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Nutritional Interventions in Primary Mitochondrial Disorders: Developing an Evidence Base[☆]

Kathryn M. Camp^{a,*}, Danuta Krotoski^b, Melissa A. Parisi^b, Katrina A. Gwinn^c, Bruce H. Cohen^d, Christine S. Cox^e, Gregory M. Enns^f, Marni J. Falk^g, Amy C. Goldstein^h, Rashmi Gopal-Srivastavaⁱ, Gráinne S. Gorman^j, Stephen P. Hersh^k, Michio Hirano^l, Freddie Ann Hoffman^m, Amel Karaaⁿ, Erin L. MacLeod^o, Robert McFarland^j, Charles Mohan^p, Andrew E. Mulberg^{q,2}, Joanne C. Odenkirchen^c, Sumit Parikh^r, Patricia J. Rutherford^s, Shawne K. Suggs-Anderson^t, W.H. Wilson Tang^u, Jerry Vockley^v, Lynne A. Wolfe^w, Steven Yannicelli^x, Philip E. Yeske^p, and Paul M. Coates^a

Kathryn M. Camp: campkm@od.nih.gov; Danuta Krotoski: krotoskd@mail.nih.gov; Melissa A. Parisi: parisima@mail.nih.gov; Katrina A. Gwinn: gwinnk@ninds.nih.gov; Bruce H. Cohen: bcohen@chmca.org; Christine S. Cox: outreach@mitoaction.org; Gregory M. Enns: genns@stanford.edu; Marni J. Falk: falkm@email.chop.edu; Amy C. Goldstein: amy.goldstein@chp.edu; Rashmi Gopal-Srivastava: rashmi.gopal-srivastava@nih.gov; Gráinne S. Gorman: grainne.gorman@newcastle.ac.uk; Stephen P. Hersh: hershs@starpower.net; Michio Hirano: mh29@cumc.columbia.edu; Freddie Ann Hoffman: fhoffman@heterogeneity-LLC.com; Amel Karaa: akaraa@mgh.harvard.edu; Erin L. MacLeod: emacleod@childrensnational.org; Robert McFarland: robert.mcfarland@newcastle.ac.uk; Charles Mohan: chuckm@umdf.org; Andrew E. Mulberg: amulberg@amicusrx.com; Joanne C. Odenkirchen: jo21x@nih.gov; Sumit Parikh: parikhs@ccf.org; Patricia J. Rutherford: triciarutherford@vitaflo.co.uk; Shawne K. Suggs-Anderson: shawne.suggs-anderson@fda.hhs.gov; W.H. Wilson Tang: tangw@ccf.org; Jerry Vockley: gerard.vockley@chp.edu; Lynne A. Wolfe:

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^{*}Corresponding author at: 6100 Executive Boulevard, Rockville, MD 20892, USA. campkm@od.nih.gov.

¹Consultant

²Employed at FDA from July 10, 2010 to July 11, 2016

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lynne.wolfe@nih.gov; Steven Yannicelli: steven.yannicelli@nutricia.com; Philip E. Yeske: philip.yeske@umdf.org; Paul M. Coates: coatesp@od.nih.gov

^aOffice of Dietary Supplements, National Institutes of Health, Bethesda, MD 20892, USA

^bEunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA

^cNational Institute of Neurological Diseases and Stroke, National Institutes of Health, Bethesda, MD 20892, USA

^dDepartment of Pediatrics, Akron Children's Hospital, Akron, OH 44308, USA

^eMitoAction, Boston, MA 02205, USA

^fDivision of Medical Genetics, Stanford University, Stanford, CA 94305, USA

^gThe Children's Hospital of Philadelphia and University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104, USA

^hDivision of Child Neurology, Children's Hospital of Pittsburgh, Pittsburgh, PA 15224, USA

ⁱNational Center for Advancing Translational Sciences, National Institutes of Health, Bethesda, MD 20892, USA

^jWellcome Trust Centre for Mitochondrial Research, Newcastle University, Newcastle upon Tyne, UK NE2 4HH

^kJ. Willard & Alice S. Marriott Foundation, Bethesda, MD 20817, USA

^lColumbia University Medical Center, New York, NY 10032, USA

^mHeteroGeneity, LLC, Washington, DC 20011, USA

ⁿGenetics Unit, Massachusetts General Hospital. Boston MA 02114, USA

^oDivision of Genetics and Metabolism, Children's National Health System, Washington, DC 20010, USA

^pUnited Mitochondrial Disease Foundation, Pittsburgh, PA 15239, USA

^qCenter for Drug Evaluation and Research, Food and Drug Administration, Silver Spring, MD 20903, USA

^rNeurosciences, Cleveland Clinic, Cleveland, OH 44195, USA

^sVitaFlo International Ltd, Liverpool L34BQ UK

^tOffice of Nutrition and Food Labeling, Food and Drug Administration, College Park, MD 20740, USA

^uCenter for Clinical Genomics, Cleveland Clinic, Cleveland, OH 44195, USA

^vUniversity of Pittsburgh School of Medicine, Pittsburgh, PA 15224, USA

^wNational Human Genome Research Institute, National Institutes of Health, Bethesda, MD 20892, USA

^xMedical and Scientific Affairs, Nutricia North America, Rockville, MD 20850, USA

Abstract

In December 2014, a workshop entitled “Nutritional Interventions in Primary Mitochondrial Disorders: Developing an Evidence Base” was convened at the NIH with the goals of exploring the use of nutritional interventions in primary mitochondrial disorders (PMD) and identifying knowledge gaps regarding their safety and efficacy; identifying research opportunities; and forging collaborations among researchers, clinicians, patient advocacy groups, and federal partners. Sponsors included the NIH, the Wellcome Trust, and the United Mitochondrial Diseases Foundation. Dietary supplements have historically been used in the management of PMD due to their potential benefits and perceived low risk, even though little evidence exists regarding their effectiveness. PMD are rare and clinically, phenotypically, and genetically heterogeneous. Thus patient recruitment for randomized controlled trials (RCTs) has proven to be challenging. Only a few RCTs examining dietary supplements, singly or in combination with other vitamins and cofactors, are reported in the literature. Regulatory issues pertaining to the use of dietary supplements as treatment modalities further complicate the research and patient access landscape. As a preface to exploring a research agenda, the workshop included presentations and discussions on what PMD are; how nutritional interventions are used in PMD; challenges and barriers to their use; new technologies and approaches to diagnosis and treatment; research opportunities and resources; and perspectives from patient advocacy, industry, and professional organizations. Seven key areas were identified during the workshop. These areas were: 1) defining the disease, 2) clinical trial design, 3) biomarker selection, 4) mechanistic approaches, 5) challenges in using dietary supplements, 6) standards of clinical care, and 7) collaboration issues. Short and long-term goals within each of these areas were identified. An example of an overarching goal is the enrollment of all individuals with PMD in a natural history study and a patient registry to enhance research capability. The workshop demonstrates an effective model for fostering and enhancing collaborations among NIH and basic research, clinical, patient, pharmaceutical industry, and regulatory stakeholders in the mitochondrial disease community to address research challenges on the use of dietary supplements in PMD.

Keywords

Primary mitochondrial disorders; Mitochondrial disease; Nutritional interventions; Dietary supplements; Medical foods; OXPHOS

1. Introduction

On December 2–3, 2014, the Office of Dietary Supplements (ODS), National Institutes of Health (NIH), sponsored a workshop in Bethesda, MD, entitled “Nutritional Interventions in Primary Mitochondrial Disorders: Developing an Evidence Base.” This workshop was developed as an outcome of the Nutrition and Dietary Supplement Interventions for Inborn Errors of Metabolism program sponsored by ODS and the NIH Office of Rare Diseases Research (ORDR), National Center for Advancing Translational Sciences (NCATS), that focuses on building an evidence base for nutritional interventions in inborn errors of metabolism (IEM) [1, 2]. Through this workshop and related activities, the organizers hoped to identify research gaps and needs, with the ultimate goal of improving patient care and the general knowledge of mitochondrial function.

The workshop was attended by researchers, health care providers, and representatives from government, advocacy organizations, and industry who have an interest in research issues pertaining to the use of nutritional interventions in primary mitochondrial disorders (PMD). Workshop co-sponsors included ORDR at the NCATS, the NIH *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD), the Wellcome Trust (London, United Kingdom), and the United Mitochondrial Diseases Foundation (UMDF).

PMD are a group of highly heterogeneous disorders typically associated with genetic dysfunction of the oxidation-phosphorylation (OXPHOS) pathway that is the major energy source for the cell. The heterogeneity of PMD results from dual genome control of the mitochondrial respiratory chain (RC) by mitochondrial DNA (mtDNA) and nuclear DNA (nDNA), where mtDNA disease presentations are further confounded by heteroplasmy, in which there is a random percent distribution of mutated mtDNA within individual cells within tissues. PMD are usually manifest in tissues with high energy requirements, and may present at any time from infancy to adulthood in an often multi-systemic and progressive manner. Signature phenotypic manifestations include lactic acidemia, skeletal myopathy, sensorineural hearing loss, vision loss, a range of central neurologic problems ranging from subacute neurodegeneration to migraines, peripheral neuropathy, intestinal dysmotility, and exercise intolerance with fatigue. Nearly all organ systems can be affected or spared, in varying combinations depending on the specific genetic disorder and mtDNA mutation heteroplasmy load. To date, there are no effective treatments for these disorders, although a number of clinical trials are now underway.

Large doses of several vitamins and other dietary ingredients are used in the management of PMD. While the evidence supporting their use is limited, these products are nonetheless widely used and often given in combination with considerable variability in the specific nutrients and brands recommended by providers, dosages and timing, and the monitoring and documentation of efficacy and adverse effects [3]. Although these products are generally viewed as well-tolerated, few double-blind, placebo controlled clinical trials document their safety and/or efficacy, particularly in children and by researchers in the United States [4]. Patient safety, health care payer reimbursement, and the ability to standardize patient care are compromised by this gap in evidence. For example, a “negative” study would decrease reliance on therapies that may have empiric rationales for their use and are readily available in the marketplace, but don’t achieve the desired outcome. Furthermore, a few high profile cases of harm to vulnerable patients from adulterated and misbranded products have come forward recently, and these further elucidate the critical need to demonstrate safety and effectiveness of specific products given to sick patients [5]. See Table 1 for key issues relevant to consideration of dietary supplement use in PMD. Note that the term “mitochondrial cocktail” has been used by clinicians and patients to refer to the combination of typically three to six compounds; however, there is large variation in the composition of combination approaches, where specific products and dosages are not standardized [3]. Thus, this terminology is non-specific, non-descriptive, and simply suggests a mixture of compounds used for intended therapeutic benefit in patients with mitochondrial disease. We have therefore, avoided using this term here except in cases where it has been used in a published research study.

This workshop focused on nutritional interventions that have a regulatory definition rather than on general nutrition-related topics such as the macronutrient composition of the diet or mode of delivery, such as whether taken by mouth or via a gastrostomy tube. Nutritional interventions for mitochondrial disorders regulated by U.S. Food and Drug Administration (FDA) that were included within the scope of this workshop fall into three general categories: (1) dietary supplements (2) medical foods, such as those used with the ketogenic diet; and (3) FDA-approved drugs that are also available as dietary supplements.

The goals of the workshop were to: (1) explore the use of nutritional interventions, including dietary supplements, in PMD; (2) identify gaps in knowledge regarding the safety and effectiveness of nutritional interventions; (3) identify research opportunities; (4) develop a research agenda towards promoting evidence-based use of nutritional interventions in PMD; and (5) forge collaborations among basic and clinical researchers, clinicians, patient advocacy groups, and federal partners.

1.1. NIH and the role of ODS

The workshop was opened by James M. Anderson, M.D., Ph.D., Director of the NIH Division of Program Coordination, Planning, and Strategic Initiatives, and Paul M. Coates, Ph.D., ODS Director. Both delineated goals of the workshop: to assess the current scientific knowledge in this area; to identify gaps and opportunities for research; and, to outline a plan forward so that therapeutics can be brought quickly and safely to individuals with mitochondrial disorders. NIH is the largest funder of biomedical research in the world; its mission is to understand human biology and translate this knowledge into prevention and treatment of human disease using an annual research budget of \$30 billion. Prioritizing how these funds are spent is a critically important activity. To help prioritize research topics, NIH carefully reviews the research areas that are currently funded and develops new priority areas by relying on workshops such as this one to engage and learn from stakeholders, including patient advocates, researchers, representatives from government agencies, and industry representatives.

The Dietary Supplement Health and Education Act (DSHEA) was passed by Congress in 1994 [6]. It established a regulatory framework through the FDA, labeling rules for dietary supplement products, and created the ODS at NIH. DSHEA defines a dietary supplement as a product intended to supplement the diet and containing one or more of the following: vitamin, mineral, amino acid, other dietary substance, herb or botanical (not tobacco) [6]. Within this context, the mission of ODS is to strengthen knowledge and understanding of dietary supplements through: (1) evaluating scientific information, (2) stimulating and supporting research, (3) disseminating research results, and (4) educating the public to foster and enhance the quality of life and health for the U.S. population.

