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Disorders of mitochondrial dynamics in peripheral neuropathy: Clues from hereditary neuropathy and diabetes

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Abstract

Peripheral neuropathy is a common and debilitating complication of diabetes and prediabetes. Recent clinical studies have identified an association between the development of neuropathy and dyslipidemia in prediabetic and diabetic patients. Despite the prevalence of this complication, studies identifying molecular mechanisms that underlie neuropathy progression in prediabetes or diabetes are limited. However, dysfunctional mitochondrial pathways in hereditary neuropathy provide feasible molecular targets for assessing mitochondrial dysfunction in neuropathy associated with prediabetes or diabetes. Recent studies suggest that elevated levels of dietary saturated fatty acid (SFAs) associated with dyslipidemia impair mitochondrial dynamics in sensory neurons by inducing mitochondrial depolarization, compromising mitochondrial bioenergetics, and impairing axonal mitochondrial transport. This causes lower neuronal ATP and apoptosis. Conversely, monounsaturated fatty acids (MUFAs) restore nerve function and sensory mitochondrial function. Understanding the mitochondrial pathways that contribute to neuropathy progression in prediabetes and diabetes may provide therapeutic targets for the treatment of this debilitating complication.

Keywords

Diabetes; Prediabetes; Hereditary Neuropathy; Charcot-Marie-Tooth Disease; Mitochondria; Bioenergetics; Fusion; Fission; Mitochondrial associated membranes; Mitochondrial Trafficking

1 Introduction

The global burden of diabetes is a pressing health concern. Over 450 million people had diabetes in 2017, and it is estimated that numbers will surpass 700 million people by 2040. This is in addition to the staggering number of people with prediabetes, a condition characterized by impaired glucose tolerance that typically precedes overt type 2 diabetes (T2D); in the United States alone, roughly 34% of adults had prediabetes in 2015. Both diabetic and prediabetic individuals are prone to an array of complications,

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the most prevalent of which is neuropathy. Approximately 60% of individuals with T2D and 30% of individuals with prediabetes manifest with distal symmetric polyneuropathy (Callaghan, Price, & Feldman, 2015; Cortez, Singleton, & Smith, 2014; O'Brien, Hinder, Callaghan, & Feldman, 2017; Tabak, Herder, Rathmann, Brunner, & Kivimaki, 2012) and exhibit the typical “stocking and glove” pattern of progressive peripheral sensory nerve loss characterized by pain, numbness, eventual loss of sensation, and increased risk for amputation, all of which lead to significant morbidity and socioeconomic burden. In 2017, the estimated costs of diabetes totaled \$327 billion USD, with \$90 billion attributed to reduced productivity. This is further compounded by the lack of treatment options for neuropathy associated with diabetes and prediabetes, as the majority of management rests on lifestyle changes (Pop-Busui et al., 2017). Consequently, current research efforts are focused on identifying the biological pathways that underlie the onset and progression of neuropathy with the goal to develop novel therapeutic strategies and improve diagnostic approaches (O'Brien et al., 2017; O'Brien, Hinder, Sakowski, & Feldman, 2014; Vas & Edmonds, 2016; Zochodne, 2014).

Diabetic and prediabetic neuropathy are secondary to a series of metabolic perturbations that link hyperglycemia, dyslipidemia, and insulin resistance with inflammation, oxidative stress, and changes in gene expression. One unifying theme underlying these perturbations is mitochondrial dysfunction, the focus of this chapter. Neurons are reliant on a continuous supply of energy and are especially susceptible to mitochondrial impairment, and conditions that impede normal mitochondrial dynamics and function can lead to axonal dysfunction, sensory neuron injury and death, and ultimately to neuropathy. Here we build on these findings. In particular, we discuss relevant insights pertaining to mitochondrial dynamics and function during normal and diseased states, with particular emphasis on mitochondrial bioenergetics, mitochondrial morphology (including fission and fusion), endoplasmic reticulum (ER)-mitochondria interactions, and mitochondrial trafficking. We then concentrate on the most recent advances in our understanding of how metabolic imbalances common in diabetes and prediabetes, specifically hyperglycemia and dyslipidemia, affect mitochondrial dynamics in the peripheral nervous system (PNS).

2 Mitochondrial dynamics

2.1 Neurons rely on mitochondrial dynamics

Neurons, including sensory PNS neurons, are especially dependent on efficient mitochondrial dynamics to fulfill their energy needs due to constraints from their unique morphology (Pareyson, Saveri, Sagnelli, & Piscosquito, 2015). PNS neuron axons can measure 3 feet in length or longer and transmit signals from the extremities to the spinal cord, and their axon length versus cell body size ratio can be in excess of 20,000 (Feldman, Nave, Jensen, & Bennett, 2017; Wang, Medress, & Barres, 2012). Therefore, they need to distribute mitochondria over large distances from their cell body along axons to their terminal(s). Furthermore, neurons require massive amounts of energy to sustain their activity; neuronal survival requires a constant adenosine triphosphate (ATP) supply (Nicholls & Budd, 2000) and a single cortical neuron utilizes approximately 4.7 billion ATPs per second in the resting human brain (X. H. Zhu et al., 2012). The reliance of neurons on ATP

for survival is highlighted by their mitochondrial density, which is greater in comparison to other cell types (Yu & Pekkurnaz, 2018). Additionally, neurons' have energy demands for diverse tasks, and among the most consuming in brain neurons are housekeeping (~25% of energy), maintenance of resting membrane potential (~15%), firing of action potentials (~16%), and synaptic transmission (~44%) (Harris, Jolivet, & Attwell, 2012). Thus, their energy needs also are highly compartmentalized and neurons need to be able to direct mitochondria to the areas of highest energy consumption, such as at their synapses (Rossi & Pekkurnaz, 2019).

The constant need for ATP to survive means that neurons, including PNS neurons, critically rely on strictly controlled and coordinated mitochondrial dynamics to ensure sustained ATP production throughout their axons and synaptic terminal(s) (Sajic, 2014). Mitochondrial dynamics comprises several aspects of mitochondria, including fission/fusion (Section 2.3), mitophagy, and transport (i.e., trafficking, Section 2.5). Mitochondrial elongation, reshaping, and/or fusion is enhanced under conditions of nutrient starvation to increase the efficiency of ATP production and continue to meet the neuron's energy demands (Yu & Pekkurnaz, 2018). Conversely, mitochondrial fission intensifies in response to increased nutrient supply and severe stress. For quality control, damaged mitochondria are cleared by mitophagy under conditions of elevated oxidative stress to enable replacement by properly functioning mitochondria. Overall, the relative extent of mitochondrial fission to fusion controls the range of mitochondrial size and morphology, which is also coordinated with mitophagy to optimize mitochondrial distribution and quality for optimal ATP production on which neurons rely.

In addition to regulation of mitochondrial turnover, neurons must ensure distribution of mitochondria from the cell body throughout the axon to guarantee they are delivered to sites in need of ATP (Pareyson et al., 2015). Thus, mitochondrial transport mechanisms must match ATP requirements within neuronal axons, otherwise loss of mitochondria results in insufficient ATP, impaired synaptic transmission, and axonal injury. Within the myelinated nerve, electrical activity regulates mitochondrial transport, suggesting that the axon's demand for energy regulates mitochondrial trafficking (Ohno et al., 2011). The essential nature of proper mitochondrial trafficking in neurons is highlighted by loss or mutation to critical mitochondrial fission/fusion, and motor proteins, which leads to neurological pathologies, such as Charcot-Marie-Tooth disease (CMT, Section 3) and motor neuron diseases, the most common of which is amyotrophic lateral sclerosis (ALS) (Baloh, Schmidt, Pestronk, & Milbrandt, 2007; De Vos et al., 2007; De Vos & Hafezparast, 2017). In a similar vein, depletion of ATP, such as through metabolically-triggered mitochondrial dysfunction associated with diabetes and prediabetes (e.g., hyperglycemia and dyslipidemia, Sections 4 and 5) could have pathological consequences, leading to neuronal cell injury and neuropathy.

2.2 Mitochondrial bioenergetics

Mitochondria are the source of ATP production, the cell's energy currency (Fig. 1). ATP is generated through a series of biochemical reactions, starting with the tricarboxylic acid cycle (TCA) (Krebs & Johnson, 1937) followed by oxidative phosphorylation (OXPHOS)

(Chaban, Boekema, & Dudkina, 2014). The TCA cycle is fueled by acetyl-coenzyme A (acetyl-CoA), which can be synthesized from glycolysis-derived pyruvate or from fatty acid (FA) β -oxidation (Houten & Wanders, 2010). The TCA cycle generates reduced nicotinamide adenine dinucleotide (NADH) and reduced flavin adenine dinucleotide (FADH₂), which drive OXPHOS and, ultimately, ATP production. Glycolytic metabolism within the cytoplasm rapidly releases 2 ATP molecules from catabolism of a single glucose molecule whereas oxygen-dependent energy conversion within mitochondria liberates, under ideal conditions, 36 additional ATP molecules by OXPHOS. The utilization of glycolysis versus OXPHOS during neuronal signal transmission is not precisely known, but evidence suggests that glycolysis serves as a readily available ATP source during periods of excessive energy demand, but that mitochondrial oxidative OXPHOS, which is more efficient, occurs during both basal conditions and periods of high neuronal activity (Rossi & Pekkurnaz, 2019).

Mitochondria are composed of an outer mitochondrial membrane (OMM) that surrounds an inner mitochondrial membrane (IMM), which folds inwardly forming cristae that expand the IMM surface area to maximize energy production. Within the cristae is the mitochondrial matrix, which is comprised of TCA components and other metabolites, lipids, ribosomes, transfer RNAs, and mitochondrial DNA, enzymes, and proteins, whereas OXPHOS enzymes reside within the IMM. The TCA cycle utilizes acetyl-CoA, which is broken down through a series of enzymatically catalyzed biochemical reactions to CO₂, NADH, FADH₂, guanosine triphosphate (GTP), and protons. Next, NADH and FADH₂ serve as cofactors to complexes I and II, respectively, which are entry points into the OXPHOS electron transport chain (Cecchini, 2003; Hirst, 2005), and which are followed by complexes III and IV (Crofts, 2004; Tsukihara et al., 1996). Electrons move down the potential energy gradient along complexes of the electron transport chain, aided by shuttle proteins, which results in a net transport of protons from the matrix to the intermembrane space (IMS) (Chaban et al., 2014). This transfer of protons across the IMM generates a proton gradient that is dissipated through complex V (ATP synthase), which produces ATP as protons are transported from the IMS to the matrix (Boyer, 1997) (Fig. 1). The proton gradient also establishes the mitochondrial membrane potential (MMP), which is necessary for mitochondrial function, regulates the direction of mitochondrial transport (Miller & Sheetz, 2004), and prevents mitochondrial damage (Sivitz & Yorek, 2010).

In addition to the TCA cycle and OXPHOS, pyruvate produced by cytoplasmic glycolysis is shuttled into the mitochondrial matrix where a three-enzyme pyruvate dehydrogenase complex (PDC) converts it into acetyl-CoA through pyruvate decarboxylation (Patel, Nemeria, Furey, & Jordan, 2014). FA acyl-L-carnitine derivatives are also shuttled into mitochondria and catabolized by β -oxidation (Houten & Wanders, 2010; Kastaniotis et al., 2017). For even-chain FAs, a repeated 4-step cycle cleaves two carbon atoms at a time from the FA, which is converted into acetyl-CoA. For odd-chain FAs, the final cleavage yields propionyl-CoA in addition to acetyl-CoA.

