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Nutritional Interventions for Mitochondrial OXPHOS Deficiencies: Mechanisms and Model Systems

Adam J. Kuszak[1], **Michael Graham Espey**[2], **Marni J. Falk**[3,4], **Marissa A. Holmbeck**[5], **Giovanni Manfredi**[6], **Gerald S. Shadel**[5,7], **Hilary J. Vernon**[8], and **Zarazuela Zolkipli-Cunningham**[9]

[1]Office of Dietary Supplements, National Institutes of Health, Bethesda, MD 20852 USA

[2]Division of Cancer Biology, National Cancer Institute, Rockville, MD 20850 USA

[3]Division of Human Genetics, Department of Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, PA 19104 USA

[4]Department of Pediatrics, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104 USA

[5]Department of Pathology, Yale School of Medicine, New Haven, CT 06510-8023 USA

[6]Brain and Mind Research Institute, Weill Cornell School of Medicine, New York, NY 10065 USA

[7]Department of Genetics, Yale School of Medicine, New Haven, CT 06520-8023 USA

[8]McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD 21205 USA

[9]Division of Neurology, Department of Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, PA 19104 USA

Abstract

Multi-system metabolic disorders caused by defects in oxidative phosphorylation (OXPHOS) are progressive and severely crippling, often lethal, conditions. Inborn errors of OXPHOS function are termed Primary Mitochondrial Disorders (PMDs), and the use of nutritional interventions is routine in PMD supportive management. However, detailed mechanistic understanding and evidence for efficacy and safety is limited. Preclinical cellular and animal models systems are important tools to investigate PMD metabolic mechanisms and therapeutic strategies. This review assesses the mechanistic rationale and experimental evidence for nutritional interventions commonly used in PMDs, including micronutrients, metabolic agents, signaling modifiers, and dietary patterns, while highlighting important gaps in knowledge gaps and impediments for randomized controlled trials. The state-of-the-science for cellular and animal model systems that recapitulate mutations and clinical manifestations of specific PMDs are evaluated for their potential in determining pathological mechanisms, elucidating therapeutic health outcomes, and investigating the value of nutritional interventions for multiple mitochondrial disease conditions.

Correspondence to: Adam J. Kuszak; Gerald S. Shadel.

Keywords

primary mitochondrial OXPHOS disorders; nutritional interventions; electron transport chain; metabolism

Introduction

The classical view of mitochondria as simply cellular “powerhouses” has given way to an appreciation of a dynamic organelle network at the nexus of energy production, metabolic signaling, and cellular homeostasis. Multi-system metabolic disorders are caused by discrete genetic mutations that disrupt oxidative phosphorylation (OXPHOS). These Primary Mitochondrial Disorders (PMDs), sometimes referred to as mitochondrial encephalopathies or myopathies, are progressive, severely crippling, and often lethal diseases that affect 1 in 4,300 people (1). First identified in the late 20th century, research on PMD mechanisms and treatments has proven challenging due to their rarity, difficulties diagnoses, and genetic and phenotypic heterogeneity (2). For example, mutations in either nuclear genes or mitochondrial (mt)DNA encoding distinct proteins involved in OXPHOS can result in similar clinical manifestations. Conversely, the same genetic mutation can result in different clinical phenotypes, where the severity of PMDs may increase with the percentage of mutant mtDNA (heteroplasmy) in an age and cell type specific manner. Consequently, preclinical identification of therapeutic targets and clinical evaluation of interventions for PMD patients represent significant challenges, and no cures exist.

The use of nutritional interventions, including dietary supplements, in the supportive management of PMDs is an established approach intended to alleviate symptoms and/or delay disease progression (3, 4). However, the efficacy of supplements for specific biological endpoints or health outcomes is usually not known (4). Marketed dosages of nutritional supplements vary greatly, and the bioavailability and metabolism of various formulations are not always known. Nutritional interventions for PMD are empirically individualized to each patient, with the literature mostly comprising observational reports of non-standardized dosing regimens and limited evidence of broad efficacy (5). The ubiquity of supplements can present challenges for compliance in use of placebo controls and randomization in clinical trials for PMDs.

An understanding of the mechanistic rationale for using nutritional interventions in PMD patients is critical for investigating efficacy and safety. While some nutritional interventions for PMDs such as nicotinic acid and L-carnitine can be prescribed in FDA-approved drug formulations, most cannot, prompting patients and caregivers to seek out dietary supplement versions for reasons related to personal preference, cost, or convenience (4, 6). To this end, the development and use of robust preclinical model systems to investigate the pathogenic mechanisms of PMDs and strategically study the impact of nutritional interventions is tantamount (4). Herein, we survey nutritional interventions utilized in clinical settings for PMDs and describe key cellular and animal model systems of OXPHOS dysfunction that can be used to further elucidate the mechanisms and health outcomes of PMDs, and point to important areas in need of additional study.

Clinical Investigations of Nutritional Interventions

Nutritional interventions can be categorized as micronutrients, metabolic-modifying agents, or signaling-pathway modulators. Those currently used for PMDs, and their mechanistic rationale, are described (Figure 1; Supplemental Table S1).

Micronutrients

Thiamine and biotin—Thiamine (vitamin B1) is a cofactor of α-ketoacid dehydrogenase complexes, which include pyruvate dehydrogenase complex (PDHc), α-ketoglutarate dehydrogenase, and branched-chain α-keto acid dehydrogenase; as well for transketolase, an enzyme of the pentose phosphate pathway, and 2-hydroxyacyl CoA lyase. While the thiamine requirement for healthy individuals is a daily intake of 1–2 mg, there is no evidence of adverse effects in patients receiving higher doses (7).

Thiamine is transported into cells by thiamine transporter 1 (THTR1) and THTR2. Mutations in the *SLC19A3* gene (encoding THTR2) results in biotin-thiamine responsive basal ganglia disease and is recognized as a treatable cause of early-onset progressive neurodegenerative Leigh syndrome. A combination of biotin (2–10 mg/day) and thiamine (100–400 mg/day) has been found to be effective in THTR2 deficiency (8, 9). In current clinical practice, empiric supplementation of thiamine and biotin in the form of a B vitamin complex is commonly used (10), based on the premise it will enhance PDHc and/or OXPHOS flux, and thiamine-responsive PDHc deficiency has been reported (7). However, these thiamine and biotin combinations have not been studied in randomized control trials (RCTs), or in mitochondrial disease models.

Riboflavin—Riboflavin (vitamin B2) is the precursor of flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), which serve as essential cofactors for numerous mitochondrial dehydrogenases (Figure 1) (11). Riboflavin (50–400 mg daily) is a common intervention intended to enhance electron transport chain (ETC) efficiency in PMDs. However, these doses are not based on evidence from RCTs (12). Beneficial responses to riboflavin supplementation have been reported in patients with primary genetic defects of riboflavin transport (13, 14). Multiple acyl CoA dehydrogenase deficiency (MADD) is an autosomal recessive disorder of fatty acid and amino acid metabolism involving a defect of electron transport from FAD-containing CoA dehydrogenases to CoQ_{10} in the mitochondrial ETC, caused by mutations in flavoprotein dehydrogenase or flavoprotein genes *ETFDH*, *ETFA* or *ETFB* (15). Oral riboflavin supplementation (100–400 mg daily) often yields muscle strength improvement in patients with MADD. Mutations in *ACAD9* (encoding acyl-CoA dehydrogenase 9) have been reported in some patients with mitochondrial ETC complex I deficiency and myopathy, and riboflavin supplementation (150–300 mg daily) yields improved exercise intolerance (16, 17). Neurological exam and muscle strength improvements, and muscle Complex I and skin fibroblast Complex II activity responses to riboflavin have been reported in some small, open label studies of patients with ETC complex I and II deficiencies (18–20). However, open-label studies in patients with a variety of PMDs (21, 22) did not show a significant response to riboflavin treatment in combination

with other vitamins, and riboflavin supplementation in PMDs has not been reported in RCTs (23).

