The function of the autonomic nervous system in type 1 diabetes and in response to physiological and pharmacological modulations

Nielsen, Thomas Arendt

DOI (link to publication from Publisher):
10.54337/aau588597746

Publication date:
2023

Document Version
Publisher's PDF, also known as Version of record

THE FUNCTION OF THE AUTONOMIC NERVOUS SYSTEM IN TYPE 1 DIABETES AND IN RESPONSE TO PHYSIOLOGICAL AND PHARMACOLOGICAL MODULATIONS

BY
THOMAS ARENDT NIELSEN

DISSERTATION SUBMITTED 2023

AALBORG UNIVERSITY
DENMARK

THE FUNCTION OF THE AUTONOMIC NERVOUS SYSTEM IN TYPE 1 DIABETES AND IN RESPONSE TO PHYSIOLOGICAL AND PHARMACOLOGICAL MODULATIONS

Ph.D. thesis
by
Thomas Arendt Nielsen

AALBORG UNIVERSITY
DENMARK

AALBORG UNIVERSITY HOSPITAL

Dissertation submitted July 2023

Dissertation submitted: 10/07/2023

PhD supervisor:: Professor Christina Brock, DVM, PhD,
Mech-Sense, Department of Gastroenterology & Hepatology, Aalborg University Hospital, Aalborg, Denmark

PhD co-supervisors: Professor Asbjørn Mohr Drewes, MD, PhD, DMSc, EDPM, R.
Director of Mech-Sense & Centre for Pancreatic Diseases, Department of Gastroenterology & Hepatology, Aalborg University Hospital, Aalborg, Denmark

Professor Henrik Vorum, MD, PhD,
Department of Ophthalmology Aalborg University Hospital, Aalborg, Denmark

PhD committee: Clinical Professor Jacob Udby Blicher (chair)
Aalborg University, Denmark

Senior Research Fellow and Consultant Autonomic Neurophysiologist Peter O. Julu
Queen Mary University of London, UK

Clinical Research Associate Professor Steffen Hamann
University of Copenhagen, Denmark

PhD Series: Faculty of Medicine, Aalborg University

Department: Department of Clinical Medicine

ISSN (online): 2246-1302
ISBN (online): 978-87-7573-667-6

Published by:
Aalborg University Press
Kroghstræde 3
DK – 9220 Aalborg Ø
Phone: +45 99407140
aauf@forlag.aau.dk
forlag.aau.dk

Printed in Denmark by Stibo Complete, 2023

CV

THOMAS ARENDT NIELSEN
Born 1990, Aalborg, Denmark

CURRENT POSITION AND WORK ADDRESS
2021-2022 Department of Gastroenterology & Hepatology, Aalborg University Hospital, Aalborg, Denmark
2022- Department of Ophthalmology, Aalborg University Hospital, Aalborg, Denmark

EDUCATION
2011-2018 Cand. Med. (MD), The Faculty of Medicine, Aalborg University

PUBLISHED PAPERS
1. Brock C, Wegeberg AM, Nielsen TA, Karout B, Hellström PM, Drewes AM, Vorum H. The Retinal Nerve Fiber Layer Thickness Is Associated with Systemic Neurodegeneration in Long-Term Type 1 Diabetes. Transl Vis Sci Technol. 2023 Jun 1;12(6):23. doi: 10.1167/tvst.12.6.23. PMID: 37367720.
2. Nielsen TA, Andersen CU, Vorum H, Riahi S, Sega R, Drewes AM, Karmisholt J, Jakobsen PE, Brock B, Brock C. Palpebral fissure height response indicates autonomic dysfunction in patients with type 1 diabetes and polyneuropathy. Invest Ophthalmol Vis Sci. 2022 Aug 2;63(9):21. doi: 10.1167/iovs.63.9.21. PMID: 35980646; PMCID: PMC9404365.
3. Nielsen TA, Sega R, Uggerhøj Andersen C, Vorum H, Drewes AM, Jakobsen PE, Brock B, Brock C. Liraglutide Treatment Does Not Induce Changes in the Peripapillary Retinal Nerve Fiber Layer Thickness in Patients with Diabetic Retinopathy. J Ocul Pharmacol Ther. 2022 Jan-Feb;38(1):114-121. doi: 10.1089/jop.2021.0055. Epub 2021 Dec 16. PMID: 34918951.
4. Arendt-Nielsen L, Carstens E, Proctor G, Boucher Y, Clavé P, Nielsen KA, Nielsen TA, Reeh PW. The Role Of Trp Channels In Nicotinic Provoked Pain And Irritation From The Oral Cavity And Throat: Translating Animal Data To Humans. Nicotine Tob Res. 2022 Feb 24:ntac054. doi: 10.1093/ntr/ntac054. PMID: 35199839.
5. Nielsen TA, Eriksen MA, Gazerani P, Andersen HH. Psychophysical and vasomotor evidence for interdependency of TRPA1 and TRPV1-evoked nociceptive responses in human skin: an experimental study. PAIN. 2018 Oct;159(10):1989-2001. doi: 10.1097/j.pain.0000000000001298. PMID: 29847470.

6. Wang K, Luo Y, Asaki T, Graven-Nielsen T, Cairns BE, Nielsen TA, Arendt-Nielsen L. Acid-induced experimental muscle pain and hyperalgesia with single and repeated infusion in human forearm. Scand J Pain. 2017 Oct;17:260-266. doi: 10.1016/j.sjpain.2017.07.012. Epub 2017 Jul 24. PMID: 29229212.

7. Asaki T, Wang K, Luo Y, Nielsen TA, Graven-Nielsen T, Arendt-Nielsen L. Acid-induced Experimental Knee Pain and Hyperalgesia in Healthy Humans. *Pain. Exp Brain Res. 2018 Feb;236(2):587-598. doi: 10.1007/s00221-017-5155-5. Epub 2017 Dec 18.* PMID: 29250706.

8. Gazerani P, Nielsen TA, and Arendt-Nielsen L. Assessing Cutaneous Sensory Function and Vasomotor Responses. In: Measuring the Skin. *ISBN: 978-3-319-26594-0 (Online). Springer International Publishing, 2016.*

9. Nielsen TA, Nielsen BP, Wang K, Arendt-Nielsen L, Boudrau S. Psychophysical and vasomotor responses of the oral tissues: a nicotine dose-response and menthol interaction study. *Nicotine Tob Res (2015) doi: 10.1093/ntr/ntv163*

10. Arendt-Nielsen L, Skou ST, Nielsen TA, Petersen KK. Altered Central Sensitization and Pain Modulation in the CNS in Chronic Joint Pain. *Curr Osteoporos Rep. 2015 Aug;13(4):225-34. doi: 10.1007/s11914-015-0276-x*

11. Petersen KK, Simonsen O, Laursen MB, Nielsen TA, Rasmussen S, Arendt-Nielsen L. Chronic Postoperative Pain Following Primary and Revision Total Knee Arthroplasty. *Clin J Pain. 2015 Jan;31(1):1-6.*

12. Arendt-Nielsen L, Nielsen TA, Gazerani P. Translational pain biomarkers in early development of new neurotherapeutics for pain management. *Expert Rev Neurother. 2014 Mar;14(3):241-54.*

13. Nielsen TA, Arendt-Nielsen L. Kroniske smerter hos artrose-patienter før og efter total knæalloplastik. *Best Practice: Reumatologi; 2013 20(3) 25-27*

14. Gram M, Graversen C, Nielsen AK, Nielsen TA, Mørch CD, Andresen T, Drewes AM.*A Novel Approach to Pharmaco-EEG for Investigating Analgesics: Assessment of Spectral Indices in Single-Sweep Evoked Brain Potentials.* Br J Clin Pharmacol. 2013 Dec;76(6):951-63

15. Mørch CD, Rahi A, Christensen L, Gazerani P, Nielsen TA, Arendt-Nielsen L. The UVB cutaneous inflammatory pain model: A reproducibility study in healthy volunteers. *Int J Physiol Pathophysiol Pharmacol. 2013 Dec 15;5(4):203-15.*

16. Nielsen TA, Bittencourt da Silva L, Arendt-Nielsen L, Gazerani P. The Effect of Topical Capsaicin-induced Sensitization on Heat Evoked Cutaneous Vasomotor *Responses. Int J Physiol Pathophysiol Pharmacol. 2013 Sep 10;5(3):148-60.*

ENGLISH SUMMARY

Type 1 diabetes is a chronic autoimmune disease caused by the immune-mediated destruction of insulin-producing pancreatic beta cells, whereas type 2 diabetes is characterized by insulin resistance alone or combined with insufficient insulin production. Both types result in hyperglycemia. Prolonged hyperglycemic exposure can lead to several life-impairing microvascular complications, such as diabetic nephropathy, neuropathy, and retinopathy.

Diabetic neuropathy is the most common complication of diabetes and can affect both peripheral and autonomic nerves and cause, e.g., distal symmetrical polyneuropathy (DSPN) and diabetic autonomic neuropathy (DAN). Whereas DSPN affects sensorimotor neurons in a “glove and stocking” pattern, DAN results in dysfunction and impaired regulation of several organ systems and autonomically innervated muscles. Diabetic neuropathies can be painful as well as non-painful.

Glucagon-like peptide 1 receptor agonists (GLP-1 RA’s) are used in the treatment of type 2 diabetes by stimulating insulin secretion and inhibiting glucagon secretion, resulting in a reduction of glucose levels. GLP-1 RA’s have several extra-pancreatic effects, including anti-inflammatory, cardioprotective, and neuroprotective effects. In mice, topical administration of a GLP-1 RA has shown to prevent thinning of the neuroretina and promote neurogenesis in retinal cells. In humans, GLP-1 RA’s are known to increase heart rate through autonomic stimulation mechanisms not yet fully understood. In this thesis, we investigate the effects of the GLP-1 RA liraglutide in individuals with long-term type 1 diabetes to assess the anti-inflammatory and neuroprotective effects, independently of the anti-hyperglycemic effects.

The overall aims of this thesis were to study:

1) The effects of long-term type 1 diabetes on autonomic nerves involved in neurocardiac regulation (Study I, III, and IV)
2) The effects of long-term type 1 diabetes on sympathetic nerves innervating the superior and inferior tarsal muscles (Study I)
3) The effects of the GLP-1 RA liraglutide on heart rate variability (HRV) and the retinal nerve fiber layer thickness in individuals with long-term type 1 diabetes and confirmed DSPN (Study II and IV)

Paper I investigated the relationship between the sympathetically innervated tarsal muscles' response to phenylephrine (assessed as changes in palpebral fissure height) and HRV measures. The study aimed to examine if the phenylephrine-induced response in palpebral fissure height was associated with HRV measures and thus could be a potential marker of autonomic dysfunction in individuals with type 1 diabetes and DSPN. The phenylephrine-induced changes in palpebral fissure height were associated with autonomic function, diabetic retinopathy stage, and disease duration.

Paper II assessed, in a randomized, double-blinded, placebo-controlled study, whether liraglutide influenced the retinal nerve fiber thickness due to anti-inflammatory and possible neurotropic actions using optical coherence tomography. The thickness of the inferior, superior, nasal, temporal, and peripapillary retinal nerve fiber layer thickness were compared in individuals with type 1 diabetes and DSPN, who were randomized to receive either liraglutide or placebo. Liraglutide did not induce measurable changes in the retinal nerve fiber layer thickness both on group level and in the subgroups constituted by proliferative and non-proliferative retinopathies.

Paper III assessed HRV in resting conditions (24-h.) and ultra-short-term HRV to assess the physiological modulation in response to tonic cold pain exposure (cold pressor test) in participants with long-term type 1 diabetes and verified DSPN. The main findings revealed adynamic sympathetic and parasympathetic HRV responses in the diabetes group when compared with healthy controls.

Paper IV investigated the effect of liraglutide/placebo on heart rate and HRV modulation before, during, and after stimulation by tonic cold pain exposure (cold pressor test). Liraglutide increased heart rate when compared to placebo. Furthermore, modulation by tonic cold pain unmasked alterations in the sympathetic response and showed an unaltered parasympathetic response, indicative of sympathetic activation.

The fundamental and clinically relevant knowledge generated in this thesis provided new insight into the effects of type 1 diabetes on autonomic nerve function, as well as the effect of liraglutide on the neurocardiac regulation and human retinal nerve fibers.

DANSK RESUME

Type 1 diabetes er en kronisk autoimmun sygdom, forårsaget af immunmedieret destruktion af de insulinproducerende beta-celler i bugspytkirtlen, hvorimod type 2 diabetes er karakteriseret af insulinresistens og insufficient insulinproduktion. Begge typer medfører i forhøjet blodsukker. Langvarig eksponering for forhøjet blodsukker niveauer kan medføre flere mikrovaskulære komplikationer, såsom diabetisk nefropati, neuropati og retinopati.

Diabetisk neuropati rammer ca. 50% af personer med diabetes og anses for at være den, der forvolder flest belastende symptomer fra både perifere og autonome nerver. De diabetes inducerede nervebeskadigelser kan føre til udvikling af distal symmetrisk polyneuropati (DSPN), samt diabetisk autonom neuropati (DAN). DSPN påvirker perifere sensomotoriske nerver, og kan medføre nedsat sensibilitet, nedsat muskelkraft og kan medføre smerter, hvorimod DAN fører til dysregulering af de fleste organsystemer samt de autonomt innerverede muskler. Diabetisk polyneuropati kan hos nogen medføre neurogene smerter men ikke hos andre.

Glukagon lignende peptid-1 receptoragonister (GLP-1RA'er) anvendes i behandlingen af type 2-diabetes, ved at stimulere frigivelsen af insulin og hæmme frigivelsen af glukagon, hvilket medføre en reduktion af blodsukkerniveauet.
Udover at virke på cellerne i bugspytkirtlen, har GLP-1 RA'er vist sig, at have anti-inflammatoriske, kardioprotektive samt neuroprotektive virkningsmekanismer. Topikal administration af øjendråber indeholdende GLP-1-RA'er har i mus vist sig at forebygge udtynding af nethinden, samt fremme nydannelsen af nerveceller i nethinden. GLP-1 RA'er har konsistent vist at øge hjertefrekvensen, omend den/de underliggende mekanisme(r) endnu ikke er fuldt belyst. I denne afhandling, undersøgte vi effekten af GLP-1 RA liraglutid i individer med langvarig type 1 diabetes, for at undersøge den anti-inflammatoriske og neuroprotektive effekt uafhængigt af den anti-hyperglykæmiske virkning.

Det overordnede formål med studierne der indgår i PhD afhandlingen er at studere:

1) Effekten af langvarig type 1 diabetes på de autonome nerver som er involveret i den neurokardielle regulering (Studie I, III og IV)
2) Effekten af langvarig type 1 diabetes på de sympatiske nerver der innerverer de superiore og inferiore tarsale muskler (Studie I)
3) Effekten af liraglutid (en GLP-1 RA) på hjertefrekvensvariation (HRV) samt på det retinale nervefiber lags tykkelse i patienter med langvarig type 1 diabetes og verificeret distal symmetrisk polyneuropati (Studie II og IV).

Artikel 1 undersøgte forholdet mellem de sympatisk innerverede tarsale musklers respons på phenylephrin (ændringer i øjenspalte højde) samt hjertevariabilitets parametre. Det blev undersøgt om den phenylephrin-inducerede respons på øjenspalte højden var associeret med hjertevariabilitets parametre og om den dermed potentielt kunne være en markør for dysfunktioner af sympatiske nerver hos personer med type

1-diabetes og verificeret polyneuropati. Studiet fandt, at de phenylephrin-inducerede ændringer i øjenspalte højden var associeret med den sympatiske funktion, graden af diabetisk retinopati, samt sygdomsvarigheden.

Artikel 2 undersøgte, i et randomiseret, double-blindet, placebo-kontrolleret studie, effekten af liraglutid på det retinale nervefiber lag. Studiet sammenlignede liraglutid og placebo's respektive effekt på tykkelsen af den inferiore, superiore, nasale, temporale, samt peripapillære retinale nervefiber lags tykkelse ved brug af optisk koherens tomografi. Liraglutid medførte ikke målbare ændringer i den retinale nervefiber lags tykkelse, hverken på gruppeniveau eller i den proliferative, eller non-proliferative undergruppe.

Formålet med artikel 3, var at vurdere effekten af langvarig type 1-diabetes på hjertevariabilitet i hvile, samt som respons på smerte-stimulering med is-vand. Studiet sammenlignede hjertevariabilitets responserne blandt personer med langvarig type 1-diabetes og raske forsøgsdeltagere, og fandt adynamiske sympatiske og parasympatiske-responser i diabetesgruppen.

I artikel 4 undersøgte, i et randomiseret, double-blindet, placebo-kontrolleret studie, effekten af liraglutid på hjertefrekvens og hjertevariabilitets parametre, som følge af smerte-stimulering med is-vand. Studiet sammenlignede effekten af liraglutid og placebo på personer med langvarig type 1-diabetes og DSPN og bekræftede at behandling med liraglutid øgede hjertefrekvensen. Stimulation is-vand tydeliggjorde ligeledes ændringer i det sympatiske respons, men ikke i parasympatiske respons.

Denne PhD afhandling bidrager til en øget klinisk og fundamental indsigt i effekten af langvarig type 1-diabetes på de autonome nervefunktioner, samt virkningen af liraglutid på den neurokardielle regulering samt på det retinale nervefiber lag.

ACKNOWLEDGEMENTS

First, I would like to show my sincere gratitude to my supervisors for their support and trust in me, both during the initiation of this thesis. Furthermore, I would like to thank my supervisors and collaborators for crucial sparring, expertise, and guidance throughout the process of this thesis.

I would like to thank Professor Christina Brock for her invaluable day-to-day sparring and support during the making of this thesis. Furthermore, I would like to thank Professor Asbjørn Mohr Drewes for being my mentor in both my scientific and clinical career. I would also like to thank Professor Henrik Vorum for his contributions and for being an inspiration in the field of ophthalmology. A special thanks to Carl Uggerhøj Andersen and Mette Kaas-Hansen for opening my eyes, and welcoming into the field of ophthalmology. A big thanks to my colleagues at Mech-Sense for welcoming me into the research group and inviting me into this supportive working environment.

Moreover, I would like to thank the Department of Ophthalmology, Aalborg University Hospital, and especially Anders Kruse for his support and trust in me.

Last but not least, I would like to thank my family for their love, support, and understanding of my continuous interest in clinical research. A special thanks to my wife, Matilde, for her understanding and support when times were tough and to my beautiful daughters, Ellie and Uma, for always making me smile and keeping my heart filled with love.

LIST OF PAPERS

This thesis is based on the following papers:

I. Nielsen TA, Andersen CU, Vorum H, Riahi S, Sega R, Drewes AM, Karmisholt J, Jakobsen PE, Brock B, Brock C. Palpebral Fissure Response to Phenylephrine Indicates Autonomic Dysfunction in Patients With Type 1 Diabetes and Polyneuropathy. Invest Ophthalmol Vis Sci. 2022 Aug 2;63(9):21. doi: 10.1167/iovs.63.9.21. PMID: 35980646; PMCID: PMC9404365.

II. Nielsen TA, Sega R, Uggerhøj Andersen C, Vorum H, Drewes AM, Jakobsen PE, Brock B, Brock C. Liraglutide Treatment Does Not Induce Changes in the Peripapillary Retinal Nerve Fiber Layer Thickness in Patients with Diabetic Retinopathy. J Ocul Pharmacol Ther. 2022 Jan-Feb;38(1):114-121. doi: 10.1089/jop.2021.0055. Epub 2021 Dec 16. PMID: 34918951

III. Nielsen TA, Lundbye-Christensen S, Dimitrova YK, Riahi S, Brock B, Drewes AM, Brock C. Adynamic response to cold pain reflects dysautonomia in type 1 diabetes and polyneuropathy. Accepted for publication in *Scientific Reports.*

IV. Nielsen TA, Lundbye-Christensen S, Dimitrova YK, Riahi S, Brock B, Drewes AM, Brock C. Cold pain exposure reveals heart rate variability alterations in liraglutide-treated individuals with type 1 diabetes. Submitted for publication in *Communications Medicine*

The above papers will be referred to as study I-IV throughout the thesis. Study I and II are published, and peer-reviewed original papers. Study III has been accepted for publication. Study IV have been submitted for publication.

ABBREVIATIONS

ANS: Autonomic nervous system

CPT: Cold pressor test

CAN: Cardiovascular autonomic neuropathy

DAN: Diabetic autonomic neuropathy

DPN: Diabetic peripheral neuropathy

DSPN: Distal symmetrical polyneuropathy

T1DM: Type 1 diabetes mellitus

T2DM: Type 2 diabetes mellitus

PFH: Palpebral fissure height

ΔPFH: Difference in PFH between phenylephrine-treated and untreated eye

RNFL: Retinal nerve fiber layer thickness

DPN: Diabetic peripheral neuropathy

DR: Diabetic retinopathy

PDR: Proliferative diabetic retinopathy

NPDR: Non-proliferative diabetic retinopathy

HR: Heart rate

HRV: Heart rate variability

VLF: Very low-frequency band (0.0033-0.04 Hz)

ΔVLF: Difference in VLF parameters between baseline and follow-up

LF: Low-frequency band (0.04-0.15 Hz)

ΔLF: Difference in LF parameters between baseline and follow-up

HF: High-frequency band 0.15-0.4 Hz)

ΔHF: Difference in HF parameters between baseline and follow-up

LF/HF: Low-frequency/high-frequency ratio

SDNN: Standard deviation of NN intervals

SDANN: Standard deviation of all NN intervals for each 5-minute for 24 hours

SDNNI: Mean of the standard deviation of all NN intervals for each 5-minute interval for 24 hours

RMSSD: Root mean square of successive RR interval differences

CBS: Cardiac sensitivity to the baroreflex

CVT: Cardiac vagal tone

GLP-1: Glucagon-like peptide 1

GLP-1 R: Glucagon-like peptide 1 receptor

GLP-1 RA: Glucagon-like peptide 1 receptor agonist

TABLE OF CONTENTS

TABLE OF FIGURES

1 INTRODUCTION

Type 1 diabetes is a chronic, metabolic disease known to cause microvascular complications such as retinopathy, neuropathy, and nephropathy. In type 1 diabetes (T1DM), the most frequent type of neuropathy is diabetic peripheral neuropathy (DPN), with a prevalence of 17,5% and an incidence of 8,8 per 1000 person-years[1,2]. DPN takes on two different forms: diabetic distal symmetrical polyneuropathy (DSPN) and diabetic autonomic neuropathy (DAN)[3,4]. While DSPN affects sensorimotor neurons in the extremities, in a "glove and stocking" pattern, DAN leads to impairment and dysregulation of the autonomic nervous system (ANS)[5,6]. When diabetic neuropathy affects the ANS, deterioration in the normal functions and regulation of, e.g., cardiovascular, gastrointestinal, urogenital, sudomotor, and neurovascular functions may appear, resulting in a reduction in quality of life[6–8]. Impairment of cardiovascular autonomic control in individuals with diabetes mellitus is known as cardiovascular autonomic neuropathy (CAN). In individuals with CAN, ANS regulation is altered, resulting in an impaired ability of the heart rate to adapt to different conditions and maintain homeostasis. Manifestations of CAN include sinus tachycardia, abnormal blood pressure regulation, orthostatic hypotension, dizziness, syncope, exercise intolerance, asymptomatic myocardial ischemia, and infarction[9]. CAN is a serious yet common complication of diabetes mellitus, associated with increased overall mortality and morbidity[10–14]. Routine clinical examinations based on existing clinical guidelines do not include a thorough investigation of the autonomic nervous system, even though CAN is associated with life-threatening complications[15].

