Kim Bhumsoo (Orcid ID: 0000-0002-2645-1673)

Evolving concepts on the role of dyslipidemia, bioenergetics, and inflammation in the pathogenesis and treatment of diabetic peripheral neuropathy

Running Title: Diabetic Neuropathy

Amro Maher Stino*, MD[1,2], Amy E. Rumora, PhD[1], Bhumsoo Kim, PhD[1], Eva L. Feldman, MD, PhD[1,2]

University of Michigan School of Medicine, [1]Department of Neurology, [2]Division of Neuromuscular Medicine, Ann Arbor, MI, USA 48109

*Corresponding author: amstino@med.umich.edu

Amro Maher Stino	amstino@med.umich.edu	0000-0002-7793-6662
Amy E Rumora	arumora@med.umich.edu	0000-0002-0788-3251
Bhumsoo Kim	bhumsoo@med.umich.edu	0000-0002-2645-1673
Eva L Feldman	efeldman@med.umich.edu	0000-0002-9162-2694

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Abstract

Diabetic peripheral neuropathy (DPN) is one of the most widespread and disabling neurological conditions, accounting for half of all neuropathy cases worldwide. Despite its high prevalence, no approved disease modifying therapies exist. There is now a growing body of evidence that DPN secondary to type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) represents different disease processes, with T2DM DPN best understood within the context of metabolic syndrome rather than hyperglycemia. In this review, we highlight currently understood mechanisms of DPN, along with their corresponding potential therapeutic targets. We frame this discussion within a practical overview of how the field evolved from initial human observations to murine pathomechanistic and therapeutic models into ongoing and human clinical trials, with particular emphasis on T2DM DPN and metabolic syndrome.

Keywords

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Introduction

Diabetic peripheral neuropathy (DPN) is a length-dependent sensory predominant axonal neuropathy that often first manifests with dysesthesias, pain, numbness, or imbalance. DPN has a prevalence ranging from 10-50% for both type 1 diabetes (T1DM) and type 2 diabetes (T2DM) The DPN annual cost, including complications, exceeds $10 billion in the United States alone [1]. DPN leads to reduced quality of life due to increased pain, falls, lower limb ulcers and amputations. While glycemic control has been shown to be effective in DPN associated with T1DM, it carries only a modest effect in DPN associated with T2DM [2,3]. Furthermore, while neuropathy associated with T1DM and T2DM has classically been categorized together, we now know that the two are largely different disease processes [4,5]. Despite recent clinical testing of contemporary drugs (**Table 1**) and decades of research on a massive global health burden, no approved disease-modifying therapies exist. Previous drug trials focused on antioxidants [6-12]; lipid lowering fibrates [10,13]; aldose reductase inhibitors [14-17]; neurotrophic factors [18-20]; GABA analogues [21]; cellular metabolism agonists [22-25]; and vasodilators [7,26] and have had limited efficacy in treating DPN associated with T1DM and T2DM [27,28]. Nevertheless, with our increased understanding of DPN pathogenesis, there is general optimism that new therapies for DPN are on the horizon. In this review, we focus on our current understanding of DPN pathogenesis and potential therapeutic targets, with a particular focus on DPN in the setting of T2DM and metabolic syndrome (MetS). We highlight how initial clinical observations in patients led to the development of murine models of T2DM and prediabetes, which guided our

understanding of pathomechanisms and potential therapies. These studies inform ongoing and future clinical trials and our approach to identifying disease modifying therapies.

Cryptogenic sensory peripheral neuropathy and metabolic syndrome

Clinicians caring for patients with cryptogenic sensory peripheral neuropathy (CSPN) in the 1990s observed that many patients without diabetes carried features similar to patients with diabetes, particularly in regards to obesity and MetS. Since then, seven international population-based studies - five cross-sectional and two-longitudinal - spanning the United States, Europe, and China have shown obesity to be the second most significant metabolic risk factor for neuropathy after diabetes [29-34]. Waist circumference and diabetes are the most significant risk factors for neuropathy [30], with waist circumference associating with the greatest number of neuropathy outcome measures [31]. In addition, the likelihood of symptomatic CSPN rises as the number of MetS features increases. Furthermore, in patients with established DPN, obesity and hyperlipidemia accelerate the rate of DPN development [35].

