Other promising candidates, the boranocarbonates, avoid potential metal toxicity, but generally release CO very rapidly. Several CORMs have been synthesized for therapeutic agents aimed at delivering controlled amounts of CO to tissues and organs [63]. CORM-1 is soluble and stable in water giving an approximate pH of 11 and decomposes rapidly releasing CO under physiological condition with slow kinetics (21 min) [64]. CORM-2 is soluble in DMSO and olive oil and releases CO by photo-dissociation [63]. CORM-3 is ruthenium-based, a water-soluble agent with half-life of 1 min [65]. CORM-3 is effective when given before or late after neuronal injury but not when given early afterwards [66]. A molybdenum-based, water-soluble CORM (ALF186) that releasing

CO in a dose- and oxygen-dependent manner after administration was shown to have protective effects *in vivo* in models of acute inflammation [67] and in the regulation of vasomotor tone [68, 69]. ALF186 inhibits IR-induced neuronal cell death via activation of soluble GC and may become a useful treatment for acute ischemic insults to the retina and the brain. These compounds generally promote vasorelaxation and hypotension via activation of GC and potassium channels, resulting in improvements in vascular function. In addition, CO released from CORMs inhibits NF- κ B-mediated inflammatory gene expression and up-regulates the expression of adaptive genes for oxidative stress. Therefore, CO-releasing compounds have been proposed as novel agents for the therapy of an array of vascular, inflammatory, and oxidative stress-induced disorders.

Hydrogen sulfide (H₂S)

Recently, a growing body of evidence has supported the idea that enzymatically-generated H₂S is an endogenous signaling gas involved in several physiological processes [69, 70] that protect organs against damage, such as I/R injury [71, 72]. In contrast, the role of the molecule in primary inflammatory states is still a matter of debate. In fact, marked pro- [73, 74] as well as anti-inflammatory effects of H₂S [75, 76] have been observed in different experimental studies.

In principle, H₂S is a potent inhibitor of OXPHOS by its well-known ability to inhibit cytochrome *c* oxidase (COX), a toxic property of many low molecular weight sulfides [77]. On the other hand, H₂S therapy has been observed to preserve mitochondrial function in the heart muscle of rodents after I/R injury [75, 78]. Similar to CO, low level H₂S administration increases the phosphorylation of protein serine/threonine kinase B (Akt) and enhances the nuclear localization of two transcription factors, nuclear respiratory factors -1 and -2 (**Figure 2**), which are involved in increasing the levels of endogenous antioxidants, attenuating apoptosis, and increasing mitochondrial biogenesis [78].

β_2 -Adrenergic Receptor Agonists (β_2 -AR)

Agonists for the β_2 -AR have been reported to modulate oxidative metabolism, energy expenditure, lipolysis, glucose transport, and glucose oxidation [79]. Mice treated with the β_2 -AR agonist formoterol showed a 5-fold induction of PGC1- α mRNA in skeletal muscle. β_2 -AR receptor agonists are airway smooth muscle relaxants and are approved for the treatment of asthma and other airways diseases. The β_2 -AR has been successfully targeted for drug discovery by using a ligand-based approach, resulting in the creation of multiple receptor-specific drugs [80, 81]. However, the therapeutic potential of established and novel β_2 -AR agonists as inducers of mitochondrial biogenesis and counter-inflammation has not fully been explored.

Triterpenoids (TPs)

TPs are a group of Nrf2-activating compounds. The Nrf2 pathway is normally activated by oxidative stress or by electrophilic compounds in cells; however, for therapeutic purposes, Nrf2 has been found to be readily activated by synthetic TP, which are olenolic acid analogs. They function as inhibitors of oxidative stress and inflammation and have been shown to be protective in cancer models [82]. For decades, many plant-derived chemical substances, or

phytochemicals, have been shown to have chemo-preventive activities, and most of these are Nrf2 inducers [83]. The most potent known phytochemical compounds that induce Nrf2 include the sulforaphanes from cruciferous vegetables [84], curcumin [85], caffeic acid phenethyl ester [85], and wasabi [86]. Recently, novel Nrf2-activating, synthetic TP, CDDO methylamide (CDDO-MA), was found to augment the expression of genes involved in mitochondria biogenesis, glutathione synthesis, and antioxidant mechanisms [87]. It is also reported that CDDO-MA has neuroprotective effects in 3-nitropropionic acid (3-NP) rat and MPTP mouse models [87]. Other intriguing neuroprotective agents that induce *Hmox1* expression by this mechanism are the electrophilic neurite outgrowth-promoting prostaglandin (NEPP) compounds [88].