Nicotinic acid and nicotinamide riboside—Niacin (vitamin B3) refers to both nicotinic acid (NA) and its amide form nicotinamide (NAM), which are precursors to the essential cofactor nicotinamide adenine dinucleotide (NAD^+) and NAD^+ phosphate ($NADP^+$) (24). In addition to crucial metabolic roles, these cofactors also play a central role in sirtuin activity and peroxisome proliferator activator receptor (PPAR) activation (25). NA is an FDA-approved drug for hypertriglyceridemia, while NAM is available as a dietary supplement in the United States.

Mitochondrial Complex I converts NADH to NAD^+, and an increased cytosolic NADH:NAD^+ ratio can shift the lactate dehydrogenase equilibrium and cause the lactic acidosis commonly seen in PMDs (26). Exercise and fasting are known to decrease the NADH/NAD^+ ratio, resulting in activation of SIRT1 that induces a metabolic-fasting response and boosts mitochondrial biogenesis, fatty acid oxidation, and ATP production (27). However, disrupted NAD^+ metabolism remains a relatively under-appreciated pathogenic factor in PMDs. NA and NR supplementation has not been reported in RCTs (28), though several studies in cellular and animal model systems indicate that niacin and its derivatives warrant further preclinical evaluation (see Sidebar).

Sidebar

Niacin and nicotinamide riboside studies in PMD model systems

Model system studies have investigated the role of NADH/NAD^+ ratio in PMDs. Leigh syndrome patient fibroblasts harboring mutations in *ND4* and *ND6* Complex I subunit genes revealed an increased relative NADH:NAD^+ ratio but decreased absolute NADH and NAD^+ concentrations. NA treatment normalized nutrient-sensing signaling network components (mTORC1 and AMPK), restored NADH/NAD^+ redox balance, and improved cellular respiratory capacity (240). NR supplementation in patient fibroblasts harboring a *NDUFS1* mutation increased cellular NAD^+ content (241). *C. elegans* research suggests NAD^+ deficiency contributors to global disruption of the nutrient-sensing network; NA treatment improved survival, decreased mitochondrial oxidant burden, normalized branched chain amino acid concentrations, and restored NADH (but not NAD^+) levels (129). Complex I-deficient patients have elevated NADPH (242), and *Ndufs4*$^{-/-}$ mice revealed $NADP^+$ levels also increase such that $NADP^+$/NADPH ratio is unchanged (243). NR supplementation in *Ndufs4*$^{-/-}$ mice partially normalized the NADH/NAD^+ ratio (244). NR induced mitochondrial biogenesis in *Deletor* mouse skeletal muscle and brown adipose tissue, preserved mitochondrial ultrastructure, and prevented mtDNA deletion accumulation (245). Collectively, these studies suggest that NADH deficiency can contribute to the phenotype of some PMDs, and that niacin supplementation might improve overall cellular function.

Folinic acid—Folinic acid (vitamin B9; 5-formyltetrahydrofolate) is a CNS-penetrant form of folic acid that functions as a B vitamin cofactor necessary for cellular one-carbon-transfer

reactions (Figure 1). Cerebral folate deficiency (CFD) is characterized by 5-methyltetrahydrofolate (5-MTHF) deficiency in the cerebral spinal fluid (CSF) with normal peripheral total folate levels (29). Kearns–Sayre syndrome patients have CFD and beneficial responses to folinic acid have been reported (30, 31). However, CFD does not occur consistently in other PMDs. Empiric supplementation is relatively common in PMD patients with neurologic symptoms, especially in those documented to have CSF 5-MTHF deficiency. Folinic acid doses range from 0.5–2.5mg/kg daily in children or 2.5–25 mg daily in adults (12), and are typically well-tolerated. To date no results of RCTs for folinic acid for PMDs have been reported.

Metabolic-modifying agents

L-arginine—Although L-arginine is synthesized endogenously, metabolic demand may exceed biosynthetic capacity under certain stress or disease conditions. L-arginine is a precursor of proteins, polyamines, and creatine, can be converted to α-ketoglutarate (32), and can be decarboxylated to form agmatine, which possess antioxidant, anti-inflammatory, and neuromodulatory properties (33). By virtue of conversion to nitric oxide, L-arginine also has widespread impacts on gene expression and metabolic profiles via activation of PPAR-α and PGC-1α (34).

Beneficial effects of L-arginine supplementation have been reported in mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS) (35), including improved serum arginine deficiency and prevention of stroke-like episodes (36). Consequently, L-arginine is considered a front-line treatment for stroke-like episodes in MELAS (10). While the initial rationale for testing L-arginine therapy in MELAS was to improve vasodilation (37), oral L-arginine (10 g/m^2 body surface area/day) in MELAS patients increased de novo arginine synthesis rate, increased arginine and citrulline flux (36), and decreased plasma lactate levels (38). L-arginine therapy in three MELAS siblings yielded increased trends in mean percent maximum work at anaerobic threshold and a small increase in their VO_{2peak}. L-arginine supplementation also slowed acidification, increased phosphorous to phosphocreatine (Pi/PCr) ratio, and decreased half-time of PCr recovery following moderate intensity exercise (39). While arginine supplementation has been investigated in open-label studies of MELAS patients, its efficacy in other PMDs has not been studied in model systems or RCTs.

L-carnitine—Carnitine is a hydrophilic quaternary amine with a major function in transporting long-chain fatty acids from the cytosol to the mitochondrial matrix for β-oxidation (Figure 1). Other functions include modulation of the acyl-CoA/CoA ratio, muscle storage of energy as acetyl-carnitine, and urinary excretion toxic acyl groups (40). L-carnitine contains a β-hydroxy moiety that can directly scavenge free radicals (41, 42).

Although carnitine is endogenously synthesized, the majority is obtained from the diet. PMD patients may have decreased free carnitine (43), which supplementation is aimed at restoring. However, circulating carnitine does not reflect tissue carnitine levels (44), and the evidence for clinical administration of L-carnitine is based only on case reports. Despite the lack of proven efficacy, L-carnitine supplementation (50–100 mg/kg/day) in PMD is

common. Recently, concerns emerged of increased risks for cardiovascular disease and adverse cardiac events in subjects with high fasting plasma carnitine and its metabolite, trimethylamine-N-oxide (TMAO) (45). In addition, chronic dietary L-carnitine supplementation in mice markedly enhanced synthesis of trimethylamine and TMAO and increased atherosclerosis (45). These findings highlight the need to carefully consider the risk:benefit ratio with regard to nutritional supplements in PMD patients, especially when the evidence for efficacy and safety from rigorous studies is limited.

Creatine—Creatine transiently stores metabolic energy via the exchange of a high-energy phosphate bond with adenosine triphosphate (ATP) by creatine kinase (CK) (46). The bulk of creatine is stored in skeletal muscle, but it is also found in other high-energy demand tissues.

