Diabetes mellitus type 2; The incretin effect and interaction with the autonomic nervous system

Sondre Vatne Meling

Thesis for the degree of Philosophiae Doctor (PhD)
University of Bergen, Norway
2023

UNIVERSITY OF BERGEN

Diabetes mellitus type 2; The incretin effect and interaction with the autonomic nervous system

Sondre Vatne Meling

Thesis for the degree of Philosophiae Doctor (PhD)
at the University of Bergen

Date of defense: 22.09.2023

Year: 2023

Title: Diabetes mellitus type 2; The incretin effect and interaction with the autonomic nervous system

Name: Sondre Vatne Meling

Print: Skipnes Kommunikasjon / University of Bergen

Scientific environment

Institute of Clinical Medicine (K2),
Faculty of Medicine and Dentistry

Department of Medicine, Sections of
Endocrinology and Gastroenterology

Department of Clinical Medicine

Department of Gastroenterology and
Hepatology, Aalborg University Hospital

Center for Clinical Metabolic Research

Acknowledgements

First, I would like to thank Eirik Søfteland, my main supervisor, for picking up the phone, the autumn of 2018, presenting me with at least six different projects he had in mind. Luckily, one of the projects, involving incretins, was a good match for the both of us, and this started off a cooperation leading us to where we are today. As main supervisor Eirik has always been available for advice and support, as a colleague we have cooperated in numerous other projects, both national and international, and as a friend letting me stay in his house during writing periods and walking together in the mountains of Bergen. I am forever grateful for giving me the opportunity to engage in the field of research and to start a lifelong relationship.

I would like to thank my co-supervisors; Niels Ejskjær, for interesting discussions and always positive feedback, your contribution to the project and papers, and expanding my international research network. Thank you also for your financial support, when the Helse Vest scholarship was emptied. Pål Njølstad, for letting us use his research infrastructure at the Center for Diabetes Research, for always keeping his door open when I have been in Bergen and supporting our work with his great experience. And in Stavanger, Siri Carlsen for daily local support, facilitating the combined clinical work, and for being a critical voice from outside the project group. In the same setting I would like to thank Erling Tjora, who in many circumstances has acted like a co-supervisor, being one of the founders of the project and main investigators, always in place for discussion or a comment, with a clever smile on his face.

I would like to thank the other main members of the project group; our study nurse Lars Johan Steinsvik, for always being available despite mainly 20% employment in the project. Without Lars Johan, there would not be a proper working online Clinical Trial Management Programme, there would be no backup data collection, no proper examination equipment in the right place at the right time, and no extra hands drawing blood from participants. To Heike Eichele, which contribution has been invaluable, not only as a main investigator keeping control of the EEG, but also

participating in drawing blood for several hours every day, revising articles and not, but least, the important everyday talk during and in between examinations.

All people working with the PanGut project and myself are in great debt to our friends, partners and colleagues in Denmark; The Mech Sense group in Aalborg with Christina Brock, Rasmus Bach Nedergaard and Asbjørn Drewes, both contributing to planning, lending us and teaching us how to use their custom-designed inflation device, evaluation of results from the evoked potential tests, interpretation, discussion and critically revising all results. To Filip Knop at Gentofte Hospital for helping plan the project, teaching us how to administer intravenous isoglycemic glucose infusion, always available for advice, discussions and interpretation of results, and last, but not least, for constantly keeping me up to date on incretins via his Twitter account.

Thanks to Liv Aasmul at the Centre for Diabetes Research, for invaluable support performing glucose tests, and for overseeing all blood samples. Also, thanks to the people working at the Research Unit for Health Surveys (FHU), University of Bergen, for providing great examination facilities and support in several of the project examinations.

Thanks to the regional Health Authorities of Western Norway for providing me with a scholarship, being able to conduct this PhD project, and also supporting the PanGut project itself. Thanks to other financial sources of the PanGut project, the Johan Selmer Kvanes Legacy and the Department of Laboratory Medicine and Pathology, Haukeland University Hospital.

A great thank you goes to all participants of the PanGut project. I am humble and deeply grateful for the people willingly volunteering to be examined in such a comprehensive way, including having me inflating a balloon inside their rectum and spending numerous hours of their time with us. Without you, there would be no PanGut project or other clinical studies.

I would like to express my gratitude to colleagues in Stavanger for working in an inspiring environment and for having a laugh at work every day. I would also like to

thank John Cooper, my former boss, colleague and mentor, for his encouraging passion for diabetes care and research for several decades.

Last but not least, I would like to thank my family: My parents for providing a safe childhood, giving me the tools to manage every challenge faced in life, and for supporting us in our daily lives. To my sister and brother for first contributing to my daily challenges, and later being friends for life. To my mother-in-law Anne who has helped almost every time I went to Bergen for the project, and father-in-law Jens Marius, just for being the person he is. To my children, Emmi, Ebbe, Eine, Eik, and Eili, for every day providing me with the true meaning of life and finally, my beloved wife Siri, who keeps up with me every day and whose daily smile is the single largest source of energy in my life. This work would not be possible without your trust and support.

Sammendrag

Bakgrunn: Inkretineffekten er kroppens evne til økt insulinsekresjon når glukose inntas peroralt sammenliknet med administrert intravenøst, utløst av spesifikke hormoner fra tarmen. En redusert inkretineffekt leder til forhøyet blodsukker etter måltid, og er et tidlig fenomen ved diabetes type 2, også påvist i forstadier til diabetes, såkalt prediabetes, og ved fedme. En bevart inkretineffekt ser delvis ut til å være avhengig av et intakt autonomt nervesystem. Autonom nevropati har vært betraktet som en sen komplikasjon til diabetes mellitus, men det er økende evidens for at nevropati også kan oppstå tidlig i forløpet. Kjennskap til disse faktorene ledet oss til en hypotese om at tidlig autonom nevropati kan bidra til den reduserte inkretineffekten ved diabetes type 2.

Mål: Vårt primære mål var å undersøke om det var assosiasjon mellom inkretineffekt og grad av autonom nevropati. Sekundære mål var å se på inkretineffekten relatert til grad av hyperglykemi og varighet av diabetes, og sammenlikne en ny test som innebærer ballongdilatasjon i rektum, som mål for tarmens sensitivitet og videre signaloverføring, med mer etablerte tester for nevropati. Et siste sekundærmål var å undersøke gjennomførbarheten av en norsk versjon av spørreskjemaet, «Composite Autonomic Symptom Score» (COMPASS) 31, som kan påvise mulige symptomer fra autonom dysfunksjon, og vi testet om dette var assosiert med øvrige nerveundersøkelser.

Metode: Tre grupper ble inkludert; en gruppe med diabetes type 2 varighet >10 år, en gruppe med nyoppdaget type 2 diabetes siste året, uten behov for medikamentell behandling, og en kontrollgruppe matchet for alder, kjønn og kroppsmasseindeks. Inkretineffekten ble kalkulert fra c-peptid (areal under kurven) ved oral glukosebelastning sammenliknet med intravenøs isoglykemisk glukose infusjon. Gastrointestinal glukose-håndtering (GIGD) ble kalkulert fra glukose gitt oralt sammenliknet med glukose tilført intravenøst. Tester for nevropati inkluderte kardiovaskulære reflekstester, hjertefrekvensvariabilitet, svettefunksjon, nerveledningshastighet i nervus suralis og monofilament test. Som mål på

gastrointestinal visceral nervefunksjon utførte vi rektal ballongdilatasjon med registrering av trykk for første følelse av dilatasjon og ubehagelig følelse av dilatasjon. Evokerte hjernepotensial ble målt som respons på ballongdilatasjon ved gjentatte stimuli av nevnte trykk. Spørreskjemaet COMPASS 31 ble besvart digitalt.

Resultat: Deltakerne med diabetes trengte høyere trykk for å oppnå første følelse av ballongdilatasjon i rektum, uavhengig av diabetesvarighet. Økt behov for trykk korrelerte med nedsatt GIGD, men ikke med inkretineffekt. Økt behov for trykk korrelerte også med nedsatt følelse på monofilament test. GIGD og inkretineffekt korrelerte signifikant med både grad av hyperglykemi og diabetesvarighet. Det ble funnet få tilfeller av nevropati totalt sett, og få forskjeller mellom gruppene. Det var en tendens til at lenger latenstid og mindre amplituder på evokerte hjernepotensial var assosiert med lavere hjertefrekvensvariabilitet og kardiovaskulære reflekstester, sural nerveledning og monofilament test, men ikke statistisk signifikant etter korreksjon for multippel testing. Høyere score på COMPASS 31 ble funnet hos dem med langvarig diabetes og hos kvinner, med best sensitivitet og negativ prediktiv verdi for score $\leq$10.

Konklusjon: Vi fant rektal hyposensitivitet både ved langvarig og tidlig type 2 diabetes og dette var assosiert med redusert GIGD, men ikke med redusert inkretineffekt. Dette kan tyde på at adekvat nervefunksjon i tarmen er viktig for andre faktorer enn inkretineffekten i håndteringen av glukose. Redusert GIGD og inkretineffekt er assosiert med økende hyperglykemi og varighet av diabetes, som viser et kontinuum i tarmens glukosehåndtering fra normo- til hyperglykemi. Vi fant klinisk plausible tegn på at sentral nerveledning er assosiert med perifer nervefunksjon, men resultatene må tolkes med forsiktighet, gitt multippel testing. Rektal ballongdilatasjon med måling av sensitivitet og evokerte hjernepotensial synes å være en lovende metode for undersøkelse av nervefunksjon i tarmen, også når øvrige autonome tester er normale. Til sist finner vi spørreskjemaet COMPASS 31 lovende til bruk både i forskning, men også i den kliniske hverdag, hvor autonome symptomer ofte er neglisjert. I en liknende populasjon som vår vil en score på 10 poeng eller mindre nærmest utelukke kardiovaskulær autonom nevropati.

Abstract

Background: The incretin effect refers to the amplified insulin response when glucose is administered orally compared to intravenously. A reduced incretin effect is found at early stages of type 2 diabetes, even in prediabetes and obesity, but the mechanisms behind are unknown. Evidence suggests that part of the effect of incretin hormones are mediated through vagal nerve transmission. Diabetic autonomic neuropathy is considered a late complication of diabetes mellitus, but there is an increasing awareness that neuropathy can exist in both prediabetes and early stages of diabetes. This led us to the hypothesis that the incretin effect could be affected by early autonomic neuropathy because of a reduced transmission of signals.

Aims: Our main objective was to explore whether a reduced incretin effect could be associated with autonomic neuropathy. Secondarily, we aimed to explore the incretin effect in relation to degree of dysglycemia and the duration of diabetes. Other secondary objectives were to explore a novel test of gut visceral sensitivity and central transmission of peripheral signals, and to compare it with established tests for diabetic neuropathy, including assessment of symptoms using the Composite Autonomic Symptom Score (COMPASS) 31.

Methods: This was case-control study including three groups of participants: People with type 2 diabetes for >10 years (longstanding), people with newly discovered type 2 diabetes within the last year, without the need for antidiabetic medication (early), and a group of matched controls in age, sex, and body mass index. An oral glucose tolerance test followed by an intravenous isoglycemic glucose infusion were performed to calculate the incretin effect (from c-peptide area under the curve). Gastrointestinal-mediated glucose disposal (GIGD) was calculated as an estimate of the body's ability to cope with the challenge of a carbohydrate ingestion. Neuropathy tests included cardiovascular reflex tests, heart rate variability, sudomotor function, sural nerve, and the monofilament test. Rapid rectal balloon distention measuring visceral sensitivity and evoked potentials was performed as a proxy for gut autonomic

nerve function. The COMPASS 31 questionnaire was distributed and answered online.

Results: Both groups of diabetes were hyposensitive to first sensation performing rapid rectal balloon distention. Also, those with reduced sensation performing the monofilament test showed hyposensitivity. A correlation was found between rectal hyposensitivity at the first sensation and reduced GIGD, but not with the incretin effect. Both GIGD and the incretin effect were found to correlate with degree of dysglycaemia and duration of diabetes, and were comparable to previous studies. Overall, few cases of confirmed neuropathy were detected, and there were few differences between groups regarding established neuropathy tests. Longer evoked potential latencies and smaller amplitudes plausibly correlated with lower heart rate variability and cardiovascular reflex test score, reduced parameters in the sural nerve test and monofilament sensation, but not statistically significant considering multiple testing. Higher scores in COMPASS 31 were correlated with longstanding diabetes and female sex. We found an acceptable negative predictive value for cardiovascular autonomic neuropathy at a 10-point cut-off .

Conclusions: Rectal hyposensitivity may be an early manifestation of type 2 diabetes, and associated with GIGD, but not with the incretin effect. GIGD and the incretin effect are associated with degree of dysglycemia and duration of diabetes, indicating a continuum in the diminished effect. Central neuronal signal processing appears to be affected in parallel with peripheral neuronal function, but the results must be interpreted with caution. In general, we found that investigating evoked potentials following rapid rectal balloon distention may be a useful research tool for evaluating gut autonomic neuropathy, also when other autonomic neuropathy tests are normal. The Norwegian version of COMPASS 31 was easy to use and for assessing autonomic neuropathy in diabetes, and we suggest a cut off at ten points for screening purposes. Symptoms of autonomic neuropathy seems to be more frequent in people with longstanding diabetes and in women.

LIST OF ABBREVIATIONS

ADA	American Diabetes Association	GAD	Glutamic acid decarboxylase
AgRP	Agouti-related protein	GCSI	Gastroparesis cardinal symptom index
AUC	Area under the curve	GI	Gastrointestinal
BMI	Body mass index	GIGD	Gastrointestinal-mediated glucose disposal
CAN	Cardiovascular autonomic neuropathy	GIP	Glucose-dependent insulinotropic peptide
CARTs	Cardiovascular reflex tests	GLP	Glucagon like peptide
CCK	Cholecystokinin	HbA1c	Haemoglobin A1c
COMPASS	Composite Autonomic Symptom Score	HRV	Heart rate variability
CNS	Central nervous system	ICA	Individual component analysis
DPN	Diabetic (distal) polyneuropathy	IGLEs	Intraganglionic laminar endings
DPP	Dipeptidyl peptidase	IMA	Intramuscular arrays
EECs	Enteroendocrine cells	LADA	Latent (late) autoimmune diabetes of adults
EEG	Electroencephalogram	MARD	Moderate age-related diabetes
eGFR	Estimated glomerular filtration rate	MOD	Moderate obesity-related diabetes
EI ratio	Expiration-Inspiration ratio	MODY	Maturity onset diabetes of the young
fMRI	Functional magnetic resonance imaging	NT	Neurotensin
FPG	Fasting plasma glucose	NTS	Nucleus tractus solitarius

GABA	Gamma-aminobutyric acid	RMSDD	Root mean square of the standard deviation from the mean heartbeat interval value
NPY	Neuropeptide Y	RS ratio	Resting to standing ratio
OGTT	Oral glucose tolerance test	SAID	Severe autoimmune diabetes
OXM	Oxyntomodulin	SDNN	Standard deviation from the mean heartbeat interval value
PAGI-SYM	Patient assessment of upper GI symptom severity index	SIDD	Severe insulin deficient diabetes
POMC	Proopiomelanocortin	SIRD	Severe insulin resistant diabetes
PP	Pancreatic polypeptide	VAS	Visual analogue scale
PYY	Peptide YY	VM ratio	Valsalva Manoeuvre ratio
mRNA	Messenger ribonucleic acid		

LIST OF PUBLICATIONS

I. **Meling S**, Tjora E, Eichele H, Nedergaard RB, Ejskjaer N, Brock C, Søfteland E. *The PanGut-study: Evoked potentials following rectal balloon distention, a way of evaluating diabetic autonomic neuropathy in the gut?* Journal of Diabetes and its Complications. 2023;37(5):108452.

II. **Meling S**, Tjora E, Eichele H, Ejskjaer N, Carlsen S, Njølstad PR, Brock C, Søfteland E. *The Composite Autonomic Symptom Score 31 Questionnaire, sensitive test to detect risk for diabetic autonomic neuropathy.* Submitted 12.05.23 to *Journal of Diabetes Research, Submission ID 4441115****. Paper published 9. Aug 2023. DOI: 10.1155/2023/4441***

III. **Meling S**, Tjora E, Eichele H, Nedergaard RB, Ejskjær N, Knop FK, Carlsen S, Njølstad PR, Brock C, Søfteland E. *Autonomic nerve function was not associated with the incretin effect, but with gastrointestinal-mediated glucose disposal.* Submitted 26.05.23 to *Diabetologia*, *Manuscript ID: Diab-23-0765*

RELATED PUBLICATION

I. **Meling S**, Bertoli D, Sangnes DA, et al. Diabetic Gastroenteropathy: Soothe the Symptoms or Unravel a Cure? Curr Diabetes Rev. 2022;18(5):e220321192412. doi:10.2174/1573399817666210322154618

Contents

1. INTRODUCTION

Type 2 diabetes is a multifactorial disease where hyperglycaemia is the defining character, and one of the mechanisms leading to deteriorating of glycaemic control is a reduced incretin effect.

The mechanisms behind the reduced incretin effect are unknown and might not only be caused by impaired secretion of incretin hormones, but also due to reduced efficacy. One way of mediating the incretin signal is through vagal transmission. Directly testing vagal nerve function in humans is difficult, and so far, only indirect tests have existed.

Diabetic neuropathy is considered a late complication of diabetes, but awareness has increased that neuropathy can be detected to a large degree at the time of diabetes diagnosis and even in prediabetic stages.

In this thesis, we investigate gut vagal function with a novel test that measures rectal sensitivity and evoked potential following rapid balloon distention in the rectum in people at different stages of type 2 diabetes. We ask the question if the reduced incretin effect found in people with type 2 diabetes could be a consequence of neural transmission failure because of early diabetic neuropathy?

1.1 Diabetes mellitus – an overview

1.1.1 Epidemiology

Diabetes mellitus is an increasing global disease burden, with the number of people living with diabetes worldwide rising from 108 million in 1980, to 537 million adults in 2021 (1). More than 95% of the people living with diabetes have type 2 diabetes (2). At the same time, it is estimated that 541 million adults have impaired glucose tolerance, often referred to as prediabetes, with a global prevalence between 7 and 8% (1). Depending on diagnostic criteria used, the prevalence of prediabetes in other studies are reported between 20 and 53% (3).

In Norway, an estimated 316 000 to 345 000 people have diabetes, approximately 23000 with type 1 diabetes, between 235 000 and 260 000 with type 2 diabetes, and approximately 60 000 having undiagnosed diabetes (4).

Of interest, the figures from Norway the last period have shown a tendency of increasing prevalence but flattening of incidence. The same trend of stabilizing or even decreasing incidence is reported in other high-income countries (5). The prevalence of diabetes is affected by both the survival of those with diabetes and the risk of developing diabetes, and incidence may be a better metric to investigate the trends of diabetes epidemiology. In both the United States and our neighbouring country Denmark, a substantial decline in the proportion of undiagnosed diabetes is reported, including a decrease in prediabetes compared to previous studies (6, 7). If this is a true decline in incidence, improved awareness of diagnosing or barely reflects a change to HbA1c diagnostics remains to be unknown, some suggesting that the latter may have changed the type 2 diabetes epidemiology (8).

1.1.2 Classifications and etiology

Diabetes mellitus has traditionally been classified as type 1 diabetes, type 2 diabetes, gestational diabetes, and other types of diabetes.

Type 1 diabetes is an autoimmune disease, eventually leading to destruction of beta cells and insulin deficiency and include latent (late) autoimmune diabetes of adults (LADA). The aetiology behind type 1 diabetes are genetic factors in combination with poorly understood environmental factors, possibly involving perinatal, viral, nutritional, or other triggers. Diagnosing LADA can often be challenging, with less classical symptoms than in children or adolescents, and phenotypically more resembling type 2 diabetes (9).

Type 2 diabetes is considered a multifactorial and heterogenous disease. There is a high degree of heritability, but genetics is poorly understood and subject to comprehensive research (10). Other factors involved are described in Figure 1, based on the “ominous octet”, previously described by DeFronzo (11). The main factor

highlighted in the development of type 2 diabetes is excess subcutaneous fat storage, with the development of ectopic fat leading to insulin resistance and hyperinsulinemia (12). Whether insulin resistance or hyperinsulinemia occurs first is still debated and remains to be fully elucidated.

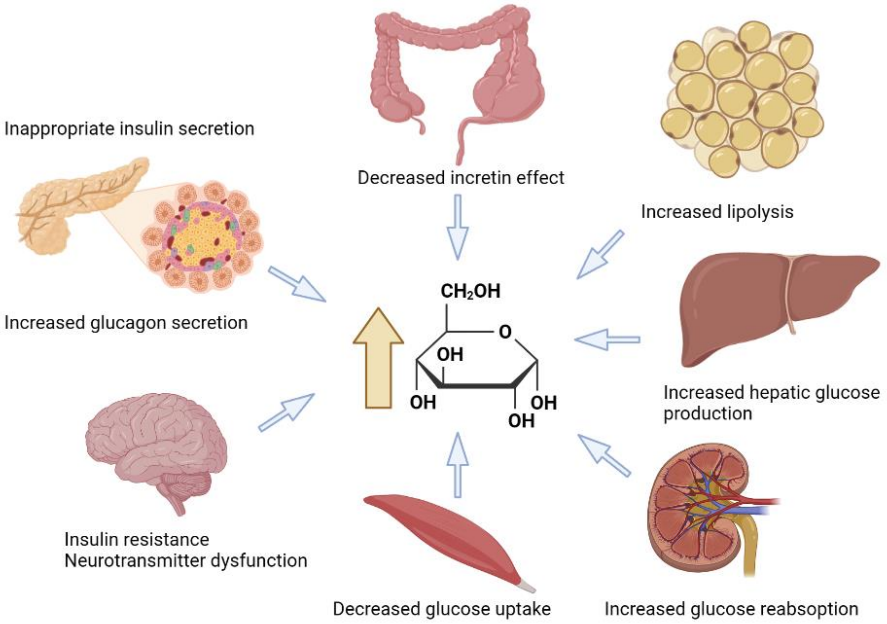


Figure 1: Factors involved in type 2 diabetes, "the ominous octet". Figure created using Biorender.com, based on DeFronzo (11).

Prediabetes, intermediate hyperglycaemia, or impaired glucose tolerance are defined as glucose values above the normal range, but not above the threshold for diabetes. Prediabetes is a heterogeneous state, and the annual conversion rate to type 2 diabetes varies in studies from 5 to 19% depending on the criteria defining the state, with an estimated 70% at some point converting to diabetes (13). The conversion rate in older age is reported to be lower, finding more people with prediabetes who convert to normoglycemia or die before developing diabetes (14). Other studies have reported 55-80% of prediabetes, based on impaired fasting glucose, converting to normoglycemia (15). Figure 2 illustrates a proposed natural history from prediabetes to type 2 diabetes, by Kendall et al (16).

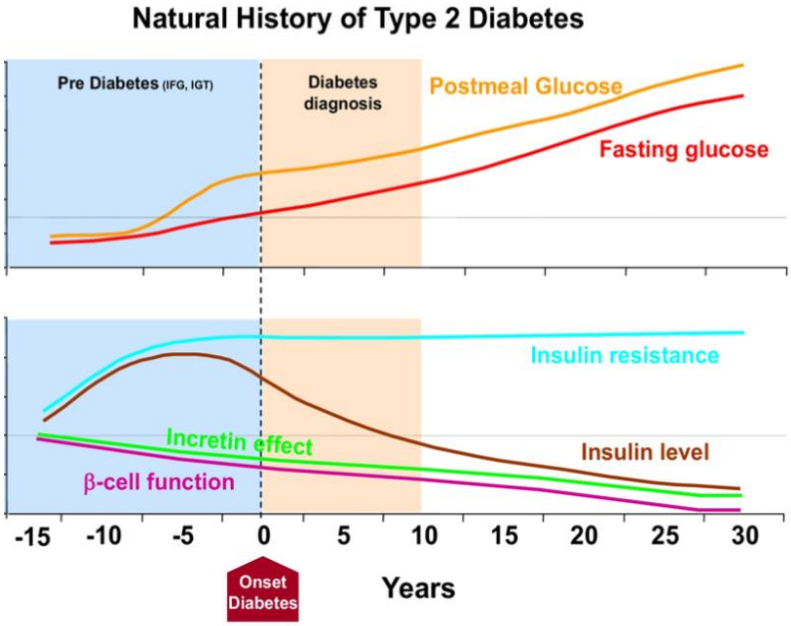


Figure 2. Representative depiction of the natural history of type 2 diabetes mellitus, regarding insulin resistance, insulin deficiency, and impaired incretin effect. Both the time course and the relative function are descriptive. IFG=impaired fasting glucose, IGT=impaired glucose tolerance. From Kendall et al. (16), printed with permission (Licence nr. 5494771179793)

In 2018, a Swedish group proposed a new classification for adult-onset diabetes mellitus, proposing five groups: Severe autoimmune diabetes (SAID), severe insulin deficient diabetes (SIDD), severe insulin resistant diabetes (SIRD), moderate obesity-related diabetes (MOD) and moderate age-related diabetes (MARD). The new classification was based on age at diagnosis, body mass index (BMI), HbA1c, c-peptide, and glutamic acid decarboxylase (GAD) antibodies. The reasons for making new classifications were to enable a more personalised approach in both treatment and prediction of possible complications. Several others have validated the new subgroups in other populations (17-19). In our experience, the new classifications have so far not been implemented to a high degree in clinical practice.

Gestational diabetes is defined as diabetes that occurs during the second or third trimester. *Other types of diabetes* include monogenic diabetes (neonatal diabetes or maturity onset diabetes of the young - MODY), several drugs leading to diabetes, post-transplantation diabetes, diabetes following disease in the exocrine pancreas, or following other diseases such as cystic fibrosis or hemochromatosis (9).

1.1.3 Diagnostic considerations

An International Expert Consensus recommended in 2009 the use of HbA1c $\geq$ 6.5% (now 48 mmol/mol) as main diagnostic criteria for diabetes mellitus (20). If no obvious symptoms of diabetes, the test is recommended repeated before confirming the diagnosis. This diagnostic criteria was implemented in Norway in 2012. Diabetes can still be diagnosed based on fasting plasma glucose (FPG) $\geq$7.0 mmol/L or based on two-hour plasma glucose $\geq$11.1 following an oral glucose tolerance test (OGTT) with 75 g of oral anhydrous glucose dissolved in water. A glucose value $\geq$11.1 is also diagnostic if randomly measured in a person with classical symptoms of hyperglycaemia.

The change of diagnostic criteria was mainly based on the OGTT being inconvenient for both patients and medical staff, requiring patient fasting, increased risk of preanalytical and biological variability, being both time dependent and time consuming and affected by stress and intercurrent disease. The results of OGTT also have considerable intra- and inter-individual variability. However, HbA1c testing may not be available in all parts of the world, is more expensive and has poor correlation with actual glucose levels, especially if other conditions affect haemoglobin. HbA1c also has a lower sensitivity, and at the defined threshold of 6.5%, it diagnoses only 30% of the diabetes cases identified by the three tests collectively (21). In people with discordance between the three tests, both the FPG and the two-hour value are considered more accurate than HbA1c, and the two-hour glucose value after OGTT diagnoses more people with prediabetes and diabetes compared to FPG and HbA1c (22, 23).

Prediabetes is defined by the World Health Organization as impaired fasting glucose between 6.1 and 6.9 mmol/l or impaired glucose tolerance with a glucose value two hours after an oral glucose tolerance test between 7.8 and 11.0 mmol/l. The American Diabetes Association (ADA) has the same definition for the two-hour glucose values, but uses an interval between 5.7 and 6.9 mmol/l for fasting glucose. The discrepancy in fasting glucose definitions was based on the ADA noticing that fewer people with prediabetes were detected using fasting glucose than diagnosed with the two-hour value and hence aimed to decrease the FGP threshold to detect a similar number of people as for the two-hour values. The ADA also included a HbA1c criteria between 5.7% and 6.4% (39-47 mmol/mol) for prediabetes (24).

One of the motives for using HbA1c when diagnosing diabetes was to reduce the number of people with undetected diabetes, and indeed, a recent population-based study in the United Kingdom found that screening with HbA1c reduced the time to diabetes diagnosis by 2.2 years compared to earlier routine care (25). Other studies report a decrease in the incidence of type 2 diabetes, which may be related to the introduction of HbA1c as a diagnostic option (5-8). At the same time, one study found increased type 2 diabetes mortality rates, indicating the selection of a higher risk diabetes population using HbA1c as diagnostic criteria, and thus, missing some of those with borderline HbA1c with increased metabolic and cardiovascular risks (8). Another study screening people with HbA1c found that the highest risk of cardiovascular outcomes and all-cause mortality was just below the diagnostic threshold, highlighting the need for increased focus on cardiovascular risk factors in prediabetes (26).

1.2 Diabetic late complications

In 2021, diabetes was estimated to be responsible for 6.7 million deaths worldwide, representing one person every five seconds (1). Still, in people with both type 1- and type 2 diabetes, cardiovascular complications and all-cause mortality have declined in recent years, in line with the general population (27, 28). Data on mortality are sparser from middle- and low-income countries.

Diabetic late complications are normally divided into macro- and microvascular complications, and do not include acute complications as diabetes ketoacidosis, diabetes hyperosmolar coma and severe hypoglycaemia.

Macrovascular complications include cardiovascular disease as myocardial infarction, ischemic stroke and peripheral arterial disease, and prevention includes treating all cardiovascular risk factors. The role of hyperglycaemia per se in the prevention of cardiovascular disease has been controversial, but the UK Prospective Diabetes Study has shown a strong legacy effect of HbA1c for both myocardial infarction and all-cause mortality (29). The guidelines of the European Society of Cardiology also include a recommendation of HbA1c $\leq$7.0% (53 mmol/mol) in reducing cardiovascular risk for the majority adults with diabetes, with an evidence level of 1, A (30). In recent years there has been increasing knowledge regarding heart failure as a diabetic complication, and there is a greater focus on preventing coexisting cardiorenal disease (31, 32).

Microvascular disease mainly includes retinopathy, nephropathy, and neuropathy. Diabetic retinopathy is still one of the main causes of "avoidable" blindness worldwide, but both prevalence and incidence have declined dramatically in recent years due to effective screening programmes and treatment options (33, 34). Diabetic kidney disease has an estimated prevalence of >25% and lifetime risk estimated to be 40% (35). Worldwide, diabetes remains the most common cause of kidney failure that requires dialysis or kidney transplantation (36). Diabetic neuropathy is discussed further in the next chapter. For microvascular disease, hyperglycaemia per