Since passage of DSHEA, estimated U.S. dietary supplement sales have increased dramatically, from \$8.6 billion in 1994 to \$34.9 billion in 2013 [7]. Most people use dietary supplements for their intended purpose, that is, to promote health and help reduce the risk of chronic disease. However, there are circumstances for which dietary supplements are used for other purposes, such as in the management of patients with IEM and PMD. While manufacturers are prohibited from marketing dietary supplements for those purposes, that

does not prevent clinicians and consumers from using them with the belief that they will help manage or alleviate symptoms of a complex disease. Research issues that pertain to dietary supplements broadly include product quality and bioavailability, product reformulations and use of different brands, lack of a single comprehensive registration database of dietary supplements, and baseline nutritional status of research participants. Additional concerns that impact research protocol development include timing and duration of the intervention, endpoints, doses and forms, and broader applicability of findings beyond the population studied.

2. Setting the stage

This session included presentations on U.S. regulatory definitions and implications for clinical development of treatments for rare diseases (Andrew Mulberg, M.D., FDA), an overview of medical foods regulation (Shawne Suggs-Anderson, M.M.Sc., R.D., FDA), and a description of mitochondrial global networks (Amy Goldstein, M.D., University of Pittsburgh). The session was moderated by Philip Yeske, Ph.D., UMD.

2.1. Regulatory definitions

Drugs and dietary supplements are regulated under the Federal Food, Drug, and Cosmetic Act (FFDCA); however, they have significant differences in their regulatory definitions. Drugs are “articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease...” and “articles (other than food) intended to affect the structure or any function of the body...”[8]. A drug is defined by intended use, not the nature of the substance. Dietary supplements, on the other hand, are intended to supplement the diet and are not intended to treat, diagnose, prevent, or cure disease [6]. If a dietary supplement is being used in some formulation to mitigate a disease and alter structure or function, then a drug definition might be applicable.

Drugs require premarket approval by the FDA. Manufacturers must demonstrate the effectiveness of their products through adequate and well-controlled studies that are able “to distinguish the effect of a drug from other influences, such as spontaneous change..., placebo effect, or biased observation” [9]. In addition, a drug manufacturer must also assure that the methods used in manufacturing are adequate to preserve the drug’s identity, strength, quality, and purity; and similar safety and efficacy can be expected with each batch. In making a final decision on a drug’s approvability, FDA considers whether the benefits of a drug outweigh its known and potential risks (see Code of Federal Regulations, Title 21 Section 312.84 [10]).

Rare diseases, also referred to as “orphan diseases,” are defined by the Orphan Drug Act (ODA) as diseases or conditions that affect fewer than 200,000 persons in the United States [11]. There are an estimated 7,000 different rare diseases that have been described that collectively affect 25–30 million Americans [12]. Approximately 80–85% of these are genetic disorders and about half of the affected patients are children [13]. Many rare diseases are chronic, progressive, serious, life-limiting, and life-threatening. Only a small number have targeted therapies approved for their treatment by the FDA. Rare disease clinical research faces a variety of clinical development challenges, including the paucity of patients

available for study, the heterogeneity of clinical presentations, and for many, poorly-understood natural history. Furthermore, well-defined endpoints, outcome measures/tools/instruments, and biomarkers are lacking for many rare diseases. Under the ODA, special status, or “orphan designation,” can be requested for drugs to treat rare diseases that meet specified criteria [14]. Under the ODA, orphan designation provides development incentives such as tax credits for qualified clinical testing.

Drugs intended to treat rare diseases are required to meet the same statutory standards of marketing approval that apply to other drugs. FDA exercises the broadest flexibility in applying the statutory standards while preserving guarantees for safety and effectiveness. Regulators working on drugs for rare diseases are well aware of and extremely sensitive to the critical nature of their mission and the impact they have on patients, and generally agree that the best access to safe and effective treatments comes from having approved products on the market.

2.2. Medical foods

Medical foods are used as the primary dietary management modality for a number of IEM, including many that are now identified via state-based universal newborn screening [15]. Several medical food products are also marketed in the U.S. for use in the ketogenic diet that has shown some utility for select PMD (see section 4.1). The historical development of medical foods and how they are defined and regulated is provided below. An understanding of these concepts and how medical foods differ from dietary supplements and FDA-approved drugs will be increasingly important as new management modalities are researched and brought into the marketplace.

The concept of specially formulated foods for seriously ill patients began in the 1940s when foods for special dietary use began to be developed. FDA proposed its first definition for Foods for Special Dietary Use (FSDU) in 1941 [16]. In 1973, the preamble to the final rule on nutrition labeling exempted two types of FSDU from the general labeling requirements and brought the term “medical foods” into being [38 FR 2124 at 2126]. The 1988 ODA Amendments created the statutory definition of medical foods:

... a food which is formulated to be consumed or administered enterally under the supervision of a physician and which is intended for the specific dietary management of a disease or condition for which distinctive nutritional requirements, based on recognized scientific principles, are established by medical evaluation” [17]

FDA considers the statutory definition of medical foods to narrowly constrain the types of products that fit within this category of food [56 FR 60366 at 60377, November 27, 1991]. In general, the category of FSDU includes formulated products designed to meet an individual’s nutritional needs; however, these nutritional needs could be met with diet modification. In contrast, medical foods are intended to meet extraordinary nutritional needs, through a specially formulated product that cannot be met with diet modification. The term “medical food” does not pertain to all foods fed to sick patients. Medical foods are foods that are specially formulated and processed (as opposed to foods from a normal or conventional diet) for the patient who requires the product as a major management modality

[56 FR 60366 at 60377]. Medical foods are regulated, as are other foods, under the provisions of the FFDCA. Medical foods must comply with all applicable requirements for the manufacture of foods, for example, current Good Manufacturing Practices (cGMP) [21 CFR 110] and specific mandatory food labeling information.

Medical foods do not undergo premarket review or approval for safety, efficacy, or label claims. Individual medical food products do not have to be registered with FDA; however, food facilities must be registered [21 CFR part 1 Subpart H] [18] and these facilities are inspected every other year or yearly if the manufacturer also produces infant formula.

2.3. Mitochondrial global networks

Mitochondrial medicine is a relatively young specialty with a limited evidence base for clinical decision-making [19]. A 2013 review of the mitochondrial disease literature conducted by Pfeffer et al. [4], with the aim of addressing the reliability of current evidence of efficacy for mitochondrial therapies revealed 1,039 publications spanning 47 years. Only 35 studies described treatment effects in 5 or more patients. The authors commented that large multicenter RCTs have been carried out for mitochondrial disease, and several others are in progress. These trials establish proof of principle that data of the highest quality can be produced to underpin mitochondrial medicine, and that such trials can be facilitated by international consortia. They also indicated that off-license prescription of medicines or use of dietary supplements could have value in a compassionate context, but the lack of objective efficacy should be made clear to patients and families [4]. The article closes with a call for mitochondrial physicians to avoid overemphasizing the theoretical benefits of unproven treatments, and to engage with patient advocacy groups and industry.

International collaborations among clinicians, researchers, and advocacy organizations are needed to accelerate discovery and treatment options [20]. A number of efforts are currently underway to advance global collaboration in mitochondrial medicine. The Mitochondrial Medicine Society (MMS) [21] was founded in 2000 and represents an international group of physicians, researchers, and clinicians working towards the better diagnosis, management, and treatment of mitochondrial diseases. The MMS is primarily based in the U.S., while actively seeking to become more of an international group, and currently has 227 registered members.

Another group dedicated to the research on and care of patients with PMD is the Mitochondrial Global Network (MitoGlobal), which is an informal network of national and international societies, networks, and initiatives [22]. Among MitoGlobal's many aims are to promote rigorous mitochondrial research from both basic and applied perspectives, improve communication among mitochondrial societies, and integrate with general scientific societies, providing a comprehensive list of mitochondrial communications networks and mitochondrial network projects. The MitoGlobal networks map [23] includes mitochondrial physicians, patient support groups, networks, metabolic societies, and the North American Mitochondrial Disease Consortium (NAMDC) sites (see below) [24]. NAMDC is part of the Rare Diseases Clinical Research Network (RDCRN) program, an initiative of ORDR/NCATS.

Through the MMS, a survey was conducted to identify international mitochondrial physicians and their practice patterns [3]. Overall, 32 practitioners responded, and in general, the results reflect current U.S. practice. There was no uniformity identified regarding recommendations for a specific combination of supplements that all patients with mitochondrial disease should take, nor global care guidelines to standardize international care. The creation of accredited U.S./international mitochondrial clinical care centers with expertise in caring for individuals with PMD was suggested, based on the success of England's National Health Service, where three centers (Newcastle, London, Oxford) have been working together to help care for patients with mitochondrial disease. These centers provide diagnostic and clinical services, management advice, specialized nursing services, education, information, and research. The Newcastle team has taken an international lead on the development of care guidelines that are published online (www.newcastle-mitochondria.com). These guidelines may serve as a future model for the care of mitochondrial patients in the United States.

The need for biomarkers and defined outcome measures to follow disease course and treatment efficacy to help in the design and conduct of global clinical trials was emphasized. NAMDC and other MitoGlobal networks can be used to help promote such trials. NAMDC includes a core group of 16 sites throughout the U.S. and Canada and is funded largely via an NIH grant mechanism (U54 NS078059). NAMDC has established a physician-entered clinical registry and biorepository, with 910 patients with mitochondrial disease enrolled (as of 7/1/16) [24]. Data collected by NAMDC and deposited into the RDCRN's Data Management and Coordinating Center represent a strong resource to identify natural histories of PMD for future studies on evaluating treatment effects in these disorders.

Promotion of international collaborations in PMD is needed along with strategies to facilitate international clinical trials. Pooling patient registries and support groups can also be used to improve international collaborative efforts and facilitate the design and conduct of robust clinical trials. The International Mito Patients organization (IMP) [25], a European patient support group, is taking a lead role. The IMP is currently focusing on developing a rehabilitation standard for patients with mitochondrial disease and seeks to work with NAMDC, UMDF, and other international registries to establish a global registry of mitochondrial patients. An exciting future international goal could be to provide every mitochondrial patient the opportunity to be enrolled in a randomized placebo-controlled clinical trial until proven effective and safe treatments are demonstrated.

With respect to the development of standardizing clinical care in the U.S., NAMDC's 16 U.S. sites could offer a starting point to establish mitochondrial research and associated care. These sites could be involved in developing practice guidelines and standardizing care, noting that developing practice guidelines is outside the scope of NIH funding.

2.4. Session discussion points

It was questioned whether products such as dietary supplements and medical foods should attain the same level of proof for use as for any new drug coming into the market, given that for some, maximum tolerated doses are known. Additionally, for nutritional therapies to undergo FDA review, changes would be required with the current regulatory framework but

with possible consideration of cGMP requirements, evidence supporting effectiveness and safety, and defined clinical endpoints and benefits. The incentive model for drug developers must change so that they can justify investing in this research. Without a change in the regulatory scheme that would more easily allow products providing nutritional therapies to be marketed, supplement manufacturers have little reason to embark in such studies.

3. Primary mitochondrial disorders

This session featured presentations on mtDNA related disorders (Michio Hirano, M.D., Columbia University Medical Center), Mendelian mitochondrial disorders (Greg Enns, MB, ChB., Stanford University and Lucile Packard Children's Hospital), and the genetics of PMD with a focus on genotype-phenotype correlations (Robert McFarland, M.A., M.B.B.S., Ph.D., M.R.C.P., MRCPCH, Wellcome Trust Centre for Mitochondrial Research/Newcastle University). It was moderated by Katrina Gwinn, M.D., of NIH's National Institute of Neurological Disorders and Stroke (NINDS).

PMD were originally defined as disorders that resulted from defects in mitochondrial RC complexes I-IV involved in oxidation and complex V involved in phosphorylation — an integrated process that serves as the primary generator of adenosine triphosphate (ATP) energy in the cell. These 5 complexes comprising the OXPHOS pathway contain more than 80 structural protein subunits that are encoded by genes located in both mtDNA and nDNA. Accordingly OXPHOS requires intact machinery for mtDNA genome replication, maintenance, transcription and translation. If there is a genetic deficiency in any one of these factors, a combined effect on many OXPHOS subunits is commonly encountered. In addition to ATP production, mitochondria perform a variety of other functions including: generation of reactive oxygen species; amino acid catabolism; pyrimidine, heme, estrogen, and testosterone synthesis; urea cycle and cholesterol metabolism; calcium homeostasis; and apoptosis [26] and all of these functions can also be affected in PMD.