Creatine monohydrate supplementation can increase intracellular phosphocreatine levels in healthy individuals (47), and since PMD patients have low phosphocreatine levels (48, 49), supplementation may help buffer energy levels. In a Cochrane review of 12 mitochondrial disease patient RCTs, three trials studied creatine monohydrate alone, and one trial studied it in combination with coenzyme Q_{10} (CoQ_{10}) and lipoic acid (23). Creatine monohydrate treatment significantly increased handgrip strength and decreased post-exercise lactate compared to placebo in a study of six MELAS and one mitochondrial myopathy patient (23). Creatine supplementation in combination with alpha-lipoic acid and CoQ_{10} in 16 patients with confirmed PMD had no clinically significant effect, despite a significant decrease in plasma lactate and urine 8-isoprostane levels (50).

Coenzyme Q_{10}—CoQ_{10} is a lipid-soluble benzoquinone that transfers electrons in the ETC from Complexes I or II to Complex III and helps maintains mitochondrial inner membrane and OXPHOS complexes stability (51). In the ubiquinol form, CoQ_{10} inhibits lipid peroxidation and can protect mitochondrial inner membrane proteins and mtDNA from oxidative damage (52). Both endogenous synthesis and dietary sources contribute to CoQ_{10} levels in plasma, but leukocyte levels may better approximate tissue levels (53).

Mitochondrial OXPHOS dysfunction commonly causes increased reactive oxygen species (ROS) which can damage mitochondrial and cellular components. Therefore, a common empiric therapy is to use antioxidants in an attempt to protect from ROS-induced oxidative damage. Indeed, as a ROS scavenger with documented safety data, CoQ_{10} and its synthetic analogs are the most commonly recommended supplement option for PMDs (54). CoQ_{10} supplementation appears to be particularly effective in primary disorders of CoQ_{10} biosynthesis, which result in severe phenotypes including encephalomyopathy, ataxia, renal diseases, cerebellar atrophy, and hyperlactatemia (11).

Despite PMD clinical trials with CoQ_{10} supplementation, its therapeutic benefit has not been definitively established (23, 55, 56). Bioavailability of different CoQ_{10} formulations is an important consideration, and several animal studies investigating absorption and distribution of CoQ_{10} supplements have been performed in rodents (57, 58). CoQ_{10} enterally administered to rodents was shown to increase CoQ_{10} levels in blood and liver, but not in other tissues (56). While there have been few studies of CoQ_{10} distribution in human tissues

following supplementation, studies in patients with neurodegenerative disorders provide evidence that uptake into brain occurs at high doses (up to 2400 mg/day) (56).

α-lipoic acid—Lipoic acid (α-LA) an essential redox cofactor of pyruvate dehydrogenase (59). It is synthesized de novo by mitochondrial lipoyl synthase (60), acts in both the cytosol and plasma membrane, readily crosses the blood–brain barrier (61), and is rapidly absorbed and reduced to dihydrolipoic acid (DHLA) in various tissues (62). The reduced form is an antioxidant (63) and also recycles other antioxidants, such as glutathione (GSH) and vitamins C and E (62). LA in combination with creatine and CoQ_{10} supplementation in a 16 patient RCT revealed no clinically significant effects (64). Given its reported potential benefits in disorders that involve mitochondrial dysfunction, such as diabetes (65) and dyslipidemia (66), further clinical and preclinical studies of α-LA in PMDs are warranted.

Signaling-pathway modulators

Resveratrol—Resveratrol is a bioactive polyphenol with reported antioxidant, anti-inflammatory, and anti-apoptotic activities (67), and the capacity to promote mitochondrial biogenesis in model organisms (68). This is thought to occur via SIRT1-dependent deacetylation of PGC-1α, (68, 69) a major regulator of mitochondrial biogenesis, although this mechanism remains controversial (70, 71). Resveratrol can correct ETC Complex I and IV defects in human fibroblasts via SIRT1 and AMPK-independent mechanisms that involve estrogen receptors (72). Additionally, the combination of resveratrol supplementation and metformin treatment stabilized OXPHOS super-complexes in HeLa cells (73). More studies into the mechanism by which resveratrol protects mitochondrial function, and might benefit PMD pathology, are clearly justified.

Ketogenic diet—A ketogenic diet (KD) modulates the amount of carbohydrates, protein, calories, and/or fats to shift metabolism towards β-oxidation and ketone body production, which in turn increase transcription of OXPHOS, TCA cycle, and glycolysis genes (74). Four KDs have been developed: the ‘classic’ KD (~90% of calories from fat); the medium-chain triglyceride diet; a low glycemic index (<50) diet; and a modified Atkins diet (≤20 g/day carbohydrates for adults) (75). A 20% fat KD was effective in reducing seizure frequency in PMD patients with OXPHOS defects (76), although no connection was found between the efficacy of the KD and changes in specific OXPHOS enzyme activities. An induction of brain mitochondrial uncoupling protein isoforms and a decrease in mitochondrial ROS was observed in mice following a classic KD, suggesting it might reduce mitochondria-mediated oxidative stress (77). However, KD remains controversial and exploratory in PMDs, with concerns of intolerance or disease exacerbation due to promoting secondary fatty acid oxidation defects and NAD^+ deficiency.

Experimental models for PMDs

In this section, we describe salient examples of cellular and animal models used to investigate OXPHOS deficiencies, focusing on their use for testing nutritional interventions for PMDs. Key features of these model systems, along with some of their strengths and limitations are also highlighted (Figure 2; Supplemental Table S2).

Cellular models

Cell culture models have been extensively used to investigate mitochondria functions and pathologic phenotypes due to OXPHOS defects. More recently, induced pluripotent stem cell (iPSC) technology has allowed the production of patient-derived replicating cells, with specific genetic OXPHOS defects, which can be differentiated into disease-relevant cell types.

Primary cells with OXPHOS defects—Primary cells from PMD patients (e.g. platelets, lymphocytes, fibroblasts, and myoblasts) provide a useful platform to characterize pathogenic mechanisms of OXPHOS deficiencies (78) and to test metabolic interventions (79). However, given that mitochondria are tailored for the specialized functions of different cell types, results from one cell type are not necessarily universal. Platelets and lymphocytes are easy to collect from peripheral blood and amenable to short-term in vitro experiments. Long-term propagation of lymphocytes in culture requires their transformation into lymphoblastoid cell lines (e.g. via Epstein-Barr virus). Lymphocytes and lymphoblasts are not ideal for certain biochemical OXPHOS studies due to their relatively low mitochondrial content. Skin fibroblasts are accessible by biopsy and can be propagated easily, and their abundant mitochondria with tubular morphology makes them well-suited for biochemical studies and investigations of mitochondrial network dynamics. Myoblasts are theoretically an optimal cell type to study mitochondrial defects because they are highly reliant on mitochondrial bioenergetics. However, myoblasts are typically procured via invasive muscle biopsy and pure cultures are not easily obtained. Myoblasts and myocytes can also be derived by transformation of primary skin fibroblasts (80).

