INTERNATIONAL DIABETIC NEUROPATHY CONSORTIUM

ANNUAL REPORT 2020-2021

AARHUS UNIVERSITY
DEPARTMENT OF CLINICAL MEDICINE

IDNC

TABLE OF CONTENTS

novo nordisk fonden

The International Diabetic Neuropathy Consortium was awarded a grant of 60 million Danish Kroner for a 6-year period from the Novo Nordisk Foundation in December 2014, with project start-up in May 2015.

Novo Nordisk Foundation: Grant number NNF14OC0011633

PREFACE

> Diabetic neuropathy continues to be a major complication of diabetes – it is associated with increased mortality and threatens life quality for millions of people world-wide and the disease is often first diagnosed when irreversible nerve damage has developed.

It is a pleasure to send you the 6th annual report of the International Diabetic Neuropathy Consortium (IDNC). But it is also a sad moment because this report also represent the closure of a consortium between 4 universities for the last 6 years. A Challenge grant from the Novo Nordisk Foundation allowed experts from four universities, the University of Michigan, Ann Arbor, USA, University of Oxford, UK the University of Southern Denmark and Aarhus University, Denmark to study diabetic neuropathy. For the past 6 years we have been able to collaborate and add new small bricks to our understanding of this debilitating disorder. IDNC has been a unique construction, where researchers from different background and different countries have joined forces to study various aspects of diabetic neuropathy ranging from basic pathophysiological, genetics, epidemiology, and diagnostic issues to preventive aspects.

A unique feature of the consortium has been the opportunity to work with large diabetes cohorts such as the ADDITION, a cohort from UK and DD2 the latter now with more than 10.000 individuals included. In the consortium, we have explored the epidemiology of diabetic neuropathy and found that obesity and in particular central obesity represent a risk factor for the development of this disease. We have had a specific interest in studying differences between painful and non-painful diabetic neuropathy. Among our findings, patients developing painful neuropathy have more severe and long lasting diabetes and show more clinical signs of nerve damage than those with non-painful neuropathy. In addition we have now within DOLORisk, an EU funded consortium been able also to look into genetic aspects of painful and non-painful diabetic neuropathy. This project includes both GWAS studies and whole genome sequencing. The genetic studies are not finished yet. However, some exciting findings have been observed including a significant linkage between the genomic locus encoding the gene KCNT2 expressed in sensory neurons and neuropathic pain. Further results from this exciting area are expected late 2022 and thereafter. Another promising area is the metabolomic changes in diabetic neuropathy. Using transcriptomics, metabolomics, and metabolic flux analysis our US colleagues are looking for nerve-specific differences in diabetic mice models and in patients with diabetes. Their recent clinical studies in man from our ADDITION cohort indicate that drivers of neuropathy include not only glucose, but also obesity and associated components of the metabolic syndrome. The clinical studies have looked at threshold changes in patients with painful and non-painful diabetic neuropathy and identification of the best diagnostic measures for small fiber damage in neuropathy.

One of the essential elements of the IDNC is the education and training of young doctors and students within the area of diabetic neuropathy and to contribute to international exchange between fellows and post docs. The IDNC has so far resulted in completion of 7 PhD studies, an additional 6 PhD studies are under way. Moreover, a number of fellows have completed post doc programs within the consortium. Within the consortium we have so far published 122 peer reviewed original paper, 15 peer-reviewed reviews and a few other papers and participated in a large number of congresses and meetings. It is expected that the ongoing PhD studies will result in a number of additional papers within the next 1-2 years.

IDNC and its members have in the last year, despite the difficulties caused by the Covid-19 pandemia in 2020 and 2021 been active in their research I would like to express my sincere thanks to all collaborators of the IDNC for their enthusiasm and for their continuing commitment to the IDNC vision. Let me also use this opportunity to thank our international scientific advisory board and Aarhus University for their support and help. Although the current IDNC will end by 2022 efforts are under way to continue the work set out by IDNC in a new program. In this respect, the establishment of Danish Steno Diabetes centers represent a particular opportunity to implement new knowledge about diabetic neuropathy into clinical practice to the benefit of patients.

Troels Staehelin Jensen
Director of the IDNC

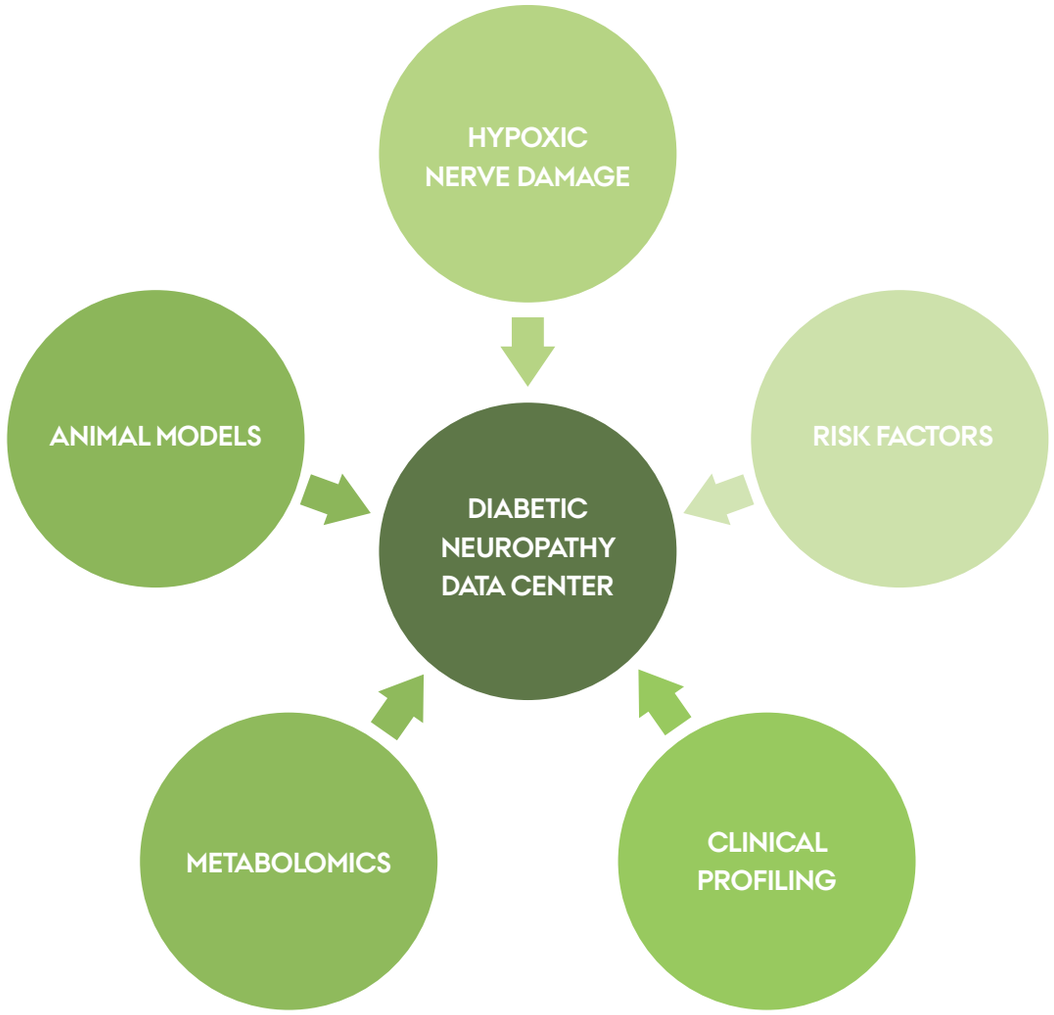
IDNC AT A GLANCE

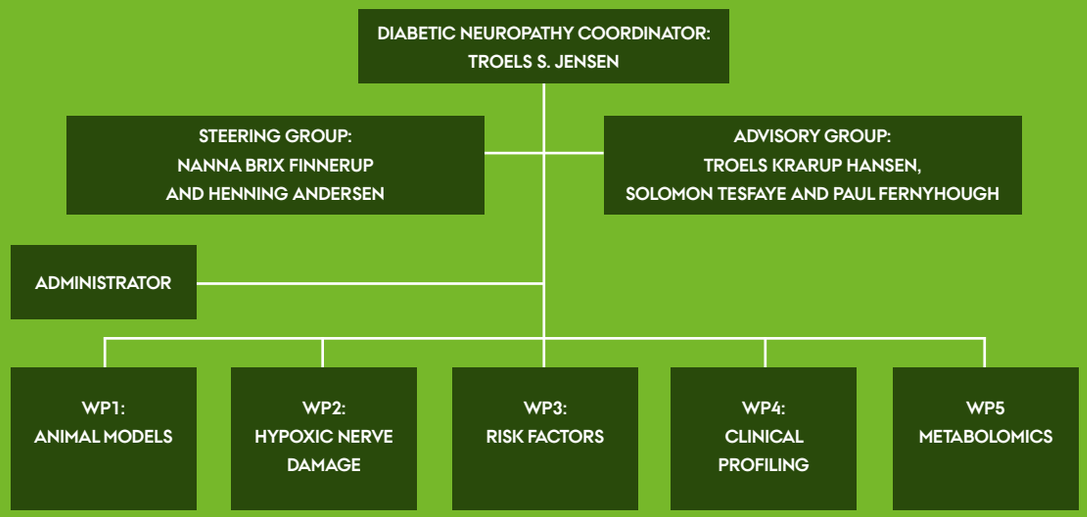
Vision: To be a leading research group on diabetic neuropathy in Denmark with an outreach to the world.

Mission: To study and unravel pathophysiological mechanisms of diabetic neuropathy, contribute to early identification, improve and develop a uniform international classification in order to better treat and prevent the detrimental consequences of diabetic neuropathy. The IDNC does so by bringing researchers and clinicians together in a stimulating and multidisciplinary environment in order to integrate and facilitate translational aspects of diabetic neuropathy.

Structure: A series of work packages in which four universities: University of Michigan, University of Oxford, South Danish University and Aarhus University work together in an effort to understand mechanisms of diabetic neuropathy, risk factors for neuropathy and pain and the clinical and metabolic profile of diabetic neuropathy.

Funding: A 6-year Novo Nordisk Foundation Challenge Program grant (Grant number NNF14OC0011633).





ORGANIZATION

The management structure of the IDNC consists of the director, the steering group and the scientific advisory board. The steering group helps to identify important research initiatives and implement them in the IDNC. The internationally renowned scientific advisory board helps identifying research questions critical to improving our understanding of diabetic neuropathy.

Aarhus University, Health hosts and supports the administration of the IDNC. The Danish Pain Research Center at Aarhus University Hospital provides housing facility for IDNC management.

DIRECTOR

PROFESSOR TROELS STAEHELIN JENSEN
Director of the INDC, Aarhus University Hospital, DK.

STEERING GROUP

PROFESSOR NANNA BRIX FINNERUP
Aarhus University, DK.

PROFESSOR HENNING ANDERSEN
Aarhus University Hospital, DK.

SCIENTIFIC ADVISORY BOARD

PROFESSOR TROELS K. HANSEN
Chairman
Aarhus University Hospital, DK.

PROFESSOR SOLOMON TESFAYE
University of Sheffield, UK.

PROFESSOR PAUL FERNYHOUGH
University of Manitoba, Canada.

RESEARCH GROUPS

HEALTH SYSTEM
UNIVERSITY OF MICHIGAN

UNIVERSITY OF MICHIGAN

Department of Neurology
Eva L. Feldman
Brian Callaghan

UNIVERSITY OF OXFORD

UNIVERSITY OF OXFORD

Nuffield Department of Clinical Neurosciences
David L. H. Bennett
Andreas Themistocleous

SDU
OUH
Odense Universitetshospital

UNIVERSITY OF SOUTHERN DENMARK AND ODENSE UNIVERSITY HOSPITAL

Department of Neurology
Søren Sindrup
Mustapha Itani
Thomas Krøigaard

AARHUS UNIVERSITY
Aarhus University Hospital

AARHUS UNIVERSITY AND AARHUS UNIVERSITY HOSPITAL

Department of Public Health
Morten Charles
Signe Toft Andersen

Department of Clinical Epidemiology
Reimar Thomsen
Diana Hedevang Christensen
Frederik Pagh Kristensen

Center of Functionally Integrative Neuroscience and MINDLab
Leif Østergaard
Anete Dudele

Department of Neurophysiology
Hatice Tankisi
Alexander Gramm Kristensen

Department of Biomedicine
Christian Vægter
Nadia Pereira Gonçalves

Department of Neurology
Karolina Snopek
Henning Andersen
Astrid Terkelsen

Comparative Medicine Lab
Michael Pedersen
Martin Nors Skov

Danish Pain Research Center
Pall Karlsson
Sandra Sif Gylfadottir
Thorsten Kamlarczyk Rasmussen
Laura Linnea Määta
Ellen Schaldemose
Vinnie Faber Rasmussen
Peter Kolind Brask

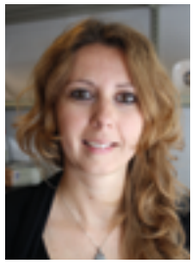
IDNC Administration and Technical Staff at Danish Pain Research Center
Bente Christensen
Rud Bugge Sørensen
Signe Toft Andersen
Irene Breinholt

WP1: ANIMAL MODELS OF DIABETIC NEUROPATHY

Mouse models of diabetic neuropathy represent important tools to understand the pathophysiological mechanisms of nerve damage in diabetes. A classical model in diabetes is the streptozotocin (STZ) model for type 1 diabetes. In the IDNC, we use mainly mice models for type 2 diabetic neuropathy.

This work package will assess the development of diabetic neuropathy over time in murine diabetes models and correlate behavioral and physiological assessments with changes in metabolism and lipid profile. In other studies, this work package focuses on Schwann cells and their relation to diabetic neuropathy.

WP1: SCHWANN CELLS AND THEIR ROLE IN DIABETIC NEUROPATHY



Nádia Pereira Gonçalves, *DVM, PhD addresses the role of Schwann cells in diabetic neuropathy in her postdoc project. Nadia´s research is now focused on studying cell-to-cell communication via exosomes and establish a new preclinical model for the study of -synuclein associated sensory nerve degeneration and neuropathic pain in Parkinson's disease Associate Professor Christian Bjerregaard Vægter led the research (Department of Biomedicine, Aarhus University, Denmark).*

Diabetic neuropathy has an incidence as high as 50% in people with diabetes and is characterized by damage to neurons, Schwann cells and blood vessels within the peripheral nervous system. The low-affinity neurotrophin receptor p75 (p75^{NTR}), particularly expressed by the Schwann cells in the peripheral nerve, has previously been reported to play a role in developmental myelination and cell survival/death. Increased levels of p75^{NTR}, in the endoneurium and plasma from people with diabetes and in rodent models have been observed, proposing that this receptor might be involved in the pathogenesis of diabetic neuropathy. Therefore, in this study, we addressed this hypothesis by utilizing a mouse model of selective nerve growth factor receptor (*Ngfr*) deletion in Schwann cells (SC-p75^{NTR}-KO). Electron microscopy of sciatic nerves from mice with high fat diet induced obesity demonstrated how loss of Schwann cell–p75NTR aggravated axonal atrophy and loss of C-fibers (Fig. 1). RNA sequencing disclosed several pre-clinical signaling alterations in the diabetic peripheral nerves, dependent on Schwann cell p75^{NTR} signaling, specially related with lysosome, phagosome, and immune pathways (Fig. 2). Morphological and biochemical analyses identified abundant lysosomes and autophagosomes in the C-fiber axoplasm of the diabetic SC-p75^{NTR}-KO nerves (Fig. 1), which together with increased Cathepsin B protein levels corroborates gene upregulation from the phagolysosomal pathways. Altogether, this study demonstrates that Schwann cell p75^{NTR} deficiency amplifies diabetic neuropathy disease by triggering over-activation of immune-related pathways and increased lysosomal stress.

Nevertheless, significant data was collected with the RNA sequencing analysis of mouse sciatic nerves, where different gene expression patterns were observed between WT mice versus WT mice fed with an HFD. In the future, we aim at evaluating protein expression levels locally in the PNS and systemically, aiming at finding a potential biomarker for diabetic neuropathy or a novel therapeutic target.

This work completed the proposed working package, it was selected for oral presentation in the PNS virtual meeting 2020, June 27th-30th, and recently published in *Glia.*

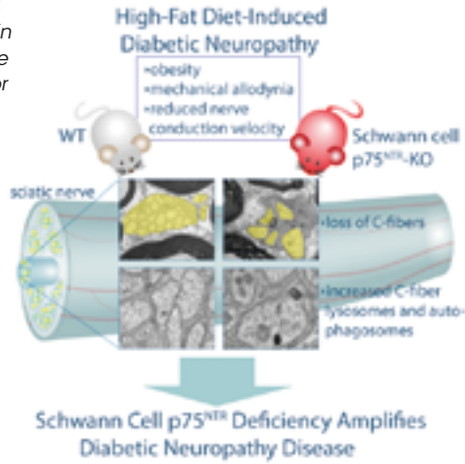


Fig. 1. *Graphical abstract denoting the approach utilized in this study and summarizing the main results. Yellow mask in the electron microscopy pictures highlights the C-fibers, which are significantly decreased in SC-p75^{NTR}-KO HFD as compared with WT HFD mice.*