Mitochondria are unique mammalian organelles in that they are the products of two genomes: nDNA and mtDNA. mtDNA is a small (~16.5KB), circular, double-stranded molecule that encodes 13 of the ~80 polypeptide subunits of the mitochondrial OXPHOS enzyme complexes as well as 22 transfer RNAs and 2 ribosomal RNAs necessary for mitochondrial protein synthesis [27]. mtDNA is required for mitochondrial ATP synthesis, and as a consequence, defects in mtDNA and the resulting deficiency of mitochondrial energy production can lead to dysfunction of virtually every organ in the body, particularly affecting tissues that have high energy dependency such as brain, peripheral nerves, and skeletal and heart muscle [28]. Sometimes, it is this unusual combination of multisystemic tissues involved when a patient presents with symptoms of mitochondrial disease that first alerts the clinician to the possible diagnosis.

3.1. mtDNA disorders

Mutations in mtDNA are typically maternally inherited as the mitochondria are transmitted exclusively through oocytes to offspring, though they may, on occasion, arise sporadically. Because hundreds to thousands of copies of mtDNA are present in each cell, the level of mtDNA mutation load (heteroplasmy) and distribution of mutant mtDNA in cells largely

dictate severity of a given disease phenotype, high levels being generally associated with more severe disease. Historically, a variety of clinical syndromes have been described in PMD including Kearns-Sayre syndrome [(ptosis, chronic progressive external ophthalmoplegia (CPEO), pigmentary retinopathy and cardiac conduction block)] and Pearson syndrome (sideroblastic anemia and exocrine pancreatic dysfunction) that result from mtDNA single deletions; myoclonus epilepsy with ragged-red fibers (MERRF); mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS); neuropathy ataxia retinitis pigmentosa (NARP); and Leigh syndrome that are caused by pathogenic mtDNA point mutations. In the last 25 years, there has been an explosion in the number of identified pathogenic mtDNA mutations (to date, over 270 point mutations as well as hundreds of deletion mutations have been identified [29]). Identifying and defining these syndromes has benefited the field significantly as it has helped clinicians identify patients, patients and families better understand their disease recurrence risk and prognosis, and facilitated the discovery of new disease mutations in phenotypically similar patients.

mtDNA disorders are not very rare, but can be difficult to diagnose; Gorman et al., have reported a mtDNA disease prevalence of 1 in 5,000 adults in the Northeast of England; while screening of umbilical cord blood samples from healthy children has revealed that 1 in 200 carries a pathogenic mtDNA mutation [30]. The low penetrance of pathogenic mtDNA mutations is likely due to the fact that many mtDNA mutation carriers have levels of mtDNA mutations that are below the threshold (typically 70% mutation, although this may vary by mutation and tissue) necessary to cause a mitochondrial disease. Further complicating the clinical recognition of mtDNA mutations are their variable clinical manifestations. The m.3243A>G mutation aptly illustrates how a single genotype can produce multiple phenotypes. The most common cause of MELAS, m.3243A>G can also cause maternally inherited diabetes and deafness, chronic progressive external ophthalmoplegia, as well as other phenotypes. The converse is also true — one clinical phenotype can be caused by multiple genotypes. For example, more than 15 mtDNA mutations have been associated with MELAS phenotype [31]. Thus, the diagnosis of mtDNA diseases is exceedingly challenging and typically requires whole mtDNA sequencing and heteroplasmy quantitation in one or more tissues.

Large cohorts of patients with the m.3243A>G mutation have been followed in both the Medical Research Council (MRC) Mitochondrial Disease Patient Cohort in Newcastle (UK) and the NICHD-funded natural history study at Columbia University. One hundred fifty-three subjects whose families have the m.3243A>G mutation have been followed and characterized genotypically for an average of 10 years in the Columbia cohort. These patients have many central nervous system (CNS) and other neurologic problems (e.g., headaches, stroke-like episodes, balance issues, dysautonomia, and neuropathy) as well as variable motor and cognitive delays, depression, hearing loss, diabetes, psychiatric issues, and gastrointestinal (GI) disturbance [32]. Many of these patients died over this interval from causes that included primarily CNS complications as well as cardiac dysfunction and GI pseudo-obstruction, adding to the body of evidence that MELAS is a devastating multisystemic disease. While heteroplasmy appears to be an important factor in determining the clinical phenotype in patients with the m.3243A>G mutation, it is clear that other, as yet unidentified, genetic and environmental factors also play a significant role. Furthermore, half

of the 129 patients in the Newcastle MRC Mitochondrial Disease Patient Cohort living with this mutation do not exhibit a “classic” syndrome and only just over 10% of recruits to the Newcastle arm of the MRC Cohort harboring this mutation had stroke-like episodes [33].

Because lactate levels in brain ventricles have been correlated with severity of MELAS disease, lactate was considered to be a good biomarker for subsequent study and potentially a target for therapy. Dichloroacetate was tried as a therapy for MELAS in a randomized, placebo-controlled study, which found that the drug did more harm than good [34]. The study, however, did demonstrate the feasibility of RCTs in these complex disorders and a number of clinical trials in PMD such as MELAS are now active (see clinical trials.gov). With support of the Marriott Foundation, a small group of investigators is taking advantage of the Columbia MELAS cohort to focus on a pipeline for MELAS drug and biomarker discovery [35].

3.2. Mendelian mitochondrial disorders

It is becoming increasingly recognized that the nuclear genome is exerting an effect on a wide range of mitochondrial processes, including the OXPHOS pathway, mitochondrial transcription, replication, and protein import [36]. More than 250 nuclear genes have been identified as being dysfunctional in mitochondrial diseases [37] and most of the disorders seen in the pediatric clinic are nuclear in origin (up to 80% of PMD seen in children) [38].

nDNA mitochondrial diseases, inherited in a Mendelian fashion, can be caused by mutations in RC subunits (e.g., Leigh syndrome secondary to complex I deficiency [39], mutations in RC ancillary or assembly proteins (e.g., SCO2 deficiency [40]), defects of mtRNA translation (e.g., DARS2 deficiency [41]), defects of the mitochondrial inner membrane lipid milieu (e.g., Barth syndrome, [42]), and defects of mitochondrial dynamics (e.g., fetal infantile encephalomyopathy and autosomal dominant optic atrophy (OPA1 mutation) [43, 44]). The subset of nDNA disorders associated with defects in mtDNA maintenance highlight the importance of mtDNA integrity [45]. Defects in these genes often cause mtDNA depletion, with or without multiple mtDNA deletions. Inheritance of nDNA mitochondrial disorders is most commonly autosomal recessive, but dominant and X-linked inheritance may occur.

When treating or developing therapeutic trials for PMD patients, it is worth noting that secondary mitochondrial involvement may be present in different diseases that do not primarily affect the mitochondrion [37]. Secondary mitochondrial disorders can have a variety of different mitochondrial mechanisms underlying clinical disease. In a paper examining the use of glutathione as a biomarker for mitochondrial disease, investigators found that across all classifications of PMD, there is a relative low redox potential and a more oxidized state when using the oxidized and reduced glutathione redox couple as a biomarker compared to normal, healthy controls [46]. Similar findings were noted in patients who have organic acidemias [47]. Many patients with organic acidemias, when in crisis, may behave similarly to patients with PMD and show a low redox potential. There is clear secondary mitochondrial involvement in at least some of the organic acidemias [48–50]. Because secondary mitochondrial dysfunction is common in many IEM, it may be that the findings from trials on PMD can be generalized to other conditions as well.

3.3. Genetics of primary mitochondrial disorders: genotype-phenotype correlations

In an effort to address some of the genotype-phenotype correlation issues associated with mitochondrial disease, the MRC Mitochondrial Disease Patient Cohort Study U.K. (n = 1,349) was established in 2009 [33]. This effort involves three U.K. sites (Newcastle, London, and Oxford) that are currently operating as clinical and diagnostic referral centers for patients with mitochondrial disease in an attempt to provide the best care to all patients. Since 2007, all patients diagnosed with and/or at risk of a mitochondrial disease in the U.K. have open access to one of these three nationally commissioned centers of excellence. Currently 850 patients are reviewed on a 6 to 12 monthly basis at the Newcastle centers alone.

The complexities of genotype-phenotype correlation for MELAS were highlighted in a previous section. Mutations in the polymerase gamma (POLG) gene can lead to a broad range of phenotypes including Alpers Huttenlocher Syndrome, Adolescent-onset Refractory Epilepsy, CPEO, Parkinsonism, premature menopause, Sensory Ataxic Neuropathy Dysarthria and Ophthalmoplegia (SANDO syndrome). In addition, different mutations can lead to the same clinical symptoms. For instance, over 75 disease genes have been reported to cause Leigh Syndrome, a progressive neurodegenerative disorder with onset in early childhood which results from both mtDNA and nDNA mutations [51].

mtDNA deletions can vary in size and are most easily detectable in muscle. Using the Newcastle Mitochondrial Disease Adult Scale (NMDAS) [52, 53] as a clinical biomarker, researchers have found that both the size of the mtDNA deletion and heteroplasmy level correlate with the score obtained on that scale [54]; however, the size of the deletion appears to exert a greater effect than heteroplasmy. This finding may allow clinicians to predict clinical progression of mitochondrial disease due to mtDNA deletion. In general, patients with high levels of heteroplasmy and larger deletions have a faster rate of progression on the NMDAS [54, 55].

To help address the complex genotype-phenotype issues associated with mitochondrial disorders, significant emphasis should be placed on the importance of clinical acumen in detailing phenotypes, enrolling patients in cohorts, as well as collaborative efforts to combine cohorts across centers and countries. Adopting such an approach will permit rare phenotypes to be recognized and allow direct comparison of next generation sequencing data from individuals on different cohorts to enable identification, or validation, of pathogenic mutations.

4. How nutritional interventions are used in PMD

This session, moderated by Kathryn Camp, M.S., R.D. (ODS), included presentations on the ketogenic diet (Ms. Camp), nutritional interventions in PMD (Bruce Cohen, M.D., Akron Children's Hospital), the MMS North American Mitochondrial Disease Survey and Consensus Project (Sumit Parikh, M.D., Cleveland Clinic), and a NAMDC patient survey on dietary supplement use (Amel Karaa, M.D., Massachusetts General Hospital).

4.1. The ketogenic diet

The ketogenic diet, developed in the 1920s, employs a very high fat, low carbohydrate distribution of macronutrients to achieve seizure control [56]. Interest in the ketogenic diet as a therapeutic option waned in the 1930s and 1940s with the advent of new antiepileptic drugs; however, due to heightened awareness of antiepileptic drug resistance and adverse effects, there was a renewed interest in the ketogenic diet starting in the 1990s [57].

Several variations of the ketogenic diet have emerged but they all share a significant reduction in dietary carbohydrate leading to the presence of ketones in the blood [58]. When dietary carbohydrate is restricted, ketones are generated in the liver through mitochondrial beta-oxidation of fatty acids. Ketones are metabolized to acetyl-CoA, which feeds into the Krebs cycle, and thus serves as a non-glucose energy source for brain, heart, and skeletal muscle. The mechanism of seizure reduction is not clear.

The classic and most restrictive ketogenic diet is a 4:1 ratio of fat to protein plus carbohydrate and derives approximately 90% of calories from fat, 7% from protein, and about 3% from carbohydrate. From a practical point of view for a school-aged child, this is roughly equivalent to the amount of fat in two sticks of butter, the amount of protein in 3 ounces of chicken, and the amount of carbohydrate in 1 tablespoon of sugar. In an effort to ease the restrictive nature of the ketogenic diet, products and recipes have been developed to improve its palatability.

The ketogenic diet for seizure control has been shown to result in short- to medium-term benefits in children and adolescents that are comparable to modern antiepileptic drugs [59]. Roughly 50–60% of children on the ketogenic diet will have at least 50% seizure reduction and one-third will become seizure-free [60] (some will remain so even after the ketogenic diet is discontinued [61]). Complications associated with the ketogenic diet include nutrient deficiencies, constipation, exacerbation of reflux, cholelithiasis, hyperlipidemia, and poor growth [62, 63]. There is growing experimental evidence for the broad neuroprotective properties of the ketogenic diet and mechanistic linkages to key cellular signaling pathways and fundamental bioenergetic processes [58]. Disorders for which there is strong or emerging evidence that the ketogenic diet may hold some benefit include diabetes, cardiovascular disease, epilepsy, obesity, acne, neurological disease, cancer, and polycystic ovary syndrome [64].