Patient-derived skin fibroblasts have proven extremely useful to model biochemical deficiencies associated with PMDs and nutritional interventions (81). For example, patient fibroblasts with primary CoQ defects and Complex I deficiency have been used to study disease-related biochemical defects and to assess rescue by CoQ_{10} analogs (82, 83) and riboflavin (84). Other examples of successful use of primary skin fibroblasts for testing metabolic therapies for PMDs include using mitochondrial-targeted versions of vitamin E and CoQ_{10} to alleviate oxidative stress in cells from Friedreich Ataxia patients (85). Human fibroblasts from PMD patients have also been used as a screening platform for potential therapeutics. For example, mitochonic acid 5, a derivative of the plant hormone indole-3-acetic acid, was found to improve the survival of MELAS patient fibroblasts under metabolic stress (86). The utility of fibroblast and other primary cell lines is limited by their availability and replicative capacity in culture, which leads to senescence and metabolic remodeling. Genetic heterogeneity between patients can also affect analyses if the sample size of patient-derived cells with similar mutations is too small to achieve statistical significance.

Cybrid models—Cytoplasmic hybrids, or “cybrids,” are widely employed to study pathogenic mtDNA mutations in a relatively homogeneous nuclear genetic background. Virtually any cell type can be used as the source of cytoplasts, but fibroblasts, white blood cells, and platelets are most frequently employed due to their relative ease of access. Human cancer cell lines are most often used as the recipient cell lines (e.g. osteosarcoma). Cybrid

technology has been used to generate immortalized human lines harboring specific pathogenic mtDNA mutations derived from PMD patients, including mt-tRNA mutations associated with MELAS (87), MERRF (myoclonic epilepsy with ragged red fibers) (88) and LHON (Leber's hereditary optic neuropathy) (89). Since many mtDNA mutations are heteroplasmic, stochastic distribution or manipulation via short-term treatment with mtDNA-depleting agents (90) allows for the analysis of cybrids with a range of mtDNA mutation loads. Cybrids have been used to screen nutrients and metabolic intermediates that improve disease phenotypes under a variety of growth or stress conditions. One common paradigm is the substitution of glucose with galactose as the main carbon source, which forces cells to rely more heavily on ATP produced by OXPHOS than aerobic glycolysis (91, 92). In heteroplasmic cybrids with a high pathogenic mutation load, a ketogenic intervention induced a shift toward accumulation of more wild-type molecules (93), presumably by selective elimination of dysfunctional mitochondria containing high levels of mutant mtDNA (94). A similar heteroplasmy shift was also produced in MELAS neuroblastoma cybrids by a treatment with low-glucose medium supplemented with 1 mM L-arginine, which putatively functions by enhancing nitric oxide synthesis to induce mitochondrial biogenesis (95). Combined glutamate and aspartate supplementation improved the survival of NARP (neuropathy, ataxia and retinitis pigmentosa) cybrids in galactose medium (96), possibly by enhancing citrate synthesis (97). Riboflavin (0.06–0.08 μM) improved proliferation, ATP levels, and complex I+III activity, as well as decreased superoxide production in MELAS cybrids (98). Finally, antioxidants, including CoQ_{10}, have also been successfully employed to improve survival and OXPHOS function in NARP and LHON cybrids (99).

Cybrid studies are useful for eliminating variation in nuclear genetic backgrounds between patient cells, but the interpretation of results can be limited if cancer cell backgrounds are employed that preferentially utilize anaerobic glycolysis over OXPHOS in culture. Thus, it is important to validate results from therapeutic intervention studies in cybrids and primary cells derived from PMD patients.

Induced pluripotent cells with OXPHOS defects—Stem cell technologies have allowed development of patient-derived iPSC lines of mitochondrial diseases (100–103) that allow for genetic manipulation and nutritional intervention studies. Importantly, iPSCs can be differentiated into disease-relevant cell types such as neurons and muscle cells. Generation and differentiation of iPSCs harboring pathogenic mtDNA mutations has allowed the study of mtDNA segregation and the effects of different mutation loads on relevant cells, such as cardiomyocytes (104). By exploiting natural mtDNA drift, combined with a contraction of mtDNA copy number upon induction of pluripotency and expansion upon differentiation, heteroplasmic iPSC lines with different proportions of mutant mtDNA can be obtained that model the variation observed in PMD patients. While these cells contain naturally occurring pathogenic mutations, they also allow for derivation of isogenic control lines via genetic correction of nuclear mutations, or by spontaneous segregation of heteroplasmic mtDNA in the parental proliferating fibroblasts. iPSCs derived from MELAS and Leigh syndrome patients have been characterized (105), and somatic cell nuclear transfer was used to replace mutant mtDNA from NARP fibroblasts to generate corrected

isogenic control iPSCs, which showed normalization of the bioenergetic defects and associated gene expression profiles. Potential limitations of iPSCs include the high costs associated with generation and maintenance, complexity of the differentiation protocols, and paucity of stem cell metabolism knowledge.

Saccharomyces cerevisiae—Fundamental knowledge about mitochondria and their role in human disease has arisen from studies in the budding yeast *Saccharomyces cerevisiae*. In fact, evidence for cytoplasmic inheritance (mtDNA genes) and some of the first direct evidence of mtDNA itself was discovered in yeast (106). Many core nuclear-encoded mitochondrial proteins are homologous between yeast and mammals, including those involved in mitochondrial protein import, metabolite transport, fission and fusion, and mtDNA maintenance (107–110).

As a facultative anaerobe *S. cerevisiae* is can survive severe OXPHOS defects when grown on a fermentable fuel source, and thus provides an invaluable model to study mitochondrial defects (111). Mutant and regulated open reading frame collections are available that allow for systematic screening for genes involved with mitochondrial function, and many yeast mutants can be functionally corrected through complementation with human homologues (112, 113). It is also possible to generate mtDNA mutations by homologous recombination-based approaches in yeast (114), a capability which is limited or non-existent in other species. Studies in *S. cerevisiae* have been instrumental in uncovering pathologic mechanisms in mitochondrial diseases, including tafazzin interactions important in Barth Syndrome (115), cytochrome oxidase assembly disruption in Leigh syndrome (116, 117), and candidate genes responsible for a form of hereditary spastic paraplegia (118). Important limitations of the study of PMDs in yeast include the lack of Complex I and a full repertoire of cell death mechanisms, which can be a detriment to understanding all functional consequences of OXPHOS dysfunction (108, 119–122). Lastly, yeast mtDNA is larger (~80-kb) than mammalian mtDNA, contains introns, encodes only seven OXPHOS proteins and a non-conserved ribosomal protein (VAR1), and is unstable when mitochondrial translation is inhibited (123–125).

Invertebrate models

Caenorhabditis elegans—The nematode *C. elegans* is a powerful genetic model system that has been used to study mitochondrial biology (126–128). Its 3-day developmental cycle, 2-week lifespan, and isogenic offspring permit the rapid, economical study of large quantities of genetically identical animals (129). The expressed *C. elegans* proteome shares greater than 83% homology with human genes (130) and includes ~80% of genes for human inborn errors of metabolism (131, 132). Their mtDNA encodes 12 of the 13 proteins found in mammalian mtDNA, lacking only ATP8 (126). Limitations of *C. elegans* for PMD research include a simple nervous system that does not model the complex network of connections and cell interactions of mammals and lack of respiratory and vascular systems, eyes and liver that are often involved in PMD pathology. Finally, all somatic tissues in adults are post-mitotic.

Despite the aforementioned limitations, *C. elegans* has proven useful as a model organism to characterize global sequelae of primary mitochondrial ETC dysfunction (26, 133), as well as to evaluate the efficacy of nutritional interventions in PMD. In a *C. elegans* model of Complex I deficiency (*NDUFV1*), riboflavin supplementation yielded enhanced assembly and improved catalytic activities of complexes I and IV (134). In another model of mitochondrial Complex I deficiency (*NDUFS2*), resveratrol treatment significantly improved shortened *gas-1(fc21)* mutant lifespan regardless of the developmental stage at initiation. These worms display compensatory up-regulation of amino acid, fat, and other nutrient pathways in response to chronic Complex I dysfunction. Resveratrol reversed this metabolic response, and restore NADH and NAD^+ levels (129) despite increasing the already elevated mitochondrial oxidant burden in this model.