Careful coordination of mitochondrial biochemical processes ensures efficient ATP generation. However, unanticipated side reactions or exogenous species can lead to suboptimal energy production or mitochondrial dysfunction, such as through the formation

of reaction oxygen species (ROS) or toxic intermediates. ROS can be inadvertently produced at the electron transport chain by premature escape of a semi-reduced oxygen species, such as the superoxide anion (Angelova & Abramov, 2018). The MMP influences ROS production; in some contexts, a low MMP slows respiration, which increases ROS production, however, in certain instances, a high MMP triggers expression of uncoupling proteins that lower ROS but also drop ATP production. Thus, MMP and ROS are intimately linked and together impact mitochondrial efficiency, ATP production, and neuronal health.

Excessive influx of long-chain saturated fatty acids (LCSFAs) into mitochondria, for instance as occurs during dyslipidemia (Section 5), interferes with the normal physiological process of FA β -oxidation. When FA uptake overcomes a mitochondria's capacity for FA β -oxidation, lipotoxic metabolic intermediates are generated, such as ceramides (Fucho, Casals, Serra, & Herrero, 2017), diacylglycerols, or cardiolipin (D'Souza, Nzirorera, & Kienesberger, 2016; DeFronzo, 2010), which negatively impact mitochondrial bioenergetics. Proteins involved in mitochondrial fission and fusion also influence mitochondrial size, and thus bioenergetic capacity. Conversely, the metabolic state (i.e., normal physiological or pathological conditions) affects mitochondrial fission and fusion (Section 2.3). Overall, mitochondrial bioenergetics and dynamics coordinate with the neuron's energy demands to sustain ATP production and neuronal health. However, this carefully orchestrated process can be disrupted by aberrant metabolic states, including diabetes or prediabetes, or hereditary mutations, such as in CMT, ultimately leading to neuropathy.

2.3 Mitochondrial morphology, fission, and fusion

Within cells, and especially neurons that are particularly reliant on continuous ATP production, mitochondrial dynamics ensure that cellular energy demands are met. The balance between mitochondrial fission and fusion regulates their number, morphology, and size distribution, whereas trafficking delivers them to the locations they are needed (Fig. 1, Section 2.5). Mitochondria also coordinate with other cellular organelles, such as the ER, for lipid biosynthesis, calcium exchange, and signaling (Section 2.4).

The major players in mitochondrial fission include mitochondrial fission factor (MFF), mitochondrial fission 1 protein (FIS1), mitochondrial elongation factor 1 and 2 (MIEF1 and MIEF2), and ganglioside induced differentiation associated protein 1 (GDAP1) localized to the OMM and dynamin 1 like (DNM1L) in the cytoplasm (Bertholet et al., 2016). The precise mechanism for fission is not known, although an interaction of GDAP1 with DNM1L and FIS1 has been proposed (Niemann, Wagner, Ruegg, & Suter, 2009; Pareyson et al., 2015). DNM1L is recruited to mitochondria by its receptor proteins MFF and FIS1, or alternatively by MIEF1 and MIEF2, that can recruit DNM1L in the absence of MFF and FIS1 (Loson, Song, Chen, & Chan, 2013). DNM1L that is recruited to the OMM forms a ring-shaped oligomer around the mitochondria, which is also wrapped by ER (Friedman et al., 2011). In MIEF1- or MIEF2-mediated fission, the DNM1L ring oligomer dissociates from the MIEF1 or MIEF2 receptors and contracts to trigger fission (Kalia et al., 2018). DNM1L activity is regulated by several post-translational modifications (Chang & Blackstone, 2010), including O-GlcNAcylation. Changes in DNM1L activity can result in mitochondrial fragmentation and a lowering of MMP (Gawlowski et al., 2012). Similar loss

of mitochondrial integrity and polarization can be triggered by hyperglycemia (Makino et al., 2011), such as occurs in diabetes.

Mitochondrial fusion is mediated by mitofusins 1 and 2 (MFN1 and MFN2), which are dynamin-related GTPases localized to the OMM, and optic atrophy 1 (OPA1), a mitochondrial dynamin-like GTPase localized to the IMM. MFN1 and MFN2 from two mitochondria form homo- and heterooligomers, that tether and initiate the fusing process of the OMM. Upon fusion, there is an interaction between MFN1 and OPA1 in the OMM that fuses the IMM (Meeusen et al., 2006; Meeusen, McCaffery, & Nunnari, 2004; Pareyson et al., 2015). The energy for the OMM fusion process derives from MFN1 and MFN2 GTPase activity whereas tethering is mediated by their C-terminal coiled-coil domain (Suen, Norris, & Youle, 2008). OPA1 is also the principle regulator of mitochondrial cristae organization, whose surface area impacts OXPHOS and therefore the efficiency of ATP production (Pernas & Scorrano, 2016). OPA1 deficiency can induce defects in mitochondrial morphology within optic nerves (Davies et al., 2007).

Neurofilaments (NFs) are cytoskeletal framework components that are specific to neurons (Gentil, Tibshirani, & Durham, 2015). They occur as light (NF-L), medium (NF-M), and heavy (NF-H) chains; the NF-H subunits run parallel to the axon, lending mechanical support, whereas the NF-M and NF-L run perpendicularly to the NF-H subunits (Pareyson et al., 2015; Sajic, 2014). NFs also organize organelles, in addition to mitochondria, whose length, rate of fusion, and motility is affected by NFs (Gentil et al., 2012).

Mutations to mitochondrial fission and fusion proteins GDAP1 and MFN2 and the gene encoding NF-L, neurofilament, light polypeptide (NEFL), and mitochondrial transport machinery (Section 2.5) are all associated with hereditary CMT neuropathy (Section 3) (Pareyson et al., 2015). Mutant OPA1 is linked to autosomal dominant optic atrophy, a heritable disease of optic nerve deterioration (Alexander et al., 2000; Delettre et al., 2000), whereas DNMI1 mutations are connected with severe infantile encephalopathy disease. In addition to their more established roles in mitochondrial fission and fusion, mutations to MFN2 (Loiseau et al., 2007; Pich et al., 2005) and OPA1 (Chevrollier et al., 2008; Zanna et al., 2008) also lead to altered mitochondrial bioenergetics, MMP, and coupling defects (Fig. 1).

2.4 ER-mitochondrial interactions regulate mitochondrial dynamics

Mitochondria-associated ER membranes (MAMs) are juxtaposed interorganellar sites (Lopez-Crisosto et al., 2015). The ER and mitochondrial membranes are brought in very close proximity through bridging by various tethering complexes, but the membranes do not actually fuse (Csordas et al., 2006). Therefore, each organelle maintains its own identity, but the MAM contact sites enable mutual regulation of both organelles. MAM contact sites allow interorganellar transfer of calcium and lipids and control diverse cellular activities, ranging from mitochondrial dynamics, autophagy, and inflammation (Marchi, Patergnani, & Pinton, 2014).

Tethering proteins are located in the OMM and ER membranes and maintain MAMs stably or transiently. Four MAM tethering complexes have been identified in mammalian cells.

MFN1 and MFN2 were originally discovered on the surface of mitochondria (Section 2.3), followed by discovery of MFN2 on ER membranes, where it helps form the MAM interface by homologous interaction with mitochondrial MFN2 or heterologous interaction with mitochondrial MFN1 (de Brito & Scorrano, 2008). This process is regulated by the mitochondrial ubiquitin ligase MITOL, which only modifies mitochondrial MFN2 at lysine 63 with a polyubiquitin chain that elicits oligomerization and subsequent tethering (Sugiura et al., 2013).

Another tethering complex is formed from the interaction between ER-localized vesicle associated membrane protein-associated protein B (VAPB) with mitochondria-localized protein tyrosine phosphatase-interacting protein 51 (PTPIP51) (De Vos et al., 2012). Voltage-dependent anion channel 1 (VDAC1) complexes with two other proteins, subtype 3 of the 1,4,5-triphosphate receptor (IP3R3) and 75 kDa glucose regulated protein (GRP75), to form another tethering complex, which regulates calcium exchange between mitochondria and ER (Szabadkai et al., 2006). A complex of ER-localized B-cell receptor associated protein 31 (BAP31), FIS1, and phosphofurin acidic cluster sorting protein-2 (PACS-2) form the tethering structure that regulates apoptosis (Iwasawa, Mahul-Mellier, Datler, Pazarentzos, & Grimm, 2011; Simmen et al., 2005). Lastly, a suite of additional proteins also associates with MAMs, including proteins or enzymes that participate in mitochondrial dynamics, lipid synthesis, and autophagy regulation, as well as ER chaperones.

MAMs perform several key functions to maintain neuronal health. Calcium transport between ER and mitochondria is primarily mediated by the GRP75-IP3R3-VDAC1 tethering complex through interaction with sigma non-opioid intracellular receptor 1 (SIGMAR1), an ER chaperone protein. SIGMAR1 blocks breakdown of IP3R3 to sustain calcium transfer across MAMs, which is essential for ER and mitochondrial function (Hayashi, Rizzuto, Hajnoczky, & Su, 2009). MAMs can also regulate autophagosome formation through the tethering proteins MFN2 (Hamasaki et al., 2013), SIGMAR1 (Vollrath et al., 2014), and VAPB-PTPIP51 (Gomez-Suaga et al., 2017), and mitophagy through PTEN-induced kinase 1 (PINK1) and Beclin-1 (BECN1) (Gelmetti et al., 2017). Importantly for mitochondrial dynamics, MAMs impact mitochondrial morphology, transport, fission, and fusion (Bernard-Marissal, Chrast, & Schneider, 2018). ER and mitochondria mutually affect each other's morphology through MAMs via MFN2. Loss of MFN2 triggers ER expansion and protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK) activation, leading to aberrant mitochondrial morphology and function (Munoz et al., 2013). MAMs influence mitochondrial transport through the interaction of MFN2 with mitochondrial Rho GTPase 1 and 2 (Miro 1 and 2, Section 2.5), receptor proteins that tether mitochondria to motor proteins in a calcium-dependent fashion. MAMs also stimulate mitochondrial fission and fusion via the tethering proteins MFN1 and MFN2, as well as through MAM-associated DNM1L (Friedman et al., 2011) or inverted formin-2 (INF2) (Korobova, Ramabhadran, & Higgs, 2013).

Overall, tethering complexes and other MAM-associated proteins coordinate to regulate calcium, auto- and mitophagy, and mitochondrial transport and dynamics (fission, fusion). Aberrant mitochondrial and ER function is an early event in the course of neuronal degeneration; in parallel, deficiencies in MAM function similarly leads to neuronal injury

(Bernard-Marissal et al., 2018). Mutation to some of these tethering and MAM-associated proteins results in CMT (Section 3.1.3) or to familial forms of ALS (Bernard-Marissal et al., 2018), underscoring the importance of MAMs to healthy neuronal function.

2.5 Mitochondrial trafficking and motor proteins

Mitochondria are transported along neuronal axons by the synchronized effort of numerous proteins involved in the motor machinery. Broadly speaking, the machinery is composed of the molecular motors that achieve step-wise movement along a microtubule track with receptor and adaptor proteins that secure the mitochondria cargo to the motors (Mishra & Chan, 2016). Mitochondria need to be transported along axons in either direction, so anterograde and retrograde transport are both essential. Kinesin-1 [KIF5A, KIF5B, KIF5C (Kawaguchi, 2013)] and dynein motor proteins conduct anterograde and retrograde mitochondrial transport, respectively (Saxton & Hollenbeck, 2012; Sheng, 2014). A canonical kinesin-1 motor is comprised of three principal domains, an ATPase motor domain that takes steps along microtubules, and a heavy and light chain, which join the motor domain to the adaptor proteins and cargo (Hirokawa & Takemura, 2005). The dynein motor is a multi-protein complex, which also contains an ATPase motor domain, in addition to heavy, intermediate, and light chain domains, and other components, including dynactin (DCTN), that join the motor domain to the adaptor proteins and mitochondrial cargo (Roberts, Kon, Knight, Sutoh, & Burgess, 2013; Sajic, 2014).