Seizures are a cardinal feature of some PMD [65], and while their exact prevalence is unknown, they have been reported to occur in 34–60% of individuals with biochemically confirmed PMD [66, 67]. Safe and effective use of the ketogenic diet has been reported in patients with RC defects; in one study, 10 of 14 patients placed on the ketogenic diet were seizure free or had a greater than 50% reduction in seizures [68]. In another study, a positive response was reported in 18 of 24 patients [69]. Twelve of the 18 patients were seizure free, and 6 had a decrease of seizure frequency between 50–90%. These studies are complicated by the fact that dietary supplements were used in conjunction with the ketogenic diet. In two studies of pyruvate dehydrogenase deficiency, 35 of 58 and 5 of 21 patients on the ketogenic diet reported benefit, [70, 71]. Additional case reports suggest beneficial effects of the ketogenic diet in patients with mitochondrial disorders [72–77]. However, the ketogenic diet

should not be used for patients who cannot comply with the dietary restrictions, and it is contraindicated in primary carnitine deficiency, beta-oxidation defects, pyruvate carboxylase deficiency, porphyria, severe gastroesophageal reflux [57], and 3-hydroxy-methylglutaryl and Co-A lyase deficiency [68].

In summary, the ketogenic diet appears to decrease seizures in at least half of treated patients with no serious side effects [60]. However, the long-term safety and effectiveness in mitochondrial disease is unknown. It may be possible to use less restrictive forms of the diet, and manipulation of pathways known to partially mediate the effects may inform better treatments. A greater understanding of the heterogeneity of mitochondrial disorders will be critical to target the use of the ketogenic diet to responsive patients [58].

4.2. Nutritional interventions in PMD: which ones are used and for what purpose?

Dr. Cohen provided a personal history of the evolution of his nutritional intervention therapy for RC deficiency. From 1989–2007, his therapy regimen included coenzyme Q₁₀ (CoQ₁₀), L-carnitine, thiamin, riboflavin, niacin, pantothenic acid, pyridoxine, vitamin B₁₂, folic acid, biotin, vitamin C, vitamin E, beta-carotene, zinc, selenium, magnesium, and N-acetyl cysteine. In 2007, based on new knowledge about disease mechanisms, he changed his treatment regimen to alpha-lipoic acid, arginine, L-carnitine, CoQ₁₀, riboflavin, creatine monohydrate, and folinic acid. Dr. Cohen indicated that one reason he now uses fewer dietary supplements than in the past is the burden of therapy (cost, complexity to the patient/family, side effects) given its unproven benefit.

In a randomized, double-blind, placebo-controlled, crossover study that included 17 patients with a definitive or probable diagnosis of mitochondrial disease, subjects were administered a combination of 3 compounds (3 g creatine monohydrate plus 300 mg alpha-lipoic acid plus 120 mg CoQ₁₀) twice daily for 2 months [78]. Following a 5-week washout period, all patients were then given placebo for 2 months. Treatment was statistically significantly associated with lower resting lactate concentrations, prevention of loss of strength at the ankle, improved fat-free mass, and a reduction in urinary 8-Isoprostane and a non-significant lowering of 8-hydroxy-2'-deoxyguanosine excretion suggestive of decreased oxidative stress. There was no change in pulmonary function tests or peak handgrip strength. Patients with MELAS showed the greatest improvement with the combination therapy, suggesting that one therapeutic strategy may not benefit all mitochondrial diseases.

In a review of clinical trials for mitochondrial disorders it has been noted that anecdotal reports do not allow evidence-based conclusions for therapy with nutritional interventions [79]. In addition, the controlled clinical trials that have been performed were viewed as weak, with studies on five or fewer patients or inadequate supplement dose. Finally, many dietary supplements in use for therapy in mitochondrial disease have not been studied at all.

A uniform multi-supplement therapy for mitochondrial therapy is not a reasonable approach for a variety of reasons. Perhaps most importantly, a single approach to treating hundreds of different diseases is not likely to be effective. Moreover, the approach is expensive, and most of the cost is borne by the patients themselves. Regimens including multiple medications and dosing patterns are only able to be carried out by highly motivated parents or patients.

Finally, aside from “n of 1” cases, opportunities to judge clinical efficacy are limited. These challenges lead to a conundrum for clinicians. Anecdotal reports of efficacy provide hope to patients, but the regimens seldom meet therapeutic goals. Expansive use of vitamins also sometimes is viewed as suspect by physicians not routinely involved in treating mitochondrial disease, especially if the patient does not have genetic proof of their illness.

4.3. The MMS North American Mitochondrial Disease Survey (2012) and Consensus Project (2013)

The practice patterns of mitochondrial physicians in North America have been evolving. As recently as 10 years ago, there were few guidelines available, little published literature, and most practitioners in this area of medicine took the same approach as their mentors. Over the last decade, however, the field has grown substantially. The MMS [21] has led efforts to examine how clinicians have treated mitochondrial disorders with the future goal of moving towards standardized care for these patients. The MMS sent invitational surveys to all of the organizations that include physicians who practice mitochondrial medicine. Thirty-two physicians and nurse practitioners completed a series of surveys related to the practice of mitochondrial medicine. It is estimated that the practice patterns of at least 90% of U.S. mitochondrial centers were captured by the surveys [3].

Overall, there was agreement in how clinics are structured and organized, acknowledgement that caring for these patients requires significant time, similar perceptions of various diagnostic laboratories, and recognition that there is a shortage of adult-trained experts in the field. However, significant variability was seen in terms of care. The diagnostic approaches used also varied substantially, as did the extent of testing prior to diagnosis, interpretation of test results, and treatment itself. Sixty-nine percent of respondents reported a unique combination of dietary supplements used for treating mitochondrial disorders, 13% used the same two compounds, while less than 10% each used the same 3 compounds or a single compound. The total number of supplements used also varied. Most reported using 3 to 6 supplements; although, 25% of mitochondrial clinicians reported using more than 6 supplements. Approximately half of survey respondents indicated that they follow either leukocyte or plasma CoQ₁₀ levels in their patients; however, the manner in which these levels are followed varied. Most clinicians recommend exercise, but again, the type of exercise varied.

The results of this survey call into question the appropriateness and efficacy of one approach over another, and spurred an effort by the MMS to develop consensus diagnostic criteria and treatment recommendations based on the existing evidence base [80]. Using the Delphi consensus methodology, agreement was reached on the use of nutritional interventions which is summarized here:

- CoQ₁₀ should be administered to most patients with a diagnosis of mitochondrial disease, and not exclusively for primary CoQ₁₀ deficiency. Reduced CoQ₁₀ (ubiquinol) is the most bioavailable form, and when used, dosing should be modified relative to other forms. Leukocyte CoQ₁₀ levels are helpful to monitor absorption and adherence to treatment whereas plasma levels are more variable and less reflective of tissue levels.

- Alpha-lipoic acid and riboflavin were the only other supplements that met some degree of consensus to recommend that they should be offered to patients with mitochondrial disorders.
- Folinic acid should be considered in mitochondrial disease patients with CNS manifestations and routinely administered to those with documented cerebral folate deficiency or with mitochondrial disease states known to be associated with folate deficiency.
- L-carnitine should only be administered when there is documented carnitine deficiency, and levels should be monitored during therapy.
- When beginning supplement therapy, one supplement at a time should be given, taking into account clinical status and avoiding a “cocktail approach”.
- There is no evidence to suggest that a patient’s diet can be adjusted on the basis of muscle or skin fibroblast mitochondrial electron transport chain analysis results alone.
- Goal levels for most dietary supplement therapies are not yet known. It is prudent, however, to correct nutrient deficiency states.
- There is a need for evidence-based guidelines.

4.4. North American Mitochondrial Disease Consortium patient survey on dietary supplement use

The NAMDC patient survey on dietary supplement use [81] was intended for patients and parents of patients who were or have taken dietary supplements as part of their treatment for mitochondrial disease. The online survey was disseminated to patients who participate in the NAMDC and/or RDCRN registries in September of 2014. At the time of the workshop presentation, 125 respondents had completed the full survey and another 35 had partially responded. The most frequent diagnoses reported were “complex I deficiency” and “mitochondrial myopathy.” The five most bothersome clinical symptoms reported were fatigue, exercise intolerance, weakness, myalgia, and constipation. Among all the patients who are or were taking dietary supplements, 64% indicated taking four or more dietary supplements at once. The five most frequently used supplements were CoQ₁₀ (28%), L-carnitine (25%), vitamin D (19%), riboflavin (16%), and vitamin C (12%). The reported doses taken for each supplement varied greatly across survey respondents. Forty-six percent of respondents reported no improvement in any of their top five symptoms; 54% of reported some improvement, generally within 2 weeks to 3 months of starting the supplements. For patients who discontinued the supplements due to various reasons (46/160), 38% noted that there was some unrecognized benefit that became noticeable once off the supplements.

All participants who were taking CoQ₁₀ believed that this supplement was the most beneficial in improving their (or their child’s) symptoms. Seventy-eight percent taking L-carnitine, 39% taking riboflavin, 28% taking creatine, and 26% taking vitamin B₁₂ believed that these supplements caused the most improvement in their symptoms. Approximately 28% reported side effects from taking supplements, the most common of which were upset

stomach, nausea, unpleasant or strong body odor, and diarrhea. Ten percent of respondents indicated that insurance covered the full cost of the supplements; 62.5% had to resubmit and/or appeal the insurance company's decision at least once. Sixty-nine percent of respondents indicated that their monthly estimated out-of-pocket expenses were less than \$200, 26% reported expenses ranging from \$200 to \$500, and 4.5% indicated spending more than \$500 out of pocket.

Thirty-four percent of respondents indicated that they were using another therapy at the same time as the supplements, including increased fluid hydration, special diet, increased physical activity, and/or another medication. Despite these other treatments, 46% of patients believed that the improved symptoms were the results of the dietary supplements alone.

Sixty-eight percent of survey respondents reported a preference to take the supplements daily, overwhelmingly noting the difficulty in keeping track of the numerous recommended supplements. Fifty-three percent of respondents indicated that supplement expenses caused their family some financial burden. Multiple suggestions were made by the respondents to improve the ability to receive and take supplements, including combining all supplements into a single small pill, improving the taste of the supplements, making less expensive supplements available, improving coverage by insurance companies, making financial assistance available, and increasing the ease of obtaining supplements from pharmacies. A need for additional research to determine the efficacy of supplements was noted.

4.5. Session discussion points

Clinicians often struggle to provide appropriate instructions to patients regarding supplements. Oftentimes, supplements may appear to improve a patient's symptoms for a period of time, but are not "changing the face of the disease." The possibility of placebo effects should be kept in mind. NAMDC survey results indicated that 90% of patients purchase supplements out of pocket from stores and the contents of these products may not be fully known, an important issue to address. More objective endpoints for treatment are needed, as are non-clinical model systems to test complex product combinations.

5. Challenges and barriers to dietary supplement use in PMD

This session featured presentations on clinical trials in mitochondrial disease (Bruce Cohen, M.D., Northeast Ohio Medical University) and mitochondrial disease treatment with a focus on scientific evidence vs. clinical practice (Jerry Vockley, M.D., Ph.D., University of Pittsburgh and Children's Hospital of Pittsburgh). It was moderated by Dr. Enns.

5.1. Clinical trials in mitochondrial disease

Obstacles facing controlled clinical trials of mitochondrial disorders include the fact that there are many diseases, confounding genotype-phenotype issues, uncertainty regarding outcome measures, long and unpredictable clinical courses, including variability in function with prolonged periods of disease inactivity, difficulty in meeting entry criteria, cost, travel, small patient populations, and other statistical challenges. The FDA ideally should be considered a partner, not an obstacle, in getting drugs into clinical trials. In evaluating efficacy, the FDA is looking for functional and clinical improvement using verified tools and

scales, and improved natural history studies. Non-clinical endpoints and biomarkers such as lower lactic acid concentrations, improved brain MRI findings, reduced ragged red fiber count or electron transport chain enzymology, or normalization of abnormal organic acids are typically not viewed as sufficient for drug approval.

To aid in defining appropriate clinical endpoints for trials, the NINDS Common Data Elements (CDE) Project [82] includes a series of mitochondrial disorder CDEs that were made available for public comment 1 week prior to this workshop. As part of this effort, the initiative's Neurological Assessments CDE Panel examined and classified every verified scale for neurological disease. It is hoped that these efforts will result in the development of a toolkit for selecting the most appropriate outcomes for ongoing studies. See section 8.2 for more information about the NINDS CDE Project.

Several clinical trials in PMD were reviewed during these session. EPI-743 (α -tocotrienol quinone) is a novel medication with a different redox potential than other available agents such as CoQ₁₀ and at the time of the workshop was in a double-blind, placebo-controlled study for treatment of PMD (see section 8.3). An open label study of the drug RP103 (cysteamine bitaurate) is currently in process in children with PMD [83]. Both studies are using the Newcastle Paediatric Mitochondrial Disease Scale as the primary outcome measure. An exploratory study of elamipretide, (SS-31) a small cell-permeable peptide of less than 10 amino acids designed to target the inner mitochondrial membrane, will soon be entering clinical trials [84]. The study will include three cohorts of 12 patients each (aged 16–65 years) with genetically confirmed PMD including mitochondrial myopathy [85]. Nine patients in each cohort will be treated with the drug, three with placebo. The drug will be given as a 2-hour continuous intravenous infusion daily for 5 consecutive days. The primary endpoint is the safety and tolerability of ascending doses; secondary endpoints include the 6-minute walk test and cardiopulmonary exercise testing. Exploratory endpoints include plasma/blood/urine biomarkers and additional measurements of mitochondrial function.