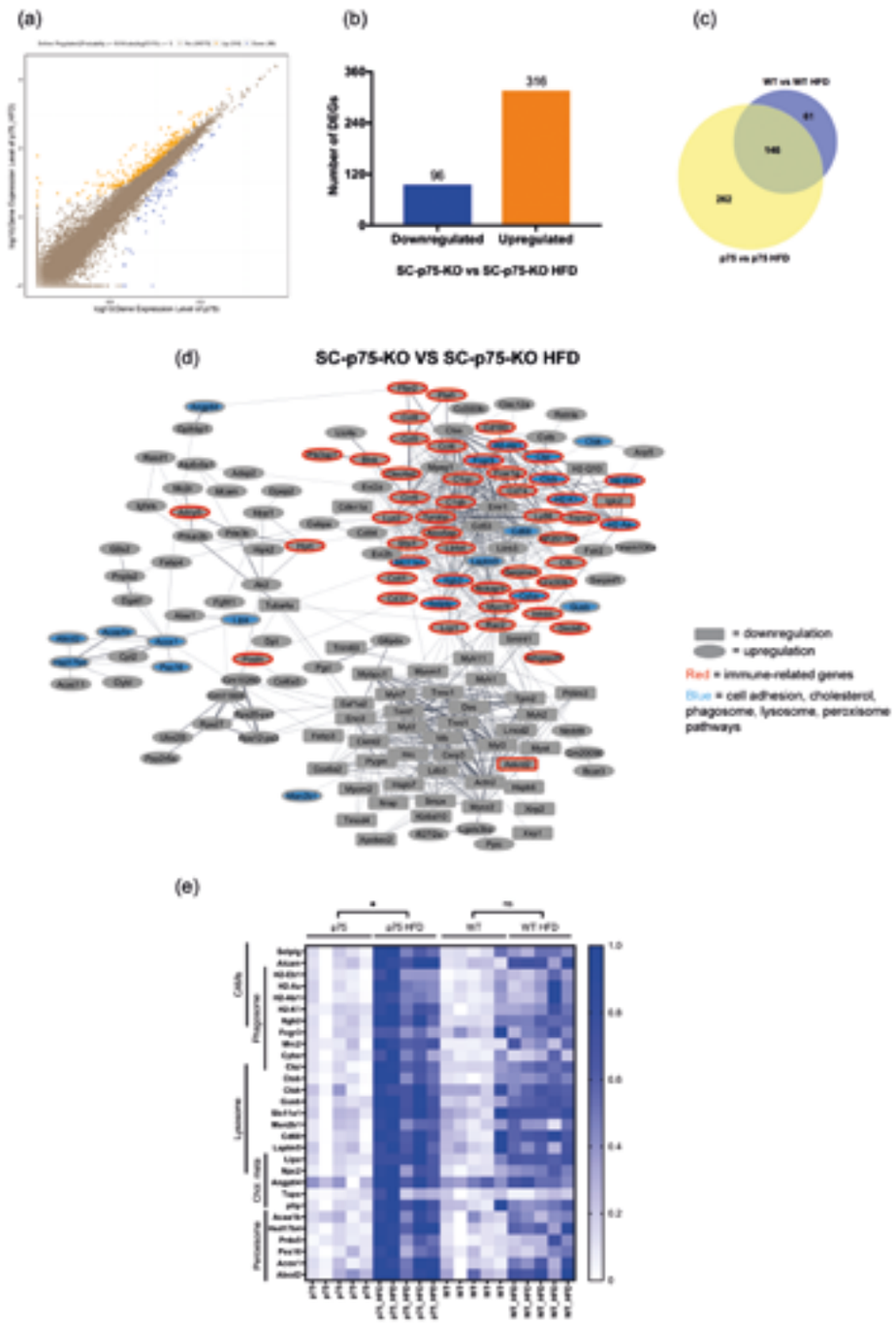
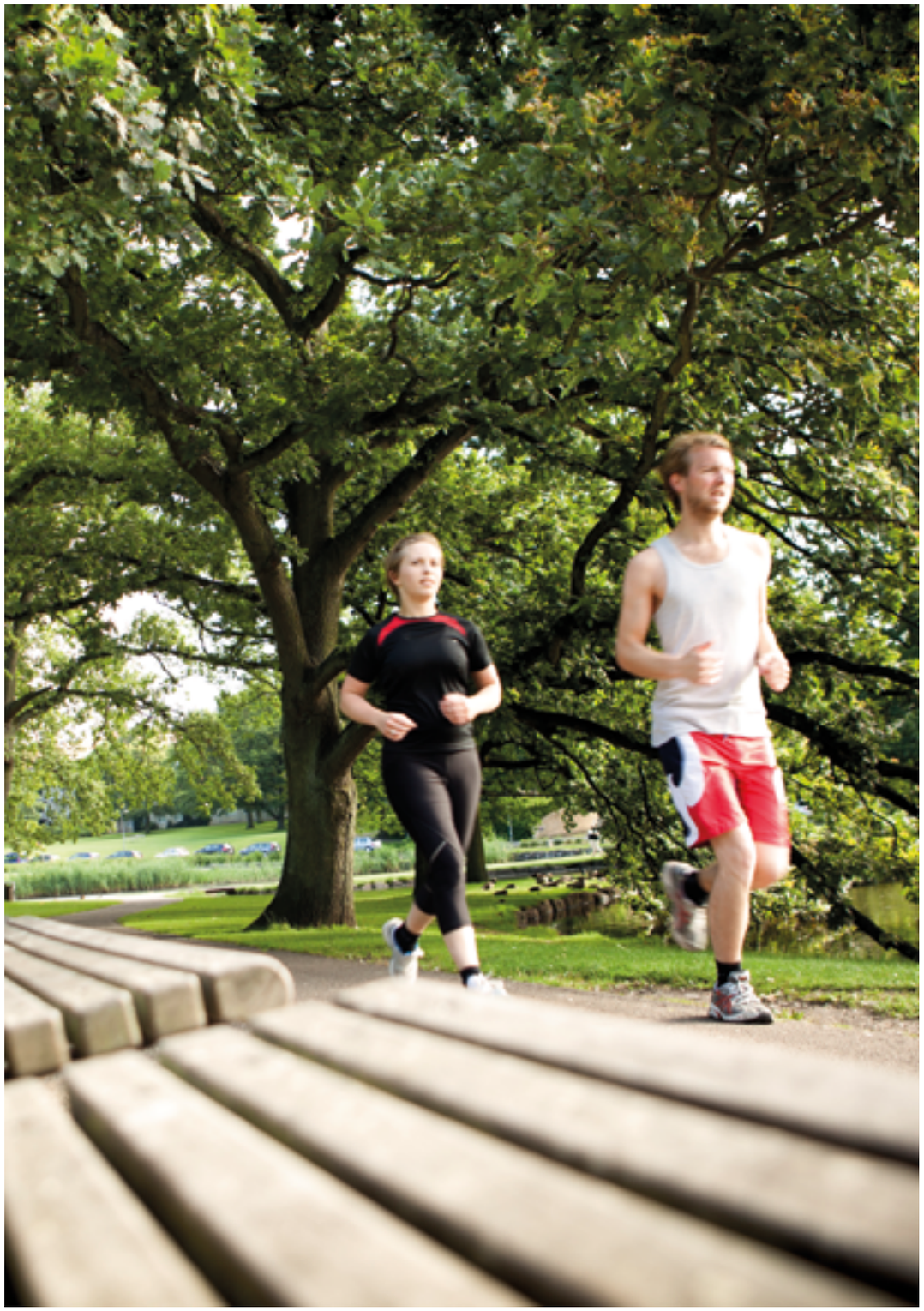


Fig. 2. *Glial p75^{NTR} modulates the inflammatory landscape and activation of lysosome and phagosome pathways in mice fed with an HFD.* ***(a)*** *log10 gene expression level graph and* ***(b)*** *a numerical graph demonstrating the upregulated (in orange) and downregulated (in blue) genes in SC-p75^{NTR}-KO mice fed with HFD relative to the SC-p75^{NTR}-KO group having the control diet.* ***(c)*** *Venn diagram illustrating WT versus WT HFD (blue) and p75 versus p75 HFD (yellow) with 146 DEG's in common. Two hundred and sixty-two genes are solely differently expressed in mice fed with HFD when Schwann cells lack p75^{NTR} while 61 genes are solely differently expressed in the WT situation and thus not depending on Schwann cell p75^{NTR} expression.* ***(d)*** *STRING network for those 262 regulated genes only activated in sciatic nerves from SC-p75^{NTR}-KO HFD mice. The 262 regulated genes produced a network of 162 genes in the STRING app in Cytoscape. The genes in the ellipse are upregulated and the genes in rectangular are downregulated in SC-p75NTR-KO HFD. Immune-related genes are depicted with a red boarder while those involved in cell adhesion, cholesterol, lysosome, phagosome and peroxisome pathways present a blue fill.* ***(e)*** *The heatmap shows FPKM values (normalized with min-max normalization) for all four analyzed groups. The genes are differently regulated only in the p75 versus p75 HFD condition and are annotated to cell adhesion (CAMs), phagosome, lysosome, cholesterol, and peroxisome-related genes. n = 5 mice per group. Statistics was based on the NOIseq method with fold changes ≥2.*



WP2: HYPOXIC NERVE DAMAGE

In this work package, the idea is that capillary flow is lost in diabetes due to endothelial glycocalyx damage, loss of pericytes, thickening of capillary basement membranes and elevated blood viscosity. Capillary flow in sural nerves of both type 1 and type 2 models for diabetic neuropathy are studied using two-photon microscopy combined with optical coherence tomography (OCT). With these methods, we test the hypothesis that elevated capillary transit time heterogeneity and reduced oxygen tension are involved in diabetic neuropathy in mice.

WP2: HYPOXIC NERVE DAMAGE

Anete Dudele *is a postdoc at the Center of Functionally Integrative neuroscience (CFIN), Aarhus University (DK) Professor Leif Østergaard leads the research.*

To study the involvement of microvascular function in development of diabetic peripheral neuropathy (DPN) in sural nerves, we used a mouse model in which DPN is a complication of type 2 diabetes produced by feeding a diet with high fat content (HFD). Using two photon in vivo laser scanning microscopy we studied sural nerve's microvascular function in mice with and without DPN. Figure 1 shows a comparison of microvascular anatomy of distal sural nerve in control and HFD mice at the age of 30 weeks. At this age HFD mice have increased body mass, blood glucose levels and signs of DPN. The anatomical differences between control and HFD mice are clearly visible in the figure. HFD mice have increased level of adipose tissue overlaying the nerve, and a more tortuous microvessel arrangement. The figure also shows an example of an in vivo two photon microscopy scan performed over a sural nerve vessel, where both vessel diameter and individual red blood cells can be visualized. This scanning modality allows to measure dynamic changes in vessel size, and in behavior of individual red blood cells – their number, movement speed and distribution.

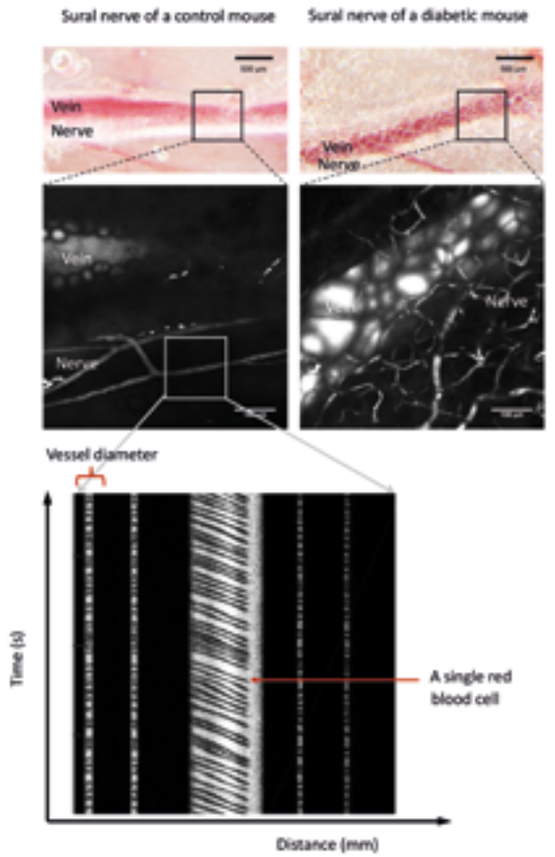


Figure 1

Using this approach, we have discovered that HFD and control mice have similar microvascular blood flow in the sural nerve during resting conditions, however, after exposure to nerve stimulation HFD mice respond differently than control mice (Figure 2). While control mice meet the increased energy demand of nerve stimulation by increasing recruitment of red blood cells to the tissue (linear density), without the accompanying increase in the red blood cell flux, HFD mice increase the overall microvascular blood flow by elevating both red blood cell velocity and flux.

Future studies will use physical activity as an intervention to study if improvement in microvascular function leads to improvement in nerve function. This will be done using voluntary exercise in mice on control diet or HFD during various stages of disease development, both as intervention and as prevention, simultaneously studying effect of exercise on nerve's microvascular function.

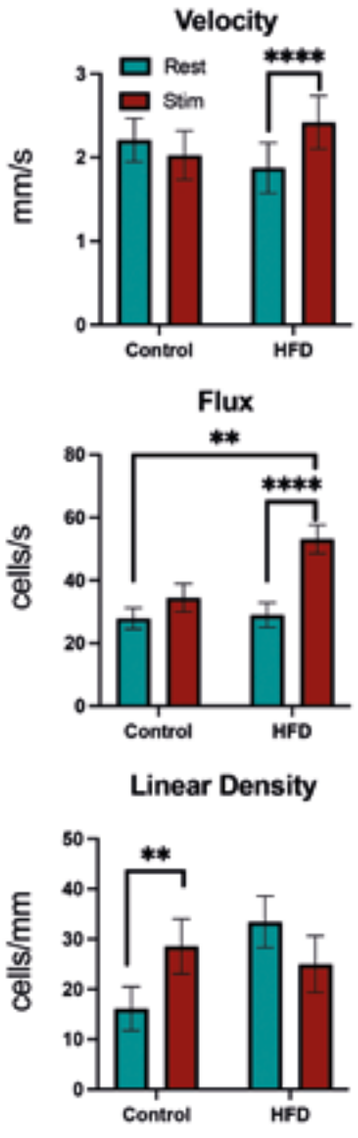


Figure 2

WP3: RISK FACTORS FOR DIABETIC NEUROPATHY

The ADDITION cohort and the DD2 cohort are the basis of this work package where we are studying:
1) the metabolic risk factors for diabetic neuropathy,
2) the effect of therapy on diabetic neuropathy and
3) the determinants for the clinical course of diabetic neuropathy and its prognosis.

The ADDITION Study (Anglo-Danish-Dutch Study of Intensive Treatment in People with Screen Detected Diabetes in Primary Care) is a study on early detection and intensive treatment of type 2 diabetes in primary care, where patients have been followed since their screen-detected diagnosis of type 2 diabetes 15 years ago.

The prospective Danish Center for Strategic Research in Type 2 Diabetes (DD2) cohort and biobank continuously enroll newly diagnosed type 2 diabetes patients throughout Denmark. The DD2 database was started in 2010 and currently holds approx. 10,000 individuals.

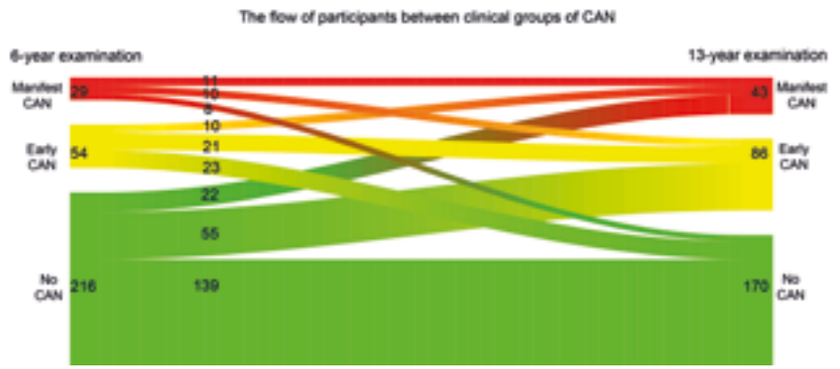
WP3: DIABETIC NEUROPATHY AND RISK STRATIFICATION IN DIABETES

***Signe Toft Andersen**, MD, PhD has continued in the IDNC as a part-time researcher and communications coordinator after she successfully defend her PhD thesis "Diabetic neuropathy and type 2 diabetes" based on data from the ADDITION-Denmark study in 2018.*

Signe completed her PhD "Diabetic neuropathy and type 2 diabetes" based on data from the ADDITION-Denmark study to study the presence and progression of diabetic neuropathy in this population of people with screen-detected type 2 diabetes. In short, lower prevalence and incidence of both diabetic polyneuropathy (DPN) and cardiovascular autonomic neuropathy (CAN) was seen compared with previous cohorts and irrespectively of using either symptom-based scores or clinical diagnostic measures to define diabetic neuropathy. Robust findings were seen across the various definitions of DPN and CAN for hyperglycemia and obesity as risk factors for the development of diabetic neuropathy. Improvements of CAN was seen over time for some participants – suggesting that CAN might be reversible (Figure).

Together with colleagues from the ADDITION-study, Signe prepares a register-based 15-year follow-up of the ADDITION-Denmark study which will provide the possibility of examining outcome-measures of people in this cohort based on their neuropathy-status (DPN and CAN) at 5- and 10-year follow-up of the cohort.

Signe is co-supervising MD Laura Linnea Määta in her PhD project on novel biomarkers of diabetic neuropathy using among others data from the ADDITION-Denmark.
At present, Signe is leading a clinical study "CANCAN study"; cardiovascular autonomic neuropathy for risk stratification in type 2 diabetes. This study assess the prevalence of subclinical cardiovascular autonomic neuropathy (CAN) in 300 people with type 2 diabetes in outpatient-clinics in Viborg, Herning and Aarhus to explore candidate measures for a future controlled trial to reduce morbidity and mortality in people with CAN and type 2 diabetes by investigating signs of undetected heart failure and adverse continuous glucose profiles. The project is based in Steno Diabetes Center Aarhus together with Professor Annelli Sandbæk. PhD student Jonas Frey Rosborg Schaarup is working with Signe on this project.



Number of participants and changes in their CAN status among defined categories; No CAN (green), early CAN (yellow) or manifest CAN (red) from 6- to the 13-year follow-up examination in ADDITION-Denmark (Andersen ST et al, Diabetes Care 2018)

WP3: DD2 COHORT AND REGISTRIES

***Diana Hedevang Christensen**, MD, PhD works as post doc at the Department of Clinical Epidemiology, Aarhus University Hospital (DK). Associate Professor Reimar W. Thomsen supervised the PhD project*

Diabetic polyneuropathy (DPN) is a devastating diabetes complication. The understanding of risk factors for DPN in type 2 diabetes and knowledge on why only some patients develop pain is limited. These topics were the focus of Diana Hedevang Christensen's PhD thesis.

The PhD thesis encompassed four studies. The first study provided a detailed description of the DD2 cohort. The second study was carried out together with MD, PhD Sif Sandra Gylfadottir using data from the IDNC-DD2 neuropathy questionnaire survey (N = 5,514). That study showed a prevalence of possible diabetic polyneuropathy (DPN) and painful DPN of 18% and 10%, respectively. Both DPN and painful DPN associated with lower quality of life and more symptoms of depression, anxiety, and sleep disturbance. The third study based on data from the IDNC-DD2 questionnaire and linked registers, investigated the association of metabolic and lifestyle factors measured at time of type 2 diabetes diagnosis with possible DPN and neuropathic pain occurrence in DPN at a median of 2.8 years later. Main findings from that study are that both BMI and central obesity measures associated with possible DPN and that central obesity associated with DPN independently of BMI. Moreover, other modifiable metabolic syndrome factors and unhealthy lifestyle habits associated with DPN. Neuropathic pain occurrence in DPN was associated with modifiable unhealthy lifestyle habits. The fourth study used register data and medical record review to examine the potential for using diagnosis and prescription codes to study non-painful and painful DPN as well as diabetic foot ulcers in future epidemiological studies. The main finding was a positive predictive value (PPV) of 74-78% for hospital-diagnosed DPN, thus, supporting a potential for register-based research on DPN risk and prognosis.

The DPN algorithm identified in Diana's fourth PhD study has been used in other register-based studies including a study by PhD student Frederik Pagh Kristensen that examined the impact of statin therapy on DPN risk. The study concluded that statin therapy is unlikely to increase or mitigate DPN risk in type 2 diabetes patients, although a small acute risk of harm cannot be excluded.

The IDNC-DD2 questionnaire data has formed the basis for several published and ongoing studies including a study by MD, PhD Karolina Snopek Khan which supported that identification of patients with symptoms of DPN by the use of the Michigan Neuropathy Screening Instrument questionnaire (MNSIq) may help in the identification of patients at risk of falls. Also, a collaborative study with MD, PhD Lasse Bjerg Hansen as first author took advantage of both DD2 and ADDITION-Denmark data and showed a markedly higher incidence rate of cardiovascular disease among those with DPN identified using the MNSIq, meta-analysis: IRR = 1.65 (95% CI: 1.41-1.95).

During Diana Hedevang Christensen's postdoc position, she has collaborated with the Danish Podiatrists to update the Danish Foot Status Database. The Danish Foot Status Database has collected data from the annual diabetes foot examinations performed by authorized Danish podiatrists since 2014, holding unique and detailed clinical foot data. Diana will soon receive data from the Danish Foot Status Database on all DD2 patients, which will enable use of the foot status data for research purposes for the first time.
Type 2 diabetes is a heterogeneous disease. In 2018, a Swedish cluster analysis identified four distinct type 2 diabetes clusters based on age at diagnosis, BMI, HbA1c, and homeostatic model assessment 2 (HOMA2) estimates of insulin resistance and beta-cell function. At the same time, a Danish study suggested the existence of three type 2 diabetes phenotypes based only on HOMA2 measures. Recently, Diana examined the subclassification of type 2 diabetes in the DD2 cohort. First, we conducted a data-driven cluster analysis of the Danish DD2 cohort (N = 3,529) using identical cluster-criteria as in the Swedish study. Next, we compared the identified clusters with the three Danish phenotypes. The two main findings of that study were: 1) we were able to replicate the four Swedish clusters, however, the Danish data seemed more compatible with three rather than four clusters, and 2) the largest overlap across the Swedish and the Danish classifications was observed for diabetes classes characterized by high insulin resistance/hyperinsulinemia. Currently, Frederik Pagh Kristensen uses the IDNC-DD2 questionnaire data to examine the association of the type 2 diabetes subclasses with DPN prevalence. This study may increase our understanding of the underlying mechanisms causing DPN.