Obesity is the main driver of MetS, producing a chronic state of low-grade metabolic inflammation and an elevated level of long-chain fatty acids that places undue burden on the peripheral nervous system [29]. In line with such findings, CSPN in the setting of obesity and MetS is now classified as obesity- or MetS-associated neuropathy. Dyslipidemia, a component of the MetS criteria, warrants particular attention. Hypertriglyceridemia independently associates with the development of CSPN [36], decreased sural nerve myelinated fiber density [37],

loss of intraepidermal nerve fiber density (IENFD) [35], the progression of established DPN [37], and the likelihood of lower limb amputations [38]. Conversely, lipid lowering therapy reduces the likelihood of the long-term development of DPN [39,40], as well as the risk of lower limb amputation [41].

In addition, impaired fasting glucose (IFG) and impaired glucose tolerance (IGT), which form part of the MetS diagnostic criteria, independently associate with the development of CSPN, and warrant discussion. Cross-sectional data from the MONICA/KORA study showed a neuropathy prevalence of 8.7% and 4.2% in patients with IGT and IFG, respectively [42]. The PROMISE longitudinal study showed that prediabetic patients (IGT or IFG) carried the same prevalence of neuropathy at 3-year follow-up (50%) as diabetics [43]. In addition, when looking at patients with established CSPN, the prevalence of IFG can be as high as 56% [44].

Somatic small unmyelinated C fibers, quantified through IENFD, are particularly sensitive at capturing early DPN. Equally important is the capacity of these somatic small fibers to regenerate, and thus to serve as primary outcome measures for clinical trials [45]. Also, corneal confocal microscopy is a rapid, validated, non-invasive and *in vivo* measure of small fiber function in diabetic neuropathy [46], which has comparable accuracy to IENFD [47]. Corneal confocal microscopy also correlates with functional measures of neuropathy severity [48] and tracks improvement in diabetic patients after simultaneous pancreas-kidney transplant [49]). Thermal perception threshold (TT) testing is a third validated and sensitive modality that captures diabetic small fiber neuropathy quite well [50], and can also be used to distinguish healthy

patients from those with small fiber neuropathy [51]. However, lack of consensus on stimulus application, location, and sensations tested hinders it more widespread adoption [52].

Dyslipidemia, bioenergetics, and inflammation

Murine models

In light of the clear clinical association between MetS and CSPN, murine models were developed with the aim of understanding pathomechanisms and developing concordant therapeutic interventions for patients with DPN. *In vitro, in vivo*, and clinical studies have focused on mechanisms underlying the pathogenic role of hyperlipidemia and hyperglycemia on neuropathy. Juvenile genetic murine models of prediabetes and T2DM arose, illustrating that hyperglycemia was not the only driver of peripheral neuropathy [53,54]. Murine models of T2DM, including leptin (*ob/ob)* and leptin receptor (*db/db)* knockout models, are used to identify molecular pathways that underlie neuropathy pathogenesis and are conserved between murine models and humans [55]. In addition, C57BL/6J diet-induced murine models now exist, in which a lard-based, high-fat diet (HFD) chow, consisting of elevated levels of long-chain fatty acids, induce prediabetes and neuropathy [56 57]. The HFD-fed C57BL/6J mice exposed to low-dose streptozotocin serve as models for T2DM [58]. All of these murine models display neuropathic features, with increased thermal latency, reduced nerve conduction velocity, and IENFD loss.

Mitochondria and bioenergetics

Numerous studies explored the effect of T2DM and MetS, particularly dyslipidemia, on the mitochondrion and bioenergetics in sensory neurons and the peripheral nerves **(Figure 1)**. Disorders of fission, fusion, oxidative phosphorylation, and mitochondrial trafficking, among others, are altered in diabetes, prediabetes, and dyslipidemia [59]. Dyslipidemia mediates changes in complex lipid synthesis, which then alters mitochondrial size, morphology, and motility [60]. Palmitate and stearate, for example, increase mitochondrial circularity and size, probably due to mitochondrial dysfunction, swelling, and loss of inner mitochondrial membrane structure [60]. Although hyperglycemia has no effect on mitochondrial trafficking in sensory neurons, increased concentrations of long-chain fatty acids impair mitochondrial trafficking and alter mitochondrial bioenergetics experimentally [61]. Palmitate, a long-chain saturated fatty acid (SFA), lowers the number and velocity of motile mitochondria and depolarizes mitochondria [62]. Palmitate also induces an increased rate of ATP turnover and basal respiration. Not unlike sensory neurons, Schwann cells also show pathogenic changes in response to dyslipidemia. Schwann cells increase the expression of medium to long-chain acylcarnitines in response to long-chain fatty acid overexposure, which in turn, induces peripheral nerve injury [63]. In parallel, sciatic nerves from HFD and HFD-STZ mice contain elevated levels of palmitate and stearate in complex lipids such as triglycerides and phospholipids [64]. Transcriptomic and lipidomic analyses on those samples showed abnormal nerve-lipid signaling, with samples showing increased expression of diacylglycerol acyltransferase 2 (DGAT2), the enzyme involved in the last step of triglyceride synthesis. This finding was corroborated with sural nerves from dyslipidemic DPN patients [64].