Securing the mitochondrial cargo to the motors are a number of receptor and adaptor proteins (Mishra & Chan, 2016). The main receptor proteins are Miro1 and Miro2, which are expressed on the outer mitochondrial membrane (Nguyen et al., 2014) and are required for mitochondrial transport (Fransson, Ruusala, & Aspenstrom, 2006). They possess calcium-binding EF-hand motifs (Lee & Lu, 2014), which are able to sense calcium levels, and regulate binding or release of mitochondria from the transport machinery. Trafficking kinesin-binding protein 1 and 2 (TRAK1 and TRAK2, also referred to as Milton) are adaptor proteins that interact with Miro and kinesins to fasten the mitochondria to the motor (Brickley & Stephenson, 2011) and are also essential for mitochondrial trafficking (van Spronsen et al., 2013). Syntaphilin is another protein that anchors mitochondria to microtubules in regions of high local calcium levels (Chen & Sheng, 2013; Kang et al., 2008).

The final component of the axonal transport machinery are the tracks themselves, the microtubules that run along axons. Microtubules form by the polymerization of α -tubulin and β -tubulin dimers (Conde & Caceres, 2009). Polymerization of the dimers instills an inherent polarity to the microtubule tracks, with a minus end on the α -tubulin side, which is usually directed towards the cell body, and a plus end on the β -tubulin side, which is distal and is generally directed towards the axon terminals. The polarity also defines the transport direction since kinesin prefers to move towards the plus end (anterograde) (Hirokawa et al., 1991) and dynein, although bidirectional (Ross, Wallace, Shuman, Goldman, & Holzbaaur, 2006), favors motion towards the negative end (retrograde) (Pilling, Horiuchi, Lively, & Saxton, 2006). Movement in either direction is carefully regulated by a number of mechanisms (Nirschl, Ghiretti, & Holzbaaur, 2016).

The concerted interaction of the mitochondria cargo, receptor and adaptor proteins, motors, and microtubule tracks ensures efficient mitochondrial trafficking. Loss of function mutations to mitochondrial trafficking proteins, such as KIF5A and TUBB3 (β -tubulin subtype) in hereditary CMT neuropathy (Section 3.1.4) (Pareyson et al., 2015). Under normal physiological conditions, a number of environmental cues, such as ATP, calcium, glucose, and oxygen levels, can regulate movement of mitochondria along axons or attachment/detachment of mitochondria to/from the motor tracks (Mishra & Chan, 2016; Saxton & Hollenbeck, 2012). Unsurprisingly, disease conditions, such as hyperglycemia (Section 4.4) or dyslipidemia (Section 5.5) in diabetic patients will disrupt regulatory mechanisms governing mitochondrial trafficking, leading to neuropathy.

3 Mitochondrial dynamics in disease

Understanding dysfunctional mitochondrial pathways associated with genetic neuropathic diseases, including hereditary neuropathies, has incredible potential to offer insight into molecular mechanisms of mitochondrial dysfunction that may be pertinent to neuropathy progression in prediabetes and diabetes. In this section, we will therefore discuss relevant advances in mitochondrial bioenergetics, mitochondrial fusion and fission, ER-mitochondrial interaction sites, and mitochondrial trafficking that have been identified through studies of mitochondrial pathways underlying other hereditary neuropathies (Table 1). These insights have paved the way for the recent progress and emerging research on mitochondrial dysfunction in neuropathy associated with diabetes and prediabetes that will be discussed in subsequent sections.

3.1 CMT, Hereditary Motor Neuropathies and Hereditary Sensory Neuropathies

CMT is a heterogeneous group of inherited neuropathies that affect both sensory and motor neurons and is also known as hereditary motor and sensory neuropathy (HMSN). Classically CMT is divided into a demyelinating form of the disease (CMT1, based on slowed motor nerve conduction velocities) and an axonal form (CMT2, based on normal nerve conduction velocities). In both CMT1 and CMT2 and their multiple respective subtypes, both motor and sensory nerves are affected. Clinical manifestations vary and include distal muscle wasting and weakness with sensory loss that most commonly begins distally in the lower limbs and progresses with age proximally, to include the upper limbs. Over 70 mutations are identified as underlying distinct forms of CMT neuropathies as well as two forms of CMT2, distal hereditary motor neuropathies (dHMN), and hereditary sensory neuropathies (HSN), which predominantly affect motor and sensory nerves, respectively (Pareyson et al., 2015; Rossor, Polke, Houlden, & Reilly, 2013). The nomenclature of the distinct types of CMT neuropathies continues to evolve even today. Historically these neuropathies were classified on a purely clinical presentation and nerve conduction studies; currently CMT neuropathies are classified based on gene mutation(s) coupled with clinical phenotypes (Pisciotta & Shy, 2018; Rossor, Tomaselli, & Reilly, 2016). Regardless, the unifying thread across all inherited mutations is that they adversely impact cellular machinery that impairs neurons and axons (Rossor et al., 2013). These impairments include defects in mitochondrial function, ER homeostasis, axonal transport, and synaptic transmission. Mutations to mitochondrial proteins or mitochondrial motor proteins lead to

altered mitochondrial bioenergetics, fission/fusion, and trafficking that make PNS neurons and axons especially susceptible to injury and degeneration (Table 1).

3.1.1 CMT mutations in genes that regulate mitochondrial bioenergetics

—Altered mitochondrial bioenergetics reduce ATP output (Section 2.2), and since neurons are reliant on a constant ATP source (Section 2.1), this scenario has negative consequences for neuronal health and survival. Mutations to several metabolic mitochondrial enzymes underlie inherited peripheral neuropathies. Apoptosis inducing factor mitochondria associated 1 (AIFM1) is an NADH oxidase encoded in the nucleus that is transported to the mitochondria (Rinaldi et al., 2012). Mutant *AIFM1* is associated with recessive X-linked CMTX4 otherwise known as Cowchock syndrome [Online Mendelian Inheritance in Man (OMIM)# 310490], which manifests with axonal neuropathy, deafness, and cognitive impairment (Rinaldi et al., 2012). Other peripheral neuropathy disorders have also been linked with mutant *AIFM1*, a rare severe, early-onset mitochondrial encephalopathy combined with OXPHOS deficiency (COXPD6, OMIM# 300816) (Ghezzi et al., 2010) and a rare infantile motor neuron disease (Diodato et al., 2016).

Mutation to another metabolic mitochondrial protein, dehydrogenase E1 and transketolase domain containing 1 (*DHTKDI*), is associated with CMT2Q (OMIM# 615025), which is similar to classical CMT2 (W. Y. Xu et al., 2012). *DHTKDI* encodes the E1 subunit of the mitochondrial amino acid degrading alpha-ketoadipic acid dehydrogenase complex (Hagen et al., 2015). Knockdown of *DHTKDI* *in vitro* decreased ATP production and mitochondrial biogenesis while increasing ROS generation, leading to a greater extent of cellular apoptosis and lower proliferation (W. Xu et al., 2013). Hexokinase 1 (HK1) phosphorylates glucose to glucose-6-phosphate (G6P), the initiation in most glucose metabolic pathways. A fraction of HK1 is bound to the OMM, where it couples cytoplasmic glycolysis to OXPHOS by interacting with VDAC and the IMM-bound adenine nucleotide translocator (ANT) (Robey & Hay, 2006). Mutant *HK1* is linked with CMT4G (OMIM# 605285) and also a severe early-onset CMT1, which is autosomal recessively inherited (Gabrikova et al., 2013).

Mitochondrial encoded ATP synthase 6 (*MT-ATP6*) is a mitochondrial DNA gene coding for the ATP6 subunit of mitochondrial ATP synthase of complex V in OXPHOS. Mutations in *MT-ATP6* leads to a form of CMT2 (Pitceathly et al., 2012) or other neuropathies, such as NARP syndrome (neurogenic muscle weakness, ataxia, and retinitis pigmentosa) (Thorburn, Rahman, & Rahman, 1993). The NARP *MT-ATP6* mutation results in lower ATP production despite proper complex assembly and normal protein expression levels (Rak et al., 2007). Another metabolic mitochondrial enzyme, pyruvate dehydrogenase kinase 3 (PDK3), is involved in peripheral neuropathy. PDK3 phosphorylates PDC, the multi-enzyme complex that generates acetyl-CoA from pyruvate coupling glycolysis to the TCA cycle (Gudi, Bowker-Kinley, Kedishvili, Zhao, & Popov, 1995). Phosphorylation of PDC thoroughly inactivates its enzymatic activity, shutting down pyruvate-derived acetyl-CoA entry into the TCA cycle. Mutant *PDK3* is associated with dominant X-linked inheritance CMTX6 (OMIM# 300905) (Kennerson et al., 2016; Kennerson et al., 2013).

3.1.2 CMT mutations in genes that regulate mitochondrial morphology, fission, and fusion—Mitochondria reshape, divide, and fuse in response to a cell's

energy needs (Section 2.3), and interference with this carefully regulated process is inevitably detrimental to cellular health. Mutation to mitochondrial fission and fusion proteins are well established causes of CMT. The most prevalent mutation in CMT patients in a mitochondrial protein is *MFN2*. Mutations in *MFN2* results in CMT2A (OMIM# 609260) (Pareyson et al., 2015). Mutant *MFN2* is also associated with autosomal recessive CMT2A (OMIM# 617087) and with autosomal dominant CMT5, a rare category characterized by pyramidal tract involvement (D. Zhu et al., 2005), and CMT6 (HSMN6, OMIM# 601152), which is accompanied by optic atrophy (Zuchner et al., 2006). Other complex phenotypes also arise more akin to mitochondrial encephalopathies (Boaretto et al., 2010). The majority of *MFN2*-linked CMT patients display a severe phenotype with early onset sensory and motor neuropathy and rapid progression although a fraction exhibit less debilitating disease. Aside from neuropathy, patients harboring mutant *MFN2* may also suffer from tremors, proximal weakness, respiratory involvement, optic atrophy, hearing loss, vocal cord palsy, and spotty white matter brain abnormalities (Pareyson et al., 2015).

Around 100 mutant *MFN2* variants have been identified, mostly as missense mutations in essential protein domains (e.g., GTPase domain, coil-coiled motifs), yet the precise mechanism that leads from mutant *MFN2* to CMT2 pathology and neuropathy is not precisely known, nor why it manifests itself in a cell-specific manner. Certain mutant *MFN2* variants negatively impact mitochondrial fusion (A. L. Misko, Sasaki, Tuck, Milbrandt, & Baloh, 2012), yet others have no effect and complementation with wild-type *MFN1* can rescue fusion defects (Detmer & Chan, 2007). Axonal transport may similarly be compromised by mutant *MFN2* (A. L. Misko et al., 2012), possibly through an interaction with Miro (A. Misko, Jiang, Wegorzewska, Milbrandt, & Baloh, 2010), as well as through loss of calcium homeostasis or ROS production.