Erythropoietin (EPO)

EPO is essential for the regulation of the mass of erythrocytes in response to changes in tissue oxygenation during hypoxia and anemia. However, EPO activity is not restricted to the erythroid lineage because the EPO receptor (EpoR) is widely expressed. The protective effects of EPO have been demonstrated in various non-erythroid tissues and experimental models of IR injury and have been attributed to its effect on non-hematopoietic metabolic adaptation, inhibition of apoptosis or stimulation of angiogenesis [89]. Recently, EPO has been reported to stimulate cardiac mitochondrial proliferation through activation of mitochondrial biogenesis, which is mediated by NRF-1 and PGC-1 α , key regulators of aerobic function [90]. Clinically, EPO reverses cardiac remodeling, improves cardiac function, and enhances the exercise tolerance and quality of life of heart patients by inducing protective effects beyond the correction of anemia [91]. These findings highlight the possibility that EPO-mediated protection may depend on its modulatory effects on intracellular energetics. EPO can stimulate proliferation of myoblasts in culture through binding to EpoR to expand the progenitor population during differentiation and may have a potential role in muscle maintenance or repair [92, 93]. Recently, EPO/EpoR signaling has been shown to increase glucose tolerance and protect against diet-induced obesity, an activity associated with skeletal muscle adaptation [94, 95]. The association of EPO treatment in mice with increased mitochondrial biogenesis and enhancement of PGC-1 α in cardiomyocytes raises the possibility that EPO may contribute directly to skeletal muscle plasticity and the balance of slow and fast-twitch fibers. Furthermore, rhEPO treatment increases OXPHOS and ETS in human skeletal muscle [96]. The enhancement and recovery of cellular functions through the stimulation of mitochondrial activity and hemoglobin production in non-hematopoietic cells by an inducer of endogenous EPO might serve as a potential therapeutic strategy for certain ischemic or mitochondrial diseases (**Figure 2**).

The Thiazolidinedione Drugs

The thiazolidinediones activate PPAR γ , a cognate target of PGC-1 α . Rosiglitazone is a plant-derived polyphenol resveratrol (3,5,4'-trihydroxystilbene) is enriched in red wine and functions as a caloric restriction mimetic via up-regulation of sirtuin-1 (Sirt1) and AMP activated protein kinase (AMPK) [97, 98]. It also enhances mitochondrial biogenesis through up-regulation of PGC1 α , NRF-1 and -2, and Tfam [99]. Thiazolidinedione modulation of mitochondrial biogenesis in the heart has not been published; however, thiazolidinedione therapy improves post-infarction cardiac remodeling and reduced infarct

size in numerous species [100, 101], but these findings have been disputed in other studies [102, 103]. Nonetheless, anti-inflammatory effects of resveratrol are well documented, and two groups have independently shown that resveratrol treatment prevents cardiac dysfunction in hypertensive rats without reduction in blood pressure [104, 105], indicating the direct influence of this compound on the heart. Importantly, mitochondrial biogenesis, mitochondrial mass, and respiration were preserved by resveratrol in salt-sensitive hypertensive rats [105] and in rats transgenic for human renin and angiotensin genes [99]. A small human clinical trial reported minor improvements in diastolic function in 40 patients with acute coronary syndrome receiving 10 mg resveratrol daily during the 3-month trial period. In addition, an improvement in endothelial function and reduction in low-density lipoprotein levels were noted in the treatment group, but mitochondrial biogenesis and function were not assessed [106]. Thus, the beneficial effects of this compound may be limited to highly selected situations, such as hypertensive and post-infarction patients. Moreover, resveratrol's therapeutic effects are limited by its short initial half-life and low in vivo bioavailability [107]. In order to differentiate the cardioprotective properties from the pleiotropic effects of resveratrol, small molecule analogs that directly activate, Sirt1 are being actively pursued [108].