***Figure 1:** In 2019, Diana defended her PhD thesis entitled "Diabetic polyneuropathy in type 2 diabetes – Prevalence, risk factors, mental health, and diagnostics validity".*

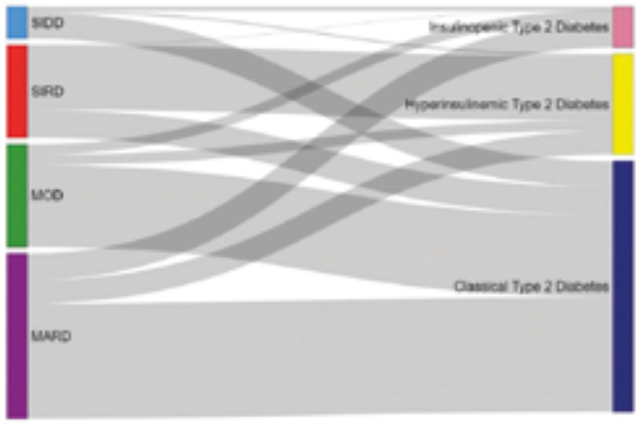
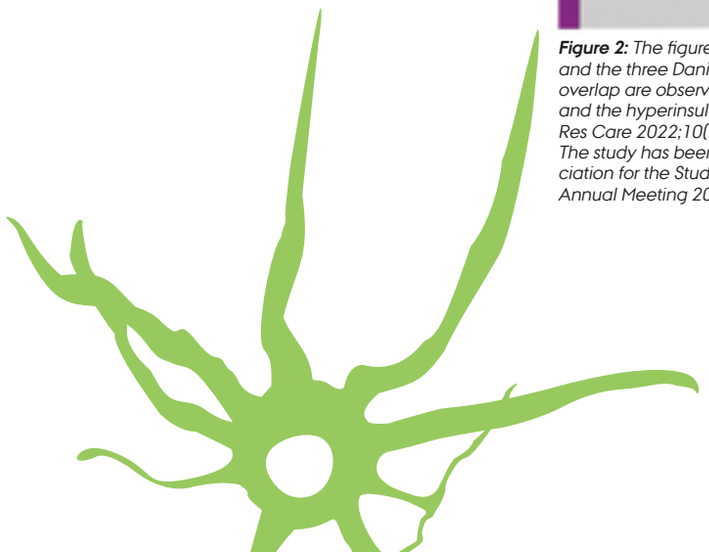


***Figure 2:** The figure shows the four replicated Swedish clusters on the left side and the three Danish phenotypes on the right. The most distinct and largest overlap are observed for the severe insulin resistant diabetes (SIRD) cluster and the hyperinsulinemic type 2 diabetes phenotype. BMJ Open Diabetes Res Care 2022;10(2):e002731.*
The study has been accepted for an oral presentation at the European Association for the Study of Diabetes (EASD) Annual Meeting 2022



WP3: DD2 COHORT AND REGISTRIES

Frederik Pagh Kristensen *is a PhD student at the Department of Clinical Epidemiology, Aarhus University Hospital (DK). Associate Professor Reimar W. Thomsen supervises the research.*

Dyslipidemia and central obesity are associated with increased risk of diabetic polyneuropathy (DPN), possibly through increased levels of reactive oxygen species and local nerve inflammation. Statins are widely used to lower cholesterol levels in type 2 diabetes patients and may reduce the risk of DPN due to lipid-lowering, anti-inflammatory, and anti-oxidative effects. However, studies have also associated statins with neurotoxicity. In *"Statin therapy and risk of polyneuropathy in type 2 diabetes: A Danish cohort study"*, a study published in Diabetes Care September 2020, Frederik Pagh Kristensen affiliated with IDNC from 2019-2020 examined whether statin therapy impacts the risk of DPN.

We used the nationwide population-based registries to conduct a cohort study of all incident type 2 diabetes patients in Denmark from 2002-2016 (N=259,625). Risk of developing DPN was assessed in non-users, new users, and prevalent users of statin therapy following patients from 6 months after diagnosed type 2 diabetes (index date). New users initiated statins between 180 days before and 180 days after their first diabetes record, while prevalent users had initiated statins before that period. Hospital-diagnosed DPN was defined using an algorithm based on hospital discharge diagnosis codes previously validated by the IDNC.

Over a median follow-up of 6.2 years (interquartile range 3.4-9.6), the cumulative incidence of DPN in the three statin user groups were similar (Figure 1). Incidence rates of DPN per 1000 person-years were 4.0 (95% CI 3.8-4.2) for new users, 3.8 (3.6-3.9) for prevalent users, and 3.8 (3.7-4.0) for non-users. Corresponding, adjusted hazard ratios (aHR) were 1.05 (95% CI, 0.98-1.11) in new users and 0.97 (95% CI, 0.91-1.04) in prevalent users, compared with non-users. New users were at slightly increased risk of DPN during the first year of follow-up (aHR 1.31 [95% CI 1.12-1.53]), this vanished after ≥2 years of follow-up (Figure 2). Findings were similar in on-treatment and propensity score-matched analyses, and with additional adjustment for pre-treatment blood lipid levels.

Overall, our study proposes that statin therapy is unlikely to increase or mitigate DPN risk in type 2 diabetes patients, although a small acute risk of harm cannot be excluded.

On January 1, 2022, Frederik Pagh Kristensen initiated a PhD about the impact of socioeconomic position, diabetes subgroups, and glucose-lowering therapy on the risk of DPN in type 2 diabetes. Using data from the Danish Centre for Strategic Research in Type 2 Diabetes (DD2), our results show that patients with hyperinsulinemia and severe insulin resistance associate with 30% higher prevalence of DPN compared to patients with low insulin levels but similar level of insulin resistance. These results confirm that hyperinsulinemia is a main driver of DPN beyond the effect of the metabolic syndrome and further reinforce the need for clinicians to consider insulin resistance and obesity as risk factors of DPN. The results have been accepted for a poster presentation at 38th International Conference on Pharmacoepidemiology & Therapeutic Risk Management (ICPE) (Saturday Aug 27 poster session B abstract 690), and as an oral presentation at the European Association for the Study of Diabetes (EASD) Annual Meeting (Thursday, oral presentation 32 "Pain or no pain?"). We expect to submit the manuscript for publication during fall 2022.

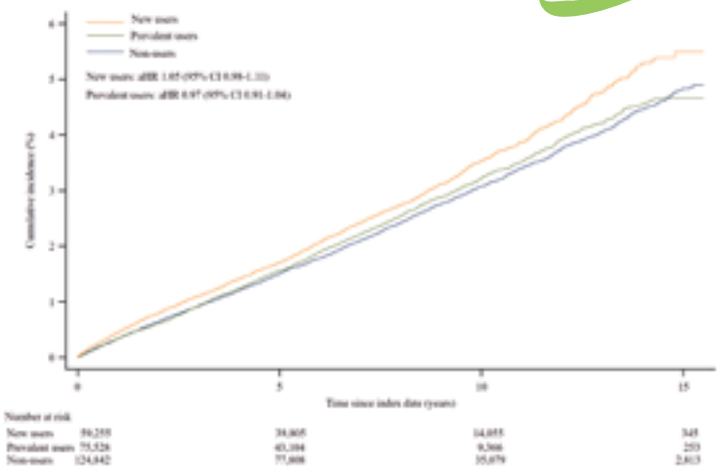


Figure 1. *Crude cumulative incidence of DPN by statin use, treating death as a competing risk.*

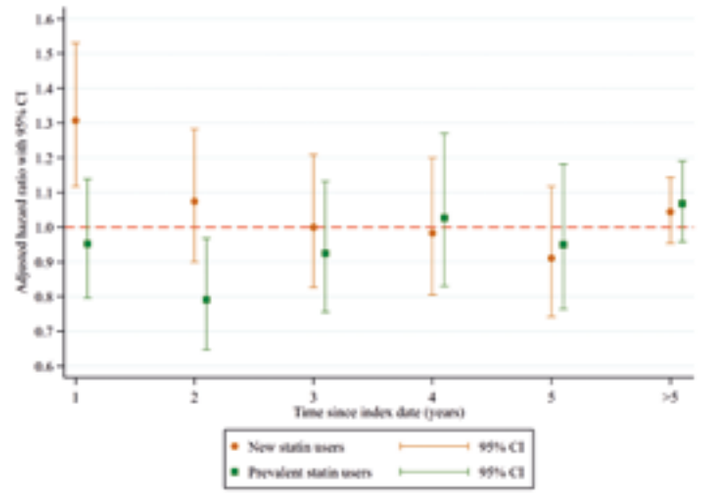


Figure 2. *Adjusted hazard ratios of DPN in one-year follow-up intervals, comparing new and prevalent statin users with statin non-users.*

WP3: A POTENTIAL NOVEL BIOMARKER OF DIABETIC NEUROPATHY

Laura Linnea Määttä *iis an MD and PhD student under IDNC and Danish Diabetes Academy with Professor Troels S. Jensen as main supervisor in collaboration with Steno Diabetes Center Aarhus and the Department of Clinical Biochemistry, Aarhus University Hospital*

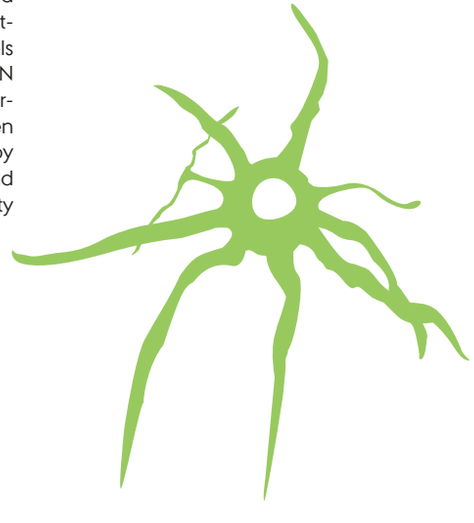
Laura works on the project "A Novel Biomarker to Assess the Presence and Progression of Diabetic Neuropathy" investigating the biomarker potential and properties of the neuronal protein neurofilament light chain (NfL) in diabetic polyneuropathy (DPN). The PhD program is co-financed by a PhD scholarship from the Danish Diabetes Academy.

We lack an easily applicable objective measure of the underlying nerve damage in DPN as the diagnosis of this complication often is delayed until late-stage disease when its symptoms and signs become clinically overt. A biomarker for DPN could thus provide a valuable tool for early detection and follow-up of DPN and focus the attention of physicians and patients on this complication.

The neuron specific structural protein NfL has gained attention for its biomarker properties within the field of neurology and recent studies have shown that levels of NfL reflect the presence and severity of certain inherited and acquired peripheral neuropathies with higher levels reflecting more severe disease. Thus, we hypothesized that the same would apply for DPN.

Findings from our proof-of-concept study on the serum levels of NfL in the ADDITON Denmark cohort indeed indicate that NfL has clear potential as a biomarker for DPN and suggest that higher NfL levels are associated with higher severity of DPN. We demonstrated a significant difference in serum NfL levels in participants with clinically confirmed DPN compared to participants without DPN. Furthermore, we show an association between NfL levels and DPN severity as reflected by nerve conduction studies (NCS) (Figure and Table). Higher levels of NCS abnormality were associated with increasing levels of NfL both for individual NCS measures and for the composite NCS scores. The two composite NCS scores consisted of established NCS criteria for DPN and of an experimental composite score designed to describe axonal abnormality. There was however no difference in the modelled increase in NfL levels per one unit increase in NCS composite score between the two scores. A manuscript for publication is in preparation.

To investigate the potential of NfL as a predictor for DPN and to characterize the longitudinal development of NfL levels in type 2 diabetes, we will analyze longitudinal levels of NfL in the same patient population of the ADDITION Denmark cohort. Further characterization of NfL levels in DN will be done in collaboration with Professor David Bennett from the Nuffield Department of Clinical Neurosciences on the PiNS/DOLORisk cohort in order to deepen description of the associations between NfL and the severity of DN.



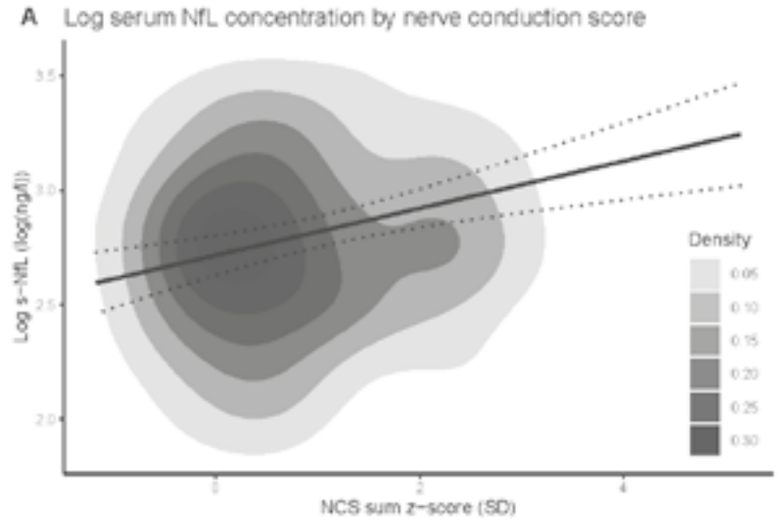


Figure. *Change in s-NfL levels by change in DPN severity as reflected by the NCS sum z-scores. Change in s-NfL concentration by change in the original NCS sum z-score (upper panel). Change in s-NfL concentration by change in the axonal NCS sum z-score (lower panel). An increase in the NCS sum z-score equals to increasing severity of nerve damage. S-NfL: serum NfL. NCS: nerve conduction study. SD: standard deviation.*

	Model 1		Model 2		Model 3		Model 4	
	ΔS-NfL (%)	95% CI	ΔS-NfL (%)	95% CI	ΔS-NfL (%)	95% CI	ΔS-NfL (%)	95% CI
NCS sum *z*-score (1 SD)	10.8*	5.2, 16.8	10.0*	5.4, 14.8	9.6*	3.0, 16.6	9.8*	3.1, 17.0
NCS axonal sum *z*-score (1 SD)	9.3†	3.9, 14.9	10.5†	6.1, 15.1	10.1†	4.5, 16.0	10.2†	4.5, 16.1

Table. *Linear models for the increase in s-NfL by change in DPN severity reflected by NCS sum z-scores. The change in s-NfL levels is expressed in percentage per 1 SD increase in nerve conduction sum z-score. Model 1: raw. Model 2: adjusted for age. Model 3: adjusted for age, sex and randomization group. Model 4: adjusted for age, sex, randomization group, eGFR and height. *p < 0.05, †p < 0.001. NCS: nerve conduction study.*

WP3: DEPRESSION IN DIABETES – POTENTIALLY RELATED WITH NEUROPATHY

Christopher Rohde *is a PhD student at the Department of Affective disorders, Aarhus University Hospital with Professor Søren Dinesen Østergaard as main supervisor*

Individuals with type 2 diabetes (T2D) are at a significantly increased risk of developing depression compared to the general population. However, it remains unknown why individuals with T2D have an increased risk of developing depression and how antidepressant treatment affects the clinical course of T2D. In my studies we have, therefore, tried to address the following aims by using data from the Danish nationwide health registers as well as from the Danish Centre for Strategic Research in Type 2 Diabetes (DD2) project cohort:

1. Is T2D a causal risk factor for developing depression?
2. Does diabetic neuropathy increase the risk of developing mental disorders?
3. How does preexisting depression affect metabolic control in T2D?
4. Why does preexisting depression associate with excess mortality in T2D?
5. How does antidepressant treatment affect metabolic control in T2D?

Our results suggest that T2D is a risk factor for developing depression, and that individuals with diabetic neuropathy have an even further increased risk of developing mental disorders. Preexisting depression in T2D associates with poor metabolic control and excess mortality. The excess mortality seems to depend on the unhealthy lifestyle and medical comorbidity accompanying depression. Lastly, antidepressant treatment initiation seems to associate with an improvement in HbA1c levels in individuals with T2D.

WP4: CLINICAL PROFILING

In this large work package, we will determine the presence of pain and sensory abnormalities in type 2 diabetes. The hypothesis is that based on a neurological history, and in particular clinical examination and detailed sensory profiling, it will be possible to find distinguishing characteristics in patients with type 2 diabetes, diabetic neuropathy and painful diabetic neuropathy.

The clinical profiling involves work done at the University of Oxford (UK), University of Southern Denmark and Aarhus University (DK). Clinical profiling in Denmark is carried out on the basis of the DD2 cohort. The examinations and profiling carried out at these three study sites are similar to those done in the major multicenter project DOLORisk, which aims to understand risk factors and determinants for neuropathic pain (dolorisk.eu). DOLORisk is funded by the European Commission Horizon 2020-PHC-2014 and is coordinated by IDNC affiliated researcher Professor David Bennett, Oxford University with Professor Nanna Brix Finnerup, Aarhus University as deputy project coordinator.

NUFFIELD DEPARTMENT OF CLINICAL NEUROSCIENCES

WP4: WHAT ARE THE DRIVERS OF NEUROPATHIC PAIN IN DIABETIC NEUROPATHY AND CAN WE BETTER STRATIFY THOSE PATIENTS WITH NEUROPATHIC PAIN?

David Bennett *is Professor of neurology and neurobiology at Nuffield Department of Clinical Neurosciences at the University of Oxford, UK working with Andreas C Themistocleous and Jishi John*

We have been continuing to investigate risk factors for the development of neuropathic pain (NeuP) in the context of diabetic neuropathy. This is being studied in the context of a highly phenotyped cohorts of patients with diabetic neuropathy (which have undergone the NeupSIG grading system for neuropathic pain) recruited in Oxford and across Europe as part of DOLORisk in collaboration with the IDNC. We have found genome wide significant linkage between the genomic locus encoding the gene KCNT2 and neuropathic pain intensity in DOLORisk (lead SNP rs79055518, P = 3.487e-08) in the gene KCNT2. This will be the largest GWAS so far conducted to assess neuropathic pain in painful diabetic neuropathy and is an exciting target given that KCNT2 is known to be highly expressed in sensory neurons. Further analysis is being undertaken on whole exome sequencing data in order to understand the impact of rare variants this will then be submitted for publication.

We have also been employing machine learning approaches in order to understand the impact of multiple factors on the risk of neuropathic pain ultimately with the aim of identifying individuals most at risk of neuropathic pain. We have now undertaken such an approach on independent discovery and replication cohorts. EQ5D index, the 10-item personality dimensions, HbA1c, Depression and Anxiety t-scores, age and Body Mass Index were consistently amongst the most powerful predictors in classifying painful vs painless Diabetic Peripheral Neuropathy. Painful DPN is associated with more depression, anxiety and certain personality traits. It is also associated with poorer self-reported quality of life, younger age, poor glucose control and high Body Mass Index (BMI). This has now been published (see Baskozos et al., 2022). We have also completed a re-phenotyping exercise in UK-BB for neuropathic pain. This data was released in 2020 and we have now undertaken an initial analysis of neuropathic pain in UK-Biobank showing that diabetes significantly increases the risk of neuropathic pain. This is the largest ever epidemiological assessment of neuropathic pain (medRxiv 2022.07.26.22278063; doi: https://doi.org/10.1101/2022.07.26.22278063) and we hope that a further data point collection in 2023 will mean that this is amenable to train machine learning approaches for prediction of neuropathic pain risk.

We have been examining biomarkers of excitability in relation to neuropathic pain using electro-physiological tools to discriminate between patients with painful and painless DPN. Threshold tracking is a neurophysiological tool that assesses large nerve fibre axonal excitability. It is an indirect measure of the ion channel excitability within myelinated nerve fibres. We have combined threshold tracking data between Oxford and Aarhus and find that threshold tracking does not discriminate between painful and painless diabetic neuropathy (Themistocleous et al., 2022). We also found that conditioned pain modulation was more efficient in patients with painful diabetic polyneuropathy than those with painless diabetic polyneuropathy (Granovsky et al., 2022).

We have also been active in clinical trials of the treatment of painful diabetic neuropathy. As part of a team led by S Tesfaye we have compared treatment pathways for painful diabetic neuropathy finding equivalent efficacy of first line analgesic agents (amitriptyline, duloxetine and pregabalin) but enhanced efficacy of combination therapy (Tesfaye et al., 2022).

WP4: CLINICAL PROFILING OF DIABETIC NEUROPATHY

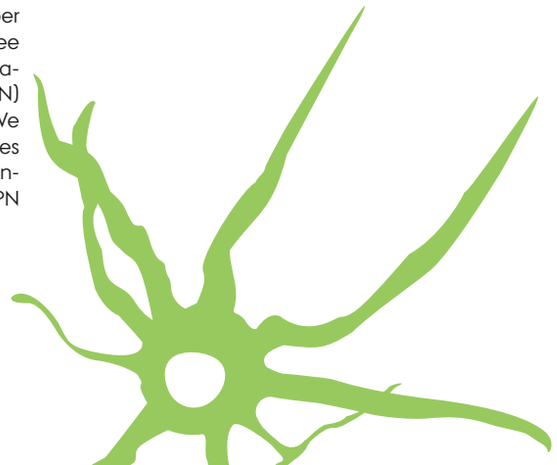
Mustapha Itani *is a neurologist at the department of neurology at Odense University Hospital. He completed his PhD study entitled "Diabetic Polyneuropathy- Prevalence, Diagnosis, Subtypes and Characteristics" at the University of Southern Denmark in May 2021 with Søren Sindrup as the main supervisor*

Mustapha Itani completed his PhD study entitled "Diabetic Polyneuropathy- Prevalence, Diagnosis, Subtypes and Characteristics" at the University of Southern Denmark in May 2021 and he has published three studies during his PhD study. The two first studies focused on the prevalence and clinical characteristics of diabetic polyneuropathy respectively while the third study compared the clinical characteristics of diabetic polyneuropathy to idiopathic polyneuropathy.