Mutant *GDAP1* is another major contributor to CMT (Cassereau, Chevrollier, Gueguen, et al., 2011), possibly the second most common type of autosomal recessive CMT and perhaps approximately 5% of all CMT2 cases (Pareyson et al., 2015). Mutations to *GDAP1* give rise to CMT subsets, either as an autosomal recessive, demyelinating CMT4A (OMIM# 214400), which is characterized by early onset, possibly with vocal cord and diaphragmatic paralysis (OMIM# 607706) (Baxter et al., 2002; Claramunt et al., 2005), or an autosomal recessive, axonal, early onset, severe CMT2K (OMIM# 607831) or autosomal dominant, late onset, less severe CMT2K (OMIM# 607831) (Cassereau, Chevrollier, Bonneau, et al., 2011).

Examination of sural nerve biopsies from patients diagnosed with dominant CMT2K confirm mitochondrial defects, characterized by aggregates of normal and abnormal mitochondria (Sivera et al., 2010). Similar mitochondrial abnormalities are a common feature in nerve biopsies from both dominant and recessive CMT2K patients (Fu et al., 2017). Dominant and recessive *GDAP1* mutations may adversely impact mitochondrial dynamics (Niemann et al., 2009). Overexpression of recessive mutant *GDAP1* in HeLa cells reduced mitochondrial fission whereas dominant mutant *GDAP1* inhibited fusion and increased ROS production and susceptibility to apoptosis. In CMT2K patient-derived fibroblasts, dominant mutant *GDAP1* induced a 40% decrease in complex I turnover and a 33 and 20% decrease in tubular mitochondria size and mitochondrial mass, respectively,

suggesting dominant mutant *GDAP1* may impact mitochondrial bioenergetics as well as morphology (Cassereau et al., 2009). In addition to altered bioenergetics, another facet of research related to mutant *GDAP1* in CMT4A and CMT2K is dysregulation of calcium dynamics (Gonzalez-Sanchez et al., 2017; Gonzalez-Sanchez, Satrustegui, Palau, & Del Arco, 2019). *GDAP1* possesses putative glutathione S-transferase (GST) binding sites and a possible role against oxidative stress has also been proposed for wild-type *GDAP1* (Noack et al., 2012).

3.1.3 CMT mutations in genes that regulate MAMs—MAMs are crucial to healthy mitochondrial function (Section 2.4), and mutations to several proteins essential for maintaining MAMs are associated with CMT (Bernard-Marissal et al., 2018). *MFN2* forges MAM tethering complexes in addition to its role in mitochondrial fusion. Although most reports have focused on its impact on mitochondrial fusion in CMT2A, a recent study examined MAM dynamics in fibroblasts derived from 3 CMT2 patients harboring mutant *MFN2* (Larrea et al., 2019). Mitochondria-ER contact distance and ER-mitochondria contact coefficient (a measure of ER-mitochondrial distance, length of contact, and mitochondrial perimeter), MAM-specific phospholipid synthesis, and capacitative calcium entry (extracellular calcium influx upon intracellular depletion) were impacted to varying extents by mutant *MFN2* depending on the disease phenotype severity. On the other hand, respiratory chain function was relatively unaffected, leading the authors to conclude that mutant *MFN2* predominantly affects MAMs but not mitochondrial bioenergetics.

SIGMAR1 is an ER chaperone protein that maintains calcium exchange between ER and mitochondria (Hayashi et al., 2009), and mutations to this gene have been linked to autosomal recessively inherited dHMN (Gregianin et al., 2016; Li et al., 2015). *In vitro* functional studies in human neuroblastoma cell lines transfected with mutant *SIGMAR1* exhibited defects in *SIGMAR1* localization, which did not accumulate at MAMs, but instead formed aggregates (Gregianin et al., 2016). Mutant *SIGMAR1* also decreased basal proliferation and survival under stress and altered cellular calcium dynamics. Autophagy was induced to a greater extent in mutant *SIGMAR1* cells, but the autophagosomes were not mobilized for clearing the *SIGMAR1* aggregates. Another study similarly found that expression of mutant *SIGMAR1* led to ER stress and elevated apoptosis (Li et al., 2015).

Solute carrier family 25 member 46 (*SLC25A46*) is a transmembrane protein located in the OMM that mediates lipid transfer between ER and mitochondria (Palmieri, 2013). It maintains mitochondrial lipid homeostasis and cristae; loss of function *SLC25A46* mutations inhibit mitochondrial respiration and trigger mitochondrial hyperfusion (Janer et al., 2016). Mutations to *SLC25A46* are linked to CMT2 and optic atrophy (Abrams et al., 2015) and to Leigh syndrome (Janer et al., 2016). Regulation of autophagy is another important MAM function; autophagosomes occur at MAMs in mammalian cells (Hamasaki et al., 2013). Autophagy related 14 (*ATG14*) and *ATG5* localize to MAMs under starvation conditions, a process that is blocked in cells with disrupted MAMs through *MFN2* knockdown. The tethering complex *VAPB-PTPIP51* also regulates autophagy at MAMs (Gomez-Suaga et al., 2017); *VAPB* or *PTPIP51* overexpression tighten MAMs, whereas knockdown loosens them, initiating autophagosome formation. The impact of *VAPB-PTPIP51* on autophagy is specifically dependent on their tethering function and involves

their role in calcium transport from ER to mitochondria. The role of autophagy, including autophagy at MAMs, is increasingly appreciated in hereditary peripheral neuropathies (Haidar & Timmerman, 2017). Although no mutation to either VAPB or PTPN12 has been linked to CMT, mutations to VAPB have been identified in patients with motor neuron diseases, including ALS (type 8, OMIM# 608627) and spinal muscular atrophy (OMIM# 182980) (Nishimura et al., 2004).

3.1.4 CMT mutations in genes that regulate mitochondrial trafficking—

Interference with the mitochondrial transport machinery (Section 2.5) jeopardizes a neuron's capacity to deliver mitochondria to areas of high energy demand. The occurrence of heritable neuropathies with mutations to key mitochondrial transport proteins highlights the essential nature of mitochondrial trafficking to neuronal health. Mutations to the key anterograde mitochondrial transport motor *KIF5A* causes hereditary spastic paraplegia 10 (SPG10, OMIM# 604187), an autosomal dominant disorder characterized by progressive gait disorder and spasticity accompanied by degeneration of corticospinal tracts and peripheral nerves (Reid et al., 2002). The disease phenotype can vary and can present as a forme-fruste of axonal CMT2 (Crimella et al., 2012). In a similar vein, mutation to kinesin-3 isoform B (*KIF1B*), a monomeric motor for anterograde mitochondrial transport (Nangaku et al., 1994), has been identified in a single family with CMT2A1 (OMIM# 118210) (C. Zhao et al., 2001). Another isoform, *KIF1A*, is a neuron-specific kinesin, which transports vesicles rather than mitochondria. Mutant *KIF1A* also leads to rare, recessively inherited neuropathies, HSN2C (OMIM# 614213) (Riviere et al., 2011) or SPG30 (OMIM# 610357) (Erlich et al., 2011).

Mutations to the retrograde dynein motor and its accessory proteins also cause heritable neuropathies. Dynein cytoplasmic 1 heavy chain 1 (*DYNC1H1*) is a dynein subunit; mutant *DYNC1H1* is linked with dominant CMT2O (OMIM# 614228) (Weedon et al., 2011) as well as dominant SMALED (spinal muscular atrophy, lower extremity predominant, OMIM# 158600), a disorder of cortical migration defects, intellectual disability, and lower-limb predominant abnormalities (Harms et al., 2012). In mice, heterozygous mutant *DYNC1H1* results in progressive motor neuron degeneration (Hafezparast et al., 2003) and deficient retrograde transport (J. Zhao et al., 2016). Fibroblasts derived from heterozygous mutant *DYNC1H1* mice display aberrant mitochondrial morphology, similar to SMALED patient-derived fibroblasts, and progressive mitochondrial dysfunction (Eschbach et al., 2013).

In addition to mutations to the motor proteins, mutations to the tracts along which mitochondria are transported can similarly lead to neuropathy. Class III β -tubulin (β 3-tubulin or β -tubulin III), is a microtubule isotype of the tubulin family, which is mostly specific to neurons and is encoded by the *TUBB3* gene (Sullivan & Cleveland, 1986). Mutant *TUBB3* results in congenital fibrosis of the extraocular muscles type 3 (CFEOM3, OMIM# 600638), an axonal sensorimotor polyneuropathy with loss of eye movement (Hong et al., 2015; Tischfield et al., 2010). *In vivo* in a knock-in mouse model, mutant *TUBB3* triggers deficiencies in axon guidance but not in cortical cell migration (Tischfield et al., 2010). *In vitro*, it interferes with tubulin heterodimerization, but mutant heterodimers that fold retain the ability to polymerize. In yeast, certain mutant *TUBB3* variants disrupt

microtubule dynamics while others perturb the microtubule-kinesin interaction. Dynamin 2 (DNM2) is a dynamin-like GTPase also linked to CMT; however, the involvement of this microtubule associated protein to mitochondrial dynamics has not been fully determined (Pareyson et al., 2015).

Mutation to cytoskeletal proteins have also been linked with mitochondrial disorders in inherited neuropathies. Mutant *NEFL* occurs in a generally autosomal dominant CMT1F (OMIM# 607734) or CMT2E (OMIM# 607684), with patients frequently suffering from early onset and severe disease, and whose sural nerve biopsies show accumulated mitochondria (Fabrizi et al., 2007). Indeed, NFs impact mitochondrial dynamics; mutant *NEFL* results in attenuated mitochondrial fusion and increased movement in primary mouse dorsal root ganglion (DRG) neurons (Gentil et al., 2012). Also, in mouse DRG neurons, expression of mutant *NEFL* resulted in rounder and shorter mitochondria preceding disruption of the neurofilament network, suggesting mitochondrial dysfunction contributes to CMT2E pathogenesis (Tradewell, Durham, Mushynski, & Gentil, 2009). Aberrant mitochondrial trafficking was also seen in motor neurons differentiated from induced pluripotent stem cells obtained from CMT2E patient fibroblasts (Saporta et al., 2015).

3.2 Other hereditary neuropathies

Mutant *OPA1* is linked to autosomal dominant optic atrophy (OMIM# 165500), which is primarily characterized by optic nerve deterioration leading to progressive vision loss (Alexander et al., 2000; Delettre et al., 2000). Around 20% of patients additionally exhibit peripheral neuropathy and are classified as autosomal dominant optic atrophy plus (OMIM# 125250) (Burte, Carelli, Chinnery, & Yu-Wai-Man, 2015). Optic atrophy 3 (OPA3) is a protein that localizes to the OMM, that regulates lipid metabolism and thermogenesis (Wells et al., 2012), and triggers mitochondrial fission (Ryu, Jeong, Choi, Karbowski, & Choi, 2010). Autosomal dominant mutant *OPA3* leads to optic atrophy and premature cataracts (OMIM# 165300) (Reynier et al., 2004), whereas autosomal recessive mutant variants are associated with 3-methylglutaconic aciduria type III (alternatively called Costeff syndrome, OMIM# 258501) (Anikster, Kleta, Shaag, Gahl, & Elpeleg, 2001). *OPA3* overexpression in cells markedly increases mitochondrial fragmentation while knockdown results in mitochondrial elongation, demonstrating an impact of *OPA3* on mitochondrial dynamics (Ryu et al., 2010). Mutant *DNM1L* results in severe infantile encephalopathy (OMIM# 614388) (Waterham et al., 2007) by altering *DNM1L* recruitment and oligomerization and hence mitochondrial fission (Chang et al., 2010; Fahrner, Liu, Perry, Klein, & Chan, 2016). Familial ALS patients harbor mutations to mitochondrial trafficking proteins that trigger neuropathy, including coiled-coil-helix-coiled-coil-helix domain containing 10 gene (*CHCHD10*), *SIGMAR1*, *VDAC1*, and *VAPB* (Bannwarth et al., 2014; Bernard-Marissal et al., 2018). A broad spectrum of additional rare neuropathies with mitochondrial defects have been documented but are outside the scope of this review. The reader is directed to other excellent reviews for further reading (Cassereau, Codron, & Funalot, 2014; Pareyson, Piscoquito, Moroni, Salsano, & Zeviani, 2013).