Drosophila melanogaster—*Drosophila melanogaster* shares a high degree of genetic conservation with mammals, and has been effectively used to study mitochondrial disease states caused by mtDNA and nuclear gene mutations. Issues unique to multi-copy mtDNA have also been studied in *Drosophila*, including transmission, heteroplasmy, and mutant mtDNA threshold effects (135). Mitochondrial-targeted restriction enzymes that cleave wild-type mtDNA molecules have allowed selection of resistant molecules and generation of flies with mutations in cytochrome c oxidase I and ND2 (135, 136). However, the ability to generate mtDNA mutations in this manner is limited to naturally occurring restriction sites. Other mtDNA-based models of mitochondrial dysfunction in flies include a mutation in the ATP6 gene that models mitochondrial encephalomyopathies and tRNA mutations that characterized how the pathogenicity of mtDNA mutations can be altered by the nuclear genetic background (135, 137, 138). Finally, there are *Drosophila* models for Friedreich ataxia (139), mitochondrial deafness (135), Autosomal Spastic Ataxia with Leukoencephalophathy (140), and Leigh syndrome (141). These models have been informative in elucidating complexities of mitochondrial pathology (e.g. tissue-specificity, oxidative stress) (142–144), however their ability to model human pathological states varies (139, 142, 145). The unique lifecycle of *Drosophila* is well-suited for controlled feeding regimens to examine the effects of supplementation and specific diets on mitochondrial pathology over time in both adults and during development.

Vertebrate models

Danio rerio—The zebrafish, *Danio rerio,* is a powerful vertebrate model system of human diseases due to its rapid development, regular production of large numbers of offspring, and relatively inexpensive maintenance (146). Zebrafish have high genetic homology to mammals, and sequence conservation of mtDNA and nucleus-encoded mitochondrial proteins make it a valid model of human mitochondrial pathology (147). Zebrafish genetic investigations have traditionally been driven by forward genetic screens, however an increasing range of reverse-genetic techniques are becoming available. Transient knockdown of gene expression is possible with morpholino oligonucleotides, which allow for rapid and effective study of gene function (148). The use of transcription activator-like effector nucleases (149), and the CRISPR-Cas system (150) can be utilized to precisely modify the sequence at a particular locus in order to generate specific models of human disease.

The zebrafish possesses key organ systems including the brain, eyes, and hematologic system (151), which makes it a good model in which to study organ-specific mitochondrial pathology. Many characteristics of the zebrafish central nervous system are also conserved with humans and they develop tactile responses throughout the first few days of life (152), allowing the modeling of motor neuron diseases, myopathy, neuropathy and behavioral and cognitive endpoints. Due to their translucency, direct in vivo visualization of organ systems and mitochondria is possible, making zebrafish an attractive model for microscopy and testing of interventions. Limitations of zebrafish for PMD research include aquatic dietary formulations and feeding strategies that differ from other model organisms and less developed techniques to assess food intake (153) and energy expenditure (154).

Mouse models of OXPHOS deficiencies—Many mouse models of OXPHOS deficiencies (155, 156) or mtDNA dysfunction (157) have been developed (Supplemental Table S2), and insights into PMD mechanisms and nutritional interventions are discussed below. Although the pathogenesis of these mouse models is well-characterized in several cases, the number of studies conducted to investigate potential therapies are limited, opening a door for future intervention studies.

Mouse models of defects in mtDNA—Like humans, mice contain a double-stranded, circular mtDNA of ~16-kb that encodes thirteen core OXPHOS components, as well as two rRNAs and twenty-two tRNAs needed for mitochondrial ribosome biogenesis and translation (158). Since all other mitochondrial proteins are encoded in the nucleus (159), a common strategy in mouse models of PMD is to alter mtDNA by knock-out or overexpression of nuclear genes encoding mtDNA regulatory factors. However, transfer of specific mutant mtDNA species from tissues or cell lines into the mouse germline has also been accomplished.

Tfam mice—Mitochondrial transcription factor A, encoded by the *Tfam* gene, is an abundant mtDNA-binding protein, originally discovered as a transcriptional activator of mtDNA promoters (160). Tfam has additional roles in mtDNA metabolism, including packaging into nucleoids, mtDNA copy number regulation, segregation, and transcriptional priming of mtDNA replication (159). Global knock-out of the *TFAM* gene in mice results in severe mtDNA depletion, loss of mitochondrial respiration, and embryonic lethality (161). Surprisingly, heterozygous knock-outs ($Tfam^{+/-}$) are viable despite having 30–70% mtDNA depletion in tissues. $Tfam^{+/-}$ mice are primed for antiviral innate immune activation due to enhanced level of cytoplasmic mtDNA (162). Thus, the $Tfam^{+/-}$ model may serve as a "sensitized" background for studying mtDNA instability in disease and the role of mtDNA in immune system activation.

To avoid embryonic lethality of global *Tfam* gene deletion, multiple tissue-specific *Tfam* KO models have been generated, and these have provided significant insight into the requirements of OXPHOS in tissues and model mitochondrial disease pathology to varying degrees (163, 164). For example, muscle-specific deletion of *Tfam* results in features that are common in human PMD including cytochrome *c* oxidase-negative and ragged-red fibers, myopathy, and hypertrophy of the heart (165, 166). The heart also displays a common metabolic switch towards glycolysis when OXPHOS is attenuated, as well as biochemical

and physiological features of dilated cardiomyopathy present in Kearns-Sayre syndrome (167). *Tfam*-knock-out models have proven valuable in mechanistic studies that show the differential requirements for mtDNA and OXPHOS, variability in mitochondrial function between different tissues, and potential therapies (163, 164, 168).

TFB1M mice—Mitochondrial transcription factor B1 (TFB1M or mtTFB1) was originally identified as a homolog of the yeast mitochondrial transcription factor Mtf1p (or sc-mtTFB) (169). The two mammalian orthologs (TFB1M and TFB2M) are homologous to a class of site-specific, rRNA N6-adenine dimethyltransferases (170, 171). Knock-out of TFB1M in mice results in severely reduced 12S rRNA methylation, destabilization of the small mitochondrial ribosome subunit, and impaired mitochondrial translation (172). Transgenic mice that overexpress TFB1M have enhanced progressive hearing loss due to tissue-specific pathology in the inner ear, which can be rescued by reducing AMP kinase activity, suggesting a potential therapeutic route for deafness involving the A1555G mtDNA mutation (173, 174).

mtDNA mutator mice—DNA polymerase gamma (POLG) functions exclusively in mitochondria to replicate and repair mtDNA. The consequences of enhanced mtDNA mutagenesis can be modeled in mice that express a proofreading-deficient allele of its catalytic subunit (175, 176). These POLG mutants (a.k.a. mtDNA mutator mice) display systemic mitochondrial dysfunction, increased apoptosis, disrupted stem cell function, and progeroid phenotypes (175, 177). Mutator mice undergo a metabolic shift toward reliance on glycolysis to compensate for mitochondrial dysfunction in some tissues (178). There is some disagreement regarding whether point mutations or deletions (and other rearrangements) in mtDNA drive the aging-like phenotypes observed in these mice (179) and the precise cause of the progeroid phenotypes remains under investigation. Interventions that improve cell and tissue phenotypes in mtDNA mutator mice include targeting catalase to mitochondria, antioxidants, and endurance exercise (180–183).