The first study was published in the European Journal of Neurology (EAN) in collaboration with Sandra Sif Gylfadottir. This study looked at the prevalence of diabetic polyneuropathy (DPN) and painful DPN. Based on a clinical diagnosis of probable and definite DPN and painful DPN, the prevalence was 43.9% for DPN and 11.5% for painful DPN. Furthermore, the study validated 2 well-known neuropathy scores, the Michigan Neuropathy Screening Instrument (MNSI) and the Dolour Neuropatique en 4 (DN4). These scores have not been validated previously in a population of type 2 diabetes. The sensitivity and specificity of MNSI ≥ 4 to detect definite DPN was 25.7% and 84.6%, respectively. The sensitivity and specificity of DN4 ≥ 3 with pain in both feet to detect definite painful DPN was 80% and 89.9%, respectively.

The second study was published in the Journal of the Peripheral Nervous System. This study examined the effect of different diagnostic models on the frequency of polyneuropathy subtypes based on nerve fiber diameter. DPN can be subtyped into three main subtypes: small fiber polyneuropathy (SFN), large fiber polyneuropathy (LFN) and mixed fiber polyneuropathy (MFN). We found a considerable variation in subtypes by applying different diagnostic models independent of the degree of certainty of DPN diagnosis. For probable and definite DPN, the frequency of subtypes across diagnostic models varied from: 1.4 to 13.1% for SFN, 9.3 to 21.5% for LFN, 51.4 to 83.2% for MFN, and 0.5 to 14.5% for non-classifiable neuropathy (NCN). For the definite DPN group, the frequency of subtypes varied from: 1.6 to 13.5% for SFN, 5.6 to 20.6% for LFN, 61.9 to 89.7% for MFN, and 0.0 to 6.3% for NCN.

The third and last study was published in British Medical Journal (BMJ) Neurology Open. This study compared the demographic, cardiovascular and neuropathy characteristics of the two most common polyneuropathies, namely DPN and idiopathic polyneuropathy (IPN). A total of 214 DPN patients were compared to a total of 88 patients with IPN. DPN was associated with a slightly higher autonomic- and total-score on the Neuropathy Symptom Score (NSS); lower frequency of hyperalgesia, allodynia, and decreased vibration on quantitative sensory testing (QST); lower intraepidermal nerve fiber density (IENFD) count and higher frequency of small fiber neuropathy (SFN).



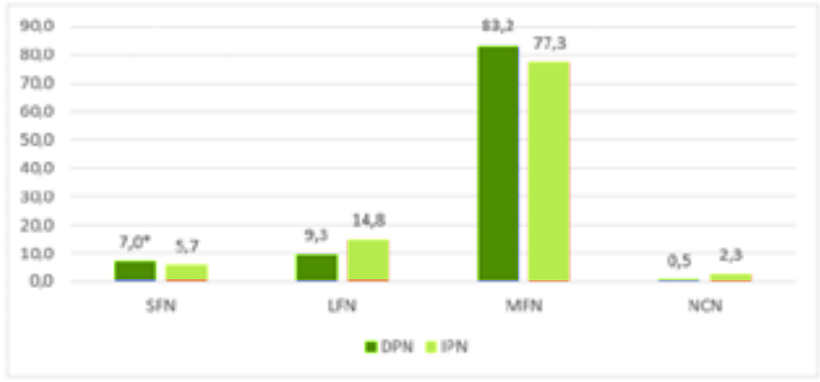
Four different models for defining polyneuropathy subtypes

Model 1	Model 2	Model 3	Model 4
Small fiber neuropathy: 1 of 4 criteria 1- Decreased or absent pinprick bedside 2- Decreased or absent thermal sensation bedside 3- Hypoesthesia on CDT or WDT 4- Abnormal IENFD **Largefiber neuropathy: 1 of 4 criteria** 1- Decreased or absent vibration bedside 2- Decreased or absent ankle reflexes 3- Hypoesthesia on VDT or MDT 4- Abnormal NCS	**Small fiber neuropathy: 2 of 4 criteria** 1- Decreased or absent pinprick bedside 2- Decreased or absent thermal sensation bedside 3- Hypoesthesia on CDT or WDT 4- Abnormal IENFD **Large fiber neuropathy: 2 of 4 criteria** 1- Decreased or absent vibration bedside 2- Decreased or absent ankle reflexes 3- Hypoesthesia on VDT or MDT 4- Abnormal NCS	**Small fiber neuropathy: All 4 criteria must be fulfilled** 1- Symptoms of thermal pain 2- Decreased or absent pinprick or temperature sensation bedside 3- Normal NCS 4- Abnormal IENFD OR abnormal CDT or WDT **Large fiber and mixed fiber neuropathy not defined**	**Small fiber neuropathy: 2 of 1-3 AND 4 must be fulfilled** 1- Decreased or absent pinprick and thermal sensation OR pinprick hyperalgesia OR thermal allodynia 2- Hypoesthesia on CDT or WDT 3- Abnormal IENFD 4- Absence of large fiber involvement (light touch OR vibratory OR proprioceptive sensory loss OR absent ankle reflexes OR muscle weakne OR abnormal NCS **Large fiber and mixed fiber neuropathy notdefined**

IENFD=Intraepidermal nerve fiber density. NCS=Nerve conduction studies. CDT=Cold detection threshold. WDT=Warm detection threshold. VDT=Vibration detection threshold. MDT= Mechanical detection threshold.

Figure from study 3:

The frequency of polyneuropathy subtypes in diabetic polyneuropathy compared to idiopathic polyneuropathy



DPN= Diabetic Polyneuropathy. IPN= Idiopathic Polyneuropathy. SFN= Small fiber polyneuropathy. LFN= Large fiber polyneuropathy. MFN= Mixed fiber polyneuropathy. NCN= Non-classifiable polyneuropathy. *: Significant difference in multivariate logistic regression.

WP4: CHALLENGES IN THE DIAGNOSIS OF DIABETIC POLYNEUROPATHY

Sandra Sif Gylfadottir *is a Neurologist and Clinical Associate Professor at Aarhus University and Department of Neurology, Aarhus University Hospital. She successfully defended her PhD Painful and non – painful diabetic polyneuropathy in March 2020 with Professor Nanna Brix Finnerup as main supervisor*

The main focus of my research is painful and non- painful diabetic polyneuropathy (DPN). In 2016 – 2018, in order to examine the prevalence of DPN, to compare patients with and without pain and address the diagnostic challenges in DPN, we invited participants from the nationwide Danish Centre for Strategic Research in type 2 Diabetes (DD2) cohort to participate in a clinical examination at two centers in Denmark, i.e. Aarhus and Odense. The Patients that were invited had participated in a questionnaire study conducted in 2016, where they answered questions on neuropathy and pain.

The examination consisted of a bedside neurological examination and history taking, nerve conduction studies (NCS), skin biopsy, quantitative sensory testing, confocal corneal microscopy (CCM) and blood samples. The participants also filled in standardized questionnaires on pain and neuropathy. For the definition of DPN we used the Toronto Consensus panel definition criteria and for painful DPN, the NeupSIG definition of neuropathic pain.

We included 389 diabetes patients and 97 controls of similar age and sex. The duration of diabetes was around 5 years and the glycemic control was excellent. The prevalence of definite DPN in this group of recently diagnosed diabetes patients was 22,7% and of them, 5.4% had painful DPN. Neuropathy scales with questions and clinical examination or clinical examination only were more accurate in the detection of definite DPN than questionnaires alone.

When we looked at patients with painful DPN compared to non painful DPN, patients with pain presented with more somatosensory nerve loss than patients with painless DPN in a bedside sensory examination. We also found that there was an intermediate group of patients with dysesthesia (unpleasant abnormal sensation), with more severe sensory loss than patients with DPN and less severe than patients with painful DPN, indicating that dyseshthetic DPN could represent a milder form of painful DPN. Lastly patients with painful DPN had worse mental health than patients with DPN and no pain.
In an additional study we assessed the diagnostic accuracy of CCM, a tool which quantifies C fibers in cornea and has been used to detect DPN. We also compared the diagnostic utility of CCM to other measures of DPN i.e. intra epidermal nerve fiber density (IENFD), cold and warm thresholds (CDT/WDT) and NCS. We included patients with probable DPN (symptoms of DPN, signs of DPN including absent or reduced ankle reflexes) in the study (Figure 1). We found a strong correlation between individual CCM measures, but weak or no correlation of CCM with cold and warm detection thresholds, IENFD or neuropathy severity (using the Toronto Clinical Neuropathy Score (TCNS)), and moderate to strong correlation between TCNS and QST and IENFD, respectively (Figure 2). The sensitivity of CCM in the diagnosis of DPN was low, but with a high specificity, where abnormal IENFD, NCS and CDT/WDT had higher sensitivities (Table 1). We concluded that CDT/WDT, IENFD and NCS can be used as supplementary measures in the diagnosis of DPN, but according to our results, the diagnostic utility of CCM alone compared to the other measures is limited (Table 2).

Future research: A follow up study of the patients and controls from the clinical cohort is ongoing. I will continue pursue research on neuropathy and pain through my work at the Danish Pain Research Center and the Headache and the Pain Clinic at Aarhus University Hospital.

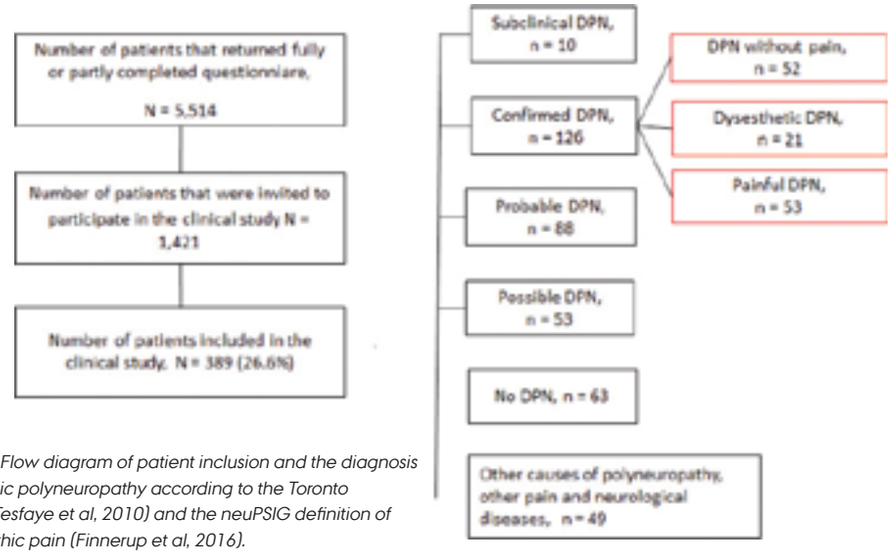


Figure 1. *Flow diagram of patient inclusion and the diagnosis of diabetic polyneuropathy according to the Toronto criteria (Tesfaye et al, 2010) and the neuPSIG definition of neuropathic pain (Finnerup et al, 2016).*

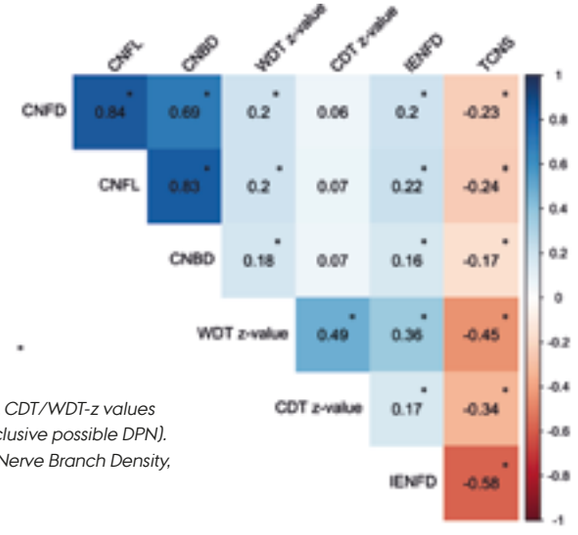


Figure 2. *Spearman's correlation matrix with CCM measures, CDT/WDT-z values and IENFD (n=374, Controls, no DPN and all DPN patients inclusive possible DPN). *p<0.05. CNFL: Corneal Nerve Fiber Length, CNBD: Corneal Nerve Branch Density, CNFD: Corneal Nerve Fiber Density. (Unpublished data)*

*	CNFL	CNBD	CNFD	CDT/WDT	IENFD	NCS
SENSITIVITY, % (95% CI)	14.4 (9.8;20.2)	8.2 (4.8:13.0)	7.7 (4.4;12.4)	30.5 (24.4;37.0)	51.1 (43.7;58.5)	37.1 (30.6;44.1)
SPECIFICITY, % (95% CI)	95.7 (88.0;99.1)	95.7 (88.0;99.1)	95.7 (86.0;99.1)	84.9 (74.6;92.2)	90.0 (79.5;96.2)	93.0 (84.3;97.7)

Table 1. *Sensitivity, specificity of CNFL, CNBD, CNFD, warm and cold thresholds and IENFD in the diagnosis of DPN (probable DPN vs no DPN). *None of the measures were a part of the DPN diagnosis.*

WP4: MOTOR DYSFUNCTION IN DIABETIC NEUROPATHY

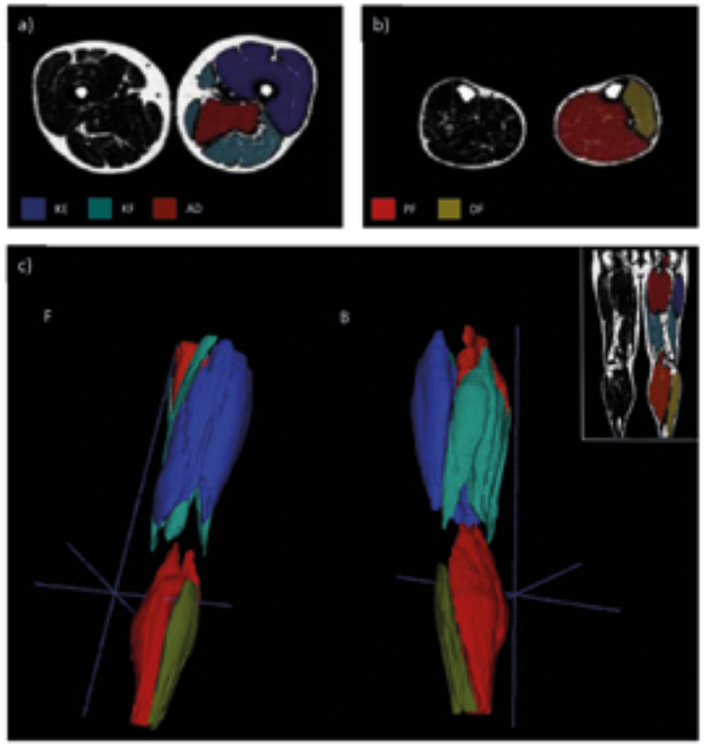
Henning Andersen, *Professor, Chair of Research, Department of Neurology, Aarhus University Hospital supervises PhD student Anders Stouge and PhD student Karolina Snopek Khan.*

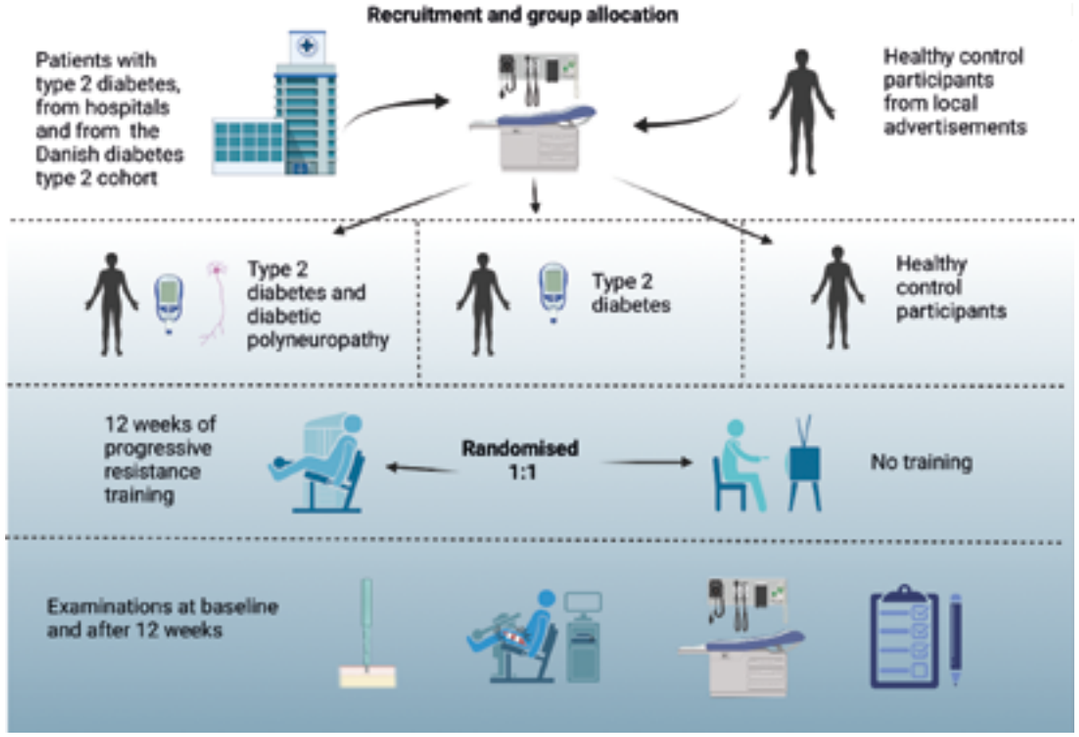
Ongoing work and future plans:
1. We are working on the last analyses of the MR images from the RCT study of training in diabetic neuropathy carried out by MD, PhD Karolina Snopek Khan and Anders Stouge This will result in a study with the following tentative title:

"Quantitative and qualitative changes in striated muscle of the lower extremities following 12 weeks high intensity resistance training – an MRI study (see Figure)."

2. We are awaiting for legal permission to perform genetic analyses (genome sequencing) of muscle biopsies from patients and controls of the RCT study. This will include biopsies taken before and following 12 weeks training. We expect to have the final permission within the next 2-3 months.

Overall, we hope to identify which genetic factors that are up- and downregulated following resistance training. This will enable a detailed understanding of the effect of training in diabetic neuropathy.





WP4: FALLS, MOTOR DYSFUNCTION AND THE EFFECTS OF RESISTANCE TRAINING IN TYPE 2 DIABETIC POLYNEUROPATHY

Karolina Snopek *Khan, MD, PhD defended her PhD entitled: Falls, motor dysfunction and the effects of resistance training in diabetic polyneuropathy in spring 2020. The project was supervised by Professor Henning Andersen, Department of Neurology, Aarhus University Hospital (DK).*

Diabetic polyneuropathy (DPN) has a negative impact on postural balance and gait kinematics combined with an increased risk of falling. DPN can lead to motor neuropathy, characterized by muscle weakness and muscular atrophy. Karolina's previous work focused on examining the incidence of falls in individuals with type 2 diabetes and DPN. It was found that individuals with newly diagnosed type 2 diabetes and possible DPN had an increased risk of falling. In individuals with type 2 diabetes and electroneurography verified DPN, the main factors associated with falls were increased postural instability, lower walking capacity and slower sit-to-stand movements. In a 12 week randomized controlled trial (RCT), the effect of progressive resistance training (PRT) in individuals with type 2 diabetes and DPN was examined. PRT improved muscle strength of the knee extensors and flexors and motor function in individuals with type 2 diabetic polyneuropathy at levels comparable with those seen in individuals with diabetes without DPN and healthy control individuals. No effect of resistance training was observed in intraepidermal nerve fiber density.

We concluded that the 6MWT, FTSST and posturography should be considered in future screening programs in identification of individuals at risk for falls. The findings from the RCT trial provide evidence for establishing PRT exercise protocols for individuals with type 2 diabetes and DPN as PRT contributes to the non-pharmacological treatment options recommended for individuals with DPN.

ONGOING AND FUTURE PROJECTS
Karolina is currently working on the analysis of the MRI images from the RCT trial to describe the changes in striated muscles of individuals with type 2 diabetes with and without DPN.

In collaboration with Kristian Vissing she is working on the analysis of genetic targets in the muscle biopsies performed in the RCT trial and in individuals with motor dysfunction. The work is anticipated to result in three papers describing NMJ stability, mitochondrial expression and proteome profiling. Together with Louise Devantier and Ina Jørgensen she is working on the last details of the manuscript describing the impact of vestibular dysfunction on falls and postural instability in individuals with DPN.

