INTERNATIONAL DIABETIC NEUROPATHY CONSORTIUM

ANNUAL REPORT 2019-2020

AARHUS UNIVERSITY
DEPARTMENT OF CLINICAL MEDICINE

IDNC

novo nordisk fonden

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> Diabetic neuropathy is a major complication of diabetes, which in addition to peripheral nervous system damage also may have deleterious effects on central nervous system functions.

PREFACE

It is a pleasure to send you the 5th annual report of the International Diabetic Neuropathy Consortium (IDNC). This consolidated collaboration between Aarhus University, the University of Southern Denmark, the University of Michigan, Ann Arbor, USA and the University of Oxford, UK to study diabetic neuropathy has now entered its 6th year. IDNC is a unique consortium founded in 2015 and among the first to be supported by a Challenge grant from the Novo Nordic Foundation (NNF) to study neuropathy in type 2 diabetes. Diabetic neuropathy was until 2015 less studied despite its high prevalence and association with increased mortality, increased morbidity and reduced quality of life. Moreover, people with this condition experience other problems besides peripheral nerve dysfunction that points towards associated central nervous system dysfunctions such as sleep disturbances, affective dysfunctions and cognitive difficulties that may be part of this complication.

The mechanisms underlying diabetic neuropathy in type 2 diabetes is still not fully understood. IDNC has taken up the challenge to study diabetic neuropathy from different angles: including the possibility that hypoxia of nerve fibers plays a role for the degeneration of nerve fibers, using new techniques to document early changes of nerve function in humans, studying how neuropathy with and without pain affects quality of life and demonstrate risk factors associated with diabetic neuropathy. PhD students and post docs in the IDNC have explored these different areas as presented in this year's annual report. Progress has indeed been made. During this annual reporting period four PhD fellows have obtained their PhD degree. However, getting closer to the end of the granting period it is also clear that new questions arise requiring future studies and some challenges remain unsolved. The collaboration between Danish universities, Steno Diabetes Centers in Denmark and prestigious universities abroad represents a unique opportunity to compile expertise from different disciplines to the benefit of each of these institutions.

IDNC and its members have in the last year, despite the difficulties caused by the Covid-19 pandemia been active in their research as reflected by finished PhD programs and published papers. I would like to express my sincere thanks to all collaborators of the IDNC for their enthusiasm and 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.

Troels Staehelin Jensen
Director of the IDNC

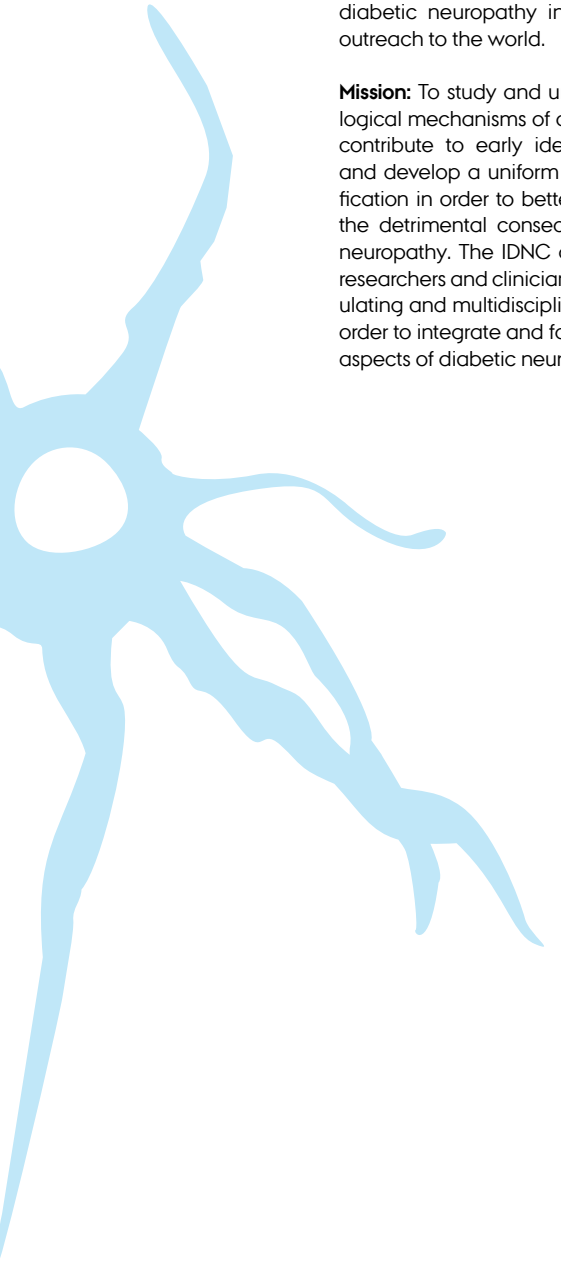
IDNC AT A GLANCE

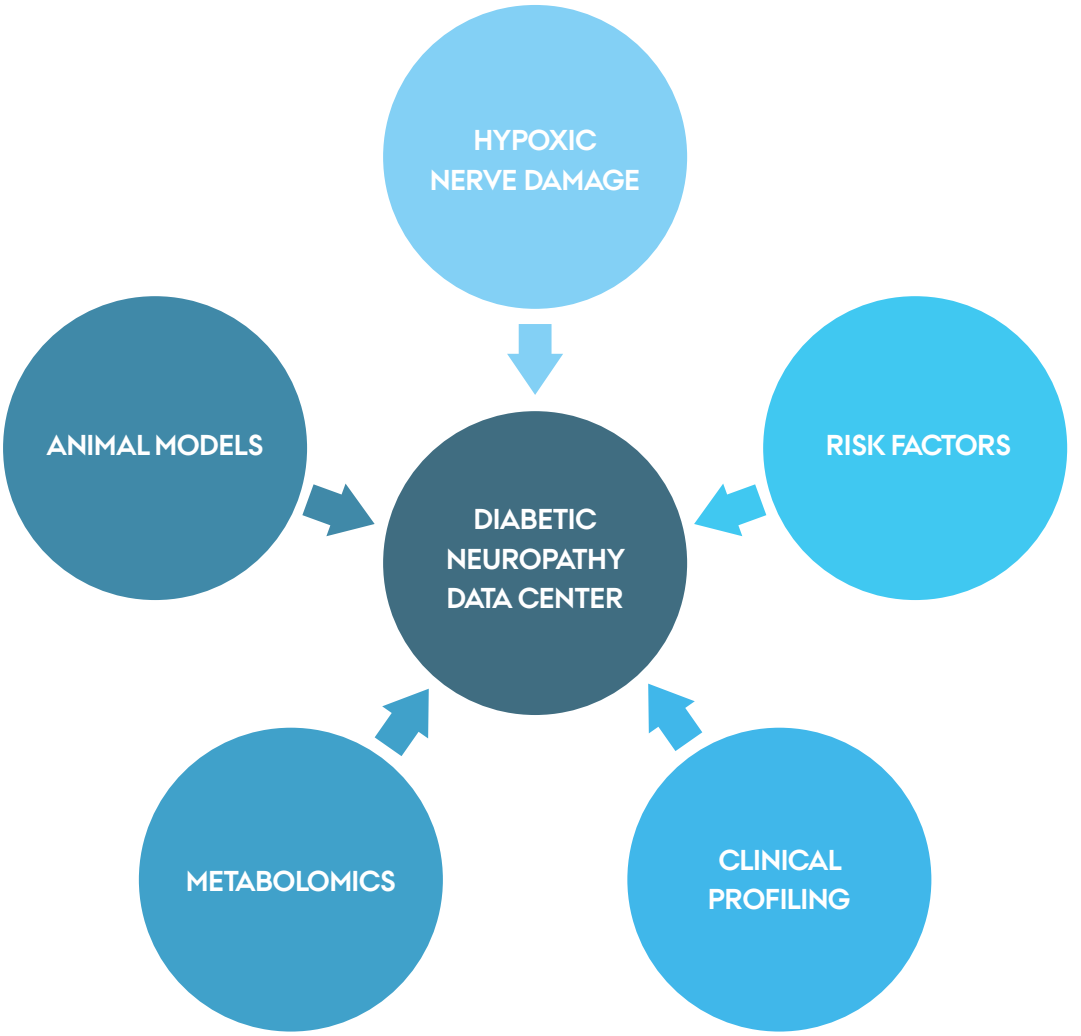
Vision: To be a leading research group on diabetic neuropathy in Denmark with an outreach to the world.

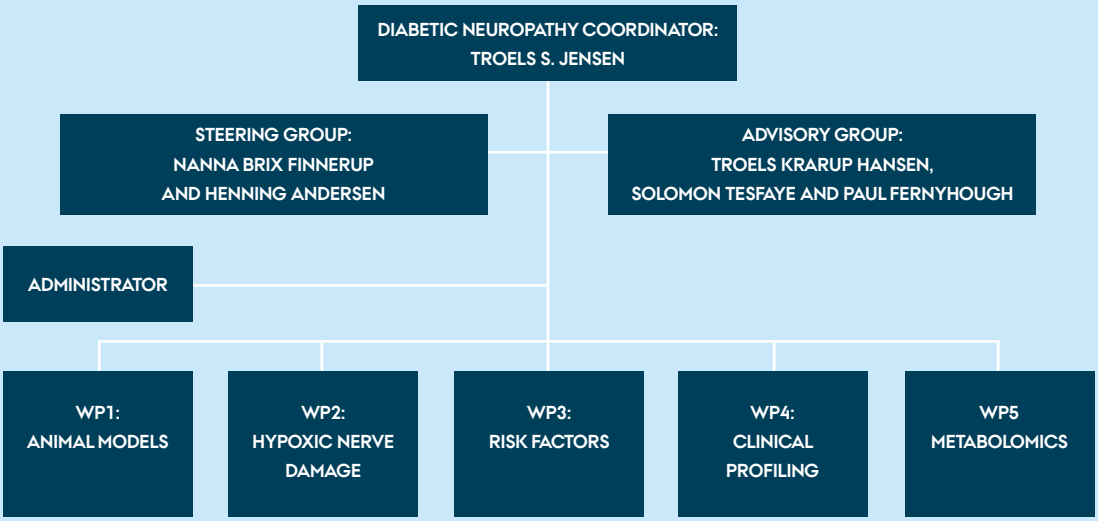
Mission: To study and unravel pathophysiological mechanisms of diabetic neuropathy, contribute to early identification, improve and develop a uniform international classification in order to better treat and prevent the detrimental consequences of diabetic neuropathy. The IDNC does so by bringing researchers 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).




HYPOXIC
NERVE DAMAGE
ANIMAL MODELS
RISK FACTORS
DIABETIC
NEUROPATHY
DATA CENTER
METABOLOMICS
CLINICAL
PROFILING



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
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DK.

SCIENTIFIC ADVISORY BOARD

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Chairman
Aarhus University Hospital,
DK.

PROFESSOR SOLOMON TESFAYE
University of Sheffield, UK.

PROFESSOR PAUL FERNYHOUGH
University of Manitoba,
Canada.

RESEARCH GROUPS

AARHUS UNIVERSITY

Aarhus University Hospital

AARHUS UNIVERSITY AND AARHUS UNIVERSITY HOSPITAL

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UNIVERSITY OF SOUTHERN DENMARK AND ODENSE UNIVERSITY HOSPITAL

Department of Neurology
Søren Sindrup
Mustapha Itani
Thomas Krøigaard

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. 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.*

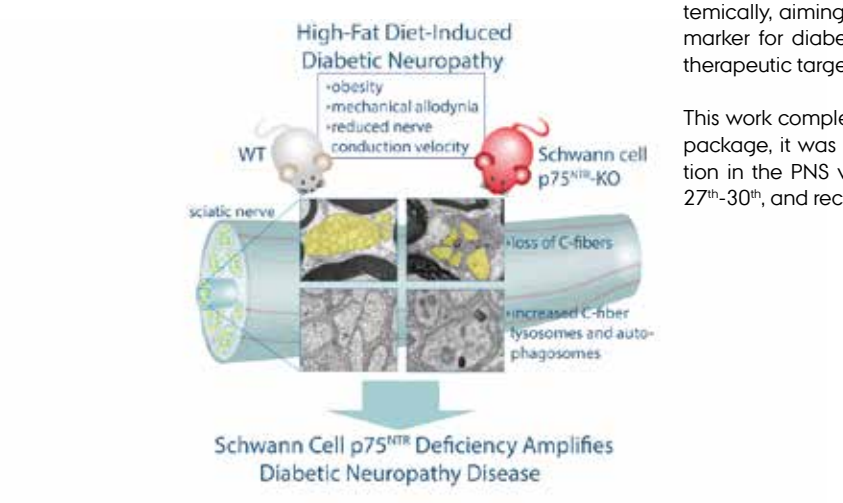


Fig. 1. *Graphical abstract denoting the approach utilized in this study and summarizing the main results. Yellow mask in the electron microscopy pictures highlights the C-fibers, which are significantly decreased in SC-$p75^{NTR}$-KO HFD as compared with WT HFD mice.*

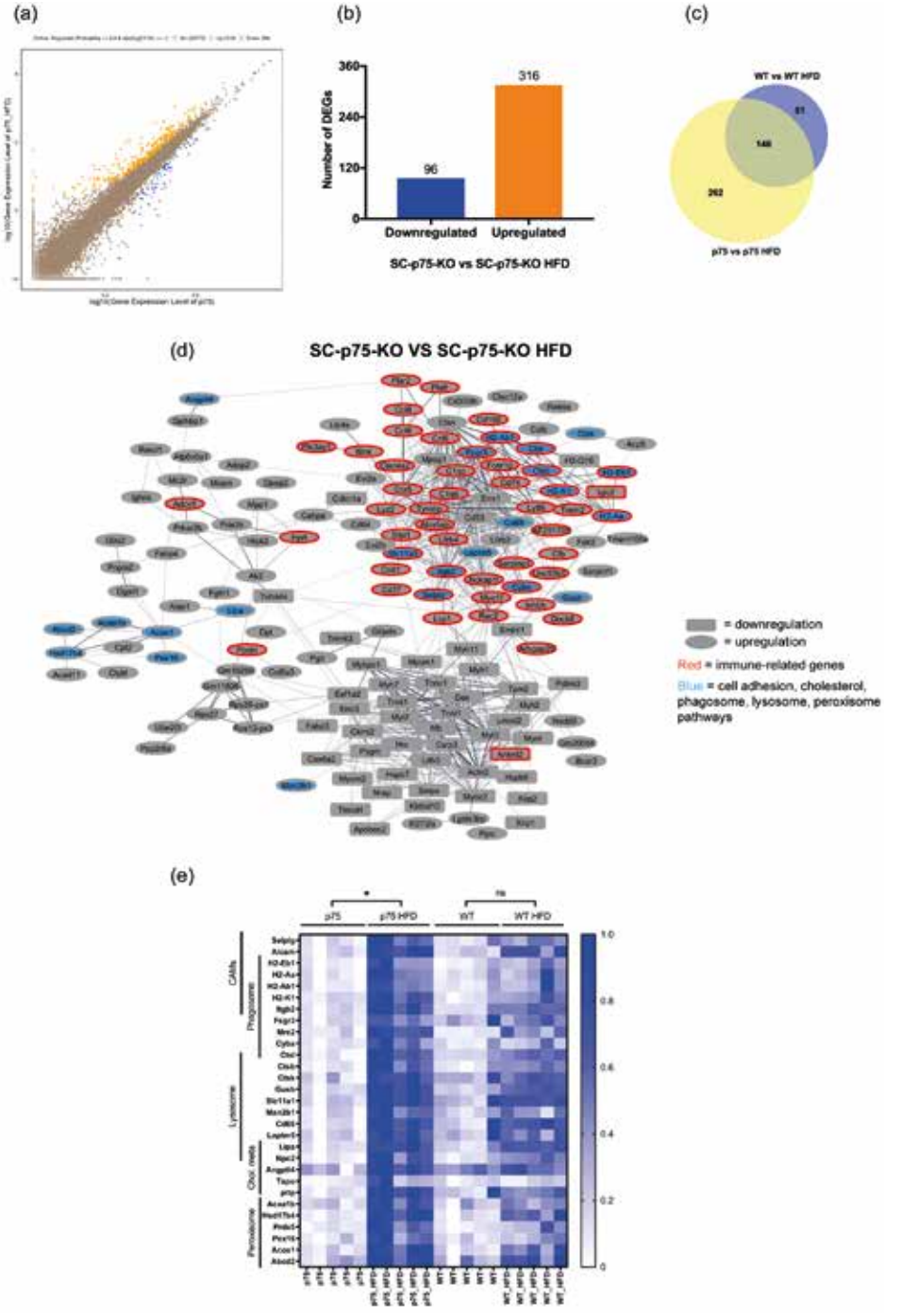


Fig. 2. *Glial p75^{NTR} modulates the inflammatory landscape and activation of lysosome and phagosome pathways in mice fed with an HFD.* ***(a)*** *log10 gene expression level graph and* ***(b)*** *a numerical graph demonstrating the upregulated (in orange) and downregulated (in blue) genes in SC-p75^{NTR}-KO 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.*

WP1: DEVELOPMENT OF A TELEMETRIC IMPLANT TO REVEAL PERIPHERAL NEUROPATHY

Martin Nors Skov *is PhD student at the Comparative Medicine Lab at Aarhus University (DK). Professor Michael Pedersen leads the research with Ass. Professor, Hatice Tankisi and Ass. Professor Vladimir Matchkov. as co-supervisors*

The project is now in its final phase, including analyses and dissemination of results. In particular, focus is put on the last (of 4) sub-studies, where electrodes are implanted in peripheral rat nerves for 8 weeks. The plan is to submit the PhD thesis ultimo 2020; the thesis will consist of 4-5 manuscripts that are in review or planned for submission to peer-review scientific journals. Except one paper, all papers are prepared with Martin Nors Skov as the first author.

We have finished the final study in which we implanted electrodes around rat nerves and received nerve measurement data up to 8 weeks after implantation. The study was designed as a biocompatibility study in order to test how the electrodes affected both the surrounding tissue and the proximal sciatic nerve. Blood samples were withdrawn weekly to evaluate the degree of inflammation associated with the implant. At the end of the study period, the sciatic nerve and surrounding tissues were dissected and histologically stained for anatomical changes. Preliminary data show that the implanted electrodes had no negative effects on nerve function nor did they cause significant inflammation. Our preliminary conclusion is that it is possible to implant novel nerve electrodes in rats without complications or nerve dysfunction.

The increasing interest in our system (particular the amplifier part) led to a secondary project, where we explored the possibility of further development of a small wireless setup for an electrophysiological system. Thus, we applied the BioInnovation Institute Proof-of Concept grant, and in 2020 we received 1 million DKR from this grant. This funding will be used for building the first clinical prototype: a wireless and battery powered full electrophysiological setup with build-in stimulator and amplifier. Comparable to a regular electrophysiological setup, our idea is to build a setup that is so small that a patient can wear it on the limb. We are pleased to note the positive response received from Hugh Bostock and James (Tim) Howells about the idea of the first prototype to incorporate Qtrac functionality. To fulfill our ambition, we have teamed up with an R&D company housed in Aarhus, DK. The first prototype is scheduled for early 2021.

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: ARE DIABETIC NERVES SUFFOCATING?

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

Using state of the art in vivo two-photon microscopy we have characterized anatomy and function of microvasculature in distal peripheral sensory nerves in mice *(Dudele, A., Rasmussen, P. M., and Østergaard, L. Sural nerve perfusion in mice. Submitted).* Our results showed that microvascular density and blood flow in these nerves are comparable to that previously reported in murine brain tissue. Additionally, the study revealed that external experimental factors, such as small variations in tissue temperature during in vivo measurements, affect neural blood flow.

We have further applied these techniques to investigate microvascular anatomy and function in sural nerves of obese mice with type 2 diabetes and diabetic peripheral neuropathy (DPN). These studies revealed that the disease changes microvascular anatomy of the sural nerve (Fig. 1). Additionally obesity and DPN also changes blood flow of the sural nerve as evidenced by an increase in red blood cell flux, particularly in response to electrical stimulation of the nerve – a change that was not present in control animals (Fig.2). Our results also showed that sural nerve vessels do not dilate in obese animals in response to stimulation, as they do in control mice (Fig.2).

These findings show microvascular involvement in DPN in mice with obesity and type 2 diabetes. Understanding these mechanisms will allow for future development of novel screening and intervention techniques and opportunities that can specifically target microvasculature of the distal peripheral sensory nerves.

The next step of the project is to assess whether the observed microvascular changes lead to hypoxia in the nerves. In the fall of 2020 a study utilizing oxygen sensitive tracer and two-photon microscopy (Fig.3), will characterize oxygen delivery and extraction in the sural nerves of control mice and obese mice with DPN under rest and electrical stimulation of the nerve.

Figure 1

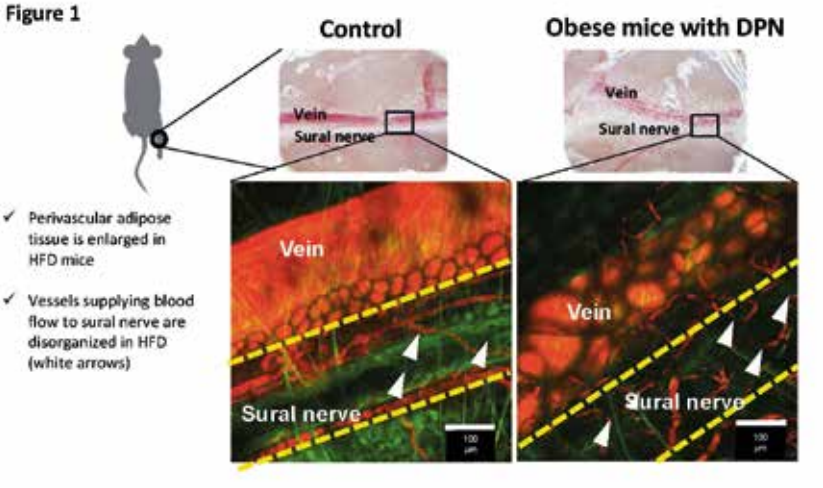


Figure 2

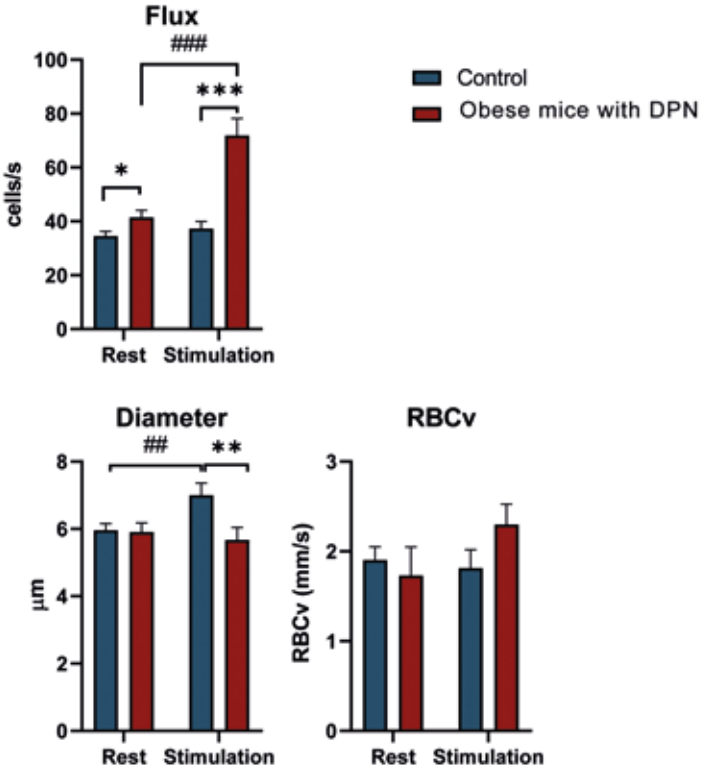
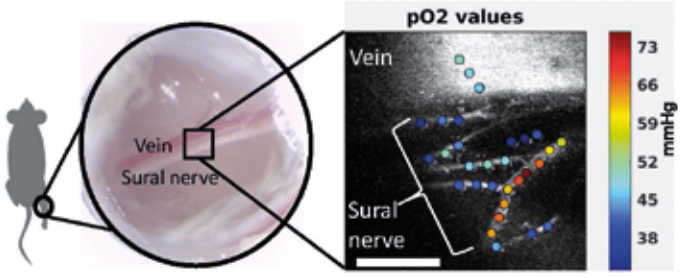
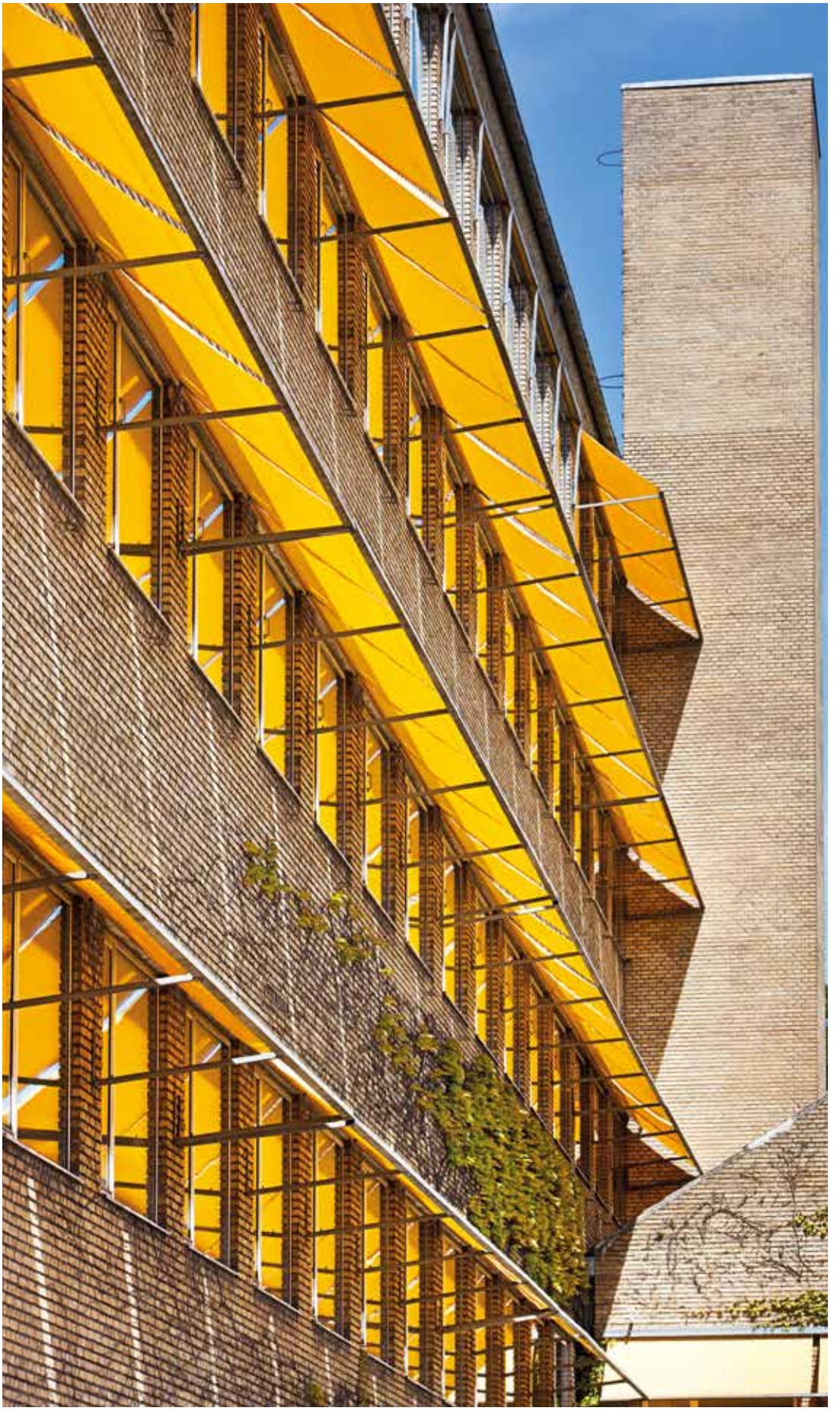


Figure 3



Intravenous injection of oxygen sensitive tracer for visualization of blood vessels (depicted in white color), and measurement of blood PO_2 in the points of interest. Points of interest here are within the microvessels supplying blood to the sural nerve, and the adjacent vein as a reference value for venous blood.
Scale bar = 100µm.



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 is involved in a number of ongoing studies based on data from ADDITION-Denmark and with the planning of a research project in general practice in collaboration with Steno Diabetes Center Aarhus. Signe has also been involved in the writing of a PhD protocol for an upcoming PhD project on novel biomarkers of diabetic neuropathy (DPN) which has received funding by the Danish Diabetes Academy and Aarhus University and will be carried out by MD Laura Linnea Määta with Signe as co-supervisor.
Signe is preparing a manuscript for a study entitled *"Subclinical cardiovascular autonomic neuropathy as a determinant of cardiovascular disease events and mortality early in the course of type 2 diabetes: ADDITION-Denmark"*.

Another study is done in a collaboration between the ADDITION-Denmark study and DD2 study with MD, PhD Lasse Bjerg Hansen as first author. This is a meta-analysis on CVD events and mortality associated with presence of DPN as defined by the Michigan Neuropathy Screening Instrument questionnaire early in the course of diabetes entitled: *"Symptoms of peripheral neuropathy early in type 2 diabetes are associated with higher risk of subsequent cardiovascular disease"* showing no significant association with mortality but a 70% higher incidence rate ratio for subsequent CVD events in people with DPN in a model adjusted for multiple confounders. The manuscript in soon to be submitted.

Signe is preparing a project: *"Screening for subclinical cardiovascular autonomic neuropathy in people with type 2 diabetes in Central Denmark Region: to improve the care and prognosis of type 2 diabetes*". This study will assess the prevalence of subclinical cardiovascular autonomic neuropathy (CAN) in 500 people representing the broad type 2 diabetes population in Denmark and measure 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. This project is under preparation in collaboration with researchers based in the Steno Diabetes Center Aarhus.

Lastly, Signe is under IDNC involved in preparing an intervention study in collaboration with Steno Diabetes Centers in Denmark to lower the risk of developing the first diabetic foot ulcer.

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*

Diana Hedevang Christensen defended her PhD thesis entitled "Diabetic polyneuropathy in type 2 diabetes – Prevalence, risk factors, mental health, and diagnostics validity" on December 20, 2019.

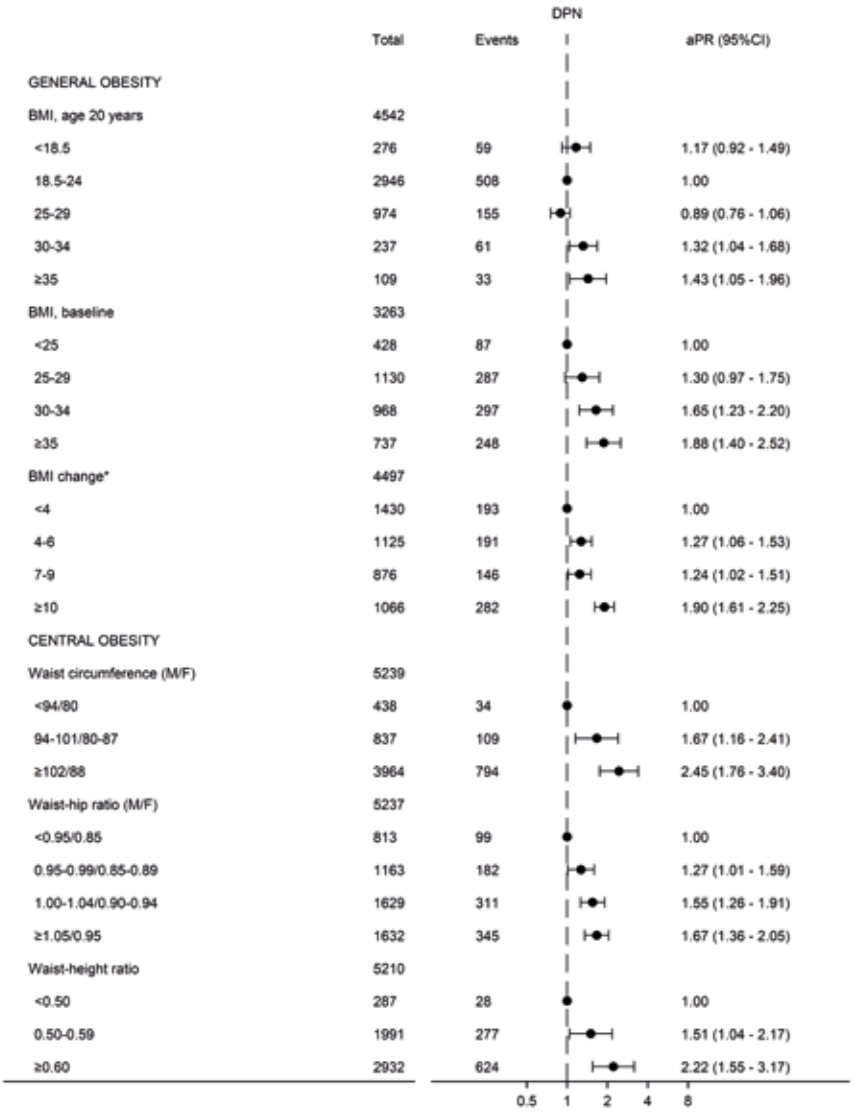
The PhD encompassed four studies. The first study provided a detailed description of the DD2 cohort. The second study was carried out together with MD, PhD Sif Sandra Gylfadottir using data from the IDNC-DD2 neuropathy questionnaire survey (N = 5,514). That study showed a prevalence of possible diabetic 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 was based on data from the IDNC-DD2 questionnaire and linked registers, and 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 (Fig). Moreover, other modifiable metabolic syndrome factors and unhealthy lifestyle habits associated with DPN. Neuropathic pain in DPN was associated with modifiable unhealthy lifestyle habits. The fourth study used register data and medical records to examine the potential for using diagnosis and prescription codes to study non-painful and painful DPN in 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.

With medical student Frederik Pagh Kristensen as first author, the study *"Statin therapy and risk of polyneuropathy in type 2 diabetes: A Danish population-based cohort study"* uses the identified DPN algorithm to examine the impact of statin therapy on DPN risk. This study concludes 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 study was recently accepted for publication in Diabetes Care. The DPN algorithm is also used in an ongoing study on the impact of incretin-based therapy on DPN risk.

The IDNC-DD2 questionnaire data has formed the basis for a study entitled *"Falls and fractures associated with type 2 diabetic polyneuropathy; a cross-sectional nationwide questionnaire study"* with MD, PhD Karolina Snopek Khan as first author, the study is currently under review. This study supports 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.

Together with MD, PhD, Lasse Bjerg Hansen, an ongoing collaborative study taking advantage of both DD2 and ADDITION-Denmark data, *"Polyneuropathy in early type 2 diabetes is associated with higher risk of subsequent cardiovascular disease: Results from two Danish cohort studies"* shows a markedly higher risk for cardiovascular disease among those with DPN identified using the MNSIq, meta-analysis: IRR = 1.65 (95% CI: 1.41-1.95).

Figure Prevalence ratios of DPN associated with general (BMI: kg/m2) and central obesity measures (waist circumference: cm)



*Abbreviations: aPR; adjusted prevalence ratio, DPN; diabetic polyneuropathy, CI; confidence interval, BMI; body mass index, M/F; male/female. All estimates are adjusted for age, sex, and diabetes duration. Baseline BMI is the BMI measured around time of type 2 diabetes diagnosis. *BMI change from age 20 to DPN assessment in 2016.* ***Christensen et al, Diabetes Care 2020***

A happy PhD candidate together with her supervisors and the assessment committee.

WP3: DD2 COHORT AND REGISTRIES

Frederik Pagh Kristensen *is a medical student and finished a research year 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 and may reduce the risk of DPN due to lipid-lowering, anti-inflammatory, and anti-oxidative effects. However, previous studies have also associated statins with neurotoxicity. In a study entitled *"Statin therapy and risk of polyneuropathy in type 2 diabetes: A Danish cohort study"*, Frederik Pagh Kristensen examined whether statin therapy impacts the risk of DPN. This study was recently accepted for publication in Diabetes Care

We used the nationwide population-based registries to conduct a cohort study of all incident type 2 diabetes patients in Denmark in 2002-2016 (N=259,625). Risk of developing DPN was assessed in new users and prevalent users of statin therapy following patients from 6 months after the diagnosis of type 2 diabetes (index date). Hospital-diagnosed DPN was defined using an algorithm based on hospital discharge diagnosis codes previously validated by the IDNC (Diana Hedevang Christiansen).

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. 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 effect vanished after ≥2 years of follow-up (Figure). Findings were similar in on-treatment and propensity score-matched analyses, and with additional adjustment for pre-treatment blood lipid levels.

We propose that previous studies investigating the association between statin therapy and DPN have been falsely inflated by a mix-up of statin-exposure groups and by non-validated diagnosis codes. Statin therapy is unlikely to increase or mitigate DPN risk in type 2 diabetes, although a small acute risk of harm cannot be ruled out.

During the fall of 2020, Frederik Pagh Kristensen will initiate a new study to investigate the association between lipid levels around the time for diagnosis of type 2 diabetes with DPN using data using the Clinical Laboratory Information System (LABKA) and linked medical registers. Moreover, Frederik is planning a PhD-study to investigate microvascular complications in diabetes after finishing his medical studies in June 2021.

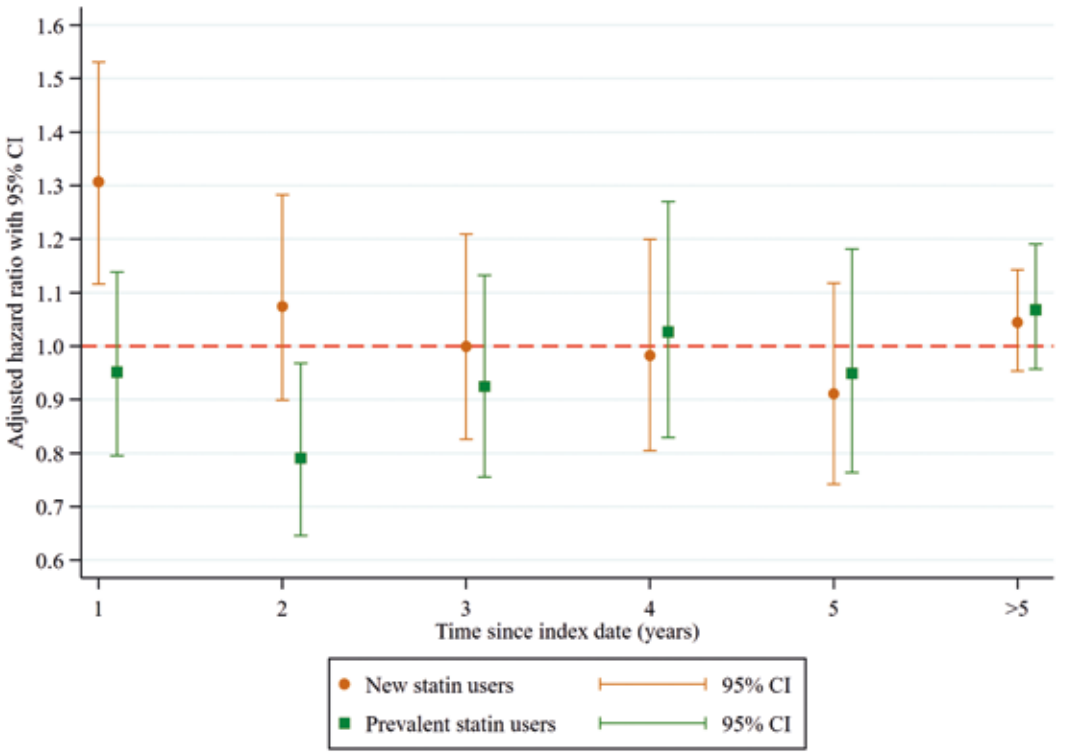


Fig. *Adjusted hazard ratios of DPN in one-year follow-up intervals, comparing new and prevalent statin users with statin non-users.*

WP3: A POTENTIAL NOVEL BIOMARKER OF DIABETIC NEUROPATHY

***Laura Linnea Määttä** is a MD, PhD student just starting her project October 2020 under IDNC 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 Linnea Määttä will work 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 neuropathy (DN) using three cohorts of people with diabetes: in the ADDITION-Denmark cohort, in the English Pain in Neuropathy Study (PiNS) and in 200 patients attending the SDCA outpatient clinic.

A biomarker for DN could provide a valuable tool for early detection and follow-up of DN and thus focus the attention of physicians and patients on this complication, as well as provide a highly needed objective intermediate outcome measure to evaluate interventions across study populations. NfL has emerged as a potential candidate biomarker for DN. NfL is a neuron-specific cytoskeletal protein confined to the intracellular environment of neurons of both the central (CNS) and peripheral nervous system (PNS), released to the extracellular environment only upon nerve fiber decay (Figure). Whereas solid evidence exists for NfL as a sensitive and broad marker of CNS diseases, the evidence for its use in the PNS is sparse. Yet, recent studies have reported serum NfL levels to reflect presence and severity of certain inherited and acquired peripheral neuropathies with higher levels reflecting more severe disease.

Thus, Laura and her group hypothesize that NfL is associated with the presence, severity and progression of DN with higher levels reflecting more progressed stages of DN. To explore this hypothesis, Laura will study both cross-sectional and longitudinal associations between NfL and DN in three study-cohorts. The PhD program includes a three-month research exchange stay at the University of Oxford, UK, under supervision of Professor David Bennett. The NfL analysis will be carried out at the Department of Clinical Biochemistry, Aarhus University Hospital, which has established and validated NfL analysis with the Single Molecule Array technology, a highly sensitive digital immunoassay, to allow accurate NfL quantification at exceedingly low levels.

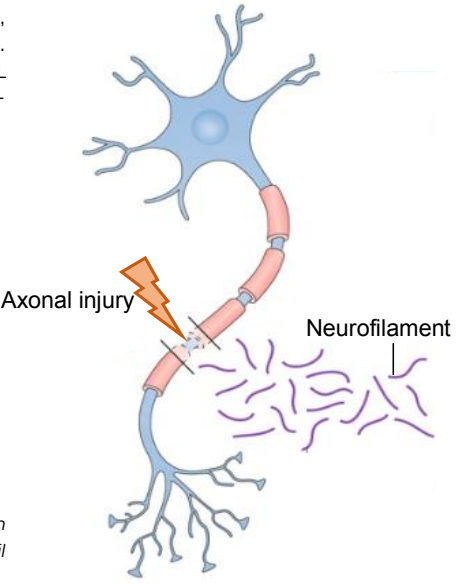


***Fig.** Illustration of neurofilament release upon axonal decay of a nerve fiber (adapted from Khalil et al. Nat Rev Neurol 2018)*

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 significantly increased risk of developing depression compared to the general population. Depressive symptoms are also common in patients with painful diabetic neuropathy. Depression comorbid to T2D can be successfully treated with antidepressants, which also represent one of the 1st choices for treating painful diabetic neuropathy. However, the beneficial effects of antidepressants in T2D may go beyond the mere treatment of depression. Indeed, we hypothesize that treatment with antidepressants can improve compliance to T2D treatment regimens, promote a healthier lifestyle, decrease the rate of major T2D-related complications including diabetic neuropathy and reduce T2D-related mortality. This project aims to test these hypotheses via a series of studies based on data from the Danish nationwide health registers as well as from the Danish Centre for Strategic Research in Type 2 Diabetes (DD2) project cohort. The following research questions will be addressed:

1. To characterize the pattern of use of antidepressants and other psychopharmacological treatments among individuals with T2D and to determine whether the choice of drugs is influenced by diabetic complications, such as neuropathy.

2. To investigate whether the use of antidepressants in T2D leads to a healthier lifestyle, e.g. increased physical activity, smoking cessation, reduced alcohol consumption, and reduced BMI.

3. To investigate whether the use of antidepressants in T2D is associated with reduced hyperglycemia and lipidemia as well as improvement in other biomarkers.

4. To investigate whether the use of antidepressants in T2D reduces the risk of major complications, such as ischemic stroke, other cardiovascular events, severe neuropathy, and dementia.

5. To investigate whether the use of antidepressants in T2D decreases mortality, and – if so – determine, which causes of death that are responsible for this decrease

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.

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*

Our priority this year has been to investigate genetic risk as a determinant of neuropathic pain (NeuP) in the context of diabetic neuropathy. This is being studied in the context of a highly phenotyped cohort of patients with diabetic neuropathy (which have undergone the NeupSIG grading system for neuropathic pain). We are also extending this genetic approach as a collaboration between the DOLORisk consortium (See Pascal et al., 2019) and IDNC. The IDNC has undertaken questionnaire based sensory phenotyping of the DD2 cohort and 688 of these subjects have evidence of diabetic neuropathy on the basis of the Michigan Neuropathy Screening Instrument questionnaire and neuropathic pain status is being determined by the DN4 questionnaire. These data will be combined with 1800 highly phenotyped diabetic neuropathy patients as part of DOLORisk. Encouraging initial results are genome wide significant linkage between the genomic locus encoding the gene KCNT2 and neuropathic pain intensity in DOLORisk and this is now being replicated in DD2 (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 diabetic neuropathy and is an exciting target given that KCNT2 is known to be highly expressed in sensory neurons. We have also completed a rephenotyping exercise in UK-BB for neuropathic pain and this data will be available for replication in Q1 next year.

As future plans we are also continuing our work on 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 recorded from 151 participants in Oxford. Preliminary data analysis of four outcomes does not show differences between participants with painful and painless DPN. We have combined this data with that collected in Aarhus and generating a joint manuscript. Over the next year we will complete the genetic analysis including GWAS and whole exome sequencing.

NUFFIELD DEPARTMENT OF CLINICAL NEUROSCIENCES

WP4: CLINICAL PROFILING OF DIABETIC NEUROPATHY

Mustapha Itani *is a neurologist at the department of neurology at Odense University Hospital (OUH) and a PhD student at the University of Southern Denmark (SDU). Professor Søren Sindrup leads the research*

Mustapha Itani has 3 distinct projects based on the detailed clinical profiling of a sample of type 2 diabetes patients from the national DD2 cohort:

The first project has just been published in the European Journal of Neurology in collaboration with other members of IDNC. This project 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 probable DPN and 11.5% for painful DPN. Furthermore, we validated 2 well known neuropathy scores, the Michigan Neuropathy Screening Instrument (MNSI) and the Dolour Neuropatique en 4 (DN4). These scores have not previously been validated in a population of people with type 2 diabetes. The sensitivity and specificity of a MNSI score ≥ 4 to detect definite DPN was 25.7% and 84.6%, respectively. The sensitivity and specificity of a DN4 score ≥ 3 with pain in both feet to detect definite painful DPN was 80% and 89.9%, respectively.

The second project will look at subtypes of DPN based on measures of 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 will examine the effect of different diagnostic models on the frequency of these subtypes and pave the way for future consensus criteria (see Figure)

The third and last project will compare demographic, cardiovascular and neuropathy characteristics of DPN in people with type 2 diabetes with idiopathic polyneuropathy (IPN) hypothesizing that considerable similarities can indicate similar pathogenesis.

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.

WP4: CHALLENGES IN THE DIAGNOSIS OF DIABETIC POLYNEUROPATHY

Sandra Sif Gylfadottir *is a MD, PhD. Her PhD project was supervised by Professor Nanna Brix Finnerup, Danish Pain Research Center, Aarhus University (DK)*

Sandra Sif Gylfadottir successfully defended her PhD project *"Painful and non-painful DPN"* in March 2020 and has now moved on to study the challenges in the diagnosis of diabetic polyneuropathy.

The commonly used gold standard for the diagnosis of diabetic polyneuropathy (DPN) is the Toronto classification of DPN comprising three levels of certainty (Tesfaye et al. 2010). Possible DPN with one of three: symptoms or signs of DPN or decreased or absent ankle reflexes, probable DPN with at least two of the aforementioned and definite DPN with at least one of three together with abnormal confirmatory nerve conduction studies or abnormal intra epidermal nerve-fiber density. Inherent in the definition is a potential large variation dependent on the combination of symptoms, signs and confirmatory tests. For example, a patient with only reduced ankle reflexes and abnormal intra epidermal nerve fiber density is classified in the same group as a patient with several symptoms, signs and abnormal IENFD. Standard neuropathy scales, can also be used in the diagnosis of DPN. They are designed to be easy to administer and to be used by various health care professionals. Examples are the Michigan Neuropathy Screening Instrument (MNSI) examination part which consists of foot examination, Toronto clinical Scoring System (TCNS) incuding both examination and symptoms and the Utah Early Neuropathy Scale (UENS) focusing on early signs of small fiber neuropathy. In Figure 1 they are plotted against the gold standard diagnosis according to the Toronto classification in a population of recently diagnosed type 2 diabetes patients from the DD2 cohort. There is a positive correlation between increasing certainty for polyneuropathy from possible to definite polyneuropathy and increasing scores on the scales, but also a large variation of scores in the DPN groups. This is most pronounced in probable and definite DPN and there is a substantial overlap between these two groups. Receiver Operating Characteristic area (ROC) area ((sensitivity + specificity)/2)) of the neuropathy scales was from 0.66-0.71 (Figure). In conclusion, this hierarchical diagnostic system is useful, but takes time and resources. The ideal alternative would be an easy to use diagnostic tool that could detect, diagnose and stage DPN with high diagnostic accuracy.

Future plans: The next step is to use the clinical data we collected together with patient reported outcomes to make a secondary analysis in order to try to make prediction models in terms of which distinctive features characterizes DPN with and without pain.

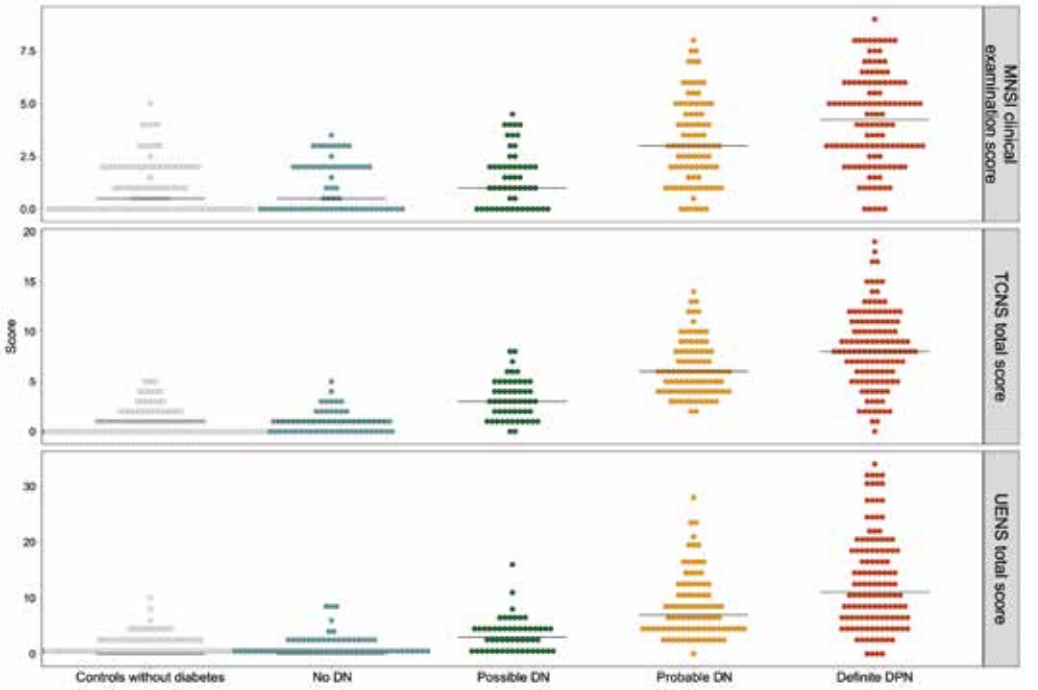


Fig. *Correlation of median scores of the MNSI examination part, TCNS and UENS and DPN groups inclusive controls without diabetes and Receiver Operating Characteristic area (ROC) area. MNSI: rs 0.61, P < 0.001, ROC area: 0.71, TCNS: rs 0.79, P < 0.001, ROC area: 0.69, UENS: rs 0.73, P < 0.001, ROC area: 0.66*

Figure from: The diagnosis and prevalence of diabetic polyneuropathy: A cross-sectional study of Danish patients with type 2 diabetes. Gylfadottir SS and Itani M et al. EJN. *In press.*

A happy PhD candidate together with (to the left) Professor Per Løgstrup Poulsen as chair of the assessment committee, (to the right) main supervisor Professor Nanna Brix Finnerup and Professor Solomon Tesfaye, UK who assessed the PhD together with Catharina G. Faber, NL. Catherine Faber was not present at the defence because of the Covid-19 situation

WP4: MOTOR DYSFUNCTION IN DIABETIC NEUROPATHY

Henning Andersen, *Professor, Chair of Research, Department of Neurology, Aarhus University Hospital supervises research of Research year 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.

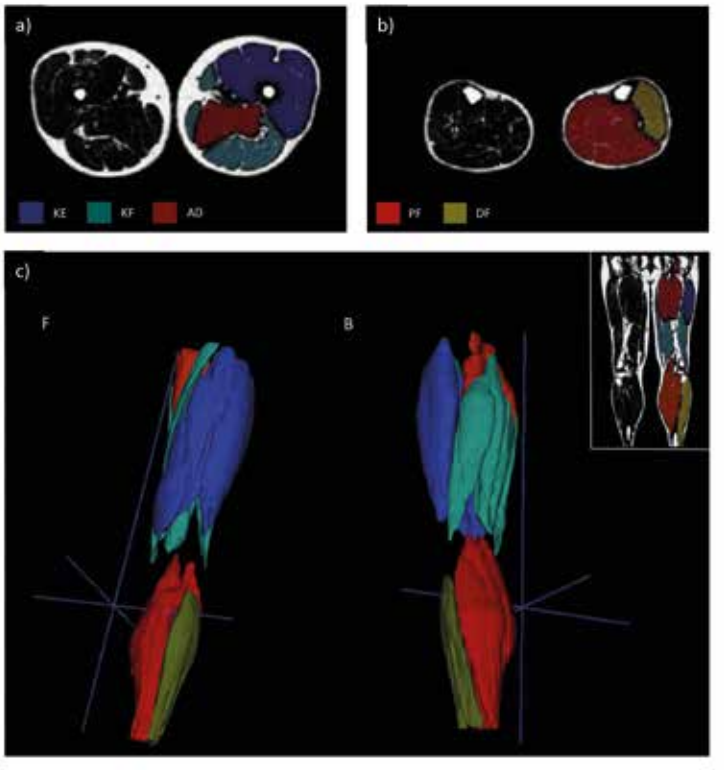


Fig. *Muscle group segmentation shown on full leg Dixon fat data of a healthy participant. (a) Segmentation of thigh muscle groups. KE = Knee extensors (left leg). KF = Knee flexors (left leg). AD = Adductors (left leg). (b) Segmentation of lower leg muscle groups. PF = Plantar flexors (left leg). DF = Dorsal flexors (left leg). (c) 3D illustration of full leg segmen-tation. Front vi ew of left leg (F). Back view of left leg (B).*

WP4: MOTOR DYSFUNCTION IN DIABETIC NEUROPATHY

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).*

Motor dysfunction is a debilitating complication of diabetic polyneuropathy (DPN) that can lead to a sedentary lifestyle that can worsen diabetes and symptoms of DPN. There is until today, no disease-modifying treatment for DPN, and medical therapy is solely aimed towards symptomatic relief. Individuals with diabetes have an increased risk of falling, and little is known about fall causation in DPN. Currently, there are no easily assessable tools to identify individuals at risk of falls with type 2 diabetes and DPN. Exercise has shown to have beneficial effects in type 2 diabetes. However, little is known about resistance training in individuals with DPN, and exercise recommendations are lacking. By understanding the underlying fall causation, the impact of motor dysfunction, and the effects of progressive resistance training (PRT) in DPN, these studies can provide insight aimed towards future treatment options and fall prevention. Furthermore, the results from the PRT trial can provide evidence for PRT and may contribute to future exercise recommendations for individuals with type 2 diabetes and DPN.

Study I: In a cross-sectional nationwide questionnaire study we examined the frequency of self-reported falls in relation to symptoms of DPN in patients with recently diagnosed type 2 diabetes. We found that falls were 2.33 times more prevalent when DPN was present, after adjusting for possible confounders aPR: 2.33 (95% confidence interval [CI] 2.06-2.63). Possible DPN was associated with a slightly although non-significantly increased risk of fractures: aPR: 1.32 (95% CI: 0.75-2.33). These results emphasize the need for preventive interventions to reduce fall risk among patients with type 2 diabetes and DPN. This study has been submitted for publication.

Study II: A total of 92 type 2 diabetes patients and 39 controls were evaluated to describe the characteristics of fallers with diabetes and the association of motor dysfunction, postural instability and DPN. Individuals with type 2 diabetes reported a higher number of falls within the preceding year compared to healthy controls, irrespective of the presence of DPN. The main risk factors associated with falls were increased postural instability, low-

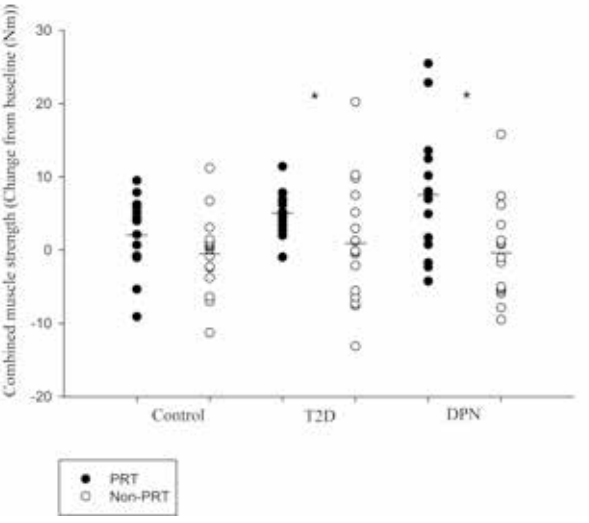


Fig. Effects of progressive resistance training on muscle strength
*Change in combined isokinetic muscle strength (% change from baseline value) following 12 weeks of progressive resistance training in patients with type 2 diabetes with DPN and without DPN (T2D) and in healthy controls (Control) according to randomization group (PRT/Non-PRT). Comparing PRT versus Non-PRT in all three groups * p<0.05.*

er walking capacity and slower sit to stand movements. – the paper of this study is currently under review

Study III and IV: We examined the effects of a 12-week progressive resistance training (PRT) in a randomized single blinded controlled trial in type 2 diabetes patients with and without DPN and healthy control individuals. A total of 90 participants completed the study including 30 participants in all three groups (DPN versus non-DPN and healthy controls).

In patients with type 2 diabetes and DPN, PRT improved strength of the knee extensors and flexors and motor function at a level comparable to both diabetes patients without DPN and healthy controls. No changes were found in postural stability index, patient complaints or symptoms of neuropathy. PRT did not result in change in skin biopsy parameters (GAP-43, IENFD, nerve fiber branching) in any of the three groups (Figure).

–This study will result in two consecutive publications and are currently under preparation for submission.

Future publications:
Karolina plan to conduct a review entitled: *"The Impact of Diabetic Neuropathy on Accidental Falls, Postural Balance and Activities of daily living"*

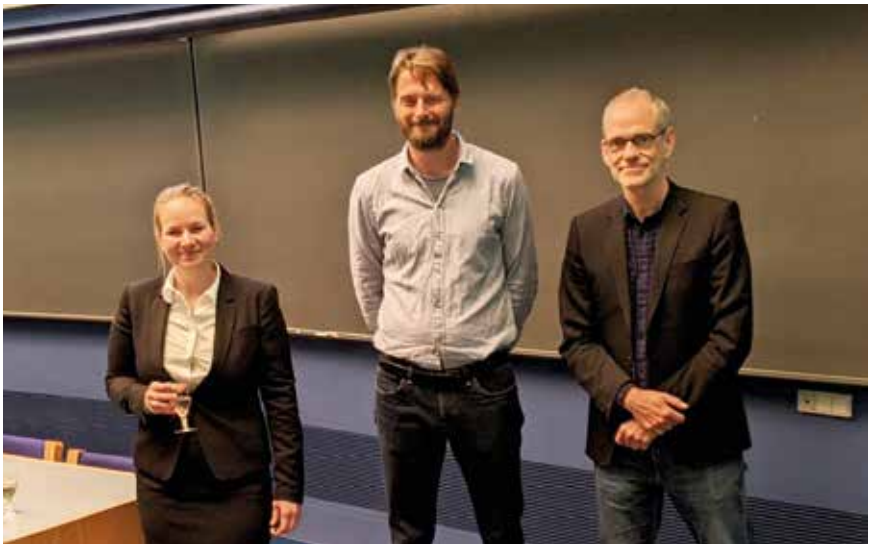


Fig. *Karolina defended her PhD on May 1st 2020. From the left Karolina, Kristian Overgaard (supervisor) and main supervisor Professor Henning Andersen. Vincenza Spallone and Jørn Wulff Helge assessed the PhD with Uffe Birk Jensen as chair.*

WP4: NEUROPHYSIOLOGY IN CLINICAL IDNC STUDIES

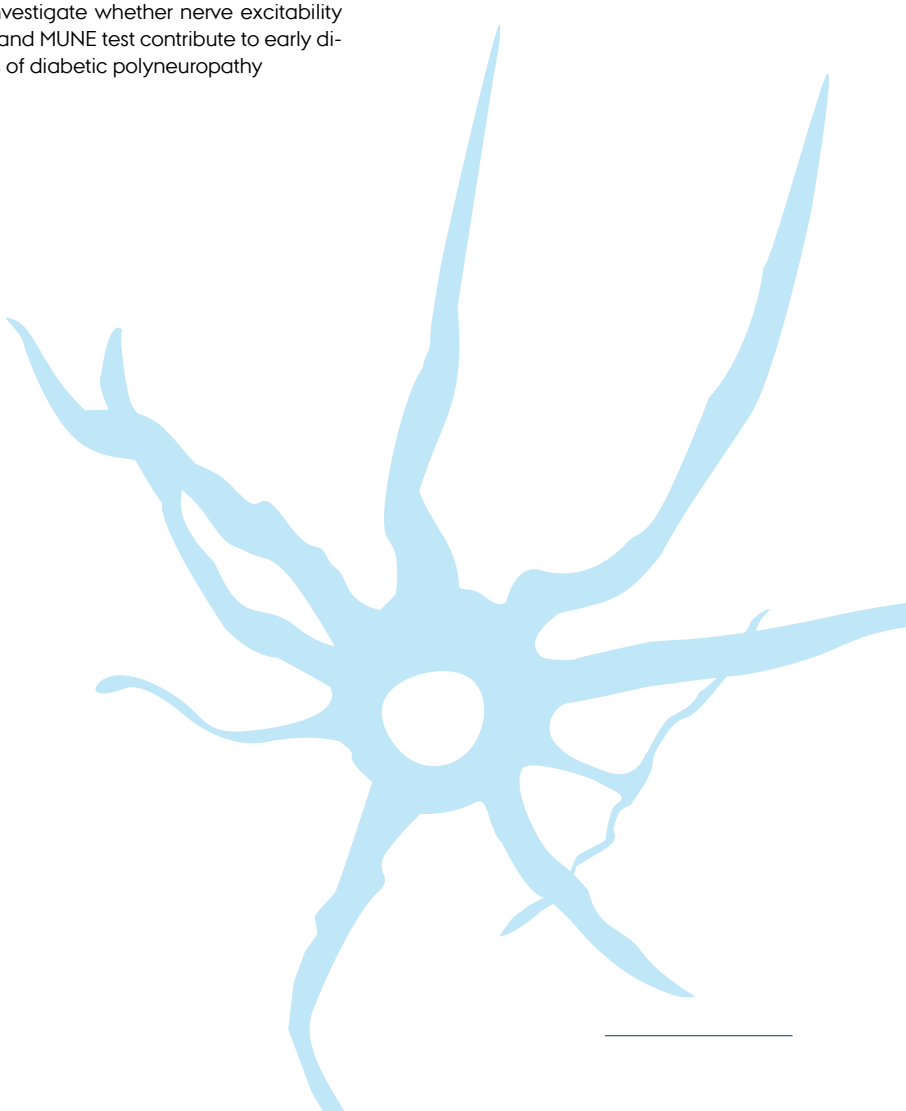
Hatice Tankisi *is an Associate Professor, Consultant, PhD at Department of Neurophysiology, Aarhus University Hospital. She supervises the research of Alexander Gram and Mustafa Aykut Kural*

Objectives:

1) To obtain the optimal diagnosis of large fiber diabetic polyneuropathy (DPN) in IDNC by use of comprehensive routine Nerve Conduction Studies (NCS)

2) To understand the mechanisms of diabetic neuropathy by use of novel electrophysiological techniques such as excitability tests called threshold tracking as well as motor unit number estimation (MUNE) tests.

3) To investigate whether nerve excitability testing and MUNE test contribute to early diagnosis of diabetic polyneuropathy



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. Associate Professor Hatice Tankisi, supervised the project.*

Alexander Gramm Kristensen conducted electrophysiological studies and defended his thesis successfully on the 7th February, 2020. Alexander wrote 3 papers as a part of his PhD thesis. Two of these papers are published and one is submitted.

In 2016-2018, more than 200 patients were included. On these patients, Alexander performed the routine NCS and nerve excitability tests and the novel MUNE method MScanFit MUNE. Mean MScanFit MUNE values in patients with diabetic neuropathy were significantly different from patients without neuropathy and healthy controls in spite of the length dependence of diabetic neuropathy and the tests were done in the hand. MScanFit MUNE is the most recent MUNE method developed by Prof. Hugh Bostock in collaboration with the Department of Clinical Neurophysiology, Aarhus University Hospital, Aarhus. Related papers were published mainly in ALS previously and Alexander contributed to one of these studies as a co-author (Jacobsen et al, 2018).

Alexander submitted his 2nd paper on the sensory and motor nerve excitability tests done in median nerve. The paper is under review. The aim of this part of the electrophysiological studies was to gain insight into the pathophysiology of DPN and examine the diagnostic value of sensory and motor axonal excitability testing. For this study, 111 patients and 60 controls were included. In addition to the comprehensive clinical examinations, NCS of lower and upper extremities and sensory and motor excitability tests of the median nerve were performed. The sensory and motor nerve excitability changes were unexpectedly mild compared to NCS. The conclusion of this study was that axonal excitability testing was not of diagnostic value or does not provide additional information on pathophysiology of DPN.

Alexander has done the MScanFit MUNE and muscle excitability tests of the PhD project by Karolina Snopek before and after training on healthy subjects and diabetics in a lower extremity nerve and muscle. Alexander analyzed the results of the baseline examinations and wrote the paper on these results as the 3rd paper in his PhD dissertation. The paper is published in Clin Neurophysiol (Kristensen et al., 2020). In this study, 79 type-2 diabetic patients were compared to 32 control subjects. All participants were examined with MScanFit MUNE and MVRCs in anterior tibial muscle. Lower limb nerve conduction studies (NCS) in peroneal, tibial and sural nerves were applied to diagnose large fiber neuropathy. NCS confirmed DPN for 47 patients (DPN+), with 32 not showing DPN (DPN-). MScanFit showed significantly decreased MUNE values and increased motor unit sizes, when comparing DPN+ patients with controls, and also when comparing DPN- patients with controls. MVRCs did not differ between groups. MScanFit is more sensitive in showing motor unit loss than NCS in type-2 diabetic patients, whereas MVRCs do not provide additional information (Figure). The MScanFit results suggested that motor changes are seen as early as sensory, and the role of axonal membrane properties in DPN pathophysiology should be revisited.

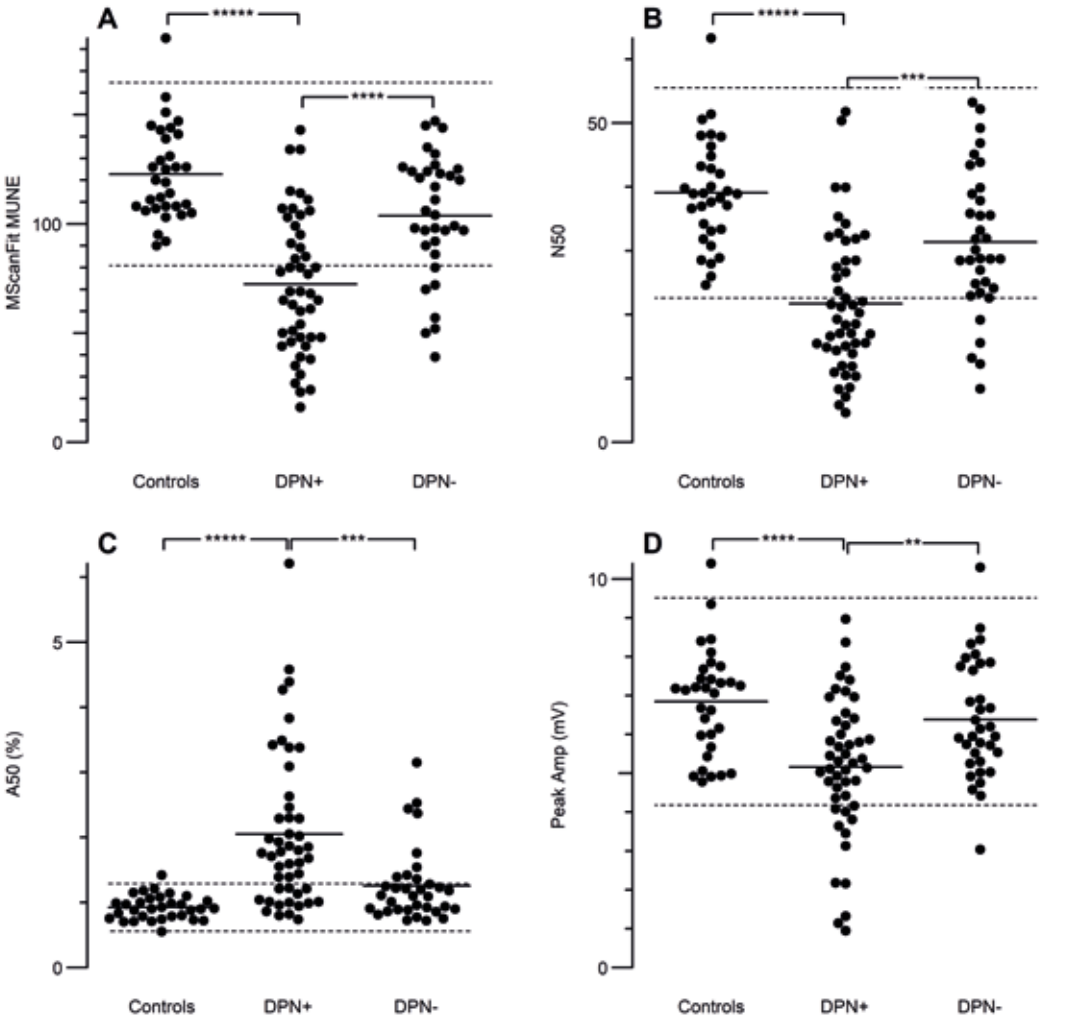


Fig. *Dotplots of the MScanFit measurements with the most significant differences between patient groups and controls. A: Number of estimated motor units. B: Number of large units that make up 50% of maximal compound muscle action potential (CMAP) amplitude. C: The smallest motor unit of the units included in N50, relative to maximal CMAP amplitude. (Note logarithmic scale to normalize distributions). D: Peak CMAP amplitude. 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). (Kristensen AG, Clin Neurophysiol 2020, Epub ahead of print)*

From left to right: Professor Michael Pedersen (chairman), Alexander Gramm Kristensen, Professor Christian Krarup, Department of Clinical Neurophysiology, University of Copenhagen (assessed the PhD), Associate Professor Hatice Tankisi and Postdoctoral Fellow James Howells, Faculty of Medicine and Health, the University of Sydney | Central Clinical School (assessed the PhD)

WP4: AUTONOMIC NEUROPATHY IN DIABETES

Astrid Juhl Terkelsen,
Associate Professor, Neurologist, Danish Pain Research Center and Department of Neurology, Aarhus University Hospital. Astrid is the main supervisor of PhD student Thorsten Rasmussen and PhD student Vinnie Faber Rasmussen

Astrid Juhl Terkelsen founded and leads the autonomic laboratory at Department of Neurology, Aarhus University Hospital. Her current clinical responsibilities comprise evaluation, diagnosis and treatment of patients with neurogenic autonomic dysfunction and small fiber polyneuropathy due to peripheral neuropathy (metabolic diseases, gammopathies etc.) and neurodegenerative diseases (pure autonomic failure, Parkinson's disease, multiple systemic atrophy, Lewy body dementia). She is both performing the neurophysiological examination at a highly specialized level and diagnosing and treating patients with neurogenic autonomic dysfunctions. She has developed tests to monitor and modulate sympathetic and parasympathetic cardiovascular functions, sweat, temperature, and skin perfusion. Thus, the autonomic laboratory is unique and the only of it kinds in Denmark and gives a unique opportunity to combine autonomic research with clinical responsibilities.

Astrid Juhl Terkelsen is main supervisor for PhD student Thorsten Rasmussen. Primary aim of the PhD project is to increase the specificity of methods used in the autonomic laboratory for patients with type 2 diabetes. Methods used to diagnose small fiber polyneuropathy is improved by combining intraepidermal nerve fiber density with functional measures (vasoconstriction and sweat) and pathology (innervation of autonomic structures in the skin). Methods to diagnose cardiovascular adrenergic dysfunction is optimized via advanced methods of blood pressure and sympathetic innervation of the heart (MIBG).

Astrid Juhl Terkelsen is also the main supervisor for Ph.D. student Vinni Faber Rasmussen. Primary aim of the PhD project is to investigate small fiber and autonomic neuropathy in adolescents with type 1 diabetes and to find a new and better screening method to detect neuropathy early in the course of this disease.

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

Thorsten Kamlarczyk Rasmussen *is a PhD student with Associate professor Astrid Juhl Terkelsen as main supervisor*

Small nerve fibers of Aδ and C type are essential for the perception of heat, cold and pain and the transmission of autonomic regulation of heart and endo- and exocrine gland functions such as sweat and smooth muscles. These autonomic small fibers ensure homeostasis through strict regulation of cardiovascular system, renal, bowel, bladder and sexual functions. Following injury to the autonomic system nerves, such as in diabetes, severe changes may occur.

Small nerve fiber damage resulting in neurogenic autonomic dysfunction and small fiber polyneuropathy is common but overseen in diabetes and associated with reduced quality of life, increased morbidity and sudden death. Patients with autonomic involvement may experience symptoms such as orthostatic hypotension or syncope, enteric dysfunction, sexual impotence, anhidrosis and urinary retention or incontinence.

This project is a two-part study with a joint focus on neurogenic adrenergic autonomic dysfunction, primarily in type 2 diabetics (DM2).

In study 1 we assessed the neuropathic impact on the adrenergic autonomic nerves in DM2 patients and matched healthy controls (HC) through assessment of a validated autonomic nervous system test-battery comprised of tilt table testing, Valsalva maneuver (Figure A and B) deep respiration, 24-hour blood pressure profiling, and the cardiovascular autonomic reflex test battery. Diabetic patients showed lower values in parasympathetic measures such as the deep breathing heart rate variability (DM2 1.12± 0.10 vs. HC 1.18± 0.13 (p = 0.02)), and the Valsalva ratio (DM2 1.54± 0.27 vs. HC 1.71± 0.29 (p = 0.02)). Although an equal proportion of DM2 and HC participants showed an abnormal composite adrenergic score (p=0.22), DM2 patients were found to have affected adrenergic regulation measured as higher nighttime blood pressure (BP) (DM2 137.9± 21.7 vs. HC 115.8± 17.8 (p = 0.00)) and a lower percentage drop in nighttime BP compared to daytime BP (DM2 8.3± 8.5 vs. HC 16.5± 7.1 (p = 0.00)). No difference in cardiovascular adrenergic markers measured through the Valsalva maneuver was found (p > 0.05).

In study 2 we aim to combine the utilization of clinical autonomic testing and skin biopsy findings for the evaluation of neuropathy in diabetics and patients with known small fiber neuropathy. We aim to quantify the innervation of cutaneous autonomic elements (sweat glands, microvascular vasomotor nerve fibers, and arrector pili) in patients with known small fiber neuropathy, compared to healthy controls. We furthermore aim to estimate the association between stereologically quantified autonomic skin biopsy markers, and their clinically measured functions. More specifically, we want to assess the association between biopsy quantified adrenergic microvascular blood vessel innervation and the microvascular blood flow rate dynamics, and the association between sweat gland innervation, and the clinical sudomotor function assessed by quantitative sudomotor axon reflex testing and skin conductance dynamics.

Collection of data in study 2 is currently ongoing.

We expect the tools and methods investigated in these studies will further promote and encourage the utilization of objectively quantifiable autonomic markers in the diagnostic assessment of diabetic neuropathy and small fiber neuropathy.

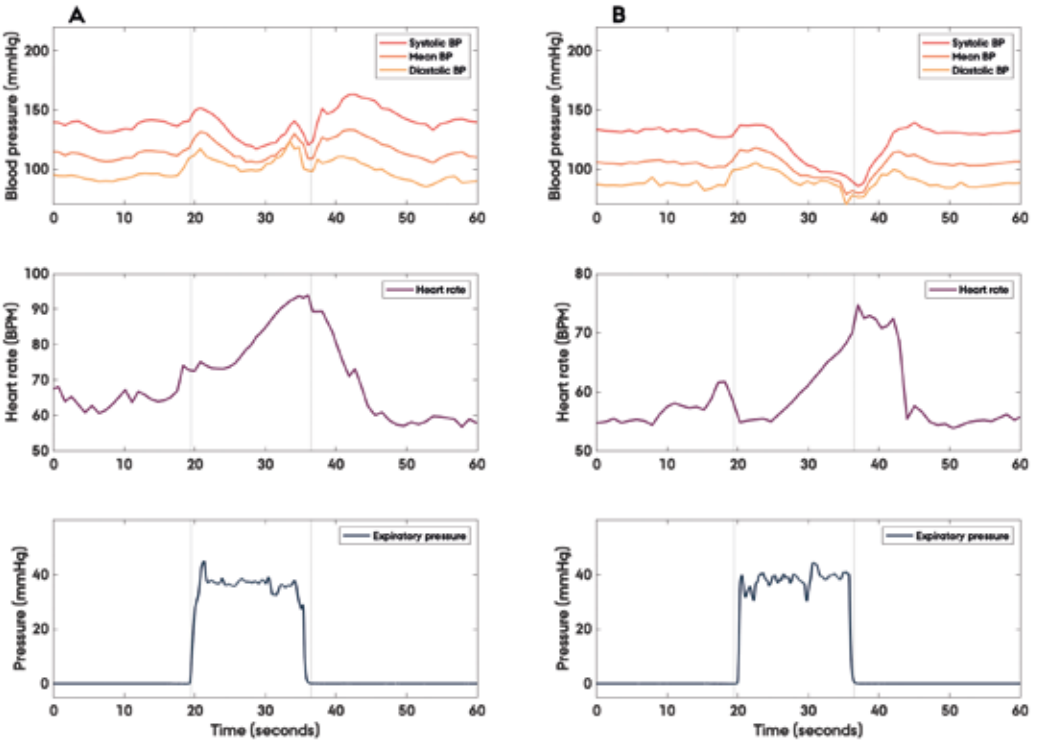


Fig. *Blood pressure, heart rate and expiratory pressure during the Valsalva maneuver. A) A normal Valsalva response – the presence of a rising blood pressure during expiration indicates a normal cardiovascular adrenergic function. The heart rate response with a significant Valsalva ratio indicates a normal cardiovagal and cardiovascular adrenergic function. B) An abnormal Valsalva response with no compensatory rise in blood pressure during the expiratory effort.*

WP4: EARLY DETECTION OF NEUROPATHY IN ADOLESCENTS WITH TYPE 1 DIABETES

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*

The aim of the PhD project is to 1) measure the presence and severity of small fibre, large fibre and autonomic neuropathy in 60 adolescents age 15-18 years with more than 5 years of type 1 diabetes and 2) provide clinicians with easy, yet reliable screening methods for better detection of early neuropathy.

Methods: Clinical research study with controlled trials. 60 adolescents with type 1 diabetes (diabetes duration ≥ 5 years, age 15-<18 years), who are treated in Denmark at Steno Diabetes Centers (Aarhus and North Jutland) or associated hospitals in Aarhus, Aalborg and Randers and 20 healthy controls will be enrolled in the study. Gold standard – and bedside methods will be conducted to assess large and small fibre - and autonomic neuropathy (please refer to figure with overview of tests). Assessment of the nerve fibre structure and functions will be analyzed in collaboration with the Mayo Clinic, Rochester, Minnesota, USA, to optimize the results.

Results: Associations between the results of neuropathy tests and metabolic control (HbA1c), body mass index, diabetes duration, quality of life, and biochemical - and genetic marker will be analysed. The bedside tests will be compared with the gold standard methods. Results from patients with type 1 diabetes will be compared with the healthy controls.

Conclusion/summary: This project will measure the presence and severity of different types of neuropathy in 60 adolescents with type 1 diabetes. The use of both gold standard tests and bedside tests may be useful to get an idea of optimal screening methods. In the future, we hopefully can follow the nerve status of the participants and develop treatment to prevent further progression of neuropathy.

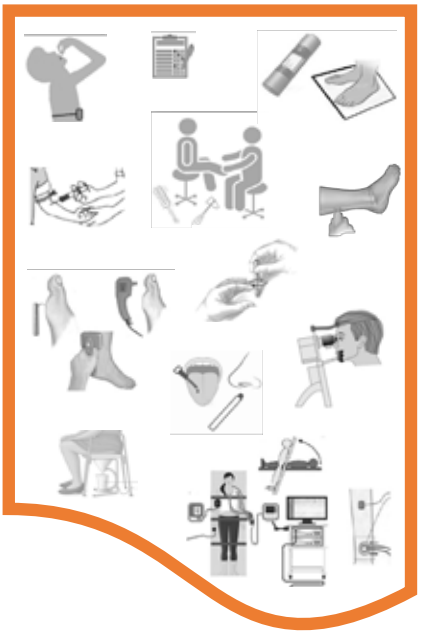


Illustration of clinical assessments in the project

WP4: DIABETIC NEUROPATHY; PATIENT PERSPECTIVES

Signe Vogel
is an anthropologist examining how life with diabetic neuropathy is experienced by patients.

Signe Vogel is studying the effects of diabetic polyneuropathy without pain (DPN) and with pain (PDPN) in patients from the WP4 studies.

Pain and other sensory disturbances correlated with DPN and PDPN can be approached as neurological findings but these sensations can also be studied as experiences in a person's life. In this study we utilized a qualitative approach in order to explore and examine important aspects of DPN and PDPN which are not accessible through quantitative research. Accordingly the main aim of this study was to gain a better understanding of the impact DPN and PDPN can have on a person's lifeworld.

We invited participants from the WP4 study to participate in an exploratory interview at the Danish Pain Research Center, Aarhus University (DK). 27 patients were interviewed. Ten were diagnosed with type II diabetes, seven were diagnosed with DPN, and ten were diagnosed with PDPN.

Findings from the study demonstrate that the burden of illness for DPN and PDPN is considerable and that it can have an impact on both physical, psychological, and social health-related quality of life. The areas of impact include fundamental aspects of everyday life such as work, domestic work, exercising, leisure activities, mood, relationships, and identity. Furthermore, clinical encounters related to DPN and PDPN are often experienced as contributing to the burden of illness instead of alleviating it.

Findings from the study have been presented on the international congress ´"Controversies in Neuropathic Pain" in Munich in 2019, and published in the Danish "Diabetes Behandlerblad", and in the international peer-reviewed journal "Practical Diabetes". Overall, these findings demonstrate that it is important to understand the full impact DPN and PDPN can have on life, not just the physical sensations and limitations but also the psychological and social consequences. It is pivotal that clinicians and researchers direct their attention to the highly complex, interactive relationship between context and experience of symptoms when trying to measure, assess, and compare DPN and PDPN symptoms and their impact on quality of life.

WP4: PEPTIDERGIC NERVE FIBRE DENSITY AS A CUTANEOUS MARKER OF PAIN IN DIABETIC SMALL FIBER NEUROPATHY

Páll Karlsson, *PhD, is an associate professor, Core Center for Molecular Morphology, Section for Stereology and Microscopy, Department of Clinical Medicine, Aarhus University*

Currently in press in the journal PAIN is a paper that aimed to identify possible pain pathophysiological biomarkers in patients with painful diabetic polyneuropathy (DPN), as that will increase our knowledge of mechanisms behind neuropathic pain and identify novel and much needed treatment targets. While decrease of intraepidermal nerve fiber density (IENFD) from skin biopsy is one of the neuropathological gold standards when diagnosing DPN, it does not explain why some patients develop neuropathic pain nor does it differentiate between patients with and without pain. We know from animal models of painful DPN that they have an increased density of peptidergic nerve fibers (Substance P (SP) and Calcitonin-gene related peptide (CGRP)), but the results have never been replicated in humans. Therefore, we set out to perform a detailed skin biopsy analysis in a well-characterized group of DPN patients, with and without pain and in healthy controls.

As expected, and shown before, while the IENFD was lower in diabetic patients compared with non-diabetic controls, there was no difference between DPN patients with and without pain. However, and importantly, patients with pain had increased density of dermal peptidergic fibers containing SP and CGRP compared to patients with painless DPN and healthy controls. Furthermore, while the nerve fibre density did not, peptidergic nerve fibre density correlated with pain ratings in patients with pain. These findings, shown for the first time in humans, provide new insight into the pathophysiological mechanisms of pain in diabetes and opens the research towards new therapeutic targets.

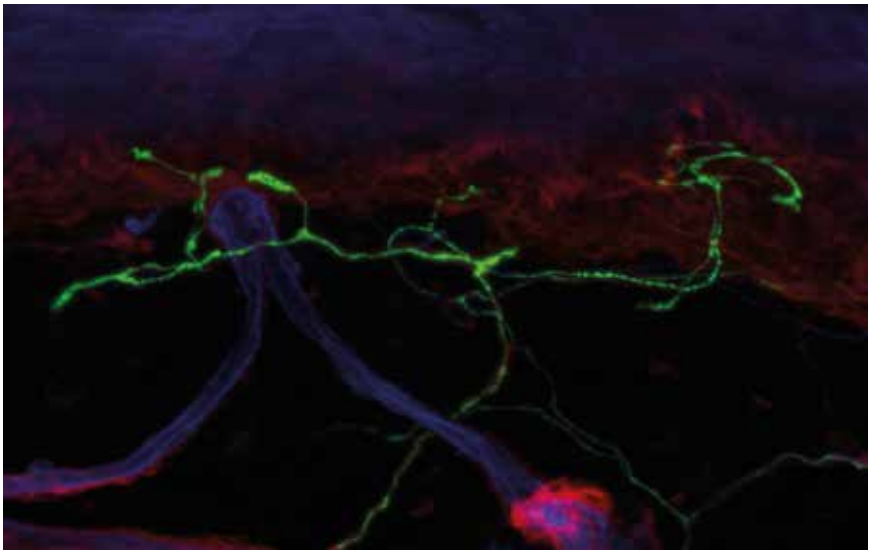


Fig. *CGRP positive nerve fibres in a skin biopsy taken from a patient with painful DPN. Green: CGRP+ nerve fibres, red: Collagen IV (basement membrane), blue: ULEX (blood vessels, endothelia and keratinocytes).*

WP4: EARLY DETECTION AND TREATMENT OF DIABETIC POLYNEUROPATHY

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

Deafferentation is the main topic of Ellen Lund Schaldemose's PhD study. She focusses on early signs of diabetic polyneuropathy. Here is a presentation of her first study about paradoxical heat sensations (PHS) in healthy individuals.

PHS is a phenomenon where cooling of the skin is described as warm. PHS can be assessed using the Thermal Sensory Limen (TSL) protocol in the German Quantitative Sensory Testing (QST).

Healthy individuals can experience PHS, but PHS is more common in patients with nervous system damage and reduced thermal sensitivity.

Since the TSL protocol does not take the difference in thermal sensitivity between individuals with normal and impaired thermal sensitivity into account, interpretation of the PHS is limited by the methodology of the protocol. We do not know to which extent PHS is experienced by healthy individuals when they receive the same warm-cold detection temperature differentials as patients with PHS.

The aim of this study is to develop a more sensitive protocol to measure PHS and to provide information about triggering factors.

We hypothesize that healthy participants will have increased PHS if they experience a cooling or heating of the skin matching the warm-cold detection differentials of patients with PHS prior to the TSL.

The study is ongoing, and currently 66 out of 100 healthy participants between the age of 18 and 65 years have been enrolled. Figure 1 and 2 illustrates the methodology of the study.

During the experiment, the skin on the dorsum of both feet is cooled to 26°C or 20°C, or heated to 38°C or 44°C prior to the TSL test and compared to baseline TSL at 32°C. Participants are instructed to press a button every time they register a change in temperature after a beep(*) and to tell whether the sensation is cold/warm or burning/prickling or if they are in doubt.

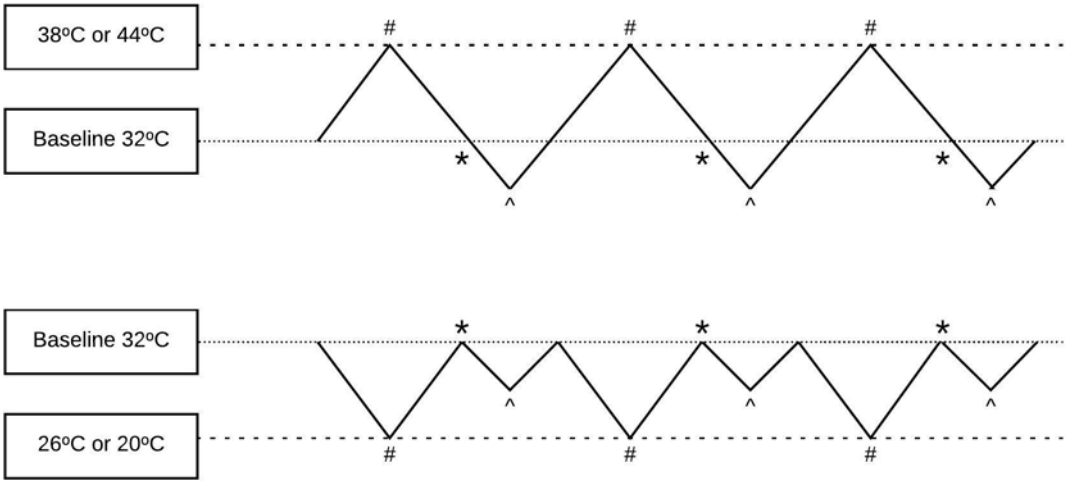


Fig. 1 *Illustration of the temperature differentials and sequences of TSL*
 # Pre-heated/cooled temperature
^ Individual warm/cold detection threshold, report of sensation
** Beep indicating the beginning of each repetition*

Fig. 2 *The experimental setup*

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 Feldman's Laboratory at the University of Michigan, Ann Arbor, USA, uses a systems approach employing transcriptomics, metabolomics, and metabolic flux analysis 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 5 years, we have summarized our approach and sentinel findings in 44 published papers, which include primary articles on our research findings along with a series of reviews focused on the clinical problem, our approach to diagnosis, and the implications our work has on both an understanding of neuropathy in patients with T1D and T2D and the development of novel treatments for these patients. Our recent clinical studies in man indicate that drivers of neuropathy include not only glucose, but obesity and associated components of the metabolic syndrome. 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 intersection of hyperglycemia and the components of the metabolic syndrome, particularly obesity, in driving the onset and progression of DPN. Our most recent advances focused on the identification of plasma metabolites that correlate with DPN in patients with T2D. Our findings are highlighted below.

Our early studies demonstrate that the metabolic syndrome (MetS) is a major independent risk factor for DPN in patients with T2D. However, the plasma metabolites associated with MetS that underlie DPN development are not completely understood.

To identify plasma metabolites that correlate with DPN in T2D, we conducted a global metabolomics analysis on plasma from 9 healthy individuals as well as from 49 T2D subjects and 48 T2D subjects with DPN from the Danish arm of the Anglo-Danish-Dutch study of Intensive Treatment in People with Screen-Detected Diabetes in Primary Care (ADDITION). Patients were attending a follow-up examination after a mean of 13 years after enrolling in ADDITION-Denmark. Each subject received anthropometric measurements, DPN assessment, and blood sample collection.

The global plasma metabolomics analysis identified 991 metabolites with 75 of the metabolites falling below the detection limit (<50%). After removing these metabolites, the remaining 916 metabolites were processed through our bioinformatics pipeline. A principle components analysis showed a clear separation between metabolites from healthy control subject plasma compared to metabolites from T2D patient plasma regardless of DPN status. To identify metabolites that distinguished T2D patients with DPN from those without, a regularized logistic regression method called elastic net was used (Figure 1). Elastic net identified 8 metabolites that separated T2D DPN patients from T2D patients without DPN. The 8 metabolites were classified as lipids, energy, amino acids, and xenobiotics. Metabolite levels for individual T2D patients showed distinct differences in metabolite abundances between T2D subjects with and without DPN (Figure 1a). The mean relative abundance of the eight metabolites indicated that five were downregulated and three were upregulated in T2D patients dependent on DPN status (Figure 1b). We then used seven machine learning algorithms to assess the importance of each metabolite in T2D DPN and found that six of the eight metabolites (Isoursodeoxycholate sulfate, N-acetyl-3-methylhistidine, tartarate, citrate, N-acetyl-beta-alanine, and ximenoylcarnitine (C26:1)) had a high importance score, indicating that they are associated with the separation between T2D and T2D DPN subjects (Figure 1c).

Based on the importance of the elastic net metabolites for mitochondrial and lipid metabolism, we next assessed changes in chain length and degree of unsaturation in plasma free fatty acid and complex lipid metabolites (Figure 2). We found that the plasma free fatty acid profile shifted from a low level of long chain saturated fatty acids

(LCSFAs) in healthy control subject plasma to an abundance of LCSFAs in T2D patient plasma (Figure 2a). This shift in free fatty acid profile occurred regardless of DPN status. Since fatty acid chain length and saturation impacts complex lipid metabolism, we next evaluated the chain length and number of double bonds in complex lipids (Figure 2b). Diacylglycerol (DAG) and phosphatidylethanolamine (PE) were higher, while phosphatidylcholine (PC), ceramides, sphingomyelin (SM), and acylcarnitines were lower in T2D versus healthy control subject plasma. These changes in complex lipid levels were even more distinct in T2D DPN patient plasma. Interestingly, 3 lipid metabolites were significantly different between T2D and T2D DPN patients (Figure 2c). These included a sphingosine metabolite related to sphingolipid metabolism and two very long-chain acylcarnitine metabolites. These results indicate that sphingolipid and mitochondrial metabolism correlated with DPN in T2D and may play a role in the progression of DPN.

Based on these results we proposed a potential mechanism for the effect of plasma metabolites on mitochondrial function within the nerve (Figure 3). First, an accumulation of LCSFAs in T2D patient plasma could lead to mitochondrial bioenergetics overload causing mitochondrial dysfunction within the nerve. Second, elevated DAG levels could stimulate de novo PE synthesis thereby altering the PC to PE ratio in the mitochondrial membrane. Since the ratio of PC to PE in the inner mitochondrial membrane is essential for mitochondrial function, alterations in this ratio through the accumulation of PE may drive mitochondrial dysfunction within the nerve. Third, N-acetyl-b-alanine is a precursor for malonyl-CoA synthesis. Malonyl-CoA is a potent inhibitor of CPT-1 and could potentially inhibit carnitine palmitoyltransferase activity within the nerve diminishing acylcarnitine levels and impairing mitochondrial ATP production. Reductions in plasma acylcarnitine and citrate levels may also impair the TCA cycle, impairing mitochondrial ATP production. Future directions will focus on testing these proposed mechanisms of mitochondrial dysfunction within the nerve.

Overall, we continue to pursue the overarching hypothesis that fatty acid uptake, lipid oxidation, and lipid biosynthetic pathways 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 first five 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 have identified specific plasma metabolites related to lipid and mitochondrial metabolism that correlate with DPN in patients with T2D from the Danish ADDITION cohort. This study parallels many of the findings from our studies involving murine models of T2D. In vitro and in murine studies, we have used a combination of mitochondrial assessments, gene expression analysis, and steady-state and dynamic fluxomics and lipidomics and have discovered effects of lipids on mitochondrial function and a downregulation of energy metabolism in the peripheral nerve in mice with diabetes and DPN. This functional downregulation of glucose and lipid metabolism supports our contention that energy failure may likely underlie the pathogenesis of DPN.

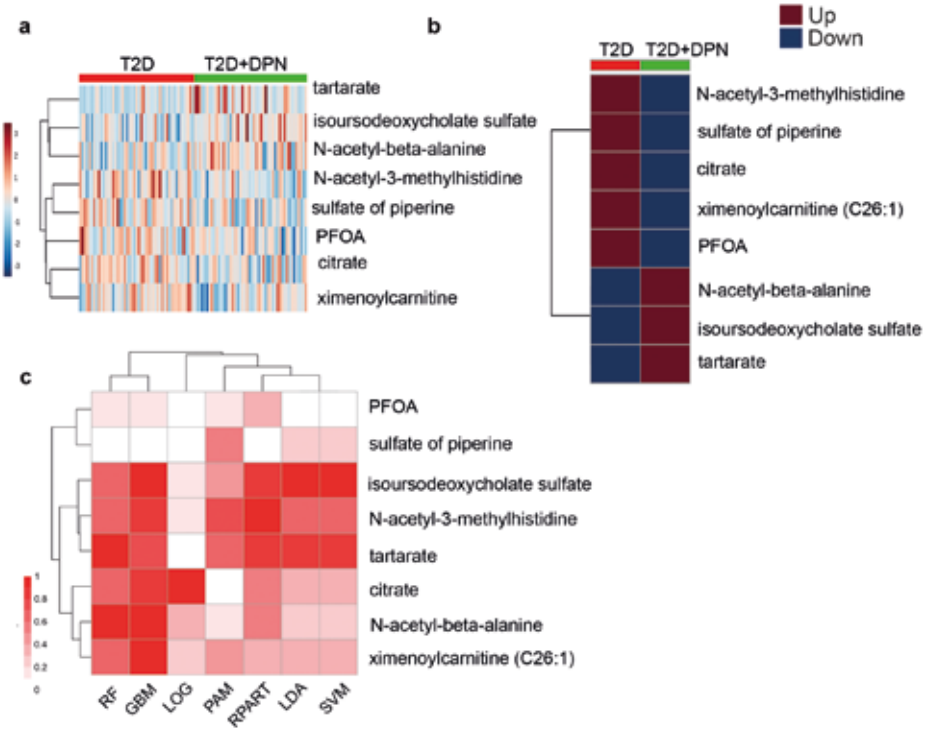


Fig. 1. *Elastic net analysis identified 8 unique metabolites that differentiate T2D and T2D DPN subjects. (A) The relative abundance of the 8 elastic net metabolites in individual subjects with T2D (left) and T2D DPN (right) plotted as a heat map show distinct differences between T2D patients with and without DPN. (B) The relative abundance of the 8 metabolites from T2D subjects (left) and T2D DPN subjects (right) were averaged and displayed as the mean value. Importantly, N-acetyl-β-alanine is elevated and ximenoylcarnitine (C26:1) and citrate are decreased in T2D patients with DPN. (C) Seven different machine learning algorithms were trained to classify T2D and T2D DPN using the 8 elastic net metabolites. The importance score of elastic net metabolites was extracted from seven different machine learning models including Linear Discriminant Analysis (LDA), Support Vector Machine (SVM), Random Forest (RF), Recursive Partitioning and Regression Trees (RPART), Prediction Analysis for Microarrays (PAM), Logistic Regression (LOG), and Gradient Boosting Machine (GBM). The importance score represents the contribution of metabolites to the performance of the machine learning model. Importance scores range from 0 representing "no importance" to 1 representing "high importance". Metabolites marked by an asterisks (*) represent uncertainties in metabolite quantification and identification. PFOA, perfluorooctanoate; sulfate of piperine, sulfate of piperine metabolite C16H19NO3.*

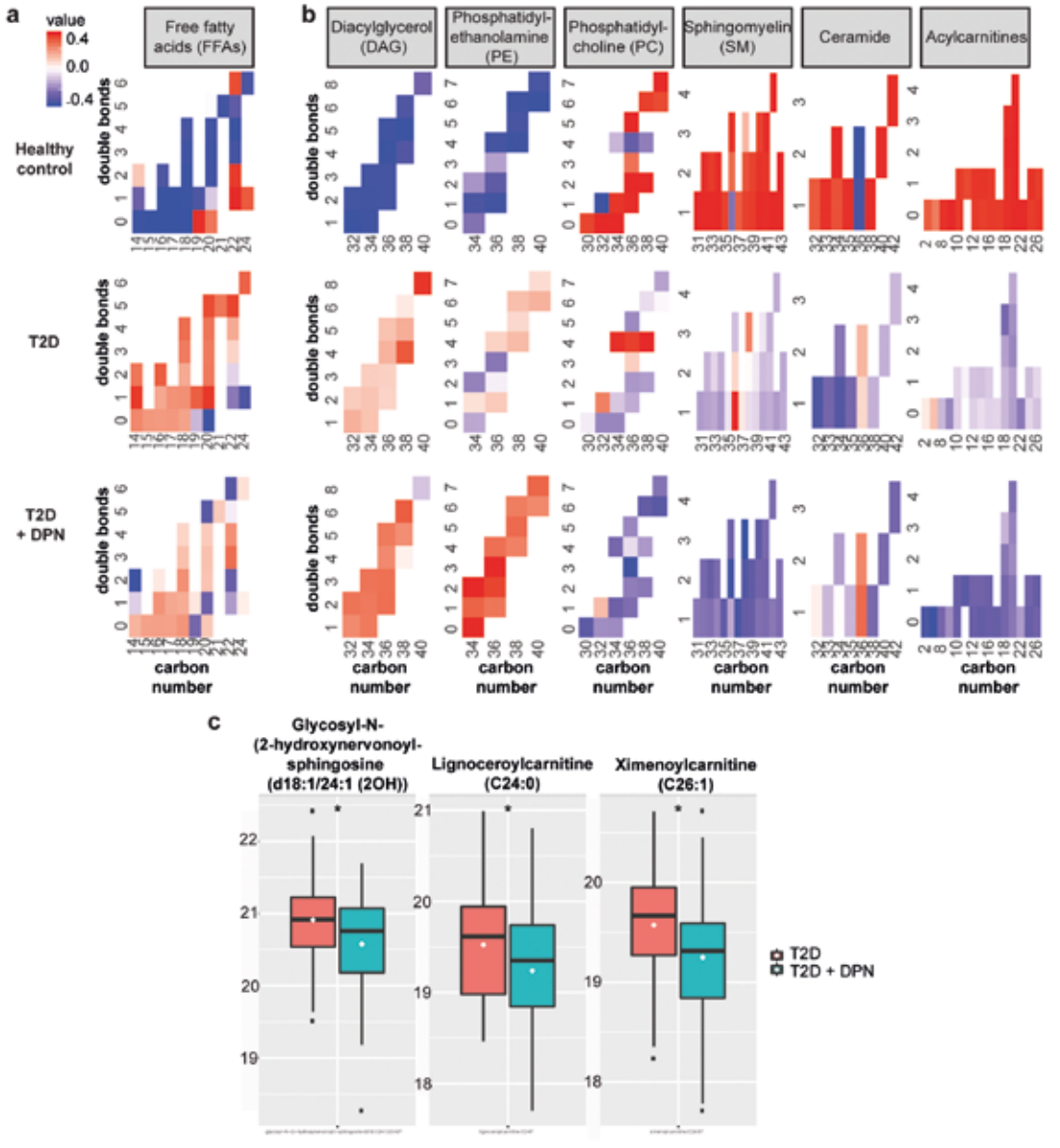


Fig. 2. *The carbon number and degree of unsaturation of free fatty acids and complex lipids is altered in plasma from T2D and T2D DPN subjects compared to healthy controls. (A) Heat maps of free fatty acid profiles show elevated levels of long chain saturated fatty acids in the plasma of T2D patients regardless of neuropathy status compared to plasma from healthy controls subjects. (B) Complex lipids, including DAG, PE, PC, SM, ceramide, and acylcarnitine metabolites, show distinct alterations in complex lipid species in T2D patient plasma compared to healthy control plasma. DAG and PE were increased whereas PC, SM, ceramides, and acylcarnitines were decreased in T2D patient plasma compared to healthy control plasma. The alterations in plasma complex lipids is further exacerbated in T2D DPN subjects. (C) Two acylcarnitine metabolites and one sphingolipid metabolite were significantly altered in T2D DPN patients versus T2D without DPN. DAG, diacylglycerol; FFA, free fatty acids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; SM, sphingomyelin*

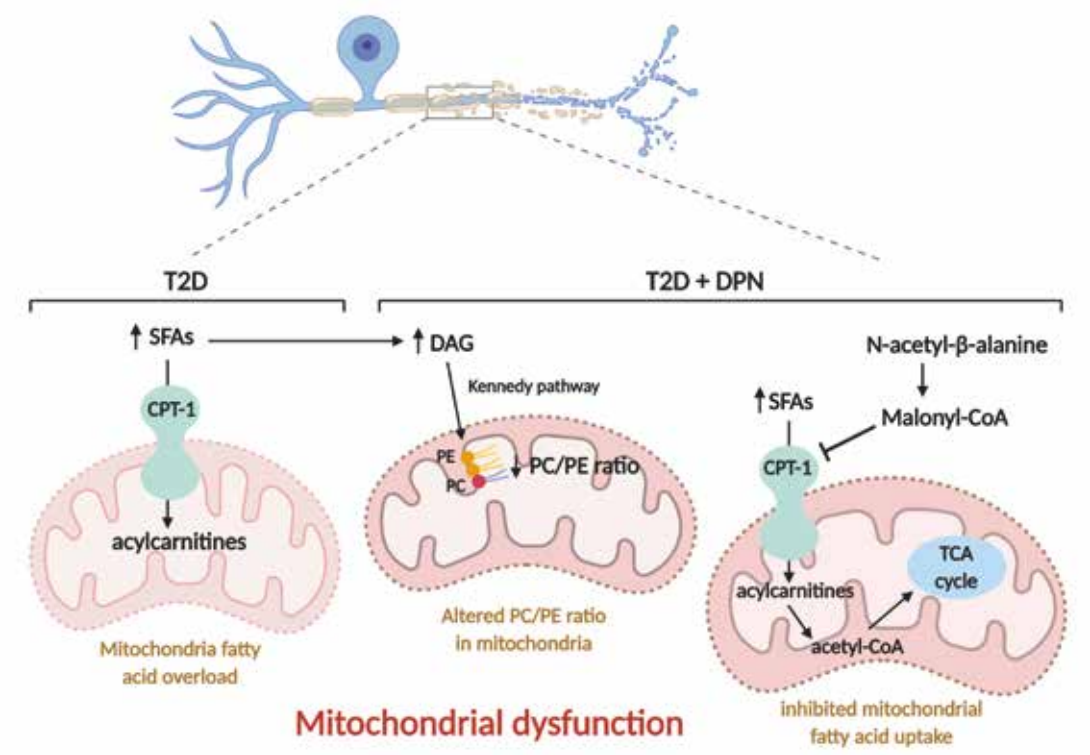
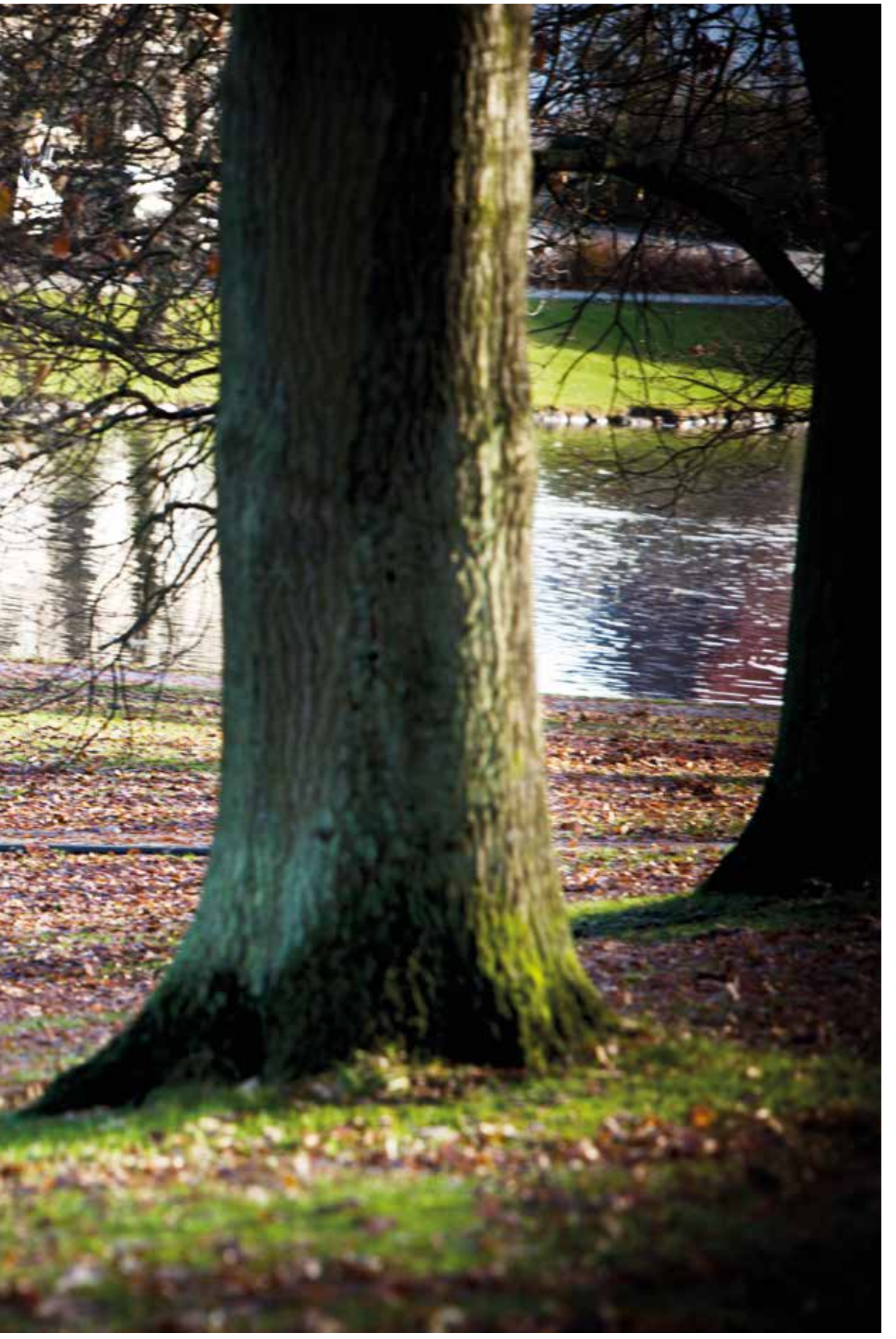


Fig. 3. *A potential mechanism for the effect of plasma metabolites on mitochondrial function within the nerve. The metabolites that are altered in T2D DPN subjects compared to T2D subjects may drive mitochondrial dysfunction in the nerve through three mechanisms. First, the shift from a higher abundance of PUFAs to elevated levels of LCSFAs in plasma from T2D subjects may cause mitochondrial overload and impair mitochondrial bioenergetics. Second, high levels of N-acetyl-β-alanine in the plasma may lead to an increased level of malonyl-CoA. Since malonyl-CoA is an inhibitor of CPT-1, N-acetyl-β-alanine may result in higher levels of malonyl-CoA decreasing the level of acylcarnitines and mitochondrial ATP production thereby triggering mitochondrial dysfunction. This loss would also result in a loss of acylcarnitines and citrate which may impair the TCA cycle and diminish mitochondrial ATP production. Third, alterations in phospholipid levels may drive mitochondrial dysfunction. Elevated DAGs are reported to stimulate de novo PE synthesis, altering the PC:PE ratio within the mitochondrial membrane. This shift in PC:PE ratio within the mitochondrial membrane may drive mitochondrial dysfunction. CPT-1, carnitine palmitoyltranferase-1; DAGs, diacylglycerols; LCSFAs, long-chain saturated fatty acids; PCs, phosphatidylcholines; PEs, phosphatidylethanolamines; PUFAs, polyunsaturated fatty acids; TCA, the citric acid cycle.*

The research group in Eva Feldman's lab



EDUCATIONAL ACTIVITIES AND NETWORKING

Educational activities and networking continue to constitute important elements of the IDNC. IDNC events are announced on the consortium's webpage (www.idnc.au.dk), university websites and mailing lists and are generally open to everyone interested – free of charge.

Collaborative workshop;
"HOW TO IMPROVE ASSESSMENT OF NERVE FIBRE DAMAGE IN DIABETES?"

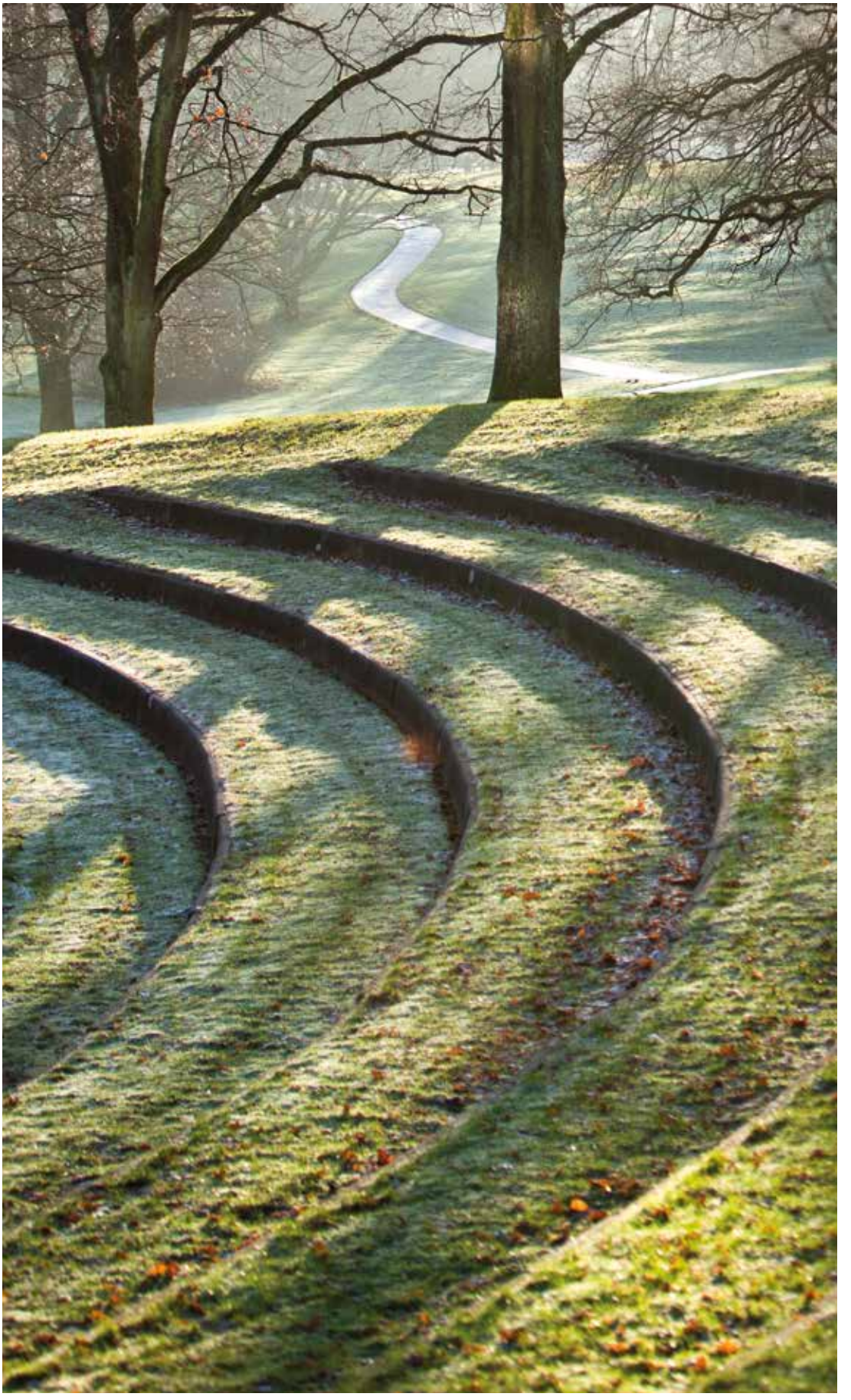
Pall Karlsson hosted an interdisciplinary, hands-on, collaborative workshop on how to assess nerve fibres in patients with polyneuropathy in collaboration with Center for Neuroplasticity and Pain, Aalborg University and the Danish Diabetes Academy 28 February 2020 at Aalborg University. The workshop was founded by the Danish Diabetes Academy and all available seats were reserved. The programme consisted of a morning session with plenary talks, followed by an afternoon session with five different stations where delegates got hands-on experience on how to assess nerve fibre function using novel equipment and methods.

Workshop "How to improve assessment of nerve fibre damage in diabetes?" February 28, Aalborg University

IDNC ANNUAL MEETING 2019

IDNC hosted the IDNC Annual Meeting 2019 "Diabetic Neuropathy and related Comorbidities" on November 8 at Aarhus University Hospital. This was a full-day meeting with a panel of invited international and national speakers and a number of short oral presentations by PhD students and postdocs from the IDNC including both basic and clinical scientists with an interest in diabetes or neuropathy. A business meeting was held the day before the annual meeting.

IDNC Annual Meeting November 8 2019



AWARDS AND PRIZES

Brian Callaghan will be receiving the 2020 Wolfe Neuropathy Research Prize from the American Neurological Association at the virtual ANA meeting

In 2020 Professor David Bennett became a Fellow of the Academy of Medical Sciences.

RESEARCH STAYS ABROAD

Karolina Snopek and **Frederik Pagh Kristensen** both had research stays at University of Michigan in 2019.

Pall Karlsson had s research visit at the Karolinska Institute, Sweden in December 2019

Troels S. Jensen receiving Honorary membership of the Danish Neurological Society in the autumn 2019.

SCIENTIFIC MEETINGS AND TEACHING ACTIVITIES

Members of the IDNC gave lectures and poster presentations at numerous key national and international scientific meetings and courses on pain and diabetic neuropathy in 2019/2020, including the following:

IASP Pain Camp
Kuching Malaysia, 6-11 April, 2019

ASEAPS congress
Kuching Malaysia, 11-14 April, 2019

International Congress on Neuropathic Pain (NEUPSIG)
London, 9-11 May 2019

American Academy of Neurology meeting
Philadelphia, USA, 6-11 May, 2019

European Diabetes Epidemiology Group 2019 (EDEG)
Mondorf-les-Bains, Luxembourg, 11-14 May 2019

17th European Congress of Clinical Neurophysiology (ECCN)
Warsaw, Poland, 5-8 June 2019

American Diabetes Association's 79th Scientific Sessions
San Francisco, California, USA, 7-11 June 2019

Active participant in the Danish "Folkemødet på Bornholm"
Svaneke, Denmark, 13-16 June 2019

PNS 2019
Genoa, Italy, 22-25 June 2019

North American Pain School Montebello
Quebec Canada 23-28 June 2019

Indonesian Neurological Association
Bali August 2019

55th EASD (European Association for the Study of Diabetes) Annual Meeting
Barcelona, Spain,16-20 September 2019

EFIC (European Federation of IASP chapters) biannual meeting
September 2019, Valencia, Spain

The International Conference on Controversies in Neuropathic Pain
Munich, Germany, 23-24 October 2019

2020 PNS Virtual Event.
27-30 June

56th EASD Annual Meeting, Virtual event
September 2020

PUBLICATIONS

PEER-REVIEWED PUBLICATIONS

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3. Bendtsen L, Zakrzewska JM, Abbott J, Braschinsky M, Di Stefano G, Donnet A, Eide PK, Leal PRL, Maarbjerg S, May A, Nurmikko T, Obermann M, Jensen TS, Cruccu G: European Academy of Neurology guideline on trigeminal neuralgia. Eur J Neurol 2019;26:831-849

4. Bjornsdottir G, Ivarsdottir EV, Bjarnadottir K, Benonisdottir S, Gylfadottir SS, Arnadottir GA, Benediktsson R, Halldorsson GH, Helgadottir A, Jonasdottir A, Jonasdottir A, Jonsdottir I, Kristinsdottir AM, Magnusson OT, Masson G, Melsted P, Rafnar T, Sigurdsson A, Sigurdsson G, Skuladottir A, Steinthorsdottir V, Styrkarsdottir U, Thorgeirsson G, Thorleifsson G, Vikingsson A, Gudbjartsson DF, Holm H, Stefansson H, Thorsteinsdottir U, Norddahl GL, Sulem P, Thorgeirsson TE, Stefansson K: A PRPH splice-donor variant associates with reduced sural nerve amplitude and risk of peripheral neuropathy. Nature communications 2019;10:1777

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8. Calvo M, Davies AJ, Hébert HL, Weir GA, Chesler EJ, Finnerup NB, Levitt RC, Smith BH, Neely GG, Costigan M, Bennett DL. The Genetics of Neuropathic Pain from Model Organisms to Clinical Application. Neuron. 2019 Nov 20;104(4):637-653. doi: 10.1016/j.neuron.2019.09.018. Review. PMID: 31751545

9. Christensen DH, Knudsen ST, Nicolaisen SK, Andersen H, Callaghan BC, Finnerup NB, Jensen TS, Thomsen RW. Can diabetic polyneuropathy and foot ulcers in patients with type 2 diabetes be accurately identified based on ICD-10 hospital diagnoses and drug prescriptions? Clin Epidemiol 2019;11:311-321.

10. Christensen DH, Knudsen ST, Gylfadottir SS, Christensen LB, Nielsen JS, Beck-Nielsen H, Sørensen HT, Andersen H, Callaghan BC, Feldman EL, Finnerup NB, Jensen TS, Thomsen RW. Metabolic Factors, Lifestyle Habits, and Possible Polyneuropathy in Early Type 2 Diabetes: A Nationwide Study of 5,249 Patients in the Danish Centre for Strategic Research in Type 2 Diabetes (DD2) Cohort. Diabetes Care 2020, dc192277; doi: 10.2337/dc19-2277

11. Costa YM*, Karlsson P*, Bonjardim LR, Conti PCR, Jensen TS, Nyengaard JR, Svensson P, Baad-Hansen L. Trigeminal nociceptive function and oral somatosensory functional and structural assessment in patients with diabetic peripheral neuropathy. Scientific Reports. 2019: 9:169. * Shared first authorship

12. Eid, S, Sas KM, Abcouwer SF, Feldman EL, Gardner TW, Pennathur S, Fort PE. New insights into the mechanisms of diabetic complication: role of lipids and lipid metabolism. Diabetologia, 62(9);1539-1549, 2019, PMCID: PMC6679814

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ACCEPTED PHD DISSERTATIONS 2019-2020

Diana Hedevang Christensen; "Diabetic polyneuropathy in type 2 diabetes - Prevalence, risk factors, mental health, and diagnostic coding", successfully defended December 20 2019

Sandra Sif Gylfadottir; "Painful- and non-painful diabetic polyneuropathy", successfully defended March 6 2020

Karolina Snopek Khan; "Falls, motor dysfunction and the effects of resistance training in type 2 diabetic polyneuropathy", successfully defended May 1 2020

Alexander Gramm Kristensen; "Early diagnosis and understanding underlying mechanisms of diabetic neuropathy", successfully defended February 7 2020

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