5.2. Mitochondrial disease treatment: scientific evidence vs. clinical practice

Dr. Vockley suggested that the significant needs facing the field of mitochondrial medicine extend to rare diseases in general. Unless the field is able to overcome some of the challenges discussed during this workshop, Dr. Vockley predicted that it will not move forward. Metabolic medicine in general and mitochondrial medicine in particular must transition from relying on individual experience to a comprehensive view of patients, conditions, and responses to potential medications that will allow clinicians to make better clinical decisions. The field of childhood cancer successfully made this transition. In 1960, childhood cancer was nearly uniformly fatal, but today, the overall 5-year survival rate is 75% and some cancers have an almost 100% cure rate. Sequential clinical trials that built on each other formed the foundation for the transition in the field of childhood cancer. Mitochondrial medicine could take a similar approach to that of the childhood cancer field, applying lessons learned to both pharmaceuticals and nutritional interventions. All individuals with mitochondrial disease should have access to a defined standard of care, and all should be offered enrollment in a clinical study to compare standard of care with one other variable. There is a need to bring together NAMDC and the other organizations

focused on patients with mitochondrial disorders for a unified approach. Having different organizations working on different approaches was suggested to be ineffective, particularly in this field, where there is a limited number of patients and a near infinite number of variables.

Potential research subjects may have misplaced biases about placebo-controlled studies (e.g., “I don’t want to be a guinea pig,” “I want to get the real treatment, not the placebo”). However, clinical trials provide the only mechanism for determining efficacy and obtaining FDA approval for new treatments. Currently, there are no FDA-approved treatments for any genetic mitochondrial disease and without patients enrolled in trials, there will be no proven treatments. Moreover, many trials with a placebo arm allow the same subjects to cross over to be in a treatment arm and hence serve as their own controls. The use of dietary supplements in this field adds an additional layer of complexity, because they are not regulated in the same fashion as drugs. However, the same level of intellectual rigor is needed in dealing with these products. This may require a new category for regulatory consideration (i.e., one that occupies the middle ground between a dietary supplement and a drug).

Lessons learned from clinical trials in other rare diseases can be applied to the field of mitochondrial medicine. For example, the use of a biomarker in a study can be acceptable and effective (e.g., blood phenylalanine concentrations as a measure of severity or control of disease in phenylketonuria). Traditional study designs may not be possible in the field of mitochondrial medicine, and more effective ways of mining already existing clinical data are needed to help make decisions on appropriate endpoints. There is a strong need for a central Institutional Review Board (IRB) or shared IRBs given the fact that studies of mitochondrial disorders generally require multicenter trials. All of these issues will become increasingly pressing as the field pursues future clinical trials on mitochondrial disorders.

Though the FDA has experience in rare diseases, including metabolic diseases, to date it has had limited experience in mitochondrial diseases. Ideally, the FDA would be able to form a group that would understand the complexities of mitochondrial diseases and could work effectively with the mitochondrial disease community in designing non-traditional trials and ensuring scientific rigor. Subsequent to the workshop, a Critical Path Innovation Meeting (CPIM) [86] was held at FDA headquarters in October 2015 to address research challenges in mitochondrial disease. A summary report of the CPIM was made available to the meeting organizers and can be found on the ODS website (<https://ods.od.nih.gov/attachments/CriticalPathInnovationMeetingSummary.pdf>).

5.3. Session discussion points

Some participants noted that qualitative research that takes into account patients’ concerns is missing in the field of mitochondrial medicine, specifically in trials of mitochondrial disorders. A mixed-methods approach that engages patients is critical for the field to move forward. The FDA has clearly delineated guidance on rare diseases, including support for patient-reported outcomes. It was suggested that FDA representatives meet with interested workshop attendees to provide additional information, discuss misperceptions regarding

FDA, and begin a dialogue on issues related to moving drugs to market for the mitochondrial disease patient population.

6. Perspectives from select patient advocacy, industry, and professional organizations panel discussion

For rare diseases like PMD that affect multiple organ systems and require a multidisciplinary team to care for patients, regular communication among researchers, physicians, patient advocacy groups, professional organizations, and the pharmaceutical industry is needed. Furthermore, collaboration among these groups will be necessary to foster research for effective nutritional interventions in PMD.

This session was moderated by Rashmi Gopal-Srivastava, Ph.D., of NIH's Office of Rare Diseases Research at NCATS. It included brief presentations by representatives from patient advocacy groups (Chuck Mohan, UMDF and Christine Cox, MitoAction), industry (Tricia Rutherford, VitaFlo International, Ltd; Steven Yannicelli, Ph.D., R.D., Nutricia North America) and professional organizations (Jerry Vockley, M.D., Ph.D., American College of Medical Genetics and Genomics and the Society of Inherited Metabolic Disorders; Erin MacLeod, Ph.D., R.D., Genetic Metabolic Dietitians International).

6.1. Patient advocacy

UMDF is an advocacy organization whose mission is to promote research and education for the diagnosis, treatment, and cure of mitochondrial disorders and provide support to affected individuals and families [87]. UMDF has stated that the use of dietary supplements is anecdotal for mitochondrial disorders. UMDF also advises individuals to check with their primary care physicians before taking any supplements. UMDF is working with other groups with similar interests and goals on a global basis. It is a founding member of the IMP (<http://www.mitopatients.org/26-about/56-organization>) and is looking at CDEs to help support patient- and/or clinician-populated registries and determine how best to get new treatments in the pipeline. A common patient-populated registry that can be adopted and accepted by all groups will help build critical momentum and benefit the overall mitochondrial community. UMDF has developed the Mitochondrial Disease Community Registry to engage as many patients, patient groups, caregivers and family members as possible.

MitoAction is a nationwide nonprofit organization focused primarily on improving the quality of life for patients and families with mitochondrial disease [88]. As part of its advocacy efforts, MitoAction has conducted patient and caregiver surveys to determine the patient perspective on dietary supplements used to treat mitochondrial disease. The most recent MitoAction survey was conducted in 2013, results of which are available on the MitoAction website (<http://www.mitoaction.org/massachusetts-mito-cocktail-mandate>). MitoAction has also developed a series of tips regarding insurance appeals for the mitochondrial cocktail and compounded prescriptions [89].

MitoAction helped introduce a bill in the Massachusetts legislature that names specific supplements for the treatment of mitochondrial disorders and requires their coverage by

health insurers [90]. An independent cost/benefit review conducted by the Massachusetts Center for Health Information and Analysis found that such a mandate in the State of Massachusetts would increase private health insurance premiums by only 0.01% [91].

6.2. Industry

VitaFlo® International, Ltd. [92], has a history of developing products for rare IEM with little or no available dietary treatments. In order to develop a product to aid in instituting nutritional therapy for patients with PMD, the company began two research programs in the U.K. The first program is charged with development of a cell-based system to investigate which RC defects may respond to a ketogenic diet. The second is a nutritional assessment program to examine patient access to nutritional therapies, which nutrition interventions have been employed, and the nutritional status of children with mitochondrial disease. VitaFlo® plans to use the results from these research projects to develop nutritional products suitable for the dietary management of mitochondrial disease.

Nutricia North America [93] specializes in medical foods and also produces a vitamin/mineral supplement designed for restrictive diets. Unlike dietary supplements, medical foods must be clinician-supervised and disease-specific. Like dietary supplements, medical foods are not prescription products. There is a need for evidence-based clinical outcomes so that coverage for the costs of medical foods would be more comprehensive.

6.3. Professional organizations

The Society of Inherited Metabolic Disorders (SIMD) [94] provides a platform for the discussion of issues related to basic and clinical science and to stimulate interactions between clinicians and investigators in the field of IEM. Additionally, SIMD strives to promote public understanding of IEM and advocates for patients and research through public policy forums. SIMD members include physicians, nutritionists, genetic counselors, and Ph.D. laboratorians. The American College of Medical Genetics and Genomics (ACMG) is a professional organization that provides education, resources, and a voice for the medical genetics profession [95]. From the perspective of mitochondrial medicine, the ACMG Therapeutics Committee can facilitate development of evidence-based clinical guidelines. An overarching clinical guideline for the treatment of mitochondrial diseases would be too broad a topic to cover. Rather, guidelines on a more focused issue such as the role of CoQ₁₀ supplementation in PMD are more practical. The ACMG can help leverage activities related to development, standardization, and recognition of clinical therapies based on its experience with other disorders. Neither group will sponsor a clinical trial per se, but they can serve as resources for identifying individuals who can conduct and design them.

Genetic Metabolic Dietitians International (GMDI) [96] is a group of dietitians focused on IEM and genetic diseases. It seeks to better serve patients and families through guidance on how to provide as close to a normal, healthy diet as possible while maximizing dietary management of their IEM. Most recently, the group has developed guidelines for various IEM using formalized evidence-based literature reviews and consensus processes. As an organization, GMDI seeks to provide a forum for guidelines development, research review,

and resources for dietitian education, including examination and compilation of existing research. Additionally, GMDI strives to be an advocate for patients and families.

6.4. Session discussion points

One of the many challenges associated with the management of PMD is that these patients are often followed by many different health care providers. Ideally, a dietitian with a genetic metabolic background would be included in the care team. A multidisciplinary team would be helpful for monitoring those PMD patients on a ketogenic diet given the risks that their condition might worsen while on it. Strong enthusiasm was voiced for the development of guidelines related to proper nutrition for patients with PMD.

Another challenge is that there is no uniform classification of mitochondrial disorders at this time, which impacts the ability to develop clinical practice guidelines and conduct drug trials. Furthermore, given that there are fewer than 150 metabolic physicians in the United States, and even fewer are comfortable treating individuals with mitochondrial disease, patients with undefined disease on mitochondrial-oriented therapies pose a challenge to the already large patient caseloads of these physicians and should be referred to other appropriate subspecialists.

7. New technologies and “omic” approaches to diagnosis, treatment, and understanding mechanisms of action

This session was moderated by Dr. Robert McFarland. It featured presentations on integrating the transcriptome, metabolome, and mitochondrial physiome to clarify disease mechanisms and treatment effects in mitochondrial RC disease (Marni Falk, M.D., Children’s Hospital of Philadelphia/University of Pennsylvania); metabolic profiling of mitochondrial disease (Vamsi Mootha, M.D., Howard Hughes Medical Institute/Massachusetts General Hospital/Harvard Medical School); an evidence-based approach to developing mitochondrial-active drugs and supplements (Gino Cortopassi, Ph.D., University of California, Davis); the gut microbiome metabolism of choline and L-carnitine and implications for mitochondrial disorders (W.H. Wilson Tang, M.D., Cleveland Clinic); and a case study highlighting patient and product safety issues (Freddie Ann Hoffman, M.D., HeteroGeneity LLC).

7.1. Integrating the transcriptome, metabolome, and mitochondrial physiome to clarify disease mechanisms and treatment effects in mitochondrial respiratory chain disease

Basic cellular physiology becomes abnormally regulated at many levels in mitochondrial disease. RC disease has a common cellular “transcriptome signature” across evolution [97–100] which includes global disruption of many core aspects of intermediary cellular metabolism. Metabolomic flux through central intermediary biochemical pathways that are involved in nutrient processing is altered in RC disease [101], including an upregulation of glycolysis and an inhibition of flux through both the tricarboxylic acid cycle and fatty acid oxidation. Mitochondrial physiology is also commonly altered in mitochondrial disease, with increased oxidative stress and variable changes in mitochondrial mass and the ability to convert nutrient-derived reducing equivalents to efficiently produce energy [102].

Recognizing these various levels of physiologic alterations that occur in the cells and tissues of patients with mitochondrial disease emphasizes the pressing need to devise effective means to provide patients' cells with proper nutrients in a way that can be effectively used to generate energy and promote growth.

The immediate biochemical pathology of RC disease can be best understood when the RC is broadly considered as a complex factory that generates four major products: ATP, nicotinic acid dinucleotide (NAD⁺), pyrimidine nucleotides (uridine), and free radicals (hydrogen peroxide). Deficiency of chemical energy is the classical hallmark of mitochondrial RC disease, since the RC is the major site of cellular ATP production. However, RC disease also results in deficiencies of NAD⁺ and uridine, as well as excessive free radical production. In particular, a relative increase in the ratio of reduced NAD⁺ (NADH) to NAD⁺ [103], as well as absolute deficiencies of both of these essential redox metabolites [104], alters flux through hundreds of downstream metabolic pathways, including amino acid metabolism [97]. Targeted blood metabolite analyses in 19 patients with RC disease identified that they have an almost two-fold increase in the ratio of several amino acids when normalized to glutamate as compared to healthy individuals [105]. Such metabolite alterations may offer a potentially useful blood-based biomarker NADH to NAD⁺ balance [97] in RC dysfunction, if prospectively validated.