Of note, mitochondrial alterations also appear in intraepidermal nerve fiber samples from human subjects with DPN [65]. Mitochondria have larger volumes at both distal thigh and distal leg sites in DPN patients relative to healthy controls, and also show a length dependent gradient, with distal leg nerves showing larger mitochondrial volume than distal thigh nerves. An earlier study also showed mitochondrial derangements in skin sections from 32 patients with small fiber neuropathy, in which mitochondrial respiratory chain complex IV (OXPHOS) fluorescence was reduced, despite preserved intraepidermal nerve fibers [66]. In addition, the normal co-localization of OXPHOS within intraepidermal and subpapillary dermal axons was also lost.

Substrate transport and uptake

Transport and substrate uptake is also dysregulated in the setting of substrate overload. Free fatty acids (FFAs) are normally β-oxidized to generate NADH and $FADH_2$ in Schwann cells, dorsal root ganglion sensory neurons, and axons. Of note, β-oxidation in Schwann cells requires the transport of long-chain FFAs across cell membranes. Subsequent to their uptake, FFAs are β-oxidized to produce acetyl-CoA, which is then shuttled to the tricarboxylic acid cycle for the generation of NADH and $FADH_2$. In T2DM, a dyslipidemic state creates FFA substrate overload, which saturates the Schwann cell transport system and causes an accumulation of acylcarnitines [63]. Schwann cells transfer accumulating acylcarnitines to sensory neurons causing axonal degeneration and mitochondrial dysfunction.

Peroxisome proliferator-activated receptors (PPARs) are also highly dysregulated in T2DM. PPARs are a superfamily of ligand-activated transcription factors of nuclear hormone receptors that perform multiple physiological functions. The PPAR- γ subclass causes enhanced glucose metabolism and insulin sensitization. Furthermore, the PPAR-γ coactivator-1α (PGC-1α) is a transcriptional co-activator and a master regulator for mitochondrial biogenesis in many tissues, including peripheral nerve, and regulates expression of proteins responsible for fatty acid uptake, particularly the fatty acid translocase CD36 [67-69]. Also, liver X receptor (LXR) activation in murine neuropathy models is central to the reduction of endoplasmic reticulum stress and restoration of myelin lipid composition [70,71]. LXRs are ligand-activated nuclear transcription factors highly linked to PPARs.

Dyslipidemia and inflammation

The interplay of dyslipidemia and inflammation serves as another focus of research interest in our laboratory and that of others. In the setting of substrate overload, oxidative phosphorylation fails, ATP production declines, reactive oxygen species (ROS) arise, and low density lipoproteins are oxidized, triggering mitochondrial dysfunction [72,73] **(Figure 1)**. Long-chain fatty acids penetrate the blood-nerve barrier, trigger neurogenic inflammation, and attract innate and adaptive cells, a finding corroborated with increased expression of TNF-α and IL-6 in DPN animal models [74]. Neuronal oxidative stress triggers a cascade of downstream cytokine and chemokine production of pro-inflammatory agents, while also producing a feed-forward loop of

injury [75]. The generation of oxidized cholesterol (oxysterol) induces tissue injury [76] by binding to the following 3 receptors: oxidized LDL receptor 1 (LOX1), a type II membrane protein located in endothelial cells [57]; toll-like receptor 4 (TLR4), a pattern-recognition receptor that initiates inflammatory and immune responses [77]; and the receptor for advanced glycation end products (RAGE), a receptor expressed in endothelial and Schwann cells which contributes to vascular injury in DPN [78]. In the *db/db* mouse model, multiple immune molecules are upregulated in both the early and later stages of disease [79]. Inflammatory dysregulation occurs as early as 5 weeks of age in *ob/ob* and *db/db* mouse models [53,54,80]. In addition, toll-like receptor signaling influences the early development of DPN in sensory neurons [81]. Several inflammatory markers increase in T2DM, including interleukin-6, sialic acid, plasminogen activator inhibitor-1, fibrinogen, and C-reactive protein [82].