AMPK Activators and mTOR

There are a number of AMPK activators known to induce mitochondrial biogenesis; here only two of the most widely studied are discussed briefly, metformin and AICAR. Metformin, an insulin-sensitizing biguanide, is currently an FDA-approved front-line oral hypoglycemic treatment for Type 2 diabetes [109]. Metformin lowers blood glucose mainly via hepatic glucose output and insulin-stimulated glucose uptake in skeletal muscle and adipocytes [110]. The molecular mechanisms behind the effects of metformin are not fully clarified. Although inhibition of Complex I in the mitochondrial electron transport chain (ETC) in cells, isolated mitochondria and muscle homogenate has been reported [111], metformin-induced activation of the 5'-AMP activated protein kinase (AMPK) has been shown in rodent muscles [112] and in human skeletal muscles from patients with type 2 diabetes [113]. Numerous studies have documented a role for AMPK in regulating mitochondrial function and biogenesis and it too leads to anti-inflammatory effects [114]. On the other hand, potential effects of metformin treatment on mitochondrial function by decreasing ROS production have been proposed [115]. Furthermore, long term metformin treatment increased PGC-1 α expression in muscle together with increased activities and protein expressions of mitochondrial marker enzymes [116]. More recent data suggest that metformin has an anti-folate function by inducing the ATM/AMPK tumor suppressor axis, secondarily altering carbon flow through folate-related single-carbon pathways [117].

5-Aminoimidazole-4-carboxamide ribotide (AICAR) was found to increase mitochondrial biogenesis, ATP content while decreasing ROS production. AICAR effects are mediated via activation of AMPK that directly regulates energy expenditure by modulating NADH⁺ dependent-type III deacetylase Sirt1, resulting in the deacetylation of downstream targets including PGC1 α , forkhead box O1 and 3 transcription factors [118]. The drug does have significant toxicity and the use of AICAR as a cardiac therapeutic target is limited in that it promotes bradycardia and hypoglycemia [119]. Other compounds such as bezafibrate and

olipraz have been seen by some as having a more favorable therapeutic index, but their anti-inflammatory properties are not dramatic.

Mitochondrial number and health are regulated by processes that degrade and recycle damaged and non-functional mitochondria by selective macroautophagy or mitophagy. Hence, mitophagy is a key determinant of mitochondrial health and apposite cell function. Faulty mitophagy has been proposed to contribute to neuronal loss in human neurodegenerative diseases, and several lines of evidence suggest that mitochondrial function substantially influences the autophagy process. The mitochondria's ability to influence and be influenced by autophagy places both mitochondria and autophagy in a position where defects in one system or the other could exacerbate various metabolic and autophagy-related diseases.

Certain autophagy induction strategies might be used appropriately to stimulate mitophagy. For example, the immunosuppressive agent rapamycin (Sirolimus), used to suppress rejection in organ transplantation, induces autophagy via inhibition of the Ser/Thr protein kinase mTOR (mammalian target of rapamycin), which down-regulates cell growth and metabolism in response to environmental cues [120]. The mTOR pathway is also disrupted in diseases such as obesity, diabetes, and in certain cancers. And rapamycin induces metabolic changes, including hyperlipidemia, glucose intolerance, decreased insulin sensitivity, and increases the propensity for diabetes [121]. Rapamycin derivatives with better pharmacokinetics (rapalogs) have been developed as chemotherapeutic agents, but despite promising results in animal models, they have generally performed poorly in human cancer trials and are currently approved only for the treatment of tuberous sclerosis, pancreatic cancer, and renal cell carcinoma [122].

This dissatisfying result has been partly attributed to the fact that rapalogs predominately inhibit mTORC1, leading to increased PI3K and Akt activity, which promotes growth. This growth response can be attenuated by the disruption of mTORC2; therefore, recent pharmaceutical interest has been focused on mTOR inhibitors that inhibit both mTORC1 and mTORC2. Interestingly, caffeine, a weak inhibitor of TOR, mediates lifespan extension in yeast [123], and the dose received from coffee might perhaps mildly affect mTOR in humans. Another common anti-inflammatory drug, aspirin, may exert protection by inhibiting the phosphorylation of TSC1 [124], although aspirin is also a weak AMPK activator. AMPK inhibits mTORC1 by the activating phosphorylation of TSC2 and by the inhibitory phosphorylation of raptor, an essential part of mTORC1 [125]. AMPK activators that specifically inhibit mTORC1 activity may be a useful strategy to favorably increase mitophagy, while stimulating mitochondrial biogenesis.