3.3 Neuropathy associated with diabetes and prediabetes

Hereditary neuropathies that exhibit mitochondrial dysfunction have provided important considerations for evaluating mitochondrial dynamics in neuropathy associated with diabetes and prediabetes. The genetic mutations to OXPHOS complexes, fission and fusion proteins, MAM proteins, and mitochondrial trafficking motor proteins in hereditary neuropathies suggest that similar pathways may also be altered in neuropathy associated with diabetes and prediabetes. Of critical importance to these studies, however, is the recent data from a Cochrane review of clinical trials with diabetic patients exhibiting neuropathy as an outcome measure. The results showed that maintaining tight glycemic control improved neuropathy outcomes in type 1 diabetes (T1D) patients but had limited efficacy in slowing the progression of neuropathy in T2D patients (Callaghan, Little, Feldman, & Hughes, 2012). Instead, a follow-up study reported that neuropathy development in T2D patients correlates with components of metabolic syndrome, including obesity and dyslipidemia (Callaghan & Feldman, 2014; Callaghan, Xia, Banerjee, et al., 2016; Lupachyk, Watcho, Hasanova, Julius, & Obrosova, 2012). Prediabetic patients with metabolic syndrome also develop neuropathy and the progression of nerve damage and sensory loss is identical to their T2D counterparts (Callaghan, Xia, Reynolds, et al., 2016). Therefore, there may be differential disease mechanisms related to hyperglycemia and dyslipidemia underlying the development of neuropathy in T1D and T2D patients, respectively (Callaghan, Hur, & Feldman, 2012). Hence, while dysfunctional mitochondrial pathways are only beginning to be addressed in the context of diabetes and prediabetes, Section 4 will highlight the effect of hyperglycemia on mitochondrial mechanisms underlying neuropathy development, and Section 5 will focus on the impact of dyslipidemia on mitochondrial function and dynamics associated with the development of neuropathy.

4. Regulation of mitochondrial dynamics by glucose

4.1 Hyperglycemia compromises DRG neuron bioenergetics

Elevated levels of glucose associated with hyperglycemia are processed by glycolysis and the TCA cycle followed by OXPHOS (Feldman et al., 2017). Excess glucose levels overload the glycolytic and TCA cycle resulting in a high proton gradient across the IMM. OXPHOS becomes compromised in the high proton gradient resulting in a decrease in ATP generation and increase in ROS production by an overloaded electron transport system.

Hyperglycemia significantly impairs mitochondrial bioenergetics in cultured DRG neurons. Elevated levels of glucose associated with hyperglycemia leads to excessive flux through the glycolytic pathway. Treatment of DRG neurons with 100 mM glucose decreases mitochondrial respiration and reduces ATP turnover (Rumora et al., 2018). Lower ATP production resulting from less efficient mitochondrial respiration suggests that DRG neuron bioenergetics are compromised by hyperglycemia. In combination with these altered bioenergetics, an increase in mitochondrial DNA indicates that mitochondrial biogenesis is occurring in DRG neurons, most likely to compensate for the increased metabolic load.

In parallel, DRG neurons from TD1 mice and rats show compromised maximal oxygen consumption rate and diminished spare respiratory capacity (S. K. Chowdhury et al., 2010;

S. R. Chowdhury et al., 2014). This decrease in mitochondrial respiratory function is accompanied by a decrease in mitochondrial electron transport chain proteins complexes I and IV and the TCA cycle protein citrate synthase (S. K. Chowdhury et al., 2010). Interestingly, mitochondrial respiration can be recovered using insulin (Aghanoori et al., 2017; S. K. Chowdhury et al., 2010), neurotrophin-3 (Huang, Sayers, Verkhatsky, & Fernyhough, 2005), or ciliary neurotrophic factor (S. R. Chowdhury et al., 2014), which correlates with improved hind paw thermal sensitivity.

4.2 Hyperglycemia induces aberrant mitochondrial fission in DRG axons

As discussed above, DRG neurons respond to oxidative stress through mitochondrial biogenesis allowing DRG neurons to increase mitochondrial mass by the division of existing mitochondria (Vincent et al., 2010). Increased mitochondrial mass is thought to improve metabolic capacity and minimize mitochondrial damage. However, excessive levels of glucose lead to proapoptotic fission of the mitochondria. Proapoptotic fission is facilitated by DNMI1 which splits the mitochondria and recruits proapoptotic protein BCL2 associated X (BAX) (Leininger, Backus, et al., 2006). This recruitment is thought to prevent mitochondrial fusion and permeabilize the mitochondrial membrane for apoptosis (Leininger, Edwards, Lipshaw, & Feldman, 2006). Knockdown of fission protein DNMI1 in glucose-treated DRG neurons results in a decrease in mitochondrial fission and an improvement in neuronal health. Collectively, these data suggest that apoptotic fission may play a key role in glucose-induced injury in DRG neurons (Russell et al., 2002; Vincent, McLean, Backus, & Feldman, 2005).

4.3 Potential for hyperglycemia to regulate ER-mitochondrial interactions DRG neurons

Although glucose-regulated MAMs have not been reported in DRG neurons or diabetic nerve damage, MAM interactions are essential for intracellular metabolic pathways and are likely to play a key role in the progression of neuropathy, as documented for the hereditary neuropathies (Section 3.1.3). Numerous reports link hyperglycemia to MAM formation in the regulation of mitochondrial function. Elevated concentrations of glucose trigger the formation of fragmented mitochondria and a decrease in MAM interactions in liver, skeletal muscle, adipose tissue, and beta cells (Rieusset et al., 2016; Theurey et al., 2016). Further research is needed in this area to understand the role of MAMs in diabetic and prediabetic neuropathy.

4.4 Hyperglycemia has no effect on mitochondrial trafficking in DRG axons

Despite the reduction in mitochondrial respiration and ATP production in glucose-treated DRG neurons, glucose has no effect on mitochondrial motility in the axons of DRG neurons cultured from adult mice (Rumora et al., 2018). Additionally, the directionality and velocity of mitochondrial transport is not altered by glucose treatment. These results suggest that axonal mitochondrial transport is maintained despite impaired mitochondrial bioenergetics. Interestingly, mitochondrial transport is altered in DRG neurons treated with dyslipidemic concentrations of LCSFAs (Section 5.5) indicating that mitochondrial transport is differentially affected by hyperglycemia and dyslipidemia.

In contrast to DRG neurons, glucose metabolism in hippocampal neurons of the central nervous system (CNS) plays a crucial role in regulating axonal mitochondrial transport through post-translational modification of the motor protein Milton. Rat hippocampal neurons treated with physiological levels of glucose display GlcNAcylation of Milton by *O*-GlcNAc transferase resulting in a significant reduction in axonal mitochondrial transport (Pekkurnaz, Trinidad, Wang, Kong, & Schwarz, 2014). This glucose-related post-translational regulation of axonal mitochondrial transport is specific to mitochondria in hippocampal neurons. These results suggest that DRG neurons may possess unique regulatory mechanisms for mitochondrial dynamics and mitochondrial transport that differ from neurons of the central nervous system. Moreover, the effect of hyperglycemia and dyslipidemia on molecular mechanisms are distinct in DRG neurons.

5. Regulation of mitochondrial dynamics by dyslipidemia

5.1 Dyslipidemia and neuropathy

Since dyslipidemia plays a central role in the pathogenesis of neuropathy in patients with prediabetes and T2D (Callaghan, Little, et al., 2012), current studies are focused on understanding the lipid-related disease mechanisms that contribute to neuropathy development. To identify pathogenic molecular mechanisms underlying the development of neuropathy, murine models of prediabetes and T2D have been used to model dysfunctional mitochondrial dynamics. Common models of T2D include leptin and leptin receptor knockout animals, the *ob/ob* and *db/db* murine models respectively, characterized by hyperglycemia, dyslipidemia, and polyneuropathy. Prediabetes is also modeled in mice, using a lard-based high fat diet (HFD) chow. HFD-fed mice develop prediabetes characterized by obesity, dyslipidemia, and neuropathy similar to their human counterparts (O'Brien, Sakowski, & Feldman, 2014).

The fatty acid composition of murine diets has recently emerged as a regulator of nerve function in prediabetic and diabetic murine models. Recent studies by the Yorek laboratory demonstrate that polyunsaturated fatty acids (PUFAs) reduce diabetes- and prediabetes-induced neuropathy. Mice fed a HFD combined with streptozotocin treatment develop T2D and neuropathy. Feeding these mice menhaden oil, an oil composed of omega-3 PUFAs, alone (Shevalye et al, 2015) or in combination with α -lipoic acid, an antioxidant, and enalapril, an angiotensin-converting enzyme inhibitor (Yorek et al., 2017), improves neuropathy phenotypes compared to untreated animals. In a rat model of T2D, menhaden oil similarly slowed nerve loss and neuropathy progression (Coppey, Davidson, Shevalye, Torres, & Yorek, 2018). Cumulatively, the results suggest that LCSFAs are pathogenic in the development of neuropathy whereas PUFAs can prevent neuropathy in prediabetic or diabetic mice.

In line with these studies, monounsaturated fatty acids (MUFAs) also restore nerve function in a murine model of prediabetes (Rumora, LoGrasso, Hayes, et al., 2019) (Fig. 2). Mice fed a LCSFA-rich HFD develop glucose intolerance, increased body weight and fat mass and exhibit deficits in both sensory and motor nerve conduction velocity. A separate group of mice fed the LCSFA-rich HFD followed by 8 weeks of a MUFA-rich HFD (HFD-MUFA) exhibited similar metabolic dysfunction but had a complete restoration in sensory and motor

nerve conduction velocities. HFD-MUFA-fed mice also exhibited a significant increase in intraepidermal nerve fiber density in footpads compared to animals who remained on the LCSFA-rich HFD. These results suggest that unsaturated fatty acids have a beneficial effect on nerve function and are likely to improve mitochondrial dynamics to repair peripheral nerve function.

5.2 Dyslipidemia and mitochondrial bioenergetics

Our laboratory has recently reported that treating *db/db* T2D mice with pioglitazone, an agonist of the peroxisome proliferator-activated receptor gamma (PPAR- γ), improves diabetic neuropathy. Transcriptomic analyses of sciatic nerves from treated animals reveal changes in expression patterns of genes related to OXPHOS, FA β -oxidation, and the TCA cycle, suggesting that improving bioenergetic pathways correlates with an improvement in nerve function (Hinder et al., 2017). Additionally, impairment of mitochondrial bioenergetics in the sciatic nerves of *db/db* mice and DRG neurons of HFD-fed mice indicate that mitochondrial energy production is compromised under dyslipidemic conditions (Sas et al., 2016; Sas et al., 2018).

To support these findings, an *in vitro* analysis of mitochondrial bioenergetics in DRG neurons revealed that diabetic levels of LCSFA palmitate caused an elevation in resting ATP generation and basal respiration (Rumora et al., 2018) (Fig. 3B). These changes in resting state bioenergetics were accompanied by a significant proton leak. Diabetic levels of LCSFA palmitate caused significant impairment of OXPHOS. These changes correlated with palmitate-induced axonal mitochondrial depolarization (Rumora et al., 2018) and of intracellular loss of ATP level (Rumora, LoGrasso, Haidar, et al., 2019).