Despite the complexity of the inflammatory process, NF-κB pathway activation is a shared pathway. NF-κB functions as a redox-sensitive transcription factor that is activated by hyperglycemia, oxidative stress, and pro-inflammatory cytokines [82]. It modulates multiple downstream inflammatory genes, notably cyclooxygenase-2 (COX-2). Of the acute phase cytokines modulated by the NF-κB pathway, tumor necrosis factor α (TNF-α) warrants particular attention, as it induces COX-2 overexpression, which, in turn, induces inflammatory changes that lead to DPN [83]. Furthermore, the heat shock chaperone proteins (HSP) 70 and 90, responsible for protein refolding and regulating proteostasis, as well as cellular protection from oxidative stress, inflammation, and apoptosis, are a potential mechanistic target in DPN. HSPs upregulate

the NF-κB pathway, TNF- α, and IL-6 by binding with high affinity to the plasma membrane [84]. These chaperones can also improve neural mitochondrial bioenergetics and improve oxidant capacity [85].

Dyslipidemic and inflammatory therapeutic targets

In light of the above work on DPN pathomechanisms in murine models, investigators explored potential therapeutic targets through dietary reversal, unsaturated fatty acid dietary intervention, and anti-inflammatory modulation (**Figure 2**). Dietary reversal in HFD mice is a natural focus. HFD mice switched to a standard chow diet for 4 weeks demonstrate normalization of neuropathy, as well as improved insulin sensitivity, increased weight loss, and restoration of LDL and oxidative LDL levels [56]. In a separate study evaluating the sciatic nerves of HFD and HFD-STZ mice, dietary reversal from a HFD to a standard chow for 8 weeks lowers levels of pathogenic palmitate and stearate in sciatic nerves [64]. The introduction of mono- and polyunsaturated fatty acids also prevents peripheral neuropathy and restores IENFD in mice fed a HFD rich in long-chain SFAs [60]. A mixture of oleate, a monounsaturated fatty acid (MUFA), and palmitate at a 2:1 molar ratio prevents palmitate-induced impairment of mitochondrial transport [60,62]. Increasing the ratio of *n-3* to *n-6* polyunsaturated fatty acids also decreases diabetic complications in T2DM adults [86,87,88]. Oleate prevents mitochondrial depolarization, preserves intracellular ATP levels, and reduces caspase activation by promoting lipid droplet (LD) formation, and thus prevents palmitate-induced apoptosis [59,89]. In response to menhaden

(fish) oil (rich in *n-3* polyunsaturated fats), HFD-STZ mice normalize motor and sensory nerve conduction velocities as well as thermal responsiveness [90]. Combinatorial therapies also provide promising therapeutic options. A diet rich in omega-3 polyunsaturated fats alone or in combination with α-lipoic acid and/or enalapril improves DPN in HFD-STZ mice as compared to untreated animals [91]. Additionally, pioglitazone, an agonist of PPAR- γ that reduces plasma levels of FFAs, improves DPN in the T2DM *db/db* mouse [92].

As for inflammatory therapeutic avenues, the NF-κB pathway has emerged as a natural target. Polyunsaturated fatty acids inhibit NF-κB activation and nuclear translocation [93,94]. In the previously cited study that showed inflammatory dysregulation as early as 5 weeks in *ob/ob* and *db/db* mice, pioglitazone and acipimox, both lipid lowering agents, improved peripheral nerve metrics [53]. COX-2 selective inhibition or gene inactivation prevents large and small nerve fiber dysfunction in murine models [83]. Interleukin-6 (IL-6) improves motor and sensory nerve conduction velocities [95 96] and corrects thermal nociception and tactile allodynia, while increasing nerve blood flow [97,98 99]. Finally, with regards to heat shock chaperone proteins, modulation of Hsp70 and Hsp90 with small molecules improves DPN in T1DM animal models [85,100].

Current state of human clinical trials and the road ahead

In light of our evolved understanding of DPN pathomechanisms and the promise of new therapeutic targets, human clinical trials have emerged to address the potential role of dietary

restriction, exercise, reduction in sedentary lifestyle, and bariatric surgery in the reversal and prevention of DPN. In addition, we highlight two ongoing drug trials, although disease-modifying drug therapeutics remains in its infancy.

