UNIVERSITÉ DE STRASBOURG

FACULTÉ DE MÉDECINE

ED414 SCIENCES DE LA VIE ET DE LA SANTÉ

THÉSE

Pour l'obtention du grade de

DOCTEUR DE UNIVERSITÉ DE STRASBOURG

Presentée et soutenue publiquement par

MARY A. HADDAD

Le 4 juin 2021

MECHANISMS OF PERIPHERAL NERVE INJURY IN DIABETES:

ROLE OF THE CYTOCHROME P450 PATHWAY

Membres du jury:
Prof. Rita AZZI-NABBOUT, Rapporteur
Prof. Nassim FARES, Rapporteur
Prof. Julien GODET, Examinateur
Prof. Hala KASSOUF, Examinateur
Prof. Erik-André SAULEAU, Directeur
Prof. Assaad A. EID, Directeur

UNIVERSITÉ DE STRASBOURG

FACULTÉ DE MÉDECINE

ED414 SCIENCES DE LA VIE ET DE LA SANTÉ

THÉSE

Pour l'obtention du grade de

DOCTEUR DE UNIVERSITÉ DE STRASBOURG

Présentée et soutenue publiquement par

MARY A. HADDAD

Le 4 juin 2021

MECANISMES DES LESIONS DES NERFS PERIPHERIQUES DANS LE DIABETES: ROLE DE LA VOIE CYTOCHROME P450

Membres du jury:
Prof. Rita AZZI-NABBOUT, Rapporteur
Prof. Nassim FARES, Rapporteur
Prof. Julien GODET, Examinateur
Prof. Hala KASSOUF, Examinateur
Prof. Erik-André SAULEAU, Directeur
Prof. Assaad A. EID, Directeur

Dedication

To my parents, Adib and Rita, my lovely brother, Elias, and my fiancé, Elio. You have taken this adventure with me from the start. You were a part of it in as much as I have. You have been nothing but patient, supportive, encouraging, and determined to help me across the rocky road. I bow before the sacrifices you have made to get me to this point. As always, I will aim higher and keep going for you. I hope I have made you proud and I hope I can one day return at least a pint of the love and care you have provided me with. I am eternally grateful.

To the blessed memory of my grandparents, Fouad and Souad Haddad and Elias and Nelly Jabbour. To the late loving memory of my dear aunts and uncles: Noha and Nehmetallah Fares, Jean Jabbour, and Marie Zoroub. Diabetes and heart failure are the genetic tax in our family. Despite the health management and the effort, this silent disease took you away in the end. Not a day went by without remembering your last words to me, which went to wish me luck through my educational endeavors. You remain the truest inspiration behind this work, every step along the way. Look down with pride, it has been done. You have sparked a light in me, I will cherish forever.

To all women in science. Always swim against the tide.

To my beloved country, Lebanon, in the most trying of times.

Acknowledgments

"As you start to walk on the way, the way appears."- Rumi.

To my thesis directors, Dr. Assaad Eid and Prof. Erik-Andre Sauleau. Thank you for granting me this opportunity and leading the first step to the 'journey of a thousand miles.' It is a great honor to be awarded a doctoral degree from the University of Strasbourg. Thank you, Dr. Erik, for the wonderful memories from my first trip to dazzling Strasbourg. Thank you for your support along the way, and for your kindness, patience and understanding. Thank you, Dr. Eid, for seeing this work through the high and low tides, your constant brotherly support, and for your trust throughout. Thank you for letting me experience many firsts, and especially the memories acquired from the better half of the decade (plus a year) I have spent at my long-standing second home, the American University of Beirut, and Lebanon.

I wish to express sincere gratitude to the thesis committee members. Thank you for taking the time to evaluate this work and return ample insight and feedback.

I would also like to express great appreciation for Dr. Frederic Harb. You have seen me through thick and thin, academically, and beyond. From listening to me rant away, to making the case with my mom to let me bring my dog home. You were a sweet friend I'm glad I met. Thank you for lending me a shoulder to lean on and for the pep-talk.

This work was not completed alone, rather with the help of a group of special individuals: the EID family. The years went by with each one of you holding a place in my memory. Wherever you are, may the best come your way. I am thankful to have met you all, and grateful for the memories; I will cherish them always.

To my partner who helped push the project a tad further in spite of the pandemic and closed off the final stages of the work. Thank you, Karen, for being with me during the tough times, and following the project for a long time. Those last few months were bearable by your presence. Thank you for being a dedicated lab partner, and a good friend.

Last but not least, I would like to extend my thanks to family and friends who encouraged me, from near or afar across the globe.

Abstract

MARY A. HADDAD FOR DOCTORATE OF PHILOSOPHY

Major: Neuroscience

TITLE: MECHANISMS OF PERIPHERAL NERVE INJURY IN DIABETES: ROLE OF THE

CYTOCHROMES P_{450} PATHWAY

Diabetes is a leading health concern due to the spectrum of metabolic disorders associated with its onset. More importantly, it is a life-threatening disease owing to the complex multiorgan complications. Among the microvascular complications associated with diabetes, diabetic neuropathy (DN) is one of most reported affecting 50% - 70% of diabetic subjects. Diabetic Peripheral Neuropathy (DPN) is characterized by peripheral nerve dysfunction. Clinically, DPN is characterized by reduced electrophysiological recordings, sensorimotor loss, and paresthesia. Research has correlated these observations with nerve fiber injury leading to degeneration, axonal atrophy, demyelination with limited regenerative potential. Oxidative stress is now considered the final common key mediator, and the overproduction of Reactive Oxygen Species (ROS) has been suggested to orchestrate diabetic complications including DPN. However, the cellular and molecular mechanisms by which diabetes contributes to oxidative stress in DPN remain unknown. Moreover, untargeted antioxidant therapy has only exhibited limited efficacy. Therefore, there is a critical need to explore specific sources of ROS that are altered in a cell-specific manner during the course of DPN so that targeted therapies can be developed. One major source is the Cytochromes P450 (CYP) family of enzymes. CYPs enzymes are shown to mediate other diabetic complications. Yet, no studies have investigated their role in DPN. This work investigates two important arms of the family, 20-HETE producing ω-hydroxylases and EET producing epoxygenases. Respectively, we examine the role of CYP4A and CYP2C in DPN in Schwann cells (SCs) and sciatic nerves from a new murine model of Type 2 Diabetes Mellitus (MKR). The data reveal that CYP protein and metabolite alteration results in a state of oxidative stress which was correlated with SC apoptosis, autophagic flux defects, disrupted myelin protein profiles, and behavioral deficits in diabetic animals. The effects of hyperglycemia and CYPs homeostatic disruption were further investigated to identify alterations in AKT and AMPK signaling pathways which are essential for peripheral nerve function and SC myelination and survival. We hypothesized that the pharmacological interception of either CYP4A/20-HETE or CYP2C/EET axes may be promising therapeutic strategy to halt the progression of DPN.

Key words: Diabetic Peripheral Neuropathy, Insulin resistance, 20-HETE, EET, CYP450, AMPK, AKT, Oxidative Stress, Autophagy

Résumé (French)

MARY A. HADDAD POUR DOCTEUR EN PHILOSOPHIE

SPECIALISATION: Neuroscience

TITRE: MECANISMES DES LESIONS DES NERFS PERIPHERIQUES DANS LE DIABETES: ROLE DE LA VOIE CYTOCHROME P450

Le diabète est un problème de santé majeur en raison du spectre des troubles métaboliques associés à son apparition. Plus important encore, il s'agit d'une maladie potentiellement mortelle en raison des complications complexes de plusieurs organes. Parmi les complications microvasculaires associées au diabète, la neuropathie diabétique (DN) est l'une des plus rapportées touchant 50% à 70% des sujets diabétiques. La neuropathie diabétique périphérique (DPN) est caractérisée par un dysfonctionnement des nerfs périphériques. Cliniquement, la DPN est caractérisée par une réduction des enregistrements électrophysiologiques, une perte sensori-motrice et une paresthésie. Les travaux de recherche ont corrélé ces observations avec des lésions des fibres nerveuses conduisant à une dégénérescence, une atrophie axonale, une démyélinisation avec un potentiel de régénération limité. Le stress oxydatif est maintenant considéré comme le médiateur clé commun final, et la surproduction des dérivés actifs de l'oxygène (ROS) a été suggérée pour orchestrer les complications diabétiques, y compris la DPN. Cependant, les mécanismes cellulaires et moléculaires par lesquels le diabète contribue au stress oxydatif dans la DPN restent inconnus. De plus, la thérapie antioxydante non ciblée n'a montré qu'une efficacité limitée. Par conséquent, il existe un besoin critique d'explorer des sources spécifiques de ROS qui sont modifiées d'une manière spécifique à la cellule au cours de la DPN afin que des thérapies ciblées puissent être développées. Une source majeure est la famille d'enzymes Cytochromes P450 (CYP). Il a été démontré que les enzymes CYP interviennent dans d'autres complications diabétiques. Pourtant, aucune étude n'a étudié leur rôle dans la DPN. Ce travail étudie deux branches importantes de la famille, 20-HETE produisant des ω-hydroxylases et EET produisant des époxygénases. Respectivement, nous examinons le rôle de CYP4A et CYP2C dans les cellules de Schwann (SC) de DPN et les nerfs sciatiques extraits d'un nouveau modèle de souris de diabète sucré de type 2 (MKR). Les données révèlent que l'altération des protéines CYP et des métabolites entraîne un état de stress oxydatif qui était corrélé à l'apoptose des SC, à des défauts de flux autophagique, à des profils de protéines de myéline perturbés et à des déficits comportementaux chez les animaux diabétiques. Les effets de l'hyperglycémie et de la perturbation homéostatique des CYP ont été étudiés de plus afin d'identifier les altérations des voies de signalisation AKT et AMPK qui sont essentielles pour la fonction nerveuse périphérique, la myélinisation des SC et la survie. Nous avons donc émis l'hypothèse que l'interception pharmacologique des axes CYP4A / 20-HETE ou CYP2C / EET pourrait être une stratégie thérapeutique prometteuse pour arrêter la progression de la DPN.

Contents

Contents (French)

List of Figures

Tables

Abbreviations

20-HETE	20-Hydroxyeicosatetraenoic acid
AICAR:	5-Aminoimidazole-4-carboxamide ribonucleotide
AMPK:	AMP-activated protein kinase
AUDA:	12-[[(tricyclo[3.3.1.13,7]dec-1-ylamino)carbonyl]amino]-dodecanoic acid
CYP P450	Cytochrome P450 enzyme
DHE	Dihydroethidium
DHET:	Dihydroxyeicosatrieonic
DN/DPN:	Diabetic Neuropathy/ Peripheral Neuropathy
EET:	Epoxyeicosatrienoic Acid
FVB:	FVB/NJ
HbA1c:	Acetylated hemoglobin
HET0016	N-Hydroxy-N'-(4-butyl-2-methylphenyl) formamidine
HPLC:	High Performance Liquid Chromatography
LC3:	Microtubule-associated protein light chain 3
MKR:	FVB-Tg(Ckm-IGF1R*K1003R)1Dlr/J
MSC80:	Mouse Schwann Cells 80
mTOR:	Mammalian Target of Rapamycin
NADPH:	Nicotinamide Adenine Dinucleotide Phosphate
NCV	Nerve conduction velocity
NG/ HG:	Normal/High Glucose
P0 or MPZ	Myelin Protein Zero

PMP22:	Peripheral Myelin Protein 22
ROS:	Reactive Oxygen Species
SC:	Schwann cell
sEH:	Soluble Epoxide Hydrolase
T2D/M:	Type 2 diabetes mellitus

Preamble

Diabetes mellitus is an emerging burden to healthcare systems worldwide especially in the Middle East and North Africa Region. In Lebanon, the prevalence of Type 2 diabetes (T2D) is alarming and in 2015, the International Diabetes Federation listed Lebanon as the leading country with elderly diagnosed with T2D. Meanwhile, France leads the charts among the European Countries with a prevalence of 7.6% among adults, and many cases among children. In fact, T2D, or non-insulin dependent diabetes, is the most widespread form. It is characterized by metabolic perturbation culminating in hyperglycemia or hyperinsulinemia which affects various organs leading to the progression of diabetic complications.

It has been estimated that up to 70% of diabetic subjects will exhibit Diabetic Peripheral Neuropathy (DPN) through the course of their disease despite proper management. DPN is characterized by paresthesia, pain, loss of sensation and motor function that are often asymptomatic until complications are severe at late stages of diabetes. DPN is associated with a pathological reduction in intraepidermal nerve fiber density, axonal degeneration, reduced nerve conduction velocity, hyperalgesia, allodynia and abnormal neurotropism. This disabling complication increases the risk of ulcers, infections, and calluses with subsiding nociception and eventually leads to gangrene and limb amputation.

In the Peripheral Nervous System (PNS), one of the critical contributors to nerve function physiology is myelination. Schwann cells (SC) of the PNS produce the myelin sheath, which is a specialized, insulating layer surrounding nerve axons, responsible for the conductance of electric potentials. Myelin is composed of lipids and proteins both of which are essential for maintaining peripheral nerve function. Extensive research has shown that DPN is characterized by nerve dysfunction triggered by hyperglycemia or dyslipidemia. At the molecular level, like other diabetic complications, a state of oxidative stress is currently recognized as the final common pathway resulting from overproduction of reactive oxygen species (ROS). In DPN, oxidative stress has been reported to mediate injury at the vasa nervorum/vascular endothelia and neurons, but limited studies investigate the effect of oxidative injury on SC physiology and myelination. **This work set out to study the effect of the Cytochromes P450 (CYPs) enzymes as cellular sources of ROS**

and their contribution to the pathophysiology of DPN *in vitro* in SCs and *in vivo* in a diabetic animal model.

CYPs are a large family of enzymes that catalyze various metabolic reactions in the body. We focus on the moonoxygenase class of CYPs which catalyzes the conversion of arachidonic acid into bioactive metabolites via the hydroxylases (CYP4A) and epoxygenases (CYP2C) subfamilies that produce 20-HETE and EET respectively. Our group and others have reported their role in diabetic complications and emphasize their homeostatic imbalance to be pathological. However, their expression has not been elucidated in the PNS or DPN yet. Our experimental approach studies CYP expression in the sciatic nerves of the FVB/MKR mouse model. This model is known to exhibit hyperglycemia and insulin resistance mimicking T2D. **The work targeted the molecular mechanisms of nerve injury associated with CYPs.**

CYP4A and CYP2C protein expression, 20-HETE and EET bioavailability reflecting CYP enzymatic activity, in addition to the oxidative status of the sciatic nerve were examined. This was further correlated with changes in myelin protein levels, autophagy flux key protein markers, and signaling pathways that may exemplify the DPN pathology. Additionally, we further aimed to **investigate the therapeutic power of intervention with pharmacologic agents that modulate CYP activity over early and late stages of diabetes.**

As for the role of glucose in the pathogenesis of diabetes-induced nerve fiber injury, several studies have described injury at the level of neuronal axons as well as the endoneurium through metabolic abnormalities and oxidative stress due to excessive glucose metabolism. However, recent evidence suggests that SCs are primarily targeted in DPN. In this spirit, **we aim to further explore the effect of hyperglycemia and therefore implement the above experiments in Schwann cells (MSC80) as an *in vitro* model.** CYP expression in SCs was explored by screening SC microsomes in normal and hyperglycemic states. Markers of SC injury such as myelin protein alteration, apoptotic death, and autophagy were also assessed.

Finally, we assessed the clinical relevance of the obtained data in human subjects. 20-HETE was analyzed as a potential biomarker of DPN in correlation with clinical parameters of T2D patients. Our data showed that urinary 20-HETE levels were significantly elevated in diabetic patients and this positively correlated with the progression of diabetic neuropathy. **Altogether, this study is**

the first that aims to highlight a link between CYPs and DPN and establish their alteration among the contributors to oxidative peripheral and SC injury.

Préambule (French)

Le diabète sucré est un fardeau émergent pour les systèmes de santé du monde entier, en particulier dans la région du Moyen-Orient et de l'Afrique du Nord. Au Liban, la prévalence du diabète de type 2 (DT2) est alarmante et en 2015, la Fédération internationale du diabète a classé le Liban comme le premier pays avec des personnes âgées diagnostiquées avec un DT2. Parallèlement, la France est en tête des classements parmi les pays européens avec une prévalence de 7,6% chez les adultes et de nombreux cas chez les enfants. En fait, le DT2, ou diabète non insulino-dépendant, est la forme la plus répandue. Elle se caractérise par une perturbation métabolique aboutissant à une hyperglycémie ou une hyperinsulinémie qui affecte divers organes conduisant à la progression des complications diabétiques.

Il a été estimé que jusqu'à 70% des sujets diabétiques présenteront une neuropathie diabétique périphérique (DPN) au cours de leur maladie malgré une prise en charge appropriée. La DPN est caractérisée par des paresthésies, des douleurs, une perte de sensation et de fonction motrice qui sont souvent asymptomatiques jusqu'à ce que les complications soient sévères aux stades avancés du diabète. La DPN est associée à une réduction pathologique de la densité des fibres nerveuses intraépidermiques, une dégénérescence axonale, une vitesse de conduction nerveuse réduite, une hyperalgésie, une allodynie et un neurotropisme anormal. Cette complication invalidante augmente le risque d'ulcères, d'infections et de callosités avec diminution de la nociception et conduit éventuellement à la gangrène et à l'amputation des membres.

Dans le système nerveux périphérique (SNP), l'un des contributeurs essentiels à la physiologie de la fonction nerveuse est la myélinisation. Les cellules de Schwann (SC) du SNP produisent la gaine de myéline, qui est une couche isolante spécialisée entourant les axones nerveux, responsable de la conductance des potentiels électriques. La myéline est composée de lipides et de protéines qui sont, tous deux, essentiels au maintien de la fonction nerveuse périphérique. Des recherches approfondies ont montré que la DPN est caractérisée par un dysfonctionnement nerveux déclenché par une hyperglycémie ou une dyslipidémie. Au niveau moléculaire, comme d'autres complications diabétiques, un état de stress oxydatif est actuellement reconnu comme la voie commune finale résultant de la surproduction des dérivés actifs de l'oxygène (ROS). Dans la DPN,

il a été rapporté que le stress oxydatif induit des lésions au niveau du vasa nervorum / de l'endothélium vasculaire et des neurones, mais des études limitées étudient l'effet de la lésion oxydative sur la physiologie et la myélinisation SC. **Ce travail vise à étudier l'effet des enzymes Cytochromes P450 (CYPs) en tant que sources cellulaires de ROS et leur contribution à la physiopathologie du DPN *in vitro* dans les SC et *in vivo* dans un modèle animal diabétique**

Les CYP sont une grande famille d'enzymes qui catalysent diverses réactions métaboliques dans le corps. Nous nous concentrons sur la classe moonoxygénase des CYP qui catalyse la conversion de l'acide arachidonique en métabolites bioactifs via les sous-familles hydroxylases (CYP4A) et époxygénases (CYP2C) qui produisent respectivement 20-HETE et EET. Notre groupe et d'autres ont rapporté leur rôle dans les complications du diabète et soulignent que leur déséquilibre homéostatique est pathologique. Cependant, leur expression n'a pas encore été élucidée dans le PNS ou le DPN. Notre approche expérimentale étudie l'expression du CYP dans les nerfs sciatiques du modèle de souris FVB / MKR. Ce modèle est connu pour présenter une hyperglycémie et une résistance à l'insuline imitant le DT2. **Les travaux cible les mécanismes moléculaires des lésions nerveuses associées aux CYP.**

L'expression des protéines CYP4A et CYP2C, la biodisponibilité du 20-HETE et de l'EET reflétant l'activité enzymatique du CYP, en plus du statut oxydatif du nerf sciatique, ont été examinées. Cela était en outre corrélé avec les changements dans les niveaux de protéines de myéline, les marqueurs de protéines clés du flux d'autophagie et les voies de signalisation qui peuvent illustrer la pathologie DPN. **De plus, nous avons également étudié le pouvoir thérapeutique de l'intervention avec des agents pharmacologiques qui modulent l'activité du CYP au cours des stades précoces et tardifs du diabète.**

Quant au rôle du glucose dans la pathogenèse des lésions des fibres nerveuses induites par le diabète, plusieurs études ont décrit des lésions au niveau des axones neuronaux ainsi que de l'endonèvre par des anomalies métaboliques et un stress oxydatif dû à un métabolisme excessif du glucose. Cependant, des preuves récentes suggèrent que les SC sont principalement ciblés dans le DPN. **Dans cet esprit, nous visons à explorer davantage l'effet de l'hyperglycémie et donc à mettre en œuvre les expériences ci-dessus dans des cellules de Schwann (MSC80) en tant que *modèle in vitro*.** L'expression du CYP dans les SC a été explorée en criblant des microsomes SC

dans des états normaux et hyperglycémiques. Les marqueurs de lésions SC telles que l'altération de la protéine myéline, la mort apoptotique et l'autophagie ont également été évalués.

Enfin, nous avons évalué la pertinence clinique des données obtenues chez des sujets humains. Le 20-HETE a été analysé en tant que biomarqueur potentiel de la DPN en corrélation avec les paramètres cliniques des patients atteints de DT2. Nos données ont montré que les taux urinaires de 20-HETE étaient significativement élevés chez les patients diabétiques, ce qui était en corrélation positive avec la progression de la neuropathie diabétique. **Dans l'ensemble, cette étude est la première qui vise à mettre en évidence un lien entre CYP et DPN et à établir leur altération parmi les contributeurs aux lésions oxydatives périphériques et SC.**

I-Introduction

A. Diabetes Overview

According to the International Diabetes Federation (IDF), diabetes mellitus (DM) currently ranks among the top global epidemics and medical challenges of the current century. Diabetes mellitus (DM) is a group of metabolic disorders that lead to the chronic and systemic disturbance in glucose metabolism due to the body's failure to use and/or produce insulin (American Diabetes Association, 2018). The risk factors that drive diabetes are plenty and complex. They include genetic predispositions, a coalition of socioeconomic, demographic and environmental factors, hypertension, obesity, dyslipidemia, and lack of physical wellness. Over time, if left untreated, the metabolic perturbations associated with diabetes are amplified, and target a wide range of organs, with long-lasting effects.

1. Epidemiology

Globally, it has been reported that 463 million adult cases of diabetes were diagnosed in 2019, with the vast majority being of the working-age population. With such a trajectory, the worldwide toll is expected to rise to 578 million by the next turn of the decade, and up to 700 million by 2045, an alarming 51% increase in incidence (International Diabetes Federation, 2019). Along this line, diabetes is considered by the World Health Organization (WHO) as one of the top four priorities in the noncommunicable disease category. That is in view of how serious and debilitating a condition diabetes is. The highest prevalence in high-income countries is noticed in China, India and the United States. As for France, the national prevalence is estimated to be around 7.6% with 3.5 million adults diagnosed, and a high rate of diagnoses among children (International Diabetes Federation, 2019).

However, the strikingly rapid increase in prevalence is imminent in the middle and low-income countries. In a timeframe spanning 25 years, the forthcoming highest increase in prevalence among the IDF regions is expected to be in Africa and MENA, with a 143% and 96% rise in incidence, respectively. In Lebanon, a striking 13% of the population among the adult group, 20-79 years of age, are diagnosed with diabetes. That is 530,000 diabetic patients, and it is approximated that 226,000 patients are undiagnosed (International Diabetes Federation, 2019). By contrast to previous decades, it now appears that this rise is due to a massive upsurge in Type 2 Diabetes

Mellitus (T2DM) cases, paralleled by swift lifestyle urbanization, dietary modifications, rising obesity, physical inactivity, and overall poor quality of life. While T2DM accounts for 95% of diagnosed cases, there is an additional significant fraction of cases with distinct types of diabetes. These include the less common Type 1 DM, targeting children and adolescents under the age of 20, gestational diabetes, and prediabetes, among others.

2. The Many Faces of Diabetes and Clinical Manifestations

Type 1 DM, also known as insulin-dependent diabetes, is characterized by the body's inability to produce insulin due to an autoimmune destruction of pancreatic β-cells. T1DM has a sudden, rapid onset and occurs primarily at a young age. It is a polygenic form of diabetes in the sense that it may be due to a defect in multiple genes. Further studies have linked T1DM occurrence with some viral infections and environmental factors. Clinically, T1DM patients present with excessive thirst, sudden weight loss, frequent urination, lethargy, blurred vision, and insatiable appetite (International Diabetes Federation, 2019). Diagnostic requirements rely on symptomatic occurrence, hyperglycemia, rapidly deficient circulating insulin, almost undetectable C-peptide levels, and positive circulating autoantibodies (O'Neal et al., 2016). The progression rate depends on age in addition to antibody number, specificity, and titer (American Diabetes Association, 2018). To date, its progression is non-preventable, however, it is manageable with a continuous supply of exogenous insulin, glucose monitoring, educated and expert medical support with healthy lifestyle interventions (International Diabetes Federation, 2019).

In addition, there is a subgroup of T1DM that may occur at an older age. Referred to as **LADA (Latent Autoimmune Diabetes of Adulthood)** or **Type 1.5 DM**, this type of diabetes is typically diagnosed in adulthood. However, unlike T1DM, it has a slower course of onset and progression. Clinically symptoms resemble T1DM, and it is often misdiagnosed as T2DM due to overlapping characteristics (Pipi et al., 2014). For instance, LADA presents with insulin resistance or a slight and gradually deficient circulating insulin level, rather than complete, lack of insulin production due to a moderate dysfunction in β-cell function. As for C-peptide levels, they record values between low to normal levels (O'Neal et al., 2016). While T1DM patients are placed on exogenous insulin immediately upon diagnosis, it may take up to 6 months for LADA patients to rely solely on insulin for survival. Like T1DM, LADA is irreversible, and the precise diagnostic measures goes a long way in the treatment of LADA.

Brittle Diabetes is a term used to describe an epiphenomenon of diabetes where patients undergo severe, erratic, and difficult to control glucose instability, even while under constant supervision and medical care. Clinically, brittle diabetes appears as episodes of severe hyperglycemia to severe hypoglycemia or diabetic ketoacidosis (DKA) due to buildup of acidic ketones. It is more prevalent among women with symptoms of delayed gastric emptying, hormonal imbalance and insulin malabsorption (Vantyghem & Press, 2006, Newman & Dinneen, 2019). Patients may end up recurrently hospitalized, in a coma, or never wake up. This encounter is life-threatening. While this form of diabetes is not as common, a subgroup of T1DM patients do experience this often, drastically disrupting daily life. It is still unclear as to what triggers this, but studies have associated it with grave psychological and psychiatric consequences of dealing with the diabetes-related distress. In other terms, the prolonged stressors from family and healthcare professionals on the diabetic individual may have adverse emotional effects triggering feelings of fear, distress, frustration, anxiety, alexithymia, and other personality disorders (Pelizza & Pupo, 2016; 2019). It was thought to be typical of T1DM patients, however, it is now generalized to all form of diabetes as severe episodes of instability that may target any diabetic patient. Physicians, in fact, have refrained from using this term of classification, and instead, treat the phenomenon as one of the serious complications associated with diabetes: aka. Hypoglycemia/DKA. This necessitated the need for insulin pumps in advanced cases, in addition to the presence of a psychiatric assessment and therapy to diabetic individuals as part of the team of experts treating a diabetic patient.

Type 2 DM, previously known as non-insulin dependent diabetes, is the more prevalent form of polygenic diabetes and is characterized by peripheral insulin resistance or a relatively reduced insulin secretion (International Diabetes Federation, 2015). The majority of T2DM individuals exhibit obesity or are overweight, with an overt abdominal body fat accumulation. Furthermore, the risk of developing T2DM rises with age, lack of exercise, and the presence of metabolic disorders such as high blood pressure and dyslipidemia. A family history and ethnicity have also been linked to onset of T2DM. However, the genetic interplay in T2DM has not been well understood. T2DM gradually progresses over time such that it may take years for it to become apparent and diagnosed. As the condition becomes more prominent, blood glucose levels rise in parallel with a deteriorating insulin secretion. By contrast to T1DM, DKA is less spontaneous in T2DM and is usually triggered by stress, infection, or other non-related medicaments. Along this

line, T2DM is manageable and preventable. Although there is no cure to date, intervention and healthy lifestyle changes are encouraged for adequate management. Nevertheless, individuals remain at risk of developing complications and premature death.

Prediabetes, or non-diabetic hyperglycemia, is an intermediary stage prior to the full onset of T2DM. It is characterized by the earliest phases of glucose irregularities. Clinical characteristics include glycemic indexes that are above normal but below the diagnostic criteria of diabetes **(Figure 1)**. Two important phenomena mark the development of prediabetes: Impaired Fasting Glucose (IFG) and Impaired Glucose Tolerance (IGT). IGT is estimated after patients ingest a glucose-load during an oral-glucose tolerance test and recording blood glucose values with values being above normal on both, 8-hours fast and 2-hours post-test. Whereas IFG is diagnosed when at-least fasting plasma glucose levels are above normal. The progression from these stages to T2D depends on the severity of hyperglycemia and risk factors that include age and weight. In fact, prediabetes is the stage that signifies the likelihood of T2D development and associated complications especially cardiovascular insults (International Diabetes Federation, 2019).

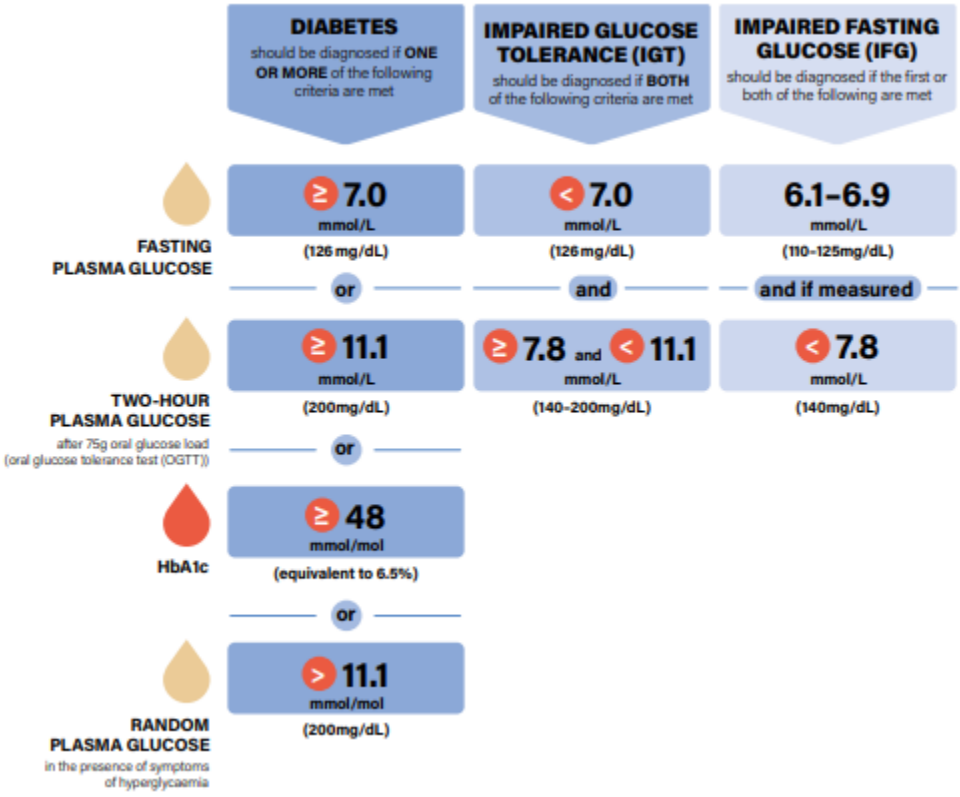


Figure 1. Summary of diabetes diagnostic criteria.

Maturity-onset Diabetes of the Young, or MODY, is a rare, strong form of familial diabetes whereby one of the parents has had diabetes passed down in at least two generations. It is an autosomal, dominant monogenic form of diabetes caused by a mutation in one out of six gene loci. Consequently, clinical manifestations may vary depending on the genetic defect inherited by the individual, and so as their mode of treatment. Some may develop hyperglycemia, others may have glucose levels that are relatively high yet stable, and others may be asymptomatic (National Institute of Diabetes and Digestive and Kidney Diseases, 2019). The unpredictability necessitates routine tests and genetic counseling. The three most common genetic abnormalities are mutations in hepatic nuclear factor HNF-1α, HNF-4α and the glucose sensor of β-cell, the Glucokinase enzyme. Patients with glucokinase-MODY usually exhibit stable hyperglycemia that is often mild, and thus rarely require intervention. The HNF-MODY's first line of therapy is sulfonylureas. Other mutations occur in transcription factors such as HNF-1β, IPF-1 and NeuroD1 (American Diabetes Association, 2018). Since it is inherited, diagnosis may be carried out prior to the development of symptoms via genetic testing of blood or saliva samples. As other forms of diabetes, MODY individuals may be prone to complications apart from asymptomatic patients.

Similarly, **Neonatal Diabetes (NDM**) is another form of monogenic diabetes, but it occurs in infants between 6 and 12 months of age. Infants with NDM may exhibit restricted growth in utero, which may be an underlying indicator of NDM. In most cases, it is temporary. However, half of the infants tend to have the condition permanently. In those who get NDM transiently, they are at a risk of redeveloping diabetes at a later stage in life (National Institute of Diabetes and Digestive and Kidney Diseases, 2019). Like MODY, NDM also has a range of genes likely to be affected. Permanent NDM is associated with mutations that target the β-cell K_{ATP} channels, INS gene, and others that are dominantly inherited. Therefore, correct and accurate diagnosis is critical in these cases for proper intervention (American Diabetes Association, 2018).

Gestational Diabetes (GDM) is a temporary form of diabetes that occurs during the second or third trimester of pregnancy, after ruling out pre-existing or undiagnosed diabetes. According to the International Diabetes Federation, 1 in 6 births are affected by GDM. Importantly, there is an added risk of developing postpartum T2DM. Therefore, women are required to test for prediabetes or diabetes in the following cases: 1- prenatally in the case of having risk factors, or 2- lifelong screening after delivery had GDM appeared during pregnancy (American Diabetes Association, 2018). After delivery, T2DM may be expected to reappear 5-10

years later. Thus, there is an added risk of imposing complications upon the pregnant mother as well as the fetus. That is due to the fact that insulin does not cross the placenta, unlike glucose. This consequently harms the fetus such that their pancreas undergoes compensatory insulin production to clear the exogenous glucose, which would then be largely stored as fat. The infant may be delivered macrosomial upon birth with a range of associated health problems such as hypoglycemia, breathing problems, and shoulder damage. In addition to that, the infant may be at risk of obesity and T2DM as adults (International Diabetes Federation, 2019).

Double Diabetes is a complicated condition first described in 1991. DD is when a diabetic patient develops a combination of T1DM and T2DM. This patient population is not well understood however research underscores the involvement of obesity in a complex interaction with high daily doses of insulin, a strong family history of T2DM, and a low glucose disposal rate (Cleland et al., 2013; Kaul et al., 2015). Other factors that feed insulin resistance in T1DM include fatty liver disease, hormonal disorders, age, and high lipid profiles. In that manner, T1DM individuals may develop insulin resistance, or T2DM may develop autoantibodies against their own β-cells in a theory known as Fertile Field. These individuals are at a significant high risk of coronary disease and hypertension. Current trials are investigating Metformin as an insulin-sparing agent as well as weight loss methods in these patients with careful attention to hypoglycemic risks (Faichney & Tate, 2003; Vella et al., 2010). In addition to that, targeted insulin therapy (for example targeting hepatic tissue rather than systemic) as a therapy is to be assessed in early trials too (Cleland et al., 2013). This condition provides evidence for the importance of understanding glucose and lipid metabolism and interaction.

Secondary Diabetes refers to diabetes that is linked to other underlying conditions. Some conditions reported include endocrinopathies such as thyroid dysfunction, pituitary malfunction and adrenal diseases (Resmini et al., 2009). This type of diabetes is associated with hormonal disorders that lead to the development of impairments in glucose tolerance or peripheral insulin resistance such as pheochromocytoma (Duncan et al., 1944) and Cushing Syndrome characterized by alarming cortisol levels and acromegaly, respectively (Mazziotti et al., 2017). In women, Polycystic ovary syndrome (PCOS) is a major contributor to secondary diabetes (Talbott et al., 2007). Further ailments include diseases that target pancreatic cells such as cystic fibrosis (Laguna et al., 2010), hemochromatosis (Mitchell & McClain, 2014), chronic pancreatitis (Larsen et al.,

1993, Hart et al., 2016), and pancreatic cancer (Magruder et al., 2011). The management of secondary diabetes depends on the respective therapeutic practices specific to each condition.

Diabetes Insipidus is a rare form of diabetes that may be mischaracterized as diabetes mellitus due to overlapping symptoms. This form of diabetes is associated with kidney malfunction in which the diabetic subject passes abnormal amounts of odorless and dilute urine. Clinically, they identify with the need to consume large amounts of liquids. However, unlike diabetes mellitus, patients of this category are normoglycemic yet exhibit defects in renal function (National Institute of Diabetes and Digestive and Kidney Diseases, 2019).

B. The Challenges: Multiorgan Diabetic Complications

1. Non-conventional

The consequences of diabetes are many and culminate in complications that largely influence the living standards of individuals and their direct community. Among the non-conventional complications that have been recently annexed with diabetes lies the features associated with **Type 3 diabetes (T3D)**. Although it is not officially recognized by health organizations as a class of diabetes, T3D has been proposed to be the 'sporadic' onset of neurodegeneration that is like that of Alzheimer's disease. In the past decade, a rising body of research lend support to 'common grounds' where pathological similarities underlying Alzheimer's disease and diabetes meet. Alterations in insulin-like growth factor and insulin signaling have shown pathophysiological effects on neuronal function and plasticity which exert adverse effects on their survival, metabolism, and genetic regulation of important cellular events. These are reflected through the formation of aggregated protein tangles that form lesions and extracellular plaques in the neural network which eventually leads to the loss of neural fibers (Blázquez et al., 2014). More importantly, it has been shown that at the metabolic level, cerebral energy production and utilization is defected leading to a state of starvation in the brain. Overtime, memory, cognition, and plasticity of the brain deteriorate as insulin/insulin-like growth factor receptor binding is reduced, and deficient insulin responsiveness ensues. From this standpoint, memory and cognitive defects as seen in Alzheimer's disease are proposed as Type 3 Diabetes. In fact, patients with overt Alzheimer's disease show innate susceptibility to insulin resistance (Gaspar et al., 2016). At the same time, patients with diabetes are increasingly shown to develop cognitive impairments. This line of research is still growing and subject to additional

investigations. In addition to that, diabetes brings about swift structural and functional changes to the brain. Clinical assessments of the brains of diabetic patients via magnetic resonance imaging identified reductions in brain volume, atrophy, and small-vessel disease. These changes occur in phases over time depending on the onset of diabetes and age, and in advanced stages, neural functional connectivity becomes compromised which manifests as cognitive decline (Biessels and Reijmer, 2014).

Another non-conventional complication that manifests along the emotional side of dealing with diabetes is **depression**. Clinical, major depression has been reported among T2D patients with adverse effects that exacerbate the risk of other complications such as advanced cardiovascular events and death (Lin et al., 2010). Complications that contribute to blindness and kidney disease were also significantly associated with depression (Ahmadieh et al., 2018). Early studies suggest the existence of both behavioral, psychological, and biological factors at play, primarily due to the daily stressors of managing diabetes or depression (Fisher et al., 2007; Katon et al., 2009). More importantly, the relationship between the occurrence of either pathologies has been dubbed bidirectional since both tend to develop impaired glycemia (Lin et al., 2010). However, the molecular connection between both diabetes and depression remains elusive.

Finally, **cancer** has also recently been added as one of the complications of diabetes. In fact, cancer rates have been reported to be higher in diabetic subjects (Gallagher & LeRoith, 2015) and to be associated with high mortality risk (Lopez et al., 2006). Additionally, this complication may affect subjects of both T1D and T2D (Gordon Dseagu et al., 2013; Carstensen et al., 2016). Increasing research correlates diabetes with the onset of many cancers that target organs such as the stomach, intestinal tract, urinary tract, and kidney (Stattin et al., 2007; Larsson & Wolk., 2011) as well as organs that strongly impact the diabetic state such as cancers of the pancreas and liver (Johnson et al., 2012). Ongoing studies examine inflammation, hyperinsulinemia, and hyperglycemia as key triggers of the ailments (Giovannucci et al., 2010; Ryu et al., 2014; Asterholm et al., 2016).

2. Conventional

Persistent hyperglycemia is implicated in macrovascular and microvascular complications provoking injuries across the entire body (American Diabetes Association, 2018). Cardiovascular disorders, heart failure, myocardial infarction, dyslipidemia, hypertension in addition to skin complications arise from damage to the macrovasculature. Consequently, diabetic subjects are at

an alarming risk for developing cerebrovascular disease that appears in the form of stroke with long-lasting effects or a transient ischemic attack that does not leave permanent damage. They may also develop peripheral arterial disease that appears in the form of intermittent claudication. In other words, they develop symptoms such as muscle pain, aches, cramps, and fatigue especially in the lower limbs of the patient's body (Lozano et al., 2014).

As for complications contributing largely to the morbidity of DM, microvascular injury manifests as nephropathy, retinopathy, and neuropathy that tend to occur collectively, and are therefore referred to as the triopathies (DCCT, 1993; Ali et al., 2013).

Diabetic nephropathy is a common complication between T1D and T2D and affects around 40% of all diabetic patients. It is the leading cause of end-stage renal disease (ERSD) (Mori et al., 2009), the number one cause of kidney transplantation, and it is among the leading causes of cardiovascular mortality (Bethesda, 2003; Gross et al., 2005; Valmadrid et al., 2000). Clinically, diabetic nephropathy is marked by the presence of more than 0.5g of protein/24 hours in the urine, which is referred to as proteinuria or macroalbuminuria. However, urinary microalbuminuria, a small quantity of albumin that cannot be detected by conventional methods, is also highly predictive of the development of advanced stages of nephropathy. Screening for urinary albumin excretion typically begins 5 years after the patient is diagnosed with T1D and immediately after T2D is diagnosed. Some patients may also exhibit reduced glomerular filtration rate (Adler et al., 2003; Caramori et al., 2003; MacIsaac et al., 2004). These functional changes are paralleled by diabetes-induced unique and characteristic changes in the structure of the kidneys. Analysis of diabetic kidneys shows an increase in the thickness of the glomerular basement membrane, hyaluronosis, microaneurysm, hyaline arteriosclerosis and mesangial fibrosis with tubular and interstitial changes (Brito et al., 1998; Katz et al., 2002; Kimmelstiel & Wilson, 1936; Mauer, Steffes, & Brown, 1981). The therapies available so far are controlling blood glucose levels and blood pressure through the inhibition of the renin-angiotensin system (RAS) or the sodium-glucose co-transporter 2 (SGLT2), which are thought to delay the onset and progression of nephropathy but have no benefit in reversing the injury (Cooper, 1998; Mori et al., 2009; Parving et al., 2001).

Diabetic retinopathy is the most common cause of blindness in adults of working age. It has an especially poor prognosis and treatment outcome. Importantly, it is an understudied complication of diabetes and the mechanism through which hyperglycemia causes damage in retinal tissue is not well understood yet (Ding & Wong, 2012; Lee et al., 2015; Sabanayagam et

al., 2016). Briefly, it involves changes in the microvasculature that lead to defects in the neural components of the retina (Lott et al., 2012; Lott et al., 2013). The microvascular component of diabetic retinopathy is characterized by macular edema and the formation of new blood vessels in the retina. The injury progresses in stages that begin with the atrophy of blood vessels. This is followed by endothelial cell junction weakening and basement membrane thickening that compromises the blood-retinal barrier. Altogether, these lead to alterations in blood supply of the retinal tissue, which leads to localized hypoxia and increased cellular secretion of a cocktail of growth factors (Ciulla et al., 2003; Ding & Wong, 2012). Local hyperpermeabilization of the vascular system and the secretion of angiogenic factors induce the formation of new blood vessels, also known as neovascularization, which is one of the hallmarks of retinopathy (Gilbert et al., 1998; Hammes et al., 1998; Witmer et al., 2002). This induces damage to endothelial cells and the pathology starts to become symptomatic. Newly formed capillaries are non-perfused and acellular leading to the increased leakage of plasma fluid into the eyes. This leads to edema that can impair the central vision (Ciulla et al., 2003; Ding & Wong, 2012; Yorston, 2014). As for the neural component of the pathology, there is a significant increase in retinal cell apoptosis and a reduction in the number of retinal ganglion cells or other types of neurons like cholinergic and dopaminergic amacrine cells triggered by diabetes (Barber et al., 2005; Martin et al., 2004; Ning et al., 2004, Gastinger et al., 2006). These changes in retinal nervous tissue precede the development of retinal vascular disease, as evidenced by the thinning of retinal nerve fiber layer in diabetic patients proportionate to fasting blood glucose levels (Takahashi et al., 2006, Peng, Lin, & Lin, 2009). Finally, neurodegenerative changes in the synapses and morphology of retinal neurons mark the final stages of diabetic retinopathy such that, if chronic, hyperglycemia may render neurodegenerative changes irreversible (Masser et al., 2014). Unfortunately, there are no drugs to date that target the neural pathology of diabetic retinopathy.

Finally**, Diabetic neuropathy (DN)** is the complication that is most frequently reported in clinical practice and is due to nerve damage that is brought about by diabetes and its metabolic dysregulation. In fact, the risk of DN rises with prolonged duration and disease severity (World Health Organization, 2016). In the nervous system, diabetes has been shown to hold various pathological outcomes demonstrated at the level of the central (CNS) and peripheral (PNS) systems. Manifestations of diabetes in the CNS include cognitive impairment, memory deficits and ischemia (Moran et al., 2013; Roberts et al., 2014; Gold et al., 2007; Zheng et al., 2017;

Pugazhenthi et al., 2017; Shukla et al., 2017). However, diabetes-induced nerve dysfunction in the PNS manifests affecting the visceral organs and peripheral innervations. DN clinically presents with heterogeneous symptoms of nerve dysfunction which include tingling, numbness, sensorimotor wasting, loss of proprioception, pain, and bone structure alteration in addition to manifestations associated with several disorders affecting the viscera such as neurogenic bladder, orthostatic hypotension and gastrointestinal disturbances. As such, DN can be categorized into peripheral, autonomic, proximal, and focal neuropathy (Tesfaye et al., 2010; Callaghan et al., 2012). Unfortunately, these diabetic complications have no direct treatment to date other than symptomatic relief of pain, and glucose management although onset remains of high risk even after glycemic control is achieved.

C. Diabetic Neuropathy

1. Classification

Diabetic neuropathies may be categorized into focal or diffuse neuropathies. On the one hand, several atypical forms of diabetic neuropathy have been identified. **Focal Neuropathies**, also known as a mononeuropathy, elicit injury to a single nerve, cranial or peripheral nerves, mostly affecting head, wrist thigh, foot, or torso nerves (National Institute of Diabetes and Digestive and Kidney Diseases, 2018). Most common mononeuropathies affect the peroneal, median, ulnar, and radial nerves. Others may affect isolated cranial nerves especially ocular nerves. These neuropathies often have a sudden nerve entrapment, and typical interventional methods involve decompression surgery (Pop-Busui et al., 2017). Mononeuritis multiplex is a rare form of mononeuropathy and involves damage to at least two peripheral nerves in isolated and different areas of the body at the same time, leading to asymmetric, painful neuropathy in patients susceptible to non-systemic vasculitis, or connective tissue disease (Kelkar & Parry, 2003). It is distinct from polyneuropathy in which multiple peripheral nerves are affected within a restricted area in the body. As for multifocal neuropathy, they are extremely rare where multiple motor nerves (mainly cranial nerves) are attacked by one's immune system and muscles gradually weaken to the point of atrophy (Said, G., 2007). The second branch of atypical neuropathies is diabetic radiculopathy which is further anatomically distributed in the thoracic, cervical, or lumbosacral plexus. Symptoms appear asymmetrically with acute or subacute onset, in addition to pain. This form of neuropathy is pathologically distinct from the peripheral neuropathies as is often

associated with rapid yet significant weight loss in T2D individuals. A major attribute of radiculopathies is that they have a clear and noticeable onset especially distally, unlike diffuse neuropathies that are gradually progressing from the proximities. In the thoracic presenting radiculopathy, a marked symptom is the band-like pain radiating from the dorsal side anteriorly towards the chest wall (Laughlin & Dyck., 2014).

By contrast, diffuse neuropathies target the whole body and multiple nerves. Diabetic Peripheral Neuropathy (DPN) and Diabetic Autonomic Neuropathy (DAN) fall within this category. Briefly, **autonomic neuropathy** affects the autonomic nervous system (parasympathetic and sympathetic neurons) and therefore manifests in a wide range of symptoms that are site-specific. The major classes are attributed to the cardiovascular, gastrointestinal, and urogenital systems. The clinical appearance of this type of neuropathy includes hypoglycemia, tachycardia, sudden arrhythmia, enteropathy or colonic hypomotility, gastroparesis, neurogenic bladder, as well as sexual and thermoregulatory dysfunction (Jensen et al., 2014; Pop-Busui et al., 2017). In addition to that, a separate form of neuropathy that is often attributed to autonomic neuropathy is treatment-induced neuropathy. Previously termed insulin neuritis, treatment-induced neuropathy is characterized by acute small-fiber painful neuropathy and degeneration (Gibbons & Freeman, 2015). It remains poorly understood, however, its onset has been linked to a slight improvement in glycemia in response to chronic hyperglycemia. This condition presents with pain and autonomic symptoms that may happen after insulin administration or upon achieving sudden improvements in glucose control. In this manner, it is considered iatrogenic, in other words, secondary to a medical intervention or treatment (Pop-Busui et al., 2017).

On the other hand, this work is centered around the most common type of neuropathy among diabetic patients which is **Diabetic Peripheral Neuropathy** (DPN). DPN is defined by the Toronto Expert Panel on Diabetic Neuropathy as diabetes-induced metabolic or micro-vessel changes brought about by hyperglycemia or 'cardiovascular risk covariates' that result in peripheral nerve damage (Tesfaye et al., 2011). Risk factors that play a role in the complexity and severity of DPN include elevated HbA1c levels, lifestyle (smoking, alcohol consumption, height, and age), diet, obesity, lipid, and blood pressure indexes (Vincent et al., 2009; Feldman et al., 2019). Moreover, clinical manifestations of DPN are characterized based on symptomatic appearance. Positive symptoms include paresthesia and pain while negative symptoms include sensitivity to non-noxious stimuli (allodynia) and intensified sensitivity to harmful stimuli

(hyperalgesia). The pathology is further exacerbated along the progression of the disease by the loss of overt sensorimotor function, muscle, and limb weakness (Andreassen et al., 2009). The incidence of DPN is often asymptomatic until complications are severe at late stages of diabetes (Genuth, S. 2006; Ziegler et al., 2014; Zhang et al., 2014). Therefore, rigorous clinical examinations are critical for the early detection of DPN. DPN affects small and large myelinated and small unmyelinated fibers in a length-dependent, symmetric, or asymmetric manner and progresses from the extremities proximally in a 'stocking glove' pattern. The predominant form of DPN is Distal Symmetric Polyneuropathy (DSPN) which is associated with a reduced intraepidermal nerve fiber density, axonal degeneration, reduced nerve conduction velocity, hyperalgesia, allodynia and abnormal neurotropism **(Figure 2)**. DSPN is a disabling complication that increases the risk of ulcers, infections and calluses with subsiding nociception and eventually leads to gangrene and limb amputation. Although the pathophysiology of DSPN is poorly understood, an increasing body of data has shown the effect of diabetes on metabolic and vascular interactions. The long-standing consensus for DPN management involves rigorous glycemic control and symptomatic relief (Rizza et al., 1985; Genuth, S. 2006). Nevertheless, the risk of microvascular complications persists even after optimal management (DCCT 1995; UKPDS 1998). Thus, further investigations are sine qua non for a better understanding of the cellular mechanisms involved in the pathogenesis of diabetic neuropathy.

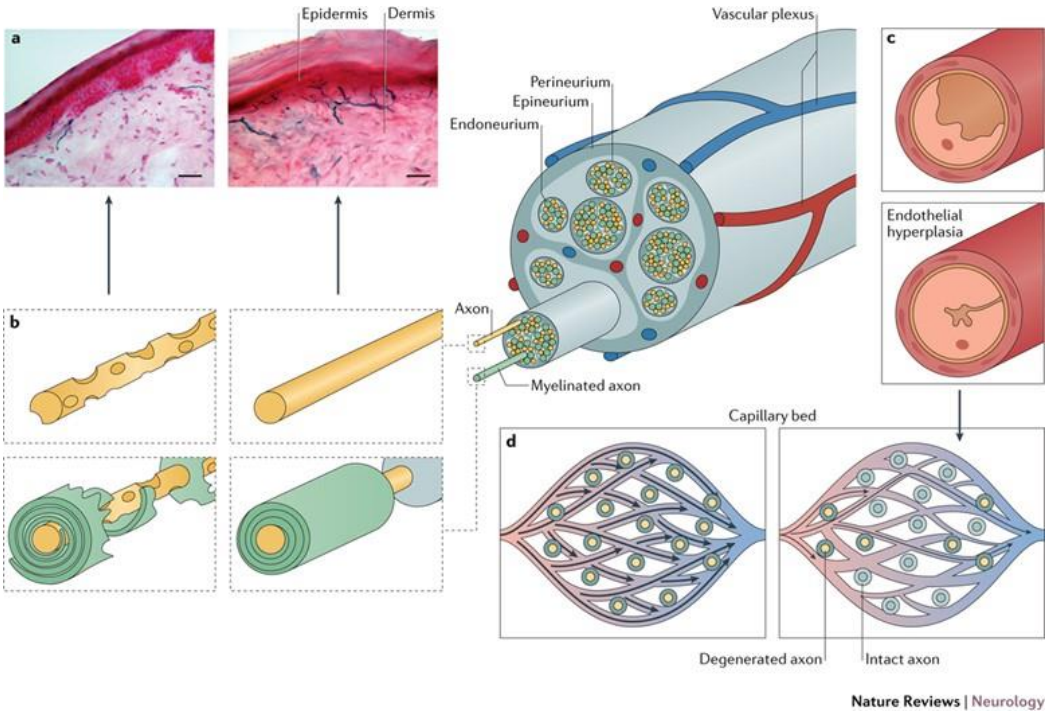


Figure 2. Overview of the axes in diabetic neuropathy pathogenesis. Complexity of nerve injury at the Schwann cell, axonal, and microvasculature levels. A) Represents intraepidermal fiber loss in diabetic subject (left) vs intact nerve fiber crossing in normal subject (right) B) Represents Schwannopathy and axonopathy, culminating in nerve fiber degeneration (left). C) Represents normal (top) vs. diabetic endoneurial capillary perfusion (bottom). Endothelial cell hyperplasia and basement membrane thickening lead to a marked reduction in capillary size resulting in D) hypoxia in capillary beds (right). (Panel a courtesy of Dr Páll Karlsson, Danish Pain Research Center, Department of Clinical Medicine, Aarhus University, Denmark.)

2. Myelinating Schwann Cells in the Peripheral Nervous System

i. *Physiological roles in the PNS*

The PNS is a remarkable tissue and functions to transport important information to and from the CNS. With input from cranial and spinal nerves, neurons and glial cells come together to transduce signals via efferent axons of motor neurons from the brain to the peripheral tissues. On the other hand, sensory information is carried from the periphery via afferent axons of sensory neurons towards the CNS. There are two main types of neurons in the PNS: Sensory and motor. Sensory neurons are fed stimuli from sensory receptors and are located beyond the blood nerve

barrier, unlike motor neurons which are located within. Furthermore, PNS nerves may be unmyelinated or myelinated fibers. Small unmyelinated axons (C-fibers) are wrapped by non-myelinating SCs, and typically branch throughout the autonomic nervous system carrying thermal and noxious stimuli. The proportion of unmyelinated fibers is much higher than myelinated fibers, which are often larger in size. Of intermediary size, Aδ fibers are small, myelinated fibers that function for pain perception, in addition to proprioceptive stimuli transmission from the periphery. Ganglia, neurons, and Schwann cells (SCs) are primary targets of metabolic dysfunction (Freeman et al., 2015). In this work, we limit the scope of the investigation on the sciatic nerve representative of the peripheral nerves as well as SCs which are thought to be primarily prone to metabolic injury in DPN.

Neural crest-derived Schwann cells (SCs) are the myelin-producing cells in the PNS. SCs play a prominent role in nerve - axon interactions, and in the regeneration, support and growth of nerve fibers. During PNS development SC division is halted in response to neuregulin, which then triggers myelination and the spiral enwrapment of SCs around axons (Michailov et al., 2004; Schulz et al., 2014). The myelin sheath is a specialized, multi-layered and insulating extension of SC membranes that engulfs a nerve axon to produce a myelinated fiber (Sherman, D. L., & Brophy, P. J. 2005). The Node of Ranvier is an unmyelinated segment where two SCs meet. It facilitates the conductance of electric potentials along nerve fibers via saltatory conduction (Garbay et al., 2000). More importantly, myelin integrity is critical for SC and nerve function. Myelin is composed of lipids (71%) and proteins (29%) that are critical for the physiology of neurons (Norton, W. T., & Cammer, W. 1984; Inouye, H., & Kirschner, D. A. 1988). Myelination is a tightly regulated process, and it is facilitated by the collaborative work of several myelin proteins. Namely, Myelin Protein Zero (P0), peripheral myelin protein 22 (PMP22), P1, P2 and myelin-associated glycoprotein (MAG) constitute the protein component and function at different stages during the myelination process and contribute to maintaining the physiology of SCs and their myelin sheaths (Bilbao, J. M., & Schmidt, R. E. 2015).

Myelin Protein Zero (P0) is the predominant protein (60%) of the PNS produced solely by SCs **(Figure 3)**. P0 is described to be a 30KDa transmembrane, adhesion protein involved in the compaction of myelin through interactions between its extracellular and cytoplasmic domains (Giese et al., 1992; Suter, U., 1997). Defects in P0 expression have been implicated in disorders due to loss of compaction such as in dysmyelinating neuropathies (Hayasaka et al., 1993; Latour

et al., 1995; Shy, ME., 2006). However, it has been reported that altered P0 expression does not lead to complete dysmyelination which suggests that P0 works in collaboration with other myelin proteins to maintain the myelin sheath's molecular architecture (Giese et al., 1992).