Lower bioenergetic energy production reduces axonal ATP levels in the nerve and affects essential ATP-driven cellular mechanisms, such as axonal mitochondrial transport (Rumora et al., 2018). The loss of functional mitochondria in the axon is likely to exacerbate the reduction in axonal ATP leading to complete bioenergetic failure (De Vos & Hafezparast, 2017). Therefore, research efforts are being directed toward understanding how certain diet-derived fatty acids are responsible for dysfunction in DRG neuron bioenergetics and these fatty acids effect neuronal and axonal mitochondrial trafficking and mitochondrial function.

5.3 Fusion and fission dynamics in dyslipidemia

Since mitochondrial bioenergetics are dependent on maintaining a balance of mitochondrial dynamics, fission and fusion of mitochondria are likely to play a key role in the pathogenesis of neuropathy. Although few studies have focused on fission and fusion in the dyslipidemic nerve, lipids are emerging as regulators of mitochondrial morphology and size. Complex lipids that are upregulated in dyslipidemia such as cardiolipin, phosphatidic acid, diacylglycerol, and phosphatidylethanolamine can regulate mitochondrial morphology (Frohman, 2015; Ha & Frohman, 2014). Cardiolipin is a negatively charged lipid found within the IMM that interacts with fusion and fission proteins. It is required for mitochondrial fusion mediated by dynamin-related GTPase MGM1 (yeast; mitochondrial dynamin like GTPase OPA1 in humans), a protein localized on the IMM (DeVay

et al., 2009). Cardiolipin may also interact with α -synuclein to induce mitochondrial fission (Nakamura et al., 2011), and also with pro-apoptotic factors, such as BAX and BH3 interacting domain death agonist (BID), which it recruits to mitochondria (Lucken-Ardjomande, Montessuit, & Martinou, 2008; Lutter et al., 2000).

Phosphatidylethanolamine is another phospholipid present within the OMM and IMM that controls membrane curvature and mitochondrial fusion. Yeast cells that lack phosphatidylserine decarboxylase 1, the enzyme that synthesizes phosphatidylethanolamine, exhibit fragmented mitochondria (Chan & McQuibban, 2012). Phosphatidic acid, which can be derived from cardiolipin, is also a negatively charged lipid that induces curvature and induces mitochondrial aggregation and fusion through DNM1L (Adachi et al., 2016; Ha & Frohman, 2014). Conversely, diacylglycerol induces mitochondrial fission, but the precise mechanism is not known (Ha & Frohman, 2014). Dyslipidemia-induced alterations to phospholipid synthesis could potentially alter mitochondrial size and morphology by changing the mitochondrial membrane lipid composition. Indeed, *in vitro* in mouse DRG neurons, palmitate and stearate increased mitochondrial circularity and shifted their size distribution towards larger mitochondria (Rumora, LoGrasso, Haidar, et al., 2019).

5.4 ER-mitochondrial interactions in dyslipidemia

Although the role of MAMs in prediabetic and diabetic neuropathy is unknown (Section 4.3), MAMs are upregulated in response to metabolic overload associated with prediabetes and T2D (Arruda et al., 2014; Lowell & Shulman, 2005). MAMs have been identified in murine models of obesity and are essential for regulating lipid synthesis pathways and maintaining mitochondrial function. MAMs are a major site for lipid biosynthesis and lipid exchange between the ER and mitochondria. The two major lipids synthesized at the MAM site are phospholipids, phosphatidylcholine and phosphatidylethanolamine (Rowland & Voeltz, 2012). Additionally, MAM formation can play a role in ceramide transfer from the ER to the mitochondria to initiate apoptotic cell death. MAMs are also major regulatory sites for regulating calcium-driven cell death. Under cellular homeostasis, calcium transfer from the ER to the mitochondria preserves normal bioenergetics (Cardenas et al., 2010). An increase of calcium flux from the ER to the mitochondria at the MAM site can trigger apoptosis (Section 2.4). Therefore, future studies on the involvement of MAMs in dyslipidemia and neuropathy may provide a novel therapeutic target for neuropathy.

5.5 The effect of dyslipidemia on axonal mitochondrial trafficking

A number of recent experiments have specifically investigated the differential impact of LCSFAs and MUFAs on mitochondrial function and trafficking in sensory neurons. Treatment of primary mouse DRG neurons with the LCSFA palmitate (C16:0) (Fig. 4D) substantially lowered the number and velocity of motile mitochondria and increased mitochondrial depolarization (Fig. 3A,B) (Rumora et al., 2018). Similar outcomes were reported upon incubation of primary mouse DRG neurons with the LCSFA stearate (C18:0) (Fig. 4E), which markedly reduced the fraction of motile mitochondria and velocity of mitochondrial trafficking (Rumora, LoGrasso, Haidar, et al., 2019). Conversely, the shorter LCSFA myristate (C14:0) (Fig. 4C) and the medium chain saturated fatty acid (MCSFA) laurate (C12:0) (Fig. 4B) did not elicit any changes to mitochondrial

trafficking. The LCSFA-induced impairment in mitochondrial trafficking correlated with a reduction in mitochondrial membrane potential, decrease in ATP levels, and activation of neuronal apoptosis (Rumora, LoGrasso, Haidar, et al., 2019) (Fig 3A,B). Overall, this body of evidence suggests LCSFAs trigger mitochondrial dysfunction supporting a role for dyslipidemia in the pathogenesis of neuropathy.

Another study compared the effect of MUFA oleate and LCSFA palmitate on mitochondrial trafficking to determine the beneficial effect of dietary MUFAs in prediabetic murine models of neuropathy (Rumora, LoGrasso, Hayes, et al., 2019). As previously reported, palmitate caused a significant reduction in the percentage of motile mitochondria in the axons of DRG neurons. Conversely, oleate had no effect on mitochondrial trafficking (Fig. 4F). Interestingly, equimolar or 2:1 mixture of oleate and palmitate completely prevented the palmitate-induced impairment in mitochondrial transport (Fig. 4G). Oleate also prevented mitochondrial depolarization, reduction in ATP levels, and caspase activation. The mechanism by which oleate prevents mitochondrial dysfunction may be related to lipid droplet formation throughout the DRG axon (Fig. 2B). The formation of lipid droplets allows for the sequestration of palmitate preventing downstream mitochondrial dysfunction.

This differential regulation of axonal mitochondrial trafficking in DRG axons suggests that LCSFAs including palmitate and stearate are particularly damaging to DRG neurons. It is likely that LCSFAs are metabolized into lipotoxic lipids, such as ceramides (Listenberger et al., 2003), diacylglycerols, or cardiolipins, that cause mitochondrial dysfunction and apoptosis in DRG neurons and axons. A MUFA-rich HFD can reverse neuropathy and restore nerve function. On a molecular level, oleate prevents impairment of mitochondrial axonal transport, retains mitochondrial membrane potential, and abolishes DRG neuron apoptosis likely by the formation of intra-axonal lipid droplets that sequester lipotoxic LCSFAs (Fig. 2B).

6 Conclusions

Peripheral neuronal architecture is essential for transmitting sensory and motor signals across long distances between the CNS and PNS, but it places unique constraints on managing their energy demands. First, neurons rely on mitochondria during basal and high activity periods for their constant ATP demand (i.e., mitochondrial bioenergetics). Second, in order to meet that continuous need, neurons are reliant on carefully orchestrated mitochondrial dynamics to ensure balanced biogenesis (i.e., fission/fusion, remodeling) for optimal energy production, and that these mitochondria are delivered to areas of high activity (i.e., trafficking/transport). Interactions with other organelles such as ER (i.e., MAMs) add a further layer of coordinated activity, in addition to clearance mechanism, which are necessary for removing and replacing damaged mitochondria. Heritable peripheral neuropathies caused by mutations in genes that regulate mitochondrial bioenergetics, fission/fusion, and transport elegantly demonstrate this critical vulnerability of neurons. Loss of well-regulated mitochondrial dynamics in these heritable neuropathies leads to aberrant mitochondrial functioning and neuronal injury and loss, underscoring the essential role of mitochondria in normal nerve function.

This particular susceptibility of neurons to mitochondrial dysfunction has led to investigations into their roles in the pathogenesis of neuropathy associated with prediabetes and diabetes. Confirming this, studies have shown that mitochondrial dysfunction underlies neuropathy onset and development in both prediabetic and diabetic patients. Hyperglycemia and dyslipidemia contribute to the pathogenesis of neuropathy but exert a differential effect on neuronal mitochondrial dynamics and function. Preliminary results suggest that impaired mitochondrial bioenergetics in prediabetes or diabetes plays a key role in the progression of neuropathy by reducing axonal energy to maintain neuronal function. To compensate for this bioenergetic reduction, DRG neurons undergo mitochondrial biogenesis under conditions of hyperglycemia or proton leak during dyslipidemia. Elevated LCSFAs associated with dyslipidemia impair mitochondrial trafficking, depolarize the MPP, induce a loss of ATP, and apoptosis. Conversely, MUFAs restore mitochondrial dynamics in DRG axons and nerve function in murine models of neuropathy. These recent developments in mitochondrial mechanisms underlying the progression of neuropathy will direct future studies with the goal of developing treatments for this prevalent and debilitating complication.

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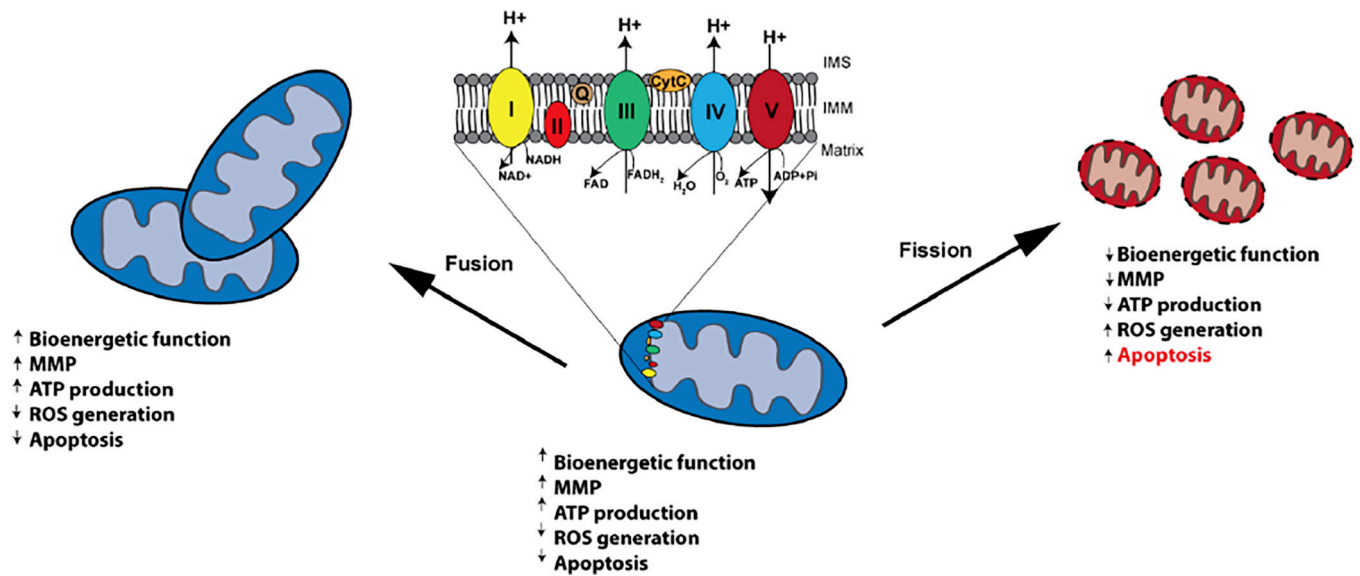


Fig. 1. Schematic of mitochondrial fission and fusion dynamics. Mitochondrial bioenergetics are regulated by mitochondrial fission and fusion events. Mitochondrial fusion events retain mitochondrial bioenergetic function, MMP, ATP production, and decrease ROS generation and apoptosis. Conversely, mitochondrial fission leads to mitochondrial depolarization which impairs mitochondrial bioenergetic function and decreases ATP production. Compromised bioenergetics lead to ROS production triggering apoptosis.