WP4: CLINICAL NEUROPHYSIOLOGICAL MEASURES IN DIABETIC NEUROPATHY

***Alexander Gramm Kristensen**, MD, PhD defended his PhD February 2020 at Department of Clinical Neurophysiology, Aarhus University Hospital. Professor Hatice Tankisi, supervised the project. Alexander is now a post doc at the Danish Pain Research Center.*

Over the course of my PhD project, I conducted three main studies examining patients with type 2 diabetes. The main goal of these studies was to provide insight into the mechanism behind the neuropathy affecting type 2 diabetic patients, using neurophysiologic methods.

The largest study examined the function of ion channels, while the other two studies examined motor nerves and the number of motor units in the upper and lower limbs, respectively.

In our first published study, we examined type 2 diabetic patients using conventional nerve conduction studies (NCS) and a novel method MScanFit MUNE.

We hypothesized that the delay between sensory and motor symptoms was due to collateral re-innervation, and not due to neuropathy targeting sensory axons preferentially.

MScanFit MUNE is a method that allows for the estimation of the number of functioning motor units in a given muscle and nerve combination. NCS was used to identify the patients with definite neuropathy.

Our results showed a clear difference in the number motor units in the patients with neuropathy, compared to the patients without (fig. 1.). Additionally, our results showed an advantage for MScanFit MUNE over conventional motor NCS in detecting changes in the motor nerve. This was in spite of examining the upper extremity, which is thought to be affected in the later stages of diabetic neuropathy.

The second study was a continuation from the results of the previous. We hypothesized that the changes seen in the upper extremity would be more pronounced in the lower extremity. We examined the anterior tibial muscle using MScanFit MUNE, to this end. In addition, we examined the same muscle using the method Muscle Velocity Recovery cycles (MVRCs). MVRCs uses electrical stimulation to examine the membrane potential of the muscle. This could provide knowledge behind the mechanisms of degeneration.
Our results showed a significantly reduced number of motor units in patients without neuropathy and, to a greater extent, patients with neuropathy (fig. 2). There was, in addition, an even larger advantage to using MScanFit MUNE to discriminate between the different groups of participants (fig. 3). This further supported our findings from the first study, and our hypothesis that motor nerve degeneration occurs earlier than previously suspected, but is masked by collateral re-innervation. We found no changes in our MVRC measures, suggesting that the degeneration is not affecting the muscle fibers. However, MVRCs has a potential bias towards only examining healthy muscle fibers and therefore no conclusion could be drawn from the non-significant changes.

The final and largest study was performed on the median nerve as well. Here, we examined both sensory and motor axons using Nerve Excitability Testing (NET). This method studies the axonal membrane in a manner similar to MVRCs, and gives insight in the function of axonal ion channels. Prior studies had shown marked differences in excitability parameters. We wished to examine a large cohort of patients and correlate our findings to clinical and para-clinical examinations in order to understand the mechanisms of diabetic neuropathy.

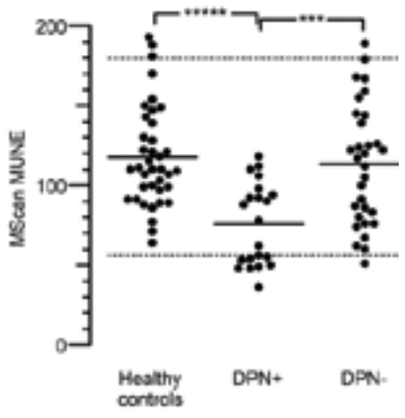


***Fig. 1.** Distributions of estimated number motor units between the 38 healthy controls, 21 patients with diabetic polyneuropathy (DPN+) and 31 patients without neuropathy (DPN-). The asterisks indicate the P values for comparison by the t-test (** = P < 0.01, *** = P < 0.001, **** = P < 0.0001, ***** = P < 0.00001). Horizontal solid lines indicate means, and dashed lines indicate 95% confidence limits for the healthy subjects.*

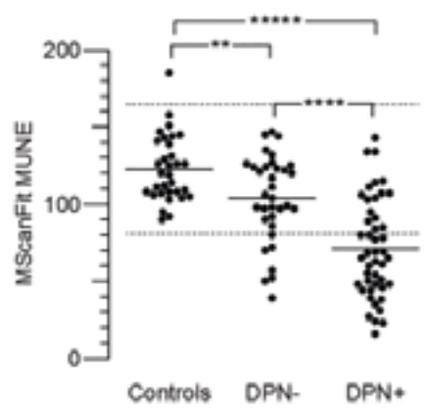


***Fig. 2.** Dotplot of the estimated number of motor units measured from the anterior tibial muscle. Solid lines are the mean of the group, dashed lines are 95% confidence limits for the control group. Asterisks indicate level of significance (** = p < 0.01, *** = p < 0.001, **** = p < 0.0001, ***** = p < 0.00001).*

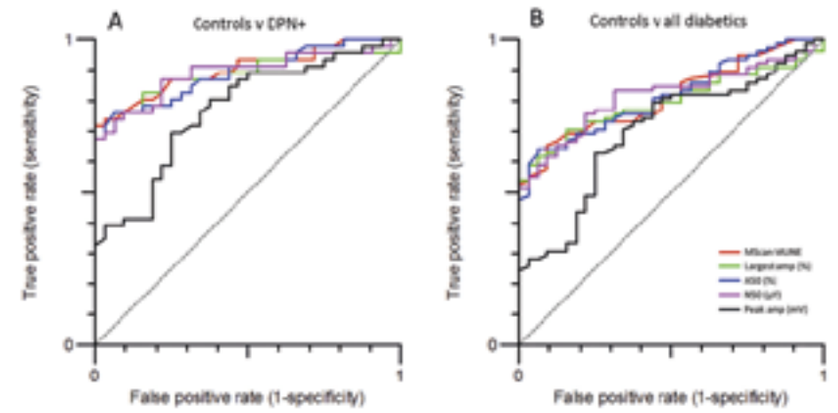


***Fig. 3.** Receiver operating characteristic (ROC) curves describing the ability of MScanFit measures and compound muscle action potential (CMAP) amplitude to discriminate A: healthy controls from diabetic polyneuropathy positive (DPN+) patients, and B: healthy controls from all the diabetic patients.*

However, our findings were sparse (fig. 4) and had weak correlations to other examinations. Upon further inspection of our results, after publication, we did find a similarity to the changes found in studies on ischemic resistance in diabetic patients.

We are contemplating a study on a number of patients with diabetic neuropathy, to compare their resistance to ischemia with control subjects, using a continuous excitability protocol while inducing short term ischemia. A finding of ischemic resitance in patients with neuropathy would support the hypothesis that neuropathy caused by diabetes could be, in part, due to decreased blood flow in the vasa nervorum.

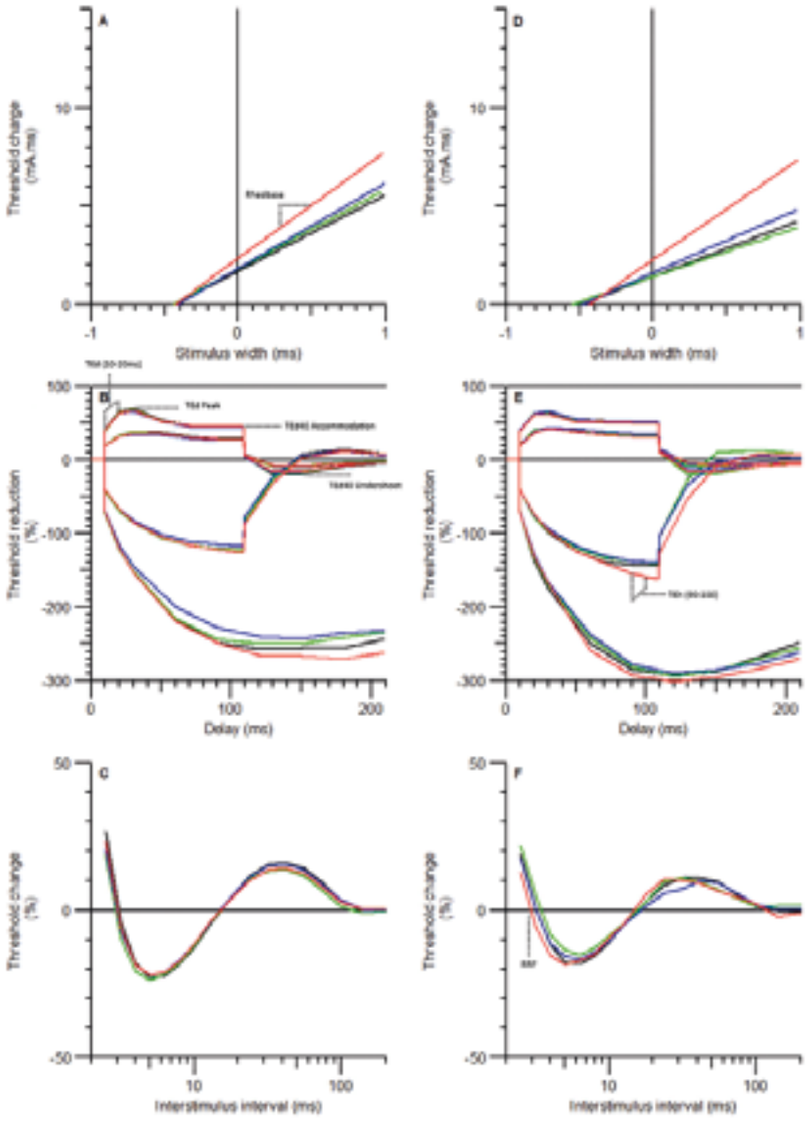
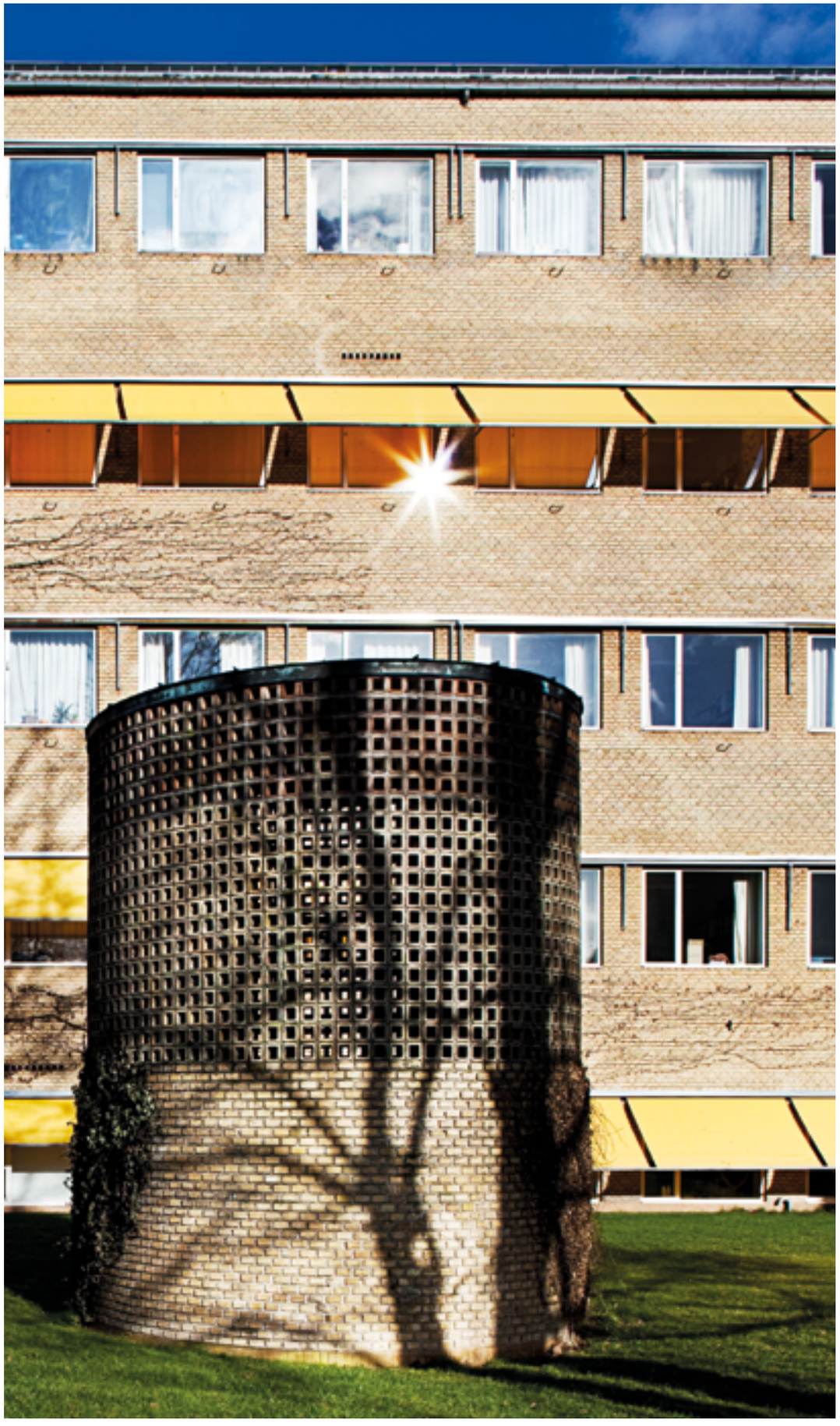


Fig. 4. *(A–C) Motor excitability results, (D–F) Sensory excitability results. A + D: Strength duration relationship, B + E: Threshold electrotonus, C + F: Recovery cycle. Black – controls, green – no neuropathy, blue – possible probable DPN, red – NCS confirmed DPN. DPN = diabetic polyneuropathy, NCS = Nerve conduction studies. The following statistically significant parameters were indicated for each curve. Rheobase is the slope of the strength duration curve. TEd (10–20 ms) is the difference in threshold between 10 and 20 ms duration of depolarizing conditioning stimulus. TEd (peak) is the maximal threshold reduction in the depolarizing measures. TEd Accommodation is the difference in threshold reduction of TEd (peak) and at the point of maximal stimulus duration of 100 ms. TEd(undershoot) is the lowest threshold reduction after insertion of a delay between the conditioning and test stimuli. TEh (90–100) is the difference in threshold reduction at 90 and 100 ms duration of the hyperpolarizing conditioning stimulus. RRP is the relative refractory period, measured as the first x-intercept of the recovery cycle.*



WP4: SMALL FIBER NEUROPATHY: CLINICAL AND PHYSIOLOGICAL CHARACTERISTICS WITH FOCUS ON ADRENERGIC DYSFUNCTION

Thorsten Kamlarczyk Rasmussen, *MD, PhD, Department of Neurology, Aarhus University successfully defended his PhD "Autonomic neuropathy in small fiber neuropathy and type 2 diabetes" in April 2022. Associate professor Astrid Juhl Terkelsen was main supervisor of the project*

Autonomic neuropathy is a common complication in a wide variety of diseases, most often assessed in patients with type 2 diabetes and patients with small fiber neuropathy, although little is known about the extent of especially sympathetic dysfunction. The aim of this project was to explore the extent of sympathetic impairment in patients with type 2 diabetes and patients with small fiber neuropathy using both established and novel methods of examination.

In the first study, 40 type 2 diabetic patients and 40 control subjects underwent a detailed assessment of sympathetic cardiovascular reflex responses. We found that type 2 diabetic patients had no impairment of sympathetic cardiovascular function and that sympathetic reflex responses were similar to healthy control subjects, although parasympathetic responses where impaired. We found an association between 24-hour blood pressure variability and hemodynamic responses during tilt table testing, suggesting these might be interchangeable markers when assessing sympathetic function.
Type 2 diabetic patients from study I were invited to undergo I123-Metaiodobenzylguanidine (MIBG) scintigraphy to assess adrenergic innervation of the cardiac tissue. The MIBG revealed that diabetic patients had significantly lower cardiac innervation compared to laboratory specific normative data from healthy control subjects. Patients with impaired adrenergic function, as assessed by cardiovascular tests, had a significantly greater adrenergic adrenergic innervation (higher heart:mediastinum ratio), and a higher turnover of noradrenaline (higher washout rate) assessed through MIBG scintigraphy. We found significant correlations between adrenergic indices of the Valsalva maneuver and the MIBG scintigraphy with impaired adrenergic function associated to higher H/M ratios and higher washout.

In the final study, we assessed peripheral sympathetic markers in 19 patients with confirmed small fiber neuropathy and 19 matched control subjects through evaluation of vascular skin responses assessed through Laser Doppler Flowmetry and evaluation of microvascular vessel and nerve density in skin biopsies. All participants underwent peripheral autonomic evaluation by quantitative sudomotor axon reflex test. We found no significant differences in vascular skin responses, in microvascular skin biopsy markers, or in sudomotor function, comparing the two groups. We found no correlation between vascular skin responses and skin biopsy indices. Furthermore, we saw no association between microvascular markers and sudomotor function, suggesting that these are independent and unrelated components of the autonomic nervous system.

In future studies we aim to further investigate the association between adrenergic innervation and cardiovascular adrenergic function in type 2 diabetic patients.

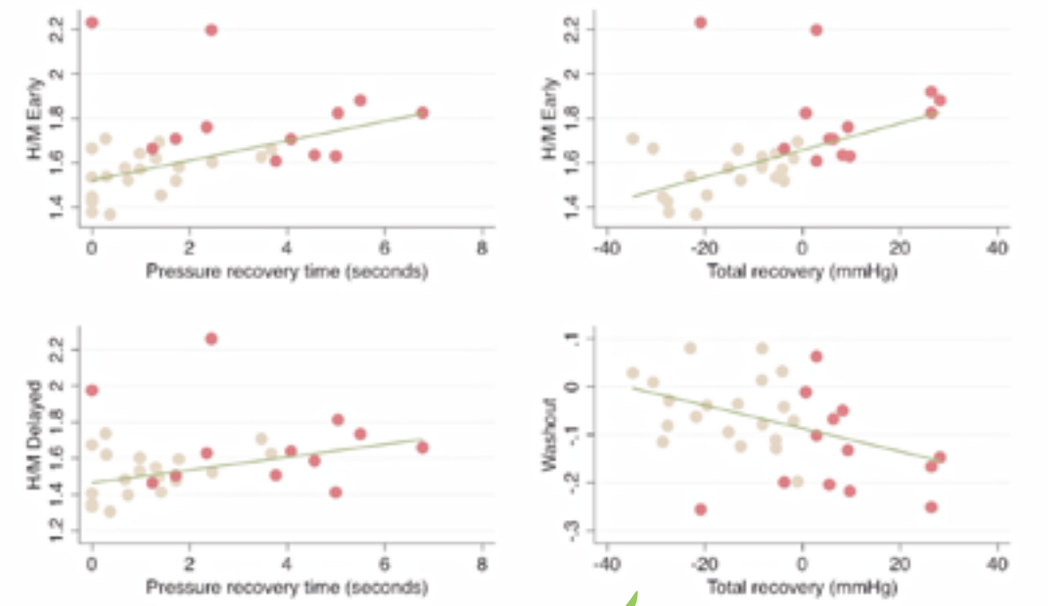
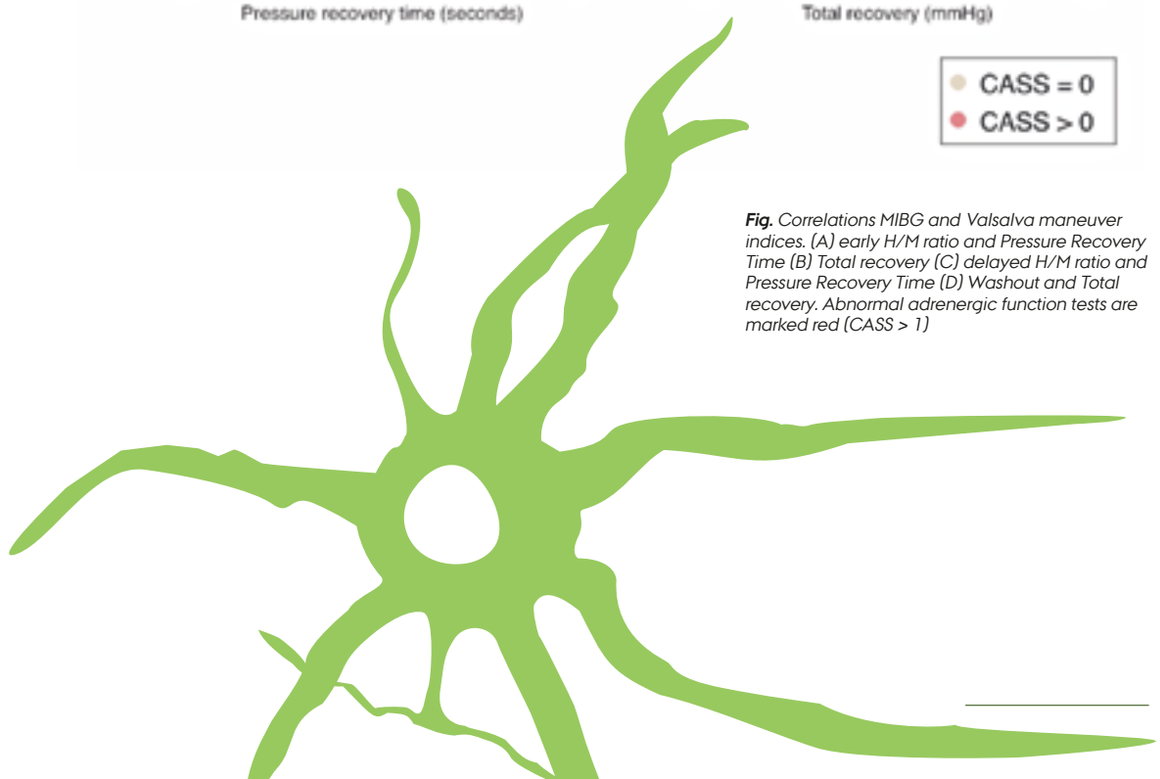