Peripheral Myelin Protein (PMP22) is another myelin protein **(Figure 3)** exclusive to the PNS whose expression is concomitant with myelin production by SCs (Snipes et al., 1992; Jetten, A. M., & Suter, U. 2000). PMP22 mutations in humans have been associated with genetic disorders such as Charcot-Marie-Tooth and inherited neuropathies (Katona et al., 2009). Studies conducted in transgenic animal models have reported that mutations in the PMP22 gene during development affect SC differentiation, myelination, and may lead to SC apoptosis (Robertson et al., 1999; Niemann et al., 2000; Sahenk et al., 2003; Jun et al., 2013). The importance of PMP22 in initiating the myelination process was investigated in PMP22 -/- mice models. Motor nerves obtained from the PMP22 deficient mice showed immature SCs with failed myelin formation (Adlkofer et al., 1995). More importantly, PMP22 is involved in heterophillic interactions with P0 (Hasse, B., & Bosse, F. 2004). Thus, P0 and PMP22 interactions determine the precise arrangement and function of myelin. Together, any alteration in expression of either protein would have direct and detrimental effects on SCs, neuronal function, as well as signal exchange to and from the PNS (D'Urso, D., Ehrhardt, P., & Müller, H. W. 1999).

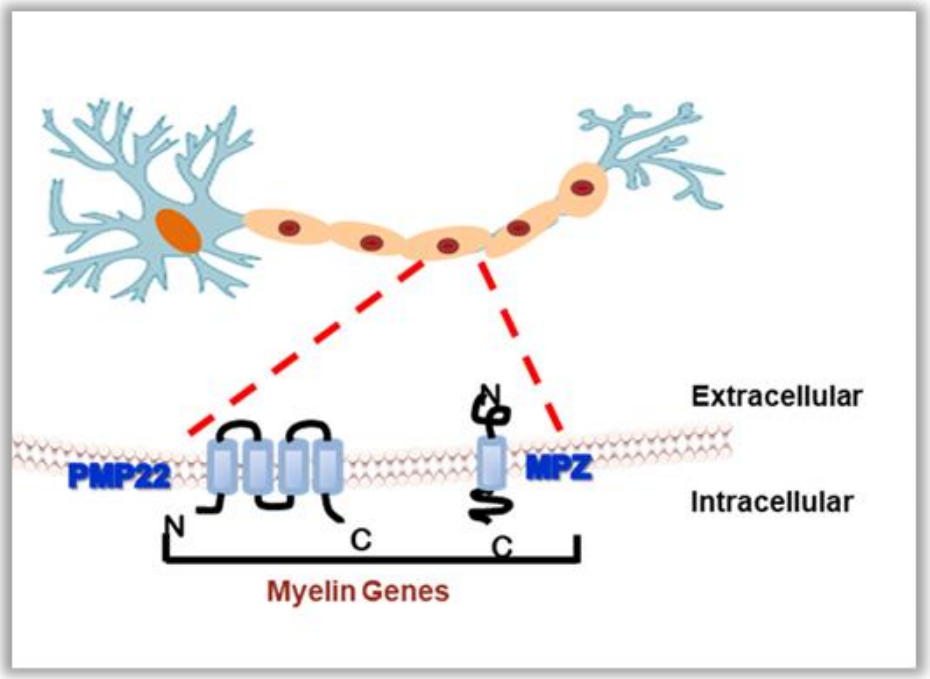


Figure 3. Schematic of important peripheral proteins produced by SCs. Myelin Protein Zero (MPZ) and Peripheral Myelin Protein 22 (PMP22). MPZ is the predominant protein of the PNS. PMP22 is involved in heterophillic interactions with MPZ. Together, they determine the precise arrangement and function of myelin. Any alteration in expression of either protein would have direct and detrimental effects on SCs, neuronal function, as well as signal exchange to and from the PNS (Eid, S. et al., 2020).

ii. *Diabetes and Schwann Cell Dysfunction*

Neural networks in the periphery are complex structures characterized by interactions with surrounding vascular endothelia. The primary source of energy for the PNS is glucose which is processed by SCs and is largely attributed to axonal repolarization (Bradbury, M.W.B., & Crowder, J. 1976). The key pathogenic factor triggering DPN is elevated blood glucose levels (Green et al., 1999). Hyperglycemia provokes injury at the level of blood vessels and capillaries through metabolic abnormalities and oxidative stress due to excessive glucose metabolism (Cameron, N. E., & Cotter, M. A. 1997; Gao et al., 2014, Cinci et al., 2015). Consequently, pathophysiology that affects the vasa nervorum around the endoneurium may affect nerve fibers (King et al., 1989). The PNS tissue has been reported to be insulin-independent and highly

vulnerable to chronic hyperglycemia due to the inefficiency in regulating glucose uptake in contrast to endothelial cells (Leinninger et al., 2006; Hinder et al., 2013). Indeed, mounting evidence suggests that SCs are primarily targeted in DPN (Magnani et al., 1998; Askwith et al., 2009; Chan et al., 2011).

In vitro studies on cultured rat primary SCs reported that hyperglycemia significantly increased apoptosis (Wu et al., 2012; Liu et al., 2016) and reduced proliferative potential (Gumy et al., 2008). Furthermore, recent investigations showed the implications of chronic hyperglycemia in SC de-differentiation due to underproduction of neurotrophic factors (Dey et al.,2013; Hao et al., 2015) showing a state of severe SC dysfunction. Similarly, morphological abnormalities were reported in nerve biopsies from diabetic animal models. Electron microscopy of sciatic nerves from diabetic rats showed disordered myelin sheaths and SCs with shrunken vacuoles corroborating previously reported SC dysfunction (Li et al., 2016). Also, glucose mediated neuronal injury was observed where neurons were described to be hypertrophic with giant vacuoles lining axons of neurons in dorsal root ganglia (DRG) of diabetic rats. SCs surrounding DRG neurons were reported to be loosely bound and misarranged relative to their controls.

Moreover, electrophysiological studies showed biphasic nerve malfunction in diabetic subjects. During the early stages of DPN, small myelinated and unmyelinated fibers are affected which manifests as hyperalgesia (Courteix et al., 1993; Ohsawa et al 2008). By the end stages of DPN, nociception is diminished and marked by reduced nerve conduction velocities (Ishii, D. N. (1995), dysmyelination, impaired and slow axonal transport (Juranek et al., 2013), axonal atrophy and degeneration (Fross, R. D., & Daube, J. R. 1987) in addition to altered sensory and motor potentials (Becker et al., 2014).

D. The Sequelae of Metabolic Imbalance in Diabetic Peripheral Neuropathy

1. Pathobiology and Biochemical Pathways

Extensive research in the field of diabetes come to the consensus that diabetic complications advance because of accumulating metabolic changes that incite pathological pathway alterations that are injurious to tissues. For that reason, diabetes has been dubbed an energy imbalance disease. These pathways revolve around modulating glucose circulation and biogenesis. In DPN, several biochemical pathways have been identified to impact the neural environment. The polyol pathway harvests excess glucose and converts it to sorbitol via aldose reductase, which insults the cell's

osmotic balance and triggers compensatory mechanisms of osmotic stress. In the peripheral nerve, this leads to myoinositol loss which in turn impacts nerve functionality. Additionally, the conversion of glucose to sorbitol depletes important cofactors such as NADPH which are essential for glutathione formation, a scavenger of reactive oxygen species (ROS) (Brownlee, M., 2005; Oates, P. J. 2008). Thus, this makes the polyol pathway among the first that lead to the accumulation of unharvested ROS. In addition to that, nitric oxide synthase enzyme is subsequently uncoupled which unfavorably impacts the endothelium by converting endothelial cells into superoxide producers rather than protective barriers (Hayden & Tyagi, 2004).

The second pathway is the Hexosamine Flux and Protein Kinase C (PKC) Activation. The primary route for glucose metabolism is the glycolytic pathway. When overwhelmed with excess glucose, the intermediary product of glycolysis, glucose-6-phosphate, is shunted into the hexosamine path where it undergoes conversion into N-acetylglucosamine (GlcNac). The GlcNac product is a sugar moiety that is capable of binding to important transcription factors that control inflammation and lipid profiles such as TGF β and others that inflict injury on the vasculature of peripheral nerves (Du et al., 2000). Another product that is the aftermath of excessive glycolysis is diacylglycerol (DAG) which feeds into the PKC path and is known to play a role in insulin resistance and lead to neuronal injury in STZ-induced diabetic animals (Geraldes & King, 2010). Furthermore, excess glucose interacts with proteins to form advanced glycated end products (AGEs) that bind to receptors and influence a series of secondary processes such as inflammatory reactions via NF- κB activation, vasoconstriction, and as a result, neuronal support is compromised (Lukic et al., 2008 Misur et al., 2004). Together, a state of 'global' inflammation is instilled with disrupted cellular bioenergetics. Subsequently, mitochondrial oxidative phosphorylation is uncoupled due to a defected proton gradient across the mitochondrial cristae, resulting in mitochondrial leakage and overload. This yields an overall reduction in ATP synthesis, while maintaining a state of oxidative and nitrosative stress.

Finally, as the glucose and lipid flux amplify, the enormity of damage by the metabolic syndrome aspects (hypertension, hyperlipidemia, defected glucose regulation and insulin resistance) gradually weaken cellular defenses until multiple injurious pathways are affected. Although SCs in particular are resilient and are distinguished by a high anti-oxidant drive, their defenses become overwhelmed under excessive nutrient flux (Vincent et al., 2011). This is further

intensified through reduced ATP synthesis and elevated ROS production, leading to the phenomenon of 'ROS begets ROS' (Fernyhough, 2015).

2. A Unified Front in Oxidative Stress: ROS begets ROS

Reactive Oxygen Species (ROS) are oxygen-containing biologically active molecules that are byproducts of ongoing cellular reactions. ROS are crucial entities for cellular physiology and are involved in gene expression, signal transduction, and homeostatic maintenance (Turpaev, K. T. 2002). Intracellular ROS signaling is well regulated via antioxidant defense mechanisms that neutralize the bioactive radicals (Bursell et al., 1999; Haak et al., 2000; Packer et al., 2001; Xu et al., 2014). Hyperglycemia has been shown to disrupt the oxidant-antioxidant balance by triggering additional ROS production (Cameron, & Cotter, 1999; King & Loeken, 2004; Singh et al., 2014). Several studies showed that blockade of ROS can be beneficial but also in some cases injurious (De Zeeuw et al., 2013). Subsequently, the identification of cellular sources of ROS is central to understanding the pathobiology of diabetes.

Intracellular glucose metabolism is associated with ROS production via glucose autoxidation, mitochondrial oxidative phosphorylation, and the production of advanced glycation end products. Additionally, a number of enzymes have been implicated in hyperglycemia induced ROS production and are reported to include nicotinamide adenine dinucleotide phosphate oxidase (NOX), cytochrome P450 monoxygenase, nitric oxide synthase, lipoxygenase, cyclooxygenase, and xanthine oxidase (Niedowicz & Daleke, 2005). When ROS overwhelm cellular defense responses, injury ensues through lipid and protein oxidation, altered metabolism, activation of intracellular signaling and transport pathways, and ultimately programmed cell death (Vincent et al., 2004).

Extensive research has shown that ROS overproduction poses a pathogenic state in multiple organs and is the common feature to the pathogenesis of diabetic complications (Lambeth et al, 2008; Maalouf et al., 2012; Naziroglu et al., 2012; Eid et al., 2013; Nayernia et al., 2014; Kowluru & Mishra, 2015; Filla & Edwards, 2016). Numerous studies have shown that inhibition of ROS sources ameliorated oxidative stress in diabetes-induced renal, cardiovascular and other system injuries (Lambeth et al, 2008; Eid et al., 2009; Eid et al., 2010; Wu et al., 2012). However, the role of, as well as knowledge of the sources, of ROS and their inhibition in the nervous system are still under investigation (Nayernia et al., 2014; Li et al., 2016). Recent studies described several mechanisms leading to ROS-induced organ damage and in some cases nerve damage. Yet, a lot of

work still needs to be done to elucidate the mechanisms of injury. Increased superoxide dismutase activity (a superoxide radical scavenger) via puerarin was shown to reduce oxidative stress and apoptosis in SCs cultured in high glucose (Wu et al, 2012). Inhibition of aldose reductase of the polyol pathway, known to be upregulated in diabetes, in both diabetic rats treated with epalrestat (Li et al., 2016) and human sural nerves treated with zenalrestat (Greene et al., 1999) showed reduced myelinated nerve fiber loss and SC injury by decreasing oxidative stress.

E. Aiming Higher: The CytochromeP450 Family

Cytochromes P450 (CYP450s) are a large family of hemoproteins responsible for metabolism of endogenous (hormones, cholesterol, fats, steroids, acids, vitamins), and exogenous molecules (toxic compounds and drugs). CYPs **(Figure 4)** have been described to be located bound to membranes of either the mitochondria or endoplasmic reticulum and to be associated with redox reactions catalyzing the oxygenation of their substrates in an NADPH-dependent manner (Rendic & Carlo, 1997; Capdevila et al., 2000). The relative contribution of CYPs to cellular processes is dependent on substrate availability, and their expression and functional roles vary based on cell-tissue-organ type, sex, species and the respective regioisomers produced (Spector & Kim., 2015; Capdevila et al., 2015). Additionally, CYPs are reported to be major sources of ROS (Figure 1) in numerous tissues (Bondy & Naderi, 1994; Puntarulo & Cederbaum, 1998; Fleming et al., 2001; Dunn et al., 2008; Medhora et al., 2008) with implications in diabetic complications (Eid et al., 2009; Eid et al., 2013). In humans, approximately 57 CYP genes have been identified (Guengerich, F. P., 2006); any polymorphism associated with a gene is directly correlated with enzyme dysfunction and a range of disorders (Panda et al., 2001; Nebert & Russell, 2002; Ohtoshi et al., 2005; Wang et al., 2010; Fan et al., 2015).

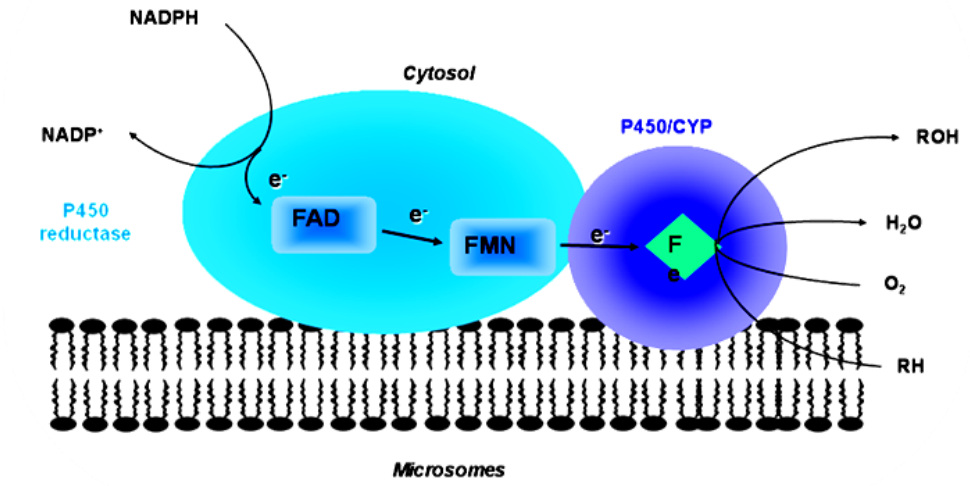


Figure 4. Reactive oxygen species production and redox cycling by CYP monooxygenases. CYPs of the monooxygenase subfamily contain a heme-based catalytic center, an NADPH reductase subunit, and an NADH/NADPH oxidase as cofactors. CYP450s are present in ferric and ferrous forms and the initial chemical reaction that takes place is the binding of a substrate to the ferric form. CYP450 reductase donates an electron to reduce the ferric form to a ferrous intermediate which then binds to an oxygen molecule to form a complex. The complex is reduced by the addition of a proton which is concomitant with H20 production and an oxenoid complex whereby activated oxygen bound to the heme moiety incorporates into the substrate to yield an oxidized form (Johnston et al., 2011; Adapted from Eid et al., 2014).

1. The Arachidonate Cascade

One of the physiologically relevant reactions catalyzed by CYP450 enzymes is arachidonic acid (AA) metabolism. AA, an ω-6 polyunsaturated fatty acid (PUFA), release is induced by the Ca^{+2} activation of phospholipase A2 from membrane phospholipids **(Figure 5).** ω-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) conversion to various metabolites parallels the AA cascade. Altogether, the free AA pool comprised of PUFA, EPA, DHA, and often linoleic acid, may be metabolized by the cyclooxygenase (COX), lipoxygenase (LOX) and moonoxygenase pathways (CYPs). In brief, the better described COXs and LOXs tend to produce a group of metabolites largely associated with inflammatory processes (Morisseau et

al., 2010). COXs produce prostaglandins, prostacyclins and thromboxanes, whereas LOXs produce leukotrienes, lipoxins, and mid-chain hydroxyeicosatetraeinoic acids (HETEs) (Marnett, L. J., 2009; Haeggström et al., 2010). While CYPs remain less known by contrast to COXs and LOXs, recent research is gaining ground in exploring their physiological importance. AA metabolism by CYPs produces both proinflammatory and anti-inflammatory mediators, with an added range of biological processes that are facilitated by their metabolites. Thus, the monooxygenases may be classified as hydroxylases or epoxygenases that yield numerous eicosanoid products.

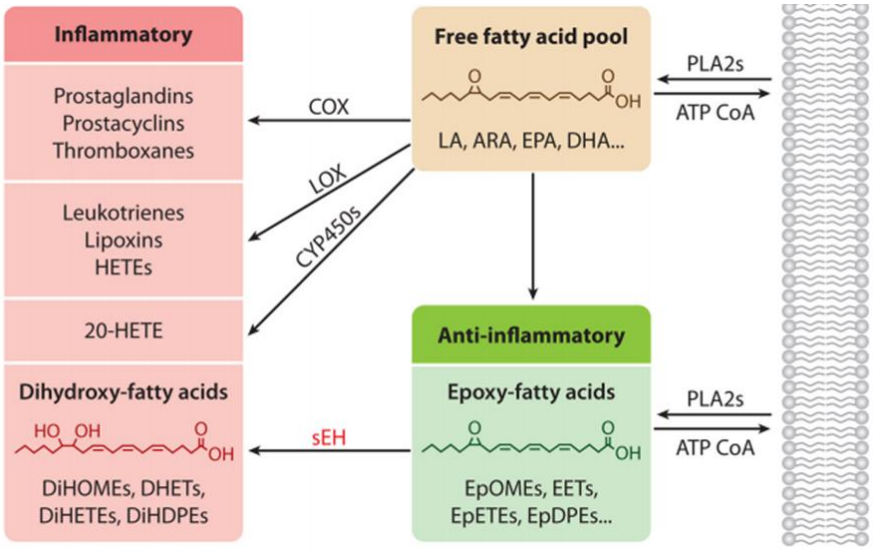


Figure 5. Summary of Arachidonic acid metabolism. Arachidonic acid is a major component of cell membranes which can be released from cell membrane phospholipids by lipases including phospholipase A2. This 20-carbon chain polyunsaturated fatty acid may be converted by a series of enzymes to numerous biologically active metabolites termed eicosanoids which are a group of lipid mediators. They are generated by the activity of three separate pathways which are the cyclooxygenases (COX), lipoxygenases (LOX) and the cytochrome P450 (CYP450) enzymes (Adapted from Morisseau & Hammock, 2013).

2. The Omega Hydroxylases

i. *Localization and characterization*

The ω -hydroxylase family **(Figure 5)** is comprised of three gene families: CYP4A, 4F, and 4B which are characterized by the ω/ ω-1-hydroxylation (addition of a hydroxyl moiety at the

terminal ω-carbon) conversion of AA which produces 19 or 20-hydroxyeicosatetraenoic acid (20-HETE), and 12-Hydroxy-5,8,4-eicosatetraeinoic acid (12-HETrE) respectively (Hardwick, J., 2006). In humans, the CYP4A and CYP4F subfamilies are encoded by CYP4A11 and CYP4F2 genes, respectively. On the other hand, murine genes are more complex and encode the hydroxylases through CYP4a12s, 4a12b, 4f14 and 4f15 (Capdevila & Falck, 2018). For the sake of this work, we will focus on 20-HETE synthase referred to henceforth as CYP4A, which is the most extensively studied hydroxylase among its family, and the most enzyme with functional selectivity and species homology for the ω–hydroxylation of AA. It is largely expressed in liver and kidney tissue, although CYP4A expression has also been observed in the brain, vascular smooth muscle, prostate, lungs, intestine, heart, and skeletal muscle (Strömstedt et al., 1990; Zhou et al., 1996; Zhu et al., 1998; Strömstedt et al., 1994; Koike et al., 1997). Yet, the cellular distribution of CYP4A, in addition to the physiological and pathophysiological role in the PNS has not been described.

20-HETE executes its biological role through binding to its recently identified G-protein coupled receptor GPR75 in the vascular endothelium (Garcia et al., 2017; Fan & Roman, 2017). The expression of GPR75 has been found in numerous tissues including heart, brain kidneys and others (https://www.ncbi.nlm.nih.gov/geo/tools/profileGraph.cgi?ID=GDS1096:220481_at). The primary site of 20-HETE production is attributed to the smooth muscle layer and vascular endothelium (Gebremedhin et al., 1998; Zhu et al., 2002), although it is also synthesized by select cells in the aforementioned tissues. Even though little is known about 20-HETE signaling mechanisms, an active body of research has recognized factors that influence 20-HETE synthesis **(Figure 6)**. For instance, androgens, high fat, ischemia, hypoxia, angiotensin, endothelin, and serotonin among other vasoactive peptides (Cambj-Sapunar et al., 2003; Lima et al., 2013; Rocic & Schwartzman, 2018), as well as hormonal factors such as parathyroid hormone and epidermal growth factor (EGF) (Ribeiro et al., 1994; Lin et al., 1995) have been shown to stimulate 20-HETE production. By contrast, carbon monoxide (Zhang et al., 2001), nitric oxide (Fan et al., 2016), superoxides generation (Gebremedhin et al., 2008) and hemeoxygenase reduce 20-HETE synthesis (Zhang et al., 2001) by binding to the heme core of CYPs in a cGMP independent manner, and thus triggering a vasodilatory response (Rocic & Schwartzman, 2018). Some studies suggest the transcriptional regulation **(Figure 7)** of CYP4A and CYP2C by androgens and peroxisome proliferator-activated receptor (PPAR-α) which is activated by ω-HEETs, the high affinity ligand

and activator of PPARα produced by the the ω-hydroxylation of EETs (Cowart et al., 2002; Hardwick, J., 2008; Corton et al., 1998, Johnson et al., 2002; Pozzi et al. 2010; Capdevila et al., 2015; Dalmasso et al., 2016; Moreno, J., 2017). However, metabolic regulatory control of 20-HETE is achieved by three main mechanisms. Alcohol dehydrogenase catalyzes the conversion of 20-HETE to carboxylic acid which is fated for β-oxidation. Another regulatory pathway is the conjugation of 20-HETE to be excreted in urine. As for the third mechanism, 20-HETE may undergo modifications by COXs and LOXs and become part of a different pathway (Alsaad et al., 2013; Kim et al., 2013; Gebremedhin et al., 2016; Fan & Roman, 2017).

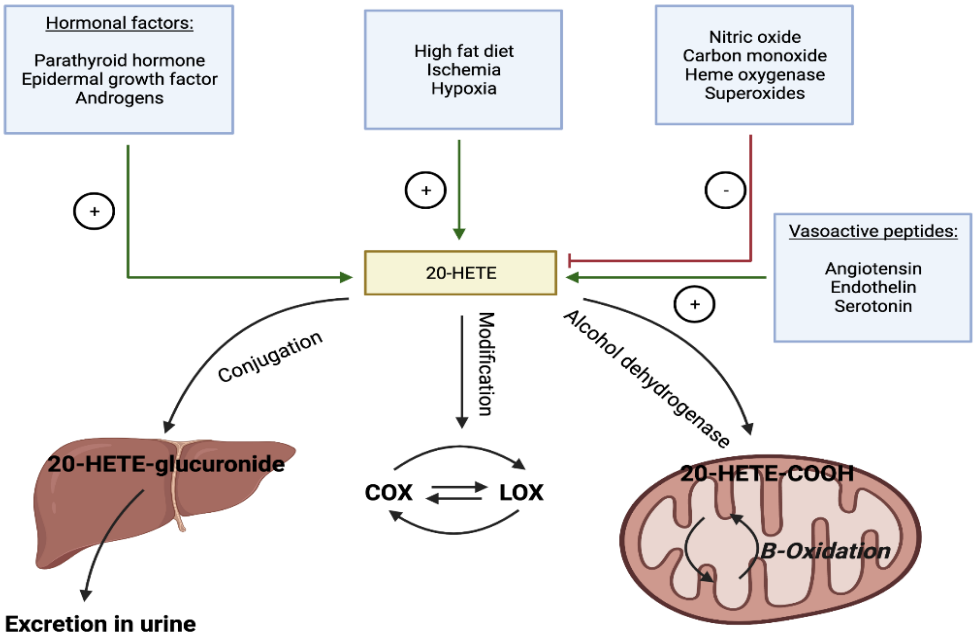


Figure 6. Metabolic regulation of 20-HETE. (Figure created by BioRender.com)

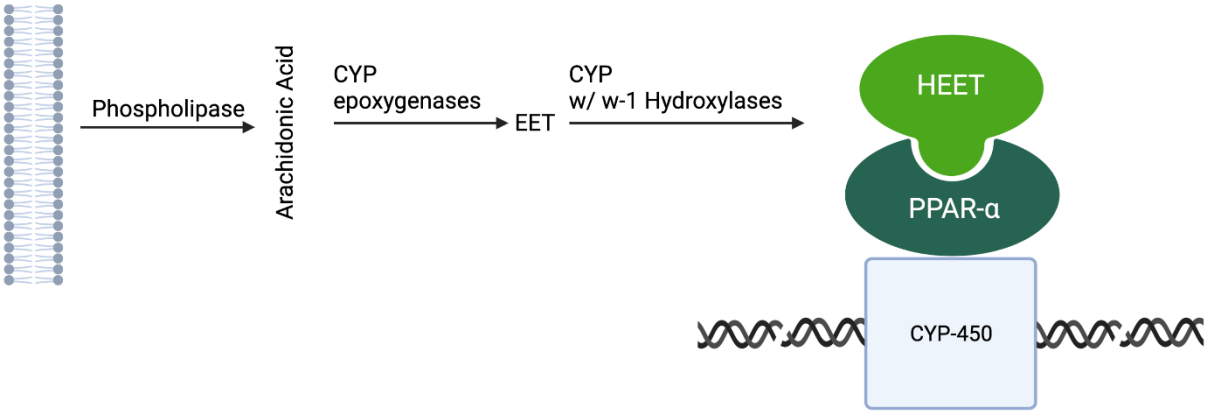


Figure 7. Transcriptional control of CYP-Hydroxylases and Epoxygenases. (Figure created by BioRender.com)

ii. *Homeostatic influence of 20-HETE*

20-HETE has been reported to have various and antagonistic functions depending on the synthesis site and target tissue (Zhu et al., 2000; McCarthy et al., 2005; Dahly-Vernon et al., 2005; Nilakantan et al., 2008; Dhanasekaran et al., 2009; Zeng et al., 2010). 20-HETE has been shown to be of significance in brain development (Anwar-Mohamed et al., 2014) and inhibition of platelet aggregation (Hill et al., 1992). Additionally, 20-HETE is a known pro-mitogenic in collaboration with epidermal growth factor in renal proximal tubules triggering cellular growth (Lin et al., 1995). 20-HETE plays a major role in circulation hemodynamics (Wang et al., 2011), vascular reactivity and remodeling (Lu et al., 2014; Ding et al., 2013), as well as angiogenesis (Carmeliet & Jain, 2011). For instance, 20-HETE was shown to facilitate renal and cerebral blood flow and pressure regulation via activating the renin-angiotensin axis as well as the inhibition of Ca^{+2} activated K^{+} channels and the consequent activation of L-type Ca^{+2} channels, which induces myogenic vasoconstriction (Alonso-Galicia et al., 2002; Roman et al., 2006; Harder et al., 2011). By contrast, 20-HETE was shown to trigger pulmonary vasodilation as a means of reducing airway resistance in lungs (Yu et al., 2002; Jacobs et al., 2006). Further studies demonstrated the activation of PKC and Rho Kinase cascades by 20-HETE as mechanisms that stimulate contractility of arteries (Fan et al., 2016). Along these lines, 20-HETE was shown to also exhibit natriuretic effects via the inhibition of Na/K-ATPase as demonstrated in renal proximal tubular cells and the loop of Henle (Ribeiro et al., 1994; Quigley et al., 2000; Yu et al., 2007; Capdevila et al., 2007) emphasizing its ability to modulate systemic arterial pressure. However, other studies discussed a beneficial role of 20-HETE in the kidney and lungs (McCarthy et al., 2005; Regner et al., 2009; Dhanasekaran et al., 2009). Bearing these significant physiological roles, alterations in 20-HETE were implicated in organ damage.

iii. *Pathological roles of 20-HETE in vascular diseases, inflammation and cancer*

In hypertensive animals, increased vascular 20-HETE levels were associated with vascular resistance and endothelial dysfunction (Garcia et al., 2017). In the kidneys, elevated 20-HETE production plays a role in the pathogenesis of polycystic and chronic kidney diseases (Park et al., 2009). This accounted for reports where 20-HETE also mediated ischemic injury, cardiovascular complexities such as restenosis and hypertrophy, stroke, subarachnoid hemorrhage (SAH) and traumatic brain injury (TBI). In SAH, extensive studies linked 20-HETE to the acute fall in

cerebral blood flow following rupture and hemorrhage of the cerebral wall (Kehl et al., 2002; Fordsmann et al., 2013). Besides, traces of 20-HETE were also detected in the cerebrospinal fluid of patients that undergo ischemic stroke following SAH (Poloyac et al., 2005). In addition to that, oxidative stress levels rise in neuronal cells triggering apoptotic death in penumbral regions exacerbating the thrombotic insult (Dunn et al., 2008). Accordingly, the blockade of 20-HETE synthesis conferred neuroprotective effects by reversing vasospasms, normalizing blood flow, and alleviating edema (Fordsmann et al., 2013; Takeuchi et al., 2005). Similarly, it has been demonstrated in experimental models of TBI that 20-HETE plays a significant role in blood brain barrier (BBB) dysfunction triggering cytotoxic edema, increased oxidative stress, and altering matrix metalloproteinase expression (Lu et al., 2017). On the other hand, the inhibition of 20-HETE was reported to suppress MMP-9 levels, alleviate edema, and this showed to be neuroprotective of the BBB (Lu et al., 2018). As for cardiac ischemic reperfusion injury, 20-HETE induces cardiomyocyte apoptosis and inflammation (Ishizuka et al., 2008; Hoff et al., 2011; Bao et al., 2011). Further studies delineate the mechanisms of injury by 20-HETE to be dependent on Rho Kinase, inflammatory responses, PKC and oxidative stress inducing cardiac hypertrophy (Zeng et al., 2010; Maalouf et al., 2012; Moreno et al., 2014). Finally, by virtue of 20-HETE stimulation by growth factors, its role in angiogenesis and cellular proliferation (Jiang et al., 2004; Chen et al., 2005; Guo et al., 2008), underscore its role in malignant tumor growth and certain cancers. In fact, ω-hydroyxlases were shown to be over-expressed in a number of human cancers (Alexanian et al., 2012). After pinpointing the activated pathway downstream of GPR75 20-HETE receptor to involve PKC, EGF, and other pathways involved in cellular proliferation, 20-HETE was depicted to promote epithelial to mesenchymal transition, and thus a more aggressive phenotype in a prostate cancer cell line (Cárdenas et al., 2020). In a triple negative breast cancer mouse model, it was demonstrated that the inhibition of CYP4A attenuated pro-angiogenic factors that reduced tumor growth (Borin et al., 2014). Similar findings were reported in gliosarcoma cells (Guo et al., 2006; 2008), colon cancer (Zhang et al., 2014), lung cancer (Yu et al., 2011), prostate cancer (Cárdenas et al., 2020), and renal adenocarcinoma (Alexanian et al., 2009). Altogether, these studies suggest a pathogenic feature of 20-HETE. However, the role of 20-HETE in DPN is not yet elucidated.

iv. *20-HETE in the neurovascular unit*

With limited reserves, the energy and oxygen requirements of the brain are supplied via the neurovascular unit; a complex meshwork of neurons, astrocytes and an endothelium lined by myocytes and pericytes (Lecrux, C., & Hamel, E. 2011). The unit is vital for monitoring and adjusting cerebral blood flow and perfusion according to neuronal activity through cerebral autoregulation mechanisms (ensuring constant pressure and flow) and neurovascular coupling (ensuring a spatial and temporal distribution between neurons) (Zonta et al., 2003; Hamel, E. 2006). It has been reported that cells of the neurovascular unit express CYP450 enzymes (Köhler et al., 1988) and are capable of 20-HETE biosynthesis (Nithipatikom et al., 2001). The vasoactive agent, 20-HETE, has been increasingly shown to play a critical role in the central nervous system (Filosa, J. A., & Iddings, J. A. 2013).

CYP4A expression is reported to be localized in arteriolar muscle tissue and astrocytes while 20-HETE to be involved in cerebral vascular tone and autoregulation (Gebremedhin et al., 2000; Kim et al., 2015; MacVicar, B. A., & Newman, E. A. 2015). Elevated 20-HETE levels have been shown to reduce cerebral blood flow (Fordsmann et al., 2013). However, the vasoconstrictive actions of 20-HETE in the brain are dynamic and are influenced by nitric oxide levels and other CYP metabolites and prostaglandins (Yu et al., 2004; Liu et al., 2008). The importance of 20-HETE in the myogenic response and BBB physiology was investigated in CYP4A1 transgenic animal models. The data showed that a genetic deficiency in 20-HETE production was detrimental and may lead to cerebral injury in hypertensive rats (Fan et al., 2015). In contrast, various studies investigated the protective role of 20-HETE inhibition via HET0016. *In vitro* studies on cultured endothelial cells have shown that 20-HETE may be involved in inflammation (Cheng et al., 2008; 2010) and oxidative stress (Toth et al., 2013; Lakhkar et al., 2016). HET0016 treatments in hypertensive rats were capable of reducing superoxide production, oxidative stress, inflammation, and restoring vasomotor function (Toth et al., 2013). A study on hippocampal slices subjected to oxygen-glucose deprivation followed by reoxygenation showed a rise in 20-HETE production in addition to ROS and caspase-dependent neuronal death. However, HET0016 was shown to be neuroprotective against ischemia (Renic et al., 2012) in addition to brain edema and blood brain barrier dysfunction following reperfusion injury (Liu et al., 2014).

The debate as to whether 20-HETE insults or compliments the CNS is ongoing and extensive *in vivo* studies are pivotal to progress further. However, with regards to the periphery,

the bulk of investigations on 20-HETE were limited to the cardiovascular and renal systems. They were conducted within a pathophysiological, vascular endothelial context such as in hypertension, acute kidney injury and diabetic complications with the exception of DPN. Indeed, vascular alterations have been shown to mediate DPN although the underlying factors influencing the pathogenesis have not yet been identified (Cameron et al., 2001).

3. The Epoxygenases

i. *Localization and characterization*

As for members of the epoxygenase family **(Figure 5)**, two major subfamilies are CYP2C and 2J (Capdevila & Falck, 2018). The human counterparts are CYP2C8, 2C9 and 2J2. Whereas the murine epoxygenases are 2c29, 2c44 and 2j3 (Capdevila & Falck, 2018). They are responsible for the epoxidation of AA into lipid mediators/epoxyfatty acid (EpFAs). Epoxygenases oxidize AA to yield epoxyeicosatetraeinoic acids (EETs), however they are also capable of metabolizing AA competitors in the pathway such as EPA, DHA and linoleic acid to yield epoxyeicosatraenoic acid (EpETE), epoxydocosapentaeinoic acid (EPDPE), epoxyoctadecanoic acid (EpOME) respectively. These products have been reported to have diverse physiological roles in inflammatory processes, pain, and cardiovascular system modulation. In this work, we focus on AA metabolism, and EET synthases which produce multiple regioisomers of EETs (5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET). In fact, EETs are a special form of lipid mediators because they are produced with high enantiofacial (R/S) selectivity which attributes specific and diverse functional roles to each regioisomer of EET. The latter two regioisomers account for up to 80% of EETs produced (Spector & Kim, 2015). In this work, the expression of CYP2C will be studied along with EETs. CYP2C expression is reported in the liver, kidney, intestines, aorta, brain, pancreas, lungs, and heart. (Zeldin et al., 1997; Klose et al., 1999; DeLozier et al., 2007; Fleming, I., 2008; Depaz et al., 2015; Drolet et al, 2017). However, like CYP4A, CYP2C expression has not been reported in the PNS.

EETs are synthesized along with other epoxyeicosanoids and are rerouted to their respective tissues/cells for utilization **(Figure 8).** Their mode of action is not fully clear yet, and the existence of an EET-specific receptor has not been identified (Campbell & Fleming, 2010), although it has been speculated that their membrane receptor mechanism may commence through a G-protein coupled receptor (Li et al., 1999). Nevertheless, EETs are esterified and incorporated with phospholipids, which is indicative of their ability to modulate membrane protein domains and

phospholipid-dependent signaling cascades (Spector, A., 2009). Other pathways EETs are fated for include glutathione conjugation, further metabolism by COXs or hydroxylases, and the β-oxidation process (Zeldin, D. C., 2001; Spector et al., 2004). Furthermore, since EETs are known to be short-lived molecules, unesterified EETs are then either stabilized and bound to fatty acid binding proteins and transported intracellularly or hydrolyzed into less active diols to be released into the extracellular fluid (dihydroxyeicosatrienoic acid – DHET) via the soluble epoxide hydrolase enzyme (sEH) (Widstrom et al., 2001; 2003). Above all, these oxylipins, including diols, may be carried by plasma lipoproteins and serve as a biosource ready for uptake when needed (Shearer & Newman, 2008). In this manner, EETs may act as intracellular signaling messengers regulating specific cascades such as cAMP, cGMP, NO and others (Capdevila et al., 2015). Furthermore, EETs may act through trans-membrane signaling either directly binding to receptors or amplifying signals (Chen et al., 2002).

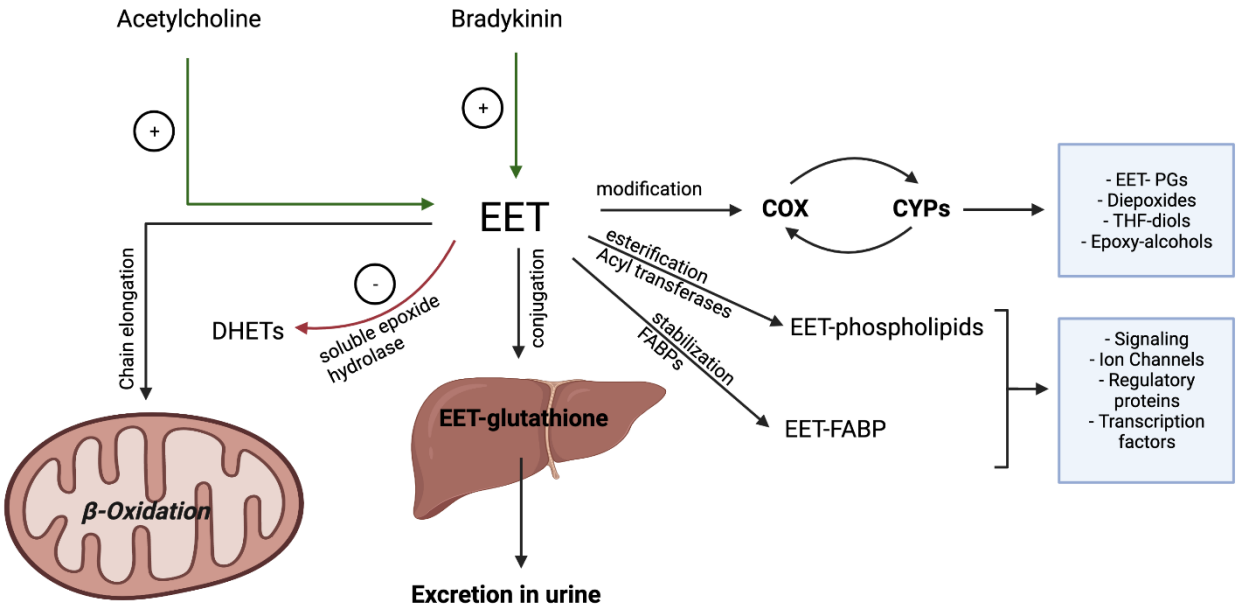


Figure 8. Metabolic regulation of EETs. (Figure created by BioRender.com)

Understanding the biological effects of EETs remains a challenge currently due to limited natural sources and the short life span of EETs making it ill-suited for in vivo studies (Newman et al., 2005). Alternative methods were adopted through the utilization of synthetic mimetics, analogs and antagonists, or inhibiting sEH pharmacologically and genetically that work to prolong the bioavailability of EETs by inhibiting their degradation (Liu et al., 2009). Major discoveries have

been made using sEH inhibitors (sEHI) which will be discussed below. Encoded by the Epxh2 gene, sEH is the regulatory enzyme responsible for clearing EETs in as much as it breaks down phosphorylated lipids. sEH is a homodimer of the α/β-hydrolase family, and it catalyzes epoxide hydrolysis with efficacy and high selectivity for 14,15, -EET, EPDPE and EpOME (Argiriadi et al., 1999; Morisseau et al., 1999; Argiriadi et al., 2000; Yu et al., 2000; Morisseau & Hammock, 2005; Spector & Kim, 2015). It is an abundantly expressed and conserved enzyme found in almost all tissues especially the liver, kidney, vasculature, intestines, and brain. The activity of sEH may be easily assessed through the EET/DHET ratio (Yu et al., 2000). In other words, once EET levels are higher than DHET, this is indicative of low sEH activity, and vice versa. In this manner, sEH modulates the bioactivity of circulating EETs.

ii. *Homeostatic influence of EETs*

A large body of research has shown EETs to evoke effects that are in opposition to 20-HETE roles. In fact, extensive studies revealed that EETs possess anti-inflammatory effects (Node et al., 1999), play a role in ion channel modulation and elevating natriuresis (Fan & Roman, 2017), regulating gene expression (Wang et al., 2006; Inceoglu et al., 2008), cell proliferation (Ma et al., 2012) and vasodilation in vascular beds which are vital regulatory mechanisms for vascular function (Campbell et al., 1996). EETs modulate Ca^{+2} flux in collaboration with TRP channels (Imig, J, D., 2012). The vasodilatory effect of EETs has been attributed to its identification as an endothelium-derived hyperpolarization factor that triggers relaxation of vascular smooth muscle cells lining blood vessels in response to acetylcholine and bradykinin independent of nitric oxide (NO) synthase and COX inhibitors (Campbell et al., 1996; Imig, J. D., 2013). In the cardiovascular system, EETs play a cardioprotective role by ameliorating hypertrophy, inflammation, hypertension, endothelial dysfunction, reactivity and myocardial preconditioning (Larsen et al., 2007). Additional studies showed anti-arrhythmic action of EETs (Westphal et al., 2011). EETs are also renoprotective and work to reduce hypertension (Imig et al., 2005). In the nervous system, EETs stimulate neurohormone release, in addition to being an important mediator of pain (Iliff et al., 2010). EETs also protect neurons from apoptosis, inflammation, and modulate their excitability and recovery post injury (Wang et al., 2018). EETs also contribute to cerebral blood flow metabolic regulation, which is the main and faster response elicited in neural tissue. Metabolic regulation caters to the tissue demands through astrocytic-endothelial cell communication and synaptic activation of metabotropic glutamate receptors that trigger Ca^{+2} influx. This subsequently

triggers the formation of EETs which influence KCa-dependent buildup of K^+ to hyperpolarize vascular smooth muscle cells or directly mediate KCa channels on vascular smooth muscles cells in a paracrine effect (MacVicar & Newman, 2015; Huang et al., 2016). Finally, EETs were shown to reduce ischemic injury in the heart and brain (Otsuka et al., 2003; Fornage et al., 2005), and abrogate hyperthermia (Kozak et al., 2000).

iii. ***Pathological roles of EETs***

EETs are heavily tied to homeostatic maintenance. Its role comes into play in several pathologies mostly from the vascular standpoint. Here, we discuss EETs in other important etiologies. As previously mentioned, EETs are anti-inflammatory in the cardiovascular, nervous, renal and pulmonary system (Specter & Kim, 2015). Studies have illustrated that EETs facilitate the initiation of a painful response in non-neuropathic pain, followed by its amplification and maintenance as long as inflammation ensues in animal models of inflammatory pain (Inceoglu et al., 2008). In an indunced inflammatory model, it was reported that EET levels were decreased which disrupts neurosteroid production and elevates prostaglandin secretion by COX2, which triggers hyperalgesia. This is followed by an increase in cAMP which stabilizes the response. However, the administration of sEHI reduced prostaglandin levels inversely to EET levels and conferred pain relief (Schmelzer et al., 2005; Inceoglu et al., 2008). It is noteworthy to mention that the nocifensive effects of sEHI were elucidated to be ineffective in the absence of a stimulus or pain induction (Inceoglu et al., 2007; 2008). Further studies showed that EETs mediate the anti-inflammatory response by inhibiting NF-kB and TNFα which consequently reduces iNOS, LOX and COX activity. This conferred protection against cardiomyocyte apoptosis and endothelial cell injury. (Zhao et al., 2012; Jiang et al., 2014). sEHI proved its efficacy in other inflammatory disease such as Irritable Bowel Disease, chronic peptic ulcers, arthritis and lytic bone disease (Wagner et al., 2017). In a hypertensive rat model, kidney fibrosis and glomerular injury was markedly reduced by the administration of an EET analog, and this was depicted by a reduction in oxidative and endoplasmic reticulum stress as well as the blockade of inflammation (Hye et al., 2013). As for the roles of EET is angiogenesis, numerous reports demonstrate the stimulation of proliferation and migration of endothelial cells in cerebral and pulmonary microvessels (Munzenmaier & Harder, 2000; Medhora et al., 2003) in addition to increased secretion of VEGF which promotes wound healing (Sander et al., 2011). Furthermore, it was elucidated in transgenic mice models that EETs act as autacoids and contribute to tissue regeneration in the kidneys, liver,

retina and lungs (Panigrahy et al., 2013). Yet, despite that, EETs play a prominent role in cancer malignancies, especially in high doses. Epoxygenase CYP2J expression was reported to be upregulated in hematologic malignancies paralleled by elevated EET levels. In vitro, the inhibition of CYP2J reduced proliferation and triggered the apoptotic death of tumorigenic cells, dwarfing the pathological growth (Chen et al., 2011). Similar reports show an EET-mediated increase in tumor growth in a breast cancer model (Mitra et al., 2011). Additional studies demonstrated on-site EET-mediated metastatic phenotypes in various tumor models (Panigrahy et al., 2012). By contrast, epoxygenase metabolites from the ω-3 arm of the pathway were depicted to be in complete opposition to EETs and downgrade cancerogenic growth, angiogenesis, and metastasis (Zhang et al., 2013, Xia et al., 2019). In brief, the body of research on EETs underscores their pleiotropic effects and their dynamic interactions with other enzymatic families and bioactive metabolites. In the next section, EETs will be overviewed in the context of neural tissue and related pathologies.

iv. ***EET in the neurovascular unit and pain***

As previously mentioned, CYP epoxygenases are expressed in the CNS in the brain. In humans, sEH expression was marked in endothelial cells, astrocytes, oligodendrocytes and neurons (Sura et al., 2008). Further investigations showed the sEH enzyme in cortical, hippocampal and sensory neurons (Abdu et al., 2011). Clusters of sEH are predominantly located in the cell bodies of cortical neurons and axonal regions of nerve fibers in white and gray matter and sensory ganglia (Iliff et al., 2009). In the periphery, sEH was discovered in sympathetic and sensory neurons (Abdu et al., 2011). EETs have been described to have distinct roles centrally and peripherally. While EETs function to modulate cerebral blood flow, trigger neurogenic vasodilation, provide cellular communication via neuroendocrine secretion from the hypothalamus and pituitary and neurovascular-glial interaction centrally (Iliff et al., 2010), EETs exhibit analgesic effects in the periphery and play a role in anti-nociception (Wagner et al., 2011).

Rising evidence shows EETs to be neuroprotective in a number of neurodegenerative diseases. For instance, sEHI reduced infarct size following ischemic brain injury and vascular occlusion by a non-vascular mechanism, which makes epoxygenases a valuable therapeutic target in stroke-related brain damage (Zhang et al., 2007). This effect was observed to occur in a manner independent of cerebral blood flow modulation. Such studies suggest that EETs may confer protection and have direct effects on neurons. This is evident in the study by Oguro et al., whereby

exogenous EETs administration *in vitro* facilitated hippocampal primary neuronal neurite outgrowth, a vital neural function in the CNS. This was mediated by EET activation of TRPV channels and the subsequent influx of Ca^{+2} (Oguro et al., 2018). In this spirit, EETs are of significance in neural plasticity which was illustrated by Minaz et al., upon inhibition of sEH to halt cognitive decline and memory deficits in a diabetic model (Minaz et al., 2018). In another study, it was reported that EETs upregulate NMDA receptors which enhance neural networks and synaptic transmission (Wu et al., 2015). Furthermore, sEH ablation proved neuroprotective against seizures by delaying onset of GABA-mediated seizures and elevating epileptogenic electrical thresholds (Inceoglu et al., 2013). Similarly, it was reported that in patients with vascular cognitive impairments, DHET levels were elevated (Nelson et al., 2014), indicative of increased sEH activity. Concomitantly, the incubation of isolated microsomes, astrocytes and neurons *in vitro* and *in vivo* with Aβ proteins typical of Alzheimers disease was shown to significantly reduce EET production suggesting a pathological interaction (Sarkar et al., 2011). As for motor neurodegenerative diseases, EETs administration alleviated Parkisons-related motricity, and protects dopaminergic neurons (Qin et al., 2015). On a more psychiatric and behavioral level, sEHI/genetic ablation have been associated with resilience, elevated BNDF levels, and sucrose preference in sEHKO animal models, and social defeat animal models, indicative of an anti-depressive like effect on mice (Ren et al., 2016).

Finally, stemming from the neuroinflammatory context linking together the mentioned CNS ailments, pain is one of the pathologies that EETs influence. Studies of inflammatory pain models such as lipopolysaccahride or carrageenan induced inflammation inflict thermal and mechanical hyperalgesia (Inceoglu et al., 2006; 2008). The topical administration of EETs or the administration of sEHI were demonstrated to effectively ameliorate hyperalgesia. EET signaling was further explored and shown to activate steroidogenic acute regulatory protein stARD1 gene expression which contributes to cholesterol transport for neurosteroid production. This is facilitated by EET binding to transmembrane TSPO receptor. (Inceoglu et al., 2006). In another study, Inceoglu et al., showed the sEHI may inhibit inflammatory pain mediated by prostaglandin release, and that sEHI necessitates an active pain state to accomplish antinociception, which is mediated by pain signaling second messenger, cAMP. By the coadministration of SEHI and a cAMP inhibitor, it was shown that epoxygenated fatty acid levels were elevated, and dramatic such that acute pain relief was rapidly achieved (Inceoglu et al., 2008; 2011). While the common

denominator among all these NS ailments revolve around neuroinflammation, it is evident that sEH modulation highlights some of the major neuroprotective regulatory processes of EETs.

4. 20-HETE and EET: Emerging leads in the diabetic race

20-HETE and EET by CYP4A and CYP2C account for up to 90% of the physiological functions and products by the CYP family (Capdevila & Falck, 2018). Although the ω-hydroxylation reaction accounts for a minor 4-15% of fatty acid metabolic pathways (Draye & Vamecq, 1989; Hardwick, J., 2008), the significance of this pathway is reflected in major physiological states such as starvation and metabolic disorders. In fact, the physiological significance of CYP450 proteins and their metabolites is under intense investigations in obesity, diabetes and diabetic complications.

Recent research has identified 20-HETE formation to be stimulated upon glucose uptake via GLUT2 by **pancreatic** β-cells, which in turn elevates 20-HETE levels that bind to the long-chain fatty acid receptor FFAR1 (GRP40) that is highly expressed in β-cells. Upon binding and activation of FFAR1, glucose-stimulated insulin secretion (GSIS) is triggered, and collaboratively induce a feed forward loop (Tunaru et al., 2018). However, in another study, the prolonged elevation of systemic 20-HETE exhibited a reduced GSIS response. The constitutive expression of CYP4F2 in the kidney yielded increased levels of 20-HETE which culminated in hypertension and hyperglycemia, and further glucose regulation malfunction as a result of defected GSIS (Zhang et al., 2016). In islets isolated from diabetic individuals, it was further shown that glucose dependent 20-HETE formation and GSIS is markedly reduced (Tunaru et al., 2018). Additional studies have associated 20-HETE levels with obesity, hyperglycemia and deficient insulin responses (Lai et al., 2012; Theken et al., 2012; Issan et al., 2013; Peterson et al., 2016). 20-HETE was also documented to contribute to adipogenesis (Kim et al., 2013) and impaired insulin signaling (Li et al., 2014). In another study, high-fat diet fed Cyp4a14 knockout mice, characterized by androgen-dependent 20-HETE overproduction, developed insulin and glucose homeostatic disruption concomitant with altered insulin receptor phosphorylation. High-fat diet feeding induced CYP4A overexpression in skeletal muscles, liver and adipose tissue, paralleled by elevated plasma 20-HETE levels. This metabolic imbalance was prevented by antagonizing with 20-SOLA, which attenuated weight gain, elevated oxygen consumption and controlled glycemic parameters (Gilani et al., 2018).

In the past decade, 20-HETE has been increasingly shown to play a role in oxidative stress (Guo et al., 2007; Cheng et al., 2008; Medhora et al., 2008; Zeng et al., 2010) and in diabetic nephropathy, cardiomyopathy and retinopathy with no description of its role in neuropathy. It has been reported that high glucose upregulates CYP4A, 20-HETE and ROS production in a sequential manner with NOX enzymes which leads t**o renal** glomerular epithelia apoptosis, tubular and glomerular hypertrophy, in addition to ischemia, leading to renal dysfunction and kidney injury. But the inhibition of 20-HETE synthesis via HET0016, a specific CYP4A inhibitor, was shown to reverse renal injury (Eid et al., 2013). In the same way, it was shown that 20-HETE production was elevated in podocytes exposed to high glucose *in vitro* which inflict podocyte injury via apoptosis and ROS production. The inhibition of CYP hydroxylases rescued the cells and alleviated diabetes-induced proteinuria in vivo in a T1DM mouse model (Eid et al., 2009). Moreover, 20-HETE is known to be a vasoconstrictor that may induce **cardiac** hypertrophy and dysfunction (Jenkins et al., 2009; Alsaad et al., 2013). In a similar fashion, 20-HETE exacerbates cardiac injury in metabolic disorders by compromising endothelial cell integrity and activating neutrophil infiltration which impairs the cardioprotective abluminal expansion of vessels (Joseph et al., 2017). The heart's functional recovery from diabetes-induced ischemia/reperfusion injury was shown to be mitigated by the blockade of 20-HETE (Yousif et al., 2009). Similarly, 20-HETE in diabetic **retinopathy** was shown to influence retinal microvascular hemodynamics. Retinal blood flow measurements in diabetic mice recorded a decreased blood flow, velocity and shear rate which may be related to clinical observations of capillary occlusion due to endothelial damage. The inhibition of 20-HETE synthesis was reported to significantly ameliorate the decreases in retinal circulatory parameters suggesting an injurious role for 20-HETE in diabetic retinopathy (Wang et al., 2011). Collectively, strong evidence points towards a pathological role of 20-HETE in the diabetic spectrum of disorders **(Table 1).**

Table 1. Summary of 20-HETE-mediated diabetic complications.

Author	Injury	Results
Eid et al., 2013 Eid et al., 2009	Renal	Nephropathy Ischemia Hypertension
Joseph et al., 2017 Alsaad et al., 2013 Issan et al., 2013 Alaeddine et al., 2021	Cardiovascular	Cardiac hypertrophy Ischemia/reperfusion injury
Zhang et al., 2016 Tunaru et al., 2018	Pancreatic	Pancreatic reduction in GSIS
Wang et al., 2011	Ocular	Retinopathy
Kim et al., 2013 Theken et al., 2012 Peterson et al., 2016	Adipose tissue	Obesity Increased adipogenesis
Lai et al., 2012 Li et al., 2014 Gilani et al., 2018	Skeletal muscle Liver Endothelium	Hyperglycemia Insulin signaling impairment

As a matter of fact, equally compelling research ascertain the role of epoxygenases in various diabetic complications. Extensive studies indicate that EET synthesis/CYP epoxygenase expression to be evidently reduced in metabolic diseases and sEH expression significantly increased (Zhao et al., 2005; Eid et al., 2013). Deficient expression of CYP2C was associated with underlying obese phenotype and insufficient hemeoxygenase (HO) activity. Importantly, restoring the interplay between CYP2C and HO via EET agonist and sEHI supplementation confers cardiovascular protection, adiposity and glucose homeostasis (Sodhi et al., 2009; 2012). Interestingly, a **polymorphism in** sEH gene G860A locus was identified to be highly correlated with insulin resistance in T2DM patients (Ohtoshi et al., 2005). Similarly, a more recent study reported a polymorphism in CYP2J2 to disrupt metabolic balance and contribute to an early onset of T2DM (Wang et al., 2010). Moreover, gene therapy was studied by Xu et al. in db/db T2DM mice such that CYP2J was genetically delivered to animals. The findings report a restoration in insulin receptor signaling and activation of the AMPK pathway which lifted insulin resistance and hypertension in diabetic animals which may be correlated with elevated EET levels in liver,

muscle, cardiac, and renal tissue. In this manner, EETs play a protective against diabetes in the periphery (Xu et al., 2010).

Resonating with these investigations, numerous diabetic complications appeared to be manifestations of pernicious epoxygenase depletion. For instance, it was demonstrated in an *ex vivo* setup of **aortic and mesenteric** artery rings from three metabolically challenged murine models that sEH activity is pathologically elevated. More importantly, the inhibition of sEH restores endothelial-dependent vascular responses concurrent with hyperglycemia attenuation (Zhang et al., 2011). The importance of the biological properties of EETs were validated in ischemia/reperfusion injury in diabetic and control rats. sEHI improved recovery post injury in both groups of animals, however, combining sEHI and 20-HETE synthesis inhibitor as a pretreatment improved the biological outcome of ischemic hearts better than the administration of each inhibitor alone (Yousif et al., 2009). In a more targeted approach, the overexpression of CYP2J in cardiac tissue (α-MHC-CYP2J2 transgenic murine model) reversed diabetic cardiomyopathic phenotype in HFD-fed STZ-induced diabetic animals. Hypertrophy and poor contractility of cardiomyocytes was improved along with the stabilization of glucose levels, insulin reactivity, and AMPK signaling (Ma et al., 2013), as well as pathological remodeling and oxidative stress induced by angiotensin (He et al., 2015). Similarly, it was demonstrated that sEHI ameliorated lipid profile alterations, adipose inflammation, and fat deposition in the liver through restoring glucose tolerance. Cardiac impairments were also improved as paralleled by elevated ventricular function (Roche et al., 2015).