The Look Ahead trial showed that intensive lifestyle intervention resulted in improvement of self-reported metrics of DPN, as measured per the Michigan Neuropathy Severity Index, and per light touch sensation on physical exam [101]. Three non-randomized trials have demonstrated the efficacy of exercise as a disease modifying intervention for patients with MetS, using IENFD as the primary outcome measure. The first showed improvement in 32 patients with impaired glucose tolerance as measured at 12 months [102]. The second showed improvement in the regenerative capacity of small fibers in 36 patients with diabetes or MetS at 4 months after start of intervention [103]. The third showed improvement in 17 DPN patients at 10 weeks after intervention [104]. It is worth noting that such improvements occurred even in the absence of significant weight loss. One randomized trial for patients with T1DM and T2DM DPN demonstrated improved nerve conduction study parameters and vibration perception thresholds after a 4-year aerobic exercise regimen, although the trial did not separate T1DM from T2DM patients nor did it designate IENFD, the current gold standard, as the primary outcome measure [105]. The ongoing ADAPT trial evaluates the effect of supervised exercise, individualized dietary counseling, and reduced sedentary behavior, as tracked by actigraphy, on the rate of progression of DPN [106].

Bariatric surgery is also a potential intervention for DPN. An ongoing randomized trial is evaluating the effect of bariatric surgery and high intensive interval training, either in combination or alone, on DPN (NCT03617185). A previous prospective 6-month cohort study assessing the impact of Roux-en-Y gastric bypass on the development of DPN in T2DM patients showed an improvement in neuropathy scores [107]. As for drug therapies, the Topiramate as a Disease Altering Therapy for Cryptogenic Neuropathy (TopCSPN) study is a multi-center, placebo-controlled, randomized trial in the United States that just completed enrollment, which aims to determine if topiramate can alter the natural history of MetS neuropathy (NCT02878798). Both trials employ IENFD as the primary outcome measure.

It is unknown whether single-target or multi-target interventions are needed to attain full disease-modifying efficacy. Pathway cross-talk perturbation modeling has been employed to identify connectivity changes induced by DPN and potential therapeutic interventions, and offers a novel approach to DPN disease modification strategy [108].

Conclusion

In this review, we explored modern concepts in our understanding of DPN, and how this can guide a more informed and precise approach to disease modifying therapy in the future. Despite the ubiquity of DPN and its economic cost, no approved disease modifying therapies exist, although exercise, dietary restriction, and bariatric surgery show some early favorable findings. Central to this understanding is a realization that T1DM and T2DM pathomechanisms

are intrinsically different, with the type 2 DPN paradigm being much more responsive to interventions aimed at MetS rather than hyperglycemia. Through a greater appreciation of DPN pathomechanisms, particularly as it pertains to T2DM and MetS, the potential for disease modifying therapies remains promising.

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Drug	Disease or Pain Modifiying	Mechanism of action	Diabetes Type	Clinical Trial Outcome for neuropathy	Reference
OnabotulintoxinA (BoNT/A)	Pain	Inhibits neurogenic inflammation from peripheral nociceptive nerve terminals	T2DM	Improved tactile and mechanical pain perception in painful DPN	[109]
Botulin toxin (BTX-A)	Pain	Potent neurotoxin, used in treatment of dystonia, muscle hyperactivity and glandular hyperactivity. BTX-A may have analgesic properties.	T2DM	Intradermal injection of BTX-A significantly improved painful DPN	[110]
Monochromatic Infrared Energy (MIRE)	Disease	Increases blood circulation	T2DM	No improvement	[111]
L-arginine	Disease	Substrate for nitric oxide synthesis to improve microcirculation	T2DM	No effect DPN	[112]
Minocycline	Disease and Pain	Anti-inflammatory and anti-apoptotic properties, suppression of microglial activation,	T2DM	Improved vibration perception threshold, reduced neuropathic symptoms, and pain disability index.	[113]
Benfotiamine	Disease	Modulates advanced glycation end	T1DM	No effect on peripheral nerve function	[114]

		products.			