RC dysfunction, likely through deficiencies of its immediate biochemical products, leads to significant dysregulation of central nodes in an integrated nutrient-sensing signaling network. This highly conserved signaling network functions to collectively sense the cellular nutrient and health status to enable cells to make the ultimate decision to either grow or die, where these essential activities are coordinated by the signaling network to mediate the specific response of hundreds of downstream biological processes and biochemical pathways [99]. For example, RC inhibition alters the expression and ability of glucose to inhibit *FOXO1* [100], activates AMPK and mTORC1 [104] and inhibits PPAR family gene activity in different tissues, key signaling and regulatory pathways for cellular function [100]. Many of these signaling changes can be reversed by treating cells with glucose [104] as well as by a range of drugs that target their specific activities [104, 106]. With these insights, providing proper cellular nutrition, such as glucose itself, can be viewed as a potential therapy to normalize cell signaling responses and thereby improve the cellular capacity to survive and function in RC disease [104]. Systematically characterizing and therapeutically targeting central alterations in the nutrient-sensing signaling network may offer a personalized means to reverse the global sequelae caused by RC dysfunction to improve patient health outcomes.

Combining therapies to treat different aspects of RC dysfunction with the goal of improving cellular and organ health in the face of chronic RC dysfunction were also discussed. Specifically, it is likely that the four major products generated by the RC will need to be replaced by one, or more likely, multiple treatments, if cellular physiology is to be effectively improved. Possibilities include (1) therapies focused on adapting nutrition to an altered cellular metabolic capacity (e.g., providing sufficient glucose to meet cellular energy production needs in the face of impaired RC function and upregulated glycolytic activity and limiting fat in the face of NAD⁺ deficiency and impaired fatty acid oxidation capacity); (2)

specific vitamin therapies (e.g., niacin formulations, such as nicotinic acid, to replenish cellular NAD⁺ levels and vitamin E to function as an antioxidant), and (3) other metabolic modifier therapies (e.g., uridine to restore pyrimidine levels) that are likely to be necessary to restore the diverse aspects of cellular metabolism that impair health in RC disease. Newer therapeutic options are also emerging that inhibit basic cellular processes such as translation and autophagy, to restore the favorable balance of basic cellular processes that regulate proteotoxic stress, which is upregulated in mitochondrial disease [106].

A new model to guide clinical trial design to develop effective therapies for individual mitochondrial disease was proposed: (1) define the disease(s), (2) prioritize outcome measures with input from patients as to what symptoms they most want treated, (3) compile a list of the available treatment options based on the prevailing pathology underlying each disease, (4) conduct *in vitro* laboratory testing of patient cells and simple animal models [107] to test the ability of drug treatment(s) to improve health and viability, (5) conduct placebo-controlled randomized clinical trials individualized to small, well-defined patient populations, and (6) develop treatment standards of care to which to iteratively compare additional treatment options over time.

7.2. Metabolic profiling of mitochondrial disease: quantitative biomarkers for precise mitochondrial medicine

Exome sequencing is emerging as a first-line diagnostic test for PMD and is transforming its molecular diagnosis [108, 109]. The cost of sequencing has dropped so significantly (nearly 1 million-fold) in the last 12 years that it is now far more time- and cost-efficient to perform whole exome sequencing rather than sequential testing of individual genes. Many new mitochondrial disease genes of nuclear origin continue to be identified using exome sequencing. It has been estimated that in roughly half of patients with strong clinical evidence of PMD, the use of exome sequencing applied to nDNA and mtDNA from blood can result in a firm molecular diagnosis [108].

Despite advances in genetics and molecular diagnosis, there remains an ongoing, unmet need for quantitative and preferably mechanistic biomarkers. Once a molecular diagnosis has been established, quantitative biomarkers can be very useful for monitoring the progression and for classifying these disorders. Such markers are also very informative in clinical trials where they can help to segment patients likely to benefit from a therapy, or be used for monitoring therapeutic response. Finally, quantitative biomarkers can provide valuable insight into pathogenesis, which can help to motivate new therapies. Currently, there are more than 250 genes that have been implicated in PMD, but how these genetic mutations lead to end-organ pathology is unclear [110] — quantitative biomarkers may connect causal genetic changes to inform mechanisms of end-organ pathology.

Mass spectrometry-based metabolomics represents a powerful method with which to discover quantitative biomarkers for PMD. While the technology can be applied to any biological specimen, when applied to blood or urine of patients, it can represent a facile and minimally invasive modality with which to monitor disease progression. Several years ago, Shaham et al. applied mass spectrometry metabolomics to identify metabolites altered in the plasma of patients with muscle manifesting PMD [111]. They began by identifying 32

metabolites that are altered in cellular models of mitochondrial myopathy. They then asked which of these 32 metabolites are altered in the plasma of patients with muscle manifesting PMD. Three of these metabolites were also found to be significantly different in patients: lactate and creatine levels were elevated, while uridine levels were reduced.

The identification of reduced uridine in mitochondrial myopathy is particularly noteworthy. Specifically, the mean and median levels of plasma uridine were reduced in patients with mitochondrial myopathy, although overlap was seen in the distribution of plasma levels between patients and controls. From a mechanistic standpoint, it had long been known that cells deficient in RC activity require exogenous uridine [112] for survival. Based on this classic observation, previous groups [103] have suggested that uridine, which is available as a dietary supplement, may be useful in treating patients with PMD. The discovery by Shaham et al. [111] of reduced uridine occurring in some (but not all) patients could be very valuable. Namely, it raises the hypothesis that the patients in whom plasma uridine levels are low may benefit from supplementation, whereas those patients in whom uridine levels are already within the normal range may not benefit. Hence the uridine plasma biomarker could help to segment those patients for whom this dietary supplement may potentially be useful. The use of such biomarkers, in principle, could help to power clinical trials to focus on those patients more likely to benefit from supplementation. In this regard, biomarker discovery may help to facilitate “precision mitochondrial medicine.”

7.3. Mitochondrial active drugs and supplements: an evidence-based approach

To develop an evidence base for drugs and dietary supplements that have an effect on mitochondrial function, and to decide which therapies to move into clinical trials, high-throughput screening strategies followed by testing in animal models of PMD have been employed. Using a biosensor-based assay of mitochondrial oxygen consumption in cell models of Leber’s hereditary optic neuropathy (LHON) and fragile X-associated tremor/ataxia syndrome (FXTAS), 1600 FDA-approved drugs were screened. Drugs with known effects on mitochondrial function, in addition to drugs not previously known to impact mitochondrial function were identified. A particularly interesting signal was identified as an increase in 24-hour oxygen consumption in a treated animal. Similarly, novel vitamins and supplements with effects on mitochondrial function have been identified, although dose-dependence has not yet been tested. A drug strategy that identifies mutation-specific defects in cells and includes a relevant animal model has produced critical lead compounds for FXTAS and LHON trials [113].

7.4. The gut microbiome metabolism of choline and L-carnitine and implications for mitochondrial disorders

The widely publicized findings [114] of an increased risk of cardiovascular disease (CVD) from dietary carnitine and carnitine caused concern in the genetic metabolic community due to the frequent use of supplemental carnitine to manage certain IEM and PMD [115]. Among other functions in the human body, carnitine transports long-chain fatty acids across the mitochondrial membrane for energy production [116] and also reacts with organic acids facilitating their excretion in the urine [117]. Thus, carnitine supplementation may have a role in enhancing energy production in mitochondrial disorders and providing a mechanism

to remove the accumulated and toxic organic acids produced in organic acidurias [117]. A recent survey found that 94% of mitochondrial disease physicians reported using supplemental carnitine in their patient management regimens [3]. The relationship between carnitine supplementation and CVD risk in individuals with IEM or PMD is largely unknown, although a recent study found markedly elevated plasma trimethylamine N-oxide (TMAO) in patients with organic acidemias given carnitine supplements over a long period of time [118]. Recent work demonstrating a mechanism by which metabolism of carnitine by the microbiome may increase CVD risk [115] was described at the workshop, and has potential implications for the practice of carnitine supplementation in patients with PMD.

The greatest environmental exposure in humans is food filtered by the gut microbiota [119]. The “food metabolome,” is defined as the component of the human metabolome that is derived from digestion and biotransformation of foods [119]. Microbial cells outnumber human cells by a factor of about 10 to 1, and roughly 80% of microbial cells are found in the gut. Emerging evidence implicates the gut microbiome as an endocrine organ that differs across individuals, households, and cultures [120] and which produces a wide range of metabolites in response to the foods we eat. The food metabolome is complex, involves many players, is not applicable to a “one-size-fits-all” approach, and comprises more than a single microbial pathway.

Research has demonstrated an association between CVD and elevated plasma concentrations of TMAO [114]. Gut microbiota play an obligatory role in TMAO generation from dietary egg yolk phosphatidylcholine in mice [121]. Furthermore, gut microbial production of TMAO from dietary phosphatidylcholine is associated with an increased risk of incident major adverse cardiovascular events in humans [121, 122]. An untargeted metabolomic survey of small molecule profiles in plasma identified three analytes that were highly inter-related and correlated to major adverse cardiovascular events—choline, betaine, and TMAO [121]. More recently, the prognostic value of plasma choline and betaine in the development of cardiovascular disease in humans has been shown to largely be due to high TMAO [123].

L-carnitine has been identified as an alternative dietary source of gut microbiota-dependent TMAO production in mice [124]. Omnivores produce more TMAO than vegans or vegetarians. Differences in TMAO production between omnivores and vegans highlight the dietary influence of gut microbiota on metabolism.

Strategies to minimize adverse consequences associated with TMAO generation are emerging, at both the microbiome and host levels. There is a need to better appreciate (and potentially monitor) off-target consequences of long-term metabolic interventions. With regard to PMD, the MMS recommended that the use of L-carnitine should only be administered when there is a documented carnitine deficiency, and carnitine concentrations should be monitored during therapy [80].

7.5. Session discussion points

Workshop participants discussed the evolving use of L-carnitine in patients with PMD. L-carnitine is not as benign as previously thought and many physicians are removing it from their treatment regimens. However, when needed, such as in patients with primary carnitine

deficiency and secondary carnitine deficiencies resulting from IEM, the benefits far outweigh any theoretical risk related to TMAO production.

7.6. Patient – product safety: a case study

Genetic urea-cycle disorders give rise to deficiencies in an endogenously-produced amino acid, L-citrulline, resulting in the abnormal plasma accumulation of ammonia, which, left untreated, can cause brain damage, coma, and death. In 2014, the FDA received two serious adverse event reports that had occurred in two patients with severe urea-cycle disorders. Both had been prescribed L-citrulline. The first was a newborn whose plasma citrulline levels were undetectable, even after dose increases. A second patient also experienced very low plasma citrulline levels following the prescribed treatment.

Investigation uncovered that both patients had been receiving compounded L-citrulline from the same ingredient lot [5]. Sample analyses from the affected lot failed to detect L-citrulline. Using several different analytical methods, the FDA identified the presence of N-acetyl-leucine, but no L-citrulline, as labeled. This finding resulted in a product recall due to the product being mislabeled and misbranded [125].

Since the prescribed product was intended to *treat* or *prevent* a medical condition, it was considered a “drug,” and the FDA Safety Alert for the suspect L-citrulline was titled a “drug recall.” Because patients experienced serious adverse events, the product recall was designated as Class I (i.e., a dangerous or defective product that predictably could cause serious problems or death) [126].

The prescribing physician apparently assumed that the compounding pharmacy would use a “drug-quality” ingredient. However, compounded drugs are not manufactured or monitored in the same way as marketed drugs. Moreover the majority of compounding pharmacies are not subject to FDA oversight. Although FDA regulates virtually all commercial pharmaceutical manufacturing and ancillary establishments involved in drug manufacturing for the United States, compounding pharmacies are under state regulation.

In its compounding of the drug, the pharmacy utilized an ingredient from a vendor that had purchased and then repackaged the L-citrulline from its original manufacturer [127]. However, neither the pharmacy nor the vendor confirmed the ingredient’s identity against its incoming label. FDA cited this omission in its Warning Letter to the intermediary vendor following the Agency’s “for cause” inspection [128].

Suppliers and manufacturers of ingredients destined for drugs or dietary supplements are responsible for conformance to drug or dietary supplement cGMP – federally mandated regulations. A commercial manufacturer must retest each ingredient lot to reconfirm the supplier’s incoming documentation, which was not done in this case.