Mouse model of MNGIE—Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is caused by a deficiency in thymidine phosphorylase (TP) resulting in cellular imbalance of dNTPs and accumulation of thymidine and deoxyuridine. This imbalance in turn results in secondary reduction of mitochondrial deoxycytidine and mtDNA depletion, deletions, and point mutations (184). A mouse model has been generated by knocking out both thymidine phosphorylase (TP) and uridine phosphorylase (UP) (185). These $TP^{-/-}UP^{-/-}$ mice display the same nucleoside accumulation as human patients and develop leukoencephalopathy, brain lesions, and in some cases OXPHOS deficiencies and brain mtDNA alterations (185, 186). Loss of motor control and gastrointestinal pathology observed in patients were not present in the disease-model mice.

Mouse models of pathogenic mtDNA mutations—Using cytoplasmic transfer of mtDNA into embryonic stem (ES) cells, chimeric mice have been generated that contain point mutations in the 16S rRNA (187, 188), OXPHOS genes (e.g., cytochrome c oxidase and ND6) (189, 190), and tRNAs (191), as well as, large-scale deletions (192, 193). These models have allowed mtDNA heteroplasmy, inheritance and pathology to be studied in vivo.

Variable percentages of mutant mtDNA are found in mice generated in this fashion (194), and the severity of pathology observed correlates with higher loads of the mutant mtDNA (192, 193). Additionally, severe mutations appear to be selected against in the germline, while milder mutations persist for several generations (189). Because the genetic background is homogeneous, these mouse models allow the pathogenic consequences of specific mtDNA mutations to be studied without confounding effects of nuclear polymorphisms. These mice recapitulate many pathologies observed in human mitochondrial diseases including OXPHOS defects, lactic acidosis, anemia, muscle weakness, and renal failure (195, 196). Some of the mutations generated contribute to characterized diseases including MERRF (191), chronic progressive external ophthalmoplegia (CPEO) (192), LHON (190), and early-onset Pearson syndrome caused by heteroplasmic mtDNA deletions (197). Some of these models have been used to test gene therapy (198), and the antioxidant N-acetylcysteine was tested in ND6 mutant mice, resulting in increased longevity and decreased incidence of lymphoma (199). The development of new approaches to generate mice with mtDNA mutations should expand the number of these invaluable models for specific mitochondrial disorders (200, 201).

The Deletor Mouse—Adult-onset autosomal progressive external ophthalmoplegia (PEO) can be caused by mutations in adenine nucleotide translocator 1 (202), POLG (203), Twinkle mtDNA helicase (204), or ribonucleotide reductase subunit p53R2 (205). Common mtDNA mutations in Twinkle (13-bp duplication and a point mutation) have been recapitulated in the mutant Twinkle^{dup} mouse (206), which accumulates mitochondrial DNA deletions in all tissues (most prominently in heart and muscle), prompting the name "*Deletor*" mouse.

Biochemical and histological analyses of *Deletor* mice demonstrated OXPHOS dysfunction, cytochrome *c* oxidase (COX)-negative muscle fibers, abnormalities in mitochondrial ultrastructure, and increased autophagy. The number of abnormal, COX-deficient muscle fibers is similar to that of human PEO patients, and the delayed development recapitulates the late-onset PEO phenotype (206). However, unlike PEO pathology in humans, *Deletor* mice have a normal lifespan and no significant compromise of physical performance. Gene expression profiling of *Deletor* mice revealed induction of folate metabolism (methylenetetrahydrofolate dehydrogenase 2, Mthfd2), fasting-related lipid metabolism (fibroblast growth factor 21, Fgf21) (207), and phosphorylation of AKT1, all reflective of modulations in nutrient-sensing signaling networks and suggesting OXPHOS dysfunction in muscle tissue results in starvation-like response. The induction of Fgf21 suggests it may be secreted by skeletal muscle cells with mitochondrial myopathy and act as a systemic mediator of metabolism. Elevated serum Fgf21 has been demonstrated in adults with mitochondrial disease (208), and studies in patients and mouse models have implicated mtDNA instability or translational defects as prerequisites for Fgf21 secretion (209, 210). Current investigations are studying the potential for Fgf21 serum level as a biomarker for muscle myopathies caused by mtDNA defects. While nucleotide pool measurements in *Deletor* mice revealed imbalanced purine metabolism and tissue-specific induction of the folate cycle, folinic acid supplementation did not reverse the pathology (211). Significant increases in mitochondrial biogenesis and improved mitochondrial ultrastructure resulted

after a two month KD in *Deletor* mice, although no alteration in the amount of mtDNA deletions nor skeletal muscle Complex I activity was observed (212). These reports prompted a pilot KD study in five mitochondrial myopathy patients and ten healthy subjects (213). While the trial was small and follow-up analyses are being performed, all of the KD subjects unexpectedly manifested muscle fiber damage after about 1.5 weeks of intervention (A. Suomalainen-Wartiovaara, personal correspondence). Therefore, while the KD may hold promise for some PMD patients, more preclinical research is needed to delineate appropriate applications.

Ndusf4$^{-/-}$ mice—Complex I dysfunction is one of the most common OXPHOS defects in PMD patients, with point mutations in NDUFS4 (NADH dehydrogenase-ubiquinone-Fe-S Protein 4) observed in patients with LHON, Leigh syndrome, and MELAS (214–216). A global knock-out mouse model for Complex I dysfunction (Ndufs4$^{-/-}$) (217) displays a phenotypic progression that resembles Leigh syndrome encephalopathy and dies prematurely by day 50, (218). Rapamycin treatment has beneficial effects in this model of Leigh syndrome (219), suggesting that nutritional interventions that inhibit the mTORC1 pathway, such as indicated for the KD (220), may be beneficial for PMDs. Ndufs4$^{+/-}$ mice are another Leigh syndrome model with a less severe reduction Complex I activity and milder phenotypes (221).

Pdss2$^{kd/kd}$ Mice—CoQ_{10} deficiency is associated with at least five heterogeneous PMDs, but for most patients the genetic basis of the deficiency is unknown (222). Primary deficiencies in CoQ_{10} levels result from mutations in various genes required for ubiquinone biosynthesis, including *PDSS1* or *PDSS2* genes, which encode prenyl diphosphate dynthase subunits (223), the *COQ2* gene, or the CoQ8/CABC1 gene. Although utilization of a ubiquinone as an electron carrier in the ETC is conserved across species, utilization of CoQ_{10} is not universal. Mice, for example, predominantly produce CoQ_9 with small amounts of CoQ_{10}.

Pdss2$^{kd/kd}$ mice, first identified by a kidney disease phenotype, harbor a homozygous mutation in PDSS2. Young *Pdss$^{kd/kd}$* mice have normal ubiquinone levels, but adults have ~15–20% of normal CoQ_9 and CoQ_{10} levels in brain, kidney, and muscle (224). Kidney Complex I+III activity is significantly reduced, although individual OXHPOS activities vary by tissue and age. Increased ROS production and oxidative stress are variably reported in kidney, but not observed in brain (224, 225). Metabolomic analyses of conditional CoQ deficiency in *Pdss2$^{kd/kd}$* mouse liver revealed upregulation of multiple pathways including OXPHOS, the TCA cycle, and pathways that provide substrates for fatty acid and amino acid metabolism (225, 226). Despite significant CoQ_9 deficiency and impaired Complex I and Complex II capacity, compensatory increases in Complex IV respiration was observed, and no overt symptomology was noted in liver-specific *Pdss2* knockout mice.