Table 1. Drug trials on peripheral neuropathy associated with T1DM and T2DM from 2010-2020

Figure 1. Pathogenic mitochondrial and inflammatory pathways associated with T2DM and dyslipidemia. SFAs associated with dyslipidemia and T2DM drive acylcarnitine and triglyceride formation in sensory neurons and peripheral nerves. Excess levels of acylcarnitine lead to increased mitochondrial dysfunction and apoptotic fission that may underlie T2DM DPN development. Dyslipidemia also causes an elevation in oxidized LDL (ox-LDL) and advanced glycation end-product LDL (AGE-LDL) that trigger pro-inflammatory signals including TNF-α, IL-6, and COX-2, perturbing mitochondrial function. Similarly, LDL is metabolized intracellularly into FFAs that increase ROS and initiate mitochondrial dysfunction and apoptosis. In addition, nuclear transcription factors regulate pro-inflammatory signals (NF-κB pathway) and FFA β-oxidation (PGC-1α, PPARγ, and LXR). Modulation of transcriptional regulation, fatty acid composition, or inflammatory pathways may offer therapeutic targets for the treatment of T2DM DPN.

Figure 2. Therapeutic targets identified by dietary intervention and inflammatory pathway studies. Molecular targets were identified in pre-clinical studies using murine models of dyslipidemia and DPN. Dietary intervention with MUFA supplementation reverses DPN progression potentially through the sequestration of SFAs into LDs in sensory neurons. Dietary reversal from a high-fat diet to a standard diet reduces the level of SFAs in sensory neurons. Subsequent to both dietary intervention paradigms, reduced levels of acylcarntine improves mitochondrial function and prevent apoptosis. Similarly, stimulation of PGC-1α, PPARγ, and LXR transcription factors by pioglitazone activates FFA β-oxidation, improving mitochondrial function and nerve function. Pioglitazone and Acipimox both inhibit NF-κB activation of pro-inflammatory pathways. The reduction in pro-inflammatory TNF-α, IL-6, COX-2, and ROS production prevents downstream mitochondrial dysfunction and sensory neuron apoptosis.

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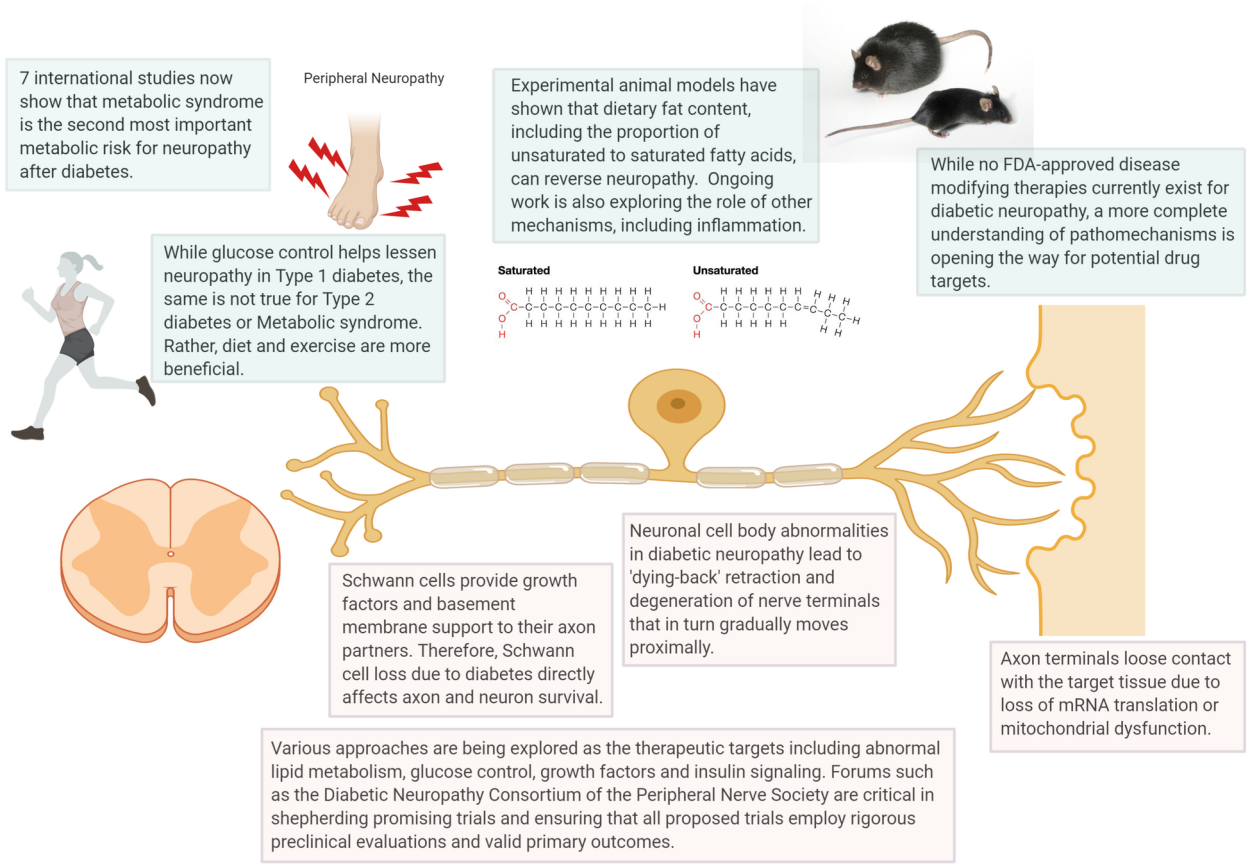