Fig. *Correlations MIBG and Valsalva maneuver indices. (A) early H/M ratio and Pressure Recovery Time (B) Total recovery (C) delayed H/M ratio and Pressure Recovery Time (D) Washout and Total recovery. Abnormal adrenergic function tests are marked red (CASS > 1)*



WP4: CONFIRMED NEUROPATHY IN ADOLESCENTS WITH TYPE 1 DIABETES AND A DIAGNOSTIC METHOD TO QUANTIFY NERVE FIBER DENSITY IN SWEAT GLANDS

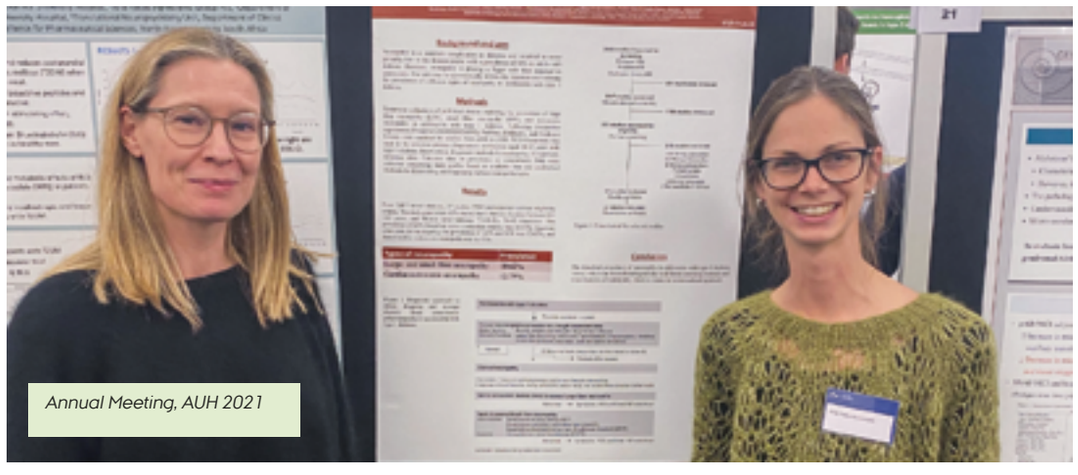
Vinnie Faber Rasmussen *is MD at the Department of Pediatrics and Adolescents, Aalborg University Hospital, Denmark and PhD student, Department of Clinical Medicine, Aarhus University, Denmark with Astrid Juhl Terkelsen as main supervisor*

I have been working on my PhD project "Early detection of neuropathy in adolescents with type 1 diabetes (T1D)". After a publication of a systemic review about the topic, I have focused on the clinical research study, where I have enrolled 60 adolescents aged 15-<19 years with diabetes duration of T1D >5 years. Neurological examination and diagnostic tests for large fiber-, small-fiber, and autonomic neuropathy were performed, including nerve conduction studies (NCS), skin biopsies determining intraepidermal nerve fiber density (IENFD), quantitative sudomotor axon reflex test (QSART), cardiovascular reflex tests (CARTs), and tilt table test analyzing orthostatic parameters. Data from adolescents with T1D was compared with own obtained normative data from 23 control subjects. In addition, screening tests were performed and compared with the diagnostic tests. Confirmed neuropathy depending on type of neuropathy was found in up to 14%.

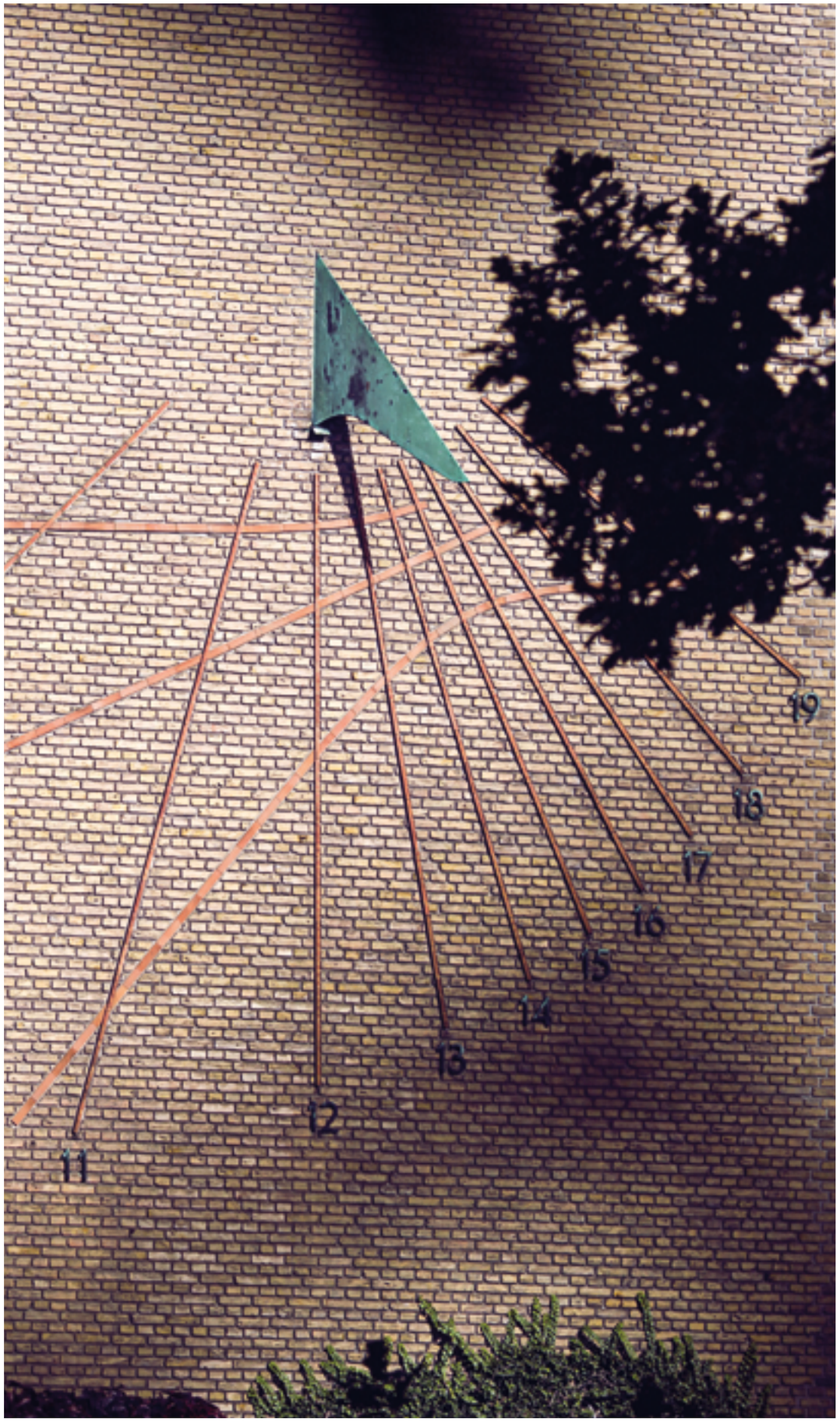
In addition, we have collaborated with Mayo Clinic, Rochester, where quantification of the sweat gland volume and 3D reconstruction of the nerve fibers was performed using a recently described quantitative, unbiassed technique. We found that the adolescents with T1D had significant reduction of maximum and mean values of total nerve fiber length (NFL) and SGNFD compared to controls.

Plans for future research

1) Map the distribution of nerve damage in adolescents with T1D, also including cranial nerves and nerves to the gastrointestinal- and bladder.
2) Continue to investigate the nerves in skin (epidermis and in sweat gland) with other tests, including investigation of genetic marker, parasympathetic versus adrenergic nerves.
3 Investigation of the blood sample for biochemical and genetic markers for neuropathy.
4) Follow-up study in 5-10 years.



Annual Meeting, AUH 2021



WP4: DETAILED SKIN BIOPSY ANALYSIS REVEALED DIFFERENCES BETWEEN PATIENTS WITH AND WITHOUT NEUROPATHIC PAIN

Páll Karlsson, *PhD, Associate professor, Danish Pain Research Centre, Core Center for Molecular Morphology, Section for Stereology and Microscopy, Department of Clinical Medicine, Aarhus University*

My research at IDNC aims to understand why some patients develop chronic neuropathic pain and to identify novel treatment targets that can be used to develop mechanism-based pharmacotherapies for the pain. Specifically, I focus on the function and pathology of the small nerve fibers in diabetic peripheral neuropathy (DPN). To do so, we take small 3 mm skin punch biopsies – a technique we implemented at Aarhus University in 2010. During IDNC, we have built up a state-of-the-art skin biopsy laboratory following international guidelines, ensuring highest quality and reproducibility. Additionally, we have further developed and optimized the technique, developed new immunological protocols and counting methods for detection of novel markers related to diabetes and pain, and thereby increased its diagnostic value and expanding its utility.

We have shown that there is a poor relationship between IENFD and the presence or intensity of neuropathic pain1. The decreased IENFD does, therefore, not explain the presence and development of neuropathic pain. The limited biomarker assessment using only PGP 9.5 antibody and the simple count of nerve fibers penetrating the epidermis and omission of more specific antibodies that visualize distinct subtypes of fibers, may be a reason for the lack of correlation between morphological findings and neuropathic pain.

Therefore, we have focused on extracting additional and perhaps more useful information from the skin biopsies, when searching for early markers of disease in the nerve fibers and markers associated with neuropathic pain. These include axonal swellings, which may be a pre-degenerative marker of nerve fibres2. We have also demonstrated higher density of peptidergic nerve fibers (nerve fibers expressing SP or CGRP) in patients with neuropathic pain, even when there is little or no difference in the IENFD3. The peptidergic fibers are a potential treatment target for neuropathic pain, and we are now launching a randomized clinical trial, assessing potential effect of CGRP monoclonal antibody on neuropathic pain in patients with painful DPN. Lastly, we have discovered that the skin in patients with painful neuropathies is to a high degree infiltrated with immune cells called macrophages that may be associated with neuropathic pain, thereby representing another potential treatment target for neuropathic pain4.

The identification of immune cells in the skin turned our focus towards other cell types as well. In a recent publication in Science5, an until now unknown organ likely playing an essential physiological role in sensing painful stimuli was identified in animal model of pain. The paper described specialized cutaneous Schwann cells that initiate pain sensation and surround themselves around the nerves in the skin and feed them with nociceptive information. However, the cells were only described in an animal model, and they were not quantified. We have now further described the Schwann cells in animal models of pain but also, for the first time, shown that they indeed are present in the human skin and quantified them6,7.

We will, in a close collaboration with our national and international collaborators, continue our efforts in understanding why some patients develop chronic neuropathic pain and to identify novel treatment targets using skin biopsies. Our attention is now on the potential role of the immune system in neuropathic pain development and the neuro-immune interaction. We are also turning our focus towards skin gene expression by using spatial transcriptomics to gain a deeper understanding of the molecular make-up on a cellular level within key regulating neurons and neighboring cells to understand both the signaling in specific cell types and in the eminent surrounding area. Lastly, we are using multiplex immunofluorescence, allowing us to stain for dozens of proteins relevant to neuropathic pain with multi targeted immunofluorescence microscopy at the same time – on the same tissue.

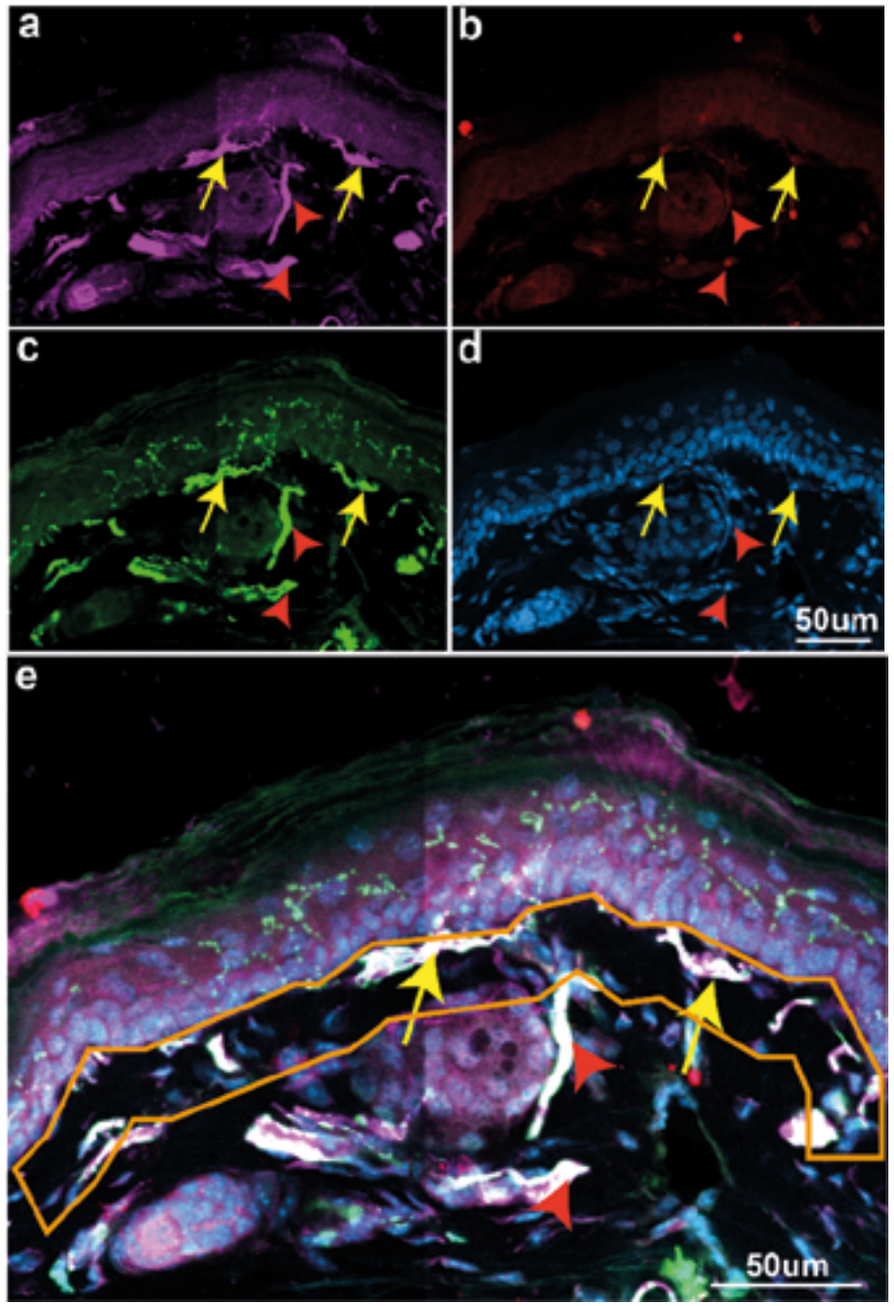


Figure. *Identification of nociceptive Schwann cells in the skin (arrows) by immunological staining of four different antibodies. A: S100, b: Sox10, c: PGP9.5, d: DAPI, e: Compose image*

WP4: CLINICAL NEUROPHYSIOLOGICAL MEASURES IN DIABETIC NEUROPATHY

Ellen Lund Schaldemose *is a MD, PhD student with Professor Nanna Brix Finnerup as main supervisor*

Even though patients with diabetic polyneuropathy mainly have sensory loss, between 5-26 % of the patients also experience painful neuropathic symptoms. The spontaneous pain is thought to develop from central or peripheral changes in the nervous system, but how these changes differentially contribute to the generation and maintenance of pain remains yet to be fully understood.
This is what Ellen Schaldemose investigates as a part of her PhD project.
Together with colleagues, she conducted a randomized, double-blind, placebo-controlled trial and aimed to evaluate the importance of the peripheral afferent input for spontaneous pain in patients with painful diabetic polyneuropathy:

PATIENTS AND METHODS
Twelve patients were included, who all underwent a detailed neurological examination followed by bilateral peripheral nerve blocks, where one leg was randomized to receive lidocaine and the other placebo (saline) (figure 1).

The primary endpoint was the leg with most pain relief 30 minutes after nerve block. Secondary endpoints included pain intensity, pain relief and intensity of cold and pinprick stimuli.

RESULTS
Nine out of the 12 patients rated the leg treated with lidocaine with most pain relief, which did not reach statistical significance (probability of 75 (43; 95) %, p = 0.15). In many patients, the pain intensity had decreased to very low levels before the nerve block and while 10/12 patients reached a pain intensity of 0 after lidocaine block (figure 2). Likewise, there was no difference in the intensity of pain reduction (mean numeric rating scale difference (range 0-10): 0.67 ± 0.98, p = 0.51) or in pain relief score (p = 0.61) between the two treatments.

CONCLUSION
In conclusion, the results of this trial could not confirm that lidocaine had more pain relieving effect than placebo in spontaneous pain in patients with painful diabetic polyneuropathy, although most patients had pain relief after lidocaine block. We can therefore only suggest that the peripheral input is important in maintaining spontaneous pain in patients with painful diabetic polyneuropathy.