L-citrulline is sold directly to consumers as dietary supplements. Dietary ingredient suppliers should be vetted by the dietary supplement manufacturers that they sell to. Physicians can legally prescribe L-citrulline compounded for their patients, but unless the ingredient supplier sells to a commercial manufacturer of finished drugs, the supplier is not subject to FDA’s direct oversight [126]. Finally, FDA does not review compounded drugs

for safety or efficacy, nor are they required to show identity, purity, stability or bioavailability. It is, therefore, risky to take or to prescribe a “nondrug” to treat or prevent a medical condition. Physicians need to become more aware that the compounding of a nondrug ingredient is not the same as the compounding of an active pharmaceutical ingredient. Administration of an “unapproved new drug” to a patient is optimally achieved under a single-patient or emergency investigational new drug application filed with the FDA.

8. Research opportunities and resources

This session provided highlights from a jointly-sponsored NIH-UMDF workshop on translational research in PMD (Danuta Krotoski, Ph.D., NICHD), the development of common data elements for mitochondrial disorders (Joanne Odenkirchen, M.P.H., NINDS), and a discussion of the EPI-743 trial as an outcome measures example (Lynne Wolfe, M.S., National Human Genome Research Institute [NHGRI]). The session was moderated by Dr. Krotoski.

8.1. NIH-UMDF Workshop on Translational Research in Primary Mitochondrial Diseases: Obstacles and Opportunities – recommendations and progress

The Translational Research in Primary Mitochondrial Diseases: Obstacles and Opportunities Workshop was held March 18–19, 2012 [20] and included leaders in mitochondrial research and clinical care, NIH and FDA staff, and industry and patient representatives. The workshop’s goals were to: (1) identify the obstacles, needs, and priorities of PMD research; (2) translate basic science discoveries into better diagnostic and therapeutic measures for patients; and (3) facilitate future collaborations.

Workshop recommendations currently guide the development of NIH-supported activities to further a better understanding of the pathophysiology of mitochondrial diseases and to develop effective interventions [129].

Outcomes include the following:

- Stable, long-term patient registries have been established through NAMDC and the UMDF. NAMDC’s patient registry contains over 910 confirmed patients with PMD serving as the basis for a longitudinal study of these disorders. In addition, the UMDF launched the Mitochondrial Disease Community Registry in 2014 that contains patient-populated information from over 1,500 patients, caregivers, and family members (as of 5/2/16). A biosample repository has been established through collaboration between The Mayo Clinic Mitochondrial Disease Biobank and NAMDC. Natural history studies to better understand the course of these individually rare diseases have been established in North America, by NAMDC for mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), Alpers-Huttenlocher Disease, Pearson Syndrome, and pyruvate dehydrogenase complex deficiency. Two other natural history studies for MELAS and Leigh Syndrome are being supported through NIH and industry, respectively. In Europe, the Wellcome Trust Centre for

Mitochondrial Research, U.K. and Radboud University, the Netherlands, also established natural history studies of patients with PMD. Moreover, Newcastle has been conducting deep phenotyping of patients with PMD and collecting natural history data for the past two decades; data collection that has now been standardized since the development of the NMDAS, in 2006.

- A genomic data resource and centralized knowledge repository for mitochondrial disorders, the Mitochondrial Disease Sequence Data Repository (MSeqDR–<https://mseqdr.org/>) [130], has been established. This resource is online and accepting data [130–132].
- Additional resources are being developed to support clinical studies in response to the workshop’s recommendations. These include CDEs for PMD, new clinical trial methodologies for rare and clinically complex diseases, and identification of outcome measures for clinical trials for PMD.

8.2. Development of common data elements for mitochondrial disorders

The NINDS CDE Project [133] is a collaborative NIH, non-profit, and international effort to identify common definitions and standardize case report forms (CRFs) and other instruments for specific diseases. The CDE Project is designed to help investigators conduct clinical research through the development of uniform formats by which clinical data can be systematically collected, analyzed, and shared across the research community. The overall impacts of the CDE Project include reducing the time and cost to develop data collection tools, reducing study start-up time, promoting data collection in a consistent format, improving data quality, and facilitating comparisons between studies and meta-analyses.

The NINDS CDE website includes a page for mitochondrial disease [82]. After an initial planning period from December 2013 through March 2014, nine Mitochondrial CDE Working Groups were formed and began their work in April 2014. Once their work was finalized, there was a 6-week public review period and final revisions were then made by the Working Groups. The recommendations were posted on the NINDS website in February 2015. The Mitochondrial CDE Working Groups developed end products including a data dictionary of elements, template CRFs, recommendations for use with existing standardized instruments and measurements (with any copyright restrictions noted), instructions and guidelines for use, and a summary of recommendations; these will be published in a peer-reviewed journal.

Recognizing the highly heterogeneous nature of these disorders, the Working Groups did not recommend a core group of CDEs or common CRFs that would be required for all mitochondrial disease studies. However, the recommended CDEs provide an important framework to compare common elements across diverse mitochondrial disorders. It is hoped that with the use of the mitochondrial CDEs in clinical research over the next 3–5 years, additional information will be obtained that will enable informed revision and updating of the CDEs. However, the currently agreed upon CDE recommendations will be maintained

until clinical data or newly validated measures suggest revisions. An external oversight committee will be formed to make recommendations to NINDS on such changes.

Moving forward, future NINDS-funded clinical trials, including PMD, will use CDEs or will be CDE-compatible. All types of clinical research can use any relevant part of the CDEs, and it is hoped that this work will accelerate clinical research progress. The NINDS encourages use of the CDEs by investigators worldwide who are conducting clinical research studies. CDEs will further increase efficiency by harmonizing data collection across the research community. NINDS CDEs can be accessed online at www.commondataelements.ninds.nih.gov.

8.3. Outcome measures example: a new approach – the EPI-743 Trial

Rare disease research limitations include small numbers of patients who are geographically dispersed, a lack of well-understood natural history data for many rare diseases, incomplete understanding of mechanisms of disease, variability of presentations, and unpredictable progression of disease [134, 135]. In rare disease study design, the main goals are to minimize the sample size requirements (e.g., through the inclusion of pre-symptomatic patients, lengthening the study duration to capture more events, etc.) and maximize the proportion of patients receiving treatment (e.g., through randomized crossover designs) [134, 135].

The intramural NHGRI Mitoworks EPI-743 study design is a double-blind, placebo-controlled, crossover trial involving 20 children with a 2-month washout period between treatment arms. “Standard of care” mitochondrial disease dietary supplement therapies are not stopped or modified throughout the trial. Main inclusion criteria include: (1) ages 2–11 years, (2) presentation consistent with a defect in energy metabolism or oxidation/reduction effect (there is no requirement for molecular or enzymology confirmation), and (3) cultured fibroblasts must exhibit susceptibility to oxidative stress and demonstrate EPI-743 rescue *in vitro* by showing improved viability under conditions of oxidative stress. As of June 2016, 17 patients (11 males, 6 females) have enrolled.

The primary outcome measures for all EPI-743 study subjects are the Newcastle Paediatric Mitochondrial Disease Scale, including the parent and patient self-report sections and reduced glutathione levels. Secondary outcome measures are patient-specific, consider patient and parent input, and are identified by mining already-available data from each subject’s medical records. Examples of current secondary outcomes include glutathione levels, chronic anemia/pancytopenia, chronic elevation of liver enzymes, seizure frequency, neuropsychiatric evaluations, progression of optic atrophy or retinitis pigmentosa, stabilization or improvement of peripheral neuropathy, and number of hospitalizations and/or length of hospital stays. The study is ongoing so summary information is not available at this time.

9. Perspectives from selected government, patient advocacy, industry, foundations panel discussion

This panel discussion featured comments from representatives of NIH (Dr. Gwinn, NINDS); the United Kingdom (Gráinne Gorman, M.D., Newcastle University); a patient advocacy group (Dr. Yeske, UMDF), industry (Matt Klein, M.D., M.S., Edison Pharmaceuticals); and a foundation (Stephen Hersh, M.D., The J. Willard and Alice S. Marriott Foundation).

9.1. U.S. federal government

This workshop has drawn attention to exciting leads that could inform the field regarding therapeutics in general and the use of dietary supplements in particular. It is a significant jump from laboratory-based studies into the clinic, and it is frustrating for patients that there are only a few clinical trials moving forward with therapeutics. However, it is likely more appropriate to have solid pre-clinical trials that generate rigorous data and move into a clinical trial at a later date rather than spend millions of dollars prematurely on a clinical trial that very likely would fail [136]. The NINDS work on CDEs harmonizes with NAMDC efforts to develop research diagnostic criteria for mitochondrial disorders. Both of these initiatives are critically important to enable progress in the field.

9.2. United Kingdom

Structured and effective integration of medical care is crucial to the successful treatment of those with PMD. The structured care pathways developed in the U.K. have been advantageous for the care of individuals with PMD. It is possible that certain dietary supplement combinations may work in subgroups of patients with certain forms of PMD, but it is not likely that a single supplement combination will be effective for all patients. There is a need to fully examine the evidence base in terms of what is happening in the laboratory and translate it effectively into clinical practice and standardized care. Only then will governments and insurance companies be persuaded to cover these therapies. Common challenges face the mitochondrial medicine field in the U.S., the U.K., and elsewhere. In the U.K., partnerships with patient advocacy groups have proven extremely valuable in helping patients with mitochondrial disorders be seen by the appropriate medical care teams, and promote enrollment in trials and registries for PMD.

9.3. Patient advocacy

Patient advocacy groups play an important role in helping steward the overall therapeutic development process by bringing together experts and resources, thereby facilitating the development of treatments and cures. From the patients' perspective, there is an understanding that there is still a long road ahead, but there is also hope. The use of dietary supplements and medical foods is currently a very important part of the symptomatic management of mitochondrial disorders. It is especially important to fully understand the safety and efficacy of dietary supplements and medical foods used in the care of mitochondrial patients to better inform decisions made by patients and clinicians about the best use of these management modalities. No single group will be able to address all of the issues related to mitochondrial disorders. This highlights the need for collaboration and

synergy among patient advocacy groups, healthcare providers, regulatory agencies, industry, and funding bodies. Patient advocacy groups can play a key role in facilitating these collaborations, and public-private partnerships are needed to adequately fund research on mitochondrial disorders moving forward.

9.4. Industry

The need for rigorous evaluation of dietary supplements is clear, not necessarily for FDA approval, but to ensure that patients are receiving safe and effective therapies. There is a strong need for well-controlled trials and studies of patients with mitochondrial diseases who are taking dietary supplements. Fundamental principles of these studies would include specific inclusion criteria, standardized dosing and duration measurements, and meaningful, clinically relevant endpoints based on the natural history of the disease. Well thought-out inclusion criteria must be carefully constructed and are critical given the heterogeneity of patients with PMD. The elements of dose and duration of treatment require a rational, safe, standardized regimen with defined start and stop times so that the association between therapy and an outcome is clear. Rigorous standardization of the doses of a dietary supplement being administered is necessary in order to determine its effect. Trials need to have a number of relevant, objectively measured endpoints in order to determine whether or not an effect was achieved. Biomarkers that correlate with clinical response or disease activity would be beneficial.

9.5. Foundation

The field of mitochondrial medicine will need to focus more on how science is communicated and information is exchanged. In terms of patient-reported outcomes, it was suggested that workshop participants review the Massachusetts General Hospital's Center for Assessment Technology and Continuous Health program [137], which uses personal communication devices as a way of gathering real-time information on patient health. Novel approaches such as this should be considered as programs are designed. The traditional models of collaboration need to be revised. It was also noted that international colleagues have much to offer in terms of lessons learned and opportunities for potential collaboration.

10. Developing a research agenda

After the sessions and panel discussions, workshop attendees participated in a discussion (facilitated by Melissa Parisi, M.D., Ph.D., NICHD, NIH) to develop an outline to inform a research agenda for PMD in general, and more specifically, for the use of nutritional interventions in PMD. Workshop participants agreed that one short-range goal for the field of mitochondrial medicine is for every patient with a PMD to be included in a natural history study and in a registry. An appropriate future goal is that every patient with a PMD will have the opportunity to be enrolled in a clinical trial (preferably a placebo-controlled randomized clinical trial) until proven treatments are identified, with a recognition that quality of life issues are important. The following seven key areas were identified as important issues to incorporate into a research agenda:

10.1. Defining the disease

The issue of defining PMD was a recurrent theme. The challenges of defining subgroups largely relate to overlapping organ system involvement and extreme heterogeneity in these conditions. Short-term actions to address these issues and challenges include:

- Using the results of the NAMDC diagnostic evaluation, and related efforts in the U.K., to inform future work on diagnostic classification strategies
- Developing strategies for the sub-classification of mitochondrial disorders, for example, by mechanism or organ system involvement, or by broader categories such as disorders of OXPHOS versus those involving genes encoding the mitochondrial proteome.
- Establishing a committee or similar body to review and share its diagnostic analysis of select challenging cases.
- Reviewing and refining the definitions on a regular basis.
- Considering opportunities for genomic nosology for PMD [138].
- Forming a working group to focus on issues related to biochemical laboratory testing standards for diagnosis of PMD.