Also, in two cardiometabolic animal models, the spontaneously hypertensive obese rats and diabetic Zucker fatty rats, it was recently demonstrated by Khan et al., that the dual modulation of sEH and PPARγ quelled the spectrum of metabolic dysfunction elicited by these animals, such as hypertension, diabetes, and insulin resistance. More importantly, **hepatic** injury was markedly reduced alongside fat accumulation. Similar **renoprotective** effects were observed whereby fibrosis and tubuloglomerular injury was subsided, showing the multifaceted benefits of this modulation (Khan et al., 2018). Furthermore, these findings were confirmed by a previous study in Goto-Kakizaki rats that exhibit diabetes with spontaneous onset of hypertension. Nephropathic progression is characteristic of this animal model such that glomerular and tubular defects are inevitable. Treatment of diabetic animals with an early generation sEHI was shown to quell the renal injury in addition to reducing pathological urinary albumin excretion and monocyte

chemoattractant protein-1 expression which is typically elevated in kidney disease concomitant with macrophage infiltration during injury (Olearczyk et al., 2009). This is further validated by Eid et al in which the prolonged incubation of proximal tubular cells with high glucose results in fluctuations in CYP2C expression as well as EET production. Also, adding an EET synthesis inhibitor *in vitro* exacerbated hypertrophy of hyperglycemic tubular cells, lending support to the protective significance of CYP2C/EETs (Eid et al., 2013).

Additional studies were performed in diabetic animals with a mutation in the insulin 2 gene $Ins2^{Akita}$ mice. sEH expression was discovered in the mouse retinas to significantly rise in a timely manner with age which was associated with the onset of diabetic **retinopathy** in these animals. In diabetic patients with non-proliferative diabetic retinopathy, sEH expression rose along the course of their disease as well (Hu et al., 2017; Sun et al., 2018). sEHI administration to diabetic animals protected pericytes and maintained vascular permeability (Hu et al., 2017), while reducing sEH expression via $Ephx2^{-/-}$ deletion improved retinal wound healing capacity in a diabetic keratopathy (corneal disorders) induced by corneal debridement in diabetic $Ephx2^{-/-}$ mice (Sun et al., 2018).

Similarly, it was demonstrated that the genetic or pharmacological ablation of sEH activity restores insulin secretion and alleviates hyperglycemia in **pancreatic** islets through restoring Ca^{+2} influx. Islets from diabetic animals were also rescued from apoptosis upon sEH knockout (Luo et al., 2010). Early studies on EETs report their role in regulating pancreatic insulin and glucagon secretions (Falck et al., 1983). Similarly, sEHI following STZ-induced diabetes led to less apoptotic death of islets, and improved glucose tolerance, hinting at a possible therapy for T1DM (Chen et al., 2013). Luria et al. further illustrate that sEHI and sEH deletion enhance the vasculature surrounding islets and increase islet size in T2DM, HFD-fed sEHKO (Luria et al.,2011).

In the CNS, deficits in cognitive and executive functions are part of the behavioral spectrum associated with diabetes. Along the course of progression, the diabetic patient is at risk of developing **cognitive** decline in addition to a deterioration in their learning and memory, often due to neuronal loss and altered neuronal activity. A recent study has explored the possibility of a neuroprotective effect by dampening sEH activity in T1DM mice. The findings by Minaz et al correlate sEHI administration with neuroprotective mechanisms such as restoration of neurotransmitter levels, and a reduction of oxidative injury. The treated diabetic animals

consequently performed better at a behavioral level as indicated by their improved spatial memory function (Minaz et al., 2018).

Although the physiology of 20-HETE is beginning to unravel in various organs, the roles of CYP4A and 20-HETE in the CNS are limited. And to our knowledge, no investigations have been conducted in the PNS and diabetic neuropathy. As for CYP2C and EETs, extensive studies outline a protective role within the pathogenesis of diabetes **(Table 2)**. In the PNS, although EETs have proved to be a vital part of anti-nociceptive strategies, they are yet to be defined within the diabetic context. In short, 20-HETE and EET remain unexplored territory in the neural tissue and merit thorough investigation, especially in DPN.

Table 2. Summary of EET roles in diabetic complications.

Author	Injury	Results
Olearczyk et al., 2009 Eid et al., 2013	Renal	Renoprotective Reduced hypertension, hypertrophy, nephropathy
Alaeddine et al., 2021 Zhang et al., 2011 Yousif et al., 2009 Ma et al., 2013 Roche et al., 2015	Cardiovascular	Cardioprotective against hypertrophy, poor contractility, and ischemia/reperfusion injury Restored endothelial-dependent vascular responses
Hu et al., 2017 Sun et al., 2018	Ocular	Wound healing Keratopathy and retinopathy
Luo et al., 2010 Luria et al., 2011 Chen et al., 2013	Pancreatic	Rescued islet secretion and apoptosis Enhanced vasculature
Theken et al., 2012 Sodhi et al., 2012	Adipose tissue Liver	Reduced inflammation, fat deposition
Ohtoshi et al., 2005 Wang et al., 2010 Xu et al., 2010	Muscle Liver Periphery	Lifted insulin resistance Restored insulin receptor signaling
Minaz et al., 2018	CNS	Neuroprotective. Enhanced cognitive function, memory, neuronal activity

F. The Lead Orchestrators AMPK, mTOR, and AKT: Pathways Tipping the Balance

1. Distinct impacts: From the failing cell to the diabetic whirlpool

Newly emerging studies exemplify that DPN is the cataclysmic outcome of disordered energy transfer to and from glia and axons (Feldman et al., 2017). In diabetic conditions, extensive literature highlight significant alterations to important pathways that modulate energy and nutrient balance. In this study, we direct our attention to the AMP-activated protein kinase (AMPK) and protein kinase AKT pathways which work collaboratively with the mammalian target of rapamycin (mTOR) pathway **(Figure 9).** So where do these pathways lie in the midst of the diabetic whirlpool?

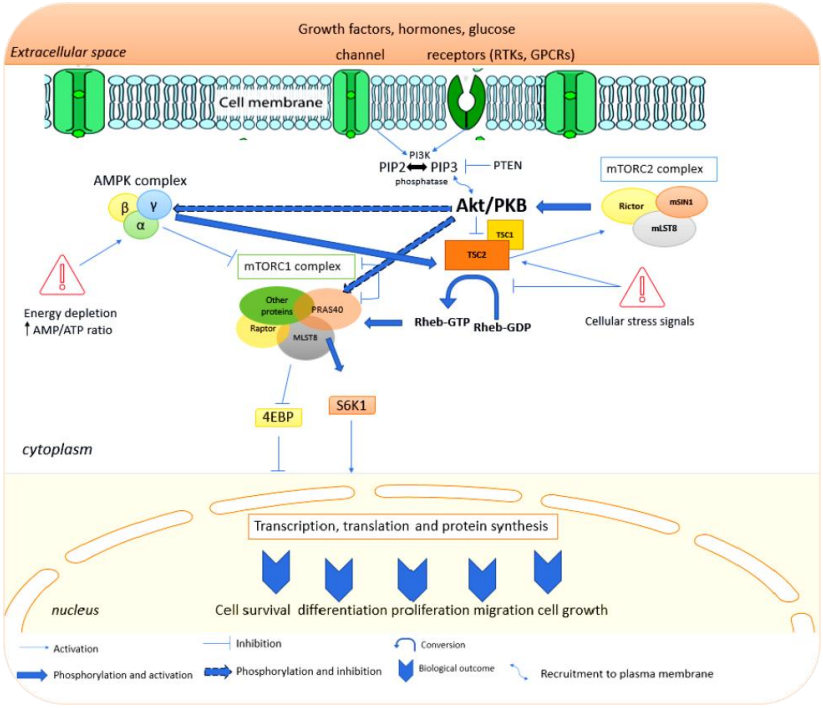


Figure 9. Interaction of essential signaling pathways mTOR, AKT and AMPK.

mTOR is part of a large family of kinases. It is a serine/threonine protein kinase involved in cellular metabolism, growth, division, aging, and survival (Bar-Peled & Sabatini, 2014; S. Eid et al., 2016; Hsu et al., 2011; Yu et al., 2011). It is a major nutrient, energy, and stress sensor such that it plays a role in diabetes onset and progression (Zoncu, R., Efeyan, A., & Sabatini, D. M. 2011). It is comprised of two functionally and structurally distinct complexes: mTOR complex 1 (mTORC1) and complex 2 (mTORC2), which are also capable of crosstalk to modulate various

cellular activities (Eid et al., 2016, Liu et al., 2017). Both complexes work in response to the integration of several signals including the bioavailability of oxygen, nutrients (glucose and amino acids), energy in the form of ATP, growth factors such as cytokines, and hormones such as insulin. To that end, mTOR plays a role in a panoply of injurious pathways. In light of the involvement of the mTOR pathway especially mTORC1 in a variety of cellular processes, it comes as no surprise that its dysregulation has been implicated in numerous metabolic disorders including insulin resistance and obesity (Zoncu et al, 2011). mTOR function is mainly dependent on tuberous sclerosis (TSC) gene products, TSC proteins, and downstream protein Rheb which is a GTPase governed by intracellular GTP/GDP ratio (Inoki et al., 2003; Dibble & Cantley, 2015). Upon activation, the downstream effectors of mTORC1 are p70S6 kinase (S6K1) and 4E-binding protein 1 (4EBP1) which interact in response to rapamycin. It has been proposed that the continuous activation of the mTORC1 pathway in diabetic conditions stands behind the development of insulin resistance. In fact, the constitutive activation of mTORC1 *in vitro* in TSC1 and TSC2 null cells has been shown to attenuate PI3K/Akt signaling in response to insulin. Normal response to insulin could only be restored upon treatment of cells with rapamycin, the mTORC1 inhibitor (Harrington et al., 2004). This was further verified by the findings of Um et al. (2004) which implicated that mTORC1 overactivation in response to nutrient abundancy such as glucose and amino acids plays a role in the development of insulin resistance and hence T2D (Um et al., 2004). By contrast, mTORC2 is rapamycin insensitive and phosphorylates Akt at its Serine473 residue fully activating it (Sarbassov et al., 2005). To date, little is known about the function of mTORC2. However, a wider pool of information is gathered from studies on its target, AKT.

AKT is another serine/threonine kinase and a key effector of mTORC2. Recent studies show that the activation of mTORC2 may be independent of nutrient status, rather it is depicted to be largely influenced by insulin in a manner that is dependent on other important pathways such as the PI3K pathway (Huang, Dibble, Matsuzaki, & Manning, 2008; Phung et al., 2006; Sarbassov et al., 2006; Zinzalla, Stracka, Oppliger, & Hall, 2011). In fact, the metabolic activity of insulin is shown to be dependent on the activation of PI3K/AKT cascades. The autophosphorylation of insulin receptors by insulin triggers the downstream conversion of substrate PIP2 to PIP3 which leads to the phosphorylation of AKT and the subsequent translocation of glucose transporters for glucose uptake (Friedrichsen et al., 2013). Further impacts of AKT include induction of glycogen synthesis in liver and skeletal muscle tissue (Manna et al., 2013). In this manner, the PI3K/AKT

axis modulates insulin-dependent metabolism in collaboration with insulin receptor substrate (IRS) proteins, PI3K regulatory subunits and AKT. Briefly, IRS blockade dwarfs cellular stimulation of insulin release, and is thus one of the signaling nodes affected in diabetes. Important players to this inhibition include the stress-induced kinases that respond to inflammatory signals and phosphorylate IRS at the serine residue, of significance to this work are the AKT, mTOR and AMPK cascades (Nandipati et al., 2017). As for PI3K, several identified subunits have been shown to be associated with actions that are responsible for the mobilization of glucose transporters and insulin-dependent glucose uptake. A significant and noteworthy regulatory mechanism associated with PI3K activation is the inverse relation between PTEN and PIP3 levels. When PIP3 levels are high, PTEN is inhibited commencing PI3K-mediated glucose metabolism (Manna et al., 2011). Like mTOR, this cascade is well regulated, and any disruption elevates the risk of disease onset. In this case, insulin resistance may also arise from a disruption in PI3K/AKT (Świderska et al., 2018).

In addition to the mentioned pathways, the **AMPK** pathway is another significant serine/threonine protein kinase that is vital for energy homeostasis. AMPK is an abundantly expressed and conserved cellular energy sensor and one of the key cellular signaling pathways that govern catabolic-anabolic reactions in response to metabolic cues, be it related to the nutrient or energy status of the cell. AMPK works closely with mTOR in maintaining a homeostatic balance. AMPK is regulated by the intracellular ratio of AMP/ATP. AMPK is activated under nutrient stress conditions and energy deficiency and in turn, inhibits mTORC1 (Andersson et al., 2004; Han et al., 2005; Kim et al., 2004; Kola et al., 2005; Minokoshi et al., 2004). AMPK is heterotrimeric with a catalytic α subunit and regulatory β and γ subunits. When AMP levels are high, the phosphorylation of the catalytic α subunit and the activation of AMPK is stimulated (Hardie, Carling, & Carlson, 1998; Kemp et al., 1999). Once activated, AMPK augments the production of ATP through enhancing catabolism and arresting energy expenditure, partially through inhibiting mTORC1 by allosteric inhibition (Gwinn et al, 2008). Therefore, the interplay between AMPK and mTORC1 allows the mammalian cell to integrate external stimuli and respond to environmental conditions that would promote survival and maintain energy homeostasis. The earliest evidence of crosstalk between the mTOR and AMPK is that AMPK can regulate S6K and 4EBP, the main downstream effectors of mTORC1, suggesting that these two axes converge at some point. AKT is one of the common players between the two axes. Studies identified that Akt,

not only lies upstream of mTORC1 but also of AMPK. AKT activates mTORC1 through the full inhibition of TSC2 and the inhibition of AMPK (Hahn-Windgassen et al., 2005 Beirowski et al., 2014). At the biological level, AMPK has been described to be associated with glucose and lipid metabolism (Eid et al., 2010; Xu et al., 2005) as well as glucose transport (Wright et al., 2004). Emerging studies point to the role of impaired AMPK activity in mediating metabolic stress and diabetes-related injuries (Eid et al.,2010; Roy Chowdhury et al., 2012; Dugan et al., 2013; Guo et al., 2015; Mroueh et al., 2019) with implications in insulin resistance and autophagy (Liu et al., 2014; Yao et al., 2016).

It is evident that these axes play a significant role in cellular metabolism and survival in a collaborative manner. Any disruption in one axis may lead to a pathological series of events that impact energy kinetics and consequently tipping the homeostatic balance. In the next section, we explore the overall consequences as well as importance of these pathways in diabetes and PNS physiology.

2. Implications in PNS Physiology and Pathophysiology

The PNS is comprised of neural tissue that infiltrates the deep innervations of the visceral and somatic tissues, and thus may affect voluntary and involuntary processes. The PNS is complex and dependent on the intricate interaction between immune cells, glial cells, and stem cells in support of transmitting information via afferents, efferents and receptors to and from the CNS (Shang et al., 2011; Sato et al., 2010; Shin et al., 2010; Krames, E., 2014). Here, we overview the roles of mTOR, AKT and AMPK that mediate cellular processes in the aforementioned cell types and are therefore major contributors to PNS myelination, regeneration, and autophagy (Nave, K., 2010; Menzies et al., 2017).

i. Affiliations in PNS myelination

After SC early life development and maturation, postnatal SC division and proliferation is halted in response to Neuregulins on the surface of axons, which are cell-signaling molecules that are ligands for ErbB receptor tyrosine kinases located on SC membranes (Michailov et al., 2004; Taveggia et al., 2005; Schulz et al., 2014; Nave and Salzer, 2006). This triggers the myelination process in SCs. Although numerous factors collectively influence SCs and are reportedly described to modulate myelination in the PNS at different stages (recently reviewed by Castelnovo et al., 2017 and Quintes and Brinkmann, 2017, Jacob, C., 2017, Gennari et al., 2017; Qian et al., 2018), one of the essential pathways implicated in the activation of the ErbB receptors is the

PI3K/AKT/mTOR cascade, which is majorly involved in myelin gene expression, myelin thickness and SC division and survival (Maurel and Salzer, 2000). This activation polarizes SCs and stimulates their membranous outgrowths via build-up and break-down of cytoskeletal filaments for myelination (Goebbels et al., 2010). The myelinating phenotype of SCs is under rigid control by the PTEN pathway. Goebbel et al showed that lifting PTEN control in PTEN knockouts lead to an overmyelination (Goebbels et al., 2010). Further to this finding, PTEN interferes with AKT to curb myelination rate postnatally. The inhibition of PTEN lifts AKT inhibition on SCs myelination programs, and in this manner, AKT is central to maintaining the myelin sheath (Cotter et al., 2010). In all, Akt modulates myelin thickness regulation, axonal wrapping, and membrane production.

As for the role of this cascade in PNS pathologies, studies demonstrate enhanced SC myelination and wrapping due to the constitutive activation of AKT through PTEN ablation to be attenuated upon treatment with mTORC1 inhibitor, rapamycin, indicative of a role of the mTOR pathway as well in myelin abnormalities (Goebbels et al., 2012). As a matter of fact, more recent studies show mTORC1 deficiency to be associated with influencing lipid-synthesizing enzyme expression, hypomyelination and reduced nerve conduction velocities (Hall, M. N., & Suter, U. 2014). Additional studies examined the effect of total mTOR prenatal ablation in SCs *in vivo*. The results of the findings from that group demonstrated poor SC growth and myelination reflected by defected axonal elongation and reduced myelin protein expression (Sherman et al., 2012). A more recent investigation attributed the observed peripheral nerve deficits in myelin by Sherman et al to mTORC1, with negligible influence by mTORC2 deficiency (Norrmén et al., 2014). These findings are consistent with a recent study describing the role of the PI3K/AKT/mTOR in SC development and myelination. Figlia et al provide evidence of differential mTORC1 activity that fluctuates with stages of SC development from radial sorting to differentiation of SCs, initiation of the myelination processes and myelin growth (Figlia et al., 2017). It was shown that mTORC1 activity in early SC development was elevated suppressing myelination onset. As differentiation of SCs progresses, mTORC1 activity gradually decreases lifting the suppression and activating mTORC1-independent downstream effectors of PI3K and AKT such as sterol regulatory-element binding protein 1 (SREBP) lipid and protein synthetic pathways (Norrmén et al., al., 2014; Sheean et al., 2014; Montani et al., 2018). By contrast, AKT hyperactivation in the early developmental stages of SCs was shown to inhibit myelination of SCs in an ex vivo experimental setup (Figlia et

al., 2017). However, constitutively active AKT was reported to lead to peripheral nerve hypermyelination in transgenic mice (Domènech-Estévez et al., 2016).

On the other hand, when SCs have differentiated and crossed their developmental path, elevated mTORC1 activity was shown to induce myelin growth (Beirowski et al., 2017). In a more recent study, additional mTORC1 modulatory pathways such as TSC1/2 in addition to PTEN were shown to interfere with the AKT and mTORC1 complexes during SC development (Wong and Beirowski, 2018). Both AKT and mTORC1 levels are reported to be elevated in early SC development whereby mTORC1 controls SC precursor proliferation until successful escape from the proliferative phase. Afterwards, AKT-mediated sorting takes place guiding SC lamellipodia to axonal targets formed 'naked axons' until both AKT and mTORC1 levels gradually lift their suppressive control on myelination and permit thickening and compaction of the myelin sheath. Thus, the roles of AKT and mTORC1 in early peripheral nerve development are that of fine-tuning. Moreover, in TSC1/2 SC-knockouts, Wong and Beirowski demonstrated reduced mTORC2 activation via its Rictor component in sciatic nerves, which is counterintuitive since mTORC1 hyperactivity induces the activation of its S6kinase effector, which is another known to activate Rictor (Wong and Beirowski, 2018). With these findings, the role of mTORC2/AKT axis remains poorly elucidated in SC physiology and in need of further studies.

ii. The autophagic flux in the PNS

From prenatal to postnatal environments, SCs undergo several distinct physiological stages that begin in the neural crest and then migrate to their target site and associate with axons on a 1:1 basis, extend their lamellipodia to larger caliber axons at the periphery through radial sorting, at which stage they are referred to as pro-myelinating SCs. Then SCs proliferate while myelinating and finally defasciculate forming complete fibers (Feltri et al., 2016). In cases of peripheral nerve injury, SCs undergo another change in differentiation state, and a repair SC profile ensues. The repair SC is activated in response to transcription factors following peripheral nerve injuries (Jessen and Mirsky, 2016) and G-protein coupled receptors (reviewed by Fernandez et al., 2017). Consequently, a myelin bolus of debris forms and myelinophagy (Gomez-sanchez et al., 2015) takes place in stages that involve first the proliferation of SCs followed by macrophage recruitment (Martini et al., 2008; Chen et al., 2015). Myelinophagy is a special type of autophagic response in the PNS and is described to be mTOR-independent. Autophagy, also known as the cell's recycling

system, is believed to classically be activated upon mTOR suppression via rapamycin or other agents.

In this spirit, the significance of autophagy as a survival mechanism in demyelinating neuropathies was demonstrated in a neuropathic mouse model characterized by PMP22 overexpression and cluster formation, rendering SCs neuropathic with unsustainable myelin sheaths. Rapamycin administration was shown to reduce elevated PMP22 levels, morphologically and functionally rescuing SCs (Rangaraju and Notterpek, 2011). However, other studies showed autophagy to ensue while mTOR is active in response to nerve transection (Kroemer et al., 2010; Ravikumar et al., 2010). In this manner, it is evident that appropriate autophagy mechanisms are necessary for PNS repair, whereby in demyelinating neuropathies, the autophagic response of peripheral nervous tissue has been reported to be defected (Gomez-sanchez et al., 2015).

On another note, evolving studies are beginning to show autophagy to be involved in PNS remyelination. Under controlled conditions, typical responses to injury in the PNS involves the migration of repair SCs to form bands of Bügner, leading axonal regeneration and flagging the site of injury for the recruitment of growth factors and essential nutrients for regrowth (Chen et al., 2007). This may be mediated by AKT which was demonstrated to mediate the migratory potential of SCs (Yu et al., 2015). At this stage, SCs differentiate into a myelinating phenotype in an autophagy-dependent manner. Jang et al have studied this phenomenon postnatally in SC-specific knockout mice for autophagy-related protein 7 (ATG7), a specific marker of macroautophagy in SCs. Electron microscopy revealed deficient autophagolysosomal activity, harvesting SC cytosol and organelles, with an electron-dense cytosol and enlarged endoplasmic reticulum morphology (Jang et al., 2015). The reduction of cytoplasmic volume is an essential stage in myelin maturation. In early postnatal periods, Rapamycin-induced activation of autophagy was shown to mediate SC cytoplasmic volume reduction. Therefore, Jang et al have shown that autophagy contributes to plasticity of the SCs rather than initiation of myelination or myelin sheath regulation such that in another study, the myelinating phenotype of SCs was shown to commence despite ATG-7 knockout. This suggests that myelination initiation/regulation is significantly different mechanistically speaking compared to nerve repair through autophagy (Jang et al., 2015; 2016). Nevertheless, it is evident that the involvement of mTOR and autophagy is essential to the regenerative potential of the PNS. Together, mTOR and the associated pathways integrating the PNS signals render the PNS physiology interactive and intricately complex. Homeostatic feedback

loops and rigid control over these pathways is significantly essential for PNS function. For that matter, the mTOR cascade is particularly addressed by virtue of its central role as the metabolic drive and functional modulator of the PNS. In the following section, the consequences of mTOR dysregulation will be discussed.

3. Implications in Diabetes: The Fate of Peripheral Nerve Innervations

Impaired insulin signaling in the PNS is central to the development of DPN (Kim et al., 2011; Grote et al., 2013). The role of the PI3K/AKT/mTOR axis in DPN pathobiology is only recently being comprehended.

In one study, the role of skin keratinocytes in mediating small fiber neuropathy was assessed and the work shows that semaphorin-3A, a factor involved in guiding cells of the nervous system, to be overexpressed when cultured in a high glucose-containing media, in addition to activated mTOR, leading to low small fiber counts in skin from diabetic subjects. Rapamycin treatment was further shown to ameliorate reductions in intra-epidermal nerve fibers, indicative of a possible therapeutic outcome in small fiber neuropathy (Wu et al., 2017). Further downstream targets to mTOR activation have been identified in the PNS. Diabetes-induced mTOR phosphorylation was also shown to modulate the function of a Nav1.8, which is a sodium ion channel expressed largely in DRG neurons that are unmyelinated and contributes to the transmission of pain (Ye et al., 2016). Activation of Nav1.8 channels was demonstrated to significantly peak at 4 weeks post-STZ induction concomitant with activated mTOR, mediating mechanical allodynia and hyperexcitability of DRG neurons. Rapamycin administration was shown to relieve diabetes-induced hyperalgesia by reducing the phosphorylation of Nav1.8 demonstrated by suppressed current densities in rapamycin-treated DRG neuronal cells compared to diabetics (He et al., 2016).

Further along the axis, neuronal hyperexcitation as a result of chronic pain may also be attenuated by AMPK activators (Melemedjian et al., 2011; Tillu et al., 2012). Furthermore, AMPK is reported to play a role in moderating neuronal functions, hyperexcitation and survival with a dual role in modulating autophagy in conditions such as chronic pain and diabetes (Atef et al., 2019; Wang et al., 2018; Roy Chowdhury et al., 2012; Ma et al., 2015; Chung et al., 2018). In support of this, the pharmacological activation of AMPK was shown to alleviate mechanical allodynia via negatively modulating the expression of membrane bound pain sensor, transient receptor potential ankyrin 1 (TRPA1) in DRG neurons in animal models of painful DPN (Wang et al., 2018). From that end, dissecting the AMPK pathway and its crosstalk with the mTOR

complexes to identify central targets such as TRPA1 is of critical value. TRPA1 channels for instance have been shown to be involved in reducing cutaneous nerve fiber innervations upon the onset of hyperglycemia, and their activity has been shown to be fueled by diabetes-evoked biochemical alterations and their intermediates (Koivisto et al., 2012). Moreover, Neuritin is another target that has been recently revealed in the context of DPN, downstream of the PI3K pathway. Insulin-like growth factor 1 (IGF-1) is a neurotrophic whose expression has been reported to be downregulated in diabetes contributing to DPN pathologies (Delaney et al., 2001; Jian-bo et al., 2010). The exogenous supplementation of IGF-1was shown to rescue primary rat SCs cultured in high glucose from apoptotic cell death, mediated by neuritin (Yan et al., 2018). Moreover, in STZ-induced diabetic animals, the direct intrathecal administration of low doses of insulin ameliorated sensorimotor conduction velocities perturbations and defects in myelinated peripheral nerves with insulin receptors likely located on perikarya of DRG neurons (Brussee et al., 2004; Guo et al., 2011). However, this may not be extrapolated to T2D (Singh et al., 2012; Kim et al., 2011). In a study by Grote et al, insulin and IGF-1 administration were reported to be ineffective in DRG and sciatic nerves where insulin receptor expression was reduced, indicative of resistance to insulin (Grote et al., 2013). This defected signaling was shown to involve impaired PI3K/AKT signaling conferring some degree of insulin resistance, and contributing to DPN (Singh et al., 2012, Grote et al., 2013; reviewed by Grote and Wright, 2016).

Likewise, mTOR, too, plays a role in insulin signaling and resistance. In a study conducted in mTORC1 and mTORC2 substrates $S6K1^{-/-}/AKT2^{-/-}$ double knockout mice, primary hepatocytes were isolated and cultured to assess insulin sensitivity, which was reported to be impaired as demonstrated by deregulated glycolytic and gluconeogenic activity in addition to insulin resistance concomitant with blunted pancreatic β-cell growth (Trein et al., 2012), further corroborating the role of mTORC2 in insulin signaling (Lamming et al., 2012). In this spirit, mTORC1 has also been reported to mediate diabetic nephropathy and neurocardiac injury (Gödel et al., 2011; Maiese, K. 2015). Ongoing studies in our laboratory investigate the role of mTORC1 in DPN. By contrast, studies on mTORC2 are very limited. Eid S et al. showed the implications of mTORC2 through an Akt dependent pathway to play a key role in diabetic kidney injury, indicative of a therapeutic potential of mTORC2 inhibition (Eid et al., 2016).

From this standpoint, speculations as to whether the mTOR, AKT and AMPK pathways may influence PNS insulin responses remain limited and poorly understood in DPN. To our knowledge,

no studies have established a link between CYP450 and the signaling pathways of mTOR, AKT, or AMPK in DPN.

G. Rationale, Hypothesis, and Aims of the Study

The PNS is one of the bodily systems that are especially vulnerable to dysmetabolism triggered by diabetes. In fact, DPN is one of the significant complications whose onset is autonomous despite strict glycemic control. To date, the pathological mechanisms underlying the progression of nerve injury in DPN is poorly understood. More importantly, due to the complexity of the neural and peripheral vasculature web, identifying specific novel targets that cater to this milieu is currently the therapeutic challenge undertaken to halt the progression of DPN. In this light, the central focus of this work is to comprehend the mechanistic progression of DPN from the standpoint of CYP enzymes. Extensive studies show a clear coalition between CYP-induced oxidative stress and diabetic complications, but the implication of CYPs as sources of oxidative stress in peripheral nerve injury is a theory subject to investigations. To our knowledge, this study is the first to highlight the pathological role of CYPs in DPN.

To investigate, the work was split into a major *in vivo* assessment followed by *in vitro* experimentation.

Aim 1: Investigate the effect of diabetes on behavioral and neurophysiological parameters such as locomotor function and sensation in diabetic mice. Then assess the mechanisms by screening for CYP4A and CYP2C expression, 20-HETE and EET levels, in addition to molecular changes in myelination and peripheral nerve physiology.

Aim 2: Assess oxidative stress and ROS production as well as identify downstream pathways implicated in the CYP-mediated injury. Utilize selective and specific pharmacological intervention with 20-HETE synthesis or sEH enzyme inhibitors to dissect the pathways altered.

Aim 3: Screen for the expression of CYPs in SCs in normal and hyperglycemic states and assess cellular damage by observing morphological, phenotypic and protein alterations in response to exogenous 20-HETE or EET administration.

The hypotheses **(Fig. 10)** were formulated as follows:

Hypothesis 1: Diabetes induces oxidative stress through altering CYP expression and activity affecting AMPK and AKT signaling. This disrupts homeostatic balance, myelin sheath

maintenance, and neural survival. These insults eventually lead to SC and peripheral nerve injury by altering myelin protein levels, autophagy and apoptotic death.

Hypothesis 2: Selectively inhibiting CYP4A/20-HETE synthesis, or EET degradation by sEH, may reverse damage at the molecular and behavioral levels, in addition to restoring 20-HETE and EET homeostatic balance.

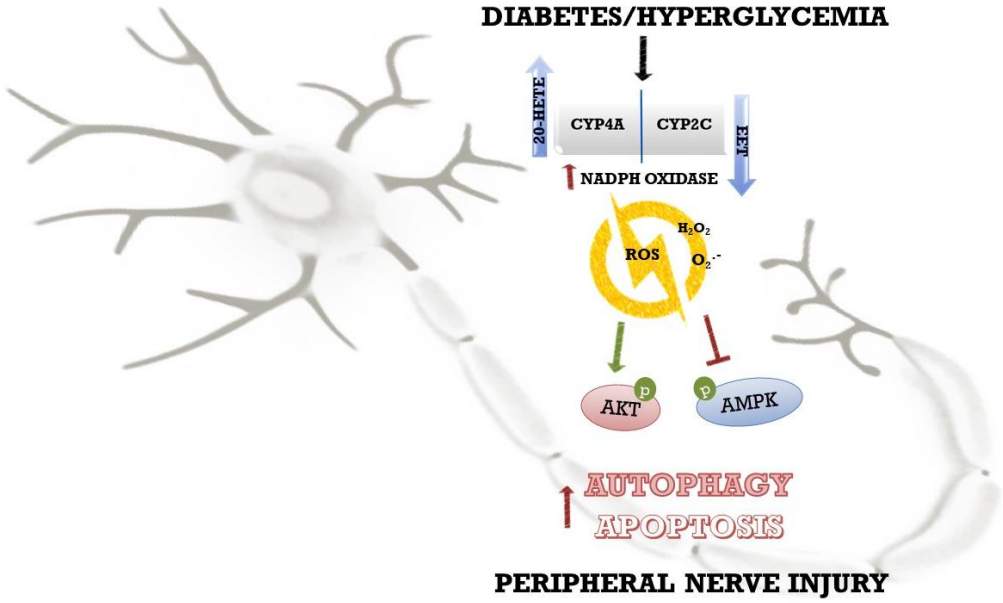


Figure 10. Hypothesis. Hyperglycemia leads to oxidative stress through alteration in CYP450 enzymes expression and activity. Increased ROS production secondary to defected AMPK and AKT signaling triggers Schwann cell and peripheral nerve injury through myelin protein alteration, defected autophagic response, and apoptosis.

II-Materials and Methods

A. Animal Studies

All animal work was conducted according to the National Institute of Health guidelines and approved by the Institutional Animal Care and Use Committee at the American University of Beirut. MKR male mice were used for this work. They are a transgenic murine model that exhibits the genotypic and phenotypic profiles of human type 2 diabetes (T2DM), and develop hyperglycemia starting 8 weeks of age (Fernández et al., 2002; Kawashima et al., 2009). Briefly, the knockdown of the MKR promoter in skeletal muscles of these animals triggers peripheral insulin resistance without the development of obesity (Fernández et al., 2002; Vaitheesvaran et al., 2010; Jackson laboratories). This feature makes the MKR murine model robust for the study of diabetic complications from the standpoint of hyperglycemia and insulin-resistance in T2DM. Age-matched FVB/NJ males served as the normoglycemic, control group. The animals were grouped into three subsets 1) non-diabetic controls treated with vehicle (FVB-Ctr) 2) untreated diabetics (MKR-Db) 3) diabetics treated with HET0016 (MKR-Db + HET0016) or Metformin (MKR-Db + Metformin) or AUDA (MKR-Db + AUDA). All animals were kept in a temperature-controlled room and on a 12/12-dark/light cycle and ad libitum access to standard chow and water. Random blood glucose levels were monitored via tail vein punctures. Prior to sacrifice, sensory motor dysfunction was assessed using the Raised Beam Walking Test and Hind paw Withdrawal Test. Neuromuscular interaction was assessed using the Grip Strength Test.

Diabetic animals were subjected to the following treatments:

-HET0016 (Cayman Chemicals) is a specific 20-HETE synthase/CYP4A inhibitor (Miyata et al., 2001) which was administered subcutaneously and daily for a period of 10 weeks at a dose of 2.5 mg/kg (Eid et al., 2009).

-AUDA (Cayman Chemicals) is a specific sEH inhibitor (Imig et al., 2005) and was administered orally with a gavage needle daily at 10 mg/kg.

-Metformin, an activator of the AMPK signaling pathway, was administered daily by intraperitoneal injection at a low dose of 150 mg/kg for 13 weeks (Mroueh et al., 2019).

NB. When drugs were administered when the MKR mice were at 10 weeks of diabetes onset, the study phase was considered as an early prevention. However, intervention studies were considered when drug administration occurred past 28 weeks of age.

B. Functional Assessment for Neuropathy

Peripheral nerve function was further assessed via electrophysiological assessment of the Nerve Conduction Velocity as previously described (Vincent et al., 2007, Eid et al., 2020). Measures of nerve conduction velocity (NCV) were performed in anaesthetized mice at 32–34°C using a heating pad. Motor Nerve Conduction Velocity (MNCV) was determined by measuring compound muscle action potentials using supramaximal stimulation distally at the ankle and proximally at the sciatic notch. The MNCV was calculated by dividing the distance between the cathode positions by subtracting the distal latency from the proximal latency. Sensory Nerve Conduction Velocity (SNCV) was recorded behind the median malleolus in the digital nerve to the second toe by stimulating with the smallest current that resulted in a maximal amplitude response.

C. Behavioral Assessment for Neuropathy

To assess motor coordination and balance, the Raised Beam Walking test was performed as previously described (Luong et al., 2011). Briefly, animals were placed on a platform above a flat surface. Animals were placed for habituation and then trained to cross the platform. Once able to perform the test, the time taken to cross the platform, the speed, the number of stops and the number of faults/slips were recorded for analysis over 3 separate trials. To assess thermal analgesia and pain perception, the Hind Paw Withdrawal test was performed (Dirig et al., 1997). The IITC plantar Analgesia meter was used. The test features a heating beam set at an idle intensity of 2% and active intensity of 25% with a cut-off time set at 20 seconds and a platform set at 32 ℃ for acclimation. The thermal stimulus was targeted at the hind paw of animals and the time to sense the heat and withdraw their paws was recorded for analysis, 6 measurements per mouse. The final test assessed muscle tone and neuromuscular strength via the Grip Strength test (Brooks & Dunnett, 2009). Animals were trained to hang using their forelimbs from a stand until their grip fails. The time spent hanging was recorded over 3 consecutive days.

D. ROS Detection by Confocal Microscopy

Dihydroethidium (DHE) is an oxidative fluorescent dye that undergoes a two-electron oxidation to form the DNA-binding fluorophoreethidium bromide. DHE staining was carried out

as previously described (Maalouf et al., 2012). Briefly, frozen sciatic nerves were cut into 4 µm thick sections and placed on glass slides. DHE (20 µmol/l) was applied to each tissue section, and the slides were incubated in a light-protected humidified chamber at 37°C for 30 min. Fluorescent images of ethidium-stained tissue were obtained with a laser-scanning confocal microscope (Zeiss, LSM 710) at t=30 mins. Fluorescence was detected at 561 nm long-pass filter. ROS production was demonstrated by red fluorescent labeling. The average of four areas per section stained with DHE was taken as the value for each animal. Quantification was done using Zen light Software.

E. ROS Detection and 20-HETE and EET production by High Performance Liquid Chromatography

Superoxide-specific production was further assessed via High Performance Liquid Chromatography as previously described **(Eid et al., 2016, Eid et al., 2020).** Sciatic nerve homogenates were washed with Hanks balanced salt solution (HBSS)-diethylenetriaminepentaacetic acid (DTPA) twice and incubated for 30 min with 50 µM DHE (Sigma-Aldrich) in HBSS–100 µM DTPA. Tissues were then processed for analysis. EOH and ethidium fluorescence was detected with excitation at 510 nm and emission at 595 nm, whereas DHE fluorescence was detected by UV absorption at 370 nm. The results are expressed as the amount of EOH produced (nmol) normalized for the amount of DHE consumed (µmol).

Levels of 20-HETE and EET were measured in sciatic nerves by HPLC. In short, [1-14C]-labeled arachidonic acid (50 –100 µmol/l) was dried down and resuspended in the reaction mix containing 50 µg microsomes, 30 mmol/l isocitrate, and 0.2 unit isocitrate dehydrogenase in reaction buffer (100 mmol/l potassium phosphate, pH 7.4, 5 mmol/l magnesium chloride, and 1 mmol/l EDTA). After incubation at 37°C for 5 min, the reaction was initiated by the addition of NADPH to a final concentration of 1 mmol/l. Aliquots were removed at 30, 60, and 90 min, and the reaction was stopped by the addition of 100% methanol. The precipitated proteins were then pelleted by centrifugation (in a microcentrifuge), and the samples were stored at -20°C until analyzed. The metabolites were separated via HPLC on a C-18 column using an acetonitrile/H20 gradient and identified by coelution with labeled standards.

F. NADPH Oxidase Activity Assay

Proteins were extracted from crushed frozen sciatic nerves in a lysis buffer (20 mM KH2PO4 (pH 7.0), 1 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, 10 µg/ml aprotinin, and 0.5 µg/ml leupeptin). The assay reaction contained 25 µg of homogenates added to 50 mM phosphate buffer

(pH 7.0), 1 mM EGTA, 150 mM sucrose, 5 μM lucigenin, and 100 μM NADPH. Photon emission expressed as relative light units (RLU) was measured every 30 s for 5 minutes in a luminometer. Superoxide production was expressed as relative light units/min/mg of protein (Eid et al., 2009; Eid et al., 2013; Eid et al., 2016).

G. Immunohistochemistry

Paraffin embedded, formalin-fixed sciatic nerves were cut into 5 μm sections and fixed onto star-frosted slides. Sections were deparaffinized by incubation at 56°C for 50 minutes. Then they were immersed in xylene, rehydrated in a descending alcohol gradient, and distilled water. Sections were then incubated with sodium citrate buffer in a humidified chamber for 1 hour for antigen retrieval. The subsequent reactions were performed using Novolink Polymer Detection Kit (Leica Biosystems) according to manufacturer instructions and at room temperature. Sections were stained overnight at 4°C with Beclin-1 and LC3B protein (Cell Signaling), processed on the following day for visualization with DAB chromogen, counterstained using hematoxylin, dehydrated, and mounted. Slides were examined using the Olympus CX41 microscope by a blinded observer and quantified using the color deconvolution plugin with NIH ImageJ software.

H. Western Blotting

Mouse sciatic nerves or MSCs were lysed using RIPA buffer containing 0.1% sodium dodecyl sulfate (SDS), 0.5% sodium deoxylate, 150 mM sodium chloride, 100 mM EDTA, 50 mM Tris-hydrochloride, 1% Tergitol (NP40), 1% of the protease and phosphatase inhibitors and 1mM phenylmethylsulfonyl fluoride. The lysates were centrifuged at 13,600 rpm for 30 minutes at 4°C. Protein concentration in the supernatants was measured using the Lowry Protein Assay. For immunoblotting, 20-40 μg of proteins were separated on 12-15% polyacrylamide gel Electrophoresis (Bio-Rad Laboratory, CA, USA) and transferred to nitrocellulose membranes (Bio-Rad Laboratory, CA, USA). The blots were blocked with 5% BSA in Tris-buffered saline and then incubated overnight with rabbit polyclonal anti-CYP4A (1:2000, Abcam), rabbit polyclonal anti-CYP2C (1:3000, Abcam), rabbit polyclonal anti-P0 (1:1000, Sigma Aldrich), rabbit polyclonal anti-PMP22 antibody (1:1000, Sigma Aldrich), Beclin-1 (1:1000, Cell Signaling), LC3B (1:500, Cell Signaling), pAKTSer473 (1:1000, Cell Signaling), pAMPKThr172(1:1000, Cell Signaling), AKT Total (1:1000, Cell Signaling), AMPK Total (1:1000, Cell Signaling). Mouse polyclonal anti-HSC70 (1:1000, Santa Cruz), mouse polyclonal anti-GAPDH (1:1, Santa Cruz) and rabbit polyclonal anti-GAPDH (1:1000, Santa Cruz) were

considered as housekeeping, loading controls. The primary antibodies were detected using horseradish peroxidase–conjugated IgG (1:1000, Bio-Rad). Bands were visualized by enhanced chemiluminescence. Densitometric analysis was performed using Image J software (Eid et al., 2009)

I. PMP22 Aggregation Assay

PMP22 is one of the aggregate-prone proteins whose accumulation has been linked to neurodegeneration (Fortun et al., 2007, Hamilton et al., 2013). In this study, we assess this aspect of injury in mouse sciatic nerves which were dounced and lysed in immunoprecipitation buffer comprised of 10 mM Tris–HCl [pH 7.5], 5 mM EDTA, 1% Nonidet P-40, 0.5% deoxycholate, 150 mM NaCl and supplemented with protease inhibitors. The lysates were microcentrifuged at 13000g for 15 minutes. The pellet formed harbors the insoluble material which was then incubated with 10 mM Tris–HCl, 3% SDS for 10 min at room temperature, then sonicated for 20 seconds at an amplitude of 30% (Optic Ivymen System). The total protein concentrations were measured using the Lowry Protein Assay and then used for Western Blot analysis in equal amounts (Hichor et al., 2017).

J. Cell Culture

Mouse Schwann cells (MSC80) were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Sigma-Aldrich, Steinheim, Germany) containing 10% Fetal Bovine Serum (FBS) (Sigma-Aldrich) and 1% Penicillin/Streptomycin (P/S) (Sigma-Aldrich). Cells were incubated at 37ºC with 5% CO2 until confluency was reached. For experimental work, cells were serum deprived overnight then treated with high glucose (25mM) in the presence or absence of 10 μM CYP4A inhibitor (HET0016) or 1 μM sEH inhibitor (AUDA), or 1.5 mM AICAR (AMPK activator). In parallel experiments, cells were treated with 1.5 μM 20-Hydroxyeicosatetraenoic acid (20-HETE, Cayman Chemicals) or 3 μM exogenous 14,15-Epoxyeicosatetraenoic (EET, Cayman Chemicals) in the presence of normal glucose (5mM). Cells incubated with 5mM glucose are euglycemic and considered controls.

K. Microsome Isolation

MSC80 cells were incubated either in normal glucose (NG), high glucose (HG), or 20-HETE and then homogenized, dounced, and sonicated in a 250 mM sucrose, 1mM EDTA, 2μg/ml aprotinin, 2μM leupeptin and 1mM PMSF. The homogenates were then centrifuged at 10,000g for 15 minutes at 4ºC. The supernatant was collected and differentially centrifuged 65000 rpm for 30

min at 4ºC. The microsomal pellet was then resuspended in a buffer consisting of 50 mM potassium phosphate of pH 7.5, containing 0.2mM EDTA and 20% glycerol. The microsomal pellet was then used for Western blotting (Eid et al., 2009).

L. Cellular DNA Fragmentation / Apoptosis Assay

Schwann cells apoptosis was investigated *in vitro* using the cellular DNA fragmentation ELISA kit (Roche Diagnostics GmbH, Mannheim, Germany). BrdU-labeled DNA fragments in. MSC80 cells were grown in 12-well tissue culture plates until 60-70% confluence and serum deprived overnight and then treated for 48 hours according to the experimental conditions. A microplate was prepared using an anti-DNA coating solution containing anti-DNA antibody adsorptively fixed to the wells of the plate. Then, BrdU-labeled DNA fragments (10 μM), which bind to the immobilized anti-DNA antibody, were added to the cultured MCT cells 12 hours before stopping the treatment. In the third step, the immunocomplexed BrdU-labeled DNA fragments were denatured and fixed on the surface of the microplate by microwave irradiation, in order to improve the accessibility of the antigen BrdU for detection by the antibody. As a final step, anti-BrdU-peroxidase conjugate reacted with the BrdU incorporated into the DNA. The amount of peroxidase bound in the immune complex was photometrically determined, after the removal of unbound peroxidase conjugates and the addition of the substrate solution. Absorbance was measured at 450 nm against a reference wavelength of 650 nm using a microplate reader (Multiskan Ex). The mean of triplicate experiments was used +/- SD.

M. Statistical Analysis

Data analysis was performed using GraphPad Prism 6. Statistical significance was assessed by one-way ANOVA with Tukey's post-test for multiple comparisons. For two group comparisons, student's unpaired t-test was used. Data are presented as mean ± standard error from multiple independent experiments. Statistical significance was determined as a probability (P value) of less than 0.05.

III-Results

PART 1: Activation of 20-HETE Synthase Triggers Oxidative Injury and Peripheral Nerve Damage in Non-Obese Type 2 Diabetic Mice

Table 3. Metabolic characteristics – HET0016 prevention

Group	*n*	HbA1c (%)	Circulating insulin (ng/mL)	Body weight (g)
FVB-Control	*11*	5.1 ± 0.1	1.0 ± 0.1	30.9 ± 0.5
MKR-Diabetic	*11*	8.26 ± 0.3*	4.5 ± 0.4*	28.8 ± 0.9
MKR-Diabetic+ HET0016	*11*	8.04 ± 0.23*	4.5 ± 0.4*	28.6 ± 0.4

Terminal body weights, glycated hemoglobin (*HbA1c)* and serum circulating insulin *levels* in 10 weeks old FVB control mice and T2DM MKR mice. T2DM MKR mice were treated with HET0016 (2.5 mg/kg) for 10 weeks. Values are the means ± SEM, HbA1c from n=5, and circulating insulin from n=4-6. *P<0.05, vs. control. #P<0.05, vs. diabetic.

Effect of HET0016 administration on the glycemic indices of MKR non-obese T2D mice.

FVB controls, MKR or MKR mice treated with the specific 20-HETE synthase/CYP4A inhibitor, HET0016 for 10 weeks were monitored for fluctuations in body weight and glucose levels (to assess progression of hyperglycemia) throughout the entire study as well as circulating insulin and Hb_{A1C} levels upon sacrifice. The data show a slight yet non-significant decrease in body weights of the animals while a significant increase in glycated hemoglobin levels and circulating insulin levels were observed in tandem with the development of hyperglycemia in diabetic animals compared to the control group **(Table 3).** Similarly, HET0016-treated diabetic animals exhibit significantly elevated HbA1c indices and insulin levels compared to controls **(Table 3).** Interestingly, HET0016 administration did not have any significant effect on the glycemic index, or insulin resistance of the diabetic animals rending it ineffective as a hypoglycemic agent. These results suggest that the effect of HET0016, if observed, is independent from any glucose regulating effect.

CYP4A inhibition attenuates sensory and motor deficits in MKR non-obese T2D mice.

To investigate the role of HET0016 in diabetes-induced nerve dysfunction, we examined various sensory and motor modalities of the peripheral nerve in the FVB controls, MKR or MKR mice treated with HET0016, via a series of neurophysiological and behavioral tests. Nerve conduction velocity measurements reflect a decrease in sensory and motor conduction in the MKR diabetic animals compared to their FVB controls. By contrast, NCV readings in the HET0016-treated group show significant neurophysiological improvements relative to the diabetic group (**Fig 11. A, B).** Moreover, sensorimotor dysfunction was reflected by the Raised Beam Walking test. Testing results show MKR animals to perform over a longer period of time **(Fig. 11C)** owing to a significantly reduced speed **(Fig. 11D)** while crossing the beam with an increased tendency to stop **(Fig. 11E)** and slip **(Fig. 11F)** in comparison to their FVB controls that seemed to exhibit minimal setbacks. Importantly, HET0016-treated MKR mice performed comparably to the FVB control littermates **(Fig. 11C-F).** We further assessed sensory nocifensive responses of the animals and predicted that mice with no peripheral nerve injury are capable of thermal sensation compared to animals with diabetes. In the Hind Paw Withdrawal test **(Fig. 11H)**, the results returned from the MKR diabetic mice show an increased withdrawal latency that achieved statistical significance by contrast to their FVB controls. Interestingly, HET0016 treated MKR mice had a significantly lower latency suggesting that the treatment alleviated deficits in thermal pain perception. Further, we examined neuromuscular strength via the Grip Strength test. MKR animals exhibit a reduced latency to fall indicative of compromised motor function. By contrast, MKR animals treated with HET0016 performed with significantly increased strength compared to the untreated MKR mice **(Fig. 11G).** Taken together, these results indicate that HET0016 treatment was effective in restoring sensorimotor coordination in type 2 diabetic animals.

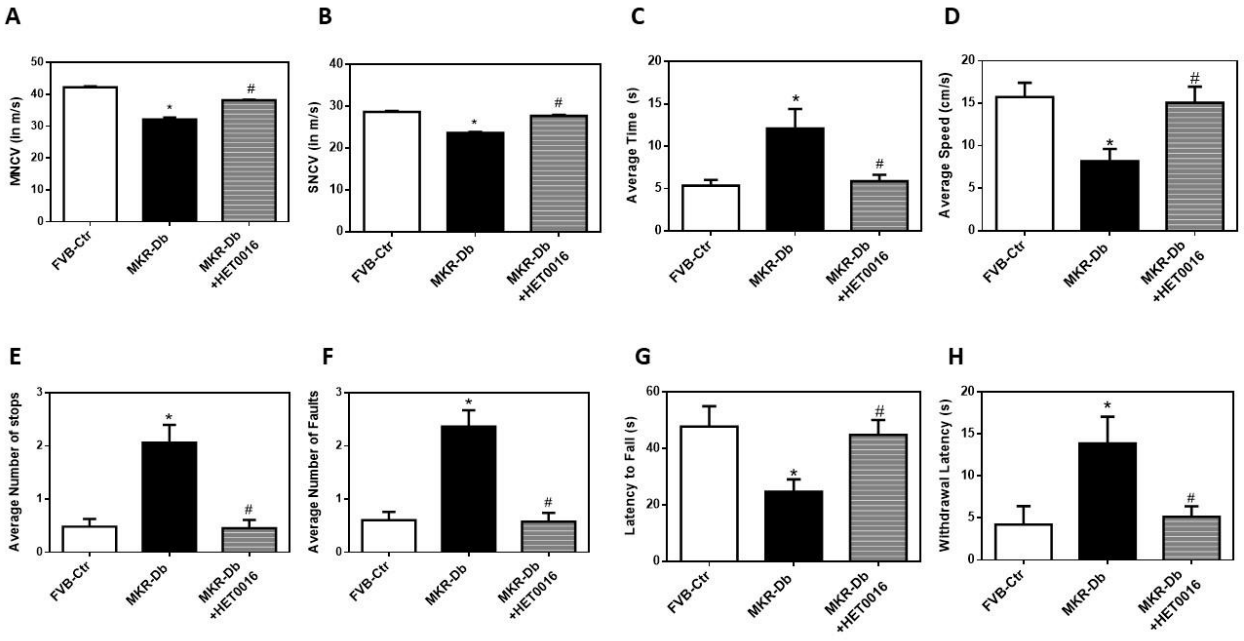


Figure 11. HET0016 administration attenuates behavioral sensory and motor deficits observed in MKR non-obese T2D mice. Diabetes brings about nerve impairments in diabetic subjects. We performed behavioral and functional assessments to phenotype neuropathy in the animal groups prior to sacrifice and after 10 weeks of HET0016 administration, respectively. Histograms representing (A) Motor and (B) Sensory nerve conduction velocities (n=5). Raised Beam Walking Test (n=11) (C-F) reflecting fine sensorimotor coordination. Histograms represent average time (C), speed (D) stops (E) and foot faults (F). Latencies reflecting (G) neuromuscular strength assessed by the Grip Strength Test (n=11) and (H) nociception latencies assessed by Thermal Hind Paw Withdrawal test (n=11). Values are the means ± SE. for all histograms except (C), ± SD. *P<0.05, vs. control. #P<0.05, vs. MKR.

CYP4A inhibition normalizes diabetes-induced alterations in myelin sheath key components in sciatic nerves of MKR non-obese T2D mice.

To account for the behavioral findings, we examined the molecular impact of HET0016 on the sciatic nerve. Peripheral Myelin Protein (PMP22) and Myelin Protein Zero (MPZ) are essential myelin proteins central to maintaining the integrity and compaction of the peripheral myelin sheath. PMP22 and MPZ were assessed as markers of peripheral nerve injury. Western blot analysis shows a significantly altered expression in the sciatic nerves of the MKR mice in both myelin proteins compared to their control littermates **(Fig. 12A, B).** Interestingly, HET0016 administration to MKR mice significantly restored myelin protein levels close to controls. We further assessed the molecular status of aggregate prone PMP22 proteins by the Aggregation

Assay. The results show PMP22 proteins to be aggregated in sciatic nerves of MKR mice by contrast to controls and HET0016-treated animals whereby PMP22 aggregation is attenuated **(Fig. 12C).** These findings suggest that myelin protein abnormalities observed in MKR non-obese T2DM mice, which are quelled upon HET0016 administration.

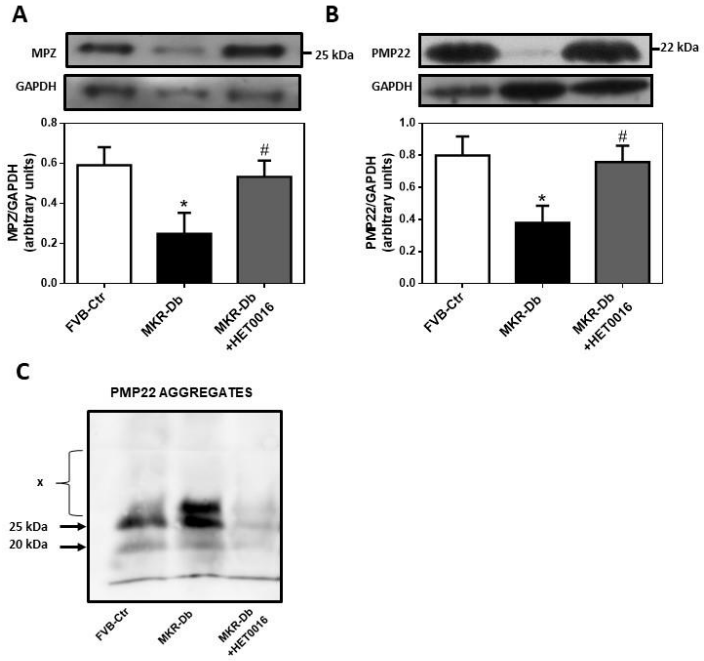


Figure 12. HET0016 administration intercepts diabetes-induced alterations in MPZ and PMP22 myelin protein profiles in the sciatic nerve of MKR non-obese T2D mice. Myelin protein soluble and insoluble profiles were assessed via western blot in sciatic nerves isolated from FVB control mice, MKR non-obese type 2 diabetic mice, and MKR non-obese type 2 diabetic mice treated with HET0016. Representative western blots with their respective densitometric quantification are shown for (A) MPZ (n=5) and (B) PMP22 (n=6-7). (C) Putative western blot of the insoluble PMP22 aggregates indicated by x. Values are the means ± SE. *P<0.05, vs. control. #P<0.05, vs. MKR.

HET0016 alleviates peripheral nerve injury by restoring CYP4A-induced oxidative stress in sciatic nerves of MKR non-obese T2D mice.

We next set out to explore the mechanism of action with which HET0016 attenuates peripheral nerve injury. HET0016 is a selective CYP4A inhibitor (Miyata et al., 2001). We thus assessed the CYP4A protein levels in sciatic nerve lysates, and the findings show a basal expression of CYP4A in control animals which was significantly increased in diabetic animals. HET0016 treatments significantly reduced CYP4A expression levels indicative of its inhibitory

effect **(Fig. 13A).** Concurrent with CYP4A overexpression in MKR animals, we measured 20-HETE levels to correlate the underlying activity of CYP4A. The results show a significant rise in 20-HETE production in the sciatic nerves of the MKR mice in parallel to increased CYP4A expression **(Fig. 13B).** The efficiency of HET0016 was further validated whereby 20-HETE levels in sciatic nerves of MKR treated animals with HET0016 was significantly reduced compared to diabetic animals **(Fig. 13B).** Together, the data suggest that peripheral nerve injury may be mediated through a CYP4A-dependent mechanism. EET levels were further assessed in sciatic nerves of diabetic animals which is hypothesized to be antagonistic to 20-HETE levels. Interestingly, EET levels were significantly decreased in diabetic and HET0016 treated animals relative to control littermates **(Fig. 13C).** However, HET0016 administration reveals a slight, yet significant elevation in EET levels compared to diabetic mice.