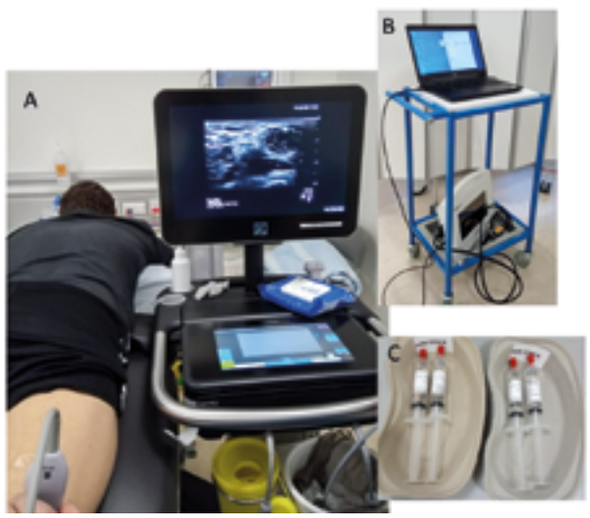


Figure 1: *The experimental setup. (A) ultrasound-guided nerve block, (B) equipment for testing of thermosensory function, (C) the medication.*

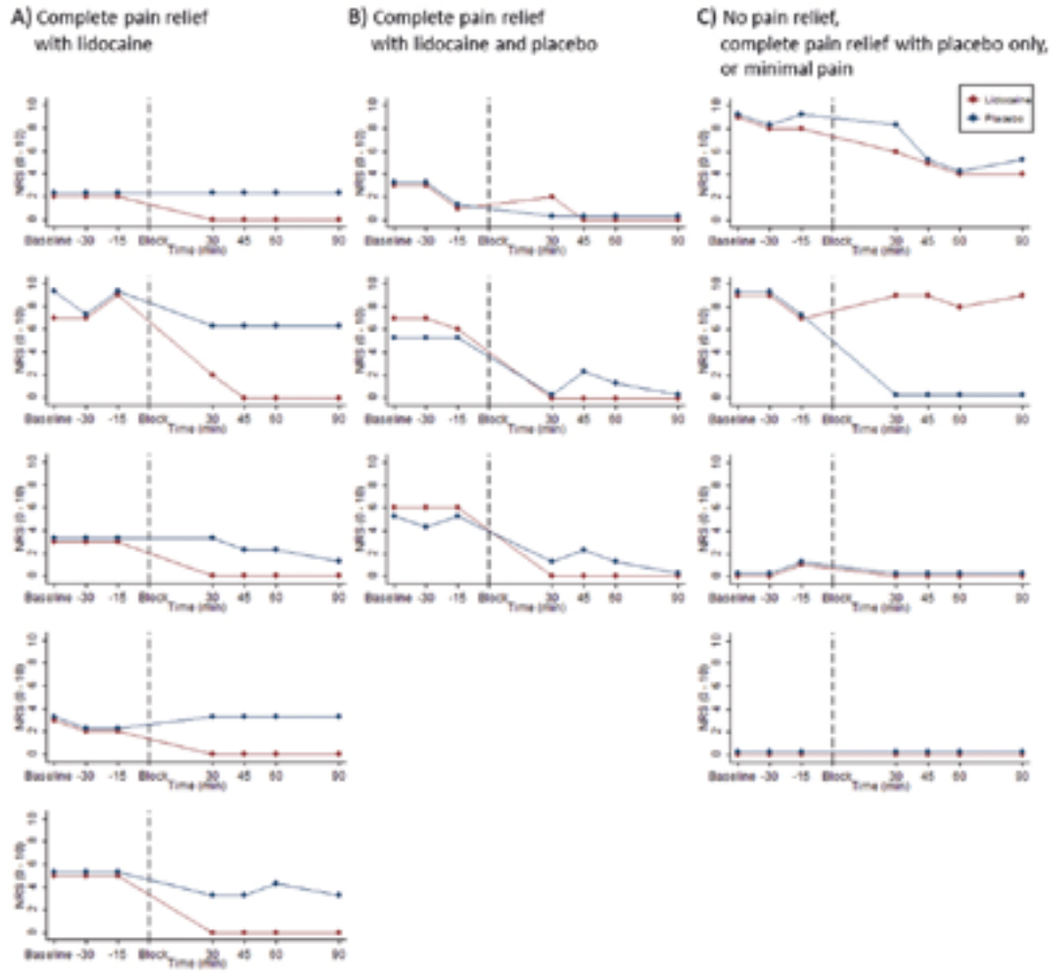


Figure 2: *Individual pain intensity scores in both legs at different time points before and after nerve block. (A) five patients with complete pain relief after lidocaine, (B) three patients with complete pain relief after both lidocaine and placebo, (C) one patient did not have any effect of the treatments, 1 patient experienced full pain relief after placebo and finally 2 patients did not have pain. Baseline measure is defined as 45 minutes before first nerve block. NRS: numerical rating scale.*

WP5: METABOLOMICS AND LIPIDOMICS

In this work package, the focus is on oxidative stress pathways and lipid metabolites for the identification and quantitation of the proteins and metabolites that are predictive of diabetic neuropathy. This work package is led by Professor Eva Feldman, Department of Neurology, University of Michigan, Ann Arbor (US).

WP5: METABOLOMICS AND LIPIDOMICS

PROFESSOR EVA L. FELDMAN, UNIVERSITY OF MICHIGAN, ANN ARBOR, USA

We use a systems approach employing transcriptomics, metabolomics, and metabolic flux analyses to identify nerve-specific differences in type 1 (T1D) and 2 diabetic (T2D) mice and man. Our goal is to identify highly conserved pathways across human and murine models that are likely to play a role in diabetic peripheral neuropathy (DPN) pathogenesis and provide new possible mechanism-based targets for DPN therapy. In the last 7 years, we have summarized our approach and sentinel findings in over 70 total published papers (see Research Outputs), which include primary articles on our research findings along with a series of reviews focused on the clinical problem, including pain and pediatric neuropathy, new risk factors, our approach to diagnosis, and mechanistic insights into DPN pathogenesis gained from pre-clinical models and systems biology, which will accelerate progress towards the development of mechanism-based therapies. Our recent clinical studies in man indicate that drivers of neuropathy include not only glucose, but obesity and associated components of the metabolic syndrome (MetS). More recently, we demonstrated that dietary weight loss in people with severe obesity stabilizes small fiber neuropathy and improves secondary outcomes including MNSI questionnaire and QST cold threshold1. These data serve as the cornerstone of our work in vitro and in murine models of T1D and T2D, where our goal is to better understand the mechanisms by which components of the MetS, particularly obesity, drive the onset and progression of DPN. Our findings over the past year are highlighted below.

To that end, we recently carried out metabolomics and targeted lipidomics plasma analyses on obese participants with (n = 44) and without (n = 44) DPN, matched for glycemic status, versus lean non-neuropathic controls (n = 43). Each subject received anthropometric measurements, DPN assessment, and blood sample collection2. We identified significant differences in gamma-glutamyl and branched-chain amino acid metabolism from metabolomics analysis and triacylglycerols (TAGs) from lipidomics between lean vs obese subjects, regardless of DPN status (Fig. 1). When obese subjects were further stratified by DPN status, we found differences in polyamine, bioenergetics defects through purine biosynthesis, and benzoate metabolism. Lipidomics uncovered diacylglycerols as the most significant subpathway distinguishing obese subjects by DPN status, with additional contributions from phosphatidylcholines, sphingomyelins, ceramides, and dihydroceramides. Stratifying the obese group by glycemic status did not affect discrimination by DPN status.

These results reinforce our preclinical findings, which revealed a dysregulation in nerve lipidome of obese, prediabetic mice, with increased TAGs and TAG synthesis in DPN progression3. Moreover, they confirm our clinical findings on the role of obesity in driving DPN and highlight the importance of plasma metabolomics and lipidomics analyses in differentiating obese individuals with and without DPN, independent of glycemic status.

We next used a bedside-to-bench approach to dissect the cellular mechanisms downstream of obesity, which will help inform the development of targeted, mechanism-based therapies for DPN treatment. We specifically aimed to understand the effect of diet-induced obesity on mitochondrial function and dynamics in sensory axons of the saphenous nerve in the high fat diet (HFD)-fed mouse model of DPN4. We found that diet-induced obesity reduced axonal mitochondrial membrane potential (MMP) and the ability of sensory neurons to conduct at physiological frequencies. While axonal mitochondria in control mice dissipated their MMP in response to increased impulse frequency (from 1 to 50 Hz), HFD mitochondria dissipated less MMP in response to axonal energy demand, suggesting a lack of reserve capacity (Fig. 2). These changes were accompanied by decreased sensory axonal Ca2+ levels and increased mitochondrial lengthening and expression

of PGC1α, a master regulator of mitochondrial biogenesis. Together, these results suggest that partial MMP loss and reduced Ca2+ levels play important roles in regulating sensory neuron axonal impulse conduction in DPN.

Overall, we continue to pursue the overarching hypothesis that fatty acid uptake, lipid oxidation, lipid biosynthetic pathways, and mitochondrial function are dysregulated by diabetes in the peripheral nervous system and that elucidation of these altered pathways will provide new mechanism-based therapeutic targets for neuropathy prevention and treatment. During the seven years of IDNC funding, we completed clinical studies that support the idea that components of the MetS, including dyslipidemia, converge with hyperglycemia to mediate nerve injury and DPN. In our most recent study, we identified specific plasma metabolites and complex lipid pathways that can differentiate obese individuals with and without PN, independent of glycemic status. This study parallels many of our findings in murine models of obesity and T2D. We also discovered in vivo mitochondrial abnormalities in the HFD mouse model, which suggest that lower MMP may result in insufficient ATP to maintain normal axonal function, further strengthening our contention that energy failure may likely underlie the pathogenesis of DPN.

REFERENCES CITED

1. Callaghan BC, Reynolds EL, Banerjee M, Akinci G, Chant E, Villegas-Umana E, Rothberg AE, Burant CF, Feldman EL. Dietary weight loss in people with severe obesity stabilizes neuropathy and improves symptomatology Obesity 2021 Dec;29(12): 2108-2118. PMC8612943
2. Guo K, Savelieff MG, Rumora AE, Alakwaa FM, Callaghan BC, Hur J, Feldman EL. Plasma Metabolomics and Lipidomics Differentiate Obese Individuals by Peripheral Neuropathy Status. JCEM. 2022 Mar 24;107(4):1091-1109. PMID: 34878536, PMCID: PMC8947234
3. O'Brien PD, Guo K, Eid SA, Rumora AE, Hinder LM, Hayes JM, Mendelson FE, Hur J, Feldman EL. Integrated lipidomic and transcriptomic analyses identify altered nerve triglycerides in mouse models of prediabetes and type 2 diabetes. Dis Model Mech. 13(2):dmm.042101, 2020. PMCID: PMC6994925.
4. Sajic M, Rumora AE, Kanhai A, Dentoni G, Varatharajah S, Casey C, Brown R, Peters F, Hinder LM, Savelieff M, Feldman EL, Smith KJ. High Dietary Fat Consumption Impairs Axonal Mitochondrial Function In Vivo. J Neuroscience. 2021 May 12;41(19):4321-4334. PMID: 33785643

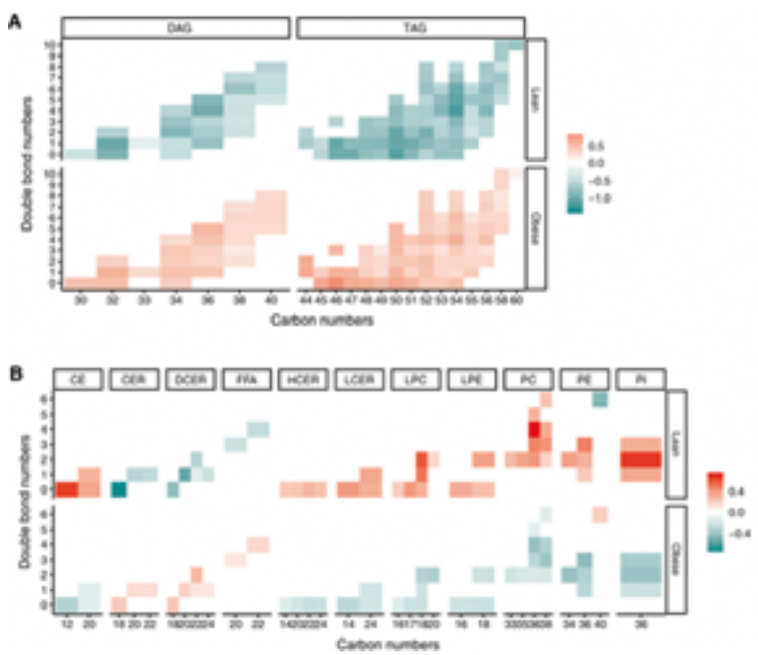


Fig. 1. Lipid abundance heat maps by chain lengths and saturation in obese vs lean participants. Heat maps of log2-transformed abundances of lipids selected by partial least squaresâ€"discriminant analysis (PLS-DA) for A, triacylglycerols (TAGs) and diacylglycerols (DAGs); and B, cholesterol esters (CE), ceramides (CER), dihydroceramides (DCER), free fatty acids (FFA), hexosylceramides (HCER), lactosylceramides (LCER), lysophosphatidylcholines (LPC), lysophosphatidylethanolamines (LPE), phosphatidylcholines (PC), phosphatidylethanolamines (PE), and phosphatidylinositols (PI).

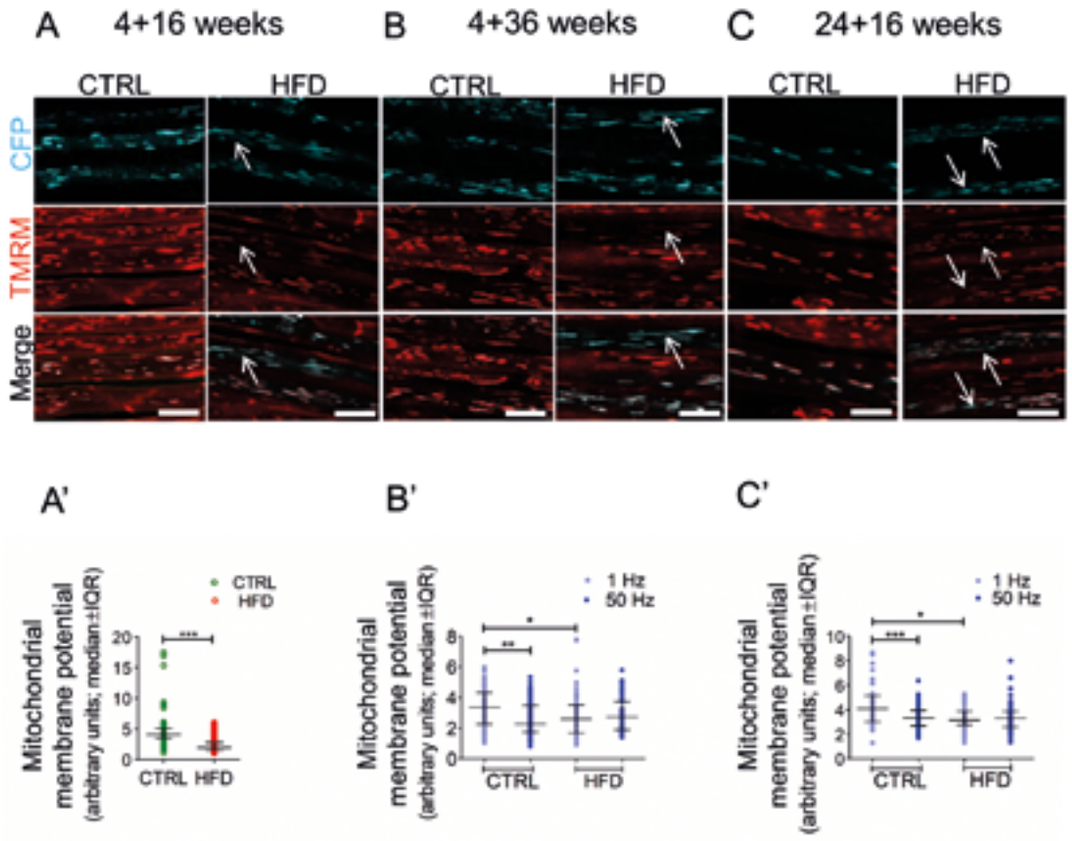


Fig. 2. *HFD mice exhibit reduced MMP in saphenous nerve axons in vivo. A–C, High-magnification confocal image of saphenous nerve axons in CFP transgenic mouse with CFP+ axonal mitochondria (blue, top) stimulated at 1 Hz. TMRM labels polarized mitochondria (red, middle). Bottom, Merged. Magenta, pink, or white represents CFP+, TMRM+ mitochondria. Red represents CFP–, TMRM+ mitochondria. In control mice, all axonal mitochondria were polarized and were magenta, pink, or white on merged images. Bottom, Red mitochondria are axonal, non-CFP expressing, or nonaxonal. In contrast, in all HFD groups, some axonal mitochondria were depolarized and appear blue on merged images (bottom, white arrows). A', The ratio of TMRM intensity in CFP+ mitochondria versus the adjacent axoplasm indicates MMP and is significantly lower in HFD 4 + 16 mice versus controls. ***p < 0.001 (unpaired Student's t test). Data are mean ± SEM. n = 4 or 5 mice per group. B', The ratio of TMRM intensity in CFP+ mitochondria versus the adjacent axoplasm indicates MMP and is significantly lower in HFD 4 + 36 mice versus controls in 1 Hz-stimulated axons. Increasing the stimulation frequency to 50 Hz lowers MMP in control mitochondria but has no effect in HFD mitochondria. *p < 0.05; **p < 0.01; ***p < 0.001; two-way ANOVA. Data are mean ± SEM. n = 4 or 5 mice per group. C', The ratio of TMRM intensity in CFP+ mitochondria versus the adjacent axoplasm indicates MMP and is significantly lower in HFD 24 + 16 mice versus controls in axons stimulated at 1 Hz. Increasing the stimulation frequency to 50 Hz lowers MMP in control mitochondria but has no effect in HFD mitochondria. *p < 0.05; **p < 0.01; ***p < 0.001; two-way ANOVA. Data are mean ± SEM. n = 4 or 5 mice per group. IQR, Interquartile range.*

REFERENCES CITED

1. Callaghan BC, Reynolds EL, Banerjee M, Akinci G, Chant E, Villegas-Umana E, Rothberg AE, Burant CF, Feldman EL. Dietary weight loss in people with severe obesity stabilizes neuropathy and improves symptomatology Obesity 2021 Dec;29(12): 2108-2118. PMC8612943
2. Guo K, Savelieff MG, Rumora AE, Alakwaa FM, Callaghan BC, Hur J, Feldman EL. Plasma Metabolomics and Lipidomics Differentiate Obese Individuals by Peripheral Neuropathy Status. JCEM. 2022 Mar 24;107(4):1091-1109. PMID: 34878536, PMCID: PMC8947234
3. O'Brien PD, Guo K, Eid SA, Rumora AE, Hinder LM, Hayes JM, Mendelson FE, Hur J, Feldman EL. Integrated lipidomic and transcriptomic analyses identify altered nerve triglycerides in mouse models of prediabetes and type 2 diabetes. Dis Model Mech. 13(2):dmm.042101, 2020. PMCID: PMC6994925.
4. Sajic M, Rumora AE, Kanhai A, Dentoni G, Varatharajah S, Casey C, Brown R, Peters F, Hinder LM, Savelieff M, Feldman EL, Smith KJ. High Dietary Fat Consumption Impairs Axonal Mitochondrial Function In Vivo. J Neuroscience. 2021 May 12;41(19):4321-4334. PMID: 33785643

PUBLICATIONS

PEER-REVIEWED PUBLICATIONS

1. Afshinnia F, Reynolds EL, Rajendiran TM, Soni T, Byun J, Savelieff MG, Looker HC, Nelson RG, Michailidis G, Callaghan BC, Pennathur S, Feldman EL. Serum lipidomic determinants of human diabetic neuropathy in type 2 diabetes. Ann Clin Transl Neurol. 2022 Aug 3. doi: 10.1002/acn3.51639. Online ahead of print. PMID: 35923113.

2. J Agergaard, S Leth, TH Pedersen, T Harbo, JU Blicher, P Karlsson, L Østergaard, H Andersen, H Tankisi. Myopathic changes in patients with long-term fatigue after COVID-19. Clin Neurophysiol. 2021, 132(8):1974-1981.

3. Akinci G, Savelieff MG, Gallagher G, Callaghan BC, Feldman EL. Diabetic neuropathy in children and youth: New and emerging risk factors. Pediatr Diabetes. 2021 Mar;22(2):132-147. PMID: 33205601

4. Baskozos G, Themistocleous AC, Hebert HL, Pascal MMV, John J, Callaghan BC, Laycock H, Granovsky Y, Crombez G, Yarnitsky D, Rice ASC, Smith BH, Bennett DLH. Classification of painful or painless diabetic peripheral neuropathy and identification of the most powerful predictors using machine learning models in large cross-sectional cohorts. BMC Med Inform Decis Mak 2022;22:144

5. N Berge, N Ferreira, TW Mikkelsen, AKO Alstrup, G Tamguney, P Karlsson, AJ Terkelsen, JR Nyengaard, PH Jensen, P Borghammer. Aging promotes pathological alpha-synuclein propagation and autonomic dysfunction in wild-type rats. Brain. 2021, 28;144(6):1853-1868.