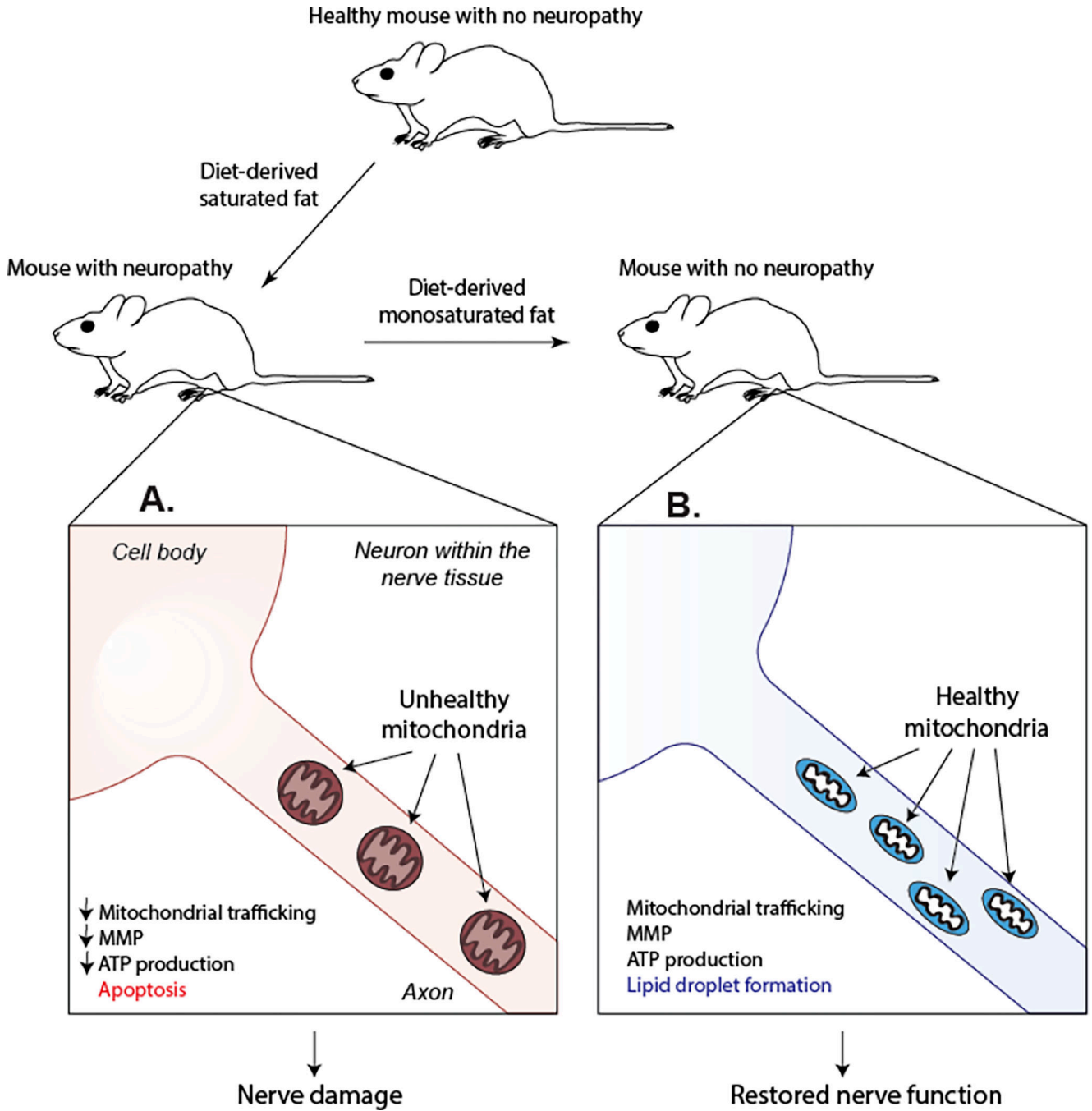


Fig. 2. A dietary switch from a SFA-rich HFD to a MUFA-rich HFD restores nerve function in a murine model of prediabetes and neuropathy. A) Mice fed a SFA-rich HFD develop robust neuropathy phenotypes including a decrease in sensory and motor nerve conduction velocity and a reduction in intraepidermal nerve fiber density. In line with these results, cultured DRG neurons treated with SFAs exhibit a decrease in axonal mitochondrial trafficking and MMP. These mitochondrial changes are associated with apoptosis and a reduction in

neuronal ATP level. B) Changing mice from a SFA-rich diet to a MUFA-rich diet restores both nerve and mitochondrial function.

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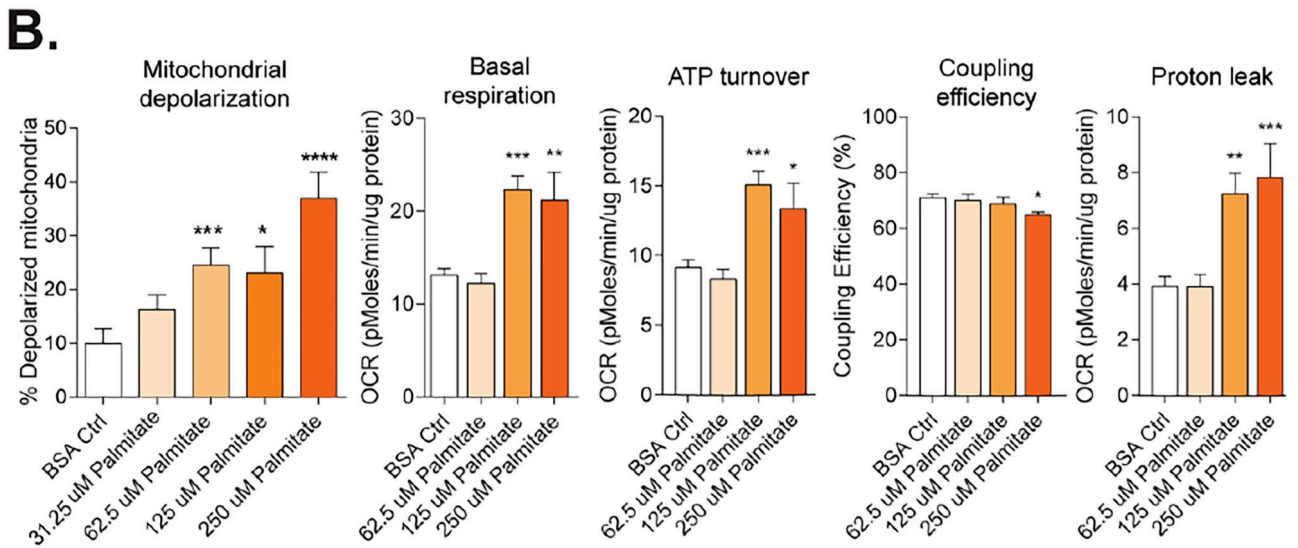
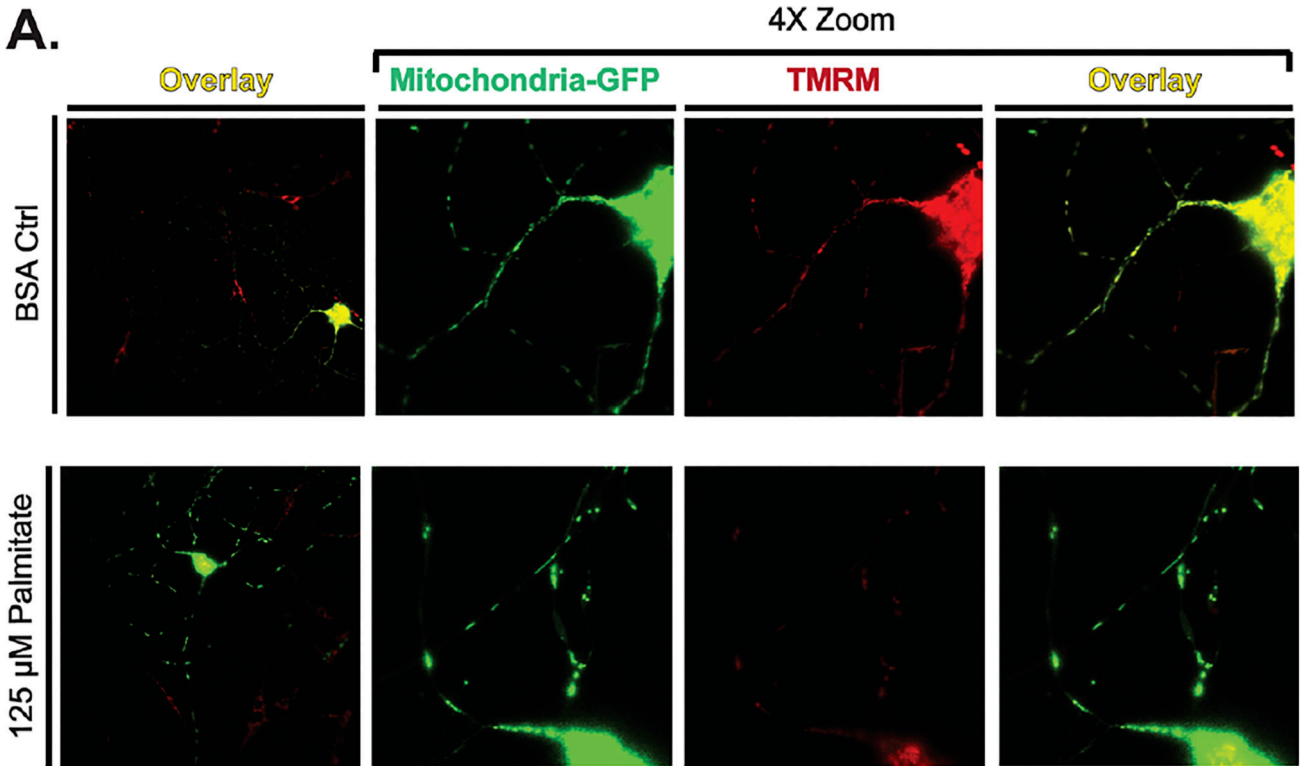


Fig. 3. Dyslipidemia decreases mitochondrial function in DRG neurons. (A) LCSFA palmitate induces a loss of MMP through mitochondrial depolarization. Mitochondria labeled with mito-GFP are stained with MMP-dependent stain TMRM. Axonal mitochondria maintain MMP in the bovine serum albumin vehicle control and appear yellow in an overlay of both signals. Palmitate-treated cells exhibit a loss in TMRM staining due to mitochondrial depolarization. (B) Palmitate impairs mitochondrial bioenergetics through mitochondrial depolarization by increasing basal respiration and ATP turn over which leads to a

decrease in coupling efficiency and increased proton leak from the electron transport chain. *Bioenergetics bar graphs included with permission from* Rumora, A.E., Lentz, S.I., Hinder, L.M., Jackson, S.W., Valesano, A., Levinson, G.E., and Feldman, E.L. (2018). *Dyslipidemia impairs mitochondrial trafficking and function in sensory neurons. Faseb j* 32, 195–207. *Mitochondrial depolarization figures adapted with permission from* Rumora, A.E., LoGrasso, G., Haidar, J.A., Dolkowski, J.J., Lentz, S.I., and Feldman, E.L. (2019). *Chain length of saturated fatty acids regulates mitochondrial trafficking and function in sensory neurons. J Lipid Res* 60, 58–70.