Pilot studies of liposomal CoQ_{10} supplementation (~200 mg/kg/d) in *Pdss$^{kd/kd}$* mice demonstrated a delayed onset of renal disease when administered from birth or weaning, and a partial rescue of proteinuria and interstitial nephritis (225, 227). However, kidney CoQ_9 and CoQ_{10} content was not significantly altered, suggesting that CoQ_{10} might exerted its effect via antioxidant activity rather than enhanced OXPHOS, although oxidative stress was

not measured. A subsequent study found limited therapeutic response to lifelong CoQ_{10} supplementation in a *Pdss2* mouse model of fatal renal disease (226). However, significant responses were seen when the endogenous synthesis of CoQ_9 and hydroquinone were increased with Probucol drug treatment, suggesting that targeting de novo supply of CoQ_{10} may be more effective than exogenous supplementation. Overall, these results suggest that understanding the mechanisms of CoQ_{10} supplementation benefits, including tissue-specific effects and the basis of responders versus non-responders, is a PMD research priority.

Barth Syndrome Mouse Model—Barth Syndrome is an X-linked disorder caused by pathogenic variants in the *TAZ* gene encoding Tafazzin (228), a transacylase that participates in acyl chain remodeling of cardiolipin, a major phospholipid of the mitochondrial inner membrane (229). Individuals with Barth Syndrome have clinical features that include early onset cardiomyopathy, neutropenia, skeletal myopathy, and growth abnormalities (228).

An inducible short hairpin RNA-induced (shRNA) mouse knockdown model of tafazzin deficiency was developed in 2011 (230, 231) with pathogenic features similar to Barth Syndrome patients, including left ventricular dilation and abnormal mass, decreased ejection fraction, stereotypical mitochondrial ultrastructural abnormalities, and abnormal cardiolipin species (230–232). However, the mice exhibit a late onset (7–8 months of age) cardiac functional abnormality, as opposed to the early onset cardiac phenotype observed in humans (233). Modifications of the shRNA induction protocol produced mice with a cardiac phenotype in utero and proved particularly useful in pinpointing the developmental stage in which cardiac non-compaction occurs in tafazzin deficiency (234). The tafazzin mouse model has proven useful in investigating other clinical phenotypes of Barth Syndrome, including exercise intolerance, OXPHOS defects, and abnormal in amino acid, folate, and lipid metabolism (232, 235–237). However the model has important limitations for studying cardiac arrhythmias (238), a major cause of morbidity and mortality in Barth Syndrome (233), and has not been used to address other cardinal features of Barth Syndrome like immune deficiency. Lastly, since this model is induced with doxycycline, potential effects of this agent on mitochondrial translation could complicate some findings (239).

Enhancing translatability of PMD model system studies

Regardless of the model system employed, rigor and reproducibility of preclinical nutritional interventions studies must be optimized such that the knowledge gained best informs approaches in PMD patients. While model systems for PMDs cannot completely recapitulate disease states observed in humans, they have the advantage of permitting detailed mechanistic experimentation in a genetically controlled population with nutritional interventions that are difficult to control for in a human PMD cohort. Preclinical nutritional intervention approaches can be reductionist, with systematic testing of single agents or combinations over time. Orthogonal testing of hypotheses in multiple models can be very powerful, as demonstrated by studies of NA and NR in patient-derived fibroblasts, *C. elegans*, *Deletor*, and Ndufs4$^{-/-}$ mice which help to elucidate the importance of NADH:NAD$^+$ balance in PMDs (Sidebar).

To best inform clinical approaches, model system investigations must carefully consider the intervention formulation, dosing, and bioavailability. Rigorous characterization of identity, purity, and composition is especially important for dietary approaches, where patients have ready access to a large variety of nutritional supplements that are often far different than formulations used in biomedical research.

Bioavailability is a major area in need of further experimental evidence to support clinical use of nutritional interventions in PMDs, in particular as it relates to understanding mechanisms of action and efficacy in humans (Table 1) and in cases where it is unclear if intake beyond recommended dietary amounts confers any added health benefit. Animal models can serve as a bridge between cellular and human investigations, and their ability to do so is strengthened when an understanding of pharmacodynamics and pharmacokinetics in the animal system inform dosing regimens in patients. Without careful consideration of the relevance of intervention concentrations and human bioavailability wherever possible, preclinical research in model systems is unlikely to inform clinical practice. Additionally, model system investigations of bioavailability should consider several experimental measurement parameters such as the specific analyte (e.g., parent compound, metabolite, biomarker), physiological compartment (e.g., plasma, serum, tissue), and quantitative analytical method used.

Conclusions

The use of nutritional interventions by the PMD community is pervasive and is often based on empirical evidence. Although the potential for metabolic cofactors as therapies is gaining emphasis, increased preclinical research on nutritional interventions is needed for the PMD patients. Cellular and animal model systems can recapitulate specific aspects of mitochondrial dysfunction and provide the means to thoroughly examine mechanistic interactions between interventions and underlying PMD biology (Figure 1). Advances in genetically engineered probes for redox imaging and multiple-omics provide new toolkits for objective measures of PMD progression at discrete sites in a controlled experimental setting. Through rigorous preclinical mechanistic studies, the potential exists to systematically evaluate specific nutrients, test dosing ranges, and query intervention efficacy and safety with enhanced confidence before initiating observational studies in patients. In some instances intervention outcomes have been unexpectedly negative, and an opportunity exists for cross-validation of both positive and negative nutritional intervention results between orthogonal PMD model systems. Moreover, indications of positive nutritional intervention effects in ‘healthy’ animals, such as resveratrol’s promotion of mitochondrial biogenesis, need to be tested in specific models of PMD to truly support their use invulnerable patient populations. Focused research in these areas will ultimately help to better inform cohort selection and outcome measurement criteria which are crucial for larger-scale RCT design for PMD interventions. Furthermore, insights gained from an enhanced emphasis on basic and preclinical research in model systems for PMDs could help build a foundation for significant advancements in our understanding of the mitochondria role in homeostasis and preventive healthcare.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acronyms/Terms

AMPK	AMP-activated protein kinase
α-LA	α-lipoic acid
CoQ_{10}	coenzyme Q_{10}
ETC	electron transport chain
iPSCs	induced pluripotent stem cells
LHON	Leber's hereditary optic neuropathy
MADD	multiple acyl CoA dehydrogenase deficiency
MELAS	mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes
MERRF	myoclonic epilepsy with ragged red fibers
MNGIE	mitochondrial neurogastrointestinal encephalomyopathy
mtDNA	mitochondrial DNA
NA	nicotinic acid
NR	nicotinamide riboside
OXPHOS	oxidative phosphorylation
PEO	progressive external ophthalmoplegia
PGC-1α	peroxisome proliferator-activated receptor gamma coactivator 1-α
PMD	primary mitochondrial disorder
POLG	DNA polymerase gamma
PPAR	peroxisome proliferator-activated receptor
ROS	reactive oxygen species