6. Bjerg L, Nicolaisen SK, Christensen DH, Nielsen JS, Andersen ST, Jørgensen ME, Jensen TS, Sandbæk A, Andersen H, Beck-Nielsen H, Sørensen HT, Witte DR, Thomsen RW, Charles M. Diabetic Polyneuropathy Early in Type 2 Diabetes Is Associated With Higher Incidence Rate of Cardiovascular Disease: Results From Two Danish Cohort Studies. Diabetes care 2021;44:1714-1721

7. Bjerg L, Laugesen E, Andersen ST, Rosborg JF, Charles M, Vistisen D, Witte DR. Long-term effects of intensive multifactorial treatment on aortic stiffness and central hemodynamics after 13 years with screen-detected type 2 diabetes: the ADDITION-Denmark trial. Diabetology & metabolic syndrome 2022;14:116

8. Callaghan BC, Reynolds E, Banerjee M, Chant E, Villegas-Umana E, Feldman EL: Central Obesity is Associated With Neuropathy in the Severely Obese, Mayo Clin Proc 95(7): 1342-1353, 2020. PMC7340115

9. Callaghan BC, Reynolds EL, Banerjee M, Akinci G, Chant E, Villegas-Umana E, Rothberg AE, Burant CF, Feldman EL. Dietary weight loss in people with severe obesity stabilizes neuropathy and improves symptomatology Obesity 2021 Dec;29(12): 2108-2118. PMC8612943

10. Callaghan BC, Armon C, Bril V, Colbert L, David WS, Del Toro DR, Fink K, Jones LK Jr, Kleemeier R, MacGregor LC, Bennett A, Shenow A: Polyneuropathy Quality Measurement Set: Quality Improvement in Neurology Neurology 98(1): 22-30, 2022. PMID: 34965986

11. Christensen DH, Nicolaisen SK, Ahlqvist E, Stidsen JV, Nielsen JS, Hojlund K, Olsen MH, García-Calzón S, Ling C, Rungby J, Brandslund I, Vestergaard P, Jessen N, Hansen T, Brøns C, Beck-Nielsen H, Sørensen HT, Thomsen RW, Vaag A. Type 2 diabetes classification: a data-driven cluster study of the Danish Centre for Strategic Research in Type 2 Diabetes (DD2) cohort. BMJ Open Diabetes Res Care 2022;10(2):e002731

12. Eid S, Hinder LM, Zhang H, Eksi R, Nair V, Eddy S, Eichinger F, Park M, Saha J, Berthier CC, Jagadish HV, Guan Y, Pennathur S, Hur J, Kretzler M, Feldman EL, Brosius FC. Gene expression profiles of diabetic kidney disease and neuropathy in eNOS knockout mice: predictors of pathology and RAAS blockade effects. FASEB J. 2021, 35(5):e21467. PMID: 33788970

13. Eid SA, O'Brien PD, Hinder LM, Hayes JM, Mendelson FE, Zhang H, Zeng L, Kretzler K, Narayanan S, Abcouwer SF, Brosius Iii FC 3rd, Pennathur S, Savelieff MG, Feldman EL. Differential effects of empagliflozin on microvascular complications in murine models of type 1 and type 2 diabetes. Biology. 9(11):347, 2020. PMCID: PMC7690408

14. Eid S, O'Brien PD, Hinder, LM, Hayes JM, Mendelson FE, Zhang H, Narayanan, S, Abcouwer SF, Brosius III FC, Pennathur S, Savelieff MG, Feldman EL. Differential effects of minocycline on microvascular complications in murine models of type 1 and type 2 diabetes. J Transl Sci. 7, 2020, DOI: 10.15761/JTS.1000431.

15. Eid SA, Feldman EL. Advances in Diet-Induced Rodent Models of Metabolically Acquired Peripheral Neuropathy. Dis Model Mech. 2021 Nov1; 14(11):dmm049337. PMC8592018

16. Eid SA, Savelieff MG, Eid AA, Feldman EL. Nox, Nox, Are You There? The Role of NADPH Oxidases in the Peripheral Nervous System. Antioxid Redox Signal. 2022 May 11. doi: 10.1089/ars.2021.0135. Online ahead of print. PMID: 34861780

17. Elafros MA, Kvalsund MP, Callaghan BC. The Global Burden of Polyneuropathy-In Need of an Accurate Assessment. JAMA Neurol. 2022 Jun 1;79(6):537-538. PMCID: PMC9197927.

18. Elzinga SE, Savelieff MG, O'Brien PD, Mendelson FE, Hayes JM, Feldman EL. Sex differences in insulin resistance but not peripheral neuropathy in a diet-induced prediabetes mouse model. Dis Models Mech, 2021 Apr 1;14(4):dmm048909. PMCID: PMC8077554

19. Feldman EL, Savelieff MG, Hayek SS, Pennathur S, Kretzler M, Pop-Busui R. COVID-19 and diabetes: A collision and collusion of two diseases. Diabetes 69(12): 2549-2565, 2020. PMC7679769

20. Ferreira N, Gonçalves NP, Jan A, Jensen NM, van der Laan A, Mohseni S, Vægter CB, Jensen PH. Trans-synaptic spreading of alpha-synuclein pathology through sensory afferents leads to sensory nerve degeneration and neuropathic pain. Acta Neuropathol Commun 2021;9:31

21. Freeman R, Gewandter JS, Faber CG, Gibbons C, Haroutounian S, Lauria G, Levine T, Malik RA, Singleton JR, Smith AG, Bell J, Dworkin RH, Feldman E, Herrmann DN, Hoke A, Kolb N, Mansikka H, Oaklander AL, Peltier A, Polydefkis M, Ritt E, Russell JW, Sainati S, Steiner D, Treister R, Uceyler N. Idiopathic distal sensory polyneuropathy: ACTTION diagnostic criteria. Neurology 95(22): 1005-1014, 2020. PMC773492

22. Fridman V, Zarini S, Sillau S, Harrison K, Bergman BC, Feldman EL, Reusch JEB, Callaghan BC. Altered plasma serine and 1-deoxydihydroceramide profiles are associated with diabetic neuropathy in type 2 diabetes and obesity. J Diabetes Complications 35(4): 107852, 2021. PMC8114795

23. E Galosi, X Hu, N Michael, JR Nyengaard, A Truini, P Karlsson. Redefining distal symmetrical polyneuropathy features in type 1 diabetes: a systematic review. Acta Diabetologica. Epub ahead of print.

24. Granovsky Y, Shafran Topaz L, Laycock H, Zubiedat R, Crystal S, Buxbaum C, Bosak N, Hadad R, Domany E, Khamaisi M, Sprecher E, Bennett DL, Rice A, Yarnitsky D. Conditioned pain modulation is more efficient in patients with painful diabetic polyneuropathy than those with nonpainful diabetic polyneuropathy. Pain 2022;163: 827-833

25. Guo K, Savelieff MG, Rumora AE, Alakwaa FM, Callaghan BC, Hur J, Feldman EL. Plasma Metabolomics and Lipidomics Differentiate Obese Individuals by Peripheral Neuropathy Status. JCEM. 2022 Mar 24;107(4):1091-1109. PMID: 34878536, PMCID: PMC8947234

26. Gylfadottir SS, Itani M, Kristensen AG, Tankisi H, Jensen TS, Sindrup SH, Bennett D, Nyengaard JR, Finnerup NB, Karlsson P. Analysis of Macrophages and Peptidergic Fibers in the Skin of Patients With Painful Diabetic Polyneuropathy. Neurol Neuroimmunol Neuroinflamm. 2021 Nov 11;9(1):e1111. doi: 10.1212/NXI.0000000000001111. PMID: 34764216; PMCID: PMC8587735.

27. Gylfadottir SS, Itani M, Kristensen AG, Karlsson P, Krøigård T, Bennett DL, Tankisi H, Andersen NT, Jensen TS, Sindrup SH, Finnerup NB. The characteristics of pain and dysesthesia in patients with diabetic polyneuropathy. PLoS One. 2022 Feb 17;17(2):e0263831. doi: 10.1371/journal.pone.0263831. eCollection 2022. PMID: 35176062

28. Henn RE, Noureldein MH, Elzinga SE, Kim B, Savelieff MG, Feldman EL. Glial-neuron crosstalk in health and disease: A focus on metabolism, obesity, and cognitive impairment. Neurobiol Dis. 2022 Aug;170:105766. PMID: 35584728

29. Itani M, Gylfadottir SS, Krøigård T, Kristensen AG, Christensen DH, Karlsson P, Möller S, Andersen H, Tankisi H, Nielsen JS, Jensen TS, Thomsen RW, Finnerup NB, Sindrup SH. Small and large fiber sensory polyneuropathy in type 2 diabetes: Influence of diagnostic criteria on neuropathy subtypes. J Peripher Nerv Syst 2021;26(1):55-65

30. Itani M, Gylfadottir S, Krøigård T, Gaist L, Holbech JV, Kristensen AG, Karlsson P, Möller S, Tankisi H, Gaist D, Jensen TS, Finnerup NB, Sindrup SH. Comparison of diabetic and idiopathic sensory polyneuropathies with respect to nerve fibre affection and risk factors. BMJ Neurol Open. 2022 Mar 14;4(1):e000247. doi: 10.1136/bmjno-2021-000247. PMID: 35360409; PMCID: PMC8921860

31. Jan A, Gonçalves NP, Vaegter CB, Jensen PH, Ferreira N. The Prion-Like Spreading of Alpha-Synuclein in Parkinson's Disease: Update on Models and Hypotheses. International journal of molecular sciences 2021;22

32. Jensen TS, Karlsson P, Gylfadottir SS, Andersen ST, Bennett DL, Tankisi H, Finnerup NB, Terkelsen AJ, Khan K, Themistocleous AC, Kristensen AG, Itani M, Sindrup SH, Andersen H, Charles M, Feldman EL, Callaghan BC: Painful and non-painful diabetic neuropathy, diagnostic challenges and implications for future management. Brain 144(6): 1632-1645, 2021. PMC8320269

33. Karlsson P, Gylfadottir SS, Kristensen AG, Ramirez JD, Cruz P, Le N, Shillo PR, Tesfaye S, Rice ASC, Tankisi H, Finnerup NB, Nyengaard JR, Jensen TS, Bennett DLH, Themistocleous AC. Axonal swellings are related to type 2 diabetes, but not to distal diabetic sensorimotor polyneuropathy. Diabetologia. 2021 Apr;64(4):923-931. doi: 10.1007/s00125-020-05352-9. Epub 2021 Jan 23. PMID: 33483760

34. P Karlsson, V Provitera, G Caporaso, A Stancanelli, AM Saltalamacchia, F Manganelli, L Santoro, TS Jensen, M Nolano. Increased Peptidergic Fibers as a Potential Cutaneous Marker Of Pain in Diabetic Small Fiber Neuropathy. Pain. 2021:162:778-786.

35. P Karlsson. Skin biopsy analysis in Diabetic Neuropathy (2022). Diabetic Neuropathy (1. Ed). Elsevier.

36. Khan KS, Christensen DH, Nicolaisen SK, Gylfadottir SS, Jensen TS, Nielsen JS, Thomsen RW, Andersen H. Falls and fractures associated with type 2 diabetic polyneuropathy: A cross-sectional nationwide questionnaire study. J Diabetes Investig. 2021;12(10):1827-1834

37. Khan KS, Pop-Busui R, Devantier L, Kristensen AG, Tankisi H, Dalgas U, Overgaard K, Andersen H. Falls in individuals with type 2 diabetes; a cross-sectional study on the impact of motor dysfunction, postural instability and diabetic polyneuropathy. Diabetic medicine : a journal of the British Diabetic Association 2021;38:e14470

38. Khan KS, Andersen H. The Impact of Diabetic Neuropathy on Activities of Daily Living, Postural Balance and Risk of Falls - A Systematic Review. Journal of diabetes science and technology 2022;16:289-294

39. Khan KS, Overgaard K, Tankisi H, Karlsson P, Devantier L, Gregersen S, Jensen TS, Finnerup NB, Pop-Busui R, Dalgas U, Andersen H. Effects of progressive resistance training in individuals with type 2 diabetic polyneuropathy: a randomised assessor-blinded controlled trial. Diabetologia 2022;65:620-631

40. Kristensen AG, Gylfadottir S, Itani M, Kuwabara S, Krøigård T, Khan KS, Finnerup NB, Andersen H, Jensen TS, Sindrup S, Tankisi H. Sensory and motor axonal excitability testing in early diabetic neuropathy. Clin Neurophysiol. 2021 Jul;132(7):1407-1415. doi: 10.1016/j.clinph.2021.02.397. Epub 2021 Apr 8. PMID: 34030050.

41. Kristensen FP, Rohde C, Østergaard SD, Thomsen RW. Four-year HbA1c and LDL-cholesterol trajectories among individuals with mental disorders and newly developed type 2 diabetes. Brain and behavior. 2021;11(11):e2372.

42. Krøigård T, Gylfadottir SS, Itani M, Khan KS, Andersen H, Sindrup SH, Jensen TS, Andersen KV, Tankisi H, Beniczky S, Kristensen AG. Normative reference values for the dorsal sural nerve derived from a large multicenter cohort. Clin Neurophysiol Pract. 2021 Sep 2;6:239-243. doi: 10.1016/j.cnp.2021.08.001. PMID: 34604609; PMCID: PMC8473014.

43. Pop-Busui R, Ang L, Boulton AJM, Feldman EL, Marcus RL, Mizokami-Stout K, Singleton JR, Ziegler D. Diagnosis and Treatment of Painful Diabetic Peripheral Neuropathy. Arlington (VA): American Diabetes Association; 2022 Feb. PMID: 35544662.

44. Price R, Smith D, Franklin G, Gronseth G, Pigone M, David WS, Armon C, Perkins BA, Bril V, Rai-Grant A, Halperin J, Licking N, O'Brien MD, Wessels SR, MacGregor LC, Fink K, Harkless LB, Colbert L, Callaghan BC: Oral and Topical Treatment of Painful Diabetic Polyneuropathy: Practice Guideline Update Summary: Report of the AAN Guideline Subcommittee Neurology 98(1): 31-43, 2022. PMID: 34965987

45. Putnam NM, Reynolds EL, Banerjee M, Mizokami-Stout K, Albright D, Lee J, Pop-Busui R, Feldman EL, Callaghan BC. Neuropsychological Outcomes in Individuals With Type 1 and Type 2 Diabetes. Front Endocrinol (Lausanne). 2022 Mar 4;13:834978. PMCID: PMC8934404.

46. Reynolds EL, Akinci G, Banerjee M, Looker HC, Patterson A, Nelson RG, Feldman EL, Callaghan BC. The determinants of complication trajectories in American Indians with type 2 diabetes. JCI Insight 6(10):e146849, 2021. PMC8262294

47. P Rinwa, L Calvo-Enrique, MD Zhang, JR Nyengaard, P Karlsson, P Ernfors. Demise of nociceptive Schwann cells causes nerve retraction and pain hyperalgesia. PAIN. 2021:162:1816-1827.

48. Rohde C, Schmitz N, Thomsen RW, Østergaard SD. Incident psychopharmacological treatment and psychiatric hospital contact in individuals with newly developed type 2 diabetes - a register-based cohort study. Acta neuropsychiatrica. 2020:1-36.

49. Rohde C, Thomsen RW, Østergaard SD. A Within-Subject Before-After Study of the Impact of Antidepressants on Hemoglobin A1c and Low-Density Lipoprotein Levels in Type 2 Diabetes. Journal of clinical psychopharmacology. 2022.

50. Rohde C, Finnerup NB, Schmitz N, Jensen TS, Thomsen RW, Østergaard SD. Is diabetic neuropathy associated with increased risk of developing mental disorders? European journal of endocrinology. 2022;186(5):K39-k43.

51. Rohde C, Nielsen JS, Knudsen JS, Thomsen RW, Østergaard SD. Risk factors associated with mortality among individuals with type 2 diabetes and depression across two cohorts. European journal of endocrinology. 2022.

52. Rumora AE, Guo K, Alakwaa FM, Andersen ST, Reynolds EL, Jorgensen ME, Witte DR, Tankisi H, Charles M, Savelieff MG, Callaghan BC, Jensen TS, Feldman EL. Plasma lipid metabolites associate with diabetic polyneuropathy in a cohort with type 2 diabetes. Ann Clin Transl Neurol. 2021 Jun;8(6):1292-1307. PMID: 33955722

53. Rumora AE, Kim B, Feldman EL. A Role for Fatty Acids in Peripheral Neuropathy Associated with Type 2 Diabetes and Prediabetes. Antioxid Redox Signal. 2022 Apr 26. doi: 10.1089/ars.2021.0155. Online ahead of print. PMID: 35152728.

54. Sajic M, Rumora AE, Kanhai A, Dentoni G, Varatharajah S, Casey C, Brown R, Peters F, Hinder LM, Savelieff M, Feldman EL, Smith KJ. High Dietary Fat Consumption Impairs Axonal Mitochondrial Function In Vivo. J Neuroscience. 2021 May 12;41(19):4321-4334. PMID: 33785643

55. Savelieff MG, Noureldein MH, Feldman EL. Systems Biology to Address Unmet Medical Needs in Neurological Disorders. Methods Mol Biol. 2022;2486:247-276. PMID: 35437727.

56. Schaldemose EL, Andersen NT, Finnerup NB, Fardo F. When cooling of the skin is perceived as warmth: Enhanced paradoxical heat sensation by pre-cooling of the skin in healthy individuals. Temperature 2022:1-16

57. Tesfaye S, Sloan G, Petrie J, White D, Bradburn M, Julious S, Rajbhandari S, Sharma S, Rayman G, Gouni R, Alam U, Cooper C, Loban A, Sutherland K, Glover R, Waterhouse S, Turton E, Horspool E, Gandhi R, Maguire D, Jude EB, Ahmed SH, Vas P, Hariman C, McDougall C, Devers M, Tsatlidis V, Johnson M, Rice ASC, Bouhassira D, Bennett DL, Selvarajahon D on behalf of the OPTION-DM trial group. Comparison of amitriptyline supplemented with pregabalin, pregabalin supplemented with amitriptyline, and duloxetine supplemented with pregabalin for the treatment of diabetic peripheral neuropathic pain (OPTION-DM): a multicentre, double-blind, randomised crossover trial. The Lancet. Aug 22nd. 2022. DOI:https://doi.org/10.1016/S0140-6736(22)01472-6.

58. Themistocleous AC, Kristensen AG, Sola R, Gylfadottir SS, Bennedsgaard K, Itani M, Krøigård T, Ventzel L, Sindrup SH, Jensen TS, Bostock H, Serra J, Finnerup NB, Tankisi H, Bennett DLH. Axonal Excitability Does Not Differ between Painful and Painless Diabetic or Chemotherapy-Induced Distal Symmetrical Polyneuropathy in a Multicenter Observational Study. Ann Neurol. 2022 Apr;91(4):506-520. doi: 10.1002/ana.26319. Epub 2022 Mar 7. PMID: 35150149

59. T Rasmussen, E Vestbo, K Krogh, P Karlsson, TS Jensen, H Andersen, AJ Terkelsen. Diabetic neuropathy caused by too rapid downregulation of blood glucose. Ugeskrift for Læger. 2021:183.

60. V Rasmussen, M Thrysoe, H Tankisi, P Karlsson, ET Vestergaard, K Kristensen, JR Nyengaard, K Krogh, C Brock, AJ Terkelsen. Treatment-induced neuropathy of diabetes in an adolescent with rapid reduction of HbA1c and weight loss: Persistent neuropathic findings at follow-up after 1.5 years. Clinical Case Reports. 2022, 10:e05415.

61. VF Rasmussen, TS Jensen, H Tankisi, P Karlsson, ET Vestergaard, K Kristensen, JR Nyengaard, AJ Terkelsen. Large fibre, small fibre and autonomic neuropathy in adolescents with type 1 diabetes: a systematic review. Journal of Diabetes and Its Complications. 2021, 35(11):108027, Epub ahead of print.

62. Wittenberg B, Svendsen TK, Gaist LM, Itani M, Gylfadottir SS, Jensen TS, Gaist D, Sindrup SH, Krøigård T. Test-retest and time dependent variation and diagnostic values of vibratory sensation determined by biothesiometer and the Rydel-Seiffer tuning fork. Brain and behavior 2021;11:e2230

63. Wittenberg B, Svendsen TK, Gaist LM, Itani M, Gylfadottir SS, Jensen TS, Gaist D, Sindrup SH, Krøigård T. Test-retest and time dependent variation and diagnostic values of vibratory sensation determined by biothesiometer and the Rydel-Seiffer tuning fork. Brain Behav. 2021 Aug;11(8):e2230. doi: 10.1002/brb3.2230. Epub 2021 Jun 4. PMID: 34087955; PMCID: PMC8413738.

64. X Hu, N Agarwal, MD Zhang, P Ernfors, R Kuner, JR Nyengaard, P Karlsson. Identification and quantification of nociceptive Schwann cells in mice with and without Streptozotocin-induced diabetes. Journal of Chemical Neuroanatomy. 2022, 123:102118.

ACCEPTED PHD DISSERTATIONS 2021-2022

Mustapha Itani; “Diabetic Polyneuropathy - Prevalence, Diagnosis, Subtypes and Characteristics” successfully defended June 11 2021

Thorsten Kamlarczyk Rasmussen; “Autonomic neuropathy in small fiber neuropathy and type 2 diabetes” successfully defended April 1 2022

Visit us at **idnc.au.dk**

Editors:
Troels Staehelin Jensen and Signe Toft Andersen,
International Diabetic Neuropathy Consortium

Layout:
Hreinn Gudlaugsson,
AU Events and Communication support

Photos:
AU Events and Communication support and private

Printing:
vahle+nikolaisen

Print run: 100

DANISH PAIN RESEARCH CENTER
Aarhus University Hospital
Palle Juul-Jensens Boulevard 165 , entrance A
DK-8200 Aarhus N

The IDNC is based at the Danish Pain Research Center.