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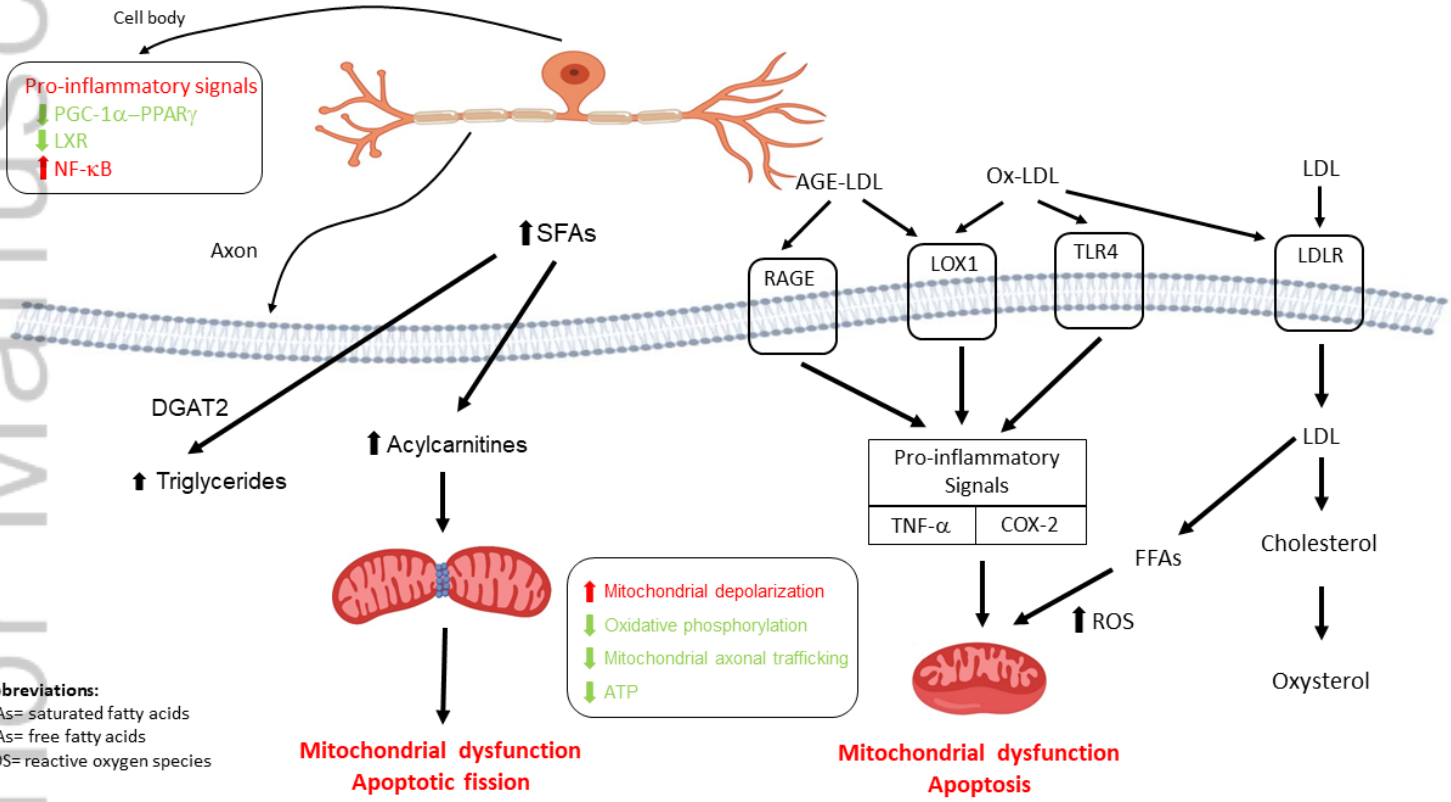