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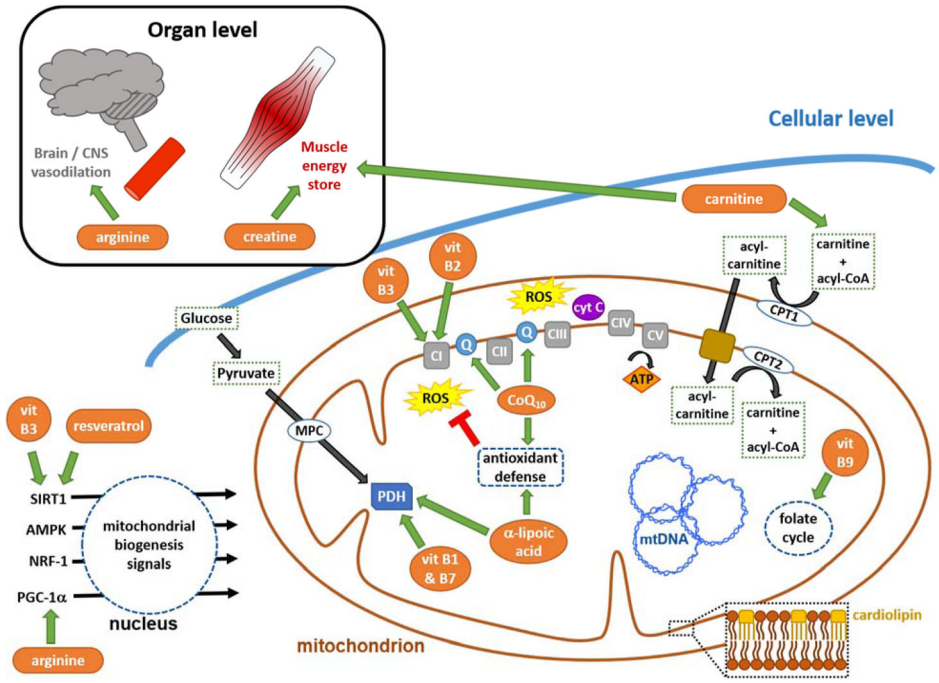

Figure 1. Mechanisms of nutritional interventions in PMDs

At the organ level, arginine and creatine are used to improve vasodilation and energy storage. At the cellular level nutrients and metabolic modifiers and signaling activators are employed to support OXPHOS (vitamins B_3 and B_2, CoQ_{10}) and metabolic pathways (vitamin B_1, B_7, and B_9, L-carnitine, and α-lipoic acid), enhance antioxidant defenses (CoQ_{10} and α-lipoic acid), and promote mitochondrial biogenesis (vitamin B_3, resveratrol, and arginine). Cardiolipin, dispersed throughout the mitochondrial inner membrane, is essential for optimal function of multiple metabolic enzymes. Abbreviations: **CI-CV**, OXPHOS complexes I-V; **Q**, ubiquinone; **CoQ_{10}**, coenzyme Q_{10}; **CPT**, carnitine palmitoyl transferase; **cyt c**, cytochrome *c*; **MPC**, mitochondrial pyruvate carrier; **mtDNA**, mitochondrial DNA; **PDH**, pyruvate dehydrogenase; **ROS**, reactive oxygen species, (superoxide, hydrogen peroxide and hydroxyl radical; **vit**, vitamin.

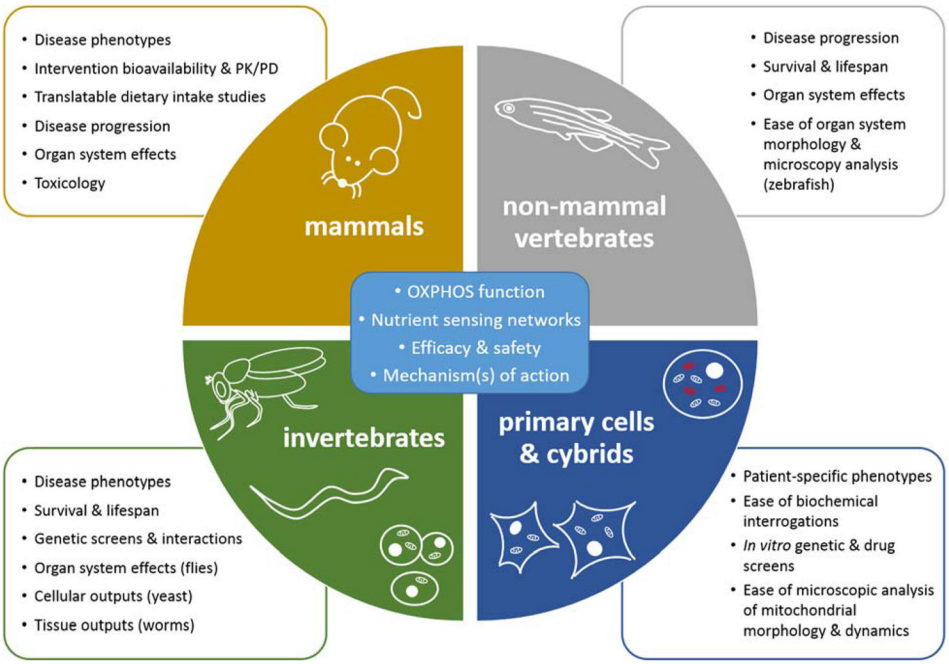

Figure 2. Key experimental strengths of model systems for PMD intervention studies

Shared among the indicated model organisms and cellular systems is an ability to investigate genetic and biochemical characteristics of specific PMDs, nutrient sensing networks, and the efficacy, safety, and mechanism(s) of action for nutritional interventions. Individual model systems also have distinct experimental advantages, described in each quadrant. For example, primary cells allow for the study of patient-specific phenotypes and straightforward analysis of mitochondrial morphology and dynamics, invertebrates are well-suited for survival and lifespan investigations, and rodents permit assessments of nutritional intervention bioavailability and toxicology more directly relevant to human PMDs.

Table 1

Considerations to advance mechanistic and clinically translatable research in model systems.

Analytical and measurement considerations	**Administration considerations and biological variables**
Research rigor and reproducibility is enhanced through analytical confirmation of intervention purity, composition, and stability • Has the intervention been independently tested and verified for batch-to-batch consistency? • Has the intervention stability over the experimental time frame been analytically demonstrated?	*Dosing* • Is the dosage delivery relevant to nutritional interventions? • Is the dosage amount relevant to nutritional interventions?
	Adsorption • Nutrient adsorption can vary whether from food or as isolated compounds • Adsorption can be linear or dose-dependent (e.g., bi-phasic, saturable)
Research comparability is enhanced with thorough reporting of intervention identity • Multiple supplement formulations can be available to patients (e.g., malate/citrate/pyruvate/α-ketoglutarate conjugates of creatine; ubiquinone/ubiquinol forms of CoQ_{10})	*Distribution* • Does the intervention reach the target tissue? • What concentrations of the intervention are specific tissues exposed to?
Experimental bioavailability quantification is dependent upon analytical methodology • Were measurements in plasma, serum, lipid compartments, or tissues? • Were measurements of a parent compound, metabolite, or biomarker? • Quantification via isotope labeling, chromatography, or another approach?	*Metabolism* • Is the intervention compound the true bioactive, or is a metabolite responsible for activity?
	Excretion • How much of the intervention is excreted without being circulated to tissues?
	Genetic variations • Can influence amount absorbed and/or metabolism • Certain interventions will only address nutrient deficiencies due to specific mutations