To further dissect the oxidative status of the peripheral nerve, cellular H_2O_2 and superoxide production were measured in sciatic nerves via DHE staining and HPLC, respectively. Sciatic nerve sections were visualized by DHE staining **(Fig. 13D, E)** and analyzed by HPLC **(Fig. 13F)** which showed a significant increase in intracellular ROS production in the MKR mice compared to their control littermates, and this was significantly reduced upon HET0016 treatment. Moreover, CYP450 enzymes have been reported to be associated with an NADPH subunit (Davydov D. R., 2001; Medhora et al., 2008; Zeng et al., 2010; Eid et al., 2014). NADPH oxidase activity correlates with a direct measure of superoxide anion production. As expected, the data show that superoxide anion production significantly increased in diabetic animals in comparison to their controls and is significantly reduced upon HET0016 treatment **(Fig. 13G).** These findings suggest that sciatic nerve injury is correlated with an increase in ROS production through an NADPH-dependent pathway.

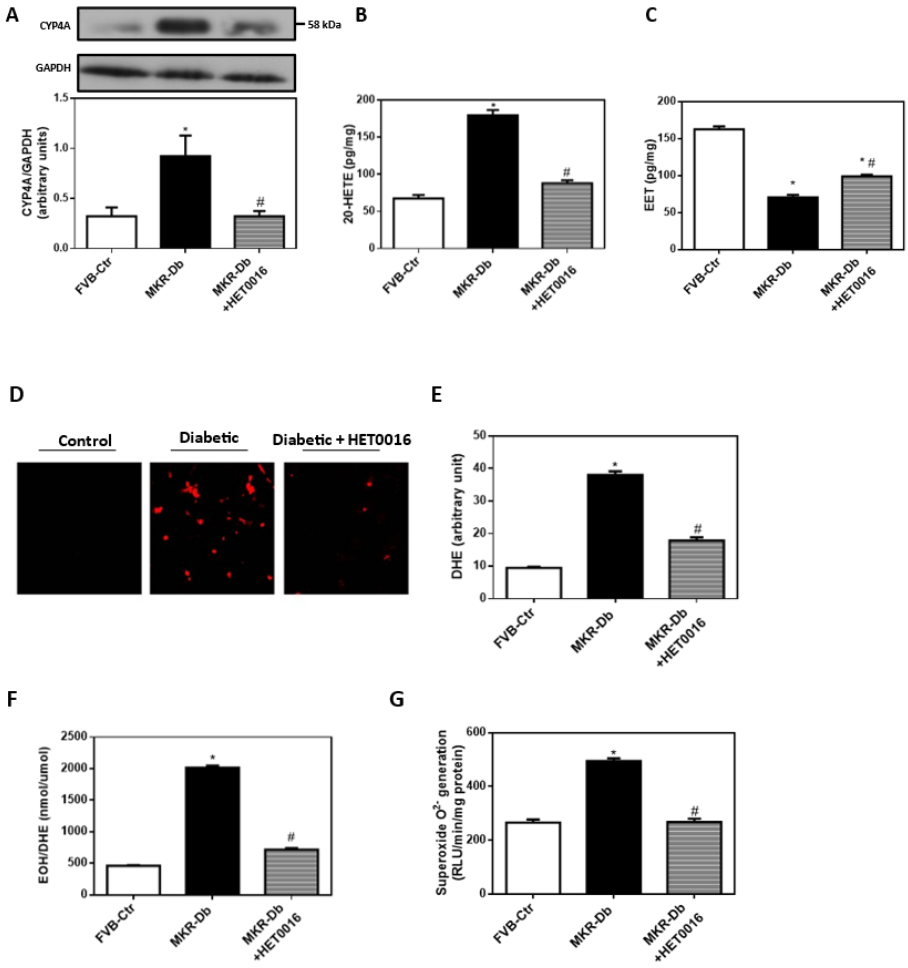


Figure 13. HET0016 administration attenuates diabetes induced, CYP4A-dependent ROS and 20-HETE overproduction in sciatic nerves of MKR non-obese T2D mice to homeostatic levels. Diabetes is associated with ROS overproduction which we assessed in sciatic nerves isolated from FVB control, MKR non-obese type 2 diabetic mice and MKR non-obese type 2 diabetic mice treated with HET0016. Sciatic nerves lysates were first examined for CYP4A expression and activity. (A) Representative western blot of CYP4A expression with the respective densitometric quantification (n=4). CYP4A activity was reflected by assessment of endogenous (B) 20-HETE and (C) EET production via HPLC analysis (n=6-7). (D) Representative images of ROS production assessed by DHE staining and the corresponding (E) quantification of (n=4) using the Image-Pro Plus 4.5 software. (F) Assessment of superoxide generation in sciatic nerves via HPLC analysis (n= 11) (G) Histograms representative of NADPH-induced ROS generation (n=4). Values are the means ± SE. *P<0.05, vs. control. #P<0.05, vs. MKR.

Hyperglycemia alters autophagic defenses, AMPK, and AKT signaling in sciatic nerves of MKR non-obese T2D mice.

Next, we sought to understand whether CYP4A inhibition impacts central cellular processes associated with myelin protein maintenance. For instance, autophagic processes are significant cellular defenses for the restoration of homeostasis, recycling nutrients or tagging cargo for lysosomal degradation. Defects in autophagy machinery are shown to be associated with various disorders, with prominent contribution to protein misfolding (Fortun et al., 2007; Gonzalez et al., 2011; Hamilton et al., 2013; Rocchi & He,, 2015; Menzies et al., 2017; Chung et al., 2018). Consequently, in order to correlate the observed alterations in myelin protein profiles and PMP22 aggregation, we next assessed protein levels of central autophagy orchestrators. Our data show a significant increase in the key regulators of autophagy, Beclin-1, expression in sciatic nerves of the MKR mice relative to their controls, and a restoration of Beclin-1 upon HET0016 administration **(Fig. 14A).** We further examined LC3B indicative of autophagosome recruitment and formation (Yerra et al., 2016). LC3B protein expression was significantly elevated in the sciatic nerve of the MKR mice by contrast to the controls, whereas HET0016-treated MKR mice exhibit reduced LC3B levels **(Fig. 14B).**

Moreover, the metabolic perturbances brought about by diabetes have been reported to trigger the alteration of signaling cascades culminating in organ and tissue injuries. In addition to that, AMPK is a known key regulator of autophagy (Kim et al., 2011). In order to cross-correlate the molecular findings and further delineate the mechanisms at play in the pathogenesis of DPN in T2D, we next assessed AMPK phosphorylation (p-AMPK). p-AMPK^{Thr172} protein expression, indicative of AMPK pathway activation, was significantly reduced in MKR mice relative to the expression observed in the FVB control mice. HET0016 administration restored p-AMPK^{Thr172} protein expression to levels close to that observed in the control FVB mice **(Fig. 14C).**

In parallel, we examined changes in AKT phosphorylation as well. The AKT pathway has been shown to be a major pathway involved in the myelination process and physiology of SCs. However, the mechanisms leading to AKT alterations are still unknown. To correlate the observed myelin protein disruption, as well as the hypophosphorylation of AMPK, AKT activation on the Serine473 residue was measured. The acquired findings correlated with a significant increase of AKT phosphorylation in diabetic sciatic nerves **(Fig. 14D).** Blockade of CYP4A using HET0016 reversed the effect of hyperglycemia-induced AKT hyperphosphorylation, possible reflecting the

amelioration of peripheral nerve injury concomitant with the restoration in AMPK levels, myelin protein alteration, and autophagic response disruption. These data suggest that phosphorylated AKT may play an injurious role in T2D, altogether in a complicated manner with attenuated AMPK signaling.

Furthermore, these findings are suggestive of significant alterations in autophaghosome recruitment and activity which may mediate the underlying neurological pathology, and possibly myelin debris accumulation in light of disrupted AKT levels. These results also suggest a potential role of AMPK in inducing peripheral nerve injury. The role of AMPK activation in diabetic complications has been rigorously studied in terms of cardiomyopathy (Xie et al., 2011), colorectal cancer (Mroueh et al., 2019) and kidney disease (Eid et al., 2010). When it comes to neuropathy, previous investigations assessed the pharmacological effect of targeting AMPK on neural inflammation and pain in streptozotocin-induced type 1 diabetes (Melemedjian et al., 2011; Yerra et al., 2017). Nevertheless, its role in peripheral nerve injury induced by diabetes needs more investigation. Therefore, we assessed if AMPK activation halts diabetes-induced peripheral nerve injury.

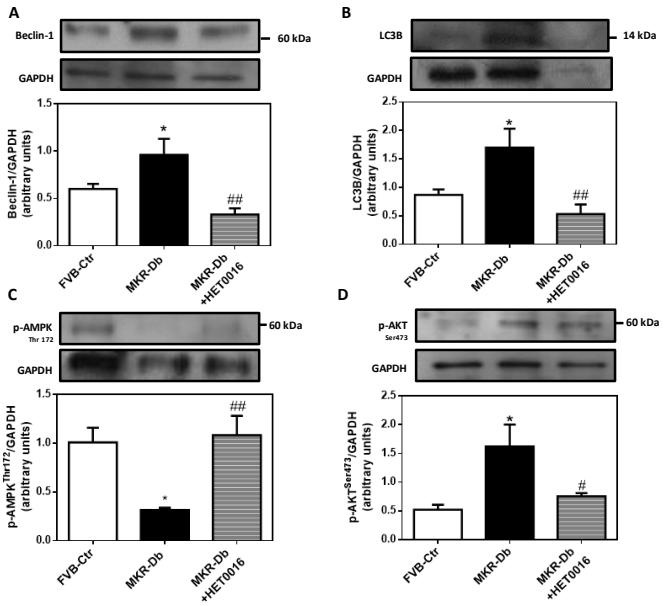
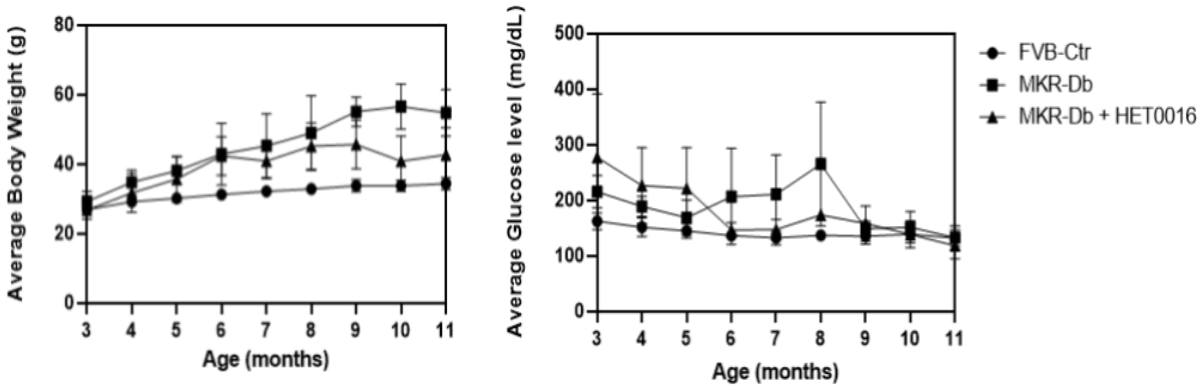


Figure 14. HET0016 administration attenuates diabetes-induced alterations in autophagic protein markers and AMPK and AKT signaling in sciatic nerves of MKR non-obese T2D mice. Key autophagy protein expression was examined in sciatic nerve lysates from FVB control mice, MKR non-obese type 2 diabetic mice, and MKR non-obese type 2 diabetic mice treated with HET0016. Representative western blots with the respective densitometric quantification are shown

for (A) Beclin-1 (n=5), (B) LC3B (n=5) (C) p-AMPKThr172 (n=5) and (D) phosphorylated AKT levels at the activation site Serin473. Values are the means ± SE. *P<0.05, vs. control. #P<0.05, vs. MKR.

Table 4. Metabolic characteristics – HET0016 intervention (Chronic)

Group	*n*	Terminal Blood glucose (mg/dl)	Terminal Body weight (g)
FVB-Control	*10*	133.5 ± 4.8	34.6 ± 0.5
MKR-Diabetic	*5*	134.2 ± 9.6	55.6 ± 2.9
MKR-Diabetic + HET0016	*6*	128 ± 5.3	43.0 ± 3.2



Random glucose levels and body weight in FVB control mice and T2DM MKR mice and T2DM MKR mice at 40 weeks of age were treated with low-dose HET0016 (2.5 mg/kg) for 10 weeks were monitored throughout the study duration. Charts represent bimonthly assessments of the metabolic parameters, whereas tabulated values represent terminal recordings. Values are the means ± SEM from 5-10 animals for each group. Values are the means ± SE. *P<0.05, vs. control. #P<0.05, vs. diabetic.

HET0016 intervention after prolonged diabetic state holds no effect on metabolic parameters.

In another set of animals, we examined the effect of CYP4A inhibition via HET0016 on an older and aged animal group. The intervention with low-dose HET0016 was altered to assess its therapeutic power over a prolonged and late stage of diabetes. Briefly, diabetic animals, 10 months of age, were treated with HET0016 to examine the therapeutic potential against DPN onset. Terminal glucose levels, on the contrary, show no significant changes among the groups **(Table 4).** By contrast, diabetic animals maintained a rise in body weight although it is not significant compared to the control group. Similarly, HET0016 treated animals showed an insignificant

increase, compared to controls, however albeit lower than the diabetic mice. However, charts from monitoring their random glucose levels on a weekly basis show severe fluctuations throughout disease progression **(Table 4)** with no alleviation in diabetic phenotype in treated animals indicating that 20-HETE synthase inhibition does not restore the diabetic phenotype in chronically diabetic MKR non-obese mice.

Delayed intervention with HET0016 alleviates behavioral deficits in chronically diabetic mice with limited outcomes.

To investigate the ability of HET0016 in reversing diabetes-induced nerve dysfunction after a chronic diabetic state in the MKR animal model, animals were first subjected to behavioral testing. Sensorimotor dysfunction was reflected by the Raised Beam Walking test. The findings reflect sensorimotor defects in performance duration, time and stops by diabetic animals, however these defects were not significant possibly due to the low sample number **(Fig. 15A-D).** As for the number of foot faults, there was a significant increase exhibited in diabetic animals, and this was restored in HET0016-treated animals. While HET0016-treated MKR mice performed comparably to the FVB control littermates, the observed improvement compared to the diabetic animals was not significant **(Fig. 15A-D).** In another attempt to verify the findings, animals were subjected to an automated version of the Beam Walking Test, known as the Rotarod Assessment. Similar results were observed whereby diabetic animals performed significantly poorly relative to controls, while HET0016-treated mice show no significant improvement **(Fig. 15G, H).** Additionally, sensory nocifensive responses of the diabetic animals reflect hypoalgesia which was significantly restored in HET0016-treated animals **(Fig. 15E)**. Similarly, data from the Grip Strength test show MKR animals to exhibit a reduced latency to fall indicative of significantly compromised motor function. By contrast, MKR animals treated with HET0016 performed with relatively better, but that did not achieve statistical significance **(Fig. 15F).** Taken together, these results indicate that HET0016 treatment was of limited potential in restoring sensorimotor coordination in chronically type 2 diabetic animals and may therefore hold promise as a preventative agent instead.

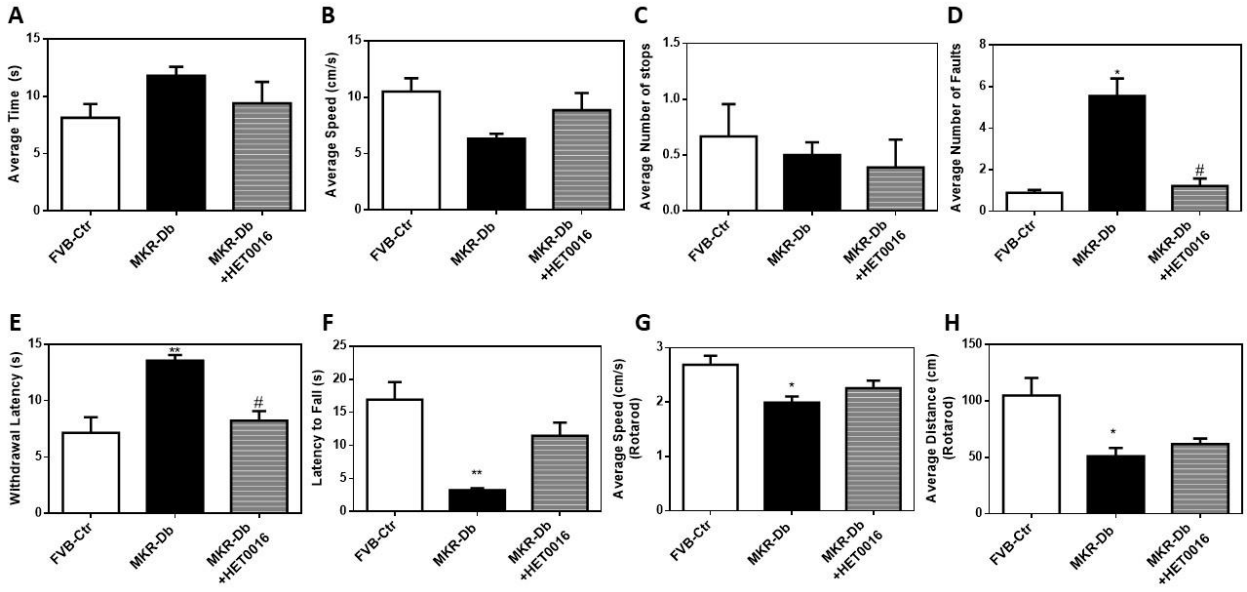


Figure 15. HET0016 administration intervention is of limited potential in ameliorating behavioral sensory and motor deficits observed in chronically diabetic MKR non-obese T2D mice. To investigate whether HET0016 administration holds a therapeutic advantage in reversing DPN-associated neurological deficits in response to chronic uncontrolled diabetes, a behavioral assessment was performed on T2DM MKR mice at 40 weeks of age and treated with low-dose HET0016 (2.5 mg/kg) for 10 weeks. Histograms representing Raised Beam Walking Test (A-D) reflecting fine sensorimotor coordination. Histograms represent average time (A), speed (B) stops (C) and foot faults (D). Latencies reflecting (E) nociception latencies assessed by Thermal Hind Paw Withdrawal test, (F) neuromuscular strength assessed by the Grip Strength Test, (G) Average Speed and (H) Average Distance via the Rotarod Assessment. Values are the means ± SE from n=6-10. *P<0.05, vs. control. #P<0.05, vs. MKR.

HET0016 reduces 20-HETE and oxidative injury in sciatic nerves of chronically diabetic MKR non-obese T2D mice.

An HPLC analysis of sciatic nerves was performed to assess the levels of CYP metabolites and oxidative stress. As expected, 20-HETE levels remained significantly high in chronically diabetic MKR mice of 50 weeks in age. HET0016-treated mice show a successful inhibition of 20-HETE synthesis as evident by the significantly reduced 20-HETE levels **(Fig. 16A).** Concurrent with this rise, we measured EET levels to correlate the underlying activity of the antagonistic CYP epoxygenases. The results show a significant reduction in EET levels in the sciatic nerves of the MKR mice which was slightly, significantly elevated upon HET0016 administration however not as close to control levels **(Fig. 16B).** Together, the data suggest that peripheral nerve injury may be mediated through a CYP4A-dependent mechanism. EET levels were further assessed in sciatic

nerves of diabetic animals which is hypothesized to be antagonistic to 20-HETE levels. Further to that, the oxidative status of the peripheral nerve of diabetic animals showed a significant increase in intracellular ROS production compared to their control littermates, and this was significantly reduced upon HET0016 treatment **(Fig. 16C)**.

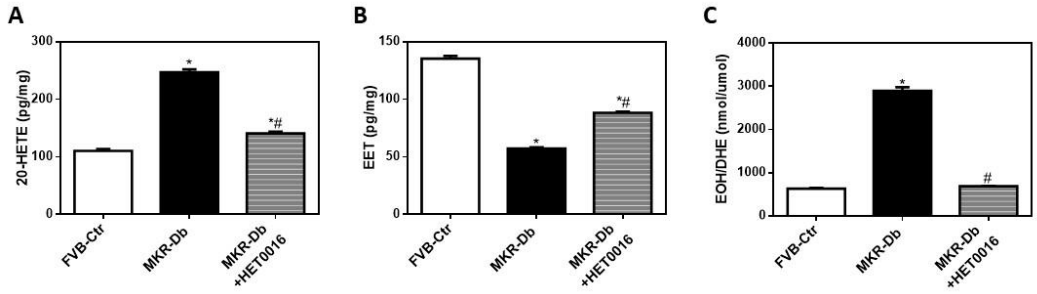


Figure 16. HET0016 administration attenuates diabetes induced 20-HETE overproduction in sciatic nerves of chronically diabetic MKR non-obese T2D mice. CYP metabolites 20-HETE and EET in addition to ROS overproduction was assessed in sciatic nerves isolated from aged FVB control, MKR non-obese type 2 diabetic mice and MKR non-obese type 2 diabetic mice treated with HET0016. CYP4A activity was reflected by assessment of endogenous (A) 20-HETE production while epoxygenase activity was assessed by (B) EET production via HPLC analysis (n=4). (C) Assessment of superoxide generation in sciatic nerves via HPLC analysis (n=4) (G) Values are the means ± SE. *P<0.05, vs. control. #P<0.05, vs. MKR.

Chronically diabetic T2D MKR mice and HET0016-treated animals exhibit reduced myelin protein expression and altered autophagy.

To be able to correlate the behavioral findings at the molecular level, MPZ and PMP22 protein expression was examined in sciatic nerves lysates. Western blot analysis data show a reduction in both MPZ and PMP22 levels in diabetic animals compared to controls. As for HET0016 treated diabetic animals, MPZ levels were significantly restored close to control levels, however, PMP22 expression was not **(Fig. 17A, B).** To further dissect the mode of injury, autophagy marker proteins Beclin-1 and LC3B were examined. Autophagy was of interest in this intervention primarily due to the chronic state of hyperglycemia as well as the age difference relative to the prevention study. Cellular autophagic responses tend to be affected with age, and it was central to investigate its implication in the severity of DPN. In this experimental study, it was apparent that there is a shy but unremarkable elevation in Beclin-1 levels in both diabetic and treated mice **(Fig. 17C).** As for LC3B expression, the results show a slight reduction in diabetic animals and a more pronounced decrease in HET0016-treated diabetic mice **(Fig. 17D).** Finally,

to better comprehend this unexpected out-turn, AKT pathway activation was further examined. Phosphorylated levels of AKT at the Serine473 activation site was analyzed and the data returned a hypophosphorylation of AKT in both diabetic and treated mice **(Fig. 17E).** Collectively, these results lend support to the limited neuroprotective potential of HET0016 administration in alleviating long-term peripheral nerve injury triggered by diabetes. In this spirit, the observed unremarkable findings returned from autophagy protein expression may point to alternative injurious pathways at play that may mediate neuronal injury, and these were further explored at a later stage in this work.

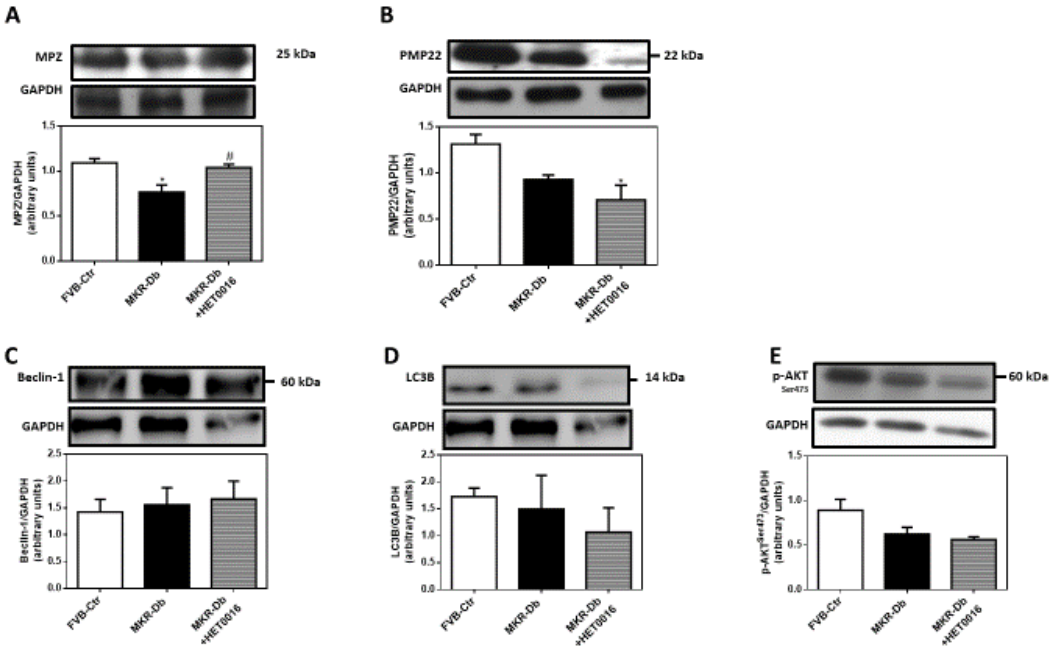


Figure 17. HET0016 administration confers limited neuroprotection at the molecular level in sciatic nerves of chronic T2D MKR mice. Myelin protein profiles and key autophagy markers protein expression was examined in sciatic nerve lysates from aged FVB control mice, MKR non-obese type 2 diabetic mice, and MKR non-obese type 2 diabetic mice treated with HET0016. Representative western blots with the respective densitometric quantification are shown for (A) MPZ (n=3) (B) PMP22 (n=3) (C) Beclin-1 (n=3), (D) LC3B (n=3). Beclin-1 and LC3B were visualized on the same gel. (E) phosphorylated AKT levels at the activation site Serine473 (n=4). Values are the means ± SE. *P<0.05, vs. control. #P<0.05, vs. MKR.

Table 5. Metabolic characteristics – Chronic low dose Metformin prevention

Group	*n*	HbA1c (%)	Blood glucose (mg/dl)	Body weight (g)
FVB-Control	*10*	5.18 ± 0.1	150.9 ± 5.9	31.5 ± 0.7
MKR-Diabetic	*11*	8.25 ± 0.2*	266.0 ± 30.9*	30.3 ± 1.1
MKR-Diabetic + Metformin	*11*	6.96 ± 0.2*$^{\#}$	153.6 ± 37.2$^{\#}$	29.3 ± 0.9

Terminal random glucose levels, body weight and glycated hemoglobin (HbA1c) in FVB control mice and T2DM MKR mice. 10 weeks old T2DM MKR mice were treated with Metformin (150 mg/kg) daily for 13 weeks. Values are the means ± SEM from10-11 animals for each group, except HbA1c from n=4-5. Values are the means ± SE. *P<0.05, vs. control. #P<0.05, vs. diabetic.

Effect of low dose Metformin administration on the glycemic index of MKR non-obese T2D mice.

10 weeks of age FVB control mice, MKR non-obese T2D mice and MKR non-obese T2D mice treated with low dose Metformin, an AMPK activator were used. Mice body weight, random blood glucose levels and glycemic index (HbA1c) were monitored throughout the study. The results show no significant differences in body weight among the groups, however there was a significant difference in HbA1c levels of diabetic animals compared to the control littermates. It is noteworthy to mention that Metformin administration showed a significant reduction in the glycemic index of diabetic animals compared to the diabetic group, verifying its anti-hyperglycemic effect. Nonetheless, these levels remained significantly higher than the controls group **(Table 5).**

Metformin administration corrects neural sensory and motor abnormalities in MKR non-obese T2D mice.

To investigate the role of AMPK activation in diabetes-induced nerve dysfunction, we examined various sensory and motor modalities of the peripheral nerve in our murine model via a series of behavioral tests. Initially, sensory and motor dysfunction was examined. The findings from the Raised Beam Walking test show an overall poor performance by the MKR non-obese type 2 diabetic mice. These mice took a significantly longer period of time to complete the test **(Fig. 18A)** with a reduced speed **(Fig. 18B)** and an increased tendency stop **(Fig. 18C)** and to slip **(Fig. 18D).** We then measured the sensory nocifensive responses of the animals via the Hind Paw Withdrawal test **(Fig. 18E)**. The data show the development of thermal hypoalgesia in the MKR

mice reflected by a significantly increased withdrawal latency relative to the FVB controls. Interestingly, MKR mice treated with Metformin had a significantly lower latency suggesting that Metformin restored thermal pain perception. We next examined neuromuscular strength via the Grip Strength test. MKR mice exhibit a reduced latency to fall indicative of compromised motor function, which was restored in Metformin treated MKR mice **(Fig. 18F).** By contrast, Metformin treated MKR mice exhibited a performance similar to the FVB control group **(Fig. 18A-F).** The results of these tests indicate that Metformin treatment improved the peripheral neuropathy-associated deficits in the MKR non-obese T2D mice.

We then examined the molecular impact of Metformin on the essential myelin proteins, MPZ and PMP22, central to maintaining the integrity and compaction of the peripheral myelin sheath. Western blot analysis of sciatic nerve lysates shows a significantly altered expression of MPZ and a tendency of an increase in PMP22 in diabetic mice compared to the controls **(Fig. 19A, B).** Interestingly, Metformin treated MKR mice exhibit a restoration of myelin protein profiles close to control levels. These alterations were as predicted and accompanied by a tendency of AMPK pathway inactivation as evidenced by the reduced expression of phosphorylated AMPK at the Threonine172 residue in sciatic nerves of MKR diabetic mice **(Fig. 19C)**. However, the possible alleviation of behavioral and molecular deficits in peripheral nerves of Metformin-treated diabetic animals may be explained by the partial restoration of AMPK activation **(Fig. 19C)**. Although these data did not achieve statistical significance, a valid argument would be due to the low sample size examined.

Metformin blunts diabetes-induced oxidative stress and peripheral nerve injury in MKR non-obese T2D mice.

We further examined if Metformin treatment can regulate hyperglycemia-induced ROS production. For that, sciatic nerve sections were analyzed by HPLC to assess superoxide generation **(Fig. 19D).** Results show a significant increase in intracellular ROS production in MKR mice compared to their control FVB littermates, and this was significantly reduced upon Metformin treatment. Moreover, NADPH-dependent ROS production was assessed in sciatic nerves of mice via the NADPH-oxidase assay. The data show that superoxide anion production significantly increased in the MKR mice in comparison to the controls and is significantly reduced upon Metformin treatment **(Fig. 19E).** These findings suggest that sciatic nerve injury and MPZ

protein alteration is correlated with an increase in ROS production through an NADPH-dependent mechanism.

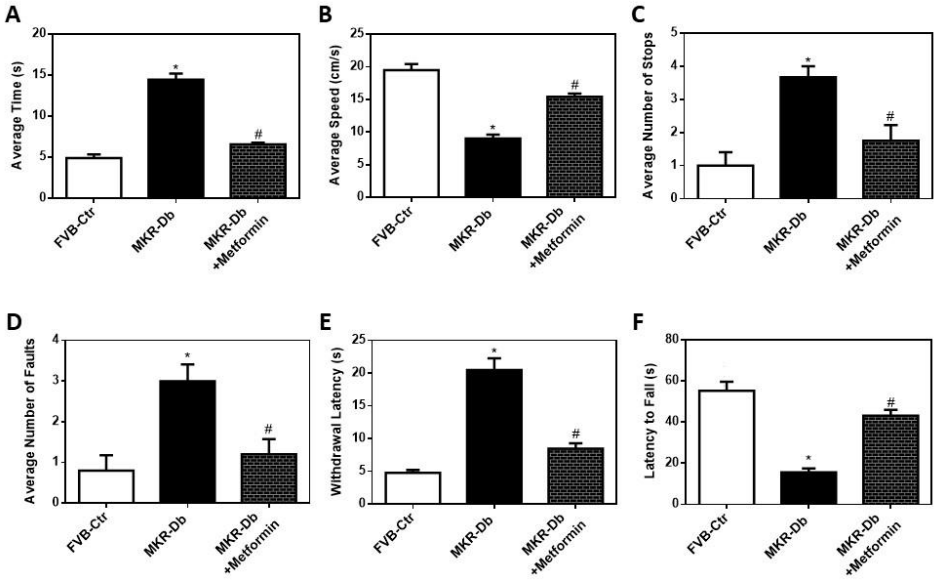


Figure 18. Treatment with Metformin abates sensorimotor deficits in MKR non-obese T2D mice. Diabetes is often associated with abnormalities in sensory and motor neural functions. We examined the behavioral performance of FVB control mice, MKR non-obese type 2 diabetic mice, and MKR non-obese type 2 diabetic mice treated with HET0016, in a series of tests to phenotype neuropathy. Histograms representing (A) thermal sensitivity to pain assessed by Thermal Hind Paw Withdrawal test (n=5) and (B) latencies reflecting neuromuscular strength assessed by the Grip Strength Test (n=5) in addition to (C-F) fine motor coordination and gait assessed by the Raised Beam Walking Test (n=5). Values are the means ± SEM. *P<0.05, vs. control. #P<0.05, vs. MKR.

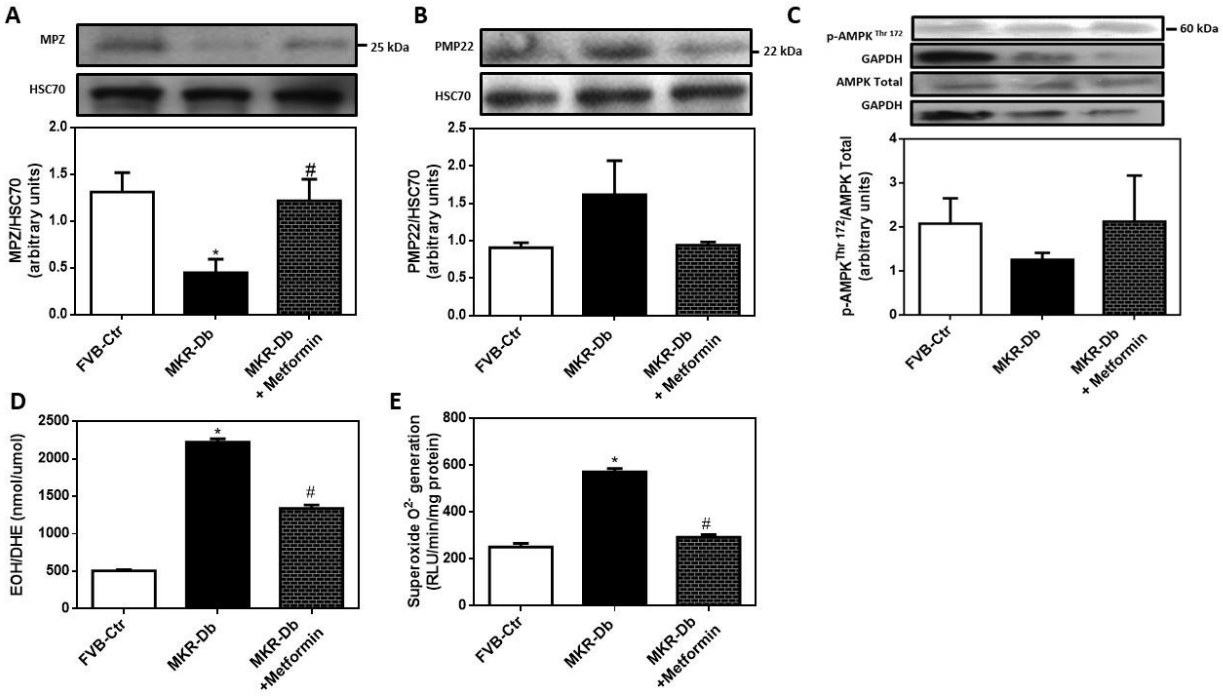


Figure 19. Metformin administration reduces peripheral nerve injury and NADPH-derived ROS production in MKR non-obese T2D mice. Sciatic nerves were isolated from FVB control, MKR non-obese type 2 diabetic mice and Metformin-treated MKR non-obese type 2 diabetic mice. MPZ and PMP22, the predominant myelin protein of the peripheral nerves, were assessed in sciatic nerve lysates as markers of peripheral nerve integrity. Representative western blot and respective densitometric quantification is shown for (A) MPZ (n=5) (B) PMP22 (n=3) and (C) p-AMPK at the activation site Threonine172. Diabetes-associated superoxide anion production was measured by (D) HPLC (n=5) and (E) NADPH oxidase Activity Assay (n=5). Values are the means ± SEM. *P<0.05, vs. control. #P<0.05, vs. MKR.

Metformin alleviates autophagy protein alterations triggered by hyperglycemia in the sciatic nerve of MKR non-obese T2D mice.

In the same spirit of our previous findings **(Fig. 14A-B),** we next examined if AMPK activation normalizes the key proteins Beclin-1 and LC3B as markers of the autophagic response in sciatic nerves of the MKR non obese T2DM mice. Immunohistochemical analysis of sciatic nerve sections show a significant increase in Beclin-1 and LC3B expression in sciatic nerves of MKR mice relative to the controls, and their respective repair in Metformin treated MKR mice **(Fig. 20A-D).**

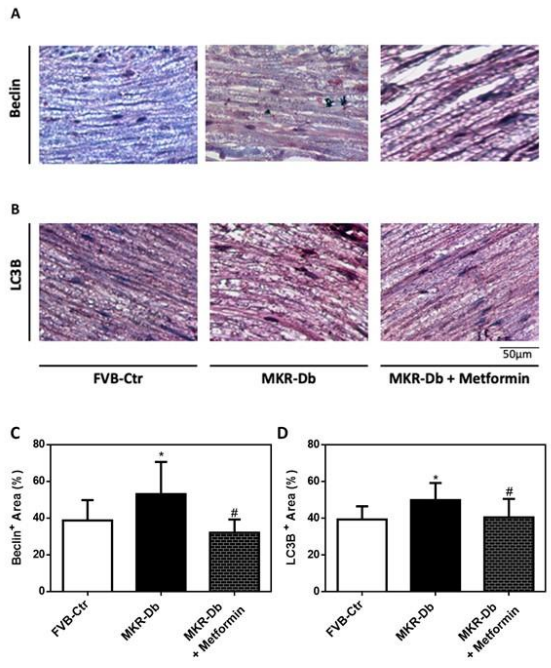


Figure 20. Diabetes-induced canonical autophagy in sciatic nerves is restored upon Metformin treatment in MKR non-obese T2D mice. Longitudinal sections of sciatic nerves longitudinal sections were examined for protein markers of autophagy. Representative immunohistochemistry images of sciatic nerves of FVB control, MKR non-obese type 2 diabetic mice and MKR non-obese type 2 diabetic mice treated with Metformin stained with (A) Beclin-1 and (B) LC3B with their respective quantifications (C, D). Values are the means ± SEM (n=5). *P<0.05, vs. control. #P<0.05, vs. MKR.

Part 2: Targeting the Soluble Epoxide Hydrolase Enzyme (sEH): Exploring the influence of EETs in DPN.

For the second part of the study, we aimed to explore the second branch of the CYP450 family: the epoxygenases that produce EETs. Since 20-HETE was established to contribute to the pathological route in DPN, the downregulated EET levels sparked interest to proceed with the investigation. As previously mentioned, 20-HETE and EET work together to maintain a homeostatic balance. The rationale behind the next series of experiments aims to exemplify the effect of restoring EET levels. Emerging literature highlight the pathological elevation of sEH enzyme activity in various ailments of the metabolic spectrum and diabetes (Luo et al., 2010; Luria et al., 2011; Zhang et al., 2011; Khan et al., 2018; Minaz et al., 2018). More importantly, the sEH enzyme is known to degrade EETs into less active diols DHETs. Consequently, to be able to

investigate further, an inhibitor of the sEH enzyme, AUDA, was administered to male MKR mice in a series of prevention or intervention studies.

Table 6. Metabolic characteristics – AUDA intervention

Group	*n*	HbA1c (%)	Body weight (g)
FVB-Control	*10*	5.05 ± 0.1	31.54 ± 1.1
MKR-Diabetic	*8*	8.06 ± 0.2*	35.6 ± 1.8
MKR-Diabetic + AUDA	*10*	7.58 ± 0.3*	31.2 ± 1.1

Terminal glycated hemoglobin levels and body weight in FVB control mice and T2DM MKR mice. 28-week-old FVB control mice were treated with vehicle while age-matched T2DM MKR mice were treated with low-dose AUDA (10 mg/kg) daily for 10 weeks. Values are the means ± SEM from 8-10 animals for each group. Values are the means ± SE. *P<0.05, vs. control. #P<0.05, vs. diabetic.

Effect of AUDA administration on the glycemic index of MKR non-obese T2D mice.

Male FVB controls and MKR diabetic animals were grouped into Control, Diabetic, and Diabetic treated with sEH inhibitor, AUDA at 10mg/kg for 10 weeks. Body weights and random blood glucose levels were monitored weekly up to 38 weeks of age. The glycemic index (HbA1c) was assessed on the final week prior to sacrifice. The results show no significant differences in body weight among the groups. However, MKR diabetic animals showed a marked increase in their HbA1c levels compared to their age-matched controls. Likewise, AUDA administration did not have any effect on the glycemic index of the diabetic animals **(Table 6).** indicative that, like HET0016, AUDA is incapable of reversing the diabetic phenotype in chronically diabetic MKR mice.

AUDA administration alleviates neural sensory and locomotor defects in MKR non-obese T2D mice.

The inhibition of sEH has been attributed to provide symptomatic relief in inflammatory pain animal models (Inceoglu et al., 2008; 2011). In this work, we next aimed to assess whether sEH inhibition may alleviate neuropathy associated with diabetes. To investigate, a series of behavioral tests were performed. The findings from the Raised Beam Walking test show an overall poor performance by the MKR non-obese type 2 diabetic mice. These mice took a significantly longer period of time to complete the test **(Fig. 21A)** with a reduced speed **(Fig. 21B)** due to a

frequent foot faults **(Fig. 21C)** and an increased tendency stop **(Fig. 21D).** We next examined neuromuscular strength via the Grip Strength test. MKR mice exhibit a reduced latency to fall indicative of compromised motor function, which was restored in AUDA treated MKR mice **(Fig. 21E).** We then measured the sensory nocifensive responses of the animals via the Hind Paw Withdrawal test **(Fig. 21F)**. The data show the development of thermal hypoalgesia in the MKR mice reflected by a significantly increased withdrawal latency relative to the FVB controls. Interestingly, MKR mice treated with AUDA had a significantly lower latency suggesting that sEH inhibition restored thermal pain perception. In all, AUDA treated MKR mice exhibited a performance similar to the FVB control group **(Fig. 21A-F).** Collectively, the results of these tests indicate that AUDA treatment improved the peripheral neuropathy-associated deficits in the MKR non-obese T2D mice.

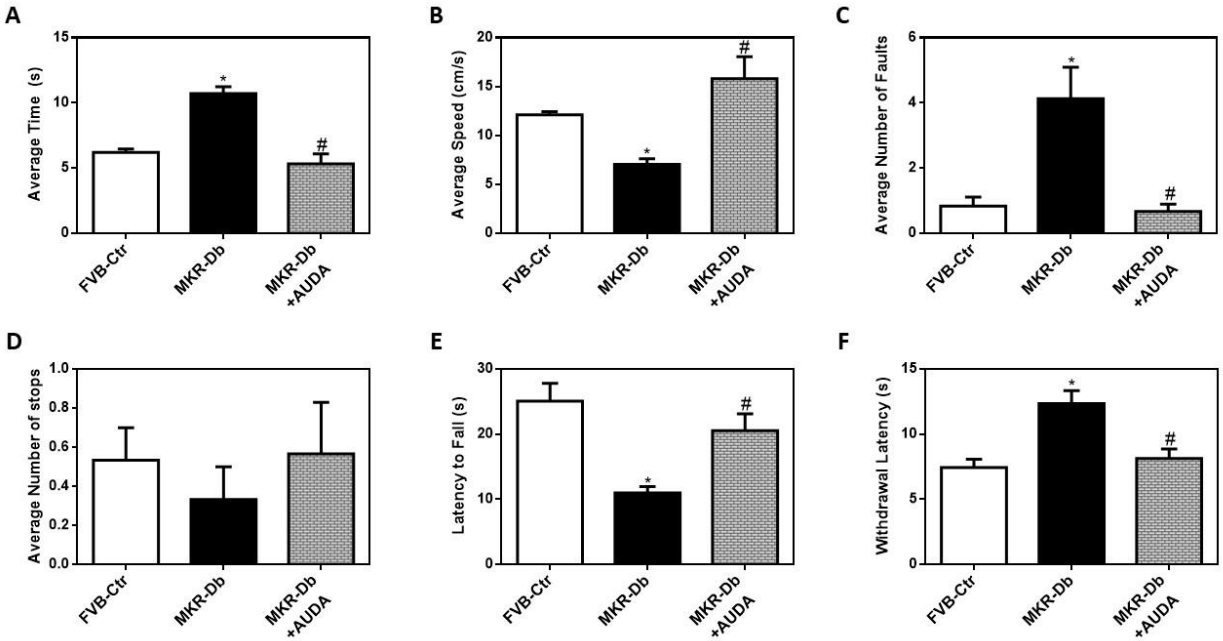


Figure 21. Assessment of sensorimotor deficits and thermal algesia in MKR non-obese T2D mice treated with sEH inhibitior, AUDA. The performance of 7 months old FVB control mice, MKR non-obese type 2 diabetic mice, and MKR non-obese type 2 diabetic mice treated with 10mg/kg of AUDA for 10 weeks was examined in a series of behavioral tests to phenotype neuropathy. Histograms representing (A-D) fine motor coordination and gait assessed by the Raised Beam Walking Test (E) latencies reflecting neuromuscular strength assessed by the Grip Strength Test in addition to (F) thermal sensitivity to pain assessed by Thermal Hind Paw Withdrawal test of 8-10 animals per group. Values are the means ± SEM. *P<0.05, vs. control. #P<0.05, vs. MKR.

AUDA administration does not result in molecular improvements in T2D animals.

In order to correlate the behavioral results and elucidate the effect of sEH inhibition in diabetic animals, further molecular tests were conducted in harvested sciatic nerves from FVB control, MKR diabetic, and MKR diabetics treated with AUDA. Our data show a significant downregulation in MPZ protein expression in diabetic mice sciatic nerves relative to the controls, and a significant reduction in MPZ upon AUDA treatment **(Fig. 22A).** Concurrent with MPZ downregulation, PMP22 protein expression was found to be slightly elevated in diabetic mice and significantly elevated in AUDA-treated diabetic littermates (**Fig. 22B).** Together, these data show a clear disruption in myelin protein profiles that were not restored upon AUDA administration.

Further molecular assessment of autophagy protein Beclin-1 was performed. The data report slight reductions in Beclin-1 in sciatic nerves isolated from the diabetic mice as well as AUDA-treated diabetic mice (**Fig. 22C).** This reduction in Beclin-1 was concurrent with a significant inactivation of AMPK signaling as evidenced by reduced p-AMPK levels. AUDA treatment slightly reversed this effect however, incomparably to control values (**Fig. 22D).** These findings prompted us to question the effectiveness of AUDA as a symptomatic therapy rather than a molecular modulator.

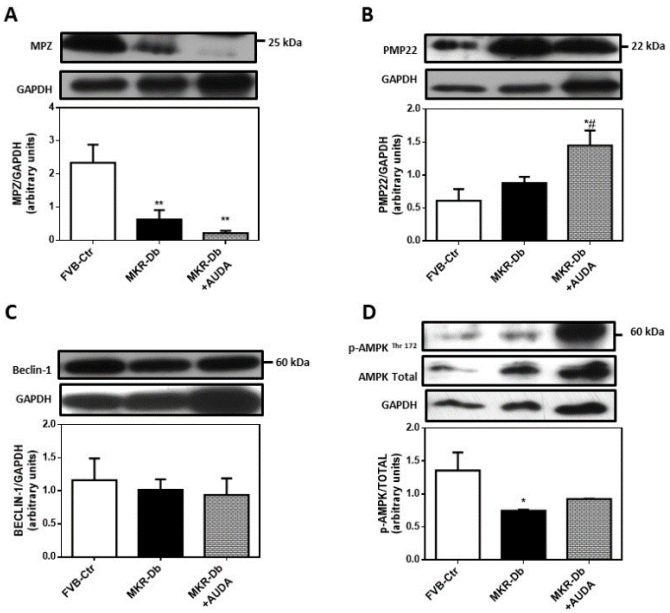


Figure 22. AUDA treatment shows ineffective neuroprotection at the molecular level in sciatic nerves of T2D MKR mice. Sciatic nerves were isolated from FVB control, MKR non-

obese type 2 diabetic mice and AUDA-treated MKR non-obese type 2 diabetic mice to assess the effectivity of 10mg/kg AUDA on nerve physiology. Representative western blots with the respective densitometric quantification are shown for (A) MPZ (n=4) (B) PMP22 (n=5-6) (C) Beclin-1 (n=6), (D) p-AMPK at the activation site Threonine172 (n=3). Values are the means ± SE. *P<0.05, vs. control. #P<0.05, vs. MKR.

AUDA restores CYP4A and CYP2C balance and alleviates CYP-mediated ROS production in sciatic nerves of MKR non-obese T2D mice.

We next set out to verify any effect of AUDA on the oxidative status of sciatic nerves from diabetic animals. We thus assessed CYP4A and CYP2C protein levels in sciatic nerve lysates, and the findings show a basal expression of CYP4A in control animals which was significantly increased in diabetic animals. AUDA treatment significantly reduced CYP4A expression levels **(Fig. 23A).** Similarly, the EET-producing epoxygenase CYP2C protein expression was markedly increased in diabetic animals and brought close to control values upon AUDA administration **(Fig. 23B)**. The upregulation of CYP4A and CYP2C were accompanied by a significant increase in intracellular superoxide production in sciatic nerves of diabetic animals. Whereas the AUDA-treated group exhibit significantly dwarfed superoxide production **(Fig. 23C).** Moreover, further blood serum examination results show that circulating 20-HETE levels were significantly increased concurrent with CYP4A overexpression in MKR animals. However, paradoxical to CYP2C upregulation, diabetic animals exhibit significantly diminished EET levels. Interestingly, AUDA treatment was capable of restoring 20-HETE and EET homeostatic levels close to control levels. However, it is noteworthy to mention that 20-HETE levels were only partially restored relative to diabetic animals **(Fig. 23D).** These results reiterate the specificity of AUDA in targeting EET stabilization rather than 20-HETE. Together, the data extend and support earlier findings whereby peripheral nerve injury may be mediated through a CYP-dependent mechanism, not limited to the ω-hydroxylase arm of CYP450. These findings emphasize the important role of EET in DPN which is yet to be elucidated further.

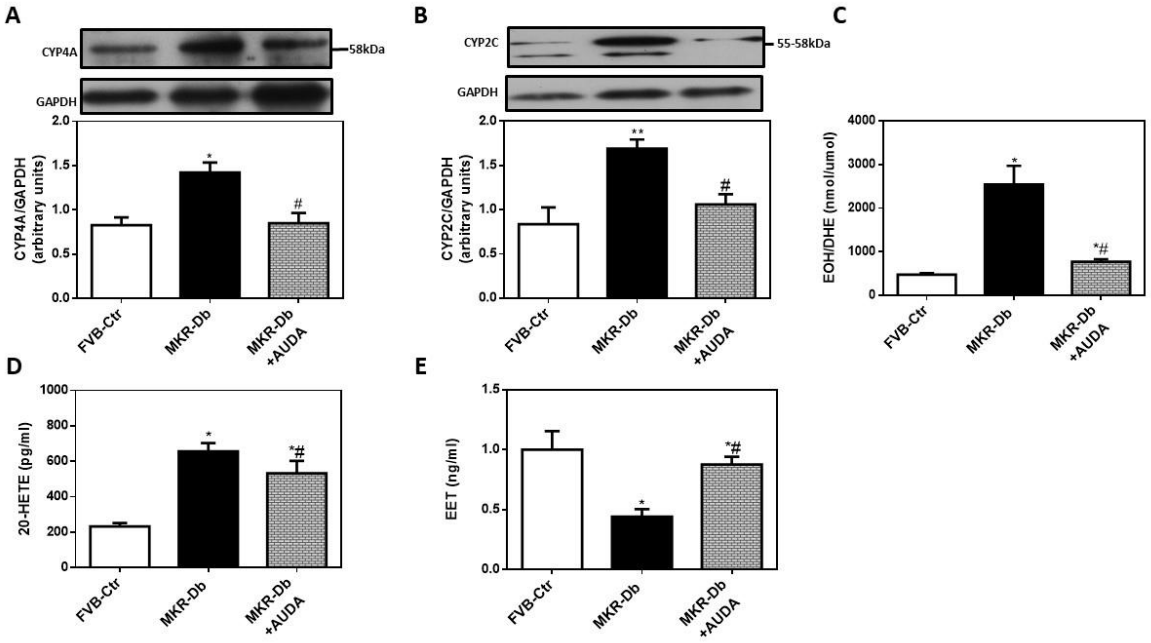


Figure 23. sEH inhibition with AUDA restores EET levels and alleviates CYP-dependent ROS overproduction in sciatic nerves of MKR non-obese T2D mice. Diabetes-associated oxidative injury was assessed in sciatic nerves isolated from FVB control, MKR non-obese type 2 diabetic mice and MKR non-obese type 2 diabetic mice treated with AUDA. The expression and activity of hydroxylase and epoxygenase CYPs was examined. Representative Western blot analysis of (A) CYP4A expression (n=3) and (B) CYP2C (n=4) with the respective densitometric quantification. (C) Assessment of superoxide generation in sciatic nerves via HPLC analysis (n=8-10). CYP activity was reflected by assessment of endogenous (D) 20-HETE and (E) EET production via HPLC analysis (n=8-10). Values are the means ± SE. *P<0.05, vs. control. #P<0.05, vs. MKR.

Collectively, the results obtained insofar point to a significant observation emanating from the *in vivo* set ups. The ω-hydroxylase CYP4A, and the epoxygenase CYP2C evidently play distinct roles in the pathogenesis of DPN. However, both CYP arms have equally relevant homeostatic roles. To that end, we next aimed to address the cell-specific effect of hyperglycemia and CYP alterations. Consequently, the work was taken to the *in vitro* perspective of Schwann cell cultures.

Part 3: Mechanisms of Schwann Cell Injury in Diabetes: Role of the Cytochromes P450 Pathways

In parallel experiments, an *in vitro* experimental approach was set up to corroborate the observed *in vivo* findings. MSC80 Schwann cells (SC) were cultured in high glucose media at different time points to study the effect of hyperglycemia on myelin proteins and SC physiology. Since CYP450 expression and activity were characterized in the sciatic nerve, the next aim was to re-establish our results at the cell-specific level.

As mentioned previously, CYP expression has not been investigated nor reported in the PNS and diabetic neuropathy. Subsequently, we first examined whether CYP4A and CYP2C are expressed in MSC80 cells. Microsomes were isolated from SCs cultured with 5 mM (normal glucose, NG) and 25mM (high glucose, HG) at 6hr, 12hr, 48hr, and 72hr timepoints **(Fig. 24 A, D).** Western blot analysis of microsomal lysates returned a basal expression of both CYP4A and CYP2C in normoglycemic SCs. This expression was shown to fluctuate in cultured SCs throughout the incubation period with HG **(Fig. 24 A, D).** Upon further assessment, it became apparent that CYP4A levels significantly peak at 6hr **(Fig. 24B)** and 48hrs **(Fig. 24C)** in hyperglycemic SCs relative to controls. Similarly, a less pronounced, however, significant increase in CYP2C expression in SCs cultured in HG media was observed at 6hr **(Fig. 24E)** and 48hr **(Fig. 24F).** Consistent with these findings, we focus on the 6- and 48-hour timepoints for further evaluation. The present work is the first to demonstrate the expression of CYP4A and CYP2C in SCs and that this expression was significantly increased 6 hours after treatment with HG and sustained until 48 hours, implicating its role in diabetes.

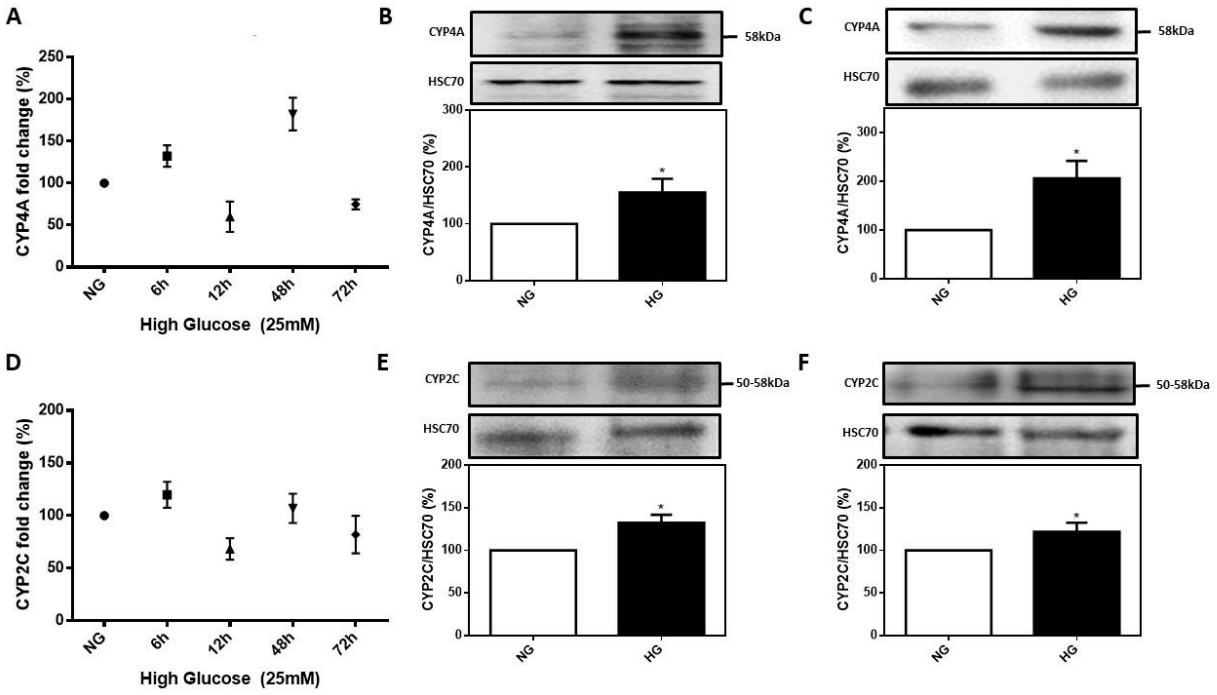


Figure 24. HG alters CYP4A and CYP2C protein expression in mouse Schwann cells. Analyses of microsomal proteins obtained from MSC80 cells in the presence of NG (5mM) or HG (25mM) showing (A) CYP4A and (D) CYP2C expression at different timepoints. Representative western blot and histogram showing the protein levels of (B) CYP4A after 6 hours and (C) CYP4A after 48 hours of HG treatment, (E) CYP2C after 6 hours and (F) CYP2C after 48 hours of HG treatment. Values of six independent experiments (n=6) are the mean ± SE. *$P<0.05$, *HG vs. NG.*

Effect of HG and exogenous 20-HETE on SC CYP4A and CYP2C protein expression.