The group agreed on the long term need for flexible diagnostic definitions, and recognized the importance of implementing personalized medicine in this area.

10.2. Biomarkers, outcome measures, and endpoints

A key question facing researchers in the field of PMD is: What biomarkers for monitoring disease exist and are feasible and clinically useful? It would be extremely beneficial for researchers conducting clinical trials to bank patient samples. This activity will allow biospecimens to be mined in the future for quantitative biomarkers of potential clinical utility. Moving forward, a good stress response paradigm for mitochondrial disease is needed, particularly in terms of intermediary metabolism. One short-term action item identified in this area was to respond to the public comment period for the NINDS CDE Project for mitochondrial disease. A long-term action item identified was to develop *in vivo* measures of metabolic response to stress.

10.3. Mechanistic approaches and preclinical studies

Efforts that include the use of approaches such as comprehensive transcriptome, proteome, metabolome, etc., may represent the next wave of understanding of these conditions at multiple physiologic levels. The value of evaluating parameter ratios was emphasized, especially since absolute levels of individual metabolites are not *a priori* broadly useful in PMD. Animal models also provide significant power in identifying potential targets for therapies. Over the last 5 years, there has been an explosion in the number of accurate small animal and mouse models for PMD. It would be extremely valuable to examine the effects of the current agents used in dietary supplement combinations for mitochondrial disease in these models to help inform which genetic subtypes respond to certain therapies. The need for preclinical rigor was also emphasized. Short-term actions identified in this area include:

(1) evaluating animal models for response to mitochondrial dietary supplements, and (2) the development of a consortium (similar to NCI's Mouse Models Consortium; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2650192/>) to allow comparison of data across laboratories.

10.4. Clinical trial design

Examples of well-designed clinical trials in the mitochondrial field were discussed throughout the workshop, but these types of trials may not always be feasible, particularly for patients already on dietary supplement combinations. Workshop participants emphasized the need to evaluate any intervention critically with regard to the inclusion criteria and stratification of subjects, dose and duration of treatment, and study endpoints. The value of crossover designs, which allow patients to serve as their own control, was noted, as was the need for creative strategies to address studies with very small enrollments such as individualized “n=1” or small cohort trials [139].

Identified short-term actions in this area include:

- Developing a prioritization scheme of interventions for this population with limited numbers of patients.
- Reaching agreement on therapeutic priorities.
- Holding annual meetings to prioritize therapies for PMD.
- Developing a central repository of comparative data related to molecules and dietary supplements used to treat PMD, possibly by expanding functionality of the existing MSeqDR resource, and developing a standard template to collect data.
- Using centralized IRBs to reduce administrative burden that can delay clinical trials.

Resources to support clinical trials

- Patient registries: UMDF (Mitochondrial Disease Community Registry), NAMDC, RDCRN, Newcastle, and others
- Mitochondrial genomic data resource and community knowledge repository: MSeqDR
- RDCRN: NAMDC natural history studies, pilot studies, DMCC, NAMDC Statistical Analysis Center,
- NeuroNEXT
- Muscular Dystrophy Association (MDA)
- Biobanks: NAMDC, Mayo Clinic, others
- CDEs and CRFs
- Department of Defense Peer Reviewed Medical Research Program
- FDA: Orphan Products Grants Program

- NIH Institutes and Centers
- NCATS: Therapeutics for Rare and Neglected Diseases services for drug development [140]; Clinical & Translational Science Awards and the Clinical & Translational Science Centers
- Industry

10.5. Challenges of nutritional interventions for PMD

As previously noted, there is a lack of consistency in what is, and what should be included in combination supplements and there is also a lack of evidence regarding the effectiveness of dietary supplements used in PMD. Furthermore, while dietary supplements are used in the management of PMD, they are not regulated as drugs and are not required to conform to the same premarket evaluation process as drugs. Translating cell-based assays of dietary supplements for use in patients is challenging, and there is limited understanding of the benefits and risks associated with long-term dietary supplement use. Issues related to access to dietary supplements include insurance coverage and the high costs to patients and families. The pharmaceutical industry needs to be sufficiently incentivized to develop the evidence base required for regulation of an existing ingredient as a drug.

Identified short-term action items in this area include:

- Clinicians treating patients with PMD who are taking dietary supplements can encourage them to make sure they choose products that have undergone a verification program to demonstrate that the content is as listed on the dietary supplement facts label.
- Developing a “toolkit” for patients, providers, and hospitals to provide helpful information about use of dietary supplements in mitochondrial diseases and disseminate these through professional organizations and patient advocacy groups.
- Exploring additional opportunities for partnership with foundations.
- Encouraging all patients to be part of a registry.
- Promoting collaborations between NIH intramural and extramural investigators through the NIH Bench-to-Bedside Program.
- Utilizing NIH’s Therapeutics for Rare and Neglected Diseases services for drug development [140].
- Meeting with FDA to discuss how to move a dietary supplement into the drug regulatory process and to seek help with clinical trial design for testing and comparing supplements.

An identified long-term action item was the development of a central pharmacy source of dietary supplements and/or external verification of existing supplements, for research and clinical use.

10.6. Standards of clinical care for patients with PMD

A number of important clinical care issues were identified that have not been rigorously studied. It was generally agreed that the baseline physiologic status of patients with PMD is poorly understood. Such an understanding is critical to optimize their nutritional status and inform the use of nutritional interventions. Some clinicians are using the ketogenic diet, yet little is known about how it should be incorporated for those with refractory epilepsy, and there are questions regarding its safety for patients with mitochondrial disease in general. The development of clinical guidelines for patients with mitochondrial disorders would provide great benefit; professional organizations such as the ACMG, SIMD, and GMDI might be able to provide assistance in this regard. A comprehensive guideline for all mitochondrial diseases is not feasible; however, it may be possible to develop guidelines in certain areas of focus, for example, for patients with a particular genotype, class of disease, or common phenotype.

Short-term action items related to developing standards of clinical care for patients with PMD include:

- Identifying and agreeing upon distinct mitochondrial disease subgroup(s) or phenotype(s) for development of a practice care guideline.
- Consider existing clinical care guidelines, for example, such as those developed by the Newcastle group to inform the development of guidelines in the U.S.

10.7. Collaboration issues

Patients with PMD are scattered throughout our national and international communities. Therefore, the development of effective nutritional, dietary supplement, and other interventions require more focused and intense collaborations than has been the norm until now. Ideally at least some of these collaborations will be international. Partners in this endeavor include basic and clinical researchers, clinicians, foundations and patient advocacy groups, international partners, regulatory bodies, government agencies, patients and families, and industry. Clear agreement on goals, targets, timelines, and formats for active, continuous communication must be included in the framework of all future collaborations.

11. Conclusion

Dietary supplements are commonly used management modalities for PMD, even though little evidence of safety or efficacy from well-designed clinical trials exists. Patient safety, health care payer reimbursement, and the ability to standardize patient care are compromised by this gap in evidence. The mitochondrial disease clinical, patient advocacy, and research communities are committed to finding safe and effective treatments for PMD through scientific discovery and the development of evidence-based clinical trials. Understanding the molecular basis of mitochondrial dysfunction and the mechanisms of action of dietary supplements through pre-clinical studies will inform the conduct of these clinical trials. The voices of patients and their families, in concert with clinicians will be paramount to selecting clinical endpoints and interventions to study. New technologies, the development of patient

registries, biobanks, and data repositories, for example, will organize and expand knowledge and ultimately lead to new therapeutic approaches. It will be through collaboration and partnerships, such as those fostered during the workshop, that evidence-based therapies for PMD will emerge and inform management strategies for mitochondrial dysfunction more generally.

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Abbreviations

ACMG	American College of Medical Genetics and Genomics
ATP	adenosine triphosphate
CDE	common data elements
cGMP	current good manufacturing practices
CNS	central nervous system
CoQ₁₀	coenzyme Q ₁₀
CPEO	chronic progressive external ophthalmoplegia
DSHEA	Dietary Supplement Health and Education Act of 1994
FDA	U.S. Food and Drug Administration
FFDCA	Federal Food, Drug, and Cosmetic Act
GMDI	Genetic Metabolic Dietitians International
FSDU	Food for Special Dietary Use
IEM	inborn errors of metabolism
IMP	International Mito Patients organization
IRB	Institutional Review Board
MELAS	mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes
MitoGlobal	Mitochondrial Global Network
MMS	Mitochondrial Medical Society
MRC	Medical Research Council
MSeqDR	Mitochondrial Disease Sequence Data Repository
mtDNA	mitochondrial DNA

NAD⁺	nicotinic acid dinucleotide
NAMDC	North American Mitochondrial Disease Consortium
nDNA	nuclear DNA
NCATS	National Center for Advancing Translational Sciences
NHGRI	National Human Genome Research Institute
NICHD	<i>Eunice Kennedy Shriver</i> National Institute of Child Health and Human Development
NIH	National Institutes of Health
NINDS	National Institute of Neurological Disorders and Stroke
NMDAS	Newcastle Mitochondrial Disease Adult Scale
ODA	Orphan Drug Act of 1983
ODS	Office of Dietary Supplements
ORDR	Office of Rare Diseases Research
OXPHOS	oxidation-phosphorylation
PMD	primary mitochondrial disorders
RC	respiratory chain
RCTs	randomized controlled trials
RDCRN	Rare Diseases Clinical Research Network
SIMD	Society for Inherited Metabolic Disorders
TMAO	trimethylamine N-oxide
UMDF	United Mitochondrial Diseases Foundation

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Highlights

- NIH convened a workshop to address the evidence base for nutritional interventions in primary mitochondrial disease (PMD).
- Dietary supplements are commonly used as management modalities for PMD despite limited evidence of safety and efficacy.
- PMD are rare and clinically, phenotypically, and genetically heterogeneous, challenging the ability to conduct clinical trials.
- Resources are being developed to support clinical trials, including patient registries, biorepositories, and common data elements for mitochondrial disease.
- Future research is needed to define the diseases; identify biomarkers, outcome measures, and endpoints.
- Collaborations among clinical researchers, advocacy and professional organizations, federal entities, and industry are needed to address research challenges in PMD.

Advantages and disadvantages of select issues relevant to consideration of dietary supplement use in primary mitochondrial diseases

Table 1

Issue	Advantages	Disadvantages
Dietary supplements are readily available in the market place	<ul style="list-style-type: none"> • No prescription needed • Multiple forms and dosages available • Does not require health care professional involvement 	<ul style="list-style-type: none"> • Costly with poor insurance reimbursement • May not have health care professional involvement to monitor adverse effects • May limit patient pool for research (e.g., patients may already be on a study intervention)
Demonstration of product safety and integrity differs from that of approved drugs	<ul style="list-style-type: none"> • Dietary supplement manufacturers are required to adhere to GMP * • Independent programs exist to verify bottle contents 	<ul style="list-style-type: none"> • Limited requirement for pre-market review of dietary supplements • FDA must inspect thousands of manufacturers • Independent programs are voluntary and expensive
Efficacy	<ul style="list-style-type: none"> • No need to conduct expensive clinical trials to prove efficacy 	<ul style="list-style-type: none"> • No pre-market evaluation of efficacy, therefore, dietary supplements may be ineffective and/or harmful • Patients may be spending money needlessly • Little incentive for insurance companies to reimburse
Dosage is often greater than Dietary Recommended Intakes (where established) **	<ul style="list-style-type: none"> • May have pharmacological effects 	<ul style="list-style-type: none"> • Effects of high doses not well studied • Interactions with drugs unknown
Multiple and multi-ingredient products often taken together	<ul style="list-style-type: none"> • Target multiple mitochondrial dysfunctions • May have synergistic effects 	<ul style="list-style-type: none"> • May not be clear which, if any, are helpful • Interactions or potentiation among multiple ingredients
Custom compounded specialty products	<ul style="list-style-type: none"> • Target multiple mitochondrial dysfunctions • May have synergistic effects • Custom tailored to an individual patient 	<ul style="list-style-type: none"> • May not be clear which, if any, are helpful • Interactions or potentiation among multiple ingredients • Stability of ingredients • Contamination and other manufacturing issues

* GMP = Good manufacturing practices (see: <http://www.fda.gov/Food/GuidanceRegulation/CGMP/ucm079496.htm>)

** Dietary Recommended Intakes have been established for a number of nutrients and provide reference values on the nutrient needs of healthy people. (see: <http://fnic.nal.usda.gov/dietary-guidance/dietary-reference-intakes>)