Several methods exist for assessing the autonomic nervous system, including evaluating cardiovascular autonomic reflex, heart rate variability (HRV), pupillomotor-, sudomotor- and pilomotor functions[16–18]. To diagnose CAN, cardiovascular autonomic reflex tests (CARTs) are needed, however, HRV is widely used to evaluate the autonomic nervous function in 24-hour Holter recordings (gold standard), but recently short-term recordings have also been used, and even ultra-short epochs of 2 minutes or less, is now being introduced.

Regulation of the heart rate results from complex autonomic interaction with primarily the brainstem, and as such, the ANS and the sympatico-vagal balance has a significant role as the heart's modulator, allowing for rapid dynamic adaptation to changes in the internal and external environment, to maintain homeostasis. Different parameters of the heart rate variability (HRV) reflect the sympathetic and parasympathetic effects on the sinoatrial node for the regulation of heart rate[19]. Based on the electrical conductivity of the heart, HRV is adopted as a specific, sensitive, and reliable method for assessing autonomic dysfunction in people with T1DM[4,20,21]. It uses the beat-to-beat variations (R-R interval) of consecutive heartbeats to quantify the functional status of the physiological control of the heart, allowing an evaluation of the ANS as a function of time and frequency. Reduction in HRV is a sign of imbalanced sympatico-vagal regulation (autonomic dysfunction) and can be used as a marker of

CAN in T1DM, even before the onset of the symptoms[22,23]. There is, however, an unmet clinical need to develop provocative tests that could reveal early signs of CAN because the current diagnostic procedure is based on abnormal reflex testing, where CAN is in its early development or manifest.

Modulation of the ANS is possible in several ways, such as by controlled deep and slow breathing activating the physiological baroreceptor response[24] or by electrical stimulation of the vagal nerve[25]. Furthermore, registered pharmacological drugs are known to block sympathetic (e.g., beta-blockers)/parasympathetic (e.g., atropine) activity or enhance sympathetic (e.g., phenylephrine, adrenalin, or dopamine)/ parasympathetic (e.g., choline esterase inhibitors or neostigmine) activity. However, glucagon-like peptide-1 receptor agonists (GLP-1 RA's) have also been shown to modulate the autonomic nervous system[26]. GLP-1 RA's act as neuropeptides by regulating the ANS and the cardiovascular system[27], and when secreted by the hypothalamic nuclei, GLP-1 has been shown to increase parasympathetic activity[28]. Furthermore, GLP-1 RA's have been shown to have antioxidative, anti-inflammatory, and neuroprotective effects on central and peripheral nerves[29,30], which may affect HRV. A recently published preclinical study found that GLP-1 receptor agonists even exert neurotrophic influence on retinal cells[31], underpinning its multifaceted actions.

The fundamental knowledge generated during this Ph.D. project will contribute to a better understanding of autonomic nervous system function in individuals with type 1 diabetes and the effect of the GLP-1 receptor agonist, liraglutide, on the neurocardiac regulation and possible neurotrophic effects on retinal nerve fibers.

The hypotheses of this PhD project are as follows:

1) Long-term type 1 diabetes causes neuropathy to the peripheral sympathetic nerve fibers innervating the superior and inferior tarsal muscles[32].
2) Treatment with liraglutide increases peripapillary retinal nerve fiber layer thickness by inducing nerve regeneration and restoring vascularization[33].
3) Exposure to tonic cold pain causes enhanced sympathetic drive and attenuates the dynamic sympatico-vagal response in participants with long-term type 1 diabetes when compared to healthy controls.
4) Treatment with liraglutide leads to increased heart rate and sympathetic drive in situational dynamic heart rate variability measures, evident as increased low-frequency and very low-frequency content.

In summary, the objectives of this PhD project are:

- To investigate the relationship between the sympathetically innervated tarsal muscle response to phenylephrine and HRV measures in individuals with long-term T1DM and polyneuropathy (Paper I)[32]
- To assess associations between the tarsal muscle response to phenylephrine and the degree of diabetic retinopathy, disease duration, as well as the degree of diabetic retinopathy and HRV measures in individuals with long-term T1DM and DSPN (Paper 1)[32]

- To compare the effect of liraglutide on retinal nerve fiber layer thickness following 26 weeks treatment in comparison to placebo in individual with long-term T1DM and DSPN (Paper II)[33]
- To assess the dynamic HRV alterations in response to cold tonic pain exposure in healthy and in individuals with long-term T1DM and DSPN (Paper III)
- To compare 24-hour HRV measures, baroreflex sensitivity and cardiac vagal tone in individual with long-term T1DM and DSPN and healthy (Paper III)
- To assess and compare the dynamic HRV responses to tonic cold pain exposure in individuals with long-term T1DM and DSPN treated following 26 weeks of treatment with liraglutide and placebo (Paper IV)

2 BACKGROUND

2.1 DIABETES MELLITUS AND ITS COMPLICATIONS

Diabetes mellitus is a chronic metabolic disease characterized by elevated blood glucose levels. It can lead to serious damage to the heart, blood vessels, eyes, kidneys, and nerves. According to WHO, diabetes affects up to 422 million individuals worldwide, and it has been estimated that up to 1.5 million deaths are directly attributed to diabetes[34].

Diabetes is caused by impairment in insulin secretion or action or a combination of the two. However, there are primarily two types of diabetes: type 1 and type 2 diabetes. Whereas type 1 diabetes is characterized by the autoimmune destruction of insulin-producing pancreatic beta cells, resulting in relative or absolute insulin deficiency, type 2 diabetes is characterized by insulin resistance alone or combined with insufficient insulin production.

Type 1 diabetes typically presents in childhood, and the incidence increases with age and peaks around 10-14 years[35]. Within the last decades, the global incidence of type 1 diabetes has risen by 3.4% annually[36]. Both genetic, epigenetic, and environmental factors are involved in developing type 1 diabetes[37]. Symptoms of type 1 diabetes include polyuria, polydipsia, weight loss, fatigue, and vision changes, and diabetes is diagnosed by the presence of elevated blood glucose levels. The progression of diabetes mellitus is associated with the development of microvascular complications, such as retinopathy, neuropathy, and nephropathy[38]. Hyperglycemia and HbA1c levels above 7% have been shown to increase the risk of developing such microvascular complications[39].

2.1.1 DIABETIC RETINOPATHY

Diabetic retinopathy (DR) is a degenerative neurovascular disease affecting retinal microvasculature. In most developed countries, DR is the leading cause of blindness in working-aged adults[40,41]. DR affects approximately 93 million people worldwide, of which 17 million are believed to have proliferative DR[40]. In a large Danish population-based cohort study of individuals with type 1 diabetes, the 25-year prevalence of diabetic retinopathy was 97%, and the incidence of proliferative retinopathy was 42.9%[42]. The prevalence of DR increases with disease duration and poor glycemic- and blood pressure control[43]. Screening for DR and improved glycemic control significantly reduces the risk of diabetes-induced blindness[44].

Diabetic retinopathy ranges from non-proliferative diabetic retinopathy (NPDR) to proliferative diabetic retinopathy (PDR). The severity of diabetic retinopathy can be staged using the International Clinical Diabetic Retinopathy Disease Severity scale[45].

NPDR is staged as mild, moderate, or severe based on the number of microaneurysms/intraretinal hemorrhages and the presence of venous beading and/or intraretinal microvascular abnormalities (see Fig. 1). PDR is characterized by the presence of neovascularization arising from the retina or optic disc and is associated with preretinal or vitreous hemorrhages. Furthermore, subsequent fibrosis may lead to tractional retinal detachment.

The development of PDR is caused by progressive retinal ischemia, which releases local growth factors, such as vascular endothelial growth factors, resulting in retinal neovascularization[46].

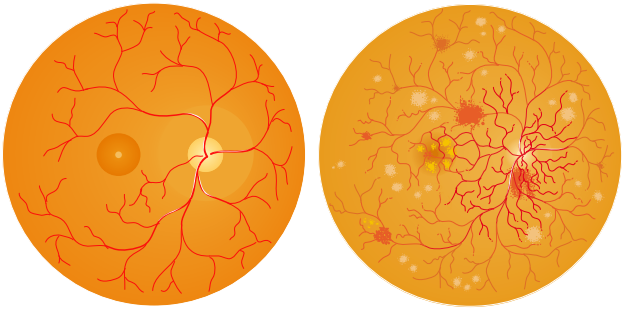

Figure 1: Graphic visualization of the retina without retinopathy (left) and with diabetic retinopathy (right), showing intraretinal hemorrhages, cotton wool spots, hard exudates, and the formation of new blood vessels (intraretinal microvascular abnormalities/ neovascularization). Graphics supplied by Servier Medical Art, licensed under a Creative Commons Attribution 3.0 unported license.

Although DR has classically been considered a microvascular disease, diabetes has also been shown to cause retinal neurodegeneration in type 1-[47,48] and type 2 diabetes[49,50]. These changes have been referred to as retinal diabetic neuropathy or diabetic retinal neurodegeneration[51,52]. Growing evidence suggests that thinning of retinal neuronal and axonal layers precedes the presence of clinically visible microvascular changes of DR[53,54]. Retinal thinning within the macula has even been shown in pre-diabetes[55].

Diabetic retinal neurodegeneration is primarily caused by dysfunction of the retinal neovascular unit[51]. Damage occurs early in disease progression, and dysfunction of the neovascular unit results in blood-retinal-barrier breakdown and vascular leakage caused by secretion of various factors, including vascular endothelial growth factors and pro-inflammatory cytokines[56–58]. As a result, apoptosis of retinal neurons results in thinning of retinal nerve fiber layers (RNFL), eventually causing loss of vision[51,58,59]. Diabetic retinal neurodegeneration may not only be an early indicator of DR but is more likely a factor in the development of DR and thus contributes to the microvascular changes[52,60]. Consequently, early therapeutic neuroprotective strategies, e.g., GLP-1 RA's[30], may not only affect diabetic retinal neurodegeneration but could, as such, also be involved in preventing the progression of DR[30,61,62].

Interestingly, diabetic retinal neurodegeneration is associated with diabetic peripheral- and autonomic neuropathy[63–67] and has been shown to predict the development of diabetic peripheral neuropathy (DPN)[68].

2.1.2 DIABETIC NEUROPATHY

Diabetic neuropathy is the most common chronic complication of diabetes[13] and affects up to 50% of individuals with diabetes[69]. It is a heterogeneous disorder that affects the peripheral, central, and autonomic nervous system in a length-dependent manner and is associated with a reduction in quality of life[13] and high healthcare costs[70]. Diabetic neuropathies can be painful as well as non-painful.

The pathogenesis of diabetic neuropathy is complex, multifactorial, and only partially understood. It involves several vascular, metabolic, inflammatory, and immune-mediated mechanisms, which result in oxidative stress, inflammatory stress, and ischemia[71]. Prolonged exposure to hyperglycemia is a key factor in developing diabetic neuropathy[71]. Other factors associated with the development of diabetic neuropathy is associated with disease duration, obesity, dyslipidemia, mitochondria dysfunction, and hypertension[71].

There are several manifestations of diabetic neuropathy[72], however, and for the sake of the scope of this thesis, the primary focus will be on distal symmetric polyneuropathy (DSPN), diabetic autonomic neuropathy (DAN), and cardiovascular autonomic neuropathy (CAN).

2.1.2.1 DISTAL SYMMETRICAL POLYNEUROPATHY

DSPN is a symmetrical, length-dependent, sensorimotor polyneuropathy affecting the peripheral sensorimotor neurons[72]. It is defined by the presence of symptoms and/or signs of peripheral nerve dysfunction in individuals with diabetes after other causes have been excluded[13]. The diagnosis of DSPN is in principal clinical and can be made after clinical examination, including pinprick, vibration, and temperature perception[13]. According to the Toronto criteria, confirmed DSPN requires the presence of nerve conduction abnormalities and symptoms and signs of neuropathy[3].

In type 1 diabetes, the 20-year prevalence of DSPN is around 20%[13]. It is the most common variety of neuropathy and accounts for up to 75% of diabetic neuropathies[72,73]. About half of the individuals with DSPN experience symptoms ranging from numbness, tingling, and pain, most commonly in the toes, feet, and legs[13]. The other half, however, may be asymptomatic (loss of protective sensation), and patients may present with painless foot ulcers. In type 1 diabetes, tight glycemic control has been shown to reduce the incidence of DSPN by up to 78%[39,74].

According to the type of sensory fiber (small or large fibers) involved, symptoms may vary. Early symptoms caused by the involvement of small sensory fibers may include

neuropathic pain, dysesthesia, paresthesia, hyperalgesia, and allodynia[13]. The involvement of large sensory fibers may cause numbness, tingling, and loss of proprioception[75].

2.1.2.2 DIABETIC AUTONOMIC NEUROPATHY

Damage to the autonomic nerves as a complication of diabetes is known as diabetic autonomic neuropathy (DAN). Such autonomic dysfunction impairs the regulatory ability of the ANS, which consequently impairs the continuous regulation of one or more organ systems, including cardiovascular, gastrointestinal, urogenital, ocular, and sudomotor function[76].

As such, the symptoms are pleomorphic and may include hypo/hyperglycemia unawareness, resting tachycardia, exercise intolerance, orthostatic hypotension, gastroparesis, constipation, diarrhea, neurogenic bladder, erectile dysfunction in men/ lacking lubrication in women, dysfunction of sweating and thermoregulation well as impaired pupillary function[15]. Such complications have a significant impact on the quality of life[15].

2.1.2.3 CARDIOVASCULAR AUTONOMIC NEUROPATHY

The prevalence of CAN in type 1 diabetes varies from 17-66%[77], and in individuals with DPN, more than 50% will have CAN[78]. The key factor for the development of CAN is hyperglycemia[74]; however, there are several risk factors for developing CAN, including increased age, disease duration, poor glycemic control, obesity, hypertension, dyslipidemia, smoking, as well as the presence of microvascular complications such as diabetic retinopathy, neuropathy, and nephropathy/microalbuminuria[11,15,79].

One manifestation of DAN is cardiovascular autonomic neuropathy (CAN) which is a serious complication of diabetes mellitus. CAN results from impairment of the autonomic regulation of the cardiovascular system, affecting heart rate control and vascular dynamics, thus challenging the maintenance of cardiovascular homeostasis[3,11]. CAN has significant clinical implications, including increased morbidity and mortality[10–14], and is associated with a range of cardiovascular symptoms and complications. One of the most common symptoms of CAN is orthostatic hypotension, which presents as dizziness and potentially syncope in response to postural changes[9]. In healthy, a compensatory vasomotor reflex mediated by baroreceptors primarily located in the aortic arch and carotid sinus increases sympathetic activity and decreases parasympathetic activity in response to a sudden drop in blood pressure, and thus maintains continuous cerebral blood flow. In diabetes however denervation of efferent sympathetic nerves results in reduced vasoconstriction in peripheral vascular beds, and thus this response is compromised[12]. Other symptoms include resting tachycardia and exercise intolerance, as well as asymptomatic myocardial ischemia and infarction, which has caused the nickname

"the silent killer"[9]. Damage to autonomic nerve fiber results in an impaired ability of the heart rate to adapt to different conditions and maintain homeostasis.

Diabetes affects autonomic nerves in a length-dependent manner[77,80]. The vagal nerve, which accounts for approximately 75% of parasympathetic activity, is the longest parasympathetic nerve, and thus, CAN often affects this nerve first[12]. Manifestations of parasympathetic denervation are resting tachycardia, as well as decreased parasympathetic activity. As a result, sympathetic predominance is present in early CAN with parasympathetic neuropathy[81], and later sympathetic nerves are also affected[12]. Thus, CAN is associated with impairment of autonomic modulation and reduced baroreflex sensitivity[82], which can even be observed in subclinical stages[83,84].

2.1.2.4 DIABETIC CENTRAL NEUROPATHY

As for the peripheral and autonomic nervous systems, the central nervous system is also affected by diabetes. Diabetes has been associated with accelerated cortical atrophy, reduced gray matter and cerebellar volume, as well as atrophy of somatosensory and motor cortices[85–87]. Moreover, cervical spine atrophy has been shown in individuals with diabetes and is more severe in individuals with DSPN than those without[88,89]. Whether or not changes in the central nervous system is a primary phenomenon or occur due to adaptive shrinking as a consequence of peripheral neuropathy remains controversial[90]. However, not only structural volumetrics are affected by diabetes; reductions in central transmission and EEG abnormalities have been shown in type 1 diabetes[91,92]. Decreased connectivity between the thalamus and superior frontal, middle frontal, and precentral cortex has also been shown in individuals with T1DM and DSPN[93]. Furthermore, type 1 diabetes has been associated with cognitive impairment[94,95].

2.1.3 DIABETIC NEPHROPATHY

Diabetic nephropathy is another common microvascular complication of diabetes and is the most common cause of chronic kidney disease, affecting up to 30% of individuals with type 1 diabetes[96]. Diabetic nephropathy is characterized by persistent (micro)albuminuria and progressive decline in renal function. Diabetic nephropathy progresses through several stages, including hyperfiltration, microalbuminuria, macroalbuminuria, and eventually leads to end-stage kidney disease[97].

2.2 THE AUTONOMIC NERVOUS SYSTEM

The ANS regulates involuntary physiologic processes. Thus, the ANS is deeply involved in every aspect of our daily life. Its ability to maintain homeostasis through changes in the sympathetic and parasympathetic branches is necessary for the appropriate functioning of most organ systems, including the cardiovascular system[98].

The ANS comprises three anatomically distinct divisions, the sympathetic, parasympathetic, and enteric nervous systems.
Activation of the sympathetic nervous system involves a “fight-and-flight” response resulting in, e.g., increased heart rate, dilated pupils, and inhibition of peristaltic movement, whereas activation of the parasympathetic nervous system involves a "rest and digest" response, which slows down the heart rate, contracts the pupils and stimulates peristaltic movements.

The ANS has traditionally been thought to be reciprocally balanced by two opposing branches, the sympathetic and parasympathetic divisions. However, as proposed by *Berntson et al.* as the doctrine of autonomic space*,* the relationship between parasympathetic and sympathetic outflow can also be described in a dimensional space, where both branches can be co-activated and co-inhibited simultaneously[99], in both a linear and non-linear fashion[100]. Even though this theory is more complex, it emphasizes that the ANS is not a zero-sum system.

The primary focus of the thesis will be on neurocardiac autonomic regulation.

2.2.1 CARDIAC AUTONOMIC NERVOUS SYSTEM

In both sympathetic preganglionic and parasympathetic preganglionic and postganglionic neurons, acetylcholine is the primary neurotransmitter; thus, these neurons are referred to as cholinergic. In sympathetic postganglionic neurons, norepinephrine is the primary neurotransmitter, and these neurons are referred to as adrenergic. Norepinephrine affects β1, β2, α1, and α2 receptors located in the cardiovascular system, and whereas α-receptors stimulate vasoconstriction, β-receptors stimulate vasodilatation allowing for increases in blood perfusion, as a result of sympathetic stimulation, heart rate, contractility, and conductivity increases[101]. In addition to autonomic modulation, heart rate is influenced by other mechanisms, including neurohumoral influence, stretching of the sinoatrial node, local temperature changes, and ionic changes within the sinoatrial node[102].

A dynamic autonomic relationship exists in healthy hearts. As a result of sympathetic and parasympathetic modulation, the beat-to-beat interval changes, and the autonomic modulation is primarily responsible for the heart rate variability (HRV)[102]. Parasympathetic nerves exert their effects more rapidly (<1 second) than sympathetic nerves (> 5 seconds), and the parasympathetic nerves have the ability to slow the heart rate down to 20-30 bpm[100]. During rest and sleep, the parasympathetic tone is the dominant influence on the sinoatrial node[103].

Short-term HRV measurements are also influenced by sinoatrial node stretching in resting conditions. In healthy individuals at rest, sinoatrial node stretching has a small influence on HRV of about 2-4%[104] however, in denervated hearts, such stretching may account for up to 100%[105].

The heart's activity is regulated through a combination of afferent and efferent neurons. Afferent neurons convey sensory information from receptors located within the cardiovascular system, including baroreceptors and chemoreceptors. Afferent information is primarily transmitted through the vagal nerve, although some information is transmitted via sympathetic spinal afferents[106]. Within the brainstem, the nucleus tractus solitarius (NTS) receives most afferent information from the heart and serves as a relay point for cardiovascular reflexes. In contrast, input from spinal afferents is received by centers in the thalamus, brainstem, and hypothalamus[107]. Through interaction between the NTS and other brainstem nuclei, reflexive autonomic motor responses are generated[108]. Furthermore, cortical control centers, such as the amygdala, and prefrontal, cingulate, and insular cortices, which are related to pain perception and emotion, are also involved in the modulation of cardiovascular responses[106].