Compounding our initial *in vitro* observation in HG-induced CYP4A and CYP2C upregulation at 6 and 48 hours, we next wanted to evaluate the impact of this upregulation in response to exogenous 20-HETE administration first. As evidenced by the in vivo findings, CYP4A overexpression was associated with elevated 20-HETE production. To further comprehend the mode of injury, MSC80 cells were incubated with 1.5μM of 20-HETE for 6 and 48 hours. Western blot analysis of SC protein lysates reveals significantly increased CYP4A and CYP2C expression in SCs cultured with HG corroborating our initial microsomal analysis. Furthermore, and to our interest, SCs incubated with 20-HETE show significantly elevated CYP4A protein expression at 6 **(Fig. 25A)** and 48 hours **(Fig. 25B).** By contrast, the results show a tendency of downregulated CYP2C expression at both timepoints, 6 hours **(Fig. 25C)** and a more pronounced reduction at 48 hours **(Fig. 25D).**

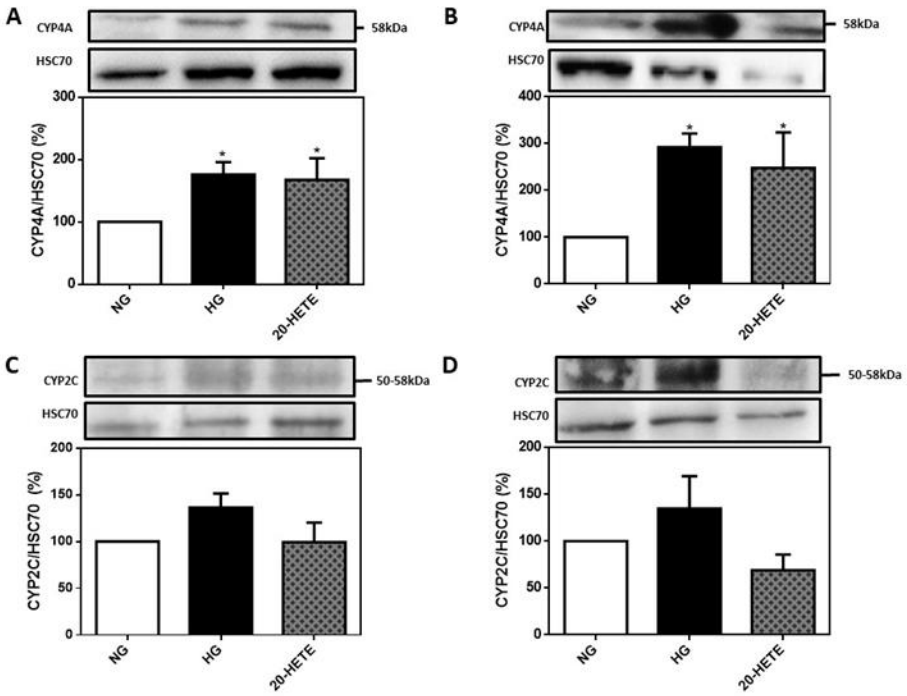


Figure 25. HG and exogenous 20-HETE alters CYP4A and CYP2C protein expression at 6- and 48-hours. To assess the effect of 20-HETE on SC CYP protein expression, western blot analysis was performed. MSC80 cells were incubated in the presence of NG (5mM) or HG (25mM) or 20-HETE (1.5μM) for 6 and 48 hours. Representative western blot and histogram showing the protein levels of (A) CYP4A after 6 hours and (B) CYP4A after 48 hours, (C) CYP2C after 6 hours and (D) CYP2C after 48 hours of HG or 20-HETE treatment. Values of eight independent experiments (n=8) are the mean ± SE. *P<0.05, HG vs. NG.

Effect of HG and exogenous 20-HETE on SC myelin proteins profile.

The role of HG-mediated CYP4A alteration as well as 20-HETE production on myelin protein expression and myelin injury has also been determined in this study. MPZ and PMP22 expression was assessed via Western blot analysis. Our findings provided evidence that SC injury could be triggered by HG treatments through an increased expression of MPZ **(Fig. 26A, B**) and PMP22 **(Fig. 26C, D)** after incubation for 6 and 48 hours, respectively. Concomitantly, 20-HETE treatments mimicked the effect of HG on SCs where MPZ expression was significantly elevated at 6 and 48 hours of treatment Similarly, PMP22 expression was increased slightly at 6 hours, and significantly at 48 hours **(Fig. 26A-D).**

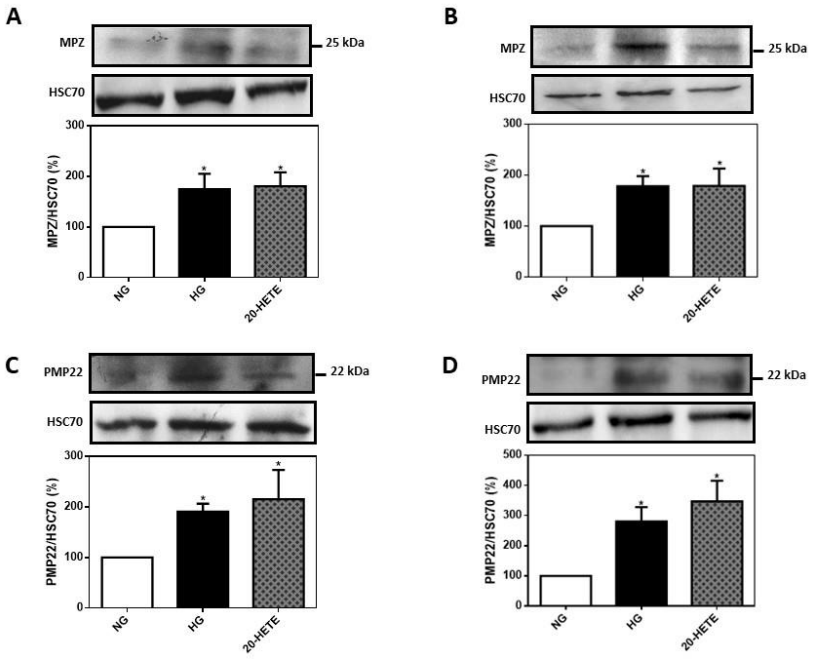


Figure 26. HG and 20-HETE lead to myelin protein alterations. Western blot analysis of MPZ and PMP22 myelin proteins from MSC80 cells exposed to NG (5mM) or HG (25mM) or 20-HETE (1.5μM) for 6 and 48 hours. Representative western blots of (A) MPZ (n=7) after 6 hours (B) MPZ after 48 hours (n=8) and (C) PMP22 after 6 hours (n=5) (D) PMP22 after 48 hours (n=8) with their respective densitometric analysis. Values are the mean ± SE.*P<0.05, high glucose or 20-HETE vs. normal glucose. **P<0.01, HG or 20-HETE vs. NG.

Effect of HG and exogenous 20-HETE on SC physiology and survival.

To further evaluate the viability of SC in response to HG-mediated CYP4A upregulation, MSC80 cells were treated with HG (25mM) or 20-HETE (1.5 μM) for 6 and 48 hours. First, autophagy protein marker, Beclin-1, levels were assessed via western blot. The results show a HG-mediated increase in Beclin-1 protein expression at 6 hours. Similarly, SCs incubated with 20-HETE for 6 hours exhibit increased Beclin-1 however, the rise at 6 hours did not achieve statistical significance **(Fig. 27A).** By contrast, Beclin-1 levels were significantly reduced after 48 hours exposure to HG or 20-HETE **(Fig. 27B).** Following this decline, cellular DNA Fragmentation ELISA Assay was used to assess apoptosis by measuring BrdU-labeled DNA fragments. The data showed a significant increase in cellular apoptosis in hyperglycemic SCs. Interestingly, a significant induction of apoptosis was observed upon exogenous 20 HETE treatment suggesting that the effect of 20-HETE mimics that of HG **(Fig. 27C).** This data is

indicative of a CYP4A-mediated injurious effect and that the blockade of CYP4A may be a promising therapeutic approach that may rescue SCs from their programmed demise.

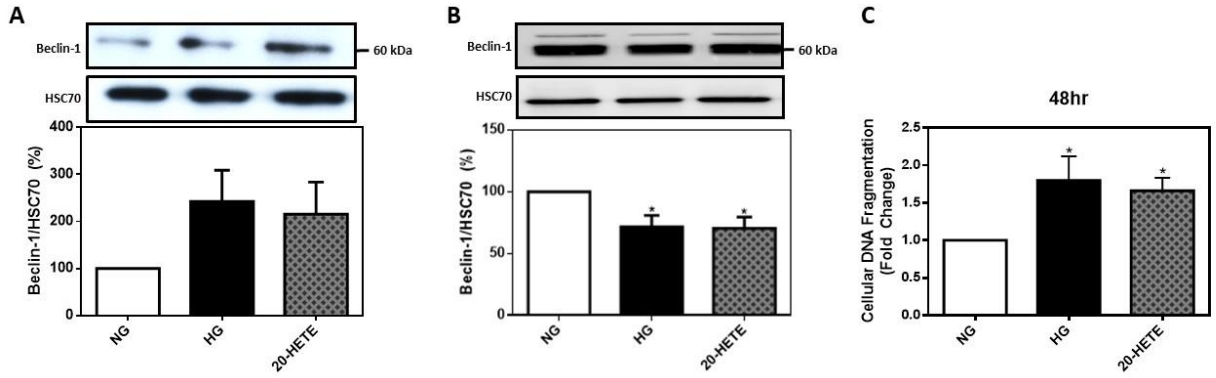


Figure 27. CYP4A-dependent 20-HETE production mediates HG triggered alteration in autophagy induction and SC apoptosis. Western blot analysis of autophagy marker Beclin-1 protein levels from MSC80 cells exposed to NG (5mM) or HG (25mM) or 20-HETE (1.5μM) for (A) 6 hours (n=4) and (B) 48 hours (n=4). In another set of experiments, MSC80 cells were serum-deprived overnight and treated with either HG (25mM) or 20-HETE (1.5μM) for 48 hours (n=4). Values are the mean ± SE. *P<0.05, ** P<0.01, HG or 20-HETE vs. NG.

Effect of HG and exogenous 14,15-EET on SC CYP4A and CYP2C protein expression.

For the final *in vitro* assessment, we wanted to determine the biological effect of exogenous administration of 14,15-EET on SCs. This approach is performed to correlate the *in vivo* findings whereby sEH inhibitor administration showed a behavioral neuroprotective advantage in diabetic animals. More importantly, we wanted to associate the reduced EET levels observed in diabetic peripheral nerves however at the cellular level and evaluate the effect of supplementing SCs incubated in HG with EETs. In this spirit, MSC80 cells were pretreated with 3μM of 14,15-EET for 6 and 48 hours. Western blot analysis of SC protein lysates reveals significantly increased CYP4A and CYP2C expression in SCs cultured with HG, once again, lending support to our microsomal analysis. Furthermore, and to our interest, SCs incubated with 14,15-EET show significantly reduced CYP4A protein expression at 6 **(Fig. 28A)** and 48 hours **(Fig. 28B).** By contrast, the results show CYP2C expression to be maintained at a relatively high level in comparison to normoglycemic SCs at both timepoints. However, the rise in CYP2C was more pronounced at 6 hours **(Fig. 28C)** rather than at 48 hours **(Fig. 28D).**

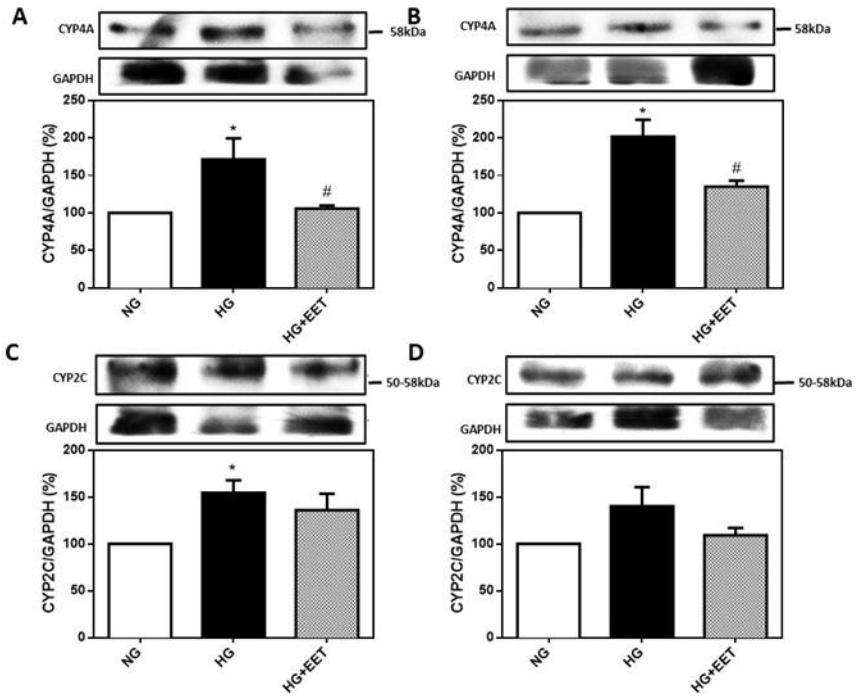


Figure 28. HG and exogenous 14,15-EET alters CYP4A and CYP2C protein expression at 6- and 48-hours. To assess the effect of 14,15-EET on SC CYP protein expression, western blot analysis was performed. MSC80 cells were incubated in the presence of NG (5mM) or HG (25mM) or HG pretreated with 14,15-EET (3μM) for 6 and 48 hours. Representative western blot and histogram showing the protein levels of (A) CYP4A after 6 hours and (B) CYP4A after 48 hours, (C) CYP2C after 6 hours and (D) CYP2C after 48 hours of HG treatment. Values of eight independent experiments (n=8) are the mean ± SE. *P<0.05, HG vs. NG.

Effect of HG and exogenous 14,15-EET on SC myelin proteins profile.

Next, we aimed to determine the role of 14,15-EET supplementation on SCs and myelin protein expression. MPZ and PMP22 expression was assessed via Western blot analysis. MPZ and CYP2C protein expression at 48 hours exposure to HG with or without EET supplementation were visualized on the same gel **(Fig. 28D and Fig. 29B).** Our findings provided evidence that SC myelin injury could be triggered by HG treatments through an increased expression of MPZ **(Fig. 29A, B**) and PMP22 **(Fig. 29C, D)** after incubation for 6 and 48 hours, respectively. Contrary to 20-HETE treatment **(Fig. 29),** incubation of hyperglycemic SCs with 14,15-EET significantly alleviated the effect of HG on SCs where myelin protein profiles were restored at both timepoints **(Fig. 29A-D).**

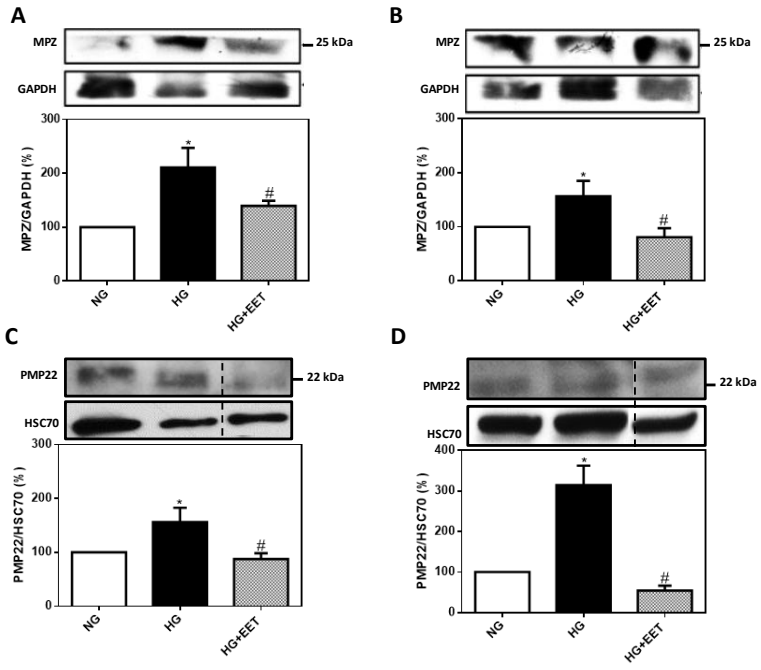


Figure 29. Supplementation with 14,15-EET restores myelin protein alterations in hyperglycemic SCs. Western blot analysis of MPZ and PMP22 myelin proteins from MSC80 cells exposed to NG (5mM) or HG (25mM) or HG pretreated with 14,15-EET (3μM) for 6 and 48 hours. Representative western blots of (A) MPZ (n=6) after 6 hours (B) MPZ after 48 hours (n=6) and (C) PMP22 after 6 hours (n=4) (D) PMP22 after 48 hours (n=3) with their respective densitometric analysis. Values are the mean ± SE.*P<0.05, high glucose or 20-HETE vs. normal glucose. **P<0.01, HG or 20-HETE vs. NG.

Part 4: Urinary 20-HETE Analysis in Type 2 Diabetic Patients

Our final aim in this study was to assess the clinical relevance of the obtained data in the human pathology. 20-HETE was analyzed as a potential biomarker of DPN in correlation with clinical parameters of T2D patients.

Study Design

This is a cross-sectional study, where patients were recruited at Hamad General Hospital in Qatar after obtaining the approval of Hamad Medical Corporation Institutional Review Board (study no. 11313/11) and experiments were conducted at the American University of Beirut, Beirut, Lebanon.

Study Subjects

Subjects were considered eligible for the study if they were between 18 and 74 years of age and able to provide an informed consent. There were no restrictions on either race or sex. Substance abuse, lactation, pregnancy, or being part of an interventional trial were the exclusion criteria. Healthy and diabetic patients were recruited (n=182) after the adherence to the exclusion criteria. Data on socio-demographics, past medical history and family history were collected from medical records. Since 20-HETE undergoes glucuronidation in the liver prior to urinary excretion, urine samples were collected to measure its levels.

Data Collection

Data on socio-demographics, past medical history and family history were collected from medical records. Type 2 diabetes was diagnosed based on American Diabetes Association (ADA) guidelines (HbA1c ≥6.5%, fasting glucose levels ≥126 mg/dl and/or random glucose level ≥200mg/dl) 14. Data regarding Diabetic Neuropathy or Retinopathy occurrence was obtained from collected medical records. Neuropathy was assessed in diabetic patients according to symptomatic reporting, physical examination, and the Michigan Diabetic Neuropathy Score (MDNS) which allows for the diagnosis and staging of diabetic neuropathy (Herman et al., 2021). Metabolic syndrome was defined based on the criteria suggested by the American Heart Association and the National Heart Lung and Blood Institute (Grundy et al., 2005). Urine samples for laboratory testing were collected at the time of the clinic visit. All data were coded using a unique identifier that kept the subject's identity anonymous.

Assessment of urinary 20-HETE levels

Urinary 20-HETE levels were measured using the 20-HETE Glucuronide ELISA kit (Detroit R&D, Inc., USA) according to the manufacturer's protocol (Liu et al., 2018). Validation experiments on 20-HETE urinary concentrations by gas chromatography-mass spectrometry (GC-MS) using electron capture negative chemical ionization, expressed in pmol/l, yielded similar values (Rivera et al., 2004). Levels were done in triplicates and the mean of the values was used to avoid any measurement bias. All analytical measurements were performed by a technician who was blinded to the study purpose.

Statistical Analysis

Data analysis was done using the Statistical Package for Social Sciences (SPSS) version 25 (IBM, USA). Baseline characteristics of the study population were determined using descriptive statistics. Results of descriptive statistics were reported using mean ± standard deviation (SD) for the continuous variables and percent for the categorical values. The association between 20-HETE levels and co-variates were evaluated using independent T test, one-way ANOVA, or correlation, depending on whether covariates were qualitative or quantitative. The second part of the analysis is the multivariate analysis. Multiple linear regression analysis with stepwise analysis was used to determine whether clinical variables had an independent effect on 20-HETE levels. Models were constructed using the variables that were significantly associated with 20-HETE levels in the univariate analysis. Two models were done using all subjects with including every variable with R>0.4 in the univariate analysis. The difference between these two models was the inclusion of the severity of albumin to creatinine ratio variable. This variable had more than 10% missing data but was considered significant when constructing the model. A subgroup analysis was done by running the model for diabetic patients and hypertensive patients. Models were considered significant if the *p* value was below 0.05. Beta estimates, confidence interval, and *p* value were reported for the retained variables. Adjusted R square was reported for each model to describe the extent of which each model is determining or explaining the 20-HETE levels. ROC curve method was used to quantify 20-HETE performance both graphically and statistically. The Area Under the Curve (AUC) was calculated **to determine whether urinary 20-HETE levels discriminate between the presence or absence of nerve injury in diabetics.**

Patients (n=182) were recruited for the study with gender ratios measuring 54.9% males and 45.1% were females. Analyzing the mean variations of 20-HETE levels with sociodemographic characteristics **(Table 7)** revealed a significant difference in 20-HETE levels with different age categories; with the highest levels observed among subjects between 45 and 74 years old compared to the group with ages ranging between 21 and 44 years old with mean values of 20-HETE increasing respectively from 4669 to 8921 reaching 19405 pmol/l in the elderly. By contrast, variations of 20-HETE levels were insignificantly affected by region, gender, or race.

20-HETE levels were next correlated with clinical parameters. Approximately 70% of patients were reported to be diagnosed with diabetes **(Table 7)**. The findings reflect a positive correlation between 20-HETE levels and diabetes onset and duration. Diabetic patients exhibit significantly higher levels of urinary 20-HETE with levels of 11081 compared to 1668 pmol/l in patients without diabetes. These findings were found to be independent of obese status in patients. More importantly, when considering microvascular complications, patients diagnosed with retinopathy, nephropathy or neuropathy had higher levels of 20-HETE with a mean difference of 7411, 8091 and 5725 pmol/l, respectively. Likewise, other co-morbidities were associated with an increase in urinary 20-HETE levels. 20-HETE levels in hypertensive patients were two-folds higher than those in non-hypertensive subjects, and patients with coronary artery disease had a 20-HETE level of 13797 pmol/l compared to 7570 pmol/l in patients without coronary artery disease. This increase was also observed with stroke, dyslipidemia, and metabolic syndrome with levels 2 times, 2.5 times and 3.5 times respectively higher 20-HETE levels in comparison to healthy individuals.

We further assessed urinary 20-HETE correlation via a univariate analysis with standard laboratory tests among all subjects. Positive correlations with 20-HETE levels were returned with blood urea nitrogen (BUN), creatinine, uric acid, urine albumin, albumin to creatinine, and protein to creatinine ratio. On the other hand, a negative correlation was observed between 20-HETE levels and serum albumin, GFR, hematocrit, or urine creatinine levels. These findings are indicative of a high sensitivity that urinary 20-HETE levels may detect microvascular dysfunction in early clinical stage which further validate the observed correlations with retinal, neural, and renal microvascular complications **(Table 8).**

To further reinforce the diagnostic and prognostic strength of 20-HETE, a multivariate regression analysis was performed **(Table 9).** focusing specifically on the diabetic patients,

diabetes duration, diabetes-induced retinopathy, family history of chronic kidney disease, proteinuria and history of stroke were significantly associated with the 20-HETE levels. Additional analysis of patients with diabetic neuropathy (n=17) shows that 20-HETE may discriminate between neuropathy occurrence among diabetic patients with an AUC of 0.76 **(Fig. 30)**. These data may hold more promise with a larger sample number.

Table 7. Association of urinary 20-HETE levels with various patient characteristics and clinical parameters. ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blockers; BMI, body mass index; HCT, hydrochlorothiazide; RAS, renin-angiotensin system.

Characteristics (n=182 unless stated)		**Patients**	**20-HETE levels Mean (pmol/l) ± SD / R Correlation Factor**	**p-value**
Age (years)	47.3±11.4		0.327	<0.001
Gender (%)	Male	100 (54.9%)	8,483±7,469	0.753
	Female	182 (45.1%)	8,051±10,784	
Comorbidities		**Patients**	**20-HETE levels Mean (pmol/l) ± SD / R Correlation Factor**	**p-value**
Diabetes	128 (70.3%)		11,081 ± 9,500	<0.001
Duration of Diabetes (years, n=126)	10.5±9.2		0.487	<0.001
HbA1c (mmol/mol) (n=124)	<53	49 (60.5)	8,697 ± 6,885	0.019
	>53	75 (39.5%)	12,803 ± 10,668	
BMI (kg/m2)	Underweight	1 (0.5%)	5,632 ± 0	0.794
	Normal	42 (23.1)	7,339 ±7,520	
	Overweight	57 (31.3%)	8,038 ± 7,978	
	Obese	82 (45.1%)	8,981 ± 10,730	
Family History of Diabetes	No history	76 (41.8%)	6,298 ± 10,723	0.044
	1st Degree	104 (57.1%)	9,681 ± 7,683	
	2nd Degree	2 (1.1%)	11,530 ± 8,341	
Hypertension	79 (43.4%)		11,575 ± 10,756	<0.001
Duration of Hypertension (years, n=79)	6.37±5.4		0.166	0.143
Coronary Artery Disease	21 (11.5%)		13,797 ± 7,452	0.003
Peripheral Vascular Disease	6 (3.3%)		15,180 ± 7,630	0.062
Stroke	5 (2.7%)		17,593.8±4,111	0.021
Dyslipidemia	71 (39%)		13,171 ± 11,104	<0.001
Metabolic syndrome	120 (65.9%)		10,853 ± 9,879	<0.001
Diabetic Neuropathy	17 (13.3%)		16,046 ± 4,362	0.02
Diabetic Retinopathy	22 (17.2%)		17,219 ± 4,560	0.001
Medications		**Patients**	**20-HETE levels Mean (pmol/l) ± SD / R Correlation Factor**	**p-value**

Oral hypoglycemic (n=128)	98 (76.6%)	10,409 ± 9,861	0.148
Insulin (n=128)	37 (28.9%)	14,930 ± 6,486	0.003
Calcium channel blocker	29 (15.9%)	12,176 ± 7,198	0.013
Beta blocker	35 (19.2%)	10,836 ± 6,601	0.068
Diuretic HCT	14 (7.7%)	13,438 ± 6,917	0.029
Drugs acting on RAS	67 (36.8%)	10,936 ± 6,845	0.003
Anti-platelet: Aspirin	48 (26.4%)	13,943 ± 12,295	<0.001
Statin	75 (41.2%)	12,184 ± 10,934	<0.001

Table 8. Association of urinary 20-HETE with patient laboratory tests and renal function tests. ACR, albumin to creatinine ratio; ALT, alanine aminotransferase; ALKP, alkaline phosphatase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; GFR, glomerular filtration rate; HDL, high density lipoprotein; LDL, low density lipoprotein; TG, triglycerides; WBCs, white blood cells.

Laboratory tests **Blood studies**	**n**	**Mean (pmol/l) ± SD**	**20-HETE levels Mean (pmol/l) ± SD / R correlation factor**	**p value**
BUN (mmol/l)	179	7.3±7.04	0.294	**<0.001**
Creatinine (umol/l)	181	97.91±65.31	0.341	**<0.001**
GFR (ml/min)	180	80.1±33.35	-0.332	**<0.001**
GFR categories (n=180)	G1	67	4873±5559	**<0.001**
	G2	69	8606±11664	
	G3a	15	10,351±7106	
	G3b	13	10926±5854	
	G4	15	17577±5490	
	G5	1	21,087 ± 0	
Serum albumin (g/l)	178	43.92±4.62	-0.372	**<0.001**
Serum total protein (g/l)	174	71.45±174.13	-0.118	0.12
Corrected serum calcium (mmol/l)	167	2.25±0.12	0.104	0.182
Serum phosphorus (mmol/l)	167	1.32±0.77	0.151	0.051
Serum sodium (mmol/l)	180	138.84±2.84	-0.099	0.187
Serum potassium (mmol/l)	178	5.33±10.09	-0.023	0.756
Serum chloride (mmol/l)	171	98.63±14.64	-0.007	0.926
Serum bicarbonate (mmol/l)	172	26.6±8.82	0	0.996
Hemoglobin (gm/dl)	169	13.86±6.87	0.041	0.595
Hematocrit (%)	169	39.84±5.4	-0.302	**<0.001**
Platelets (*1000/cmm)	169	258.93±78.26	0.083	0.286

WBC (1000/cmm)	169	10.14±16.85	0.026	0.735
Total bilirubin (umol/l)	166	9.52±18.58	0.075	0.337
ALT (U/L)	165	23.98±15.06	0.068	0.382
AST (U/L)	153	21.97±10.96	0.085	0.266
ALKP (U/L)	172	77.37±30.25	0.085	0.266
TG (mmol/l)	175	1.92±1.57	0.089	0.243
Total Cholesterol (mmol/l)	155	5.2±7.34	0.082	0.312
LDL (mmol/l)	177	2.68±0.92	-0.121	0.109
HDL (mmol/l)	177	1.17±0.48	-0.031	0.687
Uric acid (mmol/l)	142	313.26±113.04	0.255	**0.002**
Transferrin saturation (%)	144	23.05±18.47	0.11	0.189

Table 9. Various statistical models testing the predictability of urinary 20-HETE as a prognostic and diagnostic marker in diabetic complications.

a: Adjusted for age, gender, hypertension, diabetes status, coronary artery disease, stroke, family history of diabetes, family history of CKD, dyslipidemia, metabolic syndrome, CCB, diuretic, ACEI and ARB use, Antiplatelets (aspirin), statin, SBP, BUN, serum albumin, hematocrit, severity of proteinuria, severity of decrease in GFR

b: Adjusted for a and severity of albuminuria.

c: Adjusted for age, gender, hypertension, diabetes status, coronary artery disease, stroke, family history of diabetes, family history of CKD, dyslipidemia, metabolic syndrome, CCB, diuretic, ACEI and ARB use, Antiplatelets (aspirin), statin, SBP, BUN, serum albumin, hematocrit, proteinuria, severity of albuminuria, severity of decrease in GFR, diabetes duration, microvascular complications, hbA1c, anti-diabetics.

d: Adjusted for age, gender, diabetes status, coronary artery disease, stroke, family history of diabetes, family history of CKD, dyslipidemia, metabolic syndrome,

CCB, diuretic, ACEI and ARB use, Antiplatelets (aspirin), statin, SBP, BUN, serum albumin, hematocrit, severity of proteinuria, severity of decrease in GFR.

ACEI, angiotensin converting enzyme inhibitor; ACR, albumin to creatinine ratio; ARB, angiotensin II

Models		All patients [a] n=182			All patients [b] n=116			Patients with diabetes [c] n=108			Hypertensive patients [d] n=65		
Variables		**B (pmol/l)**	**CI**	***p***	**B (pmol/l)**	**CI**	***p***	**B (pmol/l)**	**CI**	***p***	**B (pmol/l)**	**CI**	***p***
Socio-demographic	Family history of CKD	11.909	9,331-14,488	<0.001	12.501	10,670-14,334	<0.001	11.204	9,514-12,894	<0.001	11.689	9,272-14,106	<0.001
Comorbidities	DM	5.3	3,096-7,503	<0.001	3.479	2,128-4,831	<0.001	-	-	-	5.424	2,322-8,627	<0.001
	Stroke	7.539	1,960-13,387	0.012	11.482	6,944-16,020		7.893	4,343-11,442	<0.001	9.766	5,157-14,375	<0.001
Medications	Anti-platelets	2.919	584-5,254	0.015	-	-	-	-	-	-	-	-	-
	Diuretic	-	-	-	3.97	1,247-6,692	0.005	-	-	-	-	-	-
Kidney function	Protein to creatinine ratio >50mg/mmol	2.974	788-5,159	0.008	-	-	-	1.997	381-3,612	0.016	2.598	65-5,132	0.045
	Stage GFR	-	-	-	1.721	1,084-2,359	<0.001	-	-	-	1.098	207-1,989	0.017
	Severity ACR	-	-	-	1.334	250-2,419	0.016	-	-	-	-	-	-
Diabetes characteristics	Duration of diabetes (years)	-	-	-	-	-	-	231	149-314	<0.001	-	-	-
	Diabetic retinopathy	-	-	-	-	-	-	2.127	256-3,999	0.026	-	-	-
Adjusted R square		0.505			0.82			0.79			0.74		

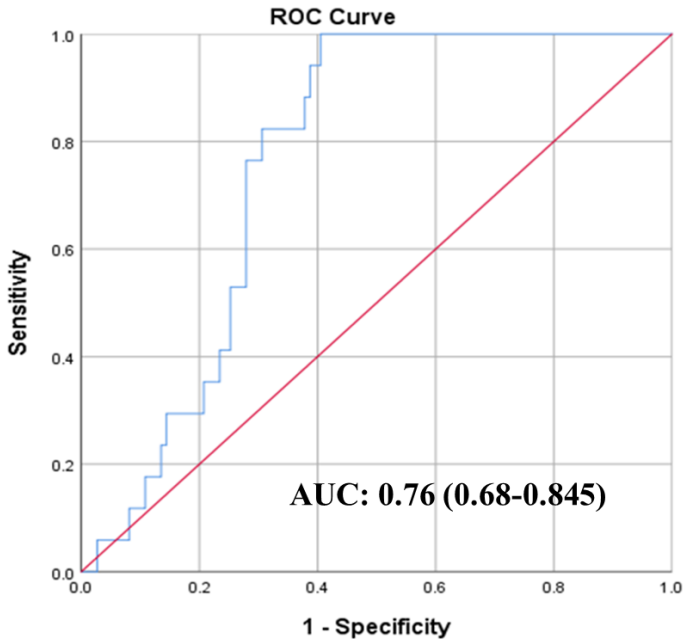


Figure 30. 20-HETE as a potential diagnostic tool for diabetic neuropathy.

IV-Discussion

Prelude

Diabetes targets a multitude of organs and organ systems including the PNS. DPN is one of the complications of diabetes with autonomous onset. DPN is characterized by nerve morphological abnormalities and physiological faults (Ohsawa et al 2008; Juranek et al., 2013; Becker et al., 2014). The clinical manifestations of DPN include sensorimotor dysfunction, reduced nerve conduction velocities, and slowed axonal transport (Johnson, P. C., Doll, S. C., & Cromey, D. W. 1986; Fross, R. D., & Daube, J. R. 1987; Ishii, D. N. 1995; Zochodne, D. W. 1999; Hill, R. E., & Williams, P. E. 2002). The myelinating cells of the PNS, Schwann cells (SCs), are also implicated in DPN pathogenesis (Askwith et al., 2009; Cinci et al., 2015). Studies have shown that SCs undergo high-glucose induced loss of axonal association (Dey et al., 2013), reduced regenerative potential (Gumy et al., 2008), apoptosis (Wu et al., 2012) in addition to demyelination and de-differentiation (Hao et al., 2015). However, the mechanisms through which hyperglycemia leads to SC alteration and dysfunction are poorly characterized. Therefore, the comprehension of the mechanistic progression of DPN is central to identify novel therapeutic targets. Glucose lowering agents are currently the primary mode of management of diabetes and diabetic complications. To date, diabetic neuropathy is among the ailments that remain entrenched among diabetic subjects despite proper management of the disease. Consequently, the largest clinical trials have urged the need for novel approaches to the management of DPN.

Numerous biochemical pathways are affected by dysmetabolism caused by hyperglycemia. A rising body of data show oxidative stress as a major mediator of diabetic complications, including DPN (Eid et al., 2009; Eid et al., 2010; Maalouf et al., 2012; Eid et al., 2014; Mroueh et al., 2019; Eid et al., 2020). Nevertheless, although several ROS sources have been identified to play a role in the pathogenesis of DPN, intercepting their activity or treatments with antioxidants have not demonstrated maximal therapeutic potential (Vincent et al., 2007; Hotta et al., 2008; Obrosova et al., 2010, Pop-Busui et al., 2013; Rumora et al., 2017). Accordingly, we set out to investigate CYP450 enzymes which are well known to be potent sources of ROS in diabetic complications, and their role in DPN has not been investigated yet.

This study provides evidence that hyperglycemia has a damaging effect on peripheral nerve integrity through the generation of a state of oxidative stress by CYP enzymes. Particularly, we

assessed physiological pillars of the CYP family, CYP4A and CYP2C, in addition to the effect of their respective metabolites 20-HETE and EET. These effects were assessed at the level of the sciatic nerve in addition to the Schwann cells (SCs). The loss of physiological balance between 20-HETE and EET was reflected in our diabetic animal model to mediate a state of ROS overproduction that triggered the alteration of AMPK and AKT signaling pathways. These pathways are dubbed essential for maintaining SC survival, myelination, and cellular energy and physiological homeostasis. Collectively, this disturbance was reflected by altered autophagic defenses and the induction of apoptotic cellular death at the cellular level. Additionally, myelin protein profile disruption was evident as hallmarks of SC and peripheral nerve injury. We first highlight the 20-HETE synthase inhibitor HET0016, as a possible pharmaceutical approach to diabetes-induced peripheral nerve injury. The impact of HET0016 was reflected in its ability to alleviate sensorimotor coordination, normalize myelin protein profiles and physiological alterations triggered by hyperglycemia. We further demonstrate that stabilizing EETs, through the pharmacological inhibition of sEH hydrolase via AUDA, possess a partial therapeutic advantage.

20-HETE in DPN: The Opening Act

We first investigated the role of CYP4A in MKR non-obese T2D at a functional and molecular level. Molecular tests in sciatic nerve tissues from all diabetic mice in our studies show increased CYP4A protein levels and 20-HETE production. This marked increase was concurrent with decreased MPZ and PMP22 protein expression. The observed decrease in MPZ levels upon diabetes induction is consistent with other studies (Kawashima et al., 2007; Cermenati et al., 2012). By contrast, our results were also in opposition with previous work that demonstrates increased MPZ and PMP22 levels in response to diabetes induction (Eid et al., 2020). However, these discrepancies may be addressed from the standpoint that myelin alteration may correlate with other factors such as the animal model assessed, type of diabetes and duration/severity of the anomaly. Indeed, diabetic neuropathy has been dubbed as a complex disease in both T1D and T2D with overlapping clinical manifestations, but with distinct underlying mechanistic insults (Callaghan et al., 2012; Feldman et al., 2017). More importantly, and cognizant of myelin protein alterations, abnormalities in PMP22 proteins were observed in the insoluble fractions of sciatic nerves of diabetic animals. Diabetic animals exhibited increased accumulation of aggregated PMP22 proteins in our study, and this lends support to previous studies that linked both protein misfolding and myelin injury in diabetes (Hamilton et al., 2013, Eid et al., 2020).

Collectively, these findings were reflected through the behavioral assessments whereby diabetic animals exhibited poor locomotion, neuromuscular strength and nociception, characteristic with the clinical manifestations of DPN (Biessels et al., 2014). In addition to that, electrophysiological recordings of the sensory and motor nerve conduction velocities of sciatic nerves from diabetic animals were significantly reduced, indicative of aberrant nerve conduction. The observed manifestations in diabetic animals mimics the clinical presentation of diabetic subjects, verifying the neuropathic phenotype in our animal model. Moreover, these findings lend support to the notion that myelin protein alterations have deleterious effects on the functional integrity of peripheral nerves (Niemann et al., 2000; Wrabetz et al., 2000; Yin et al., 2000; Niemann et al., 2006). The results are also in accordance with a large body of data that have associated alterations in myelin protein profiles with several neurodegenerative anomalies (Kawashima et al., 2007; Fortun et al., 2007; Lee et al., 2018). Interestingly, the pharmacological inhibition of CYP4A via HET0016 in diabetic animals restored myelin defects and mitigated the neurological deficits indicating a possible therapeutic benefit. Taken together, the results highlight the possible involvement of CYP4A in peripheral nerve injury in diabetes and dysmyelination.

Our group as well as others' research focuses on oxidative stress as a major mediator of diabetic complications (Eid et al., 2009; Eid et al., 2010; Eid, et al., 2013; Eid et al., 2014). From this standpoint, following the observed neuropathy alleviation afforded to diabetic animals by HET0016, we aimed to dissect its mode of action in DPN. Protein expression of the ω-hydroxylase CYP4A was significantly increased in diabetic animals and restored upon HET0016 administration. CYP4A overexpression was paralleled by a significant increase in 20-HETE levels reflecting elevated CYP4A activity. Compounding this observation, it has been shown that 20-HETE mimics TRPV1 channel agonists of sensory neurons, and that it was capable of sensitizing and activating these channels in both humans and mice. Additionally, TRPV1 channels have been described to be associated with thermal hyperalgesia (Wen et al., 2012). In our study, an important finding that merits highlighting is that HET0016-treated diabetic animals demonstrated a marked improvement in sensorimotor function and nociception compared to the diabetic group. This finding is of significance as the bioefficacy of HET0016 as a therapeutic agent may be multifaceted and subject to further experiments that correlate CYP4A/20-HETE-dependent activation of TRPV1 channels in DPN.

Moreover, previous work has correlated CYP4A upregulation induced by hyperglycemia with increased oxidative stress through 20-HETE production leading to injury in kidney tubules, podocytes (Eid et al., 2009; Eid et al., 2014) and in the lung endothelia (Medhora et al., 2008). To our knowledge, this study is the first to examine CYP4A expression in T2D within the context of DPN. The current findings have established a link between CYP4A overexpression in tandem with ROS overproduction. Notably, CYP450 enzymes are associated with a NADPH subunit which serves as the ROS-producing, catalytic center of the enzyme (Medhora et al., 2008; Zeng et al., 2010; Eid et al., 2014). By assessing the oxidative status from sciatic nerves lysates, the data show an elevated ROS production in addition to NADPH oxidase activity in the diabetic animals, in validation of oxidative peripheral nerve injury. However, CYP4A inhibition in sciatic nerves of diabetic animals significantly quelled the pathological increase in oxidative stress as well as NADPH activity. These results underscore the association of CYP4A with increased ROS production through an NADPH-dependent pathway. These data are in line with a recent study that verified the role of NADPH oxidase enzymes in the pathogenesis of DPN (Eid et al., 2020). Together, we extend the pathological pathway further to CYP4A and pinpoint a potential crosstalk between CYP450 and NADPH oxidase enzymes (Eid et al., 2009).

Emanating from this observation, this study also outlined the role of autophagy as a route implicated in hyperglycemia-induced myelin protein abnormality. Extensive literature has linked autophagy to various pathologies, with implications in diabetes (Gonzalez et al., 2011; Rocchi & He, 2015; Huang et al., 2016; Yerra et al., 2016; Chung et al., 2018) and in neurodegeneration (Menzies et al., 2017). At the physiopathological level, our results demonstrated elevated Beclin-1 and LC3B protein levels in sciatic nerves from diabetic animals, indicative of autophagy activation. Elevated Beclin-1 has been described to be secondary to the occurrence of neurodegeneration (Erlich et al., 2006). Interestingly, HET0016 treatment was capable of restoring Beclin-1 and LC3B levels to near normal which may account for the alleviation of nerve functional changes. Our results suggest that dysregulated autophagy may play a role in the pathogenesis of DPN. The demonstrated activation of autophagy in our non-obese T2D MKR model contrasts with other studies whereby a decrease in LC3 levels was observed in diabetic db/db animals (Chung et al., 2018). However, previous studies have also demonstrated a rise in autophagic vacuole formation in pancreatic beta cells of diabetic animals with insulin resistance (Ebato et al., 2008). Subsequently, in our study, the activated autophagic responses may be partly due to the

development of insulin resistance in our MKR animals. On the other hand, elevations in autophagy may also be attributed to the increase in damaged protein clearance to protect against cell death (Webb et al., 2003; Fortun et al., 2006) or it may be a transitory phase prior to apoptosis (Chung et al., 2018). This is demonstrated in a recent study in which the activation of autophagy in type 1 diabetic animals was shown to rescue myelin protein expression 12 weeks into treatment relative to the diabetic group whereby a decline in PMP22 and P0 levels was demonstrated in diabetic sciatic nerves (Liu et al., 2017).

The significance of autophagy as a survival mechanism in demyelinating neuropathies was demonstrated by Rangaraju and Notterpek. Schwann cells of the neuropathic mouse model used, which is characterized by PMP22 overexpression and cluster formation, exhibited a neuropathic phenotype marked with unsustainable myelin sheaths. Importantly, the pharmacological activation of autophagy was shown to reduce elevated PMP22 levels, morphologically and functionally rescuing SCs (Rangaraju & Notterpek, 2011). However, while some studies suggest autophagy activation in early stages of injury may be a protective mechanism to trigger recovery and regeneration (Huang et al., 2016), others relate autophagy activation as an undercover indicator of stress and a trigger for cellular recycling or death (Fortun et al., 2007, Rami et al., 2008). Our findings extend previous work that suggests autophagy activation occurs secondary to nerve injury associated with oxidative stress, although other pathologies such as inflammation, and vascular anomalies may be triggers (Huang et al., 2016; Wang et al., 2019).

The current findings have, in addition, established a link between CYP4A-induced ROS production and the mTOR/AKT pathway, pivotal for cellular survival. It has been shown that increased mTORC1 activity leads to increased myelin thickness and myelin protein levels, while the loss of mTORC1 activity is associated with hypomyelination in the central nervous system (Flores et al., 2008; Narayanan et al., 2009). Similarly, in the PNS, sciatic nerves from mTOR mouse mutants have been shown to be associated with thin myelin sheaths with abnormal elongation and SC growth in addition to reduced MPZ levels (Sherman et al., 2012). However, the involvement of mTORC1 or mTORC2 was not specified. A more recent study has set the distinction between mTORC1 and mTORC2 associations in PNS myelination and showed that mTORC1deficiency mediates hypomyelination in addition to reduction in nerve conduction velocities and not mTORC2; mTORC2 deficiency solely does not lead to hypomyelination (Norrmen et al., 2014). Nevertheless, the role of mTORC1 and mTORC2 has yet to be understood.

The downstream effector of mTORC2, AKT, has been described in the literature to enhance myelination in the central nervous system, and not PNS, when active (Flores et al., 2008). Further studies have showed that AKT plays a role in regulating myelin sheath thickness in the PNS in collaboration with basic regulatory proteins that determine cellular polarization and protein trafficking (Cotter et al., 2010). A disruption in one of the collaborative proteins that interact with AKT, such as PTEN, has been shown to increase AKT activation which leads to hypermyelination resulting in myelin pathologies such as outfoldings and tomacula (Goebbel et al., 2012; De Paula et al., 2014). Previous studies reported a decrease in phosphorylated AKT levels in SCs upon HG treatments (Ii et al., 2005). However, preliminary data from this study demonstrate hyperphosphorylation of Akt to be significantly increased in sciatic nerves from diabetic animals in parallel to CYP4A upregulation. HET0016 administration was shown to reduce both CYP4A and Akt upregulation, indicating the possibility of CYP4A-induced peripheral nerve injury through the Akt pathway.

Beyond myelination, our data are in accord with other studies that link neuropathic pain to active PI3K/AKT/mTOR signaling. Inhibition of this axis was further shown to suppress the PI3K/AKT/mTOR-induced activation of microglial infiltration in DRG, and alleviate neuropathic pain (Guo et al., 2017). Our data also support a previous report whereby AKT activation in DRG and spinal cord was depicted to play a role in the pathogenesis of neuropathic pain (Xu et al., 2007). Further to that, elevated ROS production in our model concurrent with AKT activation are in agreement with another study in which it was also demonstrated that an increase in ROS and activated AKT may contribute to the development of neuropathic pain, further suggesting a crosstalk between the two pathways (Guedes et al., 2008). Finally, more recent work by Tillu et al emphasized the complex interplay of the diverse pathways modulating mTOR and their role alongside ERK in sensitizing peripheral nociceptors in a pain model. Mechanical allodynia which was restored upon treatment with the antioxidant resveratrol, in a dose and time-dependent manner. Additionally, resveratrol was shown to inhibit nociceptor sensitization. The pharmacological mechanisms of resveratrol were displayed in vitro in trigeminal ganglion sensory neuron cultures and revealed to reduce ERK, AKT, TSC2 phosphorylation, yet activating AMPK, and thus negatively regulating mTOR signaling (Tillu et al., 2012).

To a further extent, numerous biochemical pathways are identified to be affected by dysmetabolism caused by hyperglycemia, such as AMPK signaling which is described to be

compromised in diabetes (Roy-Chowdhury et al., 2012; Eid et al., 2013; Mroueh et al., 2019). Also, AMPK is one of the pathways that are redox-regulated (Shao et al., 2014; Lin et al., 2017), and cumulative evidence shows AMPK inactivation to be associated with oxidative stress in various cell-types (Eid et al., 2010; Eid et al., 2013; Ma et al., 2015; Mroueh et al., 2019). Similarly, our results show AMPK to be dephosphorylated at the activation site in sciatic nerves of diabetic animals corroborating the myelin protein profile disruption and state of oxidative stress. Further to that, our results reflect AMPK inactivation which was relevant at 48 hours in hyperglycemic SCs. In this manner, we extend reports in which high glucose concentration and/or nutrient excess instills switching off of AMPK in several cell types (Eid et al., 2010; Chowdhury et al., 2011; Eid et al., 2013; Mroueh et al., 2019). In highly polarized cells such as neurons, this may create a metabolic problem in specific cellular compartments especially the high energy distal axons (Chowdhury et al., 2011), which require enormous amounts of ATP for their normal functioning. These data lend support to other recent studies that demonstrate AMPK inactivation in DPN to be linked to nerve defects, myelin dysfunction and oxidative injury (Yerra et al., 2017; Chung et al., 2018). Our findings are also in agreement with a previous study, in which AMPK pathway impairments in diabetic animals culminate in altered neuronal function in addition to myelinated and unmyelinated nerve fiber defects (Roy Chowdhury et al., 2012). Additionally, hyperglycemia-induced loss of AMPK in diabetic neurons extends to organelle-injury and leads to a deficiency in functional mitochondria that ultimately triggers distal axonal death, a hallmark of severe diabetic peripheral neuropathy (Emerling et al., 2009).

AMPK is an important cellular energy sensor that works closely with central pathways that modulate autophagy (Mihaylova and Shaw, 2011; Kim et al., 2011). In the nervous system, AMPK is an essential signaling pathway as it positively modulates neurite development and nerve regeneration (Melemedjian et al., 2011, Roy Chowdhury et al., 2012; Melemedjian et al., 2013). Previous work demonstrates the activation of AMPK to mitigate defective autophagic response which rescued Schwann cell and peripheral nerve injury in diabetic animals (Chung et al., 2018). Interestingly, our findings show a significant increase in AMPK activation following administration with HET0016, and this was concomitant with an attenuated autophagy protein deregulation in the MKR mice. In another study in STZ-induced diabetic animals, similar findings were demonstrated, however the role of mTOR was further expanded upon. Briefly, mTOR was reportedly phosphorylated and activated upon a hyperglycemic milieu in neuroblastoma cells and

sciatic nerves of STZ-induced diabetic animals, resulting in the deregulation of autophagy genes expression such as ATG7, Beclin-1 and LC3. These diabetes-induced alterations were concomitant with elevated oxidative species production and mitochondrial derangements. AMPK activation was shown to restore functional sensory and motor electrophysiological conduction deficits, as well as nerve blood flow, in sciatic nerves of STZ-diabetic animals. This was secondary to treatment with a sirtuin-1 activator, described to rescue etiologies related to insulin resistance (Yerra et al., 2017).

Further reports that are consistent with our finding demonstrates AMPK activation in T1D animals to alleviate changes in nociceptive parameters (Melemedjian et al., 2011; Ma et al., 2015; Atef et al., 2019). Similar findings were reflected in the current study through electrophysiological and behavioral assessments. In that manner, HET0016 exemplified a neuroprotective role, possibly through targeting the AMPK pathway. It is noteworthy to mention that an interesting finding in recent literature associates the activation of AMPK with a reduction in eicosanoid production in high-fat diet induced kidney disease (Declèves et al., 2019). Whereas, in an earlier study, 20-HETE mediated endothelial cell injury was shown to be ameliorated upon the prolonged activation of AMPK (Ward et al., 2011). The current work is particularly meritorious since, to date, we underscore for the first time a previously unreported, possible link between 20-HETE and AMPK in DPN.

Finally, in this study, we also assessed the effect of AMPK activator, Metformin, on non-obese T2D MKR mice to reiterate and verify the reported pathological axis implicated in the development of nerve injury due to hyperglycemia. In a parallel set of animals, our findings demonstrate Metformin administration to augment the diabetic phenotype of MKR mice in addition to sensory and locomotor defects associated with DPN progression. These results were in agreement with previous studies that relate AMPK inactivation with neuronal degeneration and hyperalgesia in diabetic animals (Ma et al., 2015; Atef et al., 2019) as well as another report whereby normalizing AMPK activity was shown to modulate sensory function and alleviate multiple manifestations associated with DPN (Wang et al., 2018). Further to that, our findings reflect myelin dysregulation concurrent with a state of oxidative stress in sciatic nerves of diabetic animals that subserve neural defects which were also mitigated in Metformin treated animals.

These results validate the inhibitory effect of AMPK activation against ROS overproduction triggered by diabetes and lend support to the influence of AMPK on regulating

central targets involved in diabetic neuropathy (Ma et al., 2015; Dewanjee et al., 2018). In that manner, our findings further show that AMPK activation ameliorates NADPH-derived ROS generation in diabetic animals. These results lend support to previous studies that show the activation of AMPK to reduce NADPH oxidase and high glucose-induced ROS production in endothelial cells, colorectal cancer cells, and glomerular mesangial cells (Ceolotto et al., 2007; Eid et al., 2013; Mroueh et al., 2019). Thus, an important finding in this work is that Metformin and HET0016 impact overlapping mechanisms of injury insofar intersecting at oxidative stress, NADPH-derived ROS production, and myelin protein alterations. However, our data also show Metformin administration to further alleviate alterations in Beclin-1 and LC3B levels in sciatic nerves of diabetic MKR mice, adding autophagy regulation to the downstream effectors of targeting AMPK. However, in spite of these findings, although Metformin may be closely approximated to hold promise as a therapeutic agent for DPN, there remains a dearth of clinical observations. More importantly, T2D patients on the receiving end of insulin plus Metformin have been reported to be twice as likely to develop diabetic neuropathy (Currie et al., 2013).

Taken together, despite extensive research, the pathogenic role of autophagy in diabetes is somewhat controversial and partially described and understood. It is noteworthy to mention that autophagic flux activation or suppression have been shown to culminate in diabetes-induced tissue damage. For instance, autophagy suppression was shown to contribute to cardiomyopathy in diabetic animals concurrent with AMPK signaling reduction (Xie el al., 2011). Similarly, impaired autophagy was reported to trigger pancreatic beta cell death in T2D patients, and this was reduced by Metformin (Masini et al., 2009). On the other hand, while AMPK activation via Metformin was shown in our study to reduce autophagic flux in diabetic animals, equally compelling studies show AMPK activation to increase autophagic flux (Zhou et al., 2019) or the inhibition of AMPK to induce autophagy (Vecicevic et al., 2011). These paradoxical discrepancies are attributed to the various inputs that regulate autophagy, emphasizing the complexity of autophagic defense triggers and the need to better elucidate its mechanisms. For instance, the modulation of autophagy is different depending on distinct tissues, cell-type, conditions, inputs from the mTOR or AMPK pathways, stages of autophagy and age (Bergamini et al., 2004; Kim et al., 2011; Gonzalez et al., 2011; Mihaylova & Shaw, 2011).

Having established the CYP4A/20-HETE/AMPK link, this work attempted to investigate the effect of HET0016 as an intervention to chronic diabetes in our non-obese T2D MKR model.

Our findings were of limited outcome since HET0016 administration slightly alleviated behavioral shortcomings of diabetic animals, despite it being administered for the same duration as the prevention study. Similarly, myelin protein expression in sciatic nerves showed partial improvement in MPZ levels, however PMP22 protein levels were not restored, as were autophagy proteins and AKT phosphorylation. In spite of this, 20-HETE levels were normalized as did ROS production, which is perplexing. One possible explanation may be due to the delayed or advanced progression of DPN given that the animals were considered to be of a group that is aged relative to other studies performed. It is well accepted that severe stages of DPN or diabetes are grounded with more complicated pathologies that may play a role in the pathogenesis, and it might have been the case for our animal model. In this spirit, there may be other factors that have come into play and that are not targets of HET0016. Other sources of ROS or organelle-mediated injury may also be on account here. Age, too, certainly is a compounding factor in this study despite having the animal groups age-matched. Probable solutions to this problem that need further investigation may involve upping the dose of HET0016 in these animals, prolonging the administration of HET0016 beyond the duration allocated, increasing the number of animals per sample, or assessing another mechanistic strategy as an adjunct therapy alongside HET0016. In this work, we chose to examine the second arm of the CYP450 family, the epoxygenases.

EETs in DPN: The Flipside

The second part of the work was tailored to address the flipside to 20-HETE overproduction. The 'flipside' is emphasized by cause of the significantly reduced EET levels recorded in diabetic animals, in both early onset and chronic diabetes groups, concomitant with increased 20-HETE. Although HET0016 was capable of normalizing 20-HETE, EET levels were only partially restored, significantly. These findings are suggestive of a slight effect of 20-HETE inhibition on EET levels in both the prevention, as well as aged intervention study. The pitfall in these studies is that we did not assess whether CYP2C expression was altered together with CYP4A. Bearing that in mind, the experimental approach for this part of the work accounted for both CYP4A and CYP2C expression in addition to their metabolites. Diabetic animals were treated with EET stabilizing AUDA at two doses. In the AUDA intervention study, animals showed no significant changes in body weight, however, HbA1c levels were significantly elevated. Like HET0016, AUDA showed no hypoglycemic potential and hereby these findings report that any molecular changes occurred independently from hyperglycemia. The behavioral performance of

diabetic animals treated with AUDA were remarkable indicative of successful resolution of DPN (Inceoglu et al., 2012). These results are in accord with other studies that have assessed the effect of sEHIs in PNS pathologies including inflammatory neuropathic pain (Inceoglu et al., 2011, Wagner et al., 2014). The antinociceptive effect of AUDA may be explained by the prolonged bioavailability of EETs which have been reported to commence by activating endorphins and Met-enkephalins that have an affinity to opioid receptors (Terashvili et al., 2008). On the other hand, strikingly, AUDA administration did not show any significant amelioration of myelin protein disruption nor autophagy protein deregulation. Nevertheless, ROS production was normalized concomitant with the restoration of CYP4A and CYP2C expression. Similarly, quelled EET levels in diabetic animals were normalized upon AUDA administration, yet 20-HETE levels remained significantly higher than non-diabetic controls, and slightly reduced relative to the diabetic animals despite the reduction in CYP4A expression. These findings reflect a limited molecular effect by AUDA. In actuality, little studies provide a molecular digest to its mode of action. In fact, the majority of published findings pinpoint the remedial power of sEHI. We therefore reiterate the role of AUDA to be constricted to symptomatic therapy. Likewise, a number of studies highlight the palliative ability of sEH in neuropathic pain as well as DPN (Inceoglu et al., 2012; Wagner et al., 2014; 2017).