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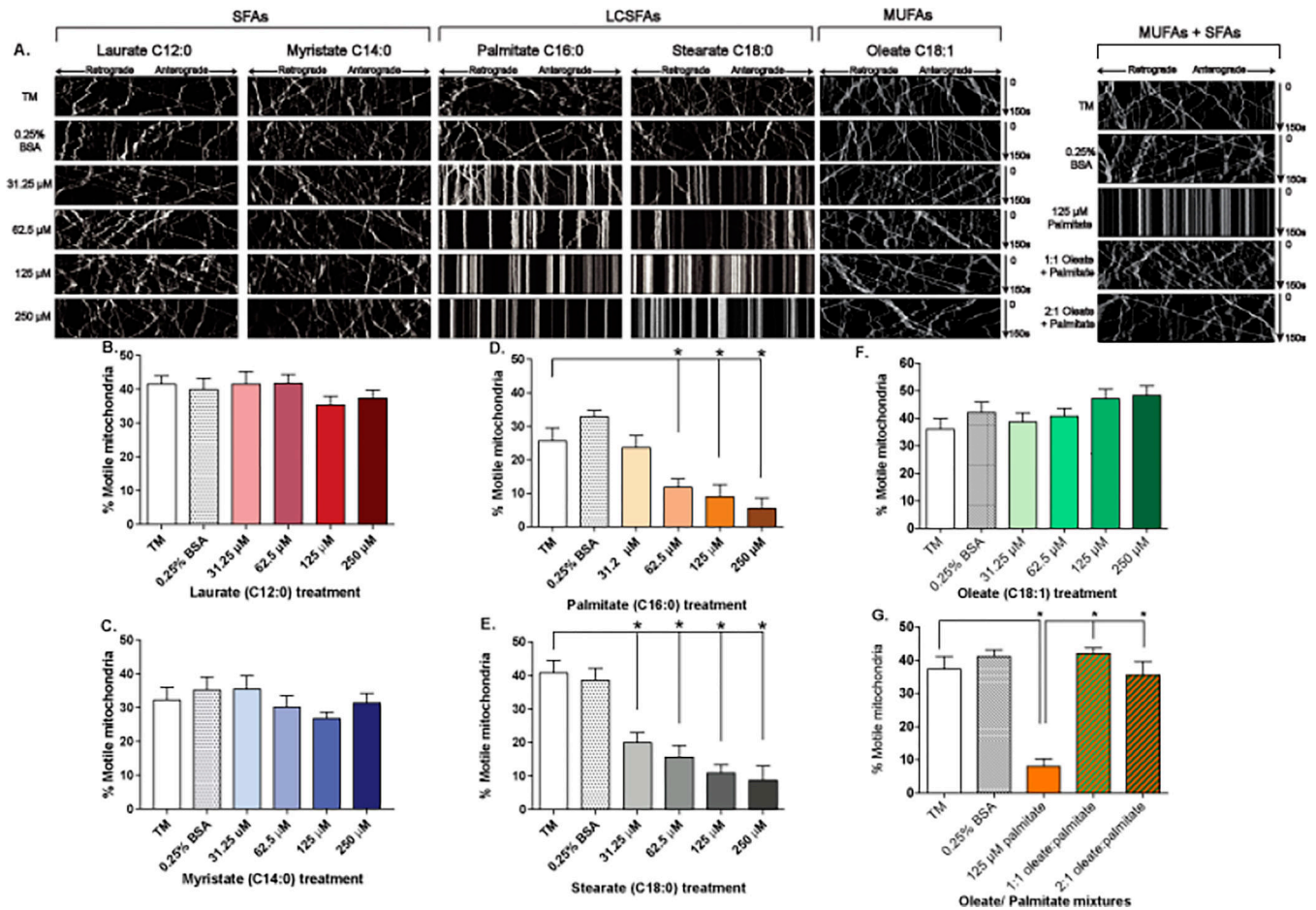


Fig. 4.

SFAs impair axonal mitochondrial transport in DRG neurons. (A) A kymograph analysis is used to record mitochondrial movement from 0–150 seconds using live-cell confocal microscopy. Straight lines indicate non-motile mitochondria. Elevated levels of exogenous short and medium chain SFAs laurate (B) and myristate (C) did not impact the number of motile mitochondria in the axon of sensory DRG neurons. LCSFAs associated with T2D and prediabetes such as palmitate (D) and stearate (E) induce a dose-dependent decrease in the percentage of motile mitochondria in the axon of sensory DRG neurons. (F) MUFA oleate had no effect on mitochondrial motility. (G) Mixtures of oleate and palmitate prevented palmitate-induced impairment of mitochondrial transport. *SFA kymographs and bar graphs modified with permission from Rumora, A.E., LoGrasso, G., Haidar, J.A., Dolkowski, J.J., Lentz, S.I., and Feldman, E.L. (2019). Chain length of saturated fatty acids regulates mitochondrial trafficking and function in sensory neurons. J Lipid Res 60, 58–70. Permission to be obtained for MUFA kymographs and bar graphs from J. Neurosci upon release of paper in press.*

Table 1. Mutations to genes involved in mitochondrial defects in CMT, other peripheral neuropathies, and optic atrophy.

| Gene (alphabetically) | Associated CMT neuropathy (OMIM#) | Mode of inheritance | Affected mitochondrial dynamics | Function |
|-----------------------|---|----------------------|---|--|
| <i>AIFM1</i> | CMTX4 (310490) | X-linked | Mitochondrial metabolism/apoptosis signaling | A FAD-dependent NADH oxidase with a C-terminal catalytic domain facing the IMS, cleaves under pro-apoptotic cues, moves to nucleus to initiate apoptosis |
| <i>DHTRKDI</i> | CMT2 (615025) | Autosomal dominant | Bioenergetics/mitochondrial metabolism | Encodes the E1 subunit of the mitochondrial amino acid degrading alpha-ketoadipic acid dehydrogenase complex |
| <i>DNM1L</i> | Severe infantile encephalopathy (614388) | AD, AR | Mitochondrial fission | Recruited to mitochondrial OMM by MFF, FIS1, MID49, or MID51 receptors, oligomerizes in a ring-like structure to trigger fission |
| <i>DYNC1H1</i> | CMT2O (614228) SMALED (158600) | Autosomal dominant | Mitochondrial trafficking | Subunit of dynein motor protein that performs retrograde axonal transport of mitochondria |
| <i>GDAP1</i> | CMT4A (214400) CMT2K (607831) CMT2K (607831) CMTRIA (608340) | AR AR AD AR | Mitochondrial fission | Forms complexes with DRP1 and FIS1 to effect mitochondrial fission |
| <i>HK1</i> | CMT4G or HMSN Russe (605285) | Autosomal recessive | Bioenergetics/mitochondrial metabolism | Phosphorylates glucose to G6P, couples glycolysis to OXPHOS on the OMM by interacting with VDAC and ANT |
| <i>KIF1B</i> | CMT2A1 (118210) | Autosomal dominant | Mitochondrial trafficking | Monomeric motor protein that performs anterograde axonal mitochondrial transport |
| <i>KIF5A</i> | SPG10 (604187) | Autosomal dominant | Mitochondrial trafficking | Kinesin-1 motor protein that performs anterograde axonal transport of mitochondria |
| <i>MFN2</i> | CMT2A (609260) CMT2A (617087) CMT5 CMT6 (601152) | AD AR AD AD | Mitochondrial fusion/MAMs interaction | OMM-localized fusion protein, forms a tethering complex by homologous interaction with ER-localized MFN2 |
| <i>MT-ATP6</i> | CMT2 | Autosomal dominant | Bioenergetics | ATP6 subunit of ATP synthase (OXPHOS complex V) |
| <i>NEFL</i> | CMT1F (607734) CMT2E (607684) | Autosomal dominant | Mitochondrial shape/mitochondrial trafficking | Neuron-specific component of the cytoskeleton that regulates mitochondrial shape and motility |
| <i>OPA1</i> | Optic atrophy (165500) Optic atrophy plus (125250) | Autosomal dominant | Mitochondrial fusion/bioenergetics | A mitochondrial fusion protein that also remodels cristae |
| <i>OPA3</i> | Optic atrophy (165300) Costeff syndrome (258501) | AD AR | Mitochondrial fission/bioenergetics | Localizes to the OMM and regulates lipid metabolism, thermogenesis, and mitochondrial fission |
| <i>PDK3</i> | CMTX6 (300905) | X-linked | Bioenergetics | Phosphorylates PDC, inactivating its activity, shutting down pyruvate-derived acetyl-CoA entry into the TCA |
| <i>SIGMAR1</i> | dHMN | Autosomal recessive | MAMs interaction | ER chaperone protein that maintains calcium exchange between ER and mitochondria |

| Gene (alphabetically) | Associated CMT neuropathy (OMIM#) | Mode of inheritance | Affected mitochondrial dynamics | Function |
|-----------------------|---|---------------------|---|--|
| <i>SLC25A46</i> | CMT2 Leigh syndrome Optic atrophy | Autosomal recessive | Mitochondrial fusion/ MAMs interaction/ bioenergetics | Transmembrane protein localized to OMM, mediates lipid transfer between ER and mitochondria, maintains mitochondrial lipid homeostasis and cristae |
| <i>TUBB3</i> | CFEOM3 (600638) | Autosomal dominant | Mitochondrial trafficking | Polymers to form axonal microtubules |
| <i>VAPB</i> | ALSS (608627) SMA (182980) | Autosomal dominant | MAMs interaction | Regulates autophagosome formation at MAMs through VAPB-PTPIP51 complex |

Abbreviations: AD = autosomal dominant; ALS8 = amyotrophic lateral sclerosis 8; ANT = adenine nucleotide translocator; AIFM1 = apoptosis inducing factor mitochondria associated 1; AR = autosomal recessive; CFEOM3 = extraocular muscles type 3; CMT = Charcot-Marie-Tooth disease; dHMN = distal hereditary motor neuropathy; DHTKDI = dehydrogenase E1 and transketolase domain containing 1; DNMI1 = dynamin 1 like; DYNC1H1 = dynein cytoplasmic 1 heavy chain 1; FIS1 = fission 1 protein; G6P = glucose-6-phosphate; GDAP1 = ganglioside induced differentiation associated protein 1; HK1 = hexokinase 1; IMS = intermembrane space; KIF1B = kinesin-3 isoform B; KIF5A = kinesin-1 isoform A; MFN2 = mitofusin 2; MT-ATP6 = mitochondrially encoded ATP synthase 6; NEFL = neurofilament, light polypeptide; OMM = outer mitochondrial membrane; OPA1 = optic atrophy 1; OPA3 = optic atrophy 3; OXPHOS = oxidative phosphorylation; PDC = pyruvate dehydrogenase complex; PDK3 = pyruvate dehydrogenase kinase 3; PTPIP51 = protein tyrosine phosphatase-interacting protein 51; SIGMAR1 = sigma non-opioid intracellular receptor 1; SLC25A46 = solute carrier family 25 member 46; SMA = spinal muscular atrophy; SMALED = spinal muscular atrophy, lower extremity predominant; SPG10 = spastic paraplegia 10; TCA = tricarboxylic acid cycle; TUBB3 = class III β -tubulin; VAPB = vesicle associated membrane protein-associated protein B; VDAC = voltage-dependent anion channel.