Natural compounds targeting mitochondrial biogenesis

Mitochondrial nutrients—Mitochondrial nutrients protect the mitochondria from oxidative damage and maintain or improve mitochondrial function. These macromolecules and nutrients are used to correct relative or absolute deficiencies of critical metabolic components or which increase the levels of critical substrates and enzymes resulting in enhanced cellular metabolic efficiency. Such nutrients also perform a number of other beneficial functions including limitation of ROS production and elimination of oxidative

stress in mitochondria and reducing acute inflammation. These micronutrients include iron-chelating agents and enzymatic and non-enzymatic radical scavengers, such as α -tocopherol [126]; enhancers of antioxidant defenses such as coenzyme Q, and lipoic acid, glutathione, and α -tocopherol, enhancers of mitochondrial metabolism via mitochondrial quality control (and increasing mitochondrial biogenesis) such as lipoic acid and acetyl-L-carnitine, creatine, pyruvate, and choline [127, 128]; protectors of mitochondrial enzymes and/or stimulators of mitochondrial enzyme activity by elevating substrate and cofactor levels (such as B vitamins) [126].

Mitochondrial-targeted ROS scavengers—The persistence of damaged mitochondria is the main source of ROS production in most aerobic cells. This has led to many attempts to target the mitochondrion with small molecule anti-oxidant drugs. The best example and most well-characterized mitochondrial-targeted antioxidant to date is MitoQ, a hydroquinone ROS-scavenging moiety linked to triphenylphosphonium (TPP), a lipophilic compound that easily crosses membranes and accumulates in the matrix as a function of membrane potential. MitoQ thus aims to mimic the role of endogenous coenzyme Q10 (CoQ10), but also to augment the antioxidant capacity of CoQ in a mitochondrial membrane potential-dependent manner. Scavenging of ROS is achieved through oxidation of MitoQ into its quinone form, which is then recycled back into the active hydroquinone by the action of mitochondrial Complex II [129]. MitoQ is orally bioavailable with no toxicity detected when administered to mice at ~20-mg/kg. Tracer studies have found the compound to be rapidly taken up into the heart, liver, brain, kidney, and muscle, with highest accumulation in the heart and liver [130]. Long-term administration of MitoQ had no effect on plasma glucose, insulin, free fatty acid, or cholesterol levels, but significantly reduced triglycerides. Affymetrix chip analysis of the heart and liver tissue of mice receiving MitoQ revealed no significant differences in gene expression profile between the treatment and control groups [131]. Thus, MitoQ is an orally available small molecule that does not significantly impact baseline physiology.

In humans, MitoQ has been studied in the treatment of Parkinson's disease (PROTECT) and in patients with chronic hepatitis C infection. The results of the PROTECT trial were negative, but it provided a wealth of data on the safety of the drug administered orally for as long as 1 year [132]. On the other hand, patients receiving 40 and 80 mg MitoQ in the chronic hepatitis C trial showed significant improvement in hepatic function [133]. Importantly, no major side effects of MitoQ were seen in either trial. Despite the significant therapeutic potential of MitoQ and other TPP-conjugated antioxidants, there are limitations. The uptake of these compounds is governed by mitochondrial membrane potential, which may be severely disrupted, for instance in failing hearts. Moreover, accumulation of cationic TPP in the matrix can potentially depolarize mitochondria, leading to unwanted side effects. Thus, this therapeutic strategy warrants further investigation.

Mitochondrial targeting using peptide mimetics or lipophilic cationic agents (MitoQ, SkQ1) or manganese superoxide (SOD2) mimetics [134-137] may also offer improved antioxidant therapies for mitochondria. However, strategies to direct these compounds to the respective organ or cell type of interest at the appropriate times are still lacking, and their impact on the redox-regulation of mitochondrial biogenesis is unknown.

In conclusion, the area of mitochondrial-based therapy to support mitochondrial biogenesis, mitochondrial QC, and anti-inflammation is still in its infancy. The recent discovery of a group of novel redox-regulated molecular pathways that protect cell viability through the support mitochondrial QC offers a plethora of new targets and the opportunity for unique small molecule treatments for diseases in which persistent mitochondrial dysfunction is a contributing factor.

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Figure 1. Schematic of cell showing the carbon substrate inputs into mitochondria and sites of action of CO and NO. Mitochondria normally release small amounts of reactive oxygen species (ROS), which can serve as signals for multiple adaptive cellular signaling processes including mitochondrial biogenesis.

Figure 2.

Simplified diagram of a generalized cell depicting the effect of activation of the membrane bound Toll-like receptor (TLR) system during inflammation on NF-κB dependent cytokine production, NO, CO and H₂S production, mitochondrial damage, and redox signaling pathways for the induction of mitochondrial biogenesis. Known targets of NO/NO donors, CO/CO-releasing molecules (CORMs), AMPK activators and other compounds discussed here are indicated.