In another group of animals, we assessed the effect of doubled doses of AUDA or HET0016. Similar findings were observed whereby CYP4A and CYP2C in diabetic animals were elevated, and successfully quelled upon treatment with either inhibitor. Unlike MPZ levels which were normalized with LC3 protein expression. These results reflect a probable benefit of higher doses of AUDA, however, our findings remain inconclusive as neither behavioral assessment was performed nor additional molecular evidence was obtained. However, contemplating the overall returns from these trials, a number of queries arise. Is EET stabilization in DPN enough to achieve neuroprotection? Our response would be a negative. EETs are short lived molecules and are produced under a homeostatic control. If we are to speculate further, inhibiting their degradation may only prolong their effect a tad longer under diabetic conditions. To be able to fully acquire the benefits associated with EETs, we anticipate that sEH inhibition together with a CYP2C activator combination may suffice. As it happens, there have been studies that venture out to examine sEHI with COX inhibitors, or 20-HETE synthase inhibitors (Zhang et al., 2014[a,b]). Our efforts focusing on each arm alone lead us to the realization that there is a distinctive, physiological

feature to the CYP pathway, specifically 20-HETE and EET interplay. This special phenomenon revolves around the counterbalance between proinflammatory 20-HETE and anti-inflammatory EETs (Luria et al., 2007). Detailed research reports that diabetes tends to tip the balance towards inflammatory pathways (Williams et al., 2010) and that the manifestation of a state of inflammation in DPN is concurrent with oxidative stress (Feldman et al., 2017). It is evident from our findings that there is a constant opposition between 20-HETE and EET in the sciatic nerves of diabetic mice. This is indicative of a 'power struggle' among the two pathways which comes into perspective while administering sEHI AUDA. AUDA fails to normalize 20-HETE levels, by contrast to HET0016 which significantly increased EET levels, albeit not to control levels. In this manner, our findings extend Williams et al 'tipped balance' towards inflammatory pathways and reiterate the need for additional ventures. In light of this, a promising future perspective is to approach DPN from this standpoint and investigate whether the inhibition of EET degradation and 20-HETE synthesis may serve as a dual, double-edged strategy in ameliorating diabetes-mediated neural damage. On a final note, efforts that attempted to inhibit ROS production indicated that high-glucose induced SC injury was ameliorated (Yang et al., 2016) in addition to the partial restoration of the perineurium morphology, nerve conduction velocity, and pain and thermal perception (Greene et al., 1999; Cotter, M. A., & Cameron, N. E. 2003; Zhao et al., 2014; Li et al., 2016). Given our findings, we next move the investigation to the cellular level.

Schwann cells and CYPs

The myelinating cells of the PNS, or Schwann cells (SCs), in particular are implicated in DPN pathogenesis (Askwith et al., 2009; Cinci et al., 2015). Studies have shown that SCs undergo high-glucose induced loss of axonal association (Dey et al., 2013), reduced regenerative potential (Gumy et al., 2008), apoptosis (Wu et al., 2012) in addition to de-myelination and de-differentiation (Hao et al., 2015). We report for the first time CYP4A and CYP2C expression in SC cultures. We show that CYP4A and CYP2C expression fluctuates in microsomal extractions from hyperglycemic SCs. The most significant upregulation of CYP4A and CYP2C was at 6 and 48 hours after exposure to HG. Concomitantly, their upregulation was associated with alterations in MPZ and PMP22 protein levels, marking SC injury. In fact, SC injury has been described to be associated with myelin dysfunction and alterations in myelin protein levels central to the proper physiology and survival of SCs (Niemann et al., 2000; Niemann, A., Berger, P., & Suter, U. 2006). Myelin proteins are crucial for maintaining myelin sheath functionality; therefore, any variation in

their expression may lead to SC dysfunction (D'Urso, D., Ehrhardt, P., & Müller, H. W. 1999). Myelin proteins upregulation *in vitro* are in opposition to our *in vivo* findings. To speculate, although the data show an upregulation in myelin protein expression at the cellular level, these proteins may be non-functional and aggregated as some studies reported (Fortun et al., 2007). Indeed, we demonstrated the aggregation of PMP22 in our animal model to further verify. However, we highlight that myelin injury is vulnerable to any alteration as extensive research has described the importance of myelin protein interaction and strict control to be able to maintain myelin sheath integrity.

Elevated CYP4A protein expression led us to hypothesize that 20-HETE levels were increased. Consequently, we tested the effect of HG and exogenous 20-HETE treatments *in vitro*. Our findings reflect that, like HG-treated SCs, 20-HETE treatment triggered an increase in CYP4A, MPZ and PMP22 levels at 6- and 48-hour exposure. These data reflect that 20-HETE mimics the effect of HG on SCs. We further validate this by assessing SC apoptotic death. The results reveal HG-induced SC death at 48 hours of exposure. Similarly, 20-HETE induced SC apoptosis at 48 hours, also demonstrating that CYP4A upregulation and 20-HETE production is deleterious. This is verified further in which SCs exposure to HG elevated autophagy protein Beclin-1 after 6 hours, which may be indicative of an early development of stress and injury, followed by the marked reduction at 48 hours, which may exemplify the failure of cellular defenses. Understanding autophagy in SCs is challenging as Beclin-1 is associated with other binding proteins which we did not assess. Nevertheless, it is evident from the *in vivo* and *in vitro* data that there is a defected autophagic response underlying the DPN pathology. Autophagy is a balance between protein synthesis and degradation. However, a disruption in this process culminates in neurodegeneration, which is reported to induce upregulation of Beclin-1(Erlich et al., 2006). Collectively, our findings reveal increased Beclin-1 in animals and in SC cultures throughout this work. In neuronal cells, increased Beclin-1 marks early stages post-injury with cells exhibiting DNA damage. Our findings indeed show the initiation of apoptotic death and elevated fragmented DNA in hyperglycemic or 20-HETE treated SCs at 48 hours. Altogether, we put forth 20-HETE-mediated SC injury as its effects on myelin proteins, autophagy initiation and apoptotic death, mimic the effects of hyperglycemia on SCs.

As for CYP2C expression, 20-HETE treated SCs show a striking downregulation in CYP2C at both timepoints, although this reduction was not statistically significant. This may in

part exemplify the attenuated EET levels observed *in vivo*. To further investigate, hyperglycemic SCs were supplemented with exogenous EET. CYP4A and CYP2C expression was elevated in hyperglycemic SCs, corroborating our previous findings. But to our surprise, CYP4A expression was significantly decreased upon treatment with EET at both 6 and 48 hours. By contrast, CYP2C expression remained elevated at 6 hours, whereas at 48 hours a non-significant reduction was observed, like our *in vivo* findings. In view of these results, we speculate that other factors related to EET degradation may play a role, such as sEH enzyme overactivity, at 48 hours. However, with regards to myelin protein profiles, hyperglycemic SCs incubated with EET show a significant restoration of protein expression. These findings are not in agreement with the molecular data from the *in vivo* setup with AUDA. These results may be indicative of the importance of a dual approach *in vivo*. One possible explanation may be that the levels of EET in culture are direct on SCs and supraphysiological in culture. Thus, in an animal model and an *in vivo* milieu, an sEHI may be more effective in collaboration with an EET or CYP epoxygenase agonist. This strategy does not only stabilize EETs, but also boosts their synthesis, and therefore assessing such a technique in our diabetic model is among our future perspectives.

Finally, in order to correlate our targeted approach *in vivo* with our SC *in vitro* model, hyperglycemic SCs were incubated with HET0016 or AUDA and assessed for CYP expression. HET0016 or AUDA were both capable of significantly normalizing HG-induced CYP4A overexpression at 6- and 48-hour timepoints. By contrast, CYP2C expression at 6 hours remained elevated upon HG and/or HET0016 supplementation, and slightly reduced upon AUDA supplementation. However, at 48 hours, CYP2C expression was significantly reduced upon exposure to HET0016, and slightly reduced upon AUDA exposure, in comparison to HG treatment. In all, CYP2C expression is seen rising along with CYP4A upregulation despite the EET levels failing. This may be indicative of a compensatory mechanism by CYP2C and perhaps other epoxygenases which we did not assess. More importantly, it may be indicative of an overactive sEH enzyme that is largely partaking in EET degradation, beyond the homeostatic efforts of CYPs. In fact, studies have shown that sEH enzyme overexpression and activity to play pathological roles (Minaz et al., 2018). In fact, dampening sEH via genetic ablation or pharmacological augmentation has proven to be effective in diabetic complications (Hu et al., 2017, Sun et al., 2018). Our future perspectives should address sEH activity and verify the effect of higher doses of AUDA along with different treatment durations and intervention.

Together, the data weigh on the notion that CYP4A may be more responsive to the modulatory effects of either 20-HETE, EET, HET0016 or AUDA relative to CYP2C. CYP2C and the status of EET and their short-lived nature is more complex compared to 20-HETE. There are alternative isoforms and regioisomers which remain poorly characterized and underexploited in the context of DPN. Additionally, sEH activity is not solely devoted to metabolizing EETs, as it also receives substrates from other pathways. Moreover, AUDA remains one of the first generation sEHI available. Its metabolic stability and effectiveness in this study is debatable as further investigations are necessary to better comprehend the effects of EETs in animals and cells. This issue may be addressed by utilizing a newer and more potent sEHI with upgraded physiochemical properties.

Last but not least, these data lend support to the hypothesis that hyperglycemia-induced injury commenced through a CYP-dependent pathway. The peripheral nerves are vulnerable to any homeostatic alterations that take place, unlike the central nervous system, which is protected by a blood barrier (Cameron, N. E., & Cotter, M. A. 1994; Morris, S. J., Shore, A. C., & Tooke, J. E. 1995; Stys, P. K. 2005). Extensive research has shown that DPN may be in part further exacerbated by peripheral vascular disease (Cameron, N. E., Eaton, S. E. M., Cotter, M. A., & Tesfaye, S. 2001) and that SCs may be the mediators (King et al., 1989). Furthermore, it has been reported that there is a diabetes-associated tendency for vasoconstriction in peripheral vasculature (Pieper, G. M.1998). In particular, *in vivo* studies in diabetic animals showed that a reduction in sciatic nerve endoneurial blood flow within a hypoxic milieu was evident in the early stages of diabetes (Tuck, R. R., Schmelzer, J. D., & Low, P. A. 1984; Cameron, N. E., Cotter, M. A., & Low, P. A. 1991). Similarly, sural nerve angiography in diabetic patients with chronic motor and sensory neuropathy reported an impaired blood flow and abnormal epineural vessel morphology (Tesfaye et al., 1993). This observation may be correlated with a dysfunctional neurovascular response by C-fibers which was evident in the diabetic foot and is concurrent with abnormal pain perception and ischemia (Ibrahim et al., 1999; Dinh, T., & Veves, A.2005). Accordingly, and given our findings, further studies would likely examine the role of 20-HETE and EET and its correlation with vascular injury in DPN.

Our clinical data verify the involvement of 20-HETE in the human pathology and associated comorbidities. The findings highlight the correlation of 20-HETE with diabetes, diabetes duration, HbA1c, and family history of diabetes that all resulted in a significant elevation of urinary 20-

HETE levels, despite obesity status (BMI levels) of the patients. Diabetes-induced nephropathy, retinopathy and neuropathy were positively correlated with increased 20-HETE levels, which may underscore the role of CYPs in microvascular complications associated with human T2D. Interestingly, the increase in urinary 20-HETE levels with diabetes duration may suggest its implication in an underlying mechanism for the development of diabetes-induced complications. However, a more comprehensive clinical assessment is necessary to further verify the role of CYPs in diabetes. Although these results highlight the utmost employment of urinary 20-HETE as both a diagnostic and prognostic biomarker for diabetes and its induced complications, further investigations are necessary. Additionally, pertinent to DPN and the clinical value of CYPs, diabetes-induced injury in peripheral nerves needs to be assessed further using intraepidermal nerve fiber density. We also need to correlate findings to biochemical analyses of myelin proteins and CYPs expression in skin biopsies from diabetic patients and investigate EETs as potential biomarkers of DPN in addition to therapeutic agents for future clinical studies.

This study opens future perspectives that include rigorous additional *in vitro* and *in vivo* testing as well as assessments of the clinical relevance of the obtained data in human subjects. 20-HETE and EET levels may be analyzed as potential biomarkers of DPN in correlation with clinical parameters of T2D patients. Firstly, the effect of HET0016 or AUDA on MSC80 cell physiology and apoptosis is a critical step to progress further in this research. A measurement of 20-HETE and EET synthesis in SCs is pivotal to verify diabetes-mediated 20-HETE-induced injury. An examination of cellular death, and autophagy using additional techniques such as flow cytometry and caspase cleavage is of major importance. It would also be of interest to investigate the short term and long-term effect of HET0016, AUDA or their combination *in vivo* to contemplate its therapeutic potential. Additionally, utilizing silencing or overactivation techniques in hyperglycemic MSC80 cells or knockout animal models is of interest to verify the outcomes further. Understanding investigation worth pursuing to fully grasp how hyperglycemia works through CYP4A. This study targets one contributor to the meshwork of pathways involved in myelination and SC physiology/pathophysiology. At the time being, dissecting the mTOR pathway and its precise roles is essential, especially in the PNS. In addition to that, the implication of mTORC2 and its target AKT in myelination is suggestive of a complex crosstalk between these pathways. More importantly, to be able to view the mTOR pathway in a more coherent manner with respect to myelination, autophagy and the role of oxidative stress and apoptotic cell death,

the need for utilizing co-cultures to replicate or mimic *in vivo* systems is emphasized. Nevertheless, mTOR is identified as a dominant and central target that integrates with homeostatic pathways and is impaired in DM, especially the PI3K/AKT/mTOR network. The impairment of this axis in response to oxidative stress is deleterious, facilitates the pathogenesis of DPN, and may be of congruent physiological value in both T1D and T2D. Being able to identify the cellular mechanistic profiles at certain stages throughout the progression of the pathology allows for the targeted manipulation of the impaired pathway and more importantly, therapeutic intervention. We believe there is a 'pathological signature' that fluctuates with disease progression and identifying that may be a more strategic approach to DPN. This paves the way for exploring the manipulation of the pathways with more than one pharmacological target, and hence, adjuvant therapy in DPN.

V-Conclusion

Studies on CYP450 enzymes in DPN have not been conducted previously. To our knowledge, this study is the first to examine CYP expression in SCs as well as T2D mice within the context of DPN. Collectively, our findings suggest that hyperglycemia triggers oxidative injury of the PNS through CYP4A/20-HETE and CYP2C/EET mediated alteration. Defected AMPK and AKT signaling exacerbate oxidative injury and culminate in disrupted autophagic defenses and induction of apoptosis. The progression of injury was shown to occur through compromised myelin protein integrity and peripheral nerve functionality. Inhibiting 20-HETE synthesis through HET0016 demonstrated neuroprotection and may be a novel, multi-targeted approach of clinical significance for the management of DPN. Likewise, sEH inhibition via AUDA demonstrated augmentation of DPN-associated behaviors (**Fig. 31**). We pinpoint for the first time a CYP-dependent mechanism of oxidative injury in the pathogenesis of DPN in T2DM. More importantly, we place CYP4A/20-HETE upstream in the pathological axis that contributes to peripheral nerve injury through AMPK inactivation, oxidative stress and autophagic dysregulation.

V-Conclusion (French)

Aucune étude sur les enzymes CYP450 dans la DPN n'a été menée auparavant. À notre connaissance, cette étude est la première à examiner l'expression de CYP dans SCs ainsi que des souris DT2 dans le contexte de DPN. Collectivement, nos résultats suggèrent que l'hyperglycémie déclenche une lésion oxydative du SNP par altération médiée par CYP4A / 20-HETE et CYP2C / EET. Les signaux AMPK et AKT défectueux exacerbent les lésions oxydatives et aboutissent à des défenses autophagiques perturbées et à l'induction de l'apoptose. Il a été démontré que la progression de la blessure se produisait en raison de l'intégrité de la protéine de myéline et de la fonctionnalité nerveuse périphérique. L'inhibition de la synthèse du 20-HETE par HET0016 a démontré une neuroprotection et peut être une nouvelle approche multi-ciblée d'importance clinique pour la gestion de la DPN. De même, l'inhibition de sEH via AUDA a démontré une augmentation des comportements associés au DPN **(Fig. 29)**. Nous identifions pour la première fois un mécanisme de lésion oxydative dépendant du CYP dans la pathogenèse du DPN dans le T2DM. Plus important encore, nous plaçons CYP4A / 20-HETE en amont dans l'axe pathologique

qui contribue à la lésion nerveuse périphérique par l'inactivation de l'AMPK, le stress oxydatif et la dérégulation autophagique.

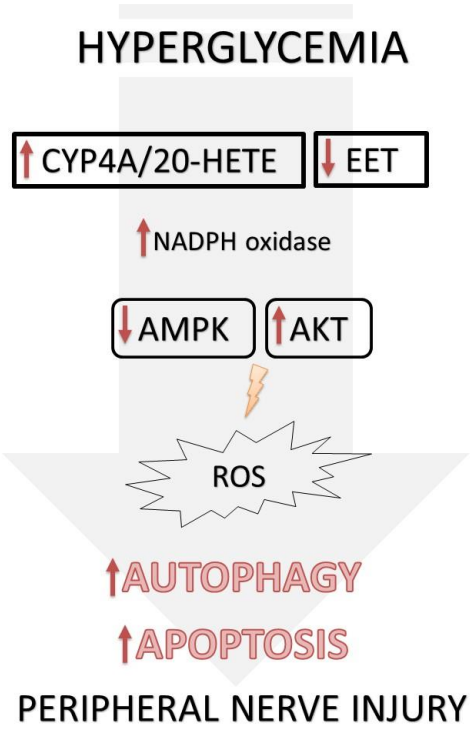


Figure 31. Summary of CYP-mediated pathogenesis of DPN.

VI-Summary of Graduate Work

Diabetes is currently a leading health concern across the world. It is a multiorgan malady and it has life-threatening complications that lead the charts in the non-communicable diseases category. If there is any health-related question asked to patients seeking medical help, it would be "Do you or any family member have diabetes?" The reason being is that diabetes is a silent killer. It complicates any medical emergency, treatment, or other diseases. To date, no cure has been formulated, and the ailment in itself is not well understood. Diabetic patients run the constant risk of developing complications from the range of disorders associated with diabetes onset, even if their indices are controlled. One of these complications is Diabetic Peripheral Neuropathy. Stemming from familial experience, my entire graduate work was devoted to understanding diabetes and DPN and placing a mark among the diabetologists in their race to the remedy. The pharmaceutical search for DPN has piqued my interest throughout the years and has made me look forward to understanding mechanisms that govern pathological induction of nerve dysfunction and identify targets that may be translated into clinical practice.

In the previous decade, a rising body of research identified the overproduction of reactive oxygen species and the induction of a state of oxidative stress to be the unifying orchestrator of diabetic complications pathologies. This is at the center of our lab's focus in addition to deciphering cellular signaling disruption in diabetes. The aim of my research is to study the contribution of the Cytochromes P450 (CYPs) enzymes to the pathophysiology of DPN as major cellular sources of ROS. We focus on the moonoxygenase class of CYPs which catalyze the conversion of arachidonic acid into bioactive metabolites via the hydroxylase (CYP4A) and epoxygenases (CYP2C) subfamilies. The conversion respectively yields eicosanoids 20-HETE and EET which are of significant physiological importance. The expression and activity of CYP4A and CYP2C has not been investigated in the PNS or in DPN. The first stage of this project was initiated during my early graduate studies which aimed to confirm and screen for CYP expression in the PNS. This work was the first to report CYP expression in sciatic nerves and Schwann cells. We selected the novel MKR non-obese T2D animal model characterized by insulin resistance, in which no previous reports assessed DPN phenotype in these animals. We were the first to highlight the involvement of CYP-mediated peripheral nerve injury while characterizing DPN in these

animals. Alongside that, CYP expression and injury was also assessed in T1D rats, and STZ-induced FVB T1D mice (incomplete work).

The work fully ventured out to hold proof throughout various stages of assessment and investigate the effect of CYP alterations in response to hyperglycemia in cultured Schwann cells by assessing myelin proteins, SC apoptosis and autophagic cellular defenses as well as mechanistic alterations in pathways essential for the myelination process and autophagic responses. These experiments were further validated *in vivo*. We used inhibitors of 20-HETE synthesis (HET0016) and soluble epoxide hydrolase enzyme (AUDA), and Metformin, to study their effect on peripheral nerve function. Animals were subjected to behavioral and electrophysiological testing to assess sensorimotor modalities and pain perception. We then correlated the findings at a molecular level. 20-HETE and EET levels, ROS production, mechanistic alterations, in addition to myelin and autophagy protein levels were examined.

- Activation of 20-HETE Synthase Triggers Oxidative Injury and Peripheral Nerve Damage in Non-Obese Type 2 Diabetic Mice - Haddad et al., 2021, (Submitted).
- Soluble Epoxide Hydrolase Inhibition in Diabetic Neuropathy: Insights into CYP epoxygenases in Peripheral Nerve Injury. Haddad et al., 2021, (In preparation).
- 20-HETE and EET: Two Overlooked Arms of the Cytochrome P450 family in the Nervous System. -Haddad et al., 2021 (In preparation).
- Affiliations of mTOR complexes in Diabetic Neuropathy and Retinopathy. Haddad et al., (In preparation).

In addition to CYPs, I was fortunate enough to work alongside my colleagues on the NADPH oxidases family of enzymes which are also major sources of ROS, and also work collaboratively with CYPs. Briefly, the work assessed the injurious role of NOXs and inhibition of Liver X Receptor signaling in nerve and Schwann cell injury from diabetic mice.

- Alteration in the Molecular Crosstalk Between NADPH oxidase-4 and Liver X Receptor Mediates Schwann Cells Injury in Diabetic Neuropathy - Eid et al., 2020.

Throughout the time allocated for the completion of my PhD, it was of interest to be able to explore DPN from a different perspective. We utilized a different mouse model (C57/B6) of T2D exhibiting obesity with severe phenotypes of diabetes (hyperglycemia and altered lipid profiles). The work assessed the effect of CYPs, HET0016 and AUDA, on DPN severity, as a more rigorous

approach to the metabolic disorder. With this added complexity of dyslipidemia, a new strategy was investigated that involves caloric restriction.

- Influence of Intermittent Fasting on Prediabetes-Induced Neuropathy: Insights on a Novel Mechanistic Pathway - Dannawi et al., 2021 (Submitted)

CYPs have largely been described to play a prominent role in modulating cerebrovascular tone and have important roles during brain development. Recent studies pinpoint insulin resistance at the heart of brain cognitive deficits and dementia owing to the breakdown of the blood brain barrier and oxidative injury in diabetic subjects. Strong evidence also points to the central affiliations of DPN. Emerging literature is consistently correlating the peripheral input to peripheral perception in the CNS. However, there is a growing body of data that suggest central alterations concomitant with peripheral injury. Thus, DPN studies may be further advanced to better understand the pathology by considering it as both, a peripheral and central disease. From this point of view, one of my major aims and future perspectives is to better comprehend the impact of CYP proteins on insulin resistance in the central nervous system.

- The Role of Insulin in Type 3 Diabetes. Ghadieh et al., 2021 (In preparation).

Last but not least, apart from DPN, I was fortunate enough to be a part of an effort to understand the molecular mechanisms underlying colorectal cancer onset in diabetes. This work combined the role of AMPK, mTOR and NOXs in the aggressiveness of the pathology in diabetic animals.

- AMPK, mTOR and NADPH-oxidase 4 Signaling Axis: at the Nexus of Diabetes and Colorectal Cancer - Mohsen et al., 2019.

VII-References

Abdu, E., Bruun, D. A., Yang, D., Yang, J., Inceoglu, B., Hammock, B. D., ... & Lein, P. J. (2011). Epoxyeicosatrienoic acids enhance axonal growth in primary sensory and cortical neuronal cell cultures. Journal of neurochemistry, 117(4), 632-642.

Adler, A. I., Stevens, R. J., Manley, S. E., Bilous, R. W., Cull, C. A., Holman, R. R., & Group, U. (2003). Development and progression of nephropathy in type 2 diabetes: the United Kingdom Prospective Diabetes Study (UKPDS 64). Kidney international, 63(1), 225-232.

Adlkofer, K., & Lai, C. (2000). Role of neuregulins in glial cell development. Glia, 29(2), 104-111.

Adlkofer, K., Martini, R., Aguzzi, A., Zielasek, J., Toyka, K. V., & Suter, U. (1995). Hypermyelination and demyelinating peripheral neuropathy in Pmp22-deficient mice. Nature genetics, 11(3), 274-280.

Ahmadieh, H., Itani, H., Itani, S., Sidani, K., Kassem, M., Farhat, K., ... & Itani, A. (2018). Diabetes and depression in Lebanon and association with glycemic control: a cross-sectional study. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy, 11, 717.

Alexanian, A., Miller, B., Roman, R. J., & Sorokin, A. (2012). 20-HETE-producing enzymes are up-regulated in human cancers. Cancer Genomics-Proteomics, 9(4), 163-169.

Alexanian, A., Rufanova, V. A., Miller, B., Flasch, A., Roman, R. J., & Sorokin, A. (2009). Down-regulation of 20-HETE synthesis and signaling inhibits renal adenocarcinoma cell proliferation and tumor growth. Anticancer research, 29(10), 3819-3824.

Ali, A., Iqbal, F., Taj, A., Iqbal, Z., Amin, M. J., & Iqbal, Q. Z. (2013). Prevalence of Microvascular Complications in Newly Diagnosed Patients with Type 2 Diabetes .Pakistan Journal of Medical Sciences, 29(4), 899–902.

Alonso-Galicia, M., Maier, K. G., Greene, A. S., Cowley, A. W., & Roman, R. J. (2002). Role of 20-hydroxyeicosatetraenoic acid in the renal and vasoconstrictor actions of angiotensin II. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 283(1), R60-R68.

Alsaad, A. M., Zordoky, B. N., Tse, M. M., & El-Kadi, A. O. (2013). Role of cytochrome P450-mediated arachidonic acid metabolites in the pathogenesis of cardiac hypertrophy. Drug metabolism reviews, 45(2), 173-195.

American Diabetes Association. (2015). Standards of medical care in diabetes—2015: summary of revisions. Diabetes care, 38(Supplement 1), S4-S4.

American Diabetes Association. (2018). 2. Classification and diagnosis of diabetes: standards of medical care in diabetes—2018. Diabetes care, 41(Supplement 1), S13-S27.

Andersson, U., Filipsson, K., Abbott, C. R., Woods, A., Smith, K., Bloom, S. R., . . . Small, C. J. (2004). AMP-activated protein kinase plays a role in the control of food intake. Journal of Biological Chemistry, 279(13), 12005-12008.

Andreassen, C. S., Jakobsen, J., Ringgaard, S., Ejskjaer, N., & Andersen, H. (2009). Accelerated atrophy of lower leg and foot muscles—a follow-up study of long-term diabetic polyneuropathy using magnetic resonance imaging (MRI). Diabetologia, 52(6), 1182-1191.

Anitha, M., Gondha, C., Sutliff, R., Parsadanian, A., Mwangi, S., Sitaraman, S. V., & Srinivasan, S. (2006). GDNF rescues hyperglycemia-induced diabetic enteric neuropathy through activation of the PI3K/Akt pathway. The Journal of clinical investigation, 116(2), 344-356.

Anwar-Mohamed, A., Elshenawy, O. H., El-Sherbeni, A. A., Abdelrady, M., & El-Kadi, A. O. (2014). Acute arsenic treatment alters arachidonic acid and its associated metabolite levels in the brain of C57BL/6 mice. Canadian journal of physiology and pharmacology, 92(8), 693-702.

Argiriadi, M. A., Morisseau, C., Goodrow, M. H., Dowdy, D. L., Hammock, B. D., & Christianson, D. W. (2000). Binding of alkylurea inhibitors to epoxide hydrolase implicates active site tyrosines in substrate activation. Journal of Biological Chemistry, 275(20), 15265-15270.

Argiriadi, M. A., Morisseau, C., Hammock, B. D., & Christianson, D. W. (1999). Detoxification of environmental mutagens and carcinogens: structure, mechanism, and evolution of liver epoxide hydrolase. Proceedings of the national academy of sciences, 96(19), 10637-10642.

Askwith, T., Zeng, W., Eggo, M. C., & Stevens, M. J. (2009). Oxidative stress and dysregulation of the taurine transporter in high-glucose-exposed human Schwann cells: implications for pathogenesis of diabetic neuropathy.American Journal of Physiology-Endocrinology and Metabolism, 297(3), E620-E628.

Asterholm, I. W., Kim-Muller, J. Y., Rutkowski, J. M., Crewe, C., Tao, C., & Scherer, P. E. (2016). Pathological Type-2 Immune Response, Enhanced Tumor Growth, and Glucose Intolerance in Retnlβ (RELMβ) Null Mice: A Model of Intestinal Immune System Dysfunction in Disease Susceptibility. The American Journal of Pathology, 186(9), 2404-2416

Atef, M. M., El-Sayed, N. M., Ahmed, A. A., & Mostafa, Y. M. (2019). Donepezil improves neuropathy through activation of AMPK signalling pathway in streptozotocin-induced diabetic mice. Biochemical pharmacology, 159, 1-10.

Bao, Y., Wang, X., Li, W., Huo, D., Shen, X., Han, Y., ... & Sun, C. (2011). 20-HETE Induces Apoptosis in Neonatal Rat Cardiomyocytes through Mitochondrial-dependent Pathways. Journal of cardiovascular pharmacology, 57(3), 294.

Barber, A. J., Antonetti, D. A., Kern, T. S., Reiter, C. E., Soans, R. S., Krady, J. K., . . . Bronson, S. K. (2005). The Ins2Akita mouse as a model of early retinal complications in diabetes. Invest Ophthalmol Vis Sci, 46(6), 2210-2218. doi:10.1167/iovs.04-1340

Becker, M., Benromano, T., Shahar, A., Nevo, Z., & Pick, C. G. (2014). Changes in the basal membrane of dorsal root ganglia schwann cells explain the biphasic pattern of the peripheral neuropathy in streptozotocin-induced diabetic rats. Journal of Molecular Neuroscience, 54(4), 704-713.

Beirowski, B., Babetto, E., Golden, J. P., Chen, Y. J., Yang, K., Gross, R. W., ... & Milbrandt, J. (2014). Metabolic regulator LKB1 is crucial for Schwann cell-mediated axon maintenance. Nature neuroscience, 17(10), 1351-1361.

Beirowski, B., Wong, K. M., Babetto, E., & Milbrandt, J. (2017). mTORC1 promotes proliferation of immature Schwann cells and myelin growth of differentiated Schwann cells. Proceedings of the National Academy of Sciences, 201620761.

Bergamini, E. T. T. O. R. E., Cavallini, G. A. B. R. I. E. L. L. A., Donati, A., & Gori, Z. I. N. A. (2004). The role of macroautophagy in the ageing process, anti-ageing intervention and age-associated diseases. The international journal of biochemistry & cell biology, 36(12), 2392-2404.

Bethesda, M. (2003). US Renal Data System: USRDS 2003 Annual Data Report: Atlas of End-Stage Renal Disease in the United States. National Institute of Health, National Institute of Diabetes and Digestive and Kidney Diseases.

Biessels, G. J., & Reijmer, Y. D. (2014). Brain changes underlying cognitive dysfunction in diabetes: what can we learn from MRI?. Diabetes, 63(7), 2244-2252.

Biessels, G. J., Bril, V., Calcutt, N. A., Cameron, N. E., Cotter, M. A., Dobrowsky, R., ... & Mizisin, A. P. (2014). Phenotyping animal models of diabetic neuropathy: a consensus statement of the diabetic neuropathy study group of the EASD (Neurodiab). Journal of the Peripheral Nervous System, 19(2), 77-87.

Bilbao, J. M., & Schmidt, R. E. (2015). Schwann Cells and Myelin in the Peripheral Nervous System. In Biopsy Diagnosis of Peripheral Neuropathy (pp. 85-109). Springer International Publishing.

Blázquez, E., Velázquez, E., Hurtado-Carneiro, V., & Ruiz-Albusac, J. M. (2014). Insulin in the brain: its pathophysiological implications for States related with central insulin resistance, type 2 diabetes and Alzheimer's disease. Frontiers in endocrinology, 5, 161.

Bondy, S. C., & Naderi, S. (1994). Contribution of hepatic cytochrome P450 systems to the generation of reactive oxygen species. Biochemical pharmacology, 48(1), 155-159.

Borin, T. F., Zuccari, D. A., Jardim-Perassi, B. V., Ferreira, L. C., Iskander, A. S. M., Varma, N. R. S., ... & Arbab, A. S. (2014). HET0016, a selective inhibitor of 20-HETE synthesis, decreases pro-angiogenic factors and inhibits growth of triple negative breast cancer in mice. PLoS One, 9(12).

Bradbury, M. W. B., & Crowder, J. (1976). Compartments and barriers in the sciatic nerve of the rabbit. Brain research, 103(3), 515-526.

Brito, P. L., Fioretto, P., Drummond, K., Kim, Y., Steffes, M. W., Basgen, J. M., . . . Mauer, M. (1998). Proximal tubular basement membrane width in insulin-dependent diabetes mellitus. Kidney international, 53(3), 754-761.

Brooks, S. P., & Dunnett, S. B. (2009). Tests to assess motor phenotype in mice: a user's guide. Nature Reviews Neuroscience, 10(7), 519-529.

Brownlee, M. (2005). The pathobiology of diabetic complications: a unifying mechanism. diabetes, 54(6), 1615-1625.

Brussee, V., Cunningham, F. A., & Zochodne, D. W. (2004). Direct insulin signaling of neurons reverses diabetic neuropathy. Diabetes, 53(7), 1824-1830.

Bursell, S. E., Clermont, A. C., Aiello, L. P., Aiello, L. M., Schlossman, D. K., Feener, E. P., ... & King, G. L. (1999). High-dose vitamin E supplementation normalizes retinal blood flow and creatinine clearance in patients with type 1 diabetes. Diabetes care, 22(8), 1245-1251.

Callaghan, B. C., Cheng, H. T., Stables, C. L., Smith, A. L., & Feldman, E. L. (2012). Diabetic neuropathy: clinical manifestations and current treatments. The Lancet Neurology, 11(6), 521-534.

Callaghan, B. C., Hur, J., & Feldman, E. L. (2012). Diabetic neuropathy: one disease or two?. Current opinion in neurology, 25(5), 536.

Cambj-Sapunar, L., Yu, M., Harder, D. R., & Roman, R. J. (2003). Contribution of 5-hydroxytryptamine1B receptors and 20-hydroxyeiscosatetraenoic acid to fall in cerebral blood flow after subarachnoid hemorrhage. Stroke, 34(5), 1269-1275.

Cameron, N. E., & Cotter, M. A. (1994). The relationship of vascular changes to metabolic factors in diabetes mellitus and their role in the development of peripheral nerve complications. Diabetes/metabolism reviews, 10(3), 189-224.

Cameron, N. E., & Cotter, M. A. (1997). Metabolic and vascular factors in the pathogenesis of diabetic neuropathy. Diabetes, 46(Supplement 2), S31-S37.

Cameron, N. E., & Cotter, M. A. (1999). Effects of antioxidants on nerve and vascular dysfunction in experimental diabetes. Diabetes research and clinical practice, 45(2), 137-146.

Cameron, N. E., Cotter, M. A., & Low, P. A. (1991). Nerve blood flow in early experimental diabetes in rats: relation to conduction deficits. American Journal of Physiology-Endocrinology and Metabolism, 261(1), E1-E8.

Cameron, N. E., Eaton, S. E. M., Cotter, M. A., & Tesfaye, S. (2001). Vascular factors and metabolic interactions in the pathogenesis of diabetic neuropathy. Diabetologia, 44(11), 1973-1988.

Campbell, W. B., & Fleming, I. (2010). Epoxyeicosatrienoic acids and endothelium-dependent responses. Pflügers Archiv-European Journal of Physiology, 459(6), 881-895.

Campbell, W. B., Gebremedhin, D., Pratt, P. F., & Harder, D. R. (1996). Identification of epoxyeicosatrienoic acids as endothelium-derived hyperpolarizing factors. Circulation research, 78(3), 415-423.

Capdevila, J. H., & Falck, J. R. (2018). The arachidonic acid monooxygenase: from biochemical curiosity to physiological/pathophysiological significance. Journal of lipid research, 59(11), 2047-2062.

Capdevila, J. H., Falck, J. R., & Harris, R. C. (2000). Cytochrome P450 and arachidonic acid bioactivation: molecular and functional properties of the arachidonate monooxygenase. Journal of lipid research, 41(2), 163-181.

Capdevila, J. H., Falck, J. R., & Imig, J. D. (2007). Roles of the cytochrome P450 arachidonic acid monooxygenases in the control of systemic blood pressure and experimental hypertension. Kidney international, 72(6), 683-689.

Capdevila, J. H., Wang, W., & Falck, J. R. (2015). Arachidonic acid monooxygenase: genetic and biochemical approaches to physiological/pathophysiological relevance. Prostaglandins & other lipid mediators, 120, 40-49.

Caramori, M. L., Fioretto, P., & Mauer, M. (2003). Low glomerular filtration rate in normoalbuminuric type 1 diabetic patients. Diabetes, 52(4), 1036-1040.

Cárdenas, S., Colombero, C., Panelo, L., Dakarapu, R., Falck, J. R., Costas, M. A., & Nowicki, S. (2020). GPR75 receptor mediates 20-HETE-signaling and metastatic features of androgen-insensitive prostate cancer cells. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 1865(2), 158573.

Carmeliet, P., & Jain, R. K. (2011). Principles and mechanisms of vessel normalization for cancer and other angiogenic diseases. Nature reviews Drug discovery, 10(6), 417-427.

Carstensen, B., Read, S. H., Friis, S., Sund, R., Keskimäki, I., Svensson, A. M., ... & Magliano,

Castelnovo, L. F., Bonalume, V., Melfi, S., Ballabio, M., Colleoni, D., & Magnaghi, V. (2017). Schwann cell development, maturation and regeneration: a focus on classic and emerging intracellular signaling pathways. Neural regeneration research, 12(7), 1013.

Ceolotto, G., Gallo, A., Papparella, I., Franco, L., Murphy, E., Iori, E., ... & Avogaro, A. (2007). Rosiglitazone reduces glucose-induced oxidative stress mediated by NAD (P) H oxidase via AMPK-dependent mechanism. Arteriosclerosis, thrombosis, and vascular biology, 27(12), 2627-2633.

Ceriello, A. (2003). New insights on oxidative stress and diabetic complications may lead to a “causal” antioxidant therapy. Diabetes care,26(5), 1589-1596.

Cermenati, G., Abbiati, F., Cermenati, S., Brioschi, E., Volonterio, A., Cavaletti, G., ... & Mitro, N. (2012). Diabetes-induced myelin abnormalities are associated with an altered lipid pattern: protective effects of LXR activation. Journal of lipid research, 53(2), 300-310.

Chan, L., Terashima, T., Urabe, H., Lin, F., & Kojima, H. (2011). Pathogenesis of diabetic neuropathy: bad to the bone. Annals of the New York Academy of Sciences, 1240(1), 70-76.

Chen, J. K., Capdevila, J., & Harris, R. C. (2002). Heparin-binding EGF-like growth factor mediates the biological effects of P450 arachidonate epoxygenase metabolites in epithelial cells. Proceedings of the National Academy of Sciences, 99(9), 6029-6034.

Chen, L., Fan, C., Zhang, Y., Bakri, M., Dong, H., Morisseau, C., ... & Wang, M. H. (2013). Beneficial effects of inhibition of soluble epoxide hydrolase on glucose homeostasis and islet damage in a streptozotocin-induced diabetic mouse model. Prostaglandins & other lipid mediators, 104, 42-48.

Chen, P., Guo, M., Wygle, D., Edwards, P. A., Falck, J. R., Roman, R. J., & Scicli, A. G. (2005). Inhibitors of cytochrome P450 4A suppress angiogenic responses. The American journal of pathology, 166(2), 615-624.

Chen, P., Piao, X., & Bonaldo, P. (2015). Role of macrophages in Wallerian degeneration and axonal regeneration after peripheral nerve injury. Acta neuropathologica, 130(5), 605-618.

Chen, Z. L., Yu, W. M., & Strickland, S. (2007). Peripheral regeneration. Annu. Rev. Neurosci., 30, 209-233.

Cheng, J., Ou, J. S., Singh, H., Falck, J. R., Narsimhaswamy, D., Pritchard, K. A., & Schwartzman, M. L. (2008). 20-hydroxyeicosatetraenoic acid causes endothelial dysfunction via eNOS uncoupling. American Journal of Physiology-Heart and Circulatory Physiology, 294(2), H1018-H1026.

Cheng, J., Wu, C. C., Gotlinger, K. H., Zhang, F., Falck, J. R., Narsimhaswamy, D., & Schwartzman, M. L. (2010). 20-Hydroxy-5, 8, 11, 14-eicosatetraenoic acid mediates endothelial dysfunction via IκB kinase-dependent endothelial nitric-oxide synthase uncoupling. Journal of Pharmacology and Experimental Therapeutics, 332(1), 57-65.

Chowdhury, S.R.; Dobrowsky, R.T.; Fernyhough, P. (2011). Nutrient excess and altered mitochondrial proteome and function contribute to neurodegeneration in diabetes. Mitochondrion , 11, 845–854.

Chung, Y. C., Lim, J. H., Oh, H. M., Kim, H. W., Kim, M. Y., Kim, E. N., ... & Park, C. W. (2018). Calcimimetic restores diabetic peripheral neuropathy by ameliorating apoptosis and improving autophagy. Cell death & disease, 9(12), 1163.

Cinci, L., Corti, F., Di Cesare Mannelli, L., Micheli, L., Zanardelli, M., & Ghelardini, C. (2015). Oxidative, metabolic, and apoptotic responses of Schwann cells to high glucose levels. Journal of biochemical and molecular toxicology, 29(6), 274-279.

Ciulla, T. A., Amador, A. G., & Zinman, B. (2003). Diabetic retinopathy and diabetic macular edema: pathophysiology, screening, and novel therapies. Diabetes Care, 26(9), 2653-2664.

Cleland, S. J., Fisher, B. M., Colhoun, H. M., Sattar, N., & Petrie, J. R. (2013). Insulin resistance in type 1 diabetes: what is 'double diabetes' and what are the risks?. Diabetologia, 56(7), 1462-1470.

Cooper, M. E. (1998). Pathogenesis, prevention, and treatment of diabetic nephropathy. The Lancet, 352(9123), 213-219.

Corton, J. C., Fan, L. Q., Brown, S., Anderson, S. P., Bocos, C., Cattley, R. C., ... & Gustafsson, J. Å. (1998). Down-regulation of cytochrome P450 2C family members and positive acute-phase response gene expression by peroxisome proliferator chemicals. Molecular pharmacology, 54(3), 463-473.

Cotter, L., Özçelik, M., Jacob, C., Pereira, J. A., Locher, V., Baumann, R., ... & Tricaud, N. (2010). Dlg1-PTEN interaction regulates myelin thickness to prevent damaging peripheral nerve overmyelination. Science, 328(5984), 1415-1418.

Cotter, M. A., & Cameron, N. E. (2003). Effect of the NAD (P) H oxidase inhibitor, apocynin, on peripheral nerve perfusion and function in diabetic rats. Life sciences, 73(14), 1813-1824.

Courteix, C., Eschalier, A., & Lavarenne, J. (1993). Streptozocin-induced diabetic rats: behavioural evidence for a model of chronic pain. Pain, 53(1), 81-88.

Cowart, L. A., Wei, S., Hsu, M. H., Johnson, E. F., Krishna, M. U., Falck, J. R., & Capdevila, J. H. (2002). The CYP4A isoforms hydroxylate epoxyeicosatrienoic acids to form high affinity peroxisome proliferator-activated receptor ligands. Journal of Biological Chemistry, 277(38), 35105-35112.

Currie, C. J., Poole, C. D., Evans, M., Peters, J. R., & Morgan, C. L. (2013). Mortality and other important diabetes-related outcomes with insulin vs other antihyperglycemic therapies in type 2 diabetes. The Journal of Clinical Endocrinology & Metabolism, 98(2), 668-677.

D'Urso, D., Ehrhardt, P., & Müller, H. W. (1999). Peripheral myelin protein 22 and protein zero: a novel association in peripheral nervous system myelin.The Journal of neuroscience, 19(9), 3396-3403.

Dahly-Vernon, A. J., Sharma, M., McCarthy, E. T., Savin, V. J., Ledbetter, S. R., & Roman, R. J. (2005). Transforming growth factor-β, 20-HETE interaction, and glomerular injury in Dahl saltsensitive rats. Hypertension,45(4), 643-648.

Dalmasso, C., Maranon, R., Patil, C., Moulana, M., Romero, D. G., & Reckelhoff, J. F. (2016). 20-HETE and CYP4A2 ω-hydroxylase contribute to the elevated blood pressure in hyperandrogenemic female rats. American Journal of Physiology-Renal Physiology, 311(1), F71-F77.

Davydov, D. R. (2001). Microsomal monooxygenase in apoptosis: another target for cytochrome c signaling?. Trends in biochemical sciences, 26(3), 155-160.

De Paula, M. L., Cui, Q. L., Hossain, S., Antel, J., & Almazan, G. (2014). The PTEN Inhibitor Bisperoxovanadium Enhances Myelination by Amplifying IGF-1 Signaling in Rat and Human Oligodendrocyte Progenitors. GLIA, 62, 64-77.

De Zeeuw, D., Akizawa, T., Audhya, P., Bakris, G. L., Chin, M., Christ-Schmidt, H., ... & McMurray, J. J. (2013). Bardoxolone methyl in type 2 diabetes and stage 4 chronic kidney disease. New England Journal of Medicine, 369(26), 2492-2503.

Declèves, A. E., Mathew, A. V., Armando, A. M., Han, X., Dennis, E. A., Quehenberger, O., & Sharma, K. (2019). AMP-activated protein kinase activation ameliorates eicosanoid dysregulation in high-fat-induced kidney disease in mice. Journal of lipid research, 60(5), 937-952.

Delaney, C. L., Russell, J. W., Cheng, H. L., & Feldman, E. L. (2001). Insulin-like growth factor-I and over-expression of Bcl-xL prevent glucose-mediated apoptosis in Schwann cells. Journal of Neuropathology & Experimental Neurology, 60(2), 147-160.

DeLozier, T. C., Kissling, G. E., Coulter, S. J., Dai, D., Foley, J. F., Bradbury, J. A., ... & Goldstein, J. A. (2007). Detection of human CYP2C8, CYP2C9, and CYP2J2 in cardiovascular tissues. Drug metabolism and disposition, 35(4), 682-688.

Depaz, I. M. B., Toselli, F., Wilce, P. A., & Gillam, E. M. (2015). Differential expression of cytochrome P450 enzymes from the CYP2C subfamily in the human brain. Drug Metabolism and Disposition, 43(3), 353-357.

Dewanjee, S., Das, S., Das, A. K., Bhattacharjee, N., Dihingia, A., Dua, T. K., ... & Manna, P. (2018). Molecular mechanism of diabetic neuropathy and its pharmacotherapeutic targets. European journal of pharmacology, 833, 472-523.

Dey, I., Midha, N., Singh, G., Forsyth, A., Walsh, S. K., Singh, B., Kumar, R., Toth, C. & Midha, R. (2013). Diabetic Schwann cells suffer from nerve growth factor and neurotrophin-3 underproduction and poor associability with axons. Glia, 61(12), 1990-1999.

Dhanasekaran, A., Bodiga, S., Gruenloh, S., Gao, Y., Dunn, L., Falck, J. R., Buonaccorsi, J.N., Medhora, M. & Jacobs, E. R. (2009). 20-HETE increases survival and decreases apoptosis in pulmonary arteries and pulmonary artery endothelial cells.American Journal of Physiology- Heart and Circulatory Physiology, 296(3), H777-H786.

Diabetes Control and Complications Trial Research Group. (1993). The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl j Med,1993(329), 977-986.

Ding, J., & Wong, T. Y. (2012). Current epidemiology of diabetic retinopathy and diabetic macular edema. Curr Diab Rep, 12(4), 346-354. doi:10.1007/s11892-012-0283-6

Ding, Y., Wu, C. C., Garcia, V., Dimitrova, I., Weidenhammer, A., Joseph, G., Ding, Y., Wu, C.C., Garcia, V., Dimitrova, I., Weidenhammer, A., Joseph, G., Zhang, F., Manthati, V.L., Falck, J.R., Capdevila, J.H. & Schwartzman, M. L. (2013). 20-HETE induces remodeling of renal resistance arteries independent of blood pressure elevation in hypertension.American Journal of Physiology-Renal Physiology, 305(5), F753-F763.

Dinh, T., & Veves, A. (2005). Microcirculation of the diabetic foot. Current pharmaceutical design, 11(18), 2301-2309.

Dirig, D. M., Salami, A., Rathbun, M. L., Ozaki, G. T., & Yaksh, T. L. (1997). Characterization of variables defining hindpaw withdrawal latency evoked by radiant thermal stimuli. Journal of neuroscience methods, 76(2), 183-191

Dolinsky, V. W., & Dyck, J. R. (2006). Role of AMP-activated protein kinase in healthy and diseased hearts. American Journal of Physiology-Heart and Circulatory Physiology, 291(6), H2557-H2569.

Domènech-Estévez, E., Baloui, H., Meng, X., Zhang, Y., Deinhardt, K., Dupree, J. L., Einheber, S., Chrast, R. & Salzer, J. L. (2016). Akt Regulates Axon Wrapping and Myelin Sheath Thickness in the PNS. The Journal of Neuroscience,36(16), 4506-4521.

Draye, J. P., & Vamecq, J. (1989). The glyconeogenicity of fatty acids in mammals. Trends in biochemical sciences, 14(12), 478-479.

Drolet, B., Pilote, S., Gélinas, C., Kamaliza, A. D., Blais-Boilard, A., Virgili, J., ... & Simard, C. (2017). Altered Protein Expression of Cardiac CYP2J and Hepatic CYP2C, CYP4A, and CYP4F in a Mouse Model of Type II Diabetes—A Link in the Onset and Development of Cardiovascular Disease?. Pharmaceutics, 9(4), 44.

Du, F., Wang, L., Qian, W., & Liu, S. (2009). Loss of enteric neurons accompanied by decreased expression of GDNF and PI3K/Akt pathway in diabetic rats. Neurogastroenterology & Motility, 21(11), 1229-e114.

Du, X. L., Edelstein, D., Rossetti, L., Fantus, I. G., Goldberg, H., Ziyadeh, F., ... & Brownlee, M. (2000). Hyperglycemia-induced mitochondrial superoxide overproduction activates the hexosamine pathway and induces plasminogen activator inhibitor-1 expression by increasing Sp1 glycosylation. Proceedings of the National Academy of Sciences, 97(22), 12222-12226.

Duncan Jr, L. E., Semans, J. H., & Howard, J. E. (1944). Adrenal medullary tumor (pheochromocytoma) and diabetes mellitus; disappearance of diabetes after removal of the tumor. Annals of Internal Medicine, 20(5), 815-821.

Dunn, K. M., Renic, M., Flasch, A. K., Harder, D. R., Falck, J., & Roman, R. J. (2008). Elevated production of 20-HETE in the cerebral vasculature contributes to severity of ischemic stroke and oxidative stress in spontaneously hypertensive rats. American Journal of Physiology-Heart and Circulatory Physiology, 295(6), H2455-H2465.

Ebato, C., Uchida, T., Arakawa, M., Komatsu, M., Ueno, T., Komiya, K., ... & Kawamori, R. (2008). Autophagy is important in islet homeostasis and compensatory increase of beta cell mass in response to high-fat diet. Cell metabolism, 8(4), 325-332.

Eid S, Abdul-Massih C, El-Khuri CM, Hamdy A, Rashid A and Eid AA. New Mechanistic Insights in the Development of Diabetic Nephropathy: Role of Cytochromes P450 and Their Metabolites. J Endocr Disord. 2014;1(2): 1006.

Eid, A. A., Ford, B. M., Bhandary, B., de Cassia Cavaglieri, R., Block, K., Barnes, J. L., ... & Abboud, H. E. (2013). Mammalian target of rapamycin regulates Nox4-mediated podocyte depletion in diabetic renal injury.Diabetes, 62(8), 2935-2947

aEid, S., Maalouf, R., Jaffa, A. A., Nassif, J., Hamdy, A., Rashid, A., Ziyadeh, F.N. & Eid, A. A. (2013). 20-HETE and EETs in diabetic nephropathy: a novel mechanistic pathway. PloS one, 8(8), e70029.

bEid, S., Abou-Kheir, W., Sabra, R., Daoud, G., Jaffa, A., Ziyadeh, F. N., Roman, L. & Eid, A. A. (2013). Involvement of renal cytochromes P450 and arachidonic acid metabolites in diabetic nephropathy. Journal of biological regulators and homeostatic agents, 27(3), 693-703.

Eid, A. A., Ford, B. M., Block, K., Kasinath, B. S., Gorin, Y., Ghosh-Choudhury, G., Barnes, J.L. & Abboud, H. E. (2010). AMP-activated protein kinase (AMPK) negatively regulates Nox4-dependent activation of p53 and epithelial cell apoptosis in diabetes. Journal of Biological Chemistry, 285(48), 37503-37512.

Eid, A. A., Gorin, Y., Fagg, B. M., Maalouf, R., Barnes, J. L., Block, K., & Abboud, H. E. (2009). Mechanisms of podocyte injury in diabetes role of cytochrome P450 and NADPH oxidases. Diabetes, 58(5), 1201-1211.

Eid, S. A., El Massry, M., Hichor, M., Haddad, M., Grenier, J., Dia, B., ... & Wiesel, P. (2020). Targeting the NADPH Oxidase-4 and Liver X Receptor Pathway Preserves Schwann Cell Integrity in Diabetic Mice. Diabetes, 69(3), 448-464.

Eid, S., Boutary, S., Braych, K., Sabra, R., Massaad, C., Hamdy, A., ... & Abboud, H. E. (2016). mTORC2 signaling regulates Nox4-induced podocyte depletion in diabetes. Antioxidants & redox signaling, 25(13), 703-719.

-induced podocyte depletion in diabetes. Antioxidants & redox signaling, 25(13), 703-719.

Emerling, B. M., Weinberg, F., Snyder, C., Burgess, Z., Mutlu, G. M., Viollet, B., ... & Chandel, N. S. (2009). Hypoxic activation of AMPK is dependent on mitochondrial ROS but independent of an increase in AMP/ATP ratio. Free Radical Biology and Medicine, 46(10), 1386-1391.

Erlich, S., Shohami, E., & Pinkas-Kramarski, R. (2006). Neurodegeneration induces upregulation of Beclin 1. Autophagy, 2(1), 49-51.

Faichney, J. D., & Tate, P. W. (2003). Metformin in type 1 diabetes: is this a good or bad idea?. Diabetes Care, 26(5), 1655-1655.

Falck, J. R., Manna, S., Moltz, J., Chacos, N., & Capdevila, J. (1983). Epoxyeicosatrienoic acids stimulate glucagon and insulin release from isolated rat pancreatic islets. Biochemical and biophysical research communications, 114(2), 743-749.

Fan, F., & Roman, R. J. (2017). Effect of cytochrome P450 metabolites of arachidonic acid in nephrology. Journal of the American Society of Nephrology, 28(10), 2845-2855.

Fan, F., & Roman, R. J. (2017). GPR75 identified as the first 20-HETE receptor: a chemokine receptor adopted by a new family.

Fan, F., Ge, Y., Lv, W., Elliott, M. R., Muroya, Y., Hirata, T., ... & Roman, R. J. (2016). Molecular mechanisms and cell signaling of 20-hydroxyeicosatetraenoic acid in vascular pathophysiology. Frontiers in bioscience (Landmark edition), 21, 1427.

Fan, F., Geurts, A. M., Murphy, S. R., Pabbidi, M. R., Jacob, H. J., & Roman, R. J. (2015). Impaired myogenic response and autoregulation of cerebral blood flow is rescued in CYP4A1 transgenic Dahl salt-sensitive rat.American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 308(5), R379-R390.

Fang, X., Hu, S., Xu, B., Snyder, G. D., Harmon, S., Yao, J., ... & Spector, A. A. (2006). 14, 15-Dihydroxyeicosatrienoic acid activates peroxisome proliferator-activated receptor-α. American Journal of Physiology-Heart and Circulatory Physiology, 290(1), H55-H63.

Feldman, E. L., Callaghan, B. C., Pop-Busui, R., Zochodne, D. W., Wright, D. E., Bennett, D. L., ... & Viswanathan, V. (2019). Diabetic neuropathy. Nature Reviews Disease Primers, 5(1), 1-18.

Feldman, E. L., Nave, K. A., Jensen, T. S., & Bennett, D. L. (2017). New horizons in diabetic neuropathy: mechanisms, bioenergetics, and pain. Neuron, 93(6), 1296-1313.

Feltri, M. L., Poitelon, Y., & Previtali, S. C. (2016). How Schwann cells sort axons: new concepts. The Neuroscientist, 22(3), 252-265.

Fernandes, D. C., Wosniak Jr, J., Pescatore, L. A., Bertoline, M. A., Liberman, M., Laurindo, F. R., & Santos, C. X. (2007). Analysis of DHE-derived oxidation products by HPLC in the assessment of superoxide production and NADPH oxidase activity in vascular systems. American Journal of Physiology-Cell Physiology, 292(1), C413-C422.

Fernández, A. M., Dupont, J., Farrar, R. P., Lee, S., Stannard, B., & Le Roith, D. (2002). Muscle-specific inactivation of the IGF-I receptor induces compensatory hyperplasia in skeletal muscle. The Journal of clinical investigation, 109(3), 347-355.

Fernández, A. M., Kim, J. K., Yakar, S., Dupont, J., Hernandez-Sanchez, C., Castle, A. L., ... & Le Roith, D. (2001). Functional inactivation of the IGF-I and insulin receptors in skeletal muscle causes type 2 diabetes. Genes & development, 15(15), 1926-1934.

Fernandez, C., Iyer, M., & Low, I. (2017). Gpr126 Is Critical for Schwann Cell Function during Peripheral Nerve Regeneration. Journal of Neuroscience, 37(12), 3106-3108.