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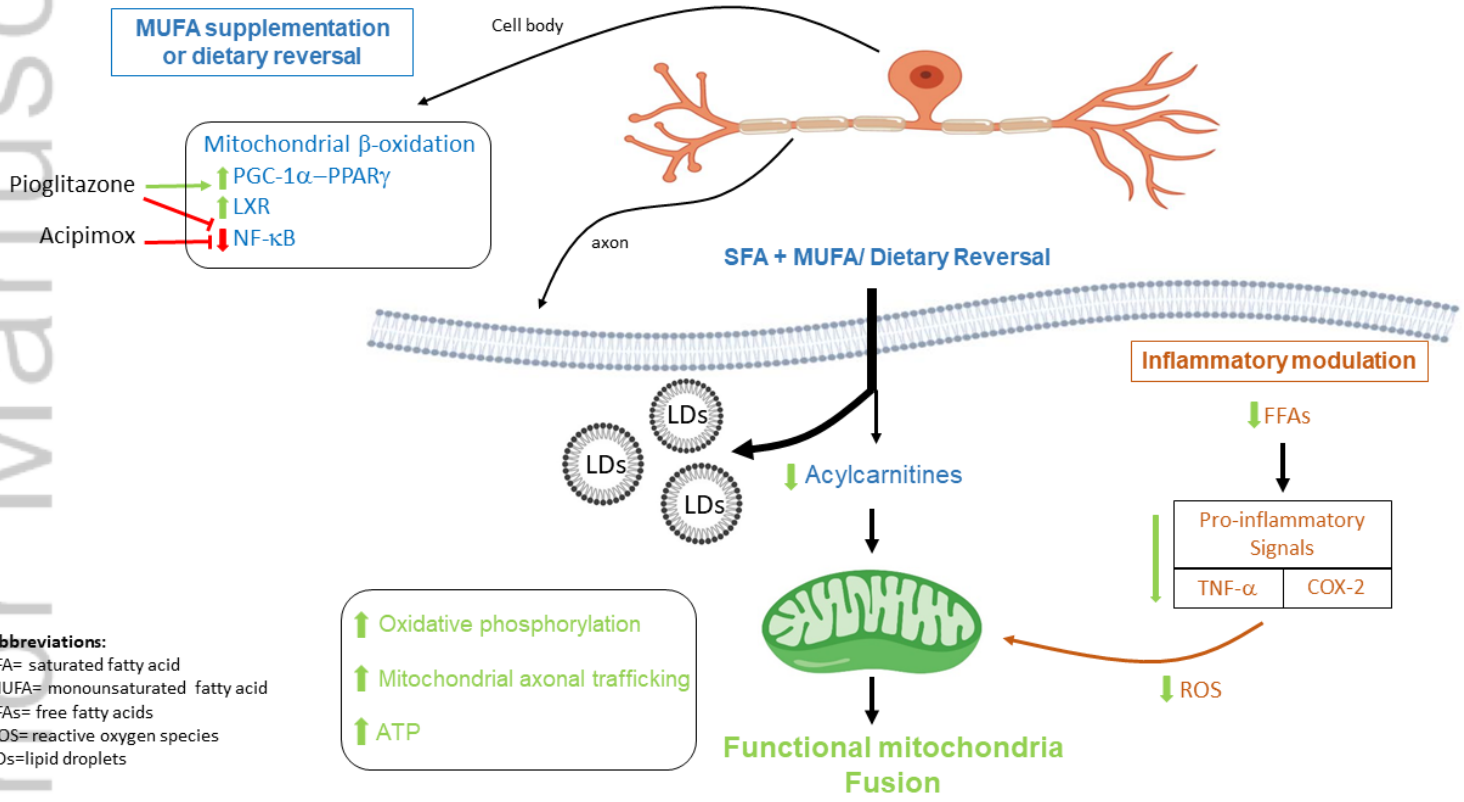

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