The efferent part of the sympathetic innervation arises from the rostral ventrolateral medulla (RVLM) and caudal ventrolateral medulla (CVLM), located in the medulla oblongata, which exerts sympatoexcitatory and sympatoinhibitory effects respectively[109]. These connect to preganglionic neurons in the intermediolateral nucleus (ILM), which leaves the spinal cord through the white rami at the T1-T4 level. After leaving the spinal cord, preganglionic neurons enter the sympathetic ganglia. From here, postganglionic neurons innervate the heart through deep and superficial cardiac plexuses, providing autonomic control of the sinoatrial node, atria, and ventricles[101].

The efferent part of the parasympathetic innervation arises primarily from the nucleus ambiguous (NA), which interacts with the vagal dorsal motor nucleus (VDMN) located in the medulla oblongata. Preganglionic neurons, primarily located in the vagal nerve (but also in part by the recurrent pharyngeal nerve[110]), synapse with postganglionic neurons in intracardiac ganglia before converging into the cardiac plexuses. For a more comprehensive overview, see Fig. 2.

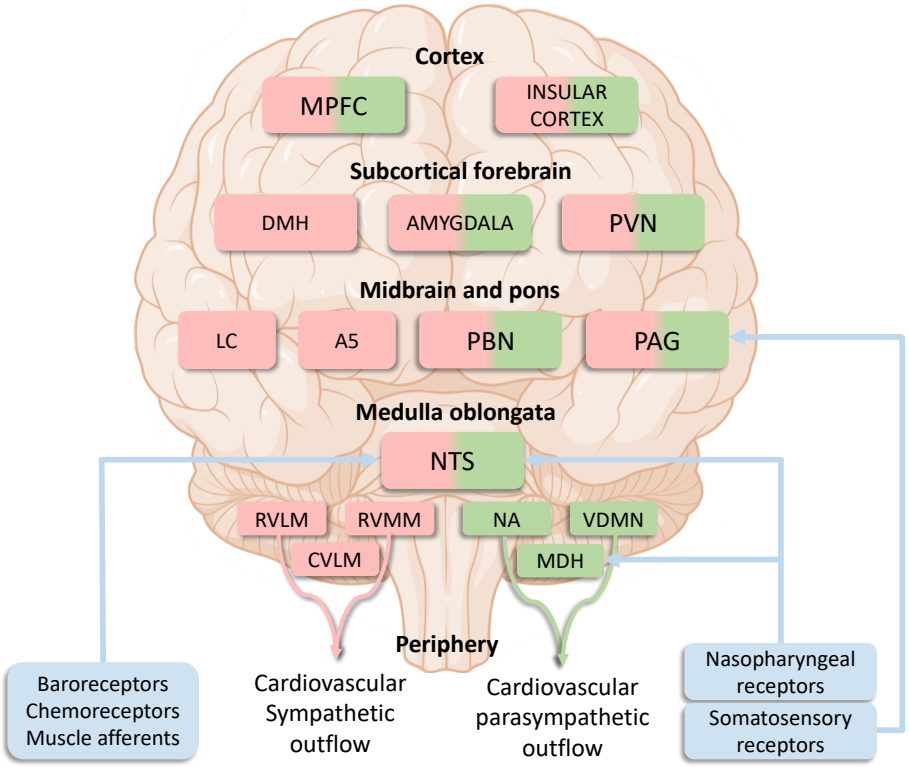

Figure 2. Schematic overview of interconnected structures involved in central autonomic cardiovascular regulation. Pink refers to areas involved in the sympathetic response, and green to areas involved in the parasympathetic response. Blue refers to peripheral afferent pathways. MPFC; medial prefrontal cortex, DMH; dorsomedial hypothalamus, PVN; hypothalamic paraventricular nucleus, LC; locus coeruleus, A5; A5 noradrenergic cell group in central pons, PBN; parabrachial nuclei, PAG; periaqueductal gray, NTS; nucleus tractus solitarius, RVLM; rostral ventrolateral medulla, RVMM; rostral ventromedial medulla, CVLM; caudal ventrolateral medulla, NA; nucleus ambiguous, VDMN; vagal dorsal motor nucleus, and MDH; medullary horn of the trigeminal nucleus. Modified from *Ruffazini et al.*[107]. Graphics supplied, in part, by Servier Medical Art, licensed under a Creative Commons Attribution 3.0 unported license.

2.2.2 PHARMACOLOGIC AND PHYSIOLOGIC MODULATION OF THE AUTONOMIC NERVOUS SYSTEM

Phenylephrine

Phenylephrine is a sympathomimetic drug commonly used topically in ophthalmology to induce dilatation of the pupil, allowing for examination of the

fundus and posterior chamber. The mechanism is through α-adrenergic stimulation of the iris dilator muscle, contraction of radial fibers occurs, resulting in dilatation of the pupil.

In addition to stimulating the iris dilator muscle, phenylephrine also stimulates the sympathetically innervated superior and inferior tarsal muscle[111], by activating α- and β-receptors[112], resulting in increased palpebral fissure height (PFH)[113–115]. Thus, phenylephrine can be used to assess the suitability of Müllers muscle-conjunctival resection surgery in patients with mild to moderate upper eyelid ptosis[116]. This test is known as the phenylephrine test[117].

In study I, we hypothesized that long-term type 1 diabetes mellitus causes neuropathy to the peripheral sympathetic nerve fibers that innervate the superior and inferior tarsal muscles. Thus, phenylephrine hydrochloride 10% was used to stimulate the superior and inferior tarsal muscles in the right eye. Measurements of PFH were performed in both the phenylephrine-stimulated and the unstimulated eye, and the difference between the two eyes (ΔPFH) was calculated and used for further analyses.

Cold Pressor Test

The cold pressor test is a validated and simple test that can be used to evaluate the cardiovascular autonomic function[118] or serve as an experimental pain stimulus used to evaluate pain treatments[119]. The cold pressor test typically involves the immersion of the hand into circulating ice water, between 1-5 °C, for a period of 1-6 minutes[120]. In healthy subjects, the cold pressor test elicits cardiac sympathetic activation, resulting in increased heart rate and blood pressure[118,121–123], possibly co-mediated by increases in neurohormones[124] such as epinephrine and norepinephrine[125–127]. Whether or not the cold pressor test also results in parasympathetic withdrawal remains controversial[118,122]. The effect of the cold pressor test, or cold tonic pain, on heart rate, is complex, and high individual variety has been reported; both cold pressor-induced increases and decreases in heart rate have been shown[120,128]. The heart rate responses have not been shown to differ between individuals with diabetes and healthy controls[127,129,130], although one study failed to show an increase in heart rate in individuals with T1DM[131]. The autonomic response may vary in case of efferent or afferent neuronal pathway damage. In individuals with DAN, one study found that the diastolic blood pressure response was lower, and heart rates were higher than in healthy controls[130]. In regards to the effect of the cold pressor test on HRV measures, both increases[132], decreases[118], and no changes[125] have been shown in low-frequency (LF) power, and high-frequency (HF) power has been shown to remain unchanged[125] or to decrease[118].

Exposure to tonic cold pain causes activation of peripheral thermo- and nociceptors, which enter the spinal cord through the dorsal root ganglion. Following contralateral crossing, they form the spinolateral tract before traveling to the thalamus. At the level of the thalamus, contralateral is sent to the reticular formation, from which these may stimulate the RVLM and cause sympathetic stimulation of the cardiovascular system[124,133]. Through other projections, cortical and subcortical structures in the hypothalamus may be affected, causing neuroendocrine responses[134].

In studies III and IV, cold tonic pain exposure was used as a provocative challenge test, equivalent to that of the treadmill test in the assessment of cardiac performance. Participants were placed in a supine position and instructed to breathe spontaneously. The left hand was then immersed in ice-chilled water (～2.0℃) for 2 minutes. In case of intolerable pain, the hand was withdrawn from the water.

2.3 GLUCAGON-LIKE PEPTIDE 1 RECEPTOR AGONISTS

Glucagon-like peptide-1 (GLP-1) is an incretin hormone secreted centrally or by enteroendocrine L-cells in the intestinal mucosa[135]. It is released in response to nutritional components, such as carbohydrates, protein, amino acids, bile acids, and fatty acids in the small intestine[136,137]. It exerts its effects by stimulating insulin secretion from pancreatic β-cells and inhibiting glucagon secretion in a glucose-dependent manner, thereby improving β-cell glucose sensitivity and consequently decreasing blood glucose levels and hemoglobin A1c levels[138–140]. Following a meal intake, a rapid increase in GLP-1 secretion occurs from the beforementioned intestinal L-cells[141], resulting in stimulation and regulation of pancreatic islet function, accounting for most of the postprandial insulin secretion[27,142]. In addition to insulin-tropic effects, GLP-1 has been shown to protect against β-cell apoptosis, stimulate β-cell proliferation and preserve β-cell mass[141] as well as delay gastric emptying, influence regulation of appetite, and induce weight loss[143].

GLP-1 receptors (GLP-1 R) are widely distributed throughout the body. GLP-1 R's are highly expressed in the pancreas but have also been reported in the lung, stomach, intestine, kidney, heart, brain, retina, and immune system[144–147]. GLP-1 receptors have been found within the heart in the myocytes of the sinoatrial node, endothelium, coronary arteries, and smooth muscle cells of the heart[145,146,148]. Furthermore, GLP-1 R's have been found on the vagal nodose ganglia neurons[149].

Several GLP-1 receptor agonists (GLP-1 RA) have been developed in the last decades, including short- and long-acting formulations. Liraglutide is a long-acting GLP-1 RA, sharing 97% amino acid sequence identity with native GLP-1[150]. In contrast to native GLP-1, liraglutide is resistant to metabolization by dipeptidyl peptidase 4, and thus the half-life of liraglutide is about 13 hours[151].

GLP-1 RA's have shown both cardioprotective[152,153154], renoprotective[154,155], neuroprotective[156–159] and anti-inflammatory effects[160]. In addition, GLP-1 RA's been associated with neurite outgrowth in sensory neurons, and in type 1 diabetic mice, the treatment improved epidermal sensory conduction, thermal sensation, and restored mechanical sensitivity[161]. In humans, however, GLP-1 RA's did not change intra-epidermal nerve fiber density[162], latencies of evoked potentials (nerve conduction)[160], or CAN measures[162,163].

2.3.1 OCULAR EFFECTS OF GLP-1 RA'S

In healthy eyes, GLP-1 R's have been shown in cells within the human retinal ganglion layer but not in eyes with PDR[144]. Other studies found GLP-Rs in microvascular endothelial cells within the human retina[164], as well as in the retinal pigment epithelium[165]. Although sparse, GLP-1 R's have also been found in the inner and outer nuclear layers[166], and GLP-1 has even been found to be locally produced within the retina[166].

Several outcome studies have found that treatment with GLP-1 was associated with a progression in DR, although these changes could potentially be attributed to rapid decreases in blood glucose levels[143,167,168]. In animal studies, topically applied liraglutide has been shown to prevent diabetic retinal neurodegeneration and vascular leakage through inhibition of endoplasmic reticulum- and oxidative stress as well as through anti-apoptotic, anti-inflammatory, and anti-VEGF effects[31,166,169,170]. Similar effects have been found for semaglutide[171]. Furthermore, GLP-1 RA's have been shown to protect the blood-retinal barrier against diabetes-induced damage[172,173]. In addition, even liraglutide-induced retinal neuro regenerative effects were found[31].

Hernandez et al. investigated both topical and systemic treatment with liraglutide, and systemic treatment was shown to prevent retinal neurodegeneration in diabetic mice[166]. Furthermore, both topical and systemic treatment prevented abnormalities in electroretinography recordings[166]. In addition, GLP-1 RA's may also have potentially beneficial neuroprotective effects in individuals with glaucoma[174].

To the best of our knowledge, it remains unknown if such effects also apply to humans with long-term type 1 diabetes.

In study II, we investigated the effects of 26 of weeks treatment with liraglutide on the retinal nerve fiber layer thickness in individuals with long-term type 1 diabetes and DSPN.

2.3.2 GLP-1 RA'S AND CARDIOVASCULAR AUTONOMIC FUNCTION

GLP-1 RA's has been shown to reduce major cardiovascular events[152,175], arteriosclerosis[176], endothelial dysfunction, inflammation, oxidative stress[177], improve lipid profiles[178,179], as well as decrease body weight and blood-pressure[153,176,180]. A recent meta-analysis of eight cardiovascular outcome trials showed that treatment with GLP-1 RA's was associated with a 13% reduction in risk of cardiovascular death,

a 16% reduction in nonfatal stroke, and a 10% reduction in heart failure hospitalization as well as a 12% reduction in all-cause mortality[154]. Such improvement can be obtained by reducing the above-mentioned risk-factors, however, it could also have a direct effect on the heart.

GLP-1 RA's have consistently been shown to increase heart rate in type 1-[181] and type-2 diabetes[152,182–189], and in a recent meta-analysis, treatment with liraglutide increased heart rate by 2.7 bpm [190], although increases up 6.4 bpm has been reported[191]. The underlying mechanism(s) are undetermined, but several mechanisms have been proposed. These include GLP-1 RA-induced activation of the sinoatrial node[184], GLP-1 RA-induced increases in sympathetic tone[192], GLP-1 RA-induced decreases in parasympathetic tone[193] or through activation of baroreceptors in response to a reduction in systemic vascular resistance[194]. Furthermore, central mechanisms may be involved, as GLP-1 RA's has been shown to activate sympathetic preganglionic neurons in the brainstem[195–197].

Although a recent meta-analysis concluded that GLP-1 RA's does not influence HRV measures[198], several studies have found GLP-1 RA-induced alteration in HRV measures (see Table 1).

In study IV, we investigated the effect of 26 weeks of treatment with liraglutide on heart rate and HRV in individuals with long-term type 1 diabetes and DSPN.

2.3.3 CENTRAL EFFECTS OF GLP-1 RA'S

In humans and rats, GLP-1 R's have been found in the cerebral cortex, thalamus, hypothalamus, medulla oblongata, hippocampus, globus pallidum, and caudate-putamen[197,199,200]. GLP-1 has been shown to be synthesized by neurons in the nucleus tractus solitarius, and GLP-1 R's have been found in both nucleus tractus solitarius and the paraventricular nucleus, both involved in the regulation of autonomic control[197,201,202]. In mice, GLP-1 RA has been found to inhibit neurotransmission to cardiac vagal neurons in the nucleus ambiguus[193].

Neuroprotective effects of GLP-1 RA's have been shown in diseases such as diabetes, Alzheimer's, and Parkinson's disease[159,203], primarily through inhibition of oxidative stress, inflammation, and apoptosis[156,204–208]. In addition, GLP-1 RA's (e.g., liraglutide) reduces systemic inflammation, increases synaptic plasticity, and stimulates neurogenesis[158,204,209,210]. Furthermore, GLP-1 is also involved in memory, learning as well as reward behavior[201]

Moreover, GLP-1 RA's has been shown to reduce the risk of stroke[175], and in animal stroke models, to reduce the volume of infarction, oxidative stress, neuroinflammation, excitotoxicity, apoptosis, blood-brain barrier leakage as well as increase neurogenesis, neuroplasticity, angiogenesis, and brain perfusion[211].

Study	Type	Subjects	Treatment	Dosage	Duration	HR	SBP	DBP	SDNN	SDANN	SDNNI	RMSSD	TP	VLF	LF	HF	LF/HF
Jaiswal et al. 2015[154]	OL. RCT	T2DM	Exenatide (n=22)	10 µg once daily	18 M	→			→			→					→
Sivalingam et al. 2022[170]	DB. RCT	T1DM	Liraglutide (n=43)	1.8 mg once daily	26 W	↑			→			→			→	→	
Brock et al 2019.[152]	DB. RCT	T1DM	Liraglutide (n=19)	1.8 mg once daily	26 W	→	→	→	→	→	→	→	→	→	→	→	→
Hansen et al. 2019[155]	DB. RCT	T1DM	Liraglutide (n=50)	1.8 mg once daily	24 W	↑			→						→	→	→
Cacciatori et al. 2018[180]	OBS.	T2DM	Exenatide ER (n=28)	2 mg once weekly	6 M	↑	↓	↓					→		↓	→	↓
Hara et al. 2015[166]	OBS.	T2DM	Liraglutide (n=7)	0.9 mg once daily	24 W	↑	↓	→	↓						↓	↓	→
Nystrøm et al. 2019[181]	OL. RCT	T2DM	Liraglutide (n=33)	1.8 mg one daily	18 W	↑			→	→	→	→	→	→	→	→	
van Ruiten et al. 2022[182]	DB. RCT	T2DM	Exenatide (n=17)	10 µg twice daily	16 W	→	→	→	↓			→					→
Kumarathurai et al. 2017[183]	DB. RCT	T2DM	Liragltide (n=30)	1.8 mg one daily	12 W	↑	→	→	↓	↓	↓	↓	↓		↓	↓	→
Smits et al. 2017[184]	DB. RCT	T2DM	Liraglutide (n=19)	1.8 mg once daily	12 W	↑	↓	→	→			→					→
Nakatani et al. 2017[165]	OL. RCT	T2DM	Liraglutide (n=30?)	0.9 mg once daily	4 W?	↑											↑
Nakatani et al. 2016[165]	OL. RCT	T2DM	Lixisenatide (n=30?)	20 µg once daily	4 W?	↑											→

Table 1: Overview of studies investigating the effect of GLP-1 RA on HRV parameters in individuals with T1DM and T2DM. DB; double-blinded, RCT; randomized (placebo) controlled trial, OL; open-label, OBS; observational study, ER; extended release, HR; heart rate, SBP; systolic blood pressure, DBP; diastolic blood pressure, SDNN; standard deviation of NN intervals, SDANN; standard deviation of all NN intervals for each 5-minute segment of a 24 hour-recording, SDNNI; mean of the standard deviation of all NN intervals for each 5-minute segment of a 24 hour-recording, RMSSD; root mean square of successive differences in NN intervals, TP; total power, VLF; very low-frequency, LF; low-frequency, HF; high-frequency, LF/HF; ratio.

3 CLINICAL ASSESSMENTS

3.1 ASSESSING DIABETIC RETINOPATHY

Diabetic retinopathy can be assessed visually, either by fundoscopy or by assessing fundus photographs.
Characteristics of diabetic retinopathy include intraretinal hemorrhages (dot-blot or flame-shaped), hard exudates, cotton wool spots, venous beading, and the presence of intraretinal microvascular abnormalities or neovascularization[45]. Furthermore, diabetic macular edema may become present as the disease progresses.
Based on the number and/or presence of these characteristics, DR can be staged as NPDR (mild, moderate, or severe, based on the number of characteristics) or PDR (presence of neovascularization)[45].

For studies I and II, evaluations of DR stage were performed by the same ophthalmologist, and photos were taken by the same photographer to avoid inter-observational bias. PDR was defined by the presence of neovascularization(s), previous or actual, assessed by visually inspecting fundus photos and/or OCT scans. Based on the absence or presence (actual or previous) of neovascularization, participants were divided into an NPDR or PDR group, respectively.

3.2 ASSESSING PALPEBRAL FISSURE HEIGHT

Palpebral fissure height (PFH) is defined as the height between the eyelid margins (upper and lower) in the pupillary plane in a primary gaze position (see Fig. 3) and is, in part, determined by the functionality of the sympathetically innervated tarsal muscles.
In healthy individuals, one study found the average PFH to be 9.9 mm[212], and phenylephrine has been shown cause increases in PFH between 1.2-1.6 mm[113–115]. In insulin-dependent diabetes, the average PFH was 8.3 mm, and the decrease was associated with the disease duration and severity of DR[212]. As such, a reduction in PFH in diabetes may, amongst other causes of ptosis, be a very visual manifestation of diabetic autonomic neuropathy. Thus, the PFH response to phenylephrine could potentially act as a surrogate measure of sympathetic nerve function.

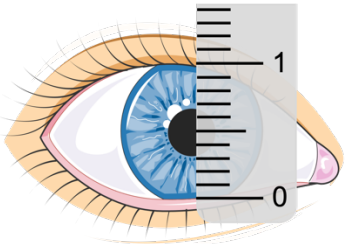

Figure 3. Assessment of palpebral fissure height (PFH).

In study I, measurements of PFH were performed on both the unstimulated left eye and phenylephrine-stimulated right eye. Measurements for both eyes were obtained from a single standardized frontal photo 15-minutes after topical application of phenylephrine hydrochloride 10% to the right eye. The difference between the two eyes (ΔPFH) was used for further analysis.

3.3 ASSESSING THE RETINAL NERVE FIBER LAYER THICKNESS

Optical coherence tomography (OCT) is a non-invasive imaging technique that allows topologic and visualization of the microstructure of individual retinal layers as well as the microvasculature (OCT-Angio). OCT is used to diagnose and manage diseases such as diabetes and glaucoma. OCT allows for the assessment of retinal nerve fiber loss, and thinning of the retinal nerve fiber layer has been associated with the severity of DPN[65].

In study II, assessments of peripapillary retinal nerve fiber layers thickness were obtained using Topcon 3D OCT-200 (see Fig. 4). Scans were fixated in the center of the optic disc, and 1024 scans were taken in a circle of 3.4 mm in diameter. RNFL thickness of the inferior, superior, nasal, and temporal quadrants was obtained at baseline and after 26 weeks of treatment with liraglutide.

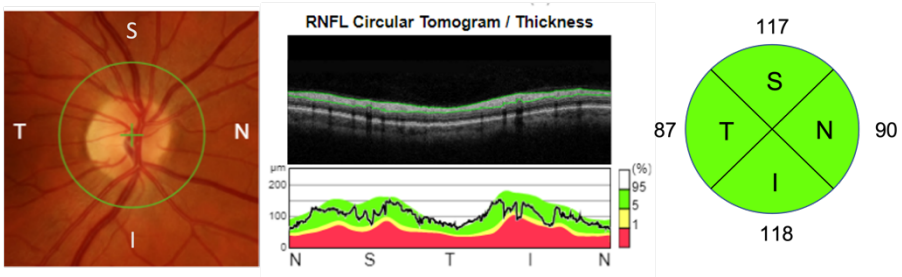

Figure 4: Assessment of peripapillary retinal nerve fiber layer (RNFL) thickness using Topcon 3D OCT. T = temporal quadrant, S = superior quadrant, I = inferior quadrant, and N = nasal quadrant.

3.4 ASSESSING DISTAL SYMMETRIC POLYNEUROPATHY

According to the Toronto Diabetic Neuropathy group[3], DPN can be classified as 1) possible DPN (presence of symptoms, such as decreased sensation or signs, such as decreased ankle reflexes), 2) probable DPN (two or more symptoms or signs), and 3) confirmed DPN (abnormal nerve conduction and a symptom or sign of DPN),

Furthermore, the quality and severity of possible neuropathic pain can be assessed by the validated Michigan neuropathy screening instrument[213,214]. Several other self-reported questionnaires, e.g., the McGill Pain Questionnaire exist[75].

Several experimental methods of assessing DSPN exist, including quantitative sensory testing, vibration threshold, electrophysiological peripheral nerve testing, and intra-epidermal nerve density in skin biopsies[75]. Ocular measures, such as small fiber nerve lengths assessed with corneal confocal microscopy, have also been used, and reduced retinal nerve fiber layer thickness can be used as surrogate markers of DSPN[75]. Whereas quantitative sensory testing, skin biopsies, and corneal confocal microscopy can be used to assess the presence of small-fiber pathology[215,216], investigations of peripheral nerve testing include nerve conduction velocity and amplitudes, and, thus, assess the presence of neuropathy in large myelinated nerve fibers[217].

In studies III and IV, peripheral nerve testing with standardized electrophysiological measures were obtained by the Department of Neurophysiology, Aalborg University Hospital.

3.5 ASSESSING CARDIOVASCULAR AUTONOMIC NEUROPATHY

The function of the ANS can be assessed by several methods, including investigation of e.g., cardiovascular, sudomotor, and pupillomotor[16].

Cardiovascular autonomic reflex tests (CARTs) represent the gold standard for CAN testing. The CAN Subcommittee of the Toronto Consensus recommends measuring changes in heart rate and blood pressure to different provocative physiological maneuvers. These maneuvers include the deep breathing test, lying-to-standing test, the Valsalva maneuver, and the blood pressure response to standing[218]. The Valsalva maneuver is, however, not advisable in patients with proliferative retinopathy due to an increased risk of retinal hemorrhage.

According to the extent of neuropathy, CAN can (☺) be classified into subclinical-, early- and advanced stages. The subclinical stage is characterized by abnormal sympathovagal balance and decreased baroreflex sensitivity but with no symptoms hereof. In the early stage, by the presence of tachycardia at rest and one abnormal cardiovascular autonomic reflex test (CARTs), and in advanced stages by exercise intolerance, orthostatic hypotension, two or more abnormal CARTs, cardiomyopathy, and asymptomatic myocardial ischemia[9,218].

Several methods for the investigation of cardiac autonomic dysfunction have been proposed in clinical research. The most sensitive and specific tests are heart rate variability (HRV), cardiac sensitivity to the baroreflex (CBS), muscle sympathetic nerve activity, measurements of plasma catecholamines, and heart sympathetic imaging[102]. HRV is widely used in clinical research and is a non-invasive method for providing information on autonomic modulation of the cardiovascular system, evaluated using time- and frequency domain indices of heart rate variability[19]. Recently HRV as shown to be a reliable measure of CAN in type 1 diabetes[219].

3.5.1 HEART RATE VARIABILITY

Whereas heart rate is a measure of heart beats per minute, HRV is defined by the fluctuations in time between consecutive heartbeats[19] and reflects the complex coupling between the brain and the heart. A healthy heart rate is non-chronometrical (high HRV), allowing for adaption to internal and external stimuli to maintain homeostasis. In contrast, a chronometrical, non-adaptive heart rate (low HRV) that barely responds to stress, exercise, sleep, etc., indicates cardiac denervation[7,10] and has been shown to predict cardiovascular events[220]. When compared to healthy, individuals with T1DM are known to have reduced HRV parameters[84,221,222].

HRV is widely used as a non-invasive assessment to characterize autonomic modulation of the heart[223]. In addition to the autonomic modulation, short-term HRV recordings (～5 min.) are influenced by respiratory-driven regulation of the heart rate, CBS, and rhythmic variations in vascular tone[100]. However, long-term HRV recordings (24-hour) are influenced by, e.g., circadian rhythms, body temperature, the renin-angiotensin system, and by metabolism[224].

HRV can be analyzed using both *time-domain-derived* HRV parameters and *frequency-derived* HRV parameters[19] (see Table 2).

HRV parameter	Units	Definition
Time-domain		
Mean RR	ms	Mean of all RR-intervals
SDNN	ms	Standard deviation of all NN-intervals (normal RR-intervals)
SDANN	ms	Standard deviation of the average all NN-intervals for each 5 minute segment of a 24 hr HRV recording
SDNNI	ms	Mean of the standard deviation of the average all NN-intervals for each 5 minute segment of a 24 hr HRV recording
RMSSD	ms	Root mean square of successive RR interval differeneces
Frequency-domain		
VLF	ms^2	Absolute power of very-low-frequency band (0.0033-0.04 Hz)
LF	ms^2	Absolute power of low-frequency band (0.04-0.15 Hz)
HF	ms^2	Absolute power of high-frequency band (0.15-0.4 Hz)
LF/HF	%	LF/HF ratio

Table 2: Overview of heart rate variability parameters used in this thesis.

Time-domain derived HRV parameters represent the total beat-to-beat variability over time. SDNN is considered an estimate of the overall adaptability of the heart, reflecting both sympathetic and parasympathetic tone[100,224], and individuals with lower SDNN values have a higher risk of mortality than those with higher values[225]. RMSSD is generally believed to reflect parasympathetic tone[19].

Frequency-domain derived parameters, however, reflect the distribution of power (signal energy) across different frequency bands (see Fig. 5) and can be assessed using power spectral analysis[19]; VLF is believed to be intrinsically generated by the heart, and the amplitude and frequency are modulated by efferent sympathetic activity[224]. LF content is believed to reflect both sympathetic and parasympathetic activity[19,100], although it has also been proposed to reflect baroreflex function. Some authors use a LF cut-off value of 0.1 Hz, as the sympathetic influence may not produce rhythms above 0.1 Hz however, parasympathetic influence may influence rhythms down to 0.05 Hz[100]. HF content is considered to reflect the parasympathetic activity and corresponds to respiratory-driven influence on heart rate[100]. LF/HF is generally believed to represent the sympathovagal balance[19], and a low LF/HF ratio reflects parasympathetic dominance[100]. The underlying influence(s) of both VLF, LF, and LF/HF ratio are, however, controversial and subject to great scientific debate[226–229].

In addition, time-domain parameters decrease with age, and HRV differences related to sex and respiration pattern have been shown[230,231].

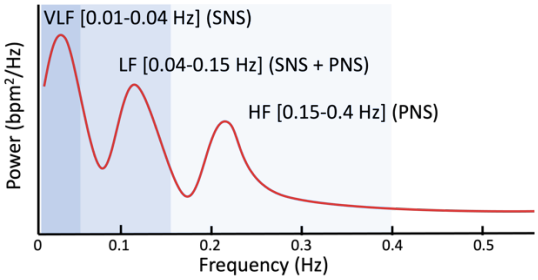

Figure 5: Visualizations of frequency-derived HRV parameters used in this thesis. SNS = sympathetic nervous system influence, PNS = parasympathetic nervous system influence.

Although 24-hour HRV recordings are considered the "gold standard" for HRV recordings, shorter, more clinically applicable HRV recordings, such as short-term (∼5 min) and ultra-short-term (<5 min), can be used to assess HRV[100]. Although no standardization of ultra-short-term measurement protocols exists, several authors have investigated the reliability and validity of ultra-short-term measurements. 10 seconds seems to be enough for valid heart rate measures; however, proposed minimum recording lengths for other parameters differ; SDNN (60-240 s.), RMSSD (10-120 s.), VLF (50-270 s.), LF (50-90 s.), HF (20-120 s.) and LF/HF (20-180 s.)[232–236].

According to the Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, a minimum of 1 and 2 minutes are required to address the LF and HF components respectively[19].

In studies III and IV, measurements of HRV were obtained using *Lifecard® CF* (Del Mar Reynolds Medical, Spacelabs Healthcare Inc.). Electrocardiographic recordings were obtained in accordance with internationally recommended standards[19], and visually inspected for artifacts, arrhythmias, ectopic beats, etc. Time-domain analysis was performed using *Pathfinder®* (Del Mar Reynolds Medical, Spacelabs Healthcare Inc.), and frequency-domain analyses was performed using *HRV Tools* (Del Mar Reynolds Medical, Spacelabs Healthcare Inc.) using Fast Fourier transformation. The derived time-domain parameters were mean RR, SDNN, SDANN, SDNNI, and RMSSD, and the following frequency-domain derived parameters were VLF, LF, HF, and the LF/HF ratio, derived with cut-off values in accordance with *Shaffer et al.*[100]. The assessment of dynamic changes in HRV in response to cold tonic pain included the division of electrocardiographic measures into two-minute segments. These segments were further grouped into pre-test (4 min. before exposure), initial response (2 min. during, and 2 min. post-exposure), sustained response (4-10 min. post-

exposure), and recovery phase (10-16 min. post-exposure). Differences between baseline and follow-up (26 weeks) were denoted as Δ.

3.5.2 CARDIAC SENSITIVITY TO THE BAROREFLEX

CBS refers to the ability of baroceptors to respond to changes in blood pressure and maintain blood pressure homeostasis. Baroreceptors, primarily located in the aortic arch and carotid sinus, are specialized nerve cells that sense changes in arterial blood pressure and work as a negative feedback system. This information is transmitted to the brainstem, which initiates an autonomic response.
Assessing CBS requires continuous and synchronized measures of heart rate and blood pressure, and CBS is widely used to quantify the parasympathetic response to changes in blood pressure[102]. In case of a sudden increase in blood pressure, an increase in parasympathetic activity and a decrease in sympathetic activity occurs, resulting in a reduction in heart rate and blood pressure[102]. A sudden decrease in blood pressure will result in the opposite response. For example, standing increases sympathetic activity and reduces CBS[237], whereas, e.g., deep-breathing increases parasympathetic activity and CBS[238].

CBS is known to be reduced in individuals with type 1 diabetes[239], even in individuals without clinical symptoms[240]. Thus measurements of CBS may provide early detection of autonomic dysfunction in individuals with diabetes[241].

In study III, CBS was assessed using Neuroscope® (Medifit Instruments, Enfield, Essex, UK)

3.5.3 CARDIAC VAGAL TONE

Cardiac vagal tone (CVT) is a validated, non-invasive, real-time measure of the brainstem's efferent parasympathetic influence on the heart[242]. Whereas CBS represents the afferent influence on the heart, CVT represents the efferent parasympathetic influence[243].

Following an increase in blood pressure, baroreceptors terminating in the nucleus tractus solitarius are activated, resulting in a decrease in sympathetic output in the RVLM and an increase in parasympathetic output from the nucleus ambiguous and VDMN, consequently decreasing heart rate[244]. As previously mentioned, the parasympathetic response is much quicker than that of the sympathetic, and thus short-term changes in heart rate are primarily attributed to changes in parasympathetic activity[100]. The relationship between baroceptor-driven changes in heart rate (RR

intervals) and parasympathetic output can be measured on a linear vagal scale, with values above zero representing efferent vagal tone[242]. The higher the values, the healthier the parasympathetic function[242,243].

In individuals with type 1 diabetes, CVT has been shown to be decreased and to correlate with 24-hour HRV measures[245]. Furthermore, CVT has been proposed as a screening tool for CAN in type 1 diabetes[246].

In study III, CVT was assessed using Neuroscope® (Medifit Instruments, Enfield, Essex, UK)

4 HYPOTHESES AND AIMS

The overall aims of this Ph.D. thesis were to investigate the effect of long-term type 1 diabetes on autonomic nerve function and the effect of liraglutide on the neurocardiac regulation and on the retinal nerve fiber layer thickness.

The hypothesis and aims for study I-IV were as follows:

STUDY I

Hypothesis:

- Long-term type 1 diabetes causes neuropathy to the peripheral sympathetic nerve fibers innervating the superior and inferior tarsal muscles[32].

Aims:

1) To measure the sympathetic paresis by assessing eye-to-eye differences in palpebral fissure height (ΔPFH) in response to topical application of 10% phenylephrine into one eye in individuals with long-term type 1 diabetes and distal symmetrical polyneuropathy[32].

2) To investigate possible associations between changes in palpebral fissure height (ΔPFH) and the severity of diabetic retinopathy, disease duration, and autonomic function[32].

3) To investigate the association between autonomic function and the severity of diabetic retinopathy[32].

STUDY II

Hypothesis:

- Treatment with liraglutide increases peripapillary retinal nerve fiber layer thickness by inducing nerve regeneration and restoring vascularization[33].

Aim:

1) To assess differences in retinal nerve fiber layer thickness between individuals with long-term type 1 diabetes, randomized to treatment with liraglutide or placebo[33].

2) To assess differences in retinal nerve fiber layer thickness between individuals with proliferative- and non-proliferative diabetic retinopathy[33].

STUDY III

Hypothesis:

- Exposure to tonic cold pain causes enhanced sympathetic drive and attenuates the dynamic sympatico-vagal response in participants with long-term type 1 diabetes when compared to healthy controls.

Aims:

1) To assess the dynamic heart rate variability response following tonic cold pain exposure in healthy individuals and in individuals with type 1 diabetes and distal symmetrical polyneuropathy.

2) To assess the differences in 24-hour heart rate variability measures, cardiac sensitivity to the baroreflex, and cardiac vagal tone between the two groups.

STUDY IV

Hypothesis:

- Treatment with liraglutide lead to increased heart rate and sympathetic drive in situational dynamic heart rate variability measures, evident as increased low-frequency and very low-frequency content

Aims:

1) To assess the heart rate variability as a proxy for neurocardiac regulation in response to tonic cold pain exposure following 26 weeks of treatment with liraglutide and placebo in individuals with long-term type 1 diabetes

2) To investigate dynamic differences in heart rate variability responses between the liraglutide-treated and placebo-treated group, based on ultra-short epochs before, during, and after exposure to tonic cold pain in order to investigate the autonomic-regulated mechanisms underlying increased heart rate during liraglutide treatment.

5 MATERIALS AND METHODS

This thesis is based on data collected at Mech-Sense, Department of Gastroenterology and Hepatology, Aalborg University Hospital. Data was collected as part of the TODINELI study (clinicaltrials.gov: NCT02138045), approved by The Scientific Ethical Committee of The North Denmark Region (N-20130077). The study was performed in accordance with the International Council for Harmonization guideline for Good Clinical Practice and the Declaration of Helsinki.

A total of 48 participants (10 female/38 male, mean age 50 years) with long-term type 1 diabetes (mean duration 32 years) and confirmed distal symmetrical polyneuropathy were enrolled in the main study. The studies were conducted in individuals with long-term type 1 diabetes to assess the anti-inflammatory and neuroprotective effects, independently of anti-hyperglycemic effects. The studies included in this thesis are based on secondary analysis of data collected as part of the TODINELI study. Inclusion and exclusion criteria can be found elsewhere[160].

Table 3 and Figure 6 provide an overview of the studies included in this thesis. Descriptions of the different clinical assessments used for these studies can be found in chapter 2: *Clinical assessments*. Description of cold tonic pain, under "*Cold pressor test*" in the "*Pharmacologic and physiologic modulation of the autonomic nervous system*"-section.

Study	Design	Subjects	Intervention	Dosage	Duration	Stimulation	Assessments
I	EX. C-S.	37 T1DM + DSPN				Ogtt. Phenylephrine 10%	PFH DR stage HRV BP
II	DB. RCT	37 T1DM + DSPN: Liraglutide (n=17) Placebo (n=20)	SC. Liraglutide	1.8 mg/day	26 weeks		RNFL DR stage HbA1c
III	EX. C-S.	48 T1DM + DSPN 21 healthy controls				Cold tonic pain exposure	HRV CBS CVT NCV Pain ratings MNSI BP Blood samples
IV	DB. RCT	32 T1DM + DSPN: Liraglutide (n=17) Placebo (n=15)	SC. Liraglutide	1.8 mg/day	26 weeks	Cold tonic pain exposure	HRV NCV Pain ratings MNSI BP Blood samples

Table 3: Overview of study design, subjects, treatment, stimulations, and assessments in the studies included in this thesis: EX. = explorative study, C-S. = cross-sectional study, DB = double blinded, RCT = randomized controlled trial, T1DM = type 1 diabetes, DPSN = distal symmetrical polyneuropathy, Ogtt. = oculogutta (eye drops), SC. = subcutaneous, PFH = palpebral fissure height, DR = diabetic retinopathy stage, HRV = heart rate variability, CBS = cardiac sensitivity to the baroreflex, CVT = cardiac vagal tone, HbA1c = glycosylated hemoglobin, NCV = nerve conduction velocity, MNSI = Michigan neuropathy screening instrument, BP = blood pressure, pain ratings; eleven point modified Likert scale, with 0 = no pain and 10 = worst imaginable pain.

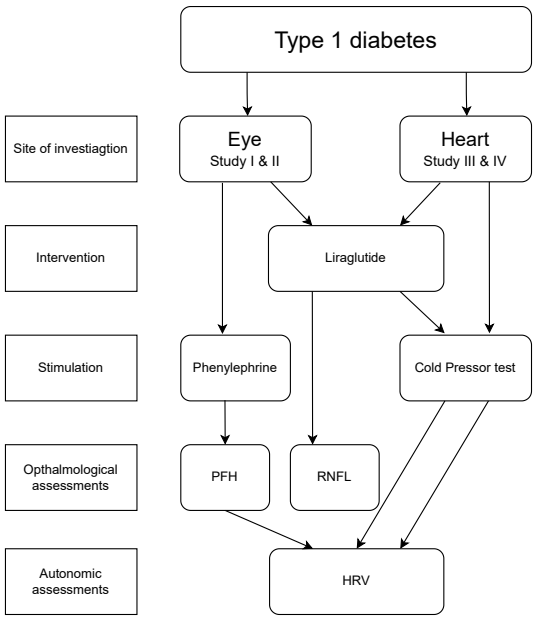

Figure 6: Graphical overview of studies included in this thesis. PFH = palpebral fissure height, RNFL = retinal nerve fiber layer, HRV = heart rate variability.

STATISTICAL ANALYSIS

For all studies, Epidata software® (The Epidata Association, Odense, Denmark) was used to manage data. In studies I and II, statistical analysis was performed using SPSS® v. 25.0.0 (IBM, Armonk, NY, and for studies III and IV, Stata® v. 17.0 (StataCorp LLC, Texas, USA) was used.

All data was tested for normality by inspecting QQ-plots, histograms, and by Shapiro-Wilk testing. Eligible data was compared using t-tests and categorical data by Spearman's rank correlation. $P<0.05$ was considered statistically significant.

STUDY I

Differences in the palpebral fissure between the phenylephrine-stimulated and non-stimulated eye (ΔPFH) were compared using independent samples t-test. Pearson's correlation coefficient was used to test for associations between ΔPFH and heart rate variability parameters. One-way analysis of variance (ANOVA) was used to test for associations between ΔPFH and the presence of NPDR or PDR[32].

STUDY II

Differences in RNFL between baseline and follow-up (Δ) were compared between groups using a two-sample t-test. Linear mixed model analysis with treatment and DR stage as independent factors were used to calculate fixed effect estimates in Δ-values. Differences in ΔHbA1c between groups were compared using independent samples t-test[33].

STUDY III

Differences in situational HRV parameters between baseline visit and follow-up visit (Δ) between groups were compared using repeated measures-ANOVA, followed by linear comparison analysis, and crude data were subsequently adjusted for BMI and age. To accommodate for violations in normality, bootstrapping with 1000 replications was performed.

STUDY IV

Differences in mean situational heart rate between baseline and follow-up for each group were compared using t-tests. Differences in heart rate and HRV between groups in response to tonic cold pain were analyzed using repeated measures-ANOVA followed by linear comparison, and crude data were adjusted for BMI and age, as well as changes in body weight between baseline and follow-up (Δkg). Correlations between mean RR/HR and HRV parameters were performed using the Pearson correlation coefficient. Pain ratings were compared using independent t-tests.

6 MAIN FINDINGS

STUDY I

Main results:

- Following the topical application of phenylephrine, the mean difference in palpebral fissure height (ΔPFH) was 1.02 ± 0.29 mm.[32].
- ΔPFH showed 1) associations with the severity of diabetic retinopathy, 2) associations with disease duration, and 3) positive associations with the low-frequency HRV content[32].
- Low ΔPFH was associated with lower HRV parameters (total power, VLF, and LF)[32].

STUDY II

Main results:

- Liraglutide treatment did not alter mean peripapillary, inferior, superior, nasal or temporal retinal nerve fiber layer thickness[33].
- Furthermore, no differences were found between the PDR or NPDR groups or associations with changes in HbA1c[33].

STUDY III

Main results:

- Following exposure to tonic cold pain, dynamic heart rate variability responses revealed adynamic patterns in the T1DM group, indicating impaired neurocardiac flexibility.
- 24-hour time- and frequency domain parameters of heart rate variability and cardiac sensitivity to the baroreflex were lower in the T1DM group, indicating diabetes-induced dysautonomia. No significant differences between groups were found for cardiac vagal tone.

STUDY IV

Main results:

- 26-week treatment with liraglutide increased overall situational heart rate by 3.6 beats per minute.
- Exposure to tonic cold pain revealed liraglutide-induced increases in SDNN, as well as decreases in VLF and LF when compared to placebo. No differences were found in parasympathetic measures of RMSSD and HF.

7 DISCUSSION

THE USE OF PALPEBRAL FISSURE HEIGHT AS A MARKER OF AUTONOMIC FUNCTION (STUDY I)

In summary, we found that stimulation with phenylephrine increased palpebral fissure height by an average of 1.02 mm compared to the untreated eye. Furthermore, we showed that ΔPFH was lower with increasing duration of diabetes, was lower in individuals with PDR, and that lower ΔPFH was associated with lower total power, VLF, and LF frequency-domain parameters of HRV. Moreover, we found that almost all HRV parameters were lower in the PDR group when compared to the NPDR group. To our knowledge, this is the first time this simple measure has been utilized in individuals with T1DM and DSPN.

A significant difference in PFH between the treated and untreated eyes (ΔPFH) was found, similar to the results reported by *Bastiaensen et al*.[212]. Compared to the PFH response found in our study, healthy individuals exhibit a response of up to 70% higher[113–115]. This shows that within our cohort, a significantly decreased excitability of the tarsal muscles to phenylephrine was present, suggesting dysfunction of the sympathetic nerves innervating these muscles. Additionally, we observed a more pronounced decrease in sympathetic capacity in the PDR group compared to the NPDR group, indicating severe microvascular complications associated with the duration of diabetes.

Moreover, our study found a positive correlation between ΔPFH and heart rate variability frequency-domain parameters VLF and LF, indicative of systemic sympathetic dysfunction. However, the interpretation of LF heart rate variability is controversial, as it may reflect both sympathetic and parasympathetic content. The absence of associations between ΔPFH and RMSSD, HF, and the LF/HF ratio, which reflect parasympathetic modulation, further support this finding. Thus, our results suggest that PFH could be a simple, easily applicable marker of sympathetic dysfunction.

Furthermore, we found that HRV measures were decreased in the PDR group compared to the NPDR group, which is in line with findings in other studies[247,248].

THE EFFECT OF LIRAGLUTIDE ON THE RETINAL NERVE FIBER LAYER THICKNESS (STUDY II)

Although GLP-1 RA's have been found to prevent thinning of RNFL and even promote neuro-regenerative effects of retinal cells[31,166,170], we found that 26-week treatment with liraglutide did not change the RNFL thickness in individuals with long-term T1DM and DSPN. Nor did it change RNFL thickness in the NPDR or PDR groups. This may, to some degree, be attributed to the loss of GLP-1 R-positive retinal cells seen in more advanced stages of DR[166,249]. Moreover, the changes in RNFL may not accurately represent the neuroregeneration, as increases in cells within the retinal ganglion layer may not necessarily reflect changes in the RNFL thickness. The same applies to GLP-1 RA-included prevention of apoptosis in inner- and outer nuclear layers[31]. Increases in RNFL may also occur due to edema within this layer and could mistakenly be interpreted as nerve regeneration. Thus, the assessment of RNFL using OCT is associated with several pitfalls. Therefore, measurements of other retinal layers, including the retinal ganglion cell layer, or even electrophysiologic tests, such as electroretinogram or visually evoked potentials, may allow for a more optimal evaluation of the possible beneficial effects of GLP-1 RA on the retina.

Although *Hernandez et al.* investigated the effects of systemically and topically administered GLP-1 RA and found no differences, most other studies have investigated the effects of topical or intravitreal administration of GLP-RA[31,166,171,250–252]. To our knowledge, differences in retinal drug concentration between systemically and topically administrated GLP-1RA have not been compared. Thus, it remains unknown if different administration methods result in different local drug concentrations.

Moreover, in our cohort with long-term T1DM, the retinal neurodegeneration, and microvascular manifestations may be too pronounced to be reversed. Thus, it would be of great interest to investigate the effect of GLP-1 RA's in earlier stages of DR or with a more extended treatment duration.

THE EFFECT OF LONG-TERM TYPE 1 DIABETES ON HEART RATE VARIABILITY PARAMETERS (STUDY III)

In study III, we investigated the effect of long-term type 1 diabetes on neurocardiac regulation during 24-hour HRV and in response to tonic cold pain exposure. We found that, compared to healthy, and when adjusting for BMI and age, the T1DM group had lower 24-hour HRV parameters, except for RMSSD and LF/HF. Moreover, CBS was lower in the T1DM group. These results indicate T1DM-induced dysautonomia, primarily related to sympathetic modulation, as parasympathetic measures (RMSSD and LF/HF) were not significantly different from healthy. The results are similar to the results found in other studies[84,221,222,253].

UTILIZING COLD TONIC PAIN EXPOSURE IN THE ASSESSMENT OF AUTONOMIC NEUROPATHY (STUDY III)

Cold tonic pain exposure was used in studies III and IV as an external stimulus, allowing for interpreting dynamic physiological stress-related responses in HRV. The concept of such challenge testing is similar to stress tests used in assessing asthma and to the treadmill test used in evaluating stress-related cardiac performance. By utilization of 2 min. ultra-short-term HRV measures, we investigated HRV in the initial, sustained, and recovery phases and compared these to pre-test measures. This allowed for assessing dynamic adaptive capacity in response to tonic cold pain exposure. In neither study III nor IV, differences were found in experienced pain between the T1DM group and healthy participants or between individuals with T1DM treated with liraglutide vs. placebo. In study III, the diabetes group experienced less unpleasantness, suggesting sensory deficits were present to some degree.

In study III, tonic cold pain exposure resulted in a decreased mean RR and an increased SDNN and RMSSD in healthy controls. Moreover, both LF and HF parameters increased, indicating increased autonomic modulation. Other studies have found both increases[254] and decreases[255] in RMSSD in healthy participants, as well as increases in both LF and HF[120,254,256]. One study found that neither SDNN nor RMSSD changed[257], and LF[254] and HF[258] have also been shown to decrease. Parasympathetic modulation is known to decrease with increasing age[231], and as both age and methodologies differ between these studies, direct comparisons are difficult.

In the T1DM group, mean RR and RMSSD increased in the initial response, indicating an increase in sympathetic modulation. Moreover, LF and LF/HF decreased, indicating a simultaneous decrease in parasympathetic modulation. Compared to healthy controls, the T1DM group exhibited adynamic responses following tonic cold pain exposure, indicating an impaired stress-related dynamic autonomic adaptability. Both mean RR, SDNN, RMSSD, total power, and LF/HF were less adaptable than in healthy. When adjusting for BMI and age, LF/HF was no longer significant, indicating that these factors confounded this measure.

THE EFFECT OF LIRAGLUTIDE ON HEART RATE AND HEART RATE VARIABILITY IN RESPONSE TO CHALLENGES TESTING (STUDY IV)

In study IV, we aimed to investigate the effect of liraglutide on HRV parameters in response to cold tonic pain exposure in patients with T1DM and DSPN. *Brock et al.* investigated the effect of liraglutide within the same cohort and found that 24-hour HRV recordings did not differ significantly compared to placebo[160]. These findings are in line with a recent meta-analysis[198], although some authors have found that GLP-1 RA's cause decreases in SDNN, RMSSD, LF, and HF in individuals with T2DM[182,185].

Following 26 weeks of treatment with liraglutide, cold tonic exposure revealed consistent increases in HR and SDNN, as well as decreases in VLF and LF compared to placebo. These findings indicate that GLP-1 RA-induced alterations in autonomic cardiac modulation may be unmasked in response to physiological stress. Moreover, we found that when compared to placebo, liraglutide increased HR by 3.6 bpm, which is in line with previous findings in T1DM[259]. As previously mentioned, the underlying mechanism(s) are not fully understood but may involve direct activation of GLP-1 R's in the sinoatrial node, decreased parasympathetic activity, activation of baroreceptors in response to a reduction in systemic vascular resistance[194], increased sympathetic activity or even via direct activation of sympathetic neurons in the brainstem.

The increase in SDNN indicates that heart rate was not chronometric, suggesting that, although direct activation of GLP-1 R's in the sinoatrial node may, in part, influence the increase in HR, some degree of preserved autonomic modulatory capacity is present. This is in line with *Baggio et al.,* who found that, in *ex vivo* studies in mice, GLP-1 RA's did not increase heart rate by itself but required neuronal inputs to do so[260]. Furthermore, we found no liraglutide-induced alterations in RMSSD and HF, indicating that parasympathetic modulation was not altered by 26 weeks of treatment with liraglutide. These findings are in line with previous findings in 24-hour recordings[160,189,261], although decreases have also been found[182].

Moreover, treatment with liraglutide decreased the dynamic VLF and LF in response to cold tonic pain. As previously mentioned, the VLF is believed to be intrinsically generated by the heart, and the amplitude and frequency are modulated by efferent sympathetic activity[224]. VLF has also been proposed to reflect thermoregulatory and sympathetic content[7,14]. LF reflects both sympathetic and parasympathetic content, and as parasympathetic measures RMSSD and HF did not change, these changes are believed to be caused by alterations in sympathetic modulation. *Mendis et al.* found that the increase in HR following GLP-1 RA's could be explained by sympathetic activation in response to vasodilatation[194], and others have found both increases in cardiac output, epinephrine levels, and blood pressure further indicative of a sympathetic response[262]. This contrasts the findings of others, who found that HR increases were not attributed to changes in sympathetic modulation[263], and *Skov et al.,* who found that a single dose of liraglutide was not accompanied by increases in the release of catacholamines[264].

In rodents, liraglutide has been shown to be able to cross the blood-brain-barrier[265], and as GLP-1 R's are widespread within the central nervous system[197,199,200], central mechanisms may also be involved in GLP-1 RA-induced HR increases. In mice, Exendin-4 (GLP-1 RA) has been shown to depress inhibitory and excitatory vagal neurons in the nucleus ambiguus[193]. Moreover, GLP-1 R's have been

found in both the nucleus tractus solitarius and the paraventricular nucleus, which are involved in autonomic regulation[197,201,202]. Additionally, GLP-1 release within the spinal cord has been shown to increase the sympathetic modulation of the heart[266].

Taken together, the underlying mechanisms of GLP-1 RA-induced HR increases are complex, and more studies are needed to further elucidate the mechanism involved.

7.1 STRENGTHS AND LIMITATIONS

The participants enrolled in these studies all had long-term type 1 diabetes, confirmed DSPN, and many had orthostatic hypotension indicative of autonomic neuropathy. As such, we know that diabetic neuropathy was present. The studies were conducted in individuals with T1DM to assess proposed liraglutide-induced anti-inflammatory and neuroprotective effects independent of glycemic modulation.

STUDY I

Strengths:

- The primary advantage of this study is its capability to link a novel measure with established validated autonomic measures of heart rate variability[32].

Limitations:

- Firstly, we did not take a frontal photograph before the phenylephrine test to assess any pre-existing asymmetry. In primary gaze, PFH asymmetry has previously not been shown to be more frequent in one eye over the other[267]. Thus, we assume that any resulting asymmetry would be equally increased or decreased and would cancel out[32].
- Secondly, the external generalizability or validity of our findings to other types or stages of diabetes remains unclear and requires further investigation[32].
- Thirdly, our cohort with severe and longstanding neuropathy may not be representative of future cohorts with prolonged duration of diabetes, as guidelines now advocate for more strict glycemic control[32].
- Finally, validating the use of PFH against sympathetic content in HRV highlights the need to examine whether reductions in PFH are associated with cardiac autonomic reflex testing, most commonly used to diagnose CAN[32].

STUDY II

Strengths:

- To our knowledge, we are the first to examine the effect of 26 weeks of treatment with liraglutide on RNFL thickness in individuals with T1DM and confirmed DSPN[33].

Limitations:

- Firstly, the intervention period in this study lasted only 26 weeks, which is relatively short compared to the typical disease duration for DR. As a result, possible anti-inflammatory and neurotrophic effects of the intervention may not have been fully reflected in the RNFL thickness measurements. Given the nonlinear nature of DR progression, a follow-up period of at least five years would have been preferable to assess the changes between the treatment and control groups[33].
- Secondly, changes in RNFL thickness should be interpreted cautiously as several factors may result in changes in RNFL thickness[33].
- Thirdly, this study only included a small number of participants, and type 2 errors cannot be excluded. Therefore, the results of this study should be considered exploratory, aimed at assessing the effect of GLP-1 on retinal nerve fibers. Further studies are necessary to fully understand if long-term treatment with GLP-1 agonists can induce anti-inflammatory and neurotrophic effects within the human retina[33].

STUDY III

Strengths:

- To our knowledge, this is the first time that the dynamic alterations in HRV, in response to tonic cold pain exposure, have been investigated in a cohort of individuals with T1DM and DSPN and compared to healthy controls.

Limitations:

- Firstly, this study was conducted on individuals with long-term type 1 diabetes and verified DSPN. 90% of the participants had orthostatic hypotension, indicative of CAN, but CAN was not evaluated using cardiovascular autonomic reflex tests. Including individuals with T1DM with no or early signs of CAN could potentially reveal discrepancies in the autonomic response to tonic cold pain exposure in early disease stages.
- Secondly, assessments of the dynamic responses were based on 2-minute ultra-short-term HRV measurements, which are known to be vulnerable to physiological noise caused by movement, coughing, or the stress response

caused by the tonic cold pain exposure. However, data was calculated within predefined intervals, so we believed this might only represent a minor issue.

- Thirdly, the inclusion of Poincaré plot may have provided additional information on changes in heart rate variability measures however, this was not included in the study.
- Fourthly, cold tonic pain exposure has been shown to have a high inter-individual variability, and conflicting results have been reported in healthy. We did, however show a consistent adynamic pattern in the T1DM group.
- Lastly, including periodic dynamic depolarization of the T-wave and deceleration capacity, which are novel parameters of sympathetic and parasympathetic activation, could potentially improve our understanding of the complex sympatico-vagal neurocardiac regulation.

STUDY IV

Strengths:

- To our knowledge, this is the first time the dynamic alterations in situational electrocardiographic HRV recordings, in response to cold tonic pain exposure, have been investigated in individuals with type 1 diabetes receiving either liraglutide or a placebo.

Limitations:

- Firstly, the HRV measures were only measured once by the same individual using validated software. However, the predefined 2-minute ultra-short-term HRV measurements likely minimized inter- and intra-observer differences.
- Secondly, the 2-minute epochs are susceptible to physiological noise, such as stress response, coughing, and movements, which may have resulted in missing data. As the conducted ECG recordings were closely inspected for artifacts, arrhythmia, etc., this problem is likely of minor significance.
- Thirdly, we used classical HRV measures, including mean RR, SDNN, RMSSD, total power, VLF, LF, HF, and LF/HF, provided by the software solution.
- Finally, the lack of measures of catecholamines may have limited the investigation of potential underlying mechanisms of GLP-1-induced neurocardiac modulation. Moreover, including periodic dynamic depolarization of the T-wave and deceleration capacity may have improved our understanding of the complex sympatico-vagal neurocardiac regulation. Further studies are needed to address these limitations and provide a more comprehensive understanding of this topic.

7.2 FUTURE STUDIES AND PERSPECTIVES

STUDY I

It would be of great interest to validate changes in PFH in response to instillation against traditional gold-standard cardiac autonomic reflex tests used for diagnosing CAN. Moreover, it would be interesting to investigate whether diminished PFH can be seen in the early stages of diabetic retinopathy and diabetic neuropathy. One way to do so would be to design a follow-up study in which cardiac autonomic reflex tests, autonomic neuropathy tests, such as electroneurographic recordings, and evaluation of diabetic retinopathy stages were assessed with meaningful intervals.

STUDY II

The assessment of retinal nerve fiber layer thickness or ganglion cell layer thickness may enable the evaluation of systemic neurodegeneration. Therefore, establishing a longitudinal database to assess neurodegenerative changes in both peripheral, autonomic, and retinal nerves would be of great interest. This would enable the evaluations of associations between RNFL or ganglion cell layer thickness and autonomic and peripheral measures and possibly allow the stratification of high risk for developing neuropathy based on changes in RNFL or ganglion cell layer changes. Furthermore, it would be interesting to evaluate the effect of liraglutide, or other potential neuroprotective agents, in earlier stages of diabetes or diabetic retinopathy.

STUDY III

It would be highly intriguing the evaluate the dynamic HRV responses, in response to cold tonic pain exposure, in earlier stages of diabetes, as this could potentially reveal early signs of alterations in neurocardiac responses in individuals with early cardiovascular autonomic neuropathy. Moreover, it would be interesting to evaluate the use of other interventions, such as vagal nerve stimulation, lifestyle changes, or cognitive therapy of the dynamic responses. Furthermore, and as previously mentioned, the inclusion of periodic dynamic depolarization of the T-wave and

deceleration capacity could potentially improve our understanding of the sympatico-vagal neurocardiac regulation.

STUDY IV

It would be of great interest to assess the effect of GLP-1 RA' on the autonomic neurocardiac regulation in earlier stages of diabetes. Moreover, it would be interesting to asses other potential neuroprotective drugs, such as Finerenone and SGLT-, or supplemens[268], such as Omega-3, in a similar randomized controlled trial design in individuals with T1DM or T2DM.

8 CONCLUSION

STUDY I

We showed that the effect of phenylephrine on palpebral fissure height was associated with the severity of DR and disease duration and validated heart rate variability parameters, total power, VLF, and LF. Hence, the effects of phenylephrine on palpebral fissure height could potentially be a simple clinical indicator of autonomic dysfunction in individuals with type 1 diabetes[32].

STUDY II

We did not find measurable changes in peripapillary RNFL thickness measures following 26 weeks of treatment with liraglutide. Not did we find differences between the NPDR and PDR groups. RNFL thickness measurements using OCT may not be sensitive enough to detect possible GLP-1 RA-induced neuro regenerative effects. The assessment of the ganglion cell layer thickness may potentially be more sensitive[33].

STUDY III

We showed that ultra-short-term HRV measures revealed complicated and dynamic centrally regulated neurocardiac responses in response to challenge testing using cold tonic pain exposure. These changes may not otherwise be present in resting conditions. We found that individuals with long-term-type 1 diabetes and DSPN showed an adynamic response to tonic cold pain exposure compared to healthy controls. Whereas tonic cold pain exposure has traditionally been considered to result in sympathetic activation, we found a more complex response, with both linear and non-linear co-activation.

STUDY IV

Challenge testing, utilizing tonic cold pain exposure, revealed alterations in dynamic HRV responses following 26 weeks of treatment with liraglutide. We showed that HR and SDNN increased, whereas VLF and LF decreased, indicating withdrawal of sympathetic activity. We found no changes in parasympathetic measures of RMSSD and HF. Moreover, we found no correlations between mean RR or HR and HRV parameters. Although our data suggest a more complex regulation than the classical

reciprocal sympathovagal balance, we cannot draw any firm conclusions about the exact underlying mechanisms of increased HR in individuals treated with liraglutide.

LITERATURE LIST

1. Sun J, Wang Y, Zhang X, Zhu S, He H. Prevalence of peripheral neuropathy in patients with diabetes: A systematic review and meta-analysis. *Prim Care Diabetes*. 2020;14(5):435-444. doi:10.1016/j.pcd.2019.12.005
2. Amutha A, Ranjit U, Anjana RM, et al. Clinical profile and incidence of microvascular complications of childhood and adolescent onset type 1 and type 2 diabetes seen at a tertiary diabetes center in India. *Pediatr Diabetes*. 2021;22(1):67-74. doi:10.1111/pedi.13033
3. Tesfaye S, Boulton AJM, Dyck PJ, et al. Diabetic neuropathies: Update on definitions, diagnostic criteria, estimation of severity, and treatments. *Diabetes Care*. 2010;33(10):2285-2293. doi:10.2337/dc10-1303
4. Vinik AI, Erbas T. Cardiovascular autonomic neuropathy: Diagnosis and management. *Curr Diab Rep*. 2006;6(6):424-430. doi:10.1007/s11892-006-0074-z
5. Vinik AI, Park TS, Stansberry KB, Pittenger GL. Diabetic neuropathies. *Diabetologia*. 2000;43(8):957-973. doi:10.1007/s001250051477
6. Yagihashi S, Yamagishi SI, Wada R. Pathology and pathogenetic mechanisms of diabetic neuropathy: Correlation with clinical signs and symptoms. *Diabetes Res Clin Pract*. 2007;77(3 SUPPL.):184-189. doi:10.1016/j.diabres.2007.01.054
7. Vinik AI, Ziegler D. Diabetic cardiovascular autonomic neuropathy. *Circulation*. 2007;115(3):387-397. doi:10.1161/CIRCULATIONAHA.106.634949
8. Brock C, Brock B, Pedersen AG, Drewes AM, Jessen N, Farmer AD. Assessment of the cardiovascular and gastrointestinal autonomic complications of diabetes. *World J Diabetes*. 2016;7(16):321. doi:10.4239/wjd.v7.i16.321
9. Serhiyenko VA, Serhiyenko AA. Cardiac autonomic neuropathy: Risk factors, diagnosis and treatment. *World J Diabetes*. 2018;9(1):1-24. doi:10.4239/wjd.v9.i1.1
10. Vinik AI, Erbas T, Casellini CM. Diabetic cardiac autonomic neuropathy, inflammation and cardiovascular disease. *J Diabetes Investig*. 2013;4(1):4-18. doi:10.1111/jdi.12042
11. Spallone V. Update on the impact, diagnosis and management of cardiovascular autonomic neuropathy in diabetes: What is defined, what is new, and what is unmet. *Diabetes Metab J*. 2019;43(1):3-30. doi:10.4093/dmj.2018.0259
12. Pop-Busui R. Cardiac autonomic neuropathy in diabetes: A clinical perspective. *Diabetes Care*. 2010;33(2):434-441. doi:10.2337/dc09-1294
13. Pop-Busui R, Boulton AJM, Feldman EL, et al. Diabetic Neuropathy: A Position Statement by the American Diabetes Association. *Diabetes Care*. 2017;40(1):136-154. doi:10.2337/dc16-2042
14. Karayannis G, Giamouzis G, Cokkinos D V, Skoularigis J, Triposkiadis F. Diabetic cardiovascular autonomic neuropathy: clinical implications. *Expert*

Rev Cardiovasc Ther. 2012;10(6):747-765. doi:10.1586/erc.12.53

15. Vinik AI, Maser RE, Mitchell BD, Freeman R. Diabetic autonomic neuropathy. *Diabetes Care*. 2003;26(5):1553-1579. doi:10.2337/diacare.26.5.1553
16. Cheshire WP, Freeman R, Gibbons CH, et al. Electrodiagnostic assessment of the autonomic nervous system: A consensus statement endorsed by the American Autonomic Society, American Academy of Neurology, and the International Federation of Clinical Neurophysiology. *Clin Neurophysiol*. 2021;132(2):666-682. doi:10.1016/j.clinph.2020.11.024
17. Hijazi MM, Buchmann SJ, Sedghi A, et al. Assessment of cutaneous axon-reflex responses to evaluate functional integrity of autonomic small nerve fibers. *Neurol Sci*. 2020;41(7):1685-1696. doi:10.1007/s10072-020-04293-w
18. Bremner F. Pupil evaluation as a test for autonomic disorders. *Clin Auton Res*. 2009;19(2):88-101. doi:10.1007/s10286-009-0515-2
19. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation*. 1996;93(5):1043-1065.
20. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation*. 1996;93(5):1043-1065. doi:10.1161/01.CIR.93.5.1043
21. Vinik AI, Erbas T. Recognizing and treating diabetic autonomic neuropathy. *Cleve Clin J Med*. 2001;68(11):928-944. doi:10.3949/ccjm.68.11.928
22. Witte DR, Tesfaye S, Chaturvedi N, Eaton SEM, Kempler P, Fuller JH. Risk factors for cardiac autonomic neuropathy in type 1 diabetes mellitus. *Diabetologia*. 2005;48(1):164-171. doi:10.1007/s00125-004-1617-y
23. Maser, Raelene E and Mitchell, Braxton D and Vinik, Aaron I and Freeman R. Autonomic Neuropathy and Mortality in Individuals with Diabetes. *Diabetes Care*. 2003;26(6):1895--1901.
24. Busch V, Magerl W, Kern U, Haas J, Hajak G, Eichhammer P. The Effect of Deep and Slow Breathing on Pain Perception, Autonomic Activity, and Mood Processing-An Experimental Study. *Pain Med*. 2012;13(2):215-228. doi:10.1111/j.1526-4637.2011.01243.x
25. He B, Lu Z, He W, Huang B, Jiang H. Autonomic Modulation by Electrical Stimulation of the Parasympathetic Nervous System: An Emerging Intervention for Cardiovascular Diseases. *Cardiovasc Ther*. 2016;34(3):167-171. doi:10.1111/1755-5922.12179
26. Wen S, Nguyen T, Gong M, et al. An overview of similarities and differences in metabolic actions and effects of central nervous system between glucagon-like peptide-1 receptor agonists (Glp-1ras) and sodium glucose co-transporter-2 inhibitors (sglt-2is). *Diabetes, Metab Syndr Obes Targets Ther*. 2021;14:2955-2972. doi:10.2147/DMSO.S312527
27. Holst JJ, Gromada J. Role of incretin hormones in the regulation of insulin secretion in diabetic and nondiabetic humans. *Am J Physiol - Endocrinol*

Metab. 2004;287(2 50-2). doi:10.1152/ajpendo.00545.2003
28. Cabou C, Campistron G, Marsollier N, et al. Brain glucagon-like peptide-1 regulates arterial blood flow, heart rate, and insulin sensitivity. *Diabetes*. 2008;57(10):2577-2587. doi:10.2337/db08-0121
29. Hölscher C. Potential role of glucagon-like peptide-1 (GLP-1) in neuroprotection. *CNS Drugs*. 2012;26(10):871-882. doi:10.2165/11635890-000000000-00000
30. Cervia, Catalani, Casini. Neuroprotective Peptides in Retinal Disease. *J Clin Med*. 2019;8(8):1146. doi:10.3390/jcm8081146
31. Sampedro J, Bogdanov P, Ramos H, et al. New Insights into the Mechanisms of Action of Topical Administration of GLP-1 in an Experimental Model of Diabetic Retinopathy. *J Clin Med*. 2019;8(3):339. doi:10.3390/jcm8030339
32. Nielsen TA, Andersen CU, Vorum H, et al. Palpebral Fissure Response to Phenylephrine Indicates Autonomic Dysfunction in Patients With Type 1 Diabetes and Polyneuropathy. *Invest Ophthalmol Vis Sci*. 2022;63(9):21. doi:10.1167/iovs.63.9.21
33. Arendt Nielsen T, Sega R, Uggerhøj Andersen C, et al. Liraglutide Treatment Does Not Induce Changes in the Peripapillary Retinal Nerve Fiber Layer Thickness in Patients with Diabetic Retinopathy. *J Ocul Pharmacol Ther Off J Assoc Ocul Pharmacol Ther*. 2022;38(1):114-121. doi:10.1089/jop.2021.0055
34. World Health Organization. Diabetes. Available online: https://www.who.int/news-room/fact-sheets/detail/diabetes (accessed on march 14th, 2023).
35. Weng J, Zhou Z, Guo L, et al. Incidence of type 1 diabetes in China, 2010-13: population based study. *BMJ*. 2018;360:j5295. doi:10.1136/bmj.j5295
36. Patterson CC, Harjutsalo V, Rosenbauer J, et al. Trends and cyclical variation in the incidence of childhood type 1 diabetes in 26 European centres in the 25 year period 1989-2013: a multicentre prospective registration study. *Diabetologia*. 2019;62(3):408-417. doi:10.1007/s00125-018-4763-3
37. Norris JM, Johnson RK, Stene LC. Type 1 diabetes-early life origins and changing epidemiology. *lancet Diabetes Endocrinol*. 2020;8(3):226-238. doi:10.1016/S2213-8587(19)30412-7
38. Dyck PJ, Kratz KM, Karnes JL, et al. The prevalence by staged severity of various types of diabetic neuropathy, retinopathy, and nephropathy in a population-based cohort: the Rochester Diabetic Neuropathy Study. *Neurology*. 1993;43(4):817-824. doi:10.1212/wnl.43.4.817
39. Nathan DM, Genuth S, Lachin J, et al. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med*. 1993;329(14):977-986. doi:10.1056/NEJM199309303291401
40. Yau JWY, Rogers SL, Kawasaki R, et al. Global prevalence and major risk factors of diabetic retinopathy. *Diabetes Care*. 2012;35(3):556-564. doi:10.2337/dc11-1909

41. Mohamed Q, Gillies MC, Wong TY. Management of diabetic retinopathy: a systematic review. *JAMA*. 2007;298(8):902-916. doi:10.1001/jama.298.8.902
42. Grauslund J, Green A, Sjølie AK. Prevalence and 25 year incidence of proliferative retinopathy among Danish type 1 diabetic patients. *Diabetologia*. 2009;52(9):1829-1835. doi:10.1007/s00125-009-1450-4
43. Klein R, Klein BE, Moss SE, Cruickshanks KJ. The Wisconsin Epidemiologic Study of Diabetic Retinopathy: XVII. The 14-year incidence and progression of diabetic retinopathy and associated risk factors in type 1 diabetes. *Ophthalmology*. 1998;105(10):1801-1815. doi:10.1016/S0161-6420(98)91020-X
44. Liew G, Michaelides M, Bunce C. A comparison of the causes of blindness certifications in England and Wales in working age adults (16-64 years), 1999-2000 with 2009-2010. *BMJ Open*. 2014;4(2):e004015. doi:10.1136/bmjopen-2013-004015
45. Wilkinson CP, Ferris FL 3rd, Klein RE, et al. Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. *Ophthalmology*. 2003;110(9):1677-1682. doi:10.1016/S0161-6420(03)00475-5
46. Adamis AP, Miller JW, Bernal MT, et al. Increased vascular endothelial growth factor levels in the vitreous of eyes with proliferative diabetic retinopathy. *Am J Ophthalmol*. 1994;118(4):445-450. doi:10.1016/s0002-9394(14)75794-0
47. Lopes de Faria JM, Russ H, Costa VP. Retinal nerve fibre layer loss in patients with type 1 diabetes mellitus without retinopathy. *Br J Ophthalmol*. 2002;86(7):725-728. doi:10.1136/bjo.86.7.725
48. Araszkiewicz A, Zozulińska-Ziółkiewicz D, Meller M, et al. Neurodegeneration of the retina in type 1 diabetic patients. *Pol Arch Med Wewn*. 2012;122(10):464-470.
49. Kim K, Kim ES, Yu S-Y. Longitudinal Relationship Between Retinal Diabetic Neurodegeneration and Progression of Diabetic Retinopathy in Patients With Type 2 Diabetes. *Am J Ophthalmol*. 2018;196:165-172. doi:10.1016/j.ajo.2018.08.053
50. Mehboob MA, Amin ZA, Islam QU. Comparison of retinal nerve fiber layer thickness between normal population and patients with diabetes mellitus using optical coherence tomography. *Pakistan J Med Sci*. 2019;35(1):29-33. doi:10.12669/pjms.35.1.65
51. Pillar S, Moisseiev E, Sokolovska J, Grzybowski A. Recent Developments in Diabetic Retinal Neurodegeneration: A Literature Review. *J Diabetes Res*. 2020;2020. doi:10.1155/2020/5728674
52. Sohn EH, van Dijk HW, Jiao C, et al. Retinal neurodegeneration may precede microvascular changes characteristic of diabetic retinopathy in diabetes mellitus. *Proc Natl Acad Sci U S A*. 2016;113(19):E2655-64. doi:10.1073/pnas.1522014113
53. Tang Z, Chan MY, Leung WY, et al. Assessment of retinal neurodegeneration with spectral-domain optical coherence tomography: a

systematic review and meta-analysis. *Eye (Lond)*. 2021;35(5):1317-1325. doi:10.1038/s41433-020-1020-z

54. Chen X, Nie C, Gong Y, et al. Peripapillary retinal nerve fiber layer changes in preclinical diabetic retinopathy: A meta-analysis. *PLoS One*. 2015;10(5):1-12. doi:10.1371/journal.pone.0125919
55. De Clerck EEB, Schouten JSAG, Berendschot TTJM, et al. Macular thinning in prediabetes or type 2 diabetes without diabetic retinopathy: the Maastricht Study. *Acta Ophthalmol*. 2018;96(2):174-182. doi:10.1111/aos.13570
56. Feng Y, Busch S, Gretz N, Hoffmann S, Hammes H-P. Crosstalk in the retinal neurovascular unit - lessons for the diabetic retina. *Exp Clin Endocrinol diabetes Off journal, Ger Soc Endocrinol [and] Ger Diabetes Assoc*. 2012;120(4):199-201. doi:10.1055/s-0032-1304571
57. Simó R, Stitt AW, Gardner TW. Neurodegeneration in diabetic retinopathy: does it really matter? *Diabetologia*. 2018;61(9):1902-1912. doi:10.1007/s00125-018-4692-1
58. Gardner TW, Davila JR. The neurovascular unit and the pathophysiologic basis of diabetic retinopathy. *Graefe's Arch Clin Exp Ophthalmol = Albr von Graefes Arch fur Klin und Exp Ophthalmol*. 2017;255(1):1-6. doi:10.1007/s00417-016-3548-y
59. Altmann C, Schmidt MHH. The Role of Microglia in Diabetic Retinopathy: Inflammation, Microvasculature Defects and Neurodegeneration. *Int J Mol Sci*. 2018;19(1). doi:10.3390/ijms19010110
60. Zafar S, Sachdeva M, Frankfort BJ, Channa R. Retinal Neurodegeneration as an Early Manifestation of Diabetic Eye Disease and Potential Neuroprotective Therapies. *Curr Diab Rep*. 2019;19(4):17. doi:10.1007/s11892-019-1134-5
61. Hernández C, Dal Monte M, Simó R, Casini G. Neuroprotection as a Therapeutic Target for Diabetic Retinopathy. *J Diabetes Res*. 2016;2016:9508541. doi:10.1155/2016/9508541
62. Simó R, Hernández C. Neurodegeneration in the diabetic eye: new insights and therapeutic perspectives. *Trends Endocrinol Metab*. 2014;25(1):23-33. doi:10.1016/j.tem.2013.09.005
63. Kim K, Yu S-Y, Kwak HW, Kim ES. Retinal Neurodegeneration Associated With Peripheral Nerve Conduction and Autonomic Nerve Function in Diabetic Patients. *Am J Ophthalmol*. 2016;170:15-24. doi:10.1016/j.ajo.2016.06.038
64. Pemp B, Palkovits S, Howorka K, et al. Correlation of retinal neurodegeneration with measures of peripheral autonomic neuropathy in type 1 diabetes. *Acta Ophthalmol*. 2018;96(7):e804-e810. doi:10.1111/aos.13733
65. Shahidi AM, Sampson GP, Pritchard N, et al. Retinal nerve fibre layer thinning associated with diabetic peripheral neuropathy. *Diabet Med*. 2012;29(7):e106-11. doi:10.1111/j.1464-5491.2012.03588.x
66. Dehghani C, Srinivasan S, Edwards K, et al. Presence of Peripheral Neuropathy Is Associated With Progressive Thinning of Retinal Nerve

Fiber Layer in Type 1 Diabetes. *Invest Ophthalmol Vis Sci*. 2017;58(6):BIO234-BIO239. doi:10.1167/iovs.17-21801

67. Brock C, Wegeberg A-M, Nielsen TA, et al. The Retinal Nerve Fiber Layer Thickness Is Associated with Systemic Neurodegeneration in Long-Term Type 1 Diabetes. *Transl Vis Sci Technol*. 2023;12(6):23. doi:10.1167/tvst.12.6.23
68. Srinivasan S, Dehghani C, Pritchard N, et al. Optical coherence tomography predicts 4-year incident diabetic neuropathy. *Ophthalmic Physiol Opt J Br Coll Ophthalmic Opt*. 2017;37(4):451-459. doi:10.1111/opo.12391
69. Ziegler D, Gries FA, Spüler M, Lessmann F. The epidemiology of diabetic neuropathy. DiaCAN Multicenter Study Group. *Diabet Med*. 1993;10 Suppl 2:82S-86S. doi:10.1111/j.1464-5491.1993.tb00208.x
70. Gordois A, Scuffham P, Shearer A, Oglesby A, Tobian JA. The health care costs of diabetic peripheral neuropathy in the US. *Diabetes Care*. 2003;26(6):1790-1795. doi:10.2337/diacare.26.6.1790
71. Callaghan BC, Cheng HT, Stables CL, Smith AL, Feldman EL. Diabetic neuropathy: clinical manifestations and current treatments. *Lancet Neurol*. 2012;11(6):521-534. doi:10.1016/S1474-4422(12)70065-0
72. Albers JW, Pop-Busui R. Diabetic neuropathy: mechanisms, emerging treatments, and subtypes. *Curr Neurol Neurosci Rep*. 2014;14(8):473. doi:10.1007/s11910-014-0473-5
73. Callaghan BC, Kerber KA, Lisabeth LL, et al. Role of neurologists and diagnostic tests on the management of distal symmetric polyneuropathy. *JAMA Neurol*. 2014;71(9):1143-1149. doi:10.1001/jamaneurol.2014.1279
74. Effect of intensive diabetes treatment on nerve conduction in the Diabetes Control and Complications Trial. *Ann Neurol*. 1995;38(6):869-880. doi:10.1002/ana.410380607
75. Iqbal Z, Azmi S, Yadav R, et al. Diabetic Peripheral Neuropathy: Epidemiology, Diagnosis, and Pharmacotherapy. *Clin Ther*. 2018;40(6):828-849. doi:10.1016/j.clinthera.2018.04.001
76. Consensus statement: Report and recommendations of the San Antonio conference on diabetic neuropathy. American Diabetes Association American Academy of Neurology. *Diabetes Care*. 1988;11(7):592-597. doi:10.2337/diacare.11.7.592
77. Fisher VL, Tahrani AA. Cardiac autonomic neuropathy in patients with diabetes mellitus: current perspectives. *Diabetes Metab Syndr Obes*. 2017;10:419-434. doi:10.2147/DMSO.S129797
78. Sudo SZ, Montagnoli TL, Rocha B de S, Santos AD, de Sá MPL, Zapata-Sudo G. Diabetes-Induced Cardiac Autonomic Neuropathy: Impact on Heart Function and Prognosis. *Biomedicines*. 2022;10(12). doi:10.3390/biomedicines10123258
79. Dafaalla MD, Nimir MN, Mohammed MI, Ali OA, Hussein A. Risk factors of diabetic cardiac autonomic neuropathy in patients with type 1 diabetes mellitus: a meta-analysis. *Open Hear*. 2016;3(2):e000336. doi:10.1136/openhrt-2015-000336
80. Balcıoğlu AS, Müderrisoğlu H. Diabetes and cardiac autonomic neuropathy:

Clinical manifestations, cardiovascular consequences, diagnosis and treatment. *World J Diabetes*. 2015;6(1):80-91. doi:10.4239/wjd.v6.i1.80

81. Williams S, Raheim SA, Khan MI, et al. Cardiac Autonomic Neuropathy in Type 1 and 2 Diabetes: Epidemiology, Pathophysiology, and Management. *Clin Ther*. 2022;44(10):1394-1416. doi:10.1016/j.clinthera.2022.09.002
82. Kuehl M, Stevens MJ. Cardiovascular autonomic neuropathies as complications of diabetes mellitus. *Nat Rev Endocrinol*. 2012;8(7):405-416. doi:10.1038/nrendo.2012.21
83. Razanskaite-Virbickiene D, Danyte E, Mockeviciene G, Dobrovolskiene R, Verkauskiene R, Zalinkevicius R. Can coefficient of variation of time-domain analysis be valuable for detecting cardiovascular autonomic neuropathy in young patients with type 1 diabetes: a case control study. *BMC Cardiovasc Disord*. 2017;17(1):34. doi:10.1186/s12872-016-0467-0
84. Turker Y, Aslantas Y, Aydin Y, et al. Heart rate variability and heart rate recovery in patients with type 1 diabetes mellitus. *Acta Cardiol*. 2013;68(2):145-150. doi:10.1080/ac.68.2.2967271
85. Erus G, Battapady H, Zhang T, et al. Spatial patterns of structural brain changes in type 2 diabetic patients and their longitudinal progression with intensive control of blood glucose. *Diabetes Care*. 2015;38(1):97-104. doi:10.2337/dc14-1196
86. Manor B, Newton E, Abduljalil A, Novak V. The relationship between brain volume and walking outcomes in older adults with and without diabetic peripheral neuropathy. *Diabetes Care*. 2012;35(9):1907-1912. doi:10.2337/dc11-2463
87. Hughes TM, Ryan CM, Aizenstein HJ, et al. Frontal gray matter atrophy in middle aged adults with type 1 diabetes is independent of cardiovascular risk factors and diabetes complications. *J Diabetes Complications*. 2013;27(6):558-564. doi:10.1016/j.jdiacomp.2013.07.001
88. Eaton SE, Harris ND, Rajbhandari SM, et al. Spinal-cord involvement in diabetic peripheral neuropathy. *Lancet (London, England)*. 2001;358(9275):35-36. doi:10.1016/S0140-6736(00)05268-5
89. Selvarajah D, Wilkinson ID, Emery CJ, et al. Early involvement of the spinal cord in diabetic peripheral neuropathy. *Diabetes Care*. 2006;29(12):2664-2669. doi:10.2337/dc06-0650
90. Ferris JK, Inglis JT, Madden KM, Boyd LA. Brain and Body: A Review of Central Nervous System Contributions to Movement Impairments in Diabetes. *Diabetes*. 2020;69(1):3-11. doi:10.2337/db19-0321
91. Nissen TD, Meldgaard T, Nedergaard RW, et al. Peripheral, synaptic and central neuronal transmission is affected in type 1 diabetes. *J Diabetes Complications*. 2020;34(9):107614. doi:10.1016/j.jdiacomp.2020.107614
92. Hyllienmark L, Maltez J, Dandenell A, Ludvigsson J, Brismar T. EEG abnormalities with and without relation to severe hypoglycaemia in adolescents with type 1 diabetes. *Diabetologia*. 2005;48(3):412-419. doi:10.1007/s00125-004-1666-2
93. Croosu SS, Hansen TM, Brock B, Mohr Drewes A, Brock C, Frøkjær JB. Altered functional connectivity between brain structures in adults with type

1 diabetes and polyneuropathy. *Brain Res*. 2022;1784:147882. doi:10.1016/j.brainres.2022.147882
94. Li W, Huang E, Gao S. Type 1 Diabetes Mellitus and Cognitive Impairments: A Systematic Review. *J Alzheimers Dis*. 2017;57(1):29-36. doi:10.3233/JAD-161250
95. Brands AMA, Biessels GJ, de Haan EHF, Kappelle LJ, Kessels RPC. The effects of type 1 diabetes on cognitive performance: a meta-analysis. *Diabetes Care*. 2005;28(3):726-735. doi:10.2337/diacare.28.3.726
96. Alicic RZ, Rooney MT, Tuttle KR. Diabetic Kidney Disease: Challenges, Progress, and Possibilities. *Clin J Am Soc Nephrol*. 2017;12(12):2032-2045. doi:10.2215/CJN.11491116
97. Papadopoulou-Marketou N, Chrousos GP, Kanaka-Gantenbein C. Diabetic nephropathy in type 1 diabetes: a review of early natural history, pathogenesis, and diagnosis. *Diabetes Metab Res Rev*. 2017;33(2). doi:10.1002/dmrr.2841
98. Jänig W. *Integrative Action of the Autonomic Nervous System: Neurobiology of Homeostasis*. Cambridge University Press; 2006. doi:DOI: 10.1017/CBO9780511541667
99. Berntson GG, Cacioppo JT, Quigley KS. Autonomic determinism: The modes of autonomic control, the doctrine of autonomic space, and the laws of autonomic constraint. *Psychol Rev*. 1991;98:459-487. doi:10.1037/0033-295X.98.4.459
100. Shaffer F, Ginsberg JP. An Overview of Heart Rate Variability Metrics and Norms. *Front Public Heal*. 2017;5(September):1-17. doi:10.3389/fpubh.2017.00258
101. Gordan R, Gwathmey JK, Xie L-H. Autonomic and endocrine control of cardiovascular function. *World J Cardiol*. 2015;7(4):204-214. doi:10.4330/wjc.v7.i4.204
102. Bernardi L, Spallone V, Stevens M, et al. Methods of investigation for cardiac autonomic dysfunction in human research studies. *Diabetes Metab Res Rev*. 2011;27(7):654-664. doi:10.1002/dmrr.1224
103. Somers VK, Dyken ME, Mark AL, Abboud FM. Sympathetic-nerve activity during sleep in normal subjects. *N Engl J Med*. 1993;328(5):303-307. doi:10.1056/NEJM199302043280502
104. Bernardi L, Bianchini B, Spadacini G, et al. Demonstrable cardiac reinnervation after human heart transplantation by carotid baroreflex modulation of RR interval. *Circulation*. 1995;92(10):2895-2903. doi:10.1161/01.cir.92.10.2895
105. Bernardi L, Salvucci F, Suardi R, et al. Evidence for an intrinsic mechanism regulating heart rate variability in the transplanted and the intact heart during submaximal dynamic exercise? *Cardiovasc Res*. 1990;24(12):969-981. doi:10.1093/cvr/24.12.969
106. Jamali HK, Waqar F, Gerson MC. Cardiac autonomic innervation. *J Nucl Cardiol*. 2017;24(5):1558-1570. doi:10.1007/s12350-016-0725-7
107. Ruffinazzi M, Dusi V. Central Nervous System Management of Autonomic Cardiovascular Control BT - Brain and Heart Dynamics. In: Govoni S,

Politi P, Vanoli E, eds. Springer International Publishing; 2020:1-27. doi:10.1007/978-3-319-90305-7_65-1

108. Ciriello J, Calaresu FR. Medullary origin of vagal preganglionic axons to the heart of the cat. *J Auton Nerv Syst*. 1982;5(1):9-22. doi:10.1016/0165-1838(82)90086-8
109. Cravo SL, Morrison SF. The caudal ventrolateral medulla is a source of tonic sympathoinhibition. *Brain Res*. 1993;621(1):133-136. doi:10.1016/0006-8993(93)90308-a
110. Armour JA, Murphy DA, Yuan BX, Macdonald S, Hopkins DA. Gross and microscopic anatomy of the human intrinsic cardiac nervous system. *Anat Rec*. 1997;247(2):289-298. doi:10.1002/(SICI)1097-0185(199702)247:2<289::AID-AR15>3.0.CO;2-L
111. Park JM, Roh MS, Kim MH, et al. Microscopic characteristics of the inferior tarsal muscle and its surroundings in Korean. *Int J Ophthalmol*. 2013;6(2):126-130. doi:10.3980/j.issn.2222-3959.2013.02.03
112. Skibell BC, Harvey JH, Oestreicher JH, et al. Adrenergic receptors in the ptotic human eyelid: correlation with phenylephrine testing and surgical success in ptosis repair. *Ophthal Plast Reconstr Surg*. 2007;23(5):367-371. doi:10.1097/IOP.0b013e3181462a2e
113. Felt DP, Frueh BR. A pharmacologic study of the sympathetic eyelid tarsal muscles. *Ophthal Plast Reconstr Surg*. 1988;4(1):15-24. doi:10.1097/00002341-198801130-00003
114. Munden PM, Kardon RH, Denison CE, Carter KD. Palpebral fissure responses to topical adrenergic drugs. *Am J Ophthalmol*. 1991;111(6):706-710. doi:10.1016/s0002-9394(14)76775-3
115. Nunes TP, Matayoshi S. Ação do colírio de fenilefrina 10% no posicionamento palpebral de indivíduos normais. *Arq Bras Oftalmol*. 2008;71(5):639-643. doi:10.1590/s0004-27492008000500006
116. Grace Lee N, Lin LW, Mehta S, Freitag SK. Response to phenylephrine testing in upper eyelids with ptosis. *Digit J Ophthalmol DJO*. 2015;21(3):1-12. doi:10.5693/djo.01.2015.05.001
117. Barsegian A, Botwinick A, Reddy HS. The Phenylephrine Test Revisited. *Ophthal Plast Reconstr Surg*. 2018;34(2):151-154. doi:10.1097/IOP.0000000000000903
118. Wirch JL, Wolfe LA, Weissgerber TL, Davies GAL. Cold pressor test protocol to evaluate cardiac autonomic function. *Appl Physiol Nutr Metab*. 2006;31(3):235-243. doi:10.1139/h05-018
119. Mitchell LA, MacDonald RAR, Brodie EE. Temperature and the cold pressor test. *J pain*. 2004;5(4):233-237. doi:10.1016/j.jpain.2004.03.004
120. Mourot L, Bouhaddi M, Regnard J. Effects of the cold pressor test on cardiac autonomic control in normal subjects. *Physiol Res*. 2009;58(1):83-91. doi:10.33549/physiolres.931360
121. Prakash K, Thakur A, Malhotra AS. Association of heart rate variability, blood pressure variability, and baroreflex sensitivity with gastric motility at rest and during cold pressor test. *Gastroenterol Hepatol from Bed to Bench*. 2021;14(2):132-140. doi:10.22037/ghfbb.v14i2.1995

122. Victor RG, Leimbach WNJ, Seals DR, Wallin BG, Mark AL. Effects of the cold pressor test on muscle sympathetic nerve activity in humans. *Hypertens (Dallas, Tex 1979)*. 1987;9(5):429-436. doi:10.1161/01.hyp.9.5.429
123. Cui J, Wilson TE, Crandall CG. Baroreflex modulation of muscle sympathetic nerve activity during cold pressor test in humans. *Am J Physiol - Hear Circ Physiol*. 2002;282(5 51-5):1717-1723. doi:10.1152/ajpheart.00899.2001
124. Velasco M, Gómez J, Blanco M, Rodriguez I. The cold pressor test: pharmacological and therapeutic aspects. *Am J Ther*. 1997;4(1):34-38. doi:10.1097/00045391-199701000-00008
125. Goldstein B, Woolf PD, Deking D, Delong DJ, Cox C, Kempski MH. Heart rate power spectrum and plasma catecholamine levels after postural change and cold pressor test. *Pediatr Res*. 1994;36(3):358-363. doi:10.1203/00006450-199409000-00016
126. Bashir M, Lone R, Salroo I, Sayeed S. Assessment of vascular reactivity in young diabetics by cold pressor test and heart rate variability. *Med J Dr DY Patil Vidyapeeth*. Published online 2019:11-15. doi:10.4103/mjdrdypu.mjdrdypu_60_18
127. Mishra N, Mahajan KK. Cardiovascular response to orthostatic stress following cold challenge in diabetics. *J Indian Med Assoc*. 1996;94(1):3-5.
128. Liao D, Nedergaard RB, Unnisa M, et al. Electrocardiography Assessment of Sympatico-Vagal Balance during Resting and Pain using the Texas Instruments ADS1299. *Bioengineering*. 2023;10(2). doi:10.3390/bioengineering10020205
129. Bognetti E, Meschi F, Rota M, Cofano D, Palermo A, Chiumello G. Cardiovascular and hormonal responses to cold pressor test in insulin-dependent diabetic adolescents with microalbuminuria. *J Diabetes Complications*. 1994;8(2):84-88. doi:10.1016/1056-8727(94)90056-6
130. Sayinalp S, Sözen T, Ozdoğan M. Cold pressor test in diabetic autonomic neuropathy. *Diabetes Res Clin Pract*. 1994;26(1):21-28. doi:10.1016/0168-8227(94)90135-x
131. Luft D, Maisch C, Hofmann-Krück V, Radjaipour M, Häring HU. Correlates of venous catecholamine concentrations in patients with type 1 diabetes during a cold pressor test. *Clin Auton Res*. 2000;10(3):131-137. doi:10.1007/BF02278017
132. Weise F, Laude D, Girard A, Zitoun P, Siché JP, Elghozi JL. Effects of the cold pressor test on short-term fluctuations of finger arterial blood pressure and heart rate in normal subjects. *Clin Auton Res*. 1993;3(5):303-310. doi:10.1007/BF01827331
133. Nakamura T, Kawabe K, Sapru HN. Cold pressor test in the rat: medullary and spinal pathways and neurotransmitters. *Am J Physiol Heart Circ Physiol*. 2008;295(4):H1780-7. doi:10.1152/ajpheart.646.2008
134. Ulrich-Lai YM, Herman JP. Neural regulation of endocrine and autonomic stress responses. *Nat Rev Neurosci*. 2009;10(6):397-409. doi:10.1038/nrn2647
135. Drucker DJ. Mechanisms of Action and Therapeutic Application of

Glucagon-like Peptide-1. *Cell Metab*. 2018;27(4):740-756. doi:10.1016/j.cmet.2018.03.001
136. Kuhre RE, Holst JJ, Kappe C. The regulation of function, growth and survival of GLP-1-producing L-cells. *Clin Sci (Lond)*. 2016;130(2):79-91. doi:10.1042/CS20150154
137. Kuhre RE, Wewer Albrechtsen NJ, Larsen O, et al. Bile acids are important direct and indirect regulators of the secretion of appetite- and metabolism-regulating hormones from the gut and pancreas. *Mol Metab*. 2018;11:84-95. doi:10.1016/j.molmet.2018.03.007
138. Holst JJ. The physiology of glucagon-like peptide 1. *Physiol Rev*. 2007;87(4):1409-1439. doi:10.1152/physrev.00034.2006
139. De Heer J, Rasmussen C, Coy DH, Holst JJ. Glucagon-like peptide-1, but not glucose-dependent insulinotropic peptide, inhibits glucagon secretion via somatostatin (receptor subtype 2) in the perfused rat pancreas. *Diabetologia*. 2008;51(12):2263-2270. doi:10.1007/s00125-008-1149-y
140. Andreasen CR, Andersen A, Knop FK, Vilsbøll T. How glucagon-like peptide 1 receptor agonists work. *Endocr Connect*. 2021;10(7):R200-R212. doi:10.1530/EC-21-0130
141. Holst JJ. The physiology of glucagon-like peptide 1. *Physiol Rev*. 2007;87(4):1409-1439. doi:10.1152/physrev.00034.2006
142. Nauck MA, Homberger E, Siegel EG, et al. Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses. *J Clin Endocrinol Metab*. 1986;63(2):492-498. doi:10.1210/jcem-63-2-492
143. Marso SP, Daniels GH, Brown-Frandsen K, et al. Liraglutide and Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med*. 2016;375(4):311-322. doi:10.1056/NEJMoa1603827
144. Hebsgaard JB, Pyke C, Yildirim E, Knudsen LB, Heegaard S, Kvist PH. Glucagon-like peptide-1 receptor expression in the human eye. *Diabetes, Obes Metab*. 2018;20(9):2304-2308. doi:10.1111/dom.13339
145. Pyke C, Heller RS, Kirk RK, et al. GLP-1 receptor localization in monkey and human tissue: novel distribution revealed with extensively validated monoclonal antibody. *Endocrinology*. 2014;155(4):1280-1290. doi:10.1210/en.2013-1934
146. Wei Y, Mojsov S. Tissue-specific expression of the human receptor for glucagon-like peptide-I: brain, heart and pancreatic forms have the same deduced amino acid sequences. *FEBS Lett*. 1995;358(3):219-224. doi:10.1016/0014-5793(94)01430-9
147. McLean BA, Wong CK, Campbell JE, Hodson DJ, Trapp S, Drucker DJ. Revisiting the Complexity of GLP-1 Action from Sites of Synthesis to Receptor Activation. *Endocr Rev*. 2021;42(2):101-132. doi:10.1210/endrev/bnaa032
148. Nyström T, Gutniak MK, Zhang Q, et al. Effects of glucagon-like peptide-1 on endothelial function in type 2 diabetes patients with stable coronary artery disease. *Am J Physiol Endocrinol Metab*. 2004;287(6):E1209-15. doi:10.1152/ajpendo.00237.2004

149. Nakagawa A, Satake H, Nakabayashi H, et al. Receptor gene expression of glucagon-like peptide-1, but not glucose-dependent insulinotropic polypeptide, in rat nodose ganglion cells. *Auton Neurosci*. 2004;110(1):36-43. doi:10.1016/j.autneu.2003.11.001
150. https://www.novo-pi.com/victoza.pdf.
151. Watson E, Jonker DM, Jacobsen L V, Ingwersen SH. Population pharmacokinetics of liraglutide, a once-daily human glucagon-like peptide-1 analog, in healthy volunteers and subjects with type 2 diabetes, and comparison to twice-daily exenatide. *J Clin Pharmacol*. 2010;50(8):886-894. doi:10.1177/0091270009354996
152. Marso SP, Daniels GH, Brown-Frandsen K, et al. Liraglutide and Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med*. 2016;375(4):311-322. doi:10.1056/NEJMoa1603827
153. Sivertsen J, Rosenmeier J, Holst JJ, Vilsbøll T. The effect of glucagon-like peptide 1 on cardiovascular risk. *Nat Rev Cardiol*. 2012;9(4):209-222. doi:10.1038/nrcardio.2011.211
154. Giugliano D, Scappaticcio L, Longo M, et al. GLP-1 receptor agonists and cardiorenal outcomes in type 2 diabetes: an updated meta-analysis of eight CVOTs. *Cardiovasc Diabetol*. 2021;20(1):1-11. doi:10.1186/s12933-021-01366-8
155. Mann JFE, Ørsted DD, Brown-Frandsen K, et al. Liraglutide and Renal Outcomes in Type 2 Diabetes. *N Engl J Med*. 2017;377(9):839-848. doi:10.1056/nejmoa1616011
156. Holst JJ, Burcelin R, Nathanson E. Neuroprotective properties of GLP-1: Theoretical and practical applications. *Curr Med Res Opin*. 2011;27(3):547-558. doi:10.1185/03007995.2010.549466
157. Wiciński M, Socha M, Malinowski B, et al. Liraglutide and its neuroprotective properties—focus on possible biochemical mechanisms in alzheimer's disease and cerebral ischemic events. *Int J Mol Sci*. 2019;20(5). doi:10.3390/ijms20051050
158. Mehta K, Behl T, Kumar A, Uddin MS, Zengin G, Arora S. Deciphering the Neuroprotective Role of Glucagon-like Peptide-1 Agonists in Diabetic Neuropathy: Current Perspective and Future Directions. *Curr Protein Pept Sci*. 2021;22(1):4-18. doi:10.2174/1389203721999201208195901
159. Erbil D, Eren CY, Demirel C, Küçüker MU, Solaroğlu I, Eser HY. GLP-1's role in neuroprotection: a systematic review. *Brain Inj*. 2019;33(6):734-819. doi:10.1080/02699052.2019.1587000
160. Brock C, Hansen CS, Karmisholt J, et al. Liraglutide treatment reduced interleukin 6 in adults with type 1 diabetes but did not improve established autonomic or polyneuropathy. *Br J Clin Pharmacol*. Published online 2019. doi:10.1111/bcp.14063
161. Kan M, Guo G, Singh B, Singh V, Zochodne DW. Glucagon-like peptide 1, insulin, sensory neurons, and diabetic neuropathy. *J Neuropathol Exp Neurol*. 2012;71(6):494-510. doi:10.1097/NEN.0b013e3182580673
162. Jaiswal M, Martin CL, Brown MB, et al. Effects of exenatide on measures of diabetic neuropathy in subjects with type 2 diabetes: results from an 18-

month proof-of-concept open-label randomized study. *J Diabetes Complications*. 2015;29(8):1287-1294. doi:10.1016/j.jdiacomp.2015.07.013

163. Hansen CS, Frandsen CS, Fleischer J, et al. Liraglutide-induced weight loss may be affected by autonomic regulation in type 1 diabetes. *Front Endocrinol (Lausanne)*. 2019;10(APR):1-9. doi:10.3389/fendo.2019.00242
164. Egholm C, Khammy MM, Dalsgaard T, et al. GLP-1 inhibits VEGFA-mediated signaling in isolated human endothelial cells and VEGFA-induced dilation of rat mesenteric arteries. *Am J Physiol Heart Circ Physiol*. 2016;311(5):H1214-H1224. doi:10.1152/ajpheart.00316.2016
165. Puddu A, Sanguineti R, Montecucco F, Viviani GL. Retinal pigment epithelial cells express a functional receptor for glucagon-like peptide-1 (GLP-1). *Mediators Inflamm*. 2013;2013:975032. doi:10.1155/2013/975032
166. Hernández C, Bogdanov P, Corraliza L, et al. Topical administration of GLP-1 receptor agonists prevents retinal neurodegeneration in experimental diabetes. *Diabetes*. 2016;65(1):172-187. doi:10.2337/db15-0443
167. Marso SP, Bain SC, Consoli A, et al. Semaglutide and Cardiovascular Outcomes in Patients with Type 2 Diabetes. *N Engl J Med*. 2016;375(19):1834-1844. doi:10.1056/nejmoa1607141
168. Simó R, Hernández C. GLP-1R as a target for the treatment of diabetic retinopathy: Friend or foe? *Diabetes*. 2017;66(6):1453-1460. doi:10.2337/db16-1364
169. Liu J, Wei L, Wang Z, et al. Protective effect of Liraglutide on diabetic retinal neurodegeneration via inhibiting oxidative stress and endoplasmic reticulum stress. *Neurochem Int*. 2020;133(November 2019):104624. doi:10.1016/j.neuint.2019.104624
170. Ramos H, Bogdanov P, Sampedro J, Huerta J, Simó R, Hernández C. Beneficial Effects of Glucagon-Like Peptide-1 (GLP-1) in Diabetes-Induced Retinal Abnormalities: Involvement of Oxidative Stress. *Antioxidants (Basel, Switzerland)*. 2020;9(9). doi:10.3390/antiox9090846
171. Simó R, Bogdanov P, Ramos H, Huerta J, Simó-Servat O, Hernández C. Effects of the topical administration of semaglutide on retinal neuroinflammation and vascular leakage in experimental diabetes. *Biomedicines*. 2021;9(8). doi:10.3390/biomedicines9080926
172. Fan Y, Liu K, Wang Q, Ruan Y, Ye W, Zhang Y. Exendin-4 alleviates retinal vascular leakage by protecting the blood-retinal barrier and reducing retinal vascular permeability in diabetic Goto-Kakizaki rats. *Exp Eye Res*. 2014;127:104-116. doi:10.1016/j.exer.2014.05.004
173. Gonçalves A, Lin CM, Muthusamy A, et al. Protective effect of a GLP-1 analog on ischemia-reperfusion induced blood–retinal barrier breakdown and inflammation. *Investig Ophthalmol Vis Sci*. 2016;57(6):2584-2592. doi:10.1167/iovs.15-19006
174. Mouhammad ZA, Vohra R, Horwitz A, et al. Glucagon-Like Peptide 1 Receptor Agonists – Potential Game Changers in the Treatment of Glaucoma? *Front Neurosci*. 2022;16(February):1-19. doi:10.3389/fnins.2022.824054
175. Bellastella G, Maiorino MI, Longo M, et al. Glucagon-Like Peptide-1

Receptor Agonists and Prevention of Stroke Systematic Review of Cardiovascular Outcome Trials With Meta-Analysis. *Stroke*. 2020;51(2):666-669. doi:10.1161/STROKEAHA.119.027557

176. Arakawa M, Mita T, Azuma K, et al. Inhibition of monocyte adhesion to endothelial cells and attenuation of atherosclerotic lesion by a glucagon-like peptide-1 receptor agonist, exendin-4. *Diabetes*. 2010;59(4):1030-1037. doi:10.2337/db09-1694
177. Ceriello A, Novials A, Ortega E, et al. Glucagon-Like Peptide 1 Reduces Endothelial Dysfunction, Inflammation, and Oxidative Stress Induced by Both Hyperglycemia and Hypoglycemia in Type 1 Diabetes. *Diabetes Care*. 2013;36(8):2346-2350. doi:10.2337/dc12-2469
178. Sun F, Wu S, Wang J, et al. Effect of glucagon-like peptide-1 receptor agonists on lipid profiles among type 2 diabetes: a systematic review and network meta-analysis. *Clin Ther*. 2015;37(1):225-241.e8. doi:10.1016/j.clinthera.2014.11.008
179. Taskinen M-R, Björnson E, Matikainen N, et al. Effects of liraglutide on the metabolism of triglyceride-rich lipoproteins in type 2 diabetes. *Diabetes Obes Metab*. 2021;23(5):1191-1201. doi:10.1111/dom.14328
180. Ussher JR, Drucker DJ. Cardiovascular actions of incretin-based therapies. *Circ Res*. 2014;114(11):1788-1803. doi:10.1161/CIRCRESAHA.114.301958
181. Dejgaard TF, Frandsen CS, Hansen TS, et al. Efficacy and safety of liraglutide for overweight adult patients with type 1 diabetes and insufficient glycaemic control (Lira-1): a randomised, double-blind, placebo-controlled trial. *lancet Diabetes Endocrinol*. 2016;4(3):221-232. doi:10.1016/S2213-8587(15)00436-2
182. Kumarathurai P, Anholm C, Larsen BS, et al. Effects of Liraglutide on Heart Rate and Heart Rate Variability: A Randomized, Double-Blind, Placebo-Controlled Crossover Study. *Diabetes Care*. 2017;40(1):117-124. doi:10.2337/dc16-1580
183. Buse JB, Rosenstock J, Sesti G, et al. Liraglutide once a day versus exenatide twice a day for type 2 diabetes: a 26-week randomised, parallel-group, multinational, open-label trial (LEAD-6). *Lancet (London, England)*. 2009;374(9683):39-47. doi:10.1016/S0140-6736(09)60659-0
184. Nakatani Y, Kawabe A, Matsumura M, et al. Effects of GLP-1 Receptor Agonists on Heart Rate and the Autonomic Nervous System Using Holter Electrocardiography and Power Spectrum Analysis of Heart Rate Variability. *Diabetes Care*. 2016;39(2):e22-3. doi:10.2337/dc15-1437
185. Hara K, Aso Y, Komatsu T, et al. Liraglutide increases 24-h heart rate by reducing the cardiac parasympathetic activity of patients with type 2 diabetes: power spectral analysis of heart rate variability on 24-h Holter ECG recordings. *Diabetol Int*. 2015;6(1):26-32. doi:10.1007/s13340-014-0170-2
186. von Scholten BJ, Lajer M, Goetze JP, Persson F, Rossing P. Time course and mechanisms of the anti-hypertensive and renal effects of liraglutide treatment. *Diabet Med*. 2015;32(3):343-352. doi:10.1111/dme.12594

187. Meier JJ, Rosenstock J, Hincelin-Méry A, et al. Contrasting Effects of Lixisenatide and Liraglutide on Postprandial Glycemic Control, Gastric Emptying, and Safety Parameters in Patients With Type 2 Diabetes on Optimized Insulin Glargine With or Without Metformin: A Randomized, Open-Label Trial. *Diabetes Care*. 2015;38(7):1263-1273. doi:10.2337/dc14-1984
188. Fonseca VA, Devries JH, Henry RR, Donsmark M, Thomsen HF, Plutzky J. Reductions in systolic blood pressure with liraglutide in patients with type 2 diabetes: insights from a patient-level pooled analysis of six randomized clinical trials. *J Diabetes Complications*. 2014;28(3):399-405. doi:10.1016/j.jdiacomp.2014.01.009
189. Sivalingam S, Hein Zobel E, Hansen CS, et al. The effect of liraglutide on cardiac autonomic function in type 2 diabetes: A prespecified secondary analysis from the LIRAFLAME randomized, double-blinded, placebo-controlled trial. *Diabetes, Obes Metab*. 2022;24(8):1638-1642. doi:10.1111/dom.14717
190. Robinson LE, Holt TA, Rees K, Randeva HS, O'Hare JP. Effects of exenatide and liraglutide on heart rate, blood pressure and body weight: systematic review and meta-analysis. *BMJ Open*. 2013;3(1). doi:10.1136/bmjopen-2012-001986
191. Kumarathurai P, Anholm C, Fabricius-Bjerre A, et al. Effects of the glucagon-like peptide-1 receptor agonist liraglutide on 24-h ambulatory blood pressure in patients with type 2 diabetes and stable coronary artery disease: a randomized, double-blind, placebo-controlled, crossover study. *J Hypertens*. 2017;35(5):1070-1078. doi:10.1097/HJH.0000000000001275
192. Bharucha AE, Charkoudian N, Andrews CN, et al. Effects of glucagon-like peptide-1, yohimbine, and nitrergic modulation on sympathetic and parasympathetic activity in humans. *Am J Physiol Integr Comp Physiol*. 2008;295(3):R874-R880. doi:10.1152/ajpregu.00153.2008
193. Griffioen KJ, Wan R, Okun E, et al. GLP-1 receptor stimulation depresses heart rate variability and inhibits neurotransmission to cardiac vagal neurons. *Cardiovasc Res*. 2011;89(1):72-78. doi:10.1093/cvr/cvq271
194. Mendis B, Simpson E, MacDonald I, Mansell P. Investigation of the haemodynamic effects of exenatide in healthy male subjects. *Br J Clin Pharmacol*. 2012;74(3):437-444. doi:10.1111/j.1365-2125.2012.04214.x
195. Yamamoto H, Lee CE, Marcus JN, et al. Glucagon-like peptide-1 receptor stimulation increases blood pressure and heart rate and activates autonomic regulatory neurons. *J Clin Invest*. 2002;110(1):43-52. doi:10.1172/JCI15595
196. Yamamoto H, Kishi T, Lee CE, et al. Glucagon-like peptide-1-responsive catecholamine neurons in the area postrema link peripheral glucagon-like peptide-1 with central autonomic control sites. *J Neurosci*. 2003;23(7):2939-2946. doi:10.1523/JNEUROSCI.23-07-02939.2003
197. Larsen PJ, Tang-Christensen M, Holst JJ, Orskov C. Distribution of glucagon-like peptide-1 and other preproglucagon-derived peptides in the rat hypothalamus and brainstem. *Neuroscience*. 1997;77(1):257-270. doi:10.1016/s0306-4522(96)00434-4

198. Greco C, Santi D, Brigante G, Pacchioni C, Simoni M. Effect of the Glucagon-Like Peptide-1 Receptor Agonists on Autonomic Function in Subjects with Diabetes: A Systematic Review and Meta-Analysis. *Diabetes Metab J*. Published online 2022:901-911. doi:10.4093/dmj.2021.0314
199. Alvarez E, Martínez MD, Roncero I, et al. The expression of GLP-1 receptor mRNA and protein allows the effect of GLP-1 on glucose metabolism in the human hypothalamus and brainstem. *J Neurochem*. 2005;92(4):798-806. doi:10.1111/j.1471-4159.2004.02914.x
200. Hölscher C. Central effects of GLP-1: new opportunities for treatments of neurodegenerative diseases. *J Endocrinol*. 2014;221(1):T31-41. doi:10.1530/JOE-13-0221
201. Müller TD, Finan B, Bloom SR, et al. Glucagon-like peptide 1 (GLP-1). *Mol Metab*. 2019;30:72-130. doi:10.1016/j.molmet.2019.09.010
202. Merchenthaler I, Lane M, Shughrue P. Distribution of pre-pro-glucagon and glucagon-like peptide-1 receptor messenger RNAs in the rat central nervous system. *J Comp Neurol*. 1999;403(2):261-280. doi:10.1002/(sici)1096-9861(19990111)403:2<261::aid-cne8>3.0.co;2-5
203. Gejl M, Gjedde A, Egefjord L, et al. In Alzheimer's Disease, 6-Month Treatment with GLP-1 Analog Prevents Decline of Brain Glucose Metabolism: Randomized, Placebo-Controlled, Double-Blind Clinical Trial. *Front Aging Neurosci*. 2016;8:108. doi:10.3389/fnagi.2016.00108
204. Cheng D, Yang S, Zhao X, Wang G. The Role of Glucagon-Like Peptide-1 Receptor Agonists (GLP-1 RA) in Diabetes-Related Neurodegenerative Diseases. 2022;(February):665-684.
205. Du H, Meng X, Yao Y, Xu J. The mechanism and efficacy of GLP-1 receptor agonists in the treatment of Alzheimer's disease. *Front Endocrinol (Lausanne)*. 2022;13:1033479. doi:10.3389/fendo.2022.1033479
206. Bassil F, Fernagut P-O, Bezard E, Meissner WG. Insulin, IGF-1 and GLP-1 signaling in neurodegenerative disorders: targets for disease modification? *Prog Neurobiol*. 2014;118:1-18. doi:10.1016/j.pneurobio.2014.02.005
207. Grieco M, Giorgi A, Gentile MC, et al. Glucagon-Like Peptide-1: A Focus on Neurodegenerative Diseases. *Front Neurosci*. 2019;13:1112. doi:10.3389/fnins.2019.01112
208. Reich N, Hölscher C. The neuroprotective effects of glucagon-like peptide 1 in Alzheimer's and Parkinson's disease: An in-depth review. *Front Neurosci*. 2022;16:970925. doi:10.3389/fnins.2022.970925
209. Li Y, Tweedie D, Mattson MP, Holloway HW, Greig NH. Enhancing the GLP-1 receptor signaling pathway leads to proliferation and neuroprotection in human neuroblastoma cells. *J Neurochem*. 2010;113(6):1621-1631. doi:10.1111/j.1471-4159.2010.06731.x
210. Gault VA, Hölscher C. GLP-1 agonists facilitate hippocampal LTP and reverse the impairment of LTP induced by beta-amyloid. *Eur J Pharmacol*. 2008;587(1-3):112-117. doi:10.1016/j.ejphar.2008.03.025
211. Vergès B, Aboyans V, Angoulvant D, et al. Protection against stroke with glucagon-like peptide-1 receptor agonists: a comprehensive review of potential mechanisms. *Cardiovasc Diabetol*. 2022;21(1):242.

doi:10.1186/s12933-022-01686-3
212. Bastiaensen LA. Narrowing of the palpebral fissure in diabetes. *Doc Ophthalmol*. 1983;56(1-2):5-10. doi:10.1007/BF00154702
213. Herman WH, Pop-Busui R, Braffett BH, et al. Use of the Michigan Neuropathy Screening Instrument as a measure of distal symmetrical peripheral neuropathy in Type 1 diabetes: results from the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications. *Diabet Med*. 2012;29(7):937-944. doi:10.1111/j.1464-5491.2012.03644.x
214. Moghtaderi A, Bakhshipour A, Rashidi H. Validation of Michigan neuropathy screening instrument for diabetic peripheral neuropathy. *Clin Neurol Neurosurg*. 2006;108(5):477-481. doi:10.1016/j.clineuro.2005.08.003
215. Lauria G, Cornblath DR, Johansson O, et al. EFNS guidelines on the use of skin biopsy in the diagnosis of peripheral neuropathy. *Eur J Neurol*. 2005;12(10):747-758. doi:10.1111/j.1468-1331.2005.01260.x
216. Petropoulos IN, Alam U, Fadavi H, et al. Corneal nerve loss detected with corneal confocal microscopy is symmetrical and related to the severity of diabetic polyneuropathy. *Diabetes Care*. 2013;36(11):3646-3651. doi:10.2337/dc13-0193
217. Mallik A, Weir AI. Nerve conduction studies: essentials and pitfalls in practice. *J Neurol Neurosurg Psychiatry*. 2005;76 Suppl 2(Suppl 2):ii23-31. doi:10.1136/jnnp.2005.069138
218. Spallone V, Ziegler D, Freeman R, et al. Cardiovascular autonomic neuropathy in diabetes: Clinical impact, assessment, diagnosis, and management. *Diabetes Metab Res Rev*. 2011;27(7):639-653. doi:10.1002/dmrr.1239
219. Pop-Busui R, Backlund JYC, Bebu I, et al. Utility of using electrocardiogram measures of heart rate variability as a measure of cardiovascular autonomic neuropathy in type 1 diabetes patients. *J Diabetes Investig*. 2022;13(1):125-133. doi:10.1111/jdi.13635
220. Maser RE, Mitchell BD, Vinik AI, Freeman R. The association between cardiovascular autonomic neuropathy and mortality in individuals with diabetes: a meta-analysis. *Diabetes Care*. 2003;26(6):1895-1901. doi:10.2337/diacare.26.6.1895
221. Wilson LC, Peebles KC, Hoye NA, et al. Resting heart rate variability and exercise capacity in Type 1 diabetes. *Physiol Rep*. 2017;5(8). doi:10.14814/phy2.13248
222. Kristiansen E, Wanby P, Åkesson K, Blomstrand P, Brudin L, Thegerström J. Assessing heart rate variability in type 1 diabetes mellitus-Psychosocial stress a possible confounder. *Ann Noninvasive Electrocardiol*. 2020;25(5):e12760. doi:10.1111/anec.12760
223. Sassi R, Cerutti S, Lombardi F, et al. Advances in heart rate variability signal analysis: joint position statement by the e-Cardiology ESC Working Group and the European Heart Rhythm Association co-endorsed by the Asia Pacific Heart Rhythm Society. *Europace*. 2015;17(9):1341-1353.

doi:10.1093/europace/euv015
224. Shaffer F, McCraty R, Zerr CL. A healthy heart is not a metronome: an integrative review of the heart's anatomy and heart rate variability. *Front Psychol*. 2014;5:1040. doi:10.3389/fpsyg.2014.01040
225. Kleiger RE, Miller JP, Bigger JTJ, Moss AJ. Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. *Am J Cardiol*. 1987;59(4):256-262. doi:10.1016/0002-9149(87)90795-8
226. Billman GE. The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance. *Front Physiol*. 2013;4:26. doi:10.3389/fphys.2013.00026
227. Goldstein DS, Bentho O, Park M-Y, Sharabi Y. Low-frequency power of heart rate variability is not a measure of cardiac sympathetic tone but may be a measure of modulation of cardiac autonomic outflows by baroreflexes. *Exp Physiol*. 2011;96(12):1255-1261. doi:10.1113/expphysiol.2010.056259
228. Taylor JA, Carr DL, Myers CW, Eckberg DL. Mechanisms underlying very-low-frequency RR-interval oscillations in humans. *Circulation*. 1998;98(6):547-555. doi:10.1161/01.cir.98.6.547
229. Hayano J, Yuda E. Assessment of autonomic function by long-term heart rate variability: beyond the classical framework of LF and HF measurements. *J Physiol Anthropol*. 2021;40(1):21. doi:10.1186/s40101-021-00272-y
230. Nunan D, Sandercock GRH, Brodie DA. A quantitative systematic review of normal values for short-term heart rate variability in healthy adults. *PACE - Pacing Clin Electrophysiol*. 2010;33(11):1407-1417. doi:10.1111/j.1540-8159.2010.02841.x
231. Geovanini GR, Vasques ER, de Oliveira Alvim R, et al. Age and Sex Differences in Heart Rate Variability and Vagal Specific Patterns – Baependi Heart Study. *Glob Heart*. 2020;15(1):1-12. doi:10.5334/GH.873
232. Baek HJ, Cho C-H, Cho J, Woo J-M. Reliability of ultra-short-term analysis as a surrogate of standard 5-min analysis of heart rate variability. *Telemed J e-health Off J Am Telemed Assoc*. 2015;21(5):404-414. doi:10.1089/tmj.2014.0104
233. Munoz ML, van Roon A, Riese H, et al. Validity of (Ultra-)Short Recordings for Heart Rate Variability Measurements. *PLoS One*. 2015;10(9):e0138921. doi:10.1371/journal.pone.0138921
234. Nussinovitch U, Elishkevitz KP, Katz K, et al. Reliability of Ultra-Short ECG Indices for Heart Rate Variability. *Ann noninvasive Electrocardiol Off J Int Soc Holter Noninvasive Electrocardiology, Inc*. 2011;16(2):117-122. doi:10.1111/j.1542-474X.2011.00417.x
235. Salahuddin L, Cho J, Jeong MG, Kim D. Ultra short term analysis of heart rate variability for monitoring mental stress in mobile settings. *Annu Int Conf IEEE Eng Med Biol Soc IEEE Eng Med Biol Soc Annu Int Conf*. 2007;2007:4656-4659. doi:10.1109/IEMBS.2007.4353378
236. Shaffer F, Shearman S, Meehan ZM. The Promise of Ultra-Short-Term (UST) Heart Rate Variability Measurements. *Biofeedback*. 2016;44(4):229-

233. doi:10.5298/1081-5937-44.3.09
237. Rosengård-Bärlund M, Bernardi L, Fagerudd J, et al. Early autonomic dysfunction in type 1 diabetes: a reversible disorder? *Diabetologia*. 2009;52(6):1164-1172. doi:10.1007/s00125-009-1340-9
238. Bernardi L, Gabutti A, Porta C, Spicuzza L. Slow breathing reduces chemoreflex response to hypoxia and hypercapnia, and increases baroreflex sensitivity. *J Hypertens*. 2001;19(12):2221-2229. doi:10.1097/00004872-200112000-00016
239. Lefrandt JD, Hoogenberg K, van Roon AM, Dullaart RP, Gans RO, Smit AJ. Baroreflex sensitivity is depressed in microalbuminuric Type I diabetic patients at rest and during sympathetic manoeuvres. *Diabetologia*. 1999;42(11):1345-1349. doi:10.1007/s001250051448
240. Weston PJ, James MA, Panerai RB, McNally PG, Potter JF, Thurston H. Evidence of defective cardiovascular regulation in insulin-dependent diabetic patients without clinical autonomic dysfunction. *Diabetes Res Clin Pract*. 1998;42(3):141-148. doi:10.1016/s0168-8227(98)00094-1
241. Frattola A, Parati G, Gamba P, et al. Time and frequency domain estimates of spontaneous baroreflex sensitivity provide early detection of autonomic dysfunction in diabetes mellitus. *Diabetologia*. 1997;40(12):1470-1475. doi:10.1007/s001250050851
242. Julu PO. A linear scale for measuring vagal tone in man. *J Auton Pharmacol*. 1992;12(2):109-115. doi:10.1111/j.1474-8673.1992.tb00368.x
243. Farmer AD, Coen SJ, Kano M, et al. Normal values and reproducibility of the real-time index of vagal tone in healthy humans: a multi-center study. *Ann Gastroenterol*. 2014;27(4):362-368.
244. Lin Y-D, Diedrich A. Editorial: Methods on the Assessment of Human Baroreflex Function. *Front Neurosci*. 2022;16:965406. doi:10.3389/fnins.2022.965406
245. Brock C, Jessen N, Brock B, et al. Cardiac vagal tone, a non-invasive measure of parasympathetic tone, is a clinically relevant tool in Type 1 diabetes mellitus. *Diabet Med*. 2017;34(10):1428-1434. doi:10.1111/dme.13421
246. Wegeberg AM, Lunde ED, Riahi S, et al. Cardiac vagal tone as a novel screening tool to recognize asymptomatic cardiovascular autonomic neuropathy: Aspects of utility in type 1 diabetes. *Diabetes Res Clin Pract*. 2020;170:108517. doi:10.1016/j.diabres.2020.108517
247. Fleischer J, Cichosz SL, Jakobsen PE, et al. The degree of autonomic modulation is associated with the severity of microvascular complications in patients with type 1 diabetes. *J Diabetes Sci Technol*. 2015;9(3):681-686. doi:10.1177/1932296814567226
248. Galicka-Latała D, Surdacki A, Konduracka E, Kozek E, Latała H. [Heart rate variability in type 1 diabetic patients with retinopathy. Part I. Frequency analysis]. *Przegl Lek*. 2010;67(12):1253-1257.
249. Obara EA, Hannibal J, Heegaard S, Fahrenkrug J. Loss of Melanopsin-Expressing Retinal Ganglion Cells in Patients With Diabetic Retinopathy. *Invest Ophthalmol Vis Sci*. 2017;58(4):2187-2192. doi:10.1167/iovs.16-

21168
250. Zhang Y, Zhang J, Wang Q, et al. Intravitreal injection of exendin-4 analogue protects retinal cells in early diabetic rats. *Investig Ophthalmol Vis Sci*. 2011;52(1):278-285. doi:10.1167/iovs.09-4727
251. Shu X, Zhang Y, Li M, et al. Topical ocular administration of the GLP-1 receptor agonist liraglutide arrests hyperphosphorylated tau-triggered diabetic retinal neurodegeneration via activation of GLP-1R/Akt/GSK3β signaling. *Neuropharmacology*. 2019;153(March):1-12. doi:10.1016/j.neuropharm.2019.04.018
252. Simó R, Hernández C, Porta M, et al. Effects of Topically Administered Neuroprotective Drugs in Early Stages of Diabetic Retinopathy: Results of the EUROCONDOR Clinical Trial. *Diabetes*. 2019;68(2):457-463. doi:10.2337/db18-0682
253. Silva AKF da, Christofaro DGD, Bernardo AFB, Vanderlei FM, Vanderlei LCM. Sensibilidade, Especificidade e Valor Preditivo dos Índices da Variabilidade da Frequência Cardíaca no Diabetes Mellitus Tipo 1. *Arq Bras Cardiol*. 2017;108(3):255-262. doi:10.5935/abc.20170024
254. Sanchez-Gonzalez MA, May RW, Koutnik AP, Kabbaj M, Fincham FD. Sympathetic vasomotor tone is associated with depressive symptoms in young females: a potential link between depression and cardiovascular disease. *Am J Hypertens*. 2013;26(12):1389-1397. doi:10.1093/ajh/hpt131
255. Jarczewski J, Furgała A, Winiarska A, Kaczmarczyk M, Poniatowski A. Cardiovascular response to different types of acute stress stimulations. *Folia Med Cracov*. 2019;59(4):95-110. doi:10.24425/fmc.2019.131383
256. Ghiasi S, Greco A, Barbieri R, Scilingo EP, Valenza G. Assessing Autonomic Function from Electrodermal Activity and Heart Rate Variability During Cold-Pressor Test and Emotional Challenge. *Sci Rep*. 2020;10(1):1-13. doi:10.1038/s41598-020-62225-2
257. MacArtney MJ, McLennan PL, Peoples GE. Heart rate variability during cardiovascular reflex testing: The importance of underlying heart rate. *J Basic Clin Physiol Pharmacol*. 2021;32(3):145-153. doi:10.1515/jbcpp-2020-0245
258. Tulppo MP, Kiviniemi AM, Hautala AJ, et al. Physiological background of the loss of fractal heart rate dynamics. *Circulation*. 2005;112(3):314-319. doi:10.1161/CIRCULATIONAHA.104.523712
259. Dejgaard TF, von Scholten BJ, Christiansen E, et al. Efficacy and safety of liraglutide in type 1 diabetes by baseline characteristics in the ADJUNCT ONE and ADJUNCT TWO randomized controlled trials. *Diabetes Obes Metab*. Published online August 2021. doi:10.1111/dom.14532
260. Baggio LL, Ussher JR, McLean BA, et al. The autonomic nervous system and cardiac GLP-1 receptors control heart rate in mice. *Mol Metab*. 2017;6(11):1339-1349. doi:10.1016/j.molmet.2017.08.010
261. Nyström T, Santos-Pardo I, Fang X, Cao Y, Hedberg F, Jendle J. Heart rate variability in type 2 diabetic subjects randomized to liraglutide or glimepiride treatment, both in combination with metformin: A randomized, open, parallel-group study. *Endocrinol diabetes Metab*. 2019;2(2):e00058.

doi:10.1002/edm2.58

262. Asmar A, Simonsen L, Asmar M, et al. Renal extraction and acute effects of glucagon-like peptide-1 on central and renal hemodynamics in healthy men. *Am J Physiol Endocrinol Metab*. 2015;308(8):E641-9. doi:10.1152/ajpendo.00429.2014
263. Smits MM, Tonneijck L, Muskiet MHA, et al. Heart rate acceleration with GLP-1 receptor agonists in type 2 diabetes patients: an acute and 12-week randomised, double-blind, placebo-controlled trial. *Eur J Endocrinol*. 2017;176(1):77-86. doi:10.1530/EJE-16-0507
264. Skov J, Pedersen M, Holst JJ, et al. Short-term effects of liraglutide on kidney function and vasoactive hormones in type 2 diabetes: a randomized clinical trial. *Diabetes Obes Metab*. 2016;18(6):581-589. doi:10.1111/dom.12651
265. Hunter K, Hölscher C. Drugs developed to treat diabetes, liraglutide and lixisenatide, cross the blood brain barrier and enhance neurogenesis. *BMC Neurosci*. 2012;13:33. doi:10.1186/1471-2202-13-33
266. Holt MK, Cook DR, Brierley DI, et al. PPG neurons in the nucleus of the solitary tract modulate heart rate but do not mediate GLP-1 receptor agonist-induced tachycardia in mice. *Mol Metab*. 2020;39:101024. doi:10.1016/j.molmet.2020.101024
267. Lam BL, Lam S, Walls RC. Prevalence of palpebral fissure asymmetry in white persons. *Am J Ophthalmol*. 1995;120(4):518-522. doi:10.1016/s0002-9394(14)72667-4
268. Okdahl T, Brock C. Molecular Aspects in the Potential of Vitamins and Supplements for Treating Diabetic Neuropathy. *Curr Diab Rep*. 2021;21(9):31. doi:10.1007/s11892-021-01397-1

ISSN (online): 2246-1302
ISBN (online): 978-87-7573-667-6

AALBORG UNIVERSITY PRESS