Fernyhough, P. (2015). Mitochondrial dysfunction in diabetic neuropathy: a series of unfortunate metabolic events. Current diabetes reports, 15(11), 89.

Figlia, G., Norrmén, C., Pereira, J. A., Gerber, D., & Suter, U. (2017). Dual function of the PI3K-Akt-mTORC1 axis in myelination of the peripheral nervous system. Elife, 6.

Filla, L. A., & Edwards, J. L. (2016). Metabolomics in diabetic complications.Molecular BioSystems, 12(4), 1090-1105.

Filosa, J. A., & Iddings, J. A. (2013). Astrocyte regulation of cerebral vascular tone. American Journal of Physiology-Heart and Circulatory Physiology, 305(5), H609-H619.

Fisher, E. B., Thorpe, C. T., McEvoy DeVellis, B., & DeVellis, R. F. (2007). Healthy coping, negative emotions, and diabetes management. The Diabetes Educator, 33(6), 1080-1103.

Fisslthaler, B., Popp, R., Kiss, L., Potente, M., Harder, D. R., Fleming, I., & Busse, R. (1999). Cytochrome P450 2C is an EDHF synthase in coronary arteries. Nature, 401(6752), 493-497.

Fleming, I. (2008). Vascular cytochrome p450 enzymes: physiology and pathophysiology. Trends in cardiovascular medicine, 18(1), 20-25.

Fleming, I., Michaelis, U. R., Bredenkötter, D., Fisslthaler, B., Dehghani, F., Brandes, R. P., & Busse, R. (2001). Endothelium-derived hyperpolarizing factor synthase (Cytochrome P450 2C9) is a functionally significant source of reactive oxygen species in coronary arteries. Circulation Research, 88(1), 44-51.

Flores, A. I., Narayanan, S. P., Morse, E. N., Shick, H. E., Yin, X., Kidd, G., ... & Macklin, W. B. (2008). Constitutively active Akt induces enhanced myelination in the CNS. The Journal of Neuroscience, 28(28), 7174-7183.

Fordsmann, J. C., Ko, R. W., Choi, H. B., Thomsen, K., Witgen, B. M., Mathiesen, C., C., Lønstrup, M., Piilgaard, H., MacVicar, B.A. & Lauritzen, M. (2013). Increased 20-HETE synthesis explains reduced cerebral blood flow but not impaired neurovascular coupling after cortical spreading depression in rat cerebral cortex. The Journal of Neuroscience, 33(6), 2562-2570.

Fornage, M., Lee, C. R., Doris, P. A., Bray, M. S., Heiss, G., Zeldin, D. C., & Boerwinkle, E. (2005). The soluble epoxide hydrolase gene harbors sequence variation associated with susceptibility to and protection from incident ischemic stroke. Human molecular genetics, 14(19), 2829-2837.

Fortun, J., Go, J. C., Li, J., Amici, S. A., Dunn Jr, W. A., & Notterpek, L. (2006). Alterations in degradative pathways and protein aggregation in a neuropathy model based on PMP22 overexpression. Neurobiology of disease, 22(1), 153-164.

Fortun, J., Verrier, J. D., Go, J. C., Madorsky, I., Dunn, W. A., & Notterpek, L. (2007). The formation of peripheral myelin protein 22 aggregates is hindered by the enhancement of autophagy and expression of cytoplasmic chaperones. Neurobiology of disease, 25(2), 252-265.

Freeman, O. J., Unwin, R. D., Dowsey, A. W., Begley, P., Ali, S., Hollywood, K. A., ... & Gardiner, N. J. (2016). Metabolic dysfunction is restricted to the sciatic nerve in experimental diabetic neuropathy. Diabetes, 65(1), 228-238.

Friedrichsen, M., Birk, J. B., Richter, E. A., Ribel-Madsen, R., Pehmøller, C., Hansen, B. F., ... & Wojtaszewski, J. F. (2013). Akt2 influences glycogen synthase activity in human skeletal muscle through regulation of NH2-terminal (sites 2+ 2a) phosphorylation. American Journal of Physiology-Endocrinology and Metabolism, 304(6), E631-E639.

Fross, R. D., & Daube, J. R. (1987). Neuropathy in the Miller Fisher syndrome Clinical and electrophysiologic findings. Neurology, 37(9), 1493-1493.

Gallagher EJ, LeRoith D. Obesity and Diabetes: The Increased Risk of Cancer and Cancer-Related Mortality. Physiol Rev. 2015 Jul;95(3):727-48.

Gao, R., Wang, L., Sun, J., Nie, K., Jian, H., Gao, L., ... & Gan, S. (2014). MiR-204 promotes apoptosis in oxidative stress-induced rat Schwann cells by suppressing neuritin expression. FEBS letters, 588(17), 3225-3232.

Garbay, B., Heape, A. M., Sargueil, F., & Cassagne, C. (2000). Myelin synthesis in the peripheral nervous system. Progress in neurobiology,61(3), 267-304.

Garcia, V., Gilani, A., Shkolnik, B., Pandey, V., Zhang, F. F., Dakarapu, R., ... & Zeldin, D. C. (2017). 20-HETE signals through G-protein–coupled receptor GPR75 (Gq) to affect vascular function and trigger hypertension. Circulation research, 120(11), 1776-1788.

Gaspar, J. M., Baptista, F. I., Macedo, M. P., & Ambrósio, A. F. (2016). Inside the diabetic brain: role of different players involved in cognitive decline. ACS Chemical Neuroscience, 7(2), 131-142.

Gastinger, M. J., Singh, R. S., & Barber, A. J. (2006). Loss of cholinergic and dopaminergic amacrine cells in streptozotocin-diabetic rat and Ins2Akita-diabetic mouse retinas. Invest Ophthalmol Vis Sci, 47(7), 3143-3150. doi:10.1167/iovs.05-1376

Gebremedhin, D., Lange, A. R., Lowry, T. F., Taheri, M. R., Birks, E. K., Hudetz, A. G., Narayanan, J., Falck, J.R., Okamoto, H., Roman, R.J. & Nithipatikom, K. (2000). Production of 20-HETE and its role in autoregulation of cerebral blood flow. Circulation research, 87(1), 60-65.

Gebremedhin, D., Lange, A. R., Narayanan, J., Aebly, M. R., Jacobs, E. R., & Harder, D. R. (1998). Cat cerebral arterial smooth muscle cells express cytochrome P450 4A2 enzyme and produce the vasoconstrictor 20-HETE which enhances L-type Ca2+ current. The Journal of physiology, 507(3), 771-781.

Gebremedhin, D., Yamaura, K., & Harder, D. R. (2008). Role of 20-HETE in the hypoxia-induced activation of Ca2+-activated K+ channel currents in rat cerebral arterial muscle cells. American Journal of Physiology-Heart and Circulatory Physiology, 294(1), H107-H120.

Gebremedhin, D., Zhang, D. X., Carver, K. A., Rau, N., Rarick, K. R., Roman, R. J., & Harder, D. R. (2016). Expression of CYP 4A ω-hydroxylase and formation of 20-hydroxyeicosatetreanoic acid (20-HETE) in cultured rat brain astrocytes. Prostaglandins & other lipid mediators, 124, 16-26.

Gennari, C. G., Cilurzo, F., Mitro, N., Caruso, D., Minghetti, P., & Magnaghi, V. (2017). In vitro and in vivo evaluation of silk fibroin functionalized with GABA and allopregnanolone for Schwann cell and neuron survival. Regenerative medicine, (0).

Genuth, S. (2006). Insights from the diabetes control and complications trial/epidemiology of diabetes interventions and complications study on the use of intensive glycemic treatment to reduce the risk of complications of type 1 diabetes. Endocrine Practice, 12(Supplement 1), 34-

Geraldes, P., & King, G. L. (2010). Activation of protein kinase C isoforms and its impact on diabetic complications. Circulation research, 106(8), 1319-1331.

Gibbons, C. H., & Freeman, R. (2015). Treatment-induced neuropathy of diabetes: an acute, iatrogenic complication of diabetes. Brain, 138(1), 43-52.

Giese, K. P., Martini, R., Lemke, G., Soriano, P., & Schachner, M. (1992). Mouse P 0 gene disruption leads to hypomyelination, abnormal expression of recognition molecules, and degeneration of myelin and axons. Cell, 71(4), 565-576.

Gilani, A., Agostinucci, K., Hossain, S., Pascale, J. V., Garcia, V., Adebesin, A. M., ... & Schwartzman, M. L. (2021). 20-HETE interferes with insulin signaling and contributes to obesity-driven insulin resistance. Prostaglandins & Other Lipid Mediators, 152, 106485.

Gilani, A., Falck, J. R., & Schwartzman, M. (2019). 20-HETE Interferes with Insulin Signaling Through GPR75. The FASEB Journal, 33(S1), 514-8.

Gilani, A., Pandey, V., Garcia, V., Agostinucci, K., Singh, S. P., Schragenheim, J., ... & Abraham, N. G. (2018). High-fat diet-induced obesity and insulin resistance in CYP4A14−/− mice is mediated by 20-HETE. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 315(5), R934-R944.

Gilbert, R. E., Vranes, D., Berka, J. L., Kelly, D. J., Cox, A., Wu, L. L., . . . Cooper, M. E. (1998). Vascular endothelial growth factor and its receptors in control and diabetic rat eyes. Lab Invest, 78(8), 1017-1027.

Giovannucci, E., Harlan, D. M., Archer, M. C., Bergenstal, R. M., Gapstur, S. M., Habel, L. A.,

Gödel, M., Hartleben, B., Herbach, N., Liu, S., Zschiedrich, S., Lu, S., ... & Wiech, T. (2011). Role of mTOR in podocyte function and diabetic nephropathy in humans and mice. The Journal of clinical investigation,121(6), 2197-2209.

Goebbels, S., Oltrogge, J. H., Kemper, R., Heilmann, I., Bormuth, I., Wolfer, S., ... & Rossner, M. J. (2010). Elevated phosphatidylinositol 3, 4, 5-trisphosphate in glia triggers cell-autonomous membrane wrapping and myelination. Journal of Neuroscience, 30(26), 8953-8964.

Goebbels, S., Oltrogge, J. H., Wolfer, S., Wieser, G. L., Nientiedt, T., Pieper, A., ... & Nave, K. A. (2012). Genetic disruption of Pten in a novel mouse model of tomaculous neuropathy. EMBO molecular medicine, 4(6), 486-499.

Gold, S. M., Dziobek, I., Sweat, V., Tirsi, A., Rogers, K., Bruehl, H., ... & Convit, A. (2007). Hippocampal damage and memory impairments as possible early brain complications of type 2 diabetes. Diabetologia, 50(4), 711-719.

Gomez-Sanchez, J. A., Carty, L., Iruarrizaga-Lejarreta, M., Palomo-Irigoyen, M., Varela-Rey, M., Griffith, M., ... & De Juan, V. G. (2015). Schwann cell autophagy, myelinophagy, initiates myelin clearance from injured nerves. J Cell Biol, 210(1), 153-168.

Gonzalez, C. D., Lee, M. S., Marchetti, P., Pietropaolo, M., Towns, R., Vaccaro, M. I., ... & Wiley, J. W. (2011). The emerging role of autophagy in the pathophysiology of diabetes mellitus. Autophagy, 7(1), 2-11.

Gordon Dseagu, V. L., Shelton, N., & Mindell, J. S. (2013). Epidemiological evidence of a

Greene, D. A., Arezzo, J. C., Brown, M. B., & Zenarestat Study Group. (1999). Effect of aldose reductase inhibition on nerve conduction and morphometry in diabetic neuropathy. Neurology, 53(3), 580-580.

Greene, D. A., Stevens, M. J., Obrosova, I., & Feldman, E. L. (1999). Glucose-induced oxidative stress and programmed cell death in dinhiiabetic neuropathy. European journal of pharmacology, 375(1), 217-223.

Gross, J. L., De Azevedo, M. J., Silveiro, S. P., Canani, L. H., Caramori, M. L., & Zelmanovitz, T. (2005). Diabetic nephropathy: diagnosis, prevention, and treatment. Diabetes care, 28(1), 164-176.

Grote, C. W., & Wright, D. E. (2016). A role for insulin in diabetic neuropathy. Frontiers in neuroscience, 10, 581.

Grote, C. W., Groover, A. L., Ryals, J. M., Geiger, P. C., Feldman, E. L., & Wright, D. E. (2013). Peripheral nervous system insulin resistance in ob/ob mice. Acta neuropathologica communications, 1(1), 15.

Grundy, S. M. et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement. Circulation 112, 2735-2752 (2005).

Guedes, R. P., Araújo, A. S., Janner, D., Belló-Klein, A., Ribeiro, M. F. M., & Partata, W. A. (2008). Increase in reactive oxygen species and activation of Akt signaling pathway in neuropathic pain. Cellular and molecular neurobiology, 28(8), 1049-1056.

Guengerich, F. P. (2006). Cytochrome P450s and other enzymes in drug metabolism and toxicity. The AAPS journal, 8(1), E101-E111.

Gumy, L. F., Bampton, E. T., & Tolkovsky, A. M. (2008). Hyperglycaemia inhibits Schwann cell proliferation and migration and restricts regeneration of axons and Schwann cells from adult murine DRG. Molecular and Cellular Neuroscience, 37(2), 298-311.

Guo, A. M., Arbab, A. S., Falck, J. R., Chen, P., Edwards, P. A., Roman, R. J., & Scicli, A. G. (2007). Activation of vascular endothelial growth factor through reactive oxygen species mediates 20-hydroxyeicosatetraenoic acid-induced endothelial cell proliferation. Journal of Pharmacology and Experimental Therapeutics, 321(1), 18-27.

Guo, A. M., Sheng, J., Scicli, G. M., Arbab, A. S., Lehman, N. L., Edwards, P. A., ... & Scicli, A. G. (2008). Expression of CYP4A1 in U251 human glioma cell induces hyperproliferative phenotype in vitro and rapidly growing tumors in vivo. Journal of Pharmacology and Experimental Therapeutics,327(1), 10-19.

Guo, G., Kan, M., Martinez, J. A., & Zochodne, D. W. (2011). Local insulin and the rapid regrowth of diabetic epidermal axons. Neurobiology of disease, 43(2), 414-421.

Guo, J. R., Wang, H., Jin, X. J., Jia, D. L., Zhou, X., & Tao, Q. (2017). Effect and mechanism of inhibition of PI3K/Akt/mTOR signal pathway on chronic neuropathic pain and spinal microglia in a rat model of chronic constriction injury. Oncotarget, 8(32), 52923.

Guo, M., Roman, R. J., Fenstermacher, J. D., Brown, S. L., Falck, J. R., Arbab, A. S., ... & Scicli, A. G. (2006). 9L gliosarcoma cell proliferation and tumor growth in rats are suppressed by N-hydroxy-N′-(4-butyl-2-methylphenol) formamidine (HET0016), a selective inhibitor of CYP4A. Journal of Pharmacology and Experimental Therapeutics, 317(1), 97-108.

Guo, Y., Yu, W., Sun, D., Wang, J., Li, C., Zhang, R., ... & Shen, M. (2015). A novel protective mechanism for mitochondrial aldehyde dehydrogenase (ALDH2) in type i diabetes-induced cardiac dysfunction: role of AMPK-regulated autophagy. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 1852(2), 319-331.

Gwinn, D. M., Shackelford, D. B., Egan, D. F., Mihaylova, M. M., Mery, A., Vasquez, D. S., ... & Shaw, R. J. (2008). AMPK phosphorylation of raptor mediates a metabolic checkpoint. Molecular cell, 30(2), 214-226.

Haak, E., Usadel, K. H., Kusterer, K., Amini, P., Frommeyer, R., Tritschler, H. J., & Haak, T. (2000). Effects of alpha-lipoic acid on microcirculation in patients with peripheral diabetic neuropathy. Experimental and clinical endocrinology & diabetes, 108(03), 168-174.

Haeggström, J. Z., Rinaldo-Matthis, A., Wheelock, C. E., & Wetterholm, A. (2010). Advances in eicosanoid research, novel therapeutic implications. Biochemical and biophysical research communications, 396(1), 135-139.

Hahn-Windgassen, A., Nogueira, V., Chen, C.-C., Skeen, J. E., Sonenberg, N., & Hay, N. (2005). Akt activates the mammalian target of rapamycin by regulating cellular ATP level and AMPK activity. Journal of Biological Chemistry, 280(37), 32081-32089.

Hall, M. N., & Suter, U. mTORC1 Controls PNS Myelination along the mTORC1-RXRg-SREBP-Lipid Biosynthesis Axis in Schwann Cells.

Hamel, E. (2006). Perivascular nerves and the regulation of cerebrovascular tone. Journal of applied physiology, 100(3), 1059-1064.

Hamilton, R. T., Bhattacharya, A., Walsh, M. E., Shi, Y., Wei, R., Zhang, Y., ... & Van Remmen, H. (2013). Elevated protein carbonylation, and misfolding in sciatic nerve from db/db and Sod1−/− mice: plausible link between oxidative stress and demyelination. PLoS One, 8(6), e65725.

Hammes, H. P., Lin, J., Bretzel, R. G., Brownlee, M., & Breier, G. (1998). Upregulation of the vascular endothelial growth factor/vascular endothelial growth factor receptor system in experimental background diabetic retinopathy of the rat. Diabetes, 47(3), 401-406.

Han, S.-M., Namkoong, C., Jang, P., Park, I., Hong, S., Katakami, H., . . . Lee, K.-U. (2005). Hypothalamic AMP-activated protein kinase mediates counter-regulatory responses to hypoglycaemia in rats. Diabetologia, 48(10), 2170-2178.

Han, X., Tai, H., Wang, X., Wang, Z., Zhou, J., Wei, X., ... & Qin, J. (2016). AMPK activation protects cells from oxidative stress-induced senescence via autophagic flux restoration and intracellular NAD+ elevation. Aging cell, 15(3), 416-427.

Hao, W., Tashiro, S., Hasegawa, T., Sato, Y., Kobayashi, T., Tando, T., Katsuyama, E., Fujie, A., Watanabe, R., Morita, M. & Miyamoto, K. (2015). Hyperglycemia promotes schwann cell de-differentiation and de-myelination via sorbitol accumulation and Igf1 protein down-regulation. Journal of Biological Chemistry, 290(28), 17106-17115.

Hardie, D. G., Carling, D., & Carlson, M. (1998). The AMP-activated/SNF1 protein kinase subfamily: metabolic sensors of the eukaryotic cell? : Annual Reviews 4139 El Camino Way, PO Box 10139, Palo Alto, CA 94303-0139, USA.

Hardwick, J. P. (2008). Cytochrome P450 omega hydroxylase (CYP4) function in fatty acid metabolism and metabolic diseases. Biochemical pharmacology, 75(12), 2263-2275.

Harrington, L. S., Findlay, G. M., Gray, A., Tolkacheva, T., Wigfield, S., Rebholz, H., . . . Shepherd, P. R. (2004). The TSC1-2 tumor suppressor controls insulin–PI3K signaling via regulation of IRS proteins. The Journal of cell biology, 166(2), 213-223.

Hart, P. A., Bellin, M. D., Andersen, D. K., Bradley, D., Cruz-Monserrate, Z., Forsmark, C. E., ... & Pandol, S. J. (2016). Type 3c (pancreatogenic) diabetes mellitus secondary to chronic pancreatitis and pancreatic cancer. The lancet Gastroenterology & hepatology, 1(3), 226-237.

Hasse, B., Bosse, F., Hanenberg, H., & Müller, H. W. (2004). Peripheral myelin protein 22 kDa and protein zero: domain specific trans-interactions.Molecular and Cellular Neuroscience, 27(4), 370-378

Hayasaka, K., Himoro, M., Sato, W., Takada, G., Uyemura, K., Shimizu, N., ... & Chance, P. F. (1993). Charcot-Marie-Tooth neuropathy type 1B is associated with mutations of the myelin P0 gene. Nature genetics, 5(1), 31-34.

Hayden, M. R., & Tyagi, S. C. (2004). Neural redox stress and remodeling in metabolic syndrome, type 2 diabetes mellitus, and diabetic neuropathy. Medical Science Monitor, 10(12), RA291-RA307.

He, W. Y., Zhang, B., Xiong, Q. M., Yang, C. X., Zhao, W. C., He, J., ... & Wang, H. B. (2016). Intrathecal administration of rapamycin inhibits the phosphorylation of DRG Nav1. 8 and attenuates STZ-induced painful diabetic neuropathy in rats. Neuroscience letters, 619, 21-28.

He, Z., Zhang, X., Chen, C., Wen, Z., Hoopes, S. L., Zeldin, D. C., & Wang, D. W. (2015). Cardiomyocyte-specific expression of CYP2J2 prevents development of cardiac remodelling induced by angiotensin II. Cardiovascular research, 105(3), 304-317.

Herman WH, Pop-Busui R, Braffett BH, et al.; DCCT/EDIC Research Group. Use of the Michigan Neuropathy Screening Instrument as a measure of distal symmetrical peripheral neuropathy in Type 1 diabetes: results from the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications. Diabet Med 2012;29:937–944

Hichor, M., Sampathkumar, N. K., Montanaro, J., Borderie, D., Petit, P. X., Gorgievski, V., ... & Massaad, C. (2017). Paraquat induces peripheral myelin disruption and locomotor defects: crosstalk with LXR and Wnt pathways. Antioxidants & redox signaling, 27(3), 168-183.

Hill, E., Fitzpatrick, F., & Murphy, R. C. (1992). Biological activity and metabolism of 20-hydroxyeicosatetraenoic acid in the human platelet. British journal of pharmacology, 106(2), 267.

Hill, R. E., & Williams, P. E. (2002). A quantitative analysis of perineurial cell basement membrane collagen IV, laminin and fibronectin in diabetic and non-diabetic human sural nerve. Journal of anatomy, 201(2), 185-192.

Hill, S. M., Wrobel, L., & Rubinsztein, D. C. (2019). Post-translational modifications of Beclin 1 provide multiple strategies for autophagy regulation. Cell Death & Differentiation, 26(4), 617-629.

Hinder, L. M., Vivekanandan-Giri, A., McLean, L. L., Pennathur, S., & Feldman, E. L. (2013). Decreased glycolytic and tricarboxylic acid cycle intermediates coincide with peripheral nervous system oxidative stress in a murine model of type 2 diabetes. Journal of Endocrinology, 216(1), 1-11.

Hoff, U., Lukitsch, I., Chaykovska, L., Ladwig, M., Arnold, C., Manthati, V. L., Fuller, T.F., Schneider, W., Gollasch, M., Muller, D.N. & Flemming, B. (2011). Inhibition of 20-HETE synthesis and action protects the kidney from ischemia/reperfusion injury. Kidney international,79(1), 57-65.

Hotta, N., Kawamori, R., Atsumi, Y., Baba, M., Kishikawa, H., Nakamura, J., ... & ADCT Study Group. (2008). Stratified analyses for selecting appropriate target patients with diabetic peripheral neuropathy for long-term treatment with an aldose reductase inhibitor, epalrestat. Diabetic medicine, 25(7), 818-825.

Hu, J., Dziumbla, S., Lin, J., Bibli, S. I., Zukunft, S., De Mos, J., ... & Cheng, Z. (2017). Inhibition of soluble epoxide hydrolase prevents diabetic retinopathy. Nature, 552(7684), 248-252.

Huang, H. C., Chen, L., Zhang, H. X., Li, S. F., Liu, P., Zhao, T. Y., & Li, C. X. (2016). Autophagy promotes peripheral nerve regeneration and motor recovery following sciatic nerve crush injury in rats. Journal of Molecular Neuroscience, 58(4), 416-423.

Huang, H., Al-Shabrawey, M., & Wang, M. H. (2016). Cyclooxygenase-and cytochrome P450-derived eicosanoids in stroke. Prostaglandins & other lipid mediators, 122, 45-53.

Huang, J., & Manning, Brendan D. (2008). The TSC1–TSC2 complex: a molecular switchboard controlling cell growth. Biochemical Journal, 412(2), 179-190. doi:10.1042/bj20080281

Hye Khan, M. A., Neckář, J., Manthati, V., Errabelli, R., Pavlov, T. S., Staruschenko, A., ... & Imig, J. D. (2013). Orally active epoxyeicosatrienoic acid analog attenuates kidney injury in hypertensive dahl salt–sensitive rat. Hypertension, 62(5), 905-913.

Ibrahim, S., Harris, N. D., Radatz, M., Selmi, F., Rajbhandari, S., Brady, L., ... & Ward, J. D. (1999). A new minimally invasive technique to show nerve ischaemia in diabetic neuropathy. Diabetologia, 42(6), 737-742.

Ii, M., Nishimura, H., Kusano, K. F., Qin, G., Yoon, Y. S., Wecker, A., ... & Losordo, D. W. (2005). Neuronal nitric oxide synthase mediates statin-induced restoration of vasa nervorum and reversal of diabetic neuropathy. Circulation, 112(1), 93-102.

Iliff, J. J., Fairbanks, S. L., Balkowiec, A., & Alkayed, N. J. (2010). Epoxyeicosatrienoic acids are endogenous regulators of vasoactive neuropeptide release from trigeminal ganglion neurons. Journal of neurochemistry, 115(6), 1530-1542.

Iliff, J. J., Jia, J., Nelson, J., Goyagi, T., Klaus, J., & Alkayed, N. J. (2010). Epoxyeicosanoid signaling in CNS function and disease. Prostaglandins & other lipid mediators, 91(3-4), 68-84.

Iliff, J. J., Wang, R., Zeldin, D. C., & Alkayed, N. J. (2009). Epoxyeicosanoids as mediators of neurogenic vasodilation in cerebral vessels. American Journal of Physiology-Heart and Circulatory Physiology, 296(5), H1352-H1363.

Imig, J. D. (2012). Epoxides and soluble epoxide hydrolase in cardiovascular physiology. Physiological reviews, 92(1), 101-130.

Imig, J. D. (2013). Epoxyeicosatrienoic acids, 20-hydroxyeicosatetraenoic acid, and renal microvascular function. Prostaglandins & other lipid mediators, 104, 2-7.

Imig, J. D., Zhao, X., Zaharis, C. Z., Olearczyk, J. J., Pollock, D. M., Newman, J. W., ... & Hammock, B. D. (2005). An orally active epoxide hydrolase inhibitor lowers blood pressure and provides renal protection in salt-sensitive hypertension. Hypertension, 46(4), 975-981.

Inceoglu, B., Jinks, S. L., Schmelzer, K. R., Waite, T., Kim, I. H., & Hammock, B. D. (2006). Inhibition of soluble epoxide hydrolase reduces LPS-induced thermal hyperalgesia and mechanical allodynia in a rat model of inflammatory pain. Life sciences, 79(24), 2311-2319.

Inceoglu, B., Jinks, S. L., Ulu, A., Hegedus, C. M., Georgi, K., Schmelzer, K. R., ... & Hammock, B. D. (2008). Soluble epoxide hydrolase and epoxyeicosatrienoic acids modulate two distinct analgesic pathways. Proceedings of the National Academy of Sciences, 105(48), 18901-18906.

Inceoglu, B., Wagner, K. M., Yang, J., Bettaieb, A., Schebb, N. H., Hwang, S. H., ... & Hammock, B. D. (2012). Acute augmentation of epoxygenated fatty acid levels rapidly reduces pain-related behavior in a rat model of type I diabetes. Proceedings of the National Academy of Sciences, 109(28), 11390-11395.

Inceoglu, B., Wagner, K., Schebb, N. H., Morisseau, C., Jinks, S. L., Ulu, A., ... & Hammock, B. D. (2011). Analgesia mediated by soluble epoxide hydrolase inhibitors is dependent on cAMP. Proceedings of the National Academy of Sciences, 108(12), 5093-5097.

Inceoglu, B., Zolkowska, D., Yoo, H. J., Wagner, K. M., Yang, J., Hackett, E., ... & Hammock, B. D. (2013). Epoxy fatty acids and inhibition of the soluble epoxide hydrolase selectively modulate GABA mediated neurotransmission to delay onset of seizures. PloS one, 8(12).

Inouye, H., & Kirschner, D. A. (1988). Membrane interactions in nerve myelin: II. Determination of surface charge from biochemical data.Biophysical Journal, 53(2), 247-260.

Ishii, D. N. (1995). Implication of insulin-like growth factors in the pathogenesis of diabetic neuropathy. Brain research reviews, 20(1), 47-67.Ziegler, D., Papanas, N., Vinik, A. I., & Shaw, J. E. (2014). Epidemiology of polyneuropathy in diabetes and prediabetes. Handbook of clinical neurology,126, 3.

Ishizuka, T., Cheng, J., Singh, H., Vitto, M. D., Manthati, V. L., Falck, J. R., & Laniado-Schwartzman, M. (2008). 20-Hydroxyeicosatetraenoic acid stimulates nuclear factor-κB activation and the production of inflammatory cytokines in human endothelial cells. Journal of Pharmacology and Experimental Therapeutics, 324(1), 103-110.

Issan, Y., Hochhauser, E., Guo, A., Gotlinger, K. H., Kornowski, R., Leshem-Lev, D., ... & Abraham, N. G. (2013). Elevated level of pro-inflammatory eicosanoids and EPC dysfunction in diabetic patients withcardiac ischemia. Prostaglandins & other lipid mediators, 100, 15-21.

Jacob, C. (2017). Chromatin-remodeling enzymes in control of Schwann cell development, maintenance and plasticity. Current opinion in neurobiology, 47, 24-30.

Jacobs, E. R., Zhu, D., Gruenloh, S., Lopez, B., & Medhora, M. (2006). VEGF-induced relaxation of pulmonary arteries is mediated by endothelial cytochrome P-450 hydroxylase. American Journal of Physiology-Lung Cellular and Molecular Physiology, 291(3), L369-L377.

Jang, S. Y., Shin, Y. K., Park, S. Y., Park, J. Y., Lee, H. J., Yoo, Y. H., ... & Park, H. T. (2016). Autophagic myelin destruction by Schwann cells during Wallerian degeneration and segmental demyelination. Glia, 64(5), 730-742.

Jang, S. Y., Shin, Y. K., Park, S. Y., Park, J. Y., Rha, S. H., Kim, J. K., ... & Park, H. T. (2015). Autophagy is involved in the reduction of myelinating Schwann cell cytoplasm during myelin maturation of the peripheral nerve. PloS one, 10(1), e0116624.

Jenkins, C. M., Cedars, A., & Gross, R. W. (2009). Eicosanoid signalling pathways in the heart. Cardiovascular research, 82(2), 240-249.

Jensen, V. F. H., Mølck, A. M., Bøgh, I. B., & Lykkesfeldt, J. (2014). Effect of insulin-induced hypoglycaemia on the peripheral nervous system: focus on adaptive mechanisms, pathogenesis and histopathological changes. Journal of Neuroendocrinology, 26(8), 482-496.

Jessen, K. R., & Mirsky, R. (2016). The repair Schwann cell and its function in regenerating nerves. The Journal of physiology, 594(13), 3521-3531.

Jetten, A. M., & Suter, U. (2000). The peripheral myelin protein 22 and epithelial membrane protein family. Progress in nucleic acid research and molecular biology, 64, 97-129.

Jian-bo, L., Cheng-ya, W., Jia-wei, C., Xiao-lu, L., Zhen-qing, F., & Hong-tai, M. (2010). The preventive efficacy of methylcobalamin on rat peripheral neuropathy influenced by diabetes via neural IGF-1 levels. Nutritional neuroscience, 13(2), 79-86.

Jiang, J. X., Zhang, S. J., Liu, Y. N., Lin, X. X., Sun, Y. H., Shen, H. J., ... & Xie, Q. M. (2014). EETs alleviate ox-LDL-induced inflammation by inhibiting LOX-1 receptor expression in rat pulmonary arterial endothelial cells. European journal of pharmacology, 727, 43-51.

Jiang, M., Mezentsev, A., Kemp, R., Byun, K., Falck, J. R., Miano, J. M., ... & Laniado-Schwartzman, M. (2004). Smooth muscle—specific expression of CYP4A1 induces endothelial sprouting in renal arterial microvessels. Circulation research, 94(2), 167-174.

Johnson, E. F., Hsu, M. H., Savas, U., & Griffin, K. J. (2002). Regulation of P450 4A expression by peroxisome proliferator activated receptors. Toxicology, 181, 203-206.

Johnson, P. C., Doll, S. C., & Cromey, D. W. (1986). Pathogenesis of diabetic neuropathy. Annals of neurology, 19(5), 450-457.

Johnston, W. A., Hunter, D. J., Noble, C. J., Hanson, G. R., Stok, J. E., Hayes, M. A., ... & Gillam, E. M. (2011). Cytochrome P450 is present in both ferrous and ferric forms in the resting state within intact Escherichia coli and hepatocytes. Journal of Biological Chemistry, 286(47), 40750-40759.

Joseph, G., Soler, A., Hutcheson, R., Hunter, I., Bradford, C., Hutcheson, B., ... & Schwartzman, M. L. (2017). Elevated 20-HETE impairs coronary collateral growth in metabolic syndrome via endothelial dysfunction. American Journal of Physiology-Heart and Circulatory Physiology, 312(3), H528-H540.

Juranek, J. K., Geddis, M. S., Rosario, R., & Schmidt, A. M. (2013). Impaired slow axonal transport in diabetic peripheral nerve is independent of RAGE. European Journal of Neuroscience, 38(8), 3159-3168.

Katon, W., Russo, J., Lin, E. H., Heckbert, S. R., Ciechanowski, P., Ludman, E. J., & Von Korff, M. (2009). Depression and diabetes: factors associated with major depression at five-year follow-up. Psychosomatics, 50(6), 570-579.

Katona, I., Wu, X., Feely, S. M., Sottile, S., Siskind, C. E., Miller, L. J., ... & Li, J. (2009). PMP22 expression in dermal nerve myelin from patients with CMT1A. Brain, awp113.

Katz, A., Caramori, M. L. A., Sisson-Ross, S., Groppoli, T., Basgen, J. M., & Mauer, M. (2002). An increase in the cell component of the cortical interstitium antedates interstitial fibrosis in type 1 diabetic patients. Kidney international, 61(6), 2058-2065.

Kaul, K., Apostolopoulou, M., & Roden, M. (2015). Insulin resistance in type 1 diabetes mellitus. Metabolism, 64(12), 1629-1639.

Kawashima, R., Kojima, H., Nakamura, K., Arahata, A., Fujita, Y., Tokuyama, Y., ... & Tamai, Y. (2007). Alterations in mRNA expression of myelin proteins in the sciatic nerves and brains of streptozotocin-induced diabetic rats. Neurochemical research, 32(6), 1002-1010.

Kawashima, Y., Fritton, J. C., Yakar, S., Epstein, S., Schaffler, M. B., Jepsen, K. J., & LeRoith, D. (2009). Type 2 diabetic mice demonstrate slender long bones with increased fragility secondary to increased osteoclastogenesis. Bone, 44(4), 648-655.

Kehl, F., Cambj-Sapunar, L., Maier, K. G., Miyata, N., Kametani, S., Okamoto, H., ... & Roman, R. J. (2002). 20-HETE contributes to the acute fall in cerebral blood flow after subarachnoid hemorrhage in the rat. American Journal of Physiology-Heart and Circulatory Physiology, 282(4), H1556-H1565.

Kelkar, P., & Parry, G. J. (2003). Mononeuritis multiplex in diabetes mellitus: evidence for underlying immune pathogenesis. Journal of Neurology, Neurosurgery & Psychiatry, 74(6), 803-806.

Kemp, B. E., Mitchelhill, K. I., Stapleton, D., Michell, B. J., Chen, Z.-P., & Witters, L. A. (1999). Dealing with energy demand: the AMP-activated protein kinase. Trends in biochemical sciences, 24(1), 22-25.

Khan, M. A. H., Kolb, L., Skibba, M., Hartmann, M., Blöcher, R., Proschak, E., & Imig, J. D. (2018). A novel dual PPAR-γ agonist/sEH inhibitor treats diabetic complications in a rat model of type 2 diabetes. Diabetologia, 61(10), 2235-2246.

Kim, B., McLean, L. L., Philip, S. S., & Feldman, E. L. (2011). Hyperinsulinemia induces insulin resistance in dorsal root ganglion neurons. Endocrinology, 152(10), 3638-3647.

Kim, D. H., Puri, N., Sodhi, K., Falck, J. R., Abraham, N. G., Shapiro, J., & Schwartzman, M. L. (2013). Cyclooxygenase-2 dependent metabolism of 20-HETE increases adiposity and adipocyte enlargement in mesenchymal stem cell-derived adipocytes. Journal of lipid research, 54(3), 786-793.

Kim, J., Kundu, M., Viollet, B., & Guan, K. L. (2011). AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nature cell biology, 13(2), 132-141.

Kim, K. J., Iddings, J. A., Stern, J. E., Blanco, V. M., Croom, D., Kirov, S. A., & Filosa, J. A. (2015). Astrocyte contributions to flow/pressure-evoked parenchymal arteriole vasoconstriction. The Journal of Neuroscience,35(21), 8245-8257.

Kim, M.-S., Park, J.-Y., Namkoong, C., Jang, P.-G., Ryu, J.-W., Song, H.-S., . . . Park, I.-S. (2004). Anti-obesity effects of α-lipoic acid mediated by suppression of hypothalamic AMP-activated protein kinase. Nature medicine, 10(7), 727-733.

Kimmelstiel, P., & Wilson, C. (1936). Intercapillary lesions in the glomeruli of the kidney. The American journal of pathology, 12(1), 83.

King, G. L., & Loeken, M. R. (2004). Hyperglycemia-induced oxidative stress in diabetic complications. Histochemistry and cell biology, 122(4), 333-338.

King, R. H. M., Llewelyn, J. G., Thomas, P. K., Gilbey, S. G., & Watkins, P. J. (1989). Diabetic neuropathy: abnormalities of Schwann cell and perineurial basal laminae. Implications for diabetic vasculopathy. Neuropathology and applied neurobiology, 15(4), 339-355.

Klose, T. S., Blaisdell, J. A., & Goldstein, J. A. (1999). Gene structure of CYP2C8 and extrahepatic distribution of the human CYP2Cs. Journal of biochemical and molecular toxicology, 13(6), 289-295.

Köhler, C., Eriksson, L. G., Hansson, T., Warner, M., & Åke-Gustafsson, J. (1988). Immunohistochemical localization of cytochrome P-450 in the rat brain. Neuroscience letters, 84(2), 109-114.

Koike, K., Kusunose, E., Nishikawa, Y., Ichihara, K., Inagaki, S., Takagi, H., . Kikuta, Y. & Kusunose, M. (1997). Purification and characterization of rabbit small intestinal cytochromes P450 belonging to CYP2J and CYP4A subfamilies.Biochemical and biophysical research communications, 232(3), 643-647.

Koivisto, A., Hukkanen, M., Saarnilehto, M., Chapman, H., Kuokkanen, K., Wei, H., ... & Pertovaara, A. (2012). Inhibiting TRPA1 ion channel reduces loss of cutaneous nerve fiber function in diabetic animals: sustained activation of the TRPA1 channel contributes to the pathogenesis of peripheral diabetic neuropathy. Pharmacological Research, 65(1), 149-158.

Kola, B., Hubina, E., Tucci, S. A., Kirkham, T. C., Garcia, E. A., Mitchell, S. E., . . . Grossman, A. B. (2005). Cannabinoids and ghrelin have both central and peripheral metabolic and cardiac effects via AMP-activated protein kinase. Journal of Biological Chemistry, 280(26), 25196-25201.

Kowluru, R. A., & Mishra, M. (2015). Oxidative stress, mitochondrial damage and diabetic retinopathy. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 1852(11), 2474- 2483.

Kozak, W., Kluger, M. J., Kozak, A., Wachulec, M., & Dokladny, K. (2000). Role of cytochrome P-450 in endogenous antipyresis. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 279(2), R455-R460.

Krames, E. S. (2014). The role of the dorsal root ganglion in the development of neuropathic pain. Pain Medicine, 15(10), 1669-1685.

Kroemer, G., Mariño, G., & Levine, B. (2010). Autophagy and the integrated stress response. Molecular cell, 40(2), 280-293.

Kuhad, A., Sharma, S., & Chopra, K. (2008). Lycopene attenuates thermal hyperalgesia in a diabetic mouse model of neuropathic pain. European Journal of Pain, 12(5), 624-632.

Laguna, T. A., Nathan, B. M., & Moran, A. (2010). Managing diabetes in cystic fibrosis. Diabetes, Obesity and Metabolism, 12(10), 858-864.

Lai, G., Wu, J., Liu, X., & Zhao, Y. (2012). 20-HETE induces hyperglycemia through the cAMP/PKA PhK-GP pathway. Molecular Endocrinology, 26(11), 1907-1916.

Lakhkar, A., Dhagia, V., Joshi, S. R., Gotlinger, K., Patel, D., Sun, D., ... & Gupte, S. A. (2016). 20-HETE-induced mitochondrial superoxide production and inflammatory phenotype in vascular smooth muscle is prevented by glucose-6-phosphate dehydrogenase inhibition. American Journal of Physiology-Heart and Circulatory Physiology, 310(9), H1107-H1117.

Lambeth, J. D., Krause, K. H., & Clark, R. A. (2008, July). NOX enzymes as novel targets for drug development. In Seminars in immunopathology (Vol. 30, No. 3, pp. 339-363). Springer-Verlag.

Lamming, D. W., Ye, L., Katajisto, P., Goncalves, M. D., Saitoh, M., Stevens, D. M., ... & Guertin, D. A. (2012). Rapamycin-induced insulin resistance is mediated by mTORC2 loss and uncoupled from longevity. Science, 335(6076), 1638-1643.

Larsen, B. T., Campbell, W. B., & Gutterman, D. D. (2007). Beyond vasodilatation: non-vasomotor roles of epoxyeicosatrienoic acids in the cardiovascular system. Trends in pharmacological sciences, 28(1), 32-38.

Larsen, S. (1993). Diabetes mellitus secondary to chronic pancreatitis. Danish medical bulletin, 40(2), 153-162.

Larsson, S. C., & Wolk, A. (2011). Diabetes mellitus and incidence of kidney cancer: a meta-analysis of cohort studies. Diabetologia, 54(5), 1013-1018.

Latour, P., et al (1995). Mutations in the myelin protein zero gene associated with Charcot-Marie-Tooth disease type 1B. Human mutation, 6(1), 50-54.

Laughlin, R. S., & Dyck, P. J. B. (2014). Diabetic radiculoplexus neuropathies. In Handbook of clinical neurology (Vol. 126, pp. 45-52). Elsevier.

Lecrux, C., & Hamel, E. (2011). The neurovascular unit in brain function and disease. Acta physiologica, 203(1), 47-59.

Lee, R., Wong, T. Y., & Sabanayagam, C. (2015). Epidemiology of diabetic retinopathy, diabetic macular edema and related vision loss. Eye Vis (Lond), 2, 17. doi:10.1186/s40662-015-0026-2

Lee, S., Bazick, H., Chittoor-Vinod, V., Al Salihi, M. O., Xia, G., & Notterpek, L. (2018). Elevated peripheral Myelin Protein 22, reduced mitotic potential, and proteasome impairment in dermal fibroblasts from Charcot-Marie-Tooth disease type 1A patients. The American journal of pathology, 188(3), 728-738.

Leinninger GM, Edwards JL, Lipshaw MJ, Feldman EL. Mechanisms of disease: mitochondria as new therapeutic targets in diabetic neuropathy. Nature clinical practice. 2006; 2:620–628.

Li, J., Parker, B., Martyn, C., Natarajan, C., & Guo, J. (2013). The PMP22 gene and its related diseases. Molecular neurobiology, 47(2), 673-698.

Li, P. L., Chen, C. L., Bortell, R., & Campbell, W. B. (1999). 11, 12-Epoxyeicosatrienoic acid stimulates endogenous mono-ADP-ribosylation in bovine coronary arterial smooth muscle. Circulation research, 85(4), 349-356.

Li, Q. R., Wang, Z., Zhou, W., Fan, S. R., Ma, R., Xue, L., Yang, L., Li, Y.S., Tan, H.L., Shao, Q.H. & Yang, H. Y. (2016). Epalrestat protects against diabetic peripheral neuropathy by alleviating oxidative stress and inhibiting polyol pathway. Neural regeneration research, 11(2), 345.

Li, X., Zhao, G., Ma, B., Li, R., Hong, J., Liu, S., & Wang, D. W. (2014). 20-Hydroxyeicosatetraenoic acid impairs endothelial insulin signaling by inducing phosphorylation of the insulin receptor substrate-1 at Ser616. PLoS One, 9(4).

Li, Y., Tennekoon, G. I., Birnbaum, M., Marchionni, M. A., & Rutkowski, J. L. (2001). Neuregulin signaling through a PI3K/Akt/Bad pathway in Schwann cell survival. Molecular and Cellular Neuroscience, 17(4), 761-767.

Lima, R., Yanes, L. L., Davis, D. D., & Reckelhoff, J. F. (2013). Roles played by 20-HETE, angiotensin II and endothelin in mediating the hypertension in aging female spontaneously hypertensive rats. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 304(3), R248-R251.

Lin, C. H., Cheng, Y. C., Nicol, C. J., Lin, K. H., Yen, C. H., & Chiang, M. C. (2017). Activation of AMPK is neuroprotective in the oxidative stress by advanced glycosylation end products in human neural stem cells. Experimental cell research, 359(2), 367-373.

Lin, E. H., Rutter, C. M., Katon, W., Heckbert, S. R., Ciechanowski, P., Oliver, M. M., ... & Von Korff, M. (2010). Depression and advanced complications of diabetes: a prospective cohort study. Diabetes care, 33(2), 264-269.

Lin, F., Rios, A., Falck, J. R., Belosludtsev, Y., & Schwartzman, M. L. (1995). 20-Hydroxyeicosatetraenoic acid is formed in response to EGF and is a mitogen in rat proximal tubule. American Journal of Physiology-Renal Physiology, 269(6), F806-F816.

Liu, D., Liang, X., & Zhang, H. (2016). Effects of High Glucose on Cell Viability and Differentiation in Primary Cultured Schwann Cells: Potential Role of ERK Signaling Pathway. Neurochemical research, 41(6), 1281-1290.

Liu, J. Y., Tsai, H. J., Hwang, S. H., Jones, P. D., Morisseau, C., & Hammock, B. D. (2009). Pharmacokinetic optimization of four soluble epoxide hydrolase inhibitors for use in a murine model of inflammation. British journal of pharmacology, 156(2), 284-296.

Liu, S. Y., Chen, L., Li, X. C., Hu, Q. K., & He, L. J. (2017). Lycium barbarum polysaccharide protects diabetic peripheral neuropathy by enhancing autophagy via mTOR/p70S6K inhibition in Streptozotocin-induced diabetic rats. Journal of chemical neuroanatomy.

Liu, H. et al. Association of a functional cytochrome P450 4F2 haplotype with urinary 20-HETE and hypertension. Journal of the American Society of Nephrology 19, 714-721 (2008).

Liu, X., Li, C., Falck, J. R., Roman, R. J., Harder, D. R., & Koehler, R. C. (2008). Interaction of nitric oxide, 20-HETE, and EETs during functional hyperemia in whisker barrel cortex. American Journal of Physiology-Heart and Circulatory Physiology, 295(2), H619-H631.

Liu, Y., Wang, D., Wang, H., Qu, Y., Xiao, X., & Zhu, Y. (2014). The protective effect of HET0016 on brain edema and blood–brain barrier dysfunction after cerebral ischemia/reperfusion. Brain research, 1544, 45-53.

Liu, Y., Zhang, Y., Schmelzer, K., Lee, T. S., Fang, X., Zhu, Y., ... & Shyy, J. Y. J. (2005). The antiinflammatory effect of laminar flow: the role of PPARγ, epoxyeicosatrienoic acids, and soluble epoxide hydrolase. Proceedings of the National Academy of Sciences, 102(46), 16747-16752.

Lott, M. E., Slocomb, J. E., Shivkumar, V., Smith, B., Gabbay, R. A., Quillen, D., . . . Bettermann, K. (2012). Comparison of retinal vasodilator and constrictor responses in type 2 diabetes. Acta Ophthalmol, 90(6), e434-441. doi:10.1111/j.1755-3768.2012.02445.x

Lott, M. E., Slocomb, J. E., Shivkumar, V., Smith, B., Quillen, D., Gabbay, R. A., . . . Bettermann, K. (2013). Impaired retinal vasodilator responses in prediabetes and type 2 diabetes. Acta Ophthalmol, 91(6), e462-469. doi:10.1111/aos.12129

Lozano, F. S., González-Porras, J. R., March, J. R., Lobos, J. M., Carrasco, E., & Ros, E. (2014). Diabetes mellitus and intermittent claudication: a cross-sectional study of 920 claudicants. Diabetology & Metabolic Syndrome, 6(1), 21.

Lu, L., Wang, M., Wei, X., & Li, W. (2018). 20-HETE Inhibition by HET0016 Decreases the Blood–Brain Barrier Permeability and Brain Edema After Traumatic Brain Injury. Frontiers in Aging Neuroscience, 10, 207.

Lu, L., Wang, M., Yuan, F., Wei, X., & Li, W. (2017). Roles of elevated 20 HETE in the breakdown of blood brain barrier and the severity of brain edema in experimental traumatic brain injury. Molecular medicine reports, 17(5), 7339-7345.

Lu, S., Zhu, C., Long, A., Tan, L., Li, Q., & Zhu, Y. (2014). Effect of 20-hydroxyeicosatetraenoic acid on biological behavior of human villous trophoblasts and uterine vascular smooth muscle cells. Molecular medicine reports, 9(5), 1889-1894.

Lukic, I. K., Humpert, P. M., Nawroth, P. P., & Bierhaus, A. (2008). The RAGE pathway: activation and perpetuation in the pathogenesis of diabetic neuropathy. Annals of the New York Academy of Sciences, 1126(1), 76-80.

Luo, P., Chang, H. H., Zhou, Y., Zhang, S., Hwang, S. H., Morisseau, C., ... & Wang, M. H. (2010). Inhibition or deletion of soluble epoxide hydrolase prevents hyperglycemia, promotes insulin secretion, and reduces islet apoptosis. Journal of Pharmacology and Experimental Therapeutics, 334(2), 430-438.

Luong, T. N., Carlisle, H. J., Southwell, A., & Patterson, P. H. (2011). Assessment of motor balance and coordination in mice using the balance beam. JoVE (Journal of Visualized Experiments), (49), e2376-e2376.

Luria, A., Bettaieb, A., Xi, Y., Shieh, G. J., Liu, H. C., Inoue, H., ... & Hammock, B. D. (2011). Soluble epoxide hydrolase deficiency alters pancreatic islet size and improves glucose homeostasis in a model of insulin resistance. Proceedings of the National Academy of Sciences, 108(22), 9038-9043.

Luria, A., Weldon, S. M., Kabcenell, A. K., Ingraham, R. H., Matera, D., Jiang, H., ... & Hammock, B. D. (2007). Compensatory mechanism for homeostatic blood pressure regulation in Ephx2 gene-disrupted mice. Journal of Biological Chemistry, 282(5), 2891-2898.

Ma, B., Xiong, X., Chen, C., Li, H., Xu, X., Li, X., ... & Wang, D. W. (2013). Cardiac-specific overexpression of CYP2J2 attenuates diabetic cardiomyopathy in male streptozotocin-induced diabetic mice. Endocrinology, 154(8), 2843-2856.

Ma, J., Yu, H., Liu, J., Chen, Y., Wang, Q., & Xiang, L. (2015). Metformin attenuates hyperalgesia and allodynia in rats with painful diabetic neuropathy induced by streptozotocin. European journal of pharmacology, 764, 599-606.

Ma, J., Zhang, L., Han, W., Shen, T., Ma, C., Liu, Y., ... & Zhu, D. (2012). Activation of JNK/c-Jun is required for the proliferation, survival, and angiogenesis induced by EET in pulmonary artery endothelial cells. Journal of lipid research, 53(6), 1093-1105.

Maalouf, R. M., Eid, A. A., Gorin, Y. C., Block, K., Escobar, G. P., Bailey, S., & Abboud, H. E. (2012). Nox4-derived reactive oxygen species mediate cardiomyocyte injury in early type 1 diabetes. American Journal of Physiology-Cell Physiology, 302(3), C597-C604.

MacIsaac, R. J., Tsalamandris, C., Panagiotopoulos, S., Smith, T. J., McNeil, K. J., & Jerums, G. (2004). Nonalbuminuric renal insufficiency in type 2 diabetes. Diabetes care, 27(1), 195-200.

MacVicar, B. A., & Newman, E. A. (2015). Astrocyte regulation of blood flow in the brain. Cold Spring Harb. Perspect. Biol, 7, a020388.

Madhavi, Y. V., Gaikwad, N., Yerra, V. G., Kalvala, A. K., Nanduri, S., & Kumar, A. (2019). Targeting AMPK in diabetes and diabetic complications: energy homeostasis, autophagy and mitochondrial health. Current medicinal chemistry.

Magnani, P., Thomas, T. P., Tennekoon, G., DeVries, G. H., Greene, D. A., & Brosius 3rd, F. C. (1997). Regulation of glucose transport in cultured Schwann cells. Journal of the peripheral nervous system: JPNS, 3(1), 28-36.

Magruder, J. T., Elahi, D., & Andersen, D. K. (2011). Diabetes and pancreatic cancer: chicken or egg?. Pancreas, 40(3), 339-351.

Maiese, K. (2015). mTOR: Driving apoptosis and autophagy for neurocardiac complications of diabetes mellitus. World J Diabetes, 6(2), 217-224.

Manna, P., & Jain, S. K. (2011). Hydrogen sulfide and L-cysteine increase phosphatidylinositol 3, 4, 5-trisphosphate (PIP3) and glucose utilization by inhibiting phosphatase and tensin homolog (PTEN) protein and activating phosphoinositide 3-kinase (PI3K)/serine/threonine protein kinase (AKT)/protein kinase Cζ/λ (PKCζ/λ) in 3T3l1 adipocytes. Journal of Biological Chemistry, 286(46), 39848-39859.

Manna, P., & Jain, S. K. (2013). PIP3 but not PIP2 increases GLUT4 surface expression and glucose metabolism mediated by AKT/PKCζ/λ phosphorylation in 3T3L1 adipocytes. Molecular and cellular biochemistry, 381(1-2), 291-299.

Marnett, L. J. (2009). The COXIB experience: a look in the rearview mirror. Annual review of pharmacology and toxicology, 49, 265-290.

Martin, P. M., Roon, P., Van Ells, T. K., Ganapathy, V., & Smith, S. B. (2004). Death of retinal neurons in streptozotocin-induced diabetic mice. Invest Ophthalmol Vis Sci, 45(9), 3330-3336. doi:10.1167/iovs.04-0247

Martini, R., Fischer, S., López-Vales, R., & David, S. (2008). Interactions between Schwann cells and macrophages in injury and inherited demyelinating disease. Glia, 56(14), 1566-1577.

Masini, M., Bugliani, M., Lupi, R., Del Guerra, S., Boggi, U., Filipponi, F., ... & Marchetti, P. (2009). Autophagy in human type 2 diabetes pancreatic beta cells. Diabetologia, 52(6), 1083-1086.

Masser, D. R., VanGuilder Starkey, H. D., Bixler, G. V., Dunton, W., Bronson, S. K., & Freeman, W. M. (2014). Insulin treatment normalizes retinal neuroinflammation but not markers of synapse loss in diabetic rats. Exp Eye Res, 125, 95-106. doi:10.1016/j.exer.2014.06.005

Mathews, E. S., & Appel, B. (2016). Cholesterol Biosynthesis Supports Myelin Gene Expression and Axon Ensheathment through Modulation of P13K/Akt/mTor Signaling. The Journal of Neuroscience, 36(29), 7628-7639.

Mauer, S. M., Steffes, M. W., & Brown, D. M. (1981). The kidney in diabetes. The American journal of medicine, 70(3), 603-612.

Maurel, P., & Salzer, J. L. (2000). Axonal regulation of Schwann cell proliferation and survival and the initial events of myelination requires PI 3-kinase activity. Journal of Neuroscience, 20(12), 4635-4645.

Mazziotti, G., Formenti, A. M., Frara, S., Maffezzoni, F., Doga, M., & Giustina, A. (2017). Diabetes in Cushing disease. Current diabetes reports, 17(5), 32.

Mccarthy, E. T., Sharma, R. A. M., & Sharma, M. (2005). Protective effect of 20-hydroxyeicosatetraenoic acid (20-HETE) on glomerular protein permeability barrier. Kidney International, 67(1), 152-156.

Medhora, M., Chen, Y., Gruenloh, S., Harland, D., Bodiga, S., Zielonka, J., Gebremedhin, D., Gao, Y., Falck, J.R., Anjaiah, S. & Jacobs, E. R. (2008). 20-HETE increases superoxide production and activates NAPDH oxidase in pulmonary artery endothelial cells. American Journal of Physiology-Lung Cellular and Molecular Physiology, 294(5), L902-L911.

Medhora, M., Daniels, J., Mundey, K., Fisslthaler, B., Busse, R., Jacobs, E. R., & Harder, D. R. (2003). Epoxygenase-driven angiogenesis in human lung microvascular endothelial cells. American Journal of Physiology-Heart and Circulatory Physiology, 284(1), H215-H224.

Melemedjian, O. K., Asiedu, M. N., Tillu, D. V., Sanoja, R., Yan, J., Lark, A., ... & Sonenberg, N. (2011). Targeting adenosine monophosphate-activated protein kinase (AMPK) in preclinical models reveals a potential mechanism for the treatment of neuropathic pain. Molecular pain, 7(1), 70.

Melemedjian, O. K., Yassine, H. N., Shy, A., & Price, T. J. (2013). Proteomic and functional annotation analysis of injured peripheral nerves reveals ApoE as a protein upregulated by injury that is modulated by metformin treatment. Molecular pain, 9, 1744-8069.

Menzies, F. M., Fleming, A., Caricasole, A., Bento, C. F., Andrews, S. P., Ashkenazi, A., ... & Licitra, F. (2017). Autophagy and neurodegeneration: pathogenic mechanisms and therapeutic opportunities. Neuron, 93(5), 1015-1034.

Menzies, F. M., Fleming, A., Caricasole, A., Bento, C. F., Andrews, S. P., Ashkenazi, A., ... & Licitra, F. (2017). Autophagy and neurodegeneration: pathogenic mechanisms and therapeutic opportunities. Neuron, 93(5), 1015-1034.

Michailov, G. V., Sereda, M. W., Brinkmann, B. G., Fischer, T. M., Haug, B., Birchmeier, C., Role, L., Lai, C., Schwab, M.H. & Nave, K. A. (2004). Axonal neuregulin-1 regulates myelin sheath thickness. Science, 304(5671), 700-703.

Mihaylova, M. M., & Shaw, R. J. (2011). The AMPK signalling pathway coordinates cell growth, autophagy and metabolism. Nature cell biology, 13(9), 1016-1023.

Minaz, N., Razdan, R., Hammock, B. D., & Goswami, S. K. (2018). An inhibitor of soluble epoxide hydrolase ameliorates diabetes-induced learning and memory impairment in rats. Prostaglandins & other lipid mediators, 136, 84-89.

Minokoshi, Y., Alquier, T., Furukawa, N., Kim, Y.-B., Lee, A., Xue, B., . . . Birnbaum, M. J. (2004). AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. Nature, 428(6982), 569-574.

Mišur, I., Žarković, K., Barada, A., Batelja, L., Miličević, Z., & Turk, Z. (2004). Advanced glycation endproducts in peripheral nerve in type 2 diabetes with neuropathy. Acta diabetologica, 41(4), 158-166.

Mitchell, T. C., & McClain, D. A. (2014). Diabetes and hemochromatosis. Current Diabetes Reports, 14(5), 488.

Mitra, R., Guo, Z., Milani, M., Mesaros, C., Rodriguez, M., Nguyen, J., ... & Donner, D. B. (2011). CYP3A4 mediates growth of estrogen receptor-positive breast cancer cells in part by inducing nuclear translocation of phospho-Stat3 through biosynthesis of (±)-14, 15-epoxyeicosatrienoic acid (EET). Journal of Biological Chemistry, 286(20), 17543-17559.

Miyata, N., Taniguchi, K., Seki, T., Ishimoto, T., Sato-Watanabe, M., Yasuda, Y., ... & Kameo, K. (2001). HET0016, a potent and selective inhibitor of 20-HETE synthesizing enzyme. British journal of pharmacology, 133(3), 325-329.

Monje, P. V., Bartlett Bunge, M., & Wood, P. M. (2006). Cyclic AMP synergistically enhances neuregulin-dependent ERK and Akt activation and cell cycle progression in Schwann cells. Glia, 53(6), 649-659.

Montani, L., Pereira, J. A., Norrmén, C., Pohl, H. B., Tinelli, E., Trötzmüller, M., ... & Jessberger, S. (2018). De novo fatty acid synthesis by Schwann cells is essential for peripheral nervous system myelination. J Cell Biol, 217(4), 1353-1368.

Moran, C., Phan, T. G., Chen, J., Blizzard, L., Beare, R., Venn, A., ... & Pearson, S. (2013). Brain atrophy in type 2 diabetes: regional distribution and influence on cognition. Diabetes care, 36(12), 4036-4042.

Moreno, J. J. (2017). Eicosanoid receptors: Targets for the treatment of disrupted intestinal epithelial homeostasis. European journal of pharmacology, 796, 7-19.

Moreno, M. U., San José, G., Pejenaute, Á., Landecho, M. F., Díez, J., Beloqui, Ó., ... & Zalba, G. (2014). Association of phagocytic NADPH oxidase activity with hypertensive heart disease: a role for cardiotrophin-1?. Hypertension, 63(3), 468-474.

Mori, H., Inoki, K., Masutani, K., Wakabayashi, Y., Komai, K., Nakagawa, R., . . . Yoshimura, A. (2009). The mTOR pathway is highly activated in diabetic nephropathy and rapamycin has a strong therapeutic potential. Biochemical and biophysical research communications, 384(4), 471-475.

Morisseau, C., & Hammock, B. D. (2005). Epoxide hydrolases: mechanisms, inhibitor designs, and biological roles. Annu. Rev. Pharmacol. Toxicol., 45, 311-333.

Morisseau, C., Goodrow, M. H., Dowdy, D., Zheng, J., Greene, J. F., Sanborn, J. R., & Hammock, B. D. (1999). Potent urea and carbamate inhibitors of soluble epoxide hydrolases. Proceedings of the National Academy of Sciences, 96(16), 8849-8854.

Morisseau, C., Inceoglu, B., Schmelzer, K., Tsai, H. J., Jinks, S. L., Hegedus, C. M., & Hammock, B. D. (2010). Naturally occurring monoepoxides of eicosapentaenoic acid and docosahexaenoic acid are bioactive antihyperalgesic lipids. Journal of lipid research, 51(12), 3481-3490.

Morris, S. J., Shore, A. C., & Tooke, J. E. (1995). Responses of the skin microcirculation to acetylcholine and sodium nitroprusside in patients with NIDDM. Diabetologia, 38(11), 1337-1344.

Mroueh, F. M., Noureldein, M., Zeidan, Y. H., Boutary, S., Irani, S. A. M., Eid, S., ... & Kanj, R. (2019). Unmasking the interplay between mTOR and Nox4: novel insights into the mechanism connecting diabetes and cancer. The FASEB Journal, 33(12), 14051-14066.

Munzenmaier, D. H., & Harder, D. R. (2000). Cerebral microvascular endothelial cell tube formation: role of astrocytic epoxyeicosatrienoic acid release. American Journal of Physiology-Heart and Circulatory Physiology, 278(4), H1163-H1167.

Nandipati, K. C., Subramanian, S., & Agrawal, D. K. (2017). Protein kinases: mechanisms and downstream targets in inflammation-mediated obesity and insulin resistance. Molecular and cellular biochemistry, 426(1-2), 27-45.

Narayanan, S. P., Flores, A. I., Wang, F., & Macklin, W. B. (2009). Akt signals through the mammalian target of rapamycin pathway to regulate CNS myelination. The Journal of Neuroscience, 29(21), 6860-6870.

Nave, K. A. (2010). Myelination and support of axonal integrity by glia. Nature, 468(7321), 244.

Nave, K. A., & Salzer, J. L. (2006). Axonal regulation of myelination by neuregulin 1. Current opinion in neurobiology, 16(5), 492-500.

Nayernia, Z., Jaquet, V., & Krause, K. H. (2014). New insights on NOX enzymes in the central nervous system. Antioxidants & redox signaling,20(17), 2815-2837.

Nazıroğlu, M., Dikici, D. M., & Dursun, Ş. (2012). Role of oxidative stress and Ca2+ signaling on molecular pathways of neuropathic pain in diabetes: focus on TRP channels. Neurochemical research, 37(10), 2065-2075.

Nebert, D. W., & Russell, D. W. (2002). Clinical importance of the cytochromes P450. The Lancet, 360(9340), 1155-1162.

Nelson, J. W., Young, J. M., Borkar, R. N., Woltjer, R. L., Quinn, J. F., Silbert, L. C., ... & Alkayed, N. J. (2014). Role of soluble epoxide hydrolase in age-related vascular cognitive decline. Prostaglandins & other lipid mediators, 113, 30-37.

Nelson, V. L., Jiang, Y. P., Dickman, K. G., Ballou, L. M., & Lin, R. Z. (2014). Adipose tissue insulin resistance due to loss of PI3K p110α leads to decreased energy expenditure and obesity. American Journal of Physiology-Endocrinology and Metabolism, 306(10), E1205-E1216.

Newman, C., & Dinneen, S. F. (2019). Brittle diabetes revisited: a description of erratic and difficult-to-control diabetes in an elderly woman with Type 1 diabetes. Diabetic Medicine.

Newman, J. W., Morisseau, C., & Hammock, B. D. (2005). Epoxide hydrolases: their roles and interactions with lipid metabolism. Progress in lipid research, 44(1), 1-51.

Niedowicz, D. M., & Daleke, D. L. (2005). The role of oxidative stress in diabetic complications. Cell biochemistry and biophysics, 43(2), 289-330.

Niemann, A., Berger, P., & Suter, U. (2006). Pathomechanisms of mutant proteins in Charcot-Marie-Tooth disease. Neuromolecular medicine, 8(1-2), 217-241.

Niemann, S., Sereda, M. W., Suter, U., Griffiths, I. R., & Nave, K. A. (2000). Uncoupling of myelin assembly and schwann cell differentiation by transgenic overexpression of peripheral myelinprotein 22. The Journal of Neuroscience, 20(11), 4120-4128.

Ning, X., Baoyu, Q., Yuzhen, L., Shuli, S., Reed, E., & Li, Q. Q. (2004). Neuro-optic cell apoptosis and microangiopathy in KKAY mouse retina. Int J Mol Med, 13(1), 87-92.

Nilakantan, V., Maenpaa, C., Jia, G., Roman, R. J., & Park, F. (2008). 20-HETE-mediated cytotoxicity and apoptosis in ischemic kidney epithelial cells. American Journal of Physiology-Renal Physiology, 294(3), F562-F570.

Nithipatikom, K., Grall, A. J., Holmes, B. B., Harder, D. R., Falck, J. R., & Campbell, W. B. (2001). Liquid chromatographic–electrospray ionization–mass spectrometric analysis of cytochrome P450 metabolites of arachidonic acid. Analytical biochemistry, 298(2), 327-336.

Node, K., Huo, Y., Ruan, X., Yang, B., Spiecker, M., Ley, K., ... & Liao, J. K. (1999). Anti-inflammatory properties of cytochrome P450 epoxygenase-derived eicosanoids. Science, 285(5431), 1276-1279.

Norrmén, C., Figlia, G., Lebrun-Julien, F., Pereira, J. A., Trötzmüller, M., Köfeler, H. C., ... & Verheijen, M. H. (2014). mTORC1 controls PNS myelination along the mTORC1-RXRγ-SREBP-lipid biosynthesis axis in Schwann cells. Cell reports, 9(2), 646-660.

O'Neal, K. S., Johnson, J. L., & Panak, R. L. (2016). Recognizing and appropriately treating latent autoimmune diabetes in adults. Diabetes Spectrum, 29(4), 249-252.

Oates, P. J. (2008). Aldose reductase, still a compelling target for diabetic neuropathy. Current drug targets, 9(1), 14-36.

Obrosova, I. G., Stavniichuk, R., Drel, V. R., Shevalye, H., Vareniuk, I., Nadler, J. L., & Schmidt, R. E. (2010). Different roles of 12/15-lipoxygenase in diabetic large and small fiber peripheral and autonomic neuropathies. The American journal of pathology, 177(3), 1436-1447.

Ogata, T., Iijima, S., Hoshikawa, S., Miura, T., Yamamoto, S. I., Oda, H., ... & Tanaka, S. (2004). Opposing extracellular signal-regulated kinase and Akt pathways control Schwann cell myelination. The Journal of neuroscience, 24(30), 6724-6732.

Ohsawa, M., & Kamei, J. (1999). Role of intracellular calcium in thermal allodynia and hyperalgesia in diabetic mice. Brain research, 833(2), 278-281.

Ohsawa, M., Miyata, S., Carlsson, A., & Kamei, J. (2008). Preventive effect of acetyl-L-carnitine on the thermal hypoalgesia in streptozotocin-induced diabetic mice. European journal of pharmacology, 588(2), 213-216.

Ohtoshi, K., Kaneto, H., Node, K., Nakamura, Y., Shiraiwa, T., Matsuhisa, M., & Yamasaki, Y. (2005). Association of soluble epoxide hydrolase gene polymorphism with insulin resistance in type 2 diabetic patients. Biochemical and biophysical research communications, 331(1), 347-350.

Olearczyk, J. J., Quigley, J. E., Mitchell, B. C., Yamamoto, T., Kim, I. H., Newman, J. W., ... & Imig, J. D. (2009). Administration of a substituted adamantyl urea inhibitor of soluble epoxide hydrolase protects the kidney from damage in hypertensive Goto–Kakizaki rats. Clinical science, 116(1), 61-70.

Otsuka, T., Sugo, N., Koehler, R. C., Hurn, P. D., Traystman, R. J., & Alkayed, N. J. (2003). Soluble epoxide hydrolase gene deletion is protective against experimental cerebral ischemia. In Stroke (Vol. 34, No. 1, pp. 301-301). 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA: LIPPINCOTT WILLIAMS & WILKINS.

Packer, L., Kraemer, K., & Rimbach, G. (2001). Molecular aspects of lipoic acid in the prevention of diabetes complications. Nutrition, 17(10), 888-895.

Palma, F. H., Antigual, D. U., Martínez, S. F., Monrroy, M. A., & Gajardo, R. E. (2013). Static balance in patients presenting diabetes mellitus type 2 with and without diabetic polyneuropathy. Arquivos Brasileiros de Endocrinologia & Metabologia, 57(9), 722-726.

Panda, D. K., Miao, D., Tremblay, M. L., Sirois, J., Farookhi, R., Hendy, G. N., & Goltzman, D. (2001). Targeted ablation of the 25-hydroxyvitamin D 1α-hydroxylase enzyme: evidence for skeletal, reproductive, and immune dysfunction. Proceedings of the National Academy of Sciences, 98(13), 7498-7503.

Panigrahy, D., Kalish, B. T., Huang, S., Bielenberg, D. R., Le, H. D., Yang, J., ... & Butterfield, C. E. (2013). Epoxyeicosanoids promote organ and tissue regeneration. Proceedings of the National Academy of Sciences, 110(33), 13528-13533.

Park, F., Sweeney Jr, W. E., Jia, G., Akbulut, T., Mueller, B., Falck, J. R., ... & Avner, E. D. (2009). Chronic blockade of 20-HETE synthesis reduces polycystic kidney disease in an orthologous rat model of ARPKD. American Journal of Physiology-Renal Physiology, 296(3), F575-F582.

Parving, H.-H., Lehnert, H., Bröchner-Mortensen, J., Gomis, R., Andersen, S., & Arner, P. (2001). The effect of irbesartan on the development of diabetic nephropathy in patients with type 2 diabetes. New England Journal of Medicine, 345(12), 870-878.

Pelizza, L., & Pupo, S. (2016). Brittle diabetes: Psychopathology and personality. Journal of Diabetes and its Complications, 30(8), 1544-1547.

Pelizza, L., & Pupo, S. (2019). Alexithymia in adults with brittle type 1 diabetes. Acta bio-medica: Atenei Parmensis, 90(2), 279.

Peng, P. H., Lin, H. S., & Lin, S. (2009). Nerve fibre layer thinning in patients with preclinical retinopathy. Can J Ophthalmol, 44(4), 417-422. doi:10.3129/i09-112

Peterson, S. J., Vanella, L., Gotlinger, K., Jiang, H., Singh, S. P., Sodhi, K., ... & Abraham, N. G. (2016). Oxidized HDL is a potent inducer of adipogenesis and causes activation of the Ang-II and 20-HETE systems in human obese females. Prostaglandins & other lipid mediators, 123, 68-77.

Phung, T. L., Ziv, K., Dabydeen, D., Eyiah-Mensah, G., Riveros, M., Perruzzi, C., . . . Nagy, J. A. (2006). Pathological angiogenesis is induced by sustained Akt signaling and inhibited by rapamycin. Cancer Cell, 10(2), 159-170.

Pieper, G. M. (1998). Review of alterations in endothelial nitric oxide production in diabetes protective role of arginine on endothelial dysfunction. Hypertension, 31(5), 1047-1060.

Pipi, E., Marketou, M., & Tsirogianni, A. (2014). Distinct clinical and laboratory characteristics of latent autoimmune diabetes in adults in relation to type 1 and type 2 diabetes mellitus. World journal of diabetes, 5(4), 505.

Poloyac, S. M., Reynolds, R. B., Yonas, H., & Kerr, M. E. (2005). Identification and quantification of the hydroxyeicosatetraenoic acids, 20-HETE and 12-HETE, in the cerebrospinal fluid after subarachnoid hemorrhage. Journal of neuroscience methods, 144(2), 257-263.

Polsky, S., & Ellis, S. L. (2015). Obesity, insulin resistance, and type 1 diabetes mellitus. Current Opinion in Endocrinology, Diabetes and Obesity, 22(4), 277-282.

Pop-Busui, R., Boulton, A. J., Feldman, E. L., Bril, V., Freeman, R., Malik, R. A., ... & Ziegler, D. (2017). Diabetic neuropathy: a position statement by the American Diabetes Association. Diabetes care, 40(1), 136-154.

Pop-Busui, R., Kellogg, A. P., & Cheng, H. T. (2008). Cyclooxygenase-2 pathway as a potential therapeutic target in diabetic peripheral neuropathy. Current drug targets, 9(1), 68-76.

Pop-Busui, R., Stevens, M. J., Raffel, D. M., White, E. A., Mehta, M., Plunkett, C. D., ... & Feldman, E. L. (2013). Effects of triple antioxidant therapy on measures of cardiovascular autonomic neuropathy and on myocardial blood flow in type 1 diabetes: a randomised controlled trial. Diabetologia, 56(8), 1835-1844.

Pozzi, A., Popescu, V., Yang, S., Mei, S., Shi, M., Puolitaival, S. M., ... & Capdevila, J. H. (2010). The anti-tumorigenic properties of peroxisomal proliferator-activated receptor α are arachidonic acid epoxygenase-mediated. Journal of Biological Chemistry, 285(17), 12840-12850.

Pugazhenthi, S., Qin, L., & Reddy, P. H. (2017). Common neurodegenerative pathways in obesity, diabetes, and Alzheimer's disease. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 1863(5), 1037-1045.

Puntarulo, S., & Cederbaum, A. I. (1998). Production of reactive oxygen species by microsomes enriched in specific human cytochrome P450 enzymes. Free Radical Biology and Medicine, 24(7), 1324-1330.

Qian, T., Wang, X., Wang, Y., Wang, P., Liu, Q., Liu, J., & Yi, S. (2018). Novel miR-sc4 regulates the proliferation and migration of Schwann cells by targeting Cdk5r1. Molecular and cellular biochemistry, 1-7.

Qin, X., Wu, Q., Lin, L., Sun, A., Liu, S., Li, X., ... & Wang, X. (2015). Soluble epoxide hydrolase deficiency or inhibition attenuates MPTP-induced parkinsonism. Molecular neurobiology, 52(1), 187-195.

Quigley, R., Baum, M., Reddy, K. M., Griener, J. C., & Falck, J. R. (2000). Effects of 20-HETE and 19 (S)-HETE on rabbit proximal straight tubule volume transport. American Journal of Physiology-Renal Physiology, 278(6), F949-F953.

Quintes, S., & Brinkmann, B. G. (2017). Transcriptional inhibition in Schwann cell development and nerve regeneration. Neural regeneration research, 12(8), 1241.

Rami, A., Langhagen, A., & Steiger, S. (2008). Focal cerebral ischemia induces upregulation of Beclin 1 and autophagy-like cell death. Neurobiology of disease, 29(1), 132-141.

Rangaraju, S., & Notterpek, L. (2011). Autophagy aids membrane expansion by neuropathic Schwann cells. Autophagy.

Ravikumar, B., Sarkar, S., Davies, J. E., Futter, M., Garcia-Arencibia, M., Green-Thompson, Z. W., ... & Massey, D. C. (2010). Regulation of mammalian autophagy in physiology and pathophysiology. Physiological reviews, 90(4), 1383-1435.

Regner, K. R., Zuk, A., Van Why, S. K., Shames, B. D., Ryan, R. P., Falck, J. R., ... & Roman, R. J. (2009). Protective effect of 20-HETE analogues in experimental renal ischemia reperfusion injury. Kidney international,75(5), 511-517.

Reljanovic, M., Reichel, G., Rett, K., Lobisch, M., Schuette, K., Möller, W., ... & Mehnert, H. (1999). Treatment of diabetic polyneuropathy with the antioxidant thioctic acid (α-lipoic acid): a two year multicenter randomized double-blind placebo-controlled trial (ALADIN II). Free radical research,31(3), 171-179.

Ren, Q., Ma, M., Ishima, T., Morisseau, C., Yang, J., Wagner, K. M., ... & Han, M. (2016). Gene deficiency and pharmacological inhibition of soluble epoxide hydrolase confers resilience to repeated social defeat stress. Proceedings of the National Academy of Sciences, 113(13), E1944-E1952.

Rendic, S., & Carlo, F. J. D. (1997). Human cytochrome P450 enzymes: a status report summarizing their reactions, substrates, inducers, and inhibitors. Drug metabolism reviews, 29(1-2), 413-580.

Renic, M., Kumar, S. N., Gebremedhin, D., Florence, M. A., Gerges, N. Z., Falck, J. R., ... & Roman, R. J. (2012). Protective effect of 20-HETE inhibition in a model of oxygen-glucose deprivation in hippocampal slice cultures. American Journal of Physiology-Heart and Circulatory Physiology, ajpheart-00340.

Resmini, E., Minuto, F., Colao, A., & Ferone, D. (2009). Secondary diabetes associated with principal endocrinopathies: the impact of new treatment modalities. Acta diabetologica, 46(2), 85-95.

Ribeiro, C. M., Dubay, G. R., Falck, J. R., & Mandel, L. J. (1994). Parathyroid hormone inhibits Na (+)-K (+)-ATPase through a cytochrome P-450 pathway. American Journal of Physiology-Renal Physiology, 266(3), F497-F505.

Rivera J, Ward N, Hodgson J, Puddey IB, Falck JR, Croft KD. Measurement of 20-hydroxyeicosatrienoic acid in human urine by gas chromatography–mass spectrometry. Clin Chem. 50: 224–226, 2004.

Rizza, R. A., Daube, J. R., O'Brien, P. C., & Dyck, P. J. (1985). Near normoglycaemia improved nerve conduction and vibration sensation in diabetic neuropathy. Diabetologia, 28(10), 722-727.

Roberts, R. O., Knopman, D. S., Przybelski, S. A., Mielke, M. M., Kantarci, K., Preboske, G. M., ... & Ivnik, R. J. (2014). Association of type 2 diabetes with brain atrophy and cognitive impairment. Neurology, 82(13), 1132-1141.

Robertson AM, Huxley C, King RH, Thomas PK (1999) Development of early postnatal peripheral nerve abnormalities in Trembler-J and PMP22 transgenic mice. J Anat 195(Pt 3):331–339

Rocchi, A., & He, C. (2015). Emerging roles of autophagy in metabolism and metabolic disorders. Frontiers in biology, 10(2), 154-164.

Roche, C., Besnier, M., Cassel, R., Harouki, N., Coquerel, D., Guerrot, D., ... & Mulder, P. (2015). Soluble epoxide hydrolase inhibition improves coronary endothelial function and prevents the development of cardiac alterations in obese insulin-resistant mice. American Journal of Physiology-Heart and Circulatory Physiology, 308(9), H1020-H1029.

Rocic, P., & Schwartzman, M. L. (2018). 20-HETE in the regulation of vascular and cardiac function. Pharmacology & therapeutics, 192, 74-87.

Roman, R. J., Renic, M., Dunn, K. M., Takeuchi, K., & Hacein-Bey, L. (2006). Evidence that 20-HETE contributes to the development of acute and delayed cerebral vasospasm. Neurological research, 28(7), 738-749.

Roy Chowdhury, S. K., Smith, D. R., Saleh, A., Schapansky, J., Marquez, A., Gomes, S., ... & Fernyhough, P. (2012). Impaired adenosine monophosphate-activated protein kinase signalling in dorsal root ganglia neurons is linked to mitochondrial dysfunction and peripheral neuropathy in diabetes. Brain, 135(6), 1751-1766.

Rumora, A. E., Lentz, S. I., Hinder, L. M., Jackson, S. W., Valesano, A., Levinson, G. E., & Feldman, E. L. (2017). Dyslipidemia impairs mitochondrial trafficking and function in sensory neurons. The FASEB Journal, 32(1), 195-207.

Ryu, T. Y., Park, J., & Scherer, P. E. (2014). Hyperglycemia as a risk factor for cancer progression. Diabetes & metabolism journal, 38(5), 330-336.

Sabanayagam, C., Yip, W., Ting, D. S., Tan, G., & Wong, T. Y. (2016). Ten Emerging Trends in the Epidemiology of Diabetic Retinopathy. Ophthalmic Epidemiol, 23(4), 209-222. doi:10.1080/09286586.2016.1193618

Sahenk, Z., Serrano-Munuera, C., Chen, L., Kakabadze, I., & Najagara, H. N. (2003). Evidence for impaired axonal regeneration in PMP22 duplication: studies in nerve xenografts. Journal of the Peripheral Nervous System, 8(2), 116-127.

Said, G. (2007). Diabetic neuropathy—a review. Nature clinical practice Neurology, 3(6), 331-340.

Said, G. (2007). Focal and multifocal diabetic neuropathies. Arquivos de neuro-psiquiatria, 65(4B), 1272-1278.

Sander, A. L., Jakob, H., Sommer, K., Sadler, C., Fleming, I., Marzi, I., & Frank, J. (2011). Cytochrome P450-derived epoxyeicosatrienoic acids accelerate wound epithelialization and neovascularization in the hairless mouse ear wound model. Langenbeck's archives of surgery, 396(8), 1245.

Sarbassov, D. D., Ali, S. M., Sengupta, S., Sheen, J.-H., Hsu, P. P., Bagley, A. F., . . . Sabatini, D. M. (2006). Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. Molecular cell, 22(2), 159-168.

Sarbassov, D. D., Guertin, D. A., Ali, S. M., & Sabatini, D. M. (2005). Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex.Science, 307(5712), 1098-1101.

Sarkar, P., Narayanan, J., & Harder, D. R. (2011). Differential effect of amyloid beta on the cytochrome P450 epoxygenase activity in rat brain. Neuroscience, 194, 241-249.

Sato, A., Sunayama, J., Matsuda, K. I., Tachibana, K., Sakurada, K., Tomiyama, A., ... & Kitanaka, C. (2010). Regulation of neural stem/progenitor cell maintenance by PI3K and mTOR. Neuroscience letters, 470(2), 115-120.

Schartner, E., Sabbir, M. G., Saleh, A., Silva, R. V., Chowdhury, S. R., Smith, D. R., & Fernyhough, P. (2018). High glucose concentration suppresses a SIRT2 regulated pathway that enhances neurite outgrowth in cultured adult sensory neurons. Experimental neurology, 309, 134-147.

Schmelzer, K. R., Kubala, L., Newman, J. W., Kim, I. H., Eiserich, J. P., & Hammock, B. D. (2005). Soluble epoxide hydrolase is a therapeutic target for acute inflammation. Proceedings of the National Academy of Sciences, 102(28), 9772-9777.

Schulz, A., Kyselyova, A., Baader, S. L., Jung, M. J., Zoch, A., Mautner, V. F., Hagel, C.& Morrison, H. (2014). Neuronal merlin influences ERBB2 receptor expression on Schwann cells through neuregulin 1 type III signalling.Brain, 137(2), 420-432.

Shang, Y. C., Chong, Z. Z., Wang, S., & Maiese, K. (2011). Erythropoietin and Wnt1 govern pathways of mTOR, Apaf-1, and XIAP in inflammatory microglia. Current neurovascular research, 8(4), 270-285.

Shao, D., Oka, S. I., Liu, T., Zhai, P., Ago, T., Sciarretta, S., ... & Sadoshima, J. (2014). A redox-dependent mechanism for regulation of AMPK activation by Thioredoxin1 during energy starvation. Cell metabolism, 19(2), 232-245.

Shearer, G. C., & Newman, J. W. (2008). Lipoprotein lipase releases esterified oxylipins from very low-density lipoproteins. Prostaglandins, leukotrienes and essential fatty acids, 79(6), 215-222.

Sheean, M. E., McShane, E., Cheret, C., Walcher, J., Müller, T., Wulf-Goldenberg, A., ... & Meijer, D. (2014). Activation of MAPK overrides the termination of myelin growth and replaces Nrg1/ErbB3 signals during Schwann cell development and myelination. Genes & development, 28(3), 290-303.

Sheng, B., Liu, J., & Li, G. H. (2012). Metformin preconditioning protects Daphnia pulex from lethal hypoxic insult involving AMPK, HIF and mTOR signaling. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 163(1), 51-58.

Sherman, D. L., & Brophy, P. J. (2005). Mechanisms of axon ensheathment and myelin growth. Nature Reviews Neuroscience, 6(9), 683-690.

Sherman, D. L., Krols, M., Wu, L. M. N., Grove, M., Nave, K. A., Gangloff, Y. G., & Brophy, P. J. (2012). Arrest of myelination and reduced axon growth when Schwann cells lack mTOR. Journal of Neuroscience, 32(5), 1817-1825.

Shin, Y. K., Jang, S. Y., Lee, H. K., Jung, J., Suh, D. J., Seo, S. Y., & Park, H. T. (2010). Pathological adaptive responses of Schwann cells to endoplasmic reticulum stress in bortezomib-induced peripheral neuropathy. Glia, 58(16), 1961-1976.

Shukla, V., Shakya, A. K., Perez-Pinzon, M. A., & Dave, K. R. (2017). Cerebral ischemic damage in diabetes: an inflammatory perspective. Journal of neuroinflammation, 14(1), 21.

Shy, M. E. (2006). Peripheral neuropathies caused by mutations in the myelin protein zero. Journal of the neurological sciences, 242(1), 55-66.

Simpson, A. E. (1997). The cytochrome P450 4 (CYP4) family. General pharmacology, 28(3), 351.

Singh, B., Xu, Y., McLaughlin, T., Singh, V., Martinez, J. A., Krishnan, A., & Zochodne, D. W. (2012). Resistance to trophic neurite outgrowth of sensory neurons exposed to insulin. Journal of neurochemistry, 121(2), 263-276.

Singh, R., Kishore, L., & Kaur, N. (2014). Diabetic peripheral neuropathy: current perspective and future directions. Pharmacological research, 80, 21-35.

Snipes, G. J., Suter, U., Welcher, A. A., & Shooter, E. M. (1992). Characterization of a novel peripheral nervous system myelin protein (PMP-22/SR13). The Journal of cell biology, 117(1), 225-238.

Sodhi, K., Inoue, K., Gotlinger, K. H., Canestraro, M., Vanella, L., Kim, D. H., ... & Abraham, N. G. (2009). Epoxyeicosatrienoic acid agonist rescues the metabolic syndrome phenotype of HO-2-null mice. Journal of Pharmacology and Experimental Therapeutics, 331(3), 906-916.

Sodhi, K., Puri, N., Inoue, K., Falck, J. R., Schwartzman, M. L., & Abraham, N. G. (2012). EET agonist prevents adiposity and vascular dysfunction in rats fed a high fat diet via a decrease in Bach 1 and an increase in HO-1 levels. Prostaglandins & other lipid mediators, 98(3-4), 133-142.

Spector, A. A. (2009). Arachidonic acid cytochrome P450 epoxygenase pathway. Journal of lipid research, 50(Supplement), S52-S56.

Spector, A. A., & Kim, H. Y. (2015). Cytochrome P450 epoxygenase pathway of polyunsaturated fatty acid metabolism. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 1851(4), 356-365.

Spector, A. A., Fang, X., Snyder, G. D., & Weintraub, N. L. (2004). Epoxyeicosatrienoic acids (EETs): metabolism and biochemical function. Progress in lipid research, 43(1), 55-90.

Stattin, P., Björ, O., Ferrari, P., Lukanova, A., Lenner, P., Lindahl, B., ... & Kaaks, R. (2007).

Strömstedt, M., Hayashi, S., Zaphiropoulos, P. G., & Gustafsson, J. A. (1990). Cloning and characterization of a novel member of the cytochrome P450 subfamily IVA in rat prostate. DNA and cell biology, 9(8), 569.

Strömstedt, M., Warner, M., & Gustafsson, J. Å. (1994). Cytochrome P450s of the 4A subfamily in the brain. Journal of neurochemistry, 63(2), 671-676.

Stys, P. K. (2005). General mechanisms of axonal damage and its prevention. Journal of the neurological sciences, 233(1), 3-13.

Sun, H., Lee, P., Yan, C., Gao, N., Wang, J., Fan, X., & Yu, F. S. (2018). Inhibition of soluble epoxide hydrolase 2 ameliorates diabetic keratopathy and impaired wound healing in mouse corneas. Diabetes, 67(6), 1162-1172.

Sura, P., Sura, R., EnayetAllah, A. E., & Grant, D. F. (2008). Distribution and expression of soluble epoxide hydrolase in human brain. Journal of Histochemistry & Cytochemistry, 56(6), 551-559.

Suter U. 1997. "Myelin: keeping nerves well wrapped up." Current biology : CB 7 (1): R21-3.

Świderska, E., Strycharz, J., Wróblewski, A., Szemraj, J., Drzewoski, J., & Śliwińska, A. (2018). Role of PI3K/AKT pathway in insulin-mediated glucose uptake. In Blood Glucose Levels. IntechOpen.

Takahashi, H., Goto, T., Shoji, T., Tanito, M., Park, M., & Chihara, E. (2006). Diabetes-associated retinal nerve fiber damage evaluated with scanning laser polarimetry. Am J Ophthalmol, 142(1), 88-94. doi:10.1016/j.ajo.2006.02.016

Takeuchi, K., Renic, M., Bohman, Q. C., Harder, D. R., Miyata, N., & Roman, R. J. (2005). Reversal of delayed vasospasm by an inhibitor of the synthesis of 20-HETE. American Journal of Physiology-Heart and Circulatory Physiology, 289(5), H2203-H2211.

Talbott, E. O., Zborowski, J. V., Rager, J. R., Kip, K. E., Xu, X., & Orchard, T. J. (2007). Polycystic ovarian syndrome (PCOS): a significant contributor to the overall burden of type 2 diabetes in women. Journal of Women's Health, 16(2), 191-197.

Taveggia, C., Zanazzi, G., Petrylak, A., Yano, H., Rosenbluth, J., Einheber, S., ... & Chao, M. V. (2005). Neuregulin-1 type III determines the ensheathment fate of axons. Neuron, 47(5), 681-694.

Terashvili, M., Tseng, L. F., Wu, H. E., Narayanan, J., Hart, L. M., Falck, J. R., ... & Harder, D. R. (2008). Antinociception produced by 14, 15-epoxyeicosatrienoic acid is mediated by the activation of β-endorphin and met-enkephalin in the rat ventrolateral periaqueductal gray. Journal of Pharmacology and Experimental Therapeutics, 326(2), 614-622.

Tesfaye, S., Boulton, A. J., Dyck, P. J., Freeman, R., Horowitz, M., Kempler, P., ... & Bernardi, L. (2010). Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. Diabetes care,33(10), 2285-2293.

Tesfaye, S., Harris, N., Jakubowski, J. J., Mody, C., Wilson, R. M., Rennie, I. G., & Ward, J. D. (1993). Impaired blood flow and arterio-venous shunting in human diabetic neuropathy: a novel technique of nerve photography and fluorescein angiography. Diabetologia, 36(12), 1266-1274.

Tesfaye, S., Vileikyte, L., Rayman, G., Sindrup, S. H., Perkins, B. A., Baconja, M., ... & Toronto Expert Panel on Diabetic Neuropathy. (2011). Painful diabetic peripheral neuropathy: consensus recommendations on diagnosis, assessment and management. Diabetes/metabolism research and reviews, 27(7), 629-638.

Theken, K. N., Deng, Y., Schuck, R. N., Oni-Orisan, A., Miller, T. M., Alison Kannon, M., ... & Lee, C. R. (2012). Enalapril reverses high-fat diet-induced alterations in cytochrome P450-mediated eicosanoid metabolism. American Journal of Physiology-Endocrinology and Metabolism, 302(5), E500-E509.

Tillu, D. V., Melemedjian, O. K., Asiedu, M. N., Qu, N., De Felice, M., Dussor, G., & Price, T. J. (2012). Resveratrol engages AMPK to attenuate ERK and mTOR signaling in sensory neurons and inhibits incision-induced acute and chronic pain. Molecular pain, 8(1), 5.

Toosizadeh, N., Mohler, J., Armstrong, D. G., Talal, T. K., & Najafi, B. (2015). The influence of diabetic peripheral neuropathy on local postural muscle and central sensory feedback balance control. PloS one, 10(8), e0135255.

Toth, P., Csiszar, A., Sosnowska, D., Tucsek, Z., Cseplo, P., Springo, Z., Tarantini, S., Sonntag, W.E., Ungvari, Z. & Koller, A. (2013). Treatment with the cytochrome P450 ω-hydroxylase inhibitor HET0016 attenuates cerebrovascular inflammation, oxidative stress and improves vasomotor function in spontaneously hypertensive rats. British journal of pharmacology, 168(8), 1878-1888.

Treins, C., Alliouachene, S., Hassouna, R., Xie, Y., Birnbaum, M. J., & Pende, M. (2012). The combined deletion of S6K1 and Akt2 deteriorates glycemic control in a high-fat diet. Molecular and cellular biology, 32(19), 4001-4011.

Tuck, R. R., Schmelzer, J. D., & Low, P. A. (1984). Endoneurial blood flow and oxygen tension in the sciatic nerves of rats with experimental diabetic neuropathy. Brain, 107(3), 935-950.

Tunaru, S., Bonnavion, R., Brandenburger, I., Preussner, J., Thomas, D., Scholich, K., & Offermanns, S. (2018). 20-HETE promotes glucose-stimulated insulin secretion in an autocrine manner through FFAR1. Nature communications, 9(1), 1-11.

Turpaev, K. T. (2002). Reactive oxygen species and regulation of gene expression. Biochemistry (Moscow), 67(3), 281-292.

UK Prospective Diabetes Study (UKPDS) Group. (1998). Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). The lancet, 352(9131), 837-853.

Um, S. H., Frigerio, F., Watanabe, M., Picard, F., Joaquin, M., Sticker, M., . . . Auwerx, J. (2004). Absence of S6K1 protects against age-and diet-induced obesity while enhancing insulin sensitivity. Nature, 431(7005), 200-205.

Vaitheesvaran, B., LeRoith, D., & Kurland, I. J. (2010). MKR mice have increased dynamic glucose disposal despite metabolic inflexibility, and hepatic and peripheral insulin insensitivity. Diabetologia, 53(10), 2224-2232.

Valmadrid, C. T., Klein, R., Moss, S. E., & Klein, B. E. (2000). The risk of cardiovascular disease mortality associated with microalbuminuria and gross proteinuria in persons with older-onset diabetes mellitus. Archives of internal medicine, 160(8), 1093-1100.

Vantyghem, M. C., & Press, M. (2006, September). Management strategies for brittle diabetes. In Annales d'endocrinologie (Vol. 67, No. 4, pp. 287-294). Elsevier Masson.

Vella, S., Buetow, L., Royle, P., Livingstone, S., Colhoun, H. M., & Petrie, J. R. (2010). The use of metformin in type 1 diabetes: a systematic review of efficacy. Diabetologia, 53(5), 809-820.

Vincent, A. M., Callaghan, B. C., Smith, A. L., & Feldman, E. L. (2011). Diabetic neuropathy: cellular mechanisms as therapeutic targets. Nature Reviews Neurology, 7(10), 573.

Vincent, A. M., Hayes, J. M., McLean, L. L., Vivekanandan-Giri, A., Pennathur, S., & Feldman, E. L. (2009). Dyslipidemia-induced neuropathy in mice: the role of oxLDL/LOX-1. Diabetes, 58(10), 2376-2385.

Vincent, A. M., Mclean, L. L., Backus, C., & Feldman, E. L. (2005). Short-term hyperglycemia produces oxidative damage and apoptosis in neurons. The FASEB Journal, 19(6), 638-640.

Vincent, A. M., Perrone, L., Sullivan, K. A., Backus, C., Sastry, A. M., Lastoskie, C., & Feldman, E. L. (2007). Receptor for advanced glycation end products activation injures primary sensory neurons via oxidative stress. Endocrinology, 148(2), 548-558.

Vincent, A. M., Russell, J. W., Low, P., & Feldman, E. L. (2004). Oxidative stress in the pathogenesis of diabetic neuropathy. Endocrine reviews, 25(4), 612-628.

Vincent, A. M., Russell, J. W., Sullivan, K. A., Backus, C., Hayes, J. M., McLean, L. L., & Feldman, E. L. (2007). SOD2 protects neurons from injury in cell culture and animal models of diabetic neuropathy. Experimental neurology, 208(2), 216-227.

Vucicevic, L., Misirkic, M., Kristina, J., Vilimanovich, U., Sudar, E., Isenovic, E., ... & Trajkovic, V. (2011). Compound C induces protective autophagy in cancer cells through AMPK inhibition-independent blockade of Akt/mTOR pathway. Autophagy, 7(1), 40-50.

Wagner, K. M., McReynolds, C. B., Schmidt, W. K., & Hammock, B. D. (2017). Soluble epoxide hydrolase as a therapeutic target for pain, inflammatory and neurodegenerative diseases. Pharmacology & therapeutics, 180, 62-76.

Wagner, K., Gilda, J., Yang, J., Wan, D., Morisseau, C., Gomes, A. V., & Hammock, B. D. (2017). Soluble epoxide hydrolase inhibition alleviates neuropathy in Akita (Ins2 Akita) mice. Behavioural brain research, 326, 69-76.

Wagner, K., Inceoglu, B., & Hammock, B. D. (2011). Soluble epoxide hydrolase inhibition, epoxygenated fatty acids and nociception. Prostaglandins & other lipid mediators, 96(1-4), 76-83.

Wagner, K., Yang, J., Inceoglu, B., & Hammock, B. D. (2014). Soluble epoxide hydrolase inhibition is antinociceptive in a mouse model of diabetic neuropathy. The Journal of Pain, 15(9), 907-914.

Wang, C. P., Hung, W. C., Yu, T. H., Chiu, C. A., Lu, L. F., Chung, F. M., ... & Lee, Y. J. (2010). Genetic variation in the G-50T polymorphism of the cytochrome P450 epoxygenase CYP2J2 gene and the risk of younger onset type 2 diabetes among Chinese population: potential interaction with body mass index and family history. Experimental and clinical endocrinology & diabetes, 118(06), 346-352.

Wang, L., Luo, G., Zhang, L. F., & Geng, H. X. (2018). Neuroprotective effects of epoxyeicosatrienoic acids. Prostaglandins & other lipid mediators, 138, 9-14.

Wang, S., Kobayashi, K., Kogure, Y., Yamanaka, H., Yamamoto, S., Yagi, H., ... & Dai, Y. (2018). Negative Regulation of TRPA1 by AMPK in Primary Sensory Neurons as a Potential Mechanism of Painful Diabetic Neuropathy. Diabetes, 67(1), 98-109.

Wang, X., Shen, C. L., Dyson, M. T., Yin, X., Schiffer, R. B., Grammas, P., & Stocco, D. M. (2006). The involvement of epoxygenase metabolites of arachidonic acid in cAMP-stimulated steroidogenesis

and steroidogenic acute regulatory protein gene expression. Journal of endocrinology, 190(3), 871-878.

Wang, X., Sun, D., Hu, Y., Xu, X., Jiang, W., Shang, H., & Cui, D. (2019). The roles of oxidative stress and Beclin-1 in the autophagosome clearance impairment triggered by cardiac arrest. Free Radical Biology and Medicine, 136, 87-95.

Wang, Z., Yadav, A. S., Leskova, W., & Harris, N. R. (2011). Inhibition of 20-HETE attenuates diabetes-induced decreases in retinal hemodynamics.Experimental eye research, 93(1), 108-113.

Ward, N. C., Chen, K., Li, C., Croft, K. D., & Keaney Jr, J. F. (2011). Chronic activation of AMP-activated protein kinase prevents 20-hydroxyeicosatetraenoic acid-induced endothelial dysfunction. Clinical and Experimental Pharmacology and Physiology, 38(5), 328-333.

Webb, J. L., Ravikumar, B., Atkins, J., Skepper, J. N., & Rubinsztein, D. C. (2003). α-Synuclein is degraded by both autophagy and the proteasome. Journal of Biological Chemistry, 278(27), 25009-25013.

Wen, H., Östman, J., Bubb, K. J., Panayiotou, C., Priestley, J. V., Baker, M. D., & Ahluwalia, A. (2012). 20-Hydroxyeicosatetraenoic acid (20-HETE) is a novel activator of transient receptor potential vanilloid 1 (TRPV1) channel. Journal of Biological Chemistry, 287(17), 13868-13876.

Westphal, C., Konkel, A., & Schunck, W. H. (2011). CYP-eicosanoids—a new link between omega-3 fatty acids and cardiac disease?. Prostaglandins & other lipid mediators, 96(1-4), 99-108.

Widstrom, R. L., Norris, A. W., & Spector, A. A. (2001). Binding of cytochrome P450 monooxygenase and lipoxygenase pathway products by heart fatty acid-binding protein. Biochemistry, 40(4), 1070-1076.

Widstrom, R. L., Norris, A. W., Van Der Veer, J., & Spector, A. A. (2003). Fatty acid-binding proteins inhibit hydration of epoxyeicosatrienoic acids by soluble epoxide hydrolase. Biochemistry, 42(40), 11762-11767.

Williams, J. M., Murphy, S., Burke, M., & Roman, R. J. (2010). 20-HETE: a new target for the treatment of hypertension. Journal of cardiovascular pharmacology, 56(4), 336.

Witmer, A. N., Blaauwgeers, H. G., Weich, H. A., Alitalo, K., Vrensen, G. F., & Schlingemann, R. O. (2002). Altered expression patterns of VEGF receptors in human diabetic retina and in experimental VEGF-induced retinopathy in monkey. Invest Ophthalmol Vis Sci, 43(3), 849-857.

Wong, K. M., & Beirowski, B. (2018). Multiple lines of inhibitory feedback on AKT kinase in Schwann cells lacking TSC1/2 hint at distinct functions of mTORC1 and AKT in nerve development. Communicative & Integrative Biology, 11(1), e1433441.

Wrabetz L, Feltri ML, Quattrini A, Imperiale D, Previtali S, D'Antonio M, Martini R, Yin X, Trapp BD, Zhou L, Chiu SY, Messing A (2000) P(0) glycoprotein overexpression causes congenital hypomyelination of pe- ripheral nerves. J Cell Biol 148:1021–1034

Wu, H. F., Yen, H. J., Huang, C. C., Lee, Y. C., Wu, S. Z., Lee, T. S., & Lin, H. C. (2015). Soluble epoxide hydrolase inhibitor enhances synaptic neurotransmission and plasticity in mouse prefrontal cortex. Journal of biomedical science, 22(1), 94.

Wu, L. Y., Li, M., Qu, M. L., Li, X., Pi, L. H., Chen, Z., ... & Wang, S. (2017). High glucose up-regulates Semaphorin 3A expression via the mTOR signaling pathway in keratinocytes: A potential mechanism and therapeutic target for diabetic small fiber neuropathy. Molecular and cellular endocrinology.

Wu, Y., Xue, B., Li, X., & Liu, H. (2012). Puerarin prevents high glucose-induced apoptosis of Schwann cells by inhibiting oxidative stress. Neural Regeneration Research, 7(33), 2583–2591.

Xia, R., Sun, L., Liao, J., Li, H., You, X., Xu, D., ... & Yang, G. Y. (2019). Inhibition of Pancreatic Carcinoma Growth Through Enhancing ω-3 Epoxy Polyunsaturated Fatty Acid Profile by Inhibition of Soluble Epoxide Hydrolase. Anticancer research, 39(7), 3651-3660.

Xie, Z., Lau, K., Eby, B., Lozano, P., He, C., Pennington, B., ... & Kem, D. (2011). Improvement of cardiac functions by chronic metformin treatment is associated with enhanced cardiac autophagy in diabetic OVE26 mice. Diabetes, 60(6), 1770-1778.

Xu, J. T., Tu, H. Y., Xin, W. J., Liu, X. G., Zhang, G. H., & Zhai, C. H. (2007). Activation of phosphatidylinositol 3-kinase and protein kinase B/Akt in dorsal root ganglia and spinal cord contributes to the neuropathic pain induced by spinal nerve ligation in rats. Experimental neurology, 206(2), 269-279.

Xu, X., Zhao, C. X., Wang, L., Tu, L., Fang, X., Zheng, C., ... & Wang, D. W. (2010). Increased CYP2J3 expression reduces insulin resistance in fructose-treated rats and db/db mice. Diabetes, 59(4), 997-1005.

Xu, Y. J., Tappia, P. S., Neki, N. S., & Dhalla, N. S. (2014). Prevention of diabetes-induced cardiovascular complications upon treatment with antioxidants. Heart failure reviews, 19(1), 113-121.

Yan, L., Xie, M., Lu, H., Zhang, H., Shi, M., Zhang, Y., ... & Yang, T. (2018). Anti-apoptotic effect of IGF1 on Schwann exposed to hyperglycemia is mediated by neuritin, a novel neurotrophic factor. Molecular neurobiology, 55(1), 495-505.

Yang, X., Yao, W., Shi, H., Liu, H., Li, Y., Gao, Y., ... & Xu, L. (2016). Paeoniflorin protects Schwann cells against high glucose induced oxidative injury by activating Nrf2/ARE pathway and inhibiting apoptosis.Journal of ethnopharmacology, 185, 361-369.

Yao, F., Zhang, M., & Chen, L. (2016). 5'-Monophosphate-activated protein kinase (AMPK) improves autophagic activity in diabetes and diabetic complications. Acta Pharmaceutica Sinica B, 6(1), 20-25.

Yao, R., & Cooper, G. M. (1995). Requirement for phosphatidylinositol-3 kinase in the prevention of apoptosis by nerve growth factor. Science,267(5206), 2003.

Ye, P., Hua, L., Jiao, Y., Li, Z., Qin, S., Fu, J., ... & Ji, Y. (2016). Functional up-regulation of Nav1. 8 sodium channel on dorsal root ganglia neurons contributes to the induction of scorpion sting pain. Acta biochimica et biophysica Sinica, 48(2), 132-144.

Yerra, V. G., Gundu, C., Bachewal, P., & Kumar, A. (2016). Autophagy: the missing link in diabetic neuropathy?. Medical hypotheses, 86, 120-128.

Yerra, V. G., Kalvala, A. K., & Kumar, A. (2017). Isoliquiritigenin reduces oxidative damage and alleviates mitochondrial impairment by SIRT1 activation in experimental diabetic neuropathy. The Journal of nutritional biochemistry, 47, 41-52.

Yin X, Kidd GJ, Wrabetz L, Feltri ML, Messing A, Trapp BD (2000) Schwann cell myelination requires timely and precise targeting of P(0) protein. J Cell Biol 148:1009 –1020.

Yousif, M. H. M., Benter, I. F., & Roman, R. J. (2009). Cytochrome P450 metabolites of arachidonic acid play a role in the enhanced cardiac dysfunction in diabetic rats following ischaemic reperfusion injury. Autonomic and Autacoid Pharmacology, 29(1-2), 33-41.

Yorston, D. (2014). Anti-VEGF drugs in the prevention of blindness. Community Eye Health, 27(87), 44-46.

Yu, H., Zhu, L., Li, C., Sha, D., Pan, H., & Wang, N. (2015). ERK1/2 and AKT are vital factors in regulation of the migration of rat Schwann cells. Journal of Veterinary Medical Science, 77(4), 427-432.

Yu, H., Zhu, L., Li, C., Sha, D., Pan, H., Wang, N., & Ma, S. (2015). ERK1/2 and AKT are vital factors in regulation of the migration of rat Schwann cells. The Journal of veterinary medical science/the Japanese Society of Veterinary Science, 77(4), 427-432

Yu, M., Cambj-Sapunar, L., Kehl, F., Maier, K. G., Takeuchi, K., Miyata, N., Ishimoto, T., Reddy, L.M., Falck, J.R., Gebremedhin, D. & Harder, D. R. (2004). Effects of a 20-HETE antagonist and agonists on cerebral vascular tone. European journal of pharmacology, 486(3), 297-306.

Yu, M., Lopez, B., Dos Santos, E. A., Falck, J. R., & Roman, R. J. (2007). Effects of 20-HETE on Na+ transport and Na+-K+-ATPase activity in the thick ascending loop of Henle. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 292(6), R2400-R2405.

Yu, M., McAndrew, R. P., Al-Saghir, R., Maier, K. G., Medhora, M., Roman, R. J., & Jacobs, E. R. (2002). Nitric oxide contributes to 20-HETE-induced relaxation of pulmonary arteries. Journal of Applied Physiology, 93(4), 1391-1399.

Yu, W., Chen, L., Yang, Y. Q., Falck, J. R., Guo, A. M., Li, Y., & Yang, J. (2011). Cytochrome P450 ω-hydroxylase promotes angiogenesis and metastasis by upregulation of VEGF and MMP-9 in non-small cell lung cancer. Cancer chemotherapy and pharmacology, 68(3), 619-629.

Yu, Z., Xu, F., Huse, L. M., Morisseau, C., Draper, A. J., Newman, J. W., ... & Zeldin, D. C. (2000). Soluble epoxide hydrolase regulates hydrolysis of vasoactive epoxyeicosatrienoic acids. Circulation research, 87(11), 992-998.

Zeldin, D. C. (2001). Epoxygenase pathways of arachidonic acid metabolism. Journal of Biological Chemistry, 276(39), 36059-36062.

Zeldin, D. C., Foley, J., Boyle, J. E., Moomaw, C. R., Tomer, K. B., Parker, C., ... & Wu, S. (1997). Predominant expression of an arachidonate epoxygenase in islets of Langerhans cells in human and rat pancreas. Endocrinology, 138(3), 1338-1346.

Zeng, Q., Han, Y., Bao, Y., Li, W., Li, X., Shen, X., ... & Sun, C. (2010). 20-HETE increases NADPH oxidase-derived ROS production and stimulates the L-type Ca2+ channel via a PKC-dependent

mechanism in cardiomyocytes. American Journal of Physiology-Heart and Circulatory Physiology, 299(4), H1109-H1117.

aZhang, Y., Hoda, M. N., Zheng, X., Li, W., Luo, P., Maddipati, K. R., ... & Wang, M. H. (2014). Combined therapy with COX-2 inhibitor and 20-HETE inhibitor reduces colon tumor growth and the adverse effects of ischemic stroke associated with COX-2 inhibition. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 307(6), R693-R703.

bZhang, G., Panigrahy, D., Hwang, S. H., Yang, J., Mahakian, L. M., Wettersten, H. I., ... & Hammock, B. D. (2014). Dual inhibition of cyclooxygenase-2 and soluble epoxide hydrolase synergistically suppresses primary tumor growth and metastasis. Proceedings of the National Academy of Sciences, 111(30), 11127-11132.

cZhang, Y., Li, J., Wang, T., & Wang, J. (2014). Amplitude of sensory nerve action potential in early stage diabetic peripheral neuropathy: an analysis of 500 cases. Neural regeneration research, 9(14), 1389.

Zhang, B., Lai, G., Wu, J., Sun, R., Xu, R., Yang, X., ... & Zhao, Y. (2016). 20-HETE attenuates the response of glucose-stimulated insulin secretion through the AKT/GSK-3β/Glut2 pathway. Endocrine, 54(2), 371-382.

Zhang, F., Kaide, J. I., Rodriguez-Mulero, F., Abraham, N. G., & Nasjletti, A. (2001). Vasoregulatory function of the heme-heme oxygenase-carbon monoxide system. American journal of hypertension, 14(S3), 62S-67S.

Zhang, G., Panigrahy, D., Mahakian, L. M., Yang, J., Liu, J. Y., Lee, K. S. S., ... & Hwang, S. H. (2013). Epoxy metabolites of docosahexaenoic acid (DHA) inhibit angiogenesis, tumor growth, and metastasis. Proceedings of the national academy of sciences, 110(16), 6530-6535.

Zhang, L. N., Vincelette, J., Chen, D., Gless, R. D., Anandan, S. K., Rubanyi, G. M., ... & Wang, Y. X. J. (2011). Inhibition of soluble epoxide hydrolase attenuates endothelial dysfunction in animal models of diabetes, obesity and hypertension. European journal of pharmacology, 654(1), 68-74.

Zhang, Y., Li, J., Wang, T., & Wang, J. (2014). Amplitude of sensory nerve action potential in early stage diabetic peripheral neuropathy: an analysis of 500 cases. Neural regeneration research, 9(14), 1389.

Zhao, G., Wang, J., Xu, X., Jing, Y., Tu, L., Li, X., ... & Zeldin, D. C. (2012). Epoxyeicosatrienoic acids protect rat hearts against tumor necrosis factor-α-induced injury. Journal of lipid research, 53(3), 456-466.

Zhao, W. C., Zhang, B., Liao, M. J., Zhang, W. X., He, W. Y., Wang, H. B., & Yang, C. X. (2014). Curcumin ameliorated diabetic neuropathy partially by inhibition of NADPH oxidase mediating oxidative stress in the spinal cord.Neuroscience letters, 560, 81-85.

Zhao, X., Dey, A., Romanko, O. P., Stepp, D. W., Wang, M. H., Zhou, Y., ... & Imig, J. D. (2005). Decreased epoxygenase and increased epoxide hydrolase expression in the mesenteric artery of obese Zucker rats. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 288(1), R188-R196.

Zheng, H., Zheng, Y., Zhao, L., Chen, M., Bai, G., Hu, Y., ... & Gao, H. (2017). Cognitive decline in type 2 diabetic db/db mice may be associated with brain region-specific metabolic disorders. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 1863(1), 266-273.

Zhou, D., Zhou, M., Wang, Z., Fu, Y., Jia, M., Wang, X., ... & Lu, Y. (2019). Progranulin alleviates podocyte injury via regulating CAMKK/AMPK-mediated autophagy under diabetic conditions. Journal of Molecular Medicine, 97(11), 1507-1520.

Zhu, D., Birks, E. K., Dawson, C. A., Patel, M., Falck, J. R., Presberg, K., Roman, R.J. & Jacobs, E. R. (2000). Hypoxic pulmonary vasoconstriction is modified byP-450 metabolites. American Journal of Physiology-Heart and Circulatory Physiology, 279(4), H1526-H1533.

Zhu, D., Effros, R. M., Harder, D. R., Roman, R. J., & Jacobs, E. R. (1998). Tissue sources of cytochrome P450 4A and 20-HETE synthesis in rabbit lungs. American journal of respiratory cell and molecular biology, 19(1), 121-128.

Zhu, D., Zhang, C., Medhora, M., & Jacobs, E. R. (2002). CYP4A mRNA, protein, and product in rat lungs: novel localization in vascular endothelium. Journal of Applied Physiology, 93(1), 330-337.

Ziegler, D., Papanas, N., Vinik, A. I., & Shaw, J. E. (2014). Epidemiology of polyneuropathy in diabetes and prediabetes. Handbook of clinical neurology,126, 3.

Zinzalla, V., Stracka, D., Oppliger, W., & Hall, M. N. (2011). Activation of mTORC2 by association with the ribosome. Cell, 144(5), 757-768.

Zochodne, D. W. (1999). Diabetic neuropathies: features and mechanisms. Brain pathology, 9(2), 369-391.

Zoncu, R., Efeyan, A., & Sabatini, D. M. (2011). mTOR: from growth signal integration to cancer, diabetes and ageing. Nature reviews Molecular cell biology, 12(1), 21-35.

Zonta, M., Angulo, M. C., Gobbo, S., Rosengarten, B., Hossmann, K. A., Pozzan, T., & Carmignoto, G. (2003). Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation. Nature neuroscience, 6(1), 43-50.

Zou, A. P., Fleming, J. T., Falck, J. R., Jacobs, E. R., Gebremedhin, D., Harder, D. R., & Roman, R. J. (1996). 20-HETE is an endogenous inhibitor of the large-conductance Ca (2+)-activated K+ channel in renal arterioles. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 270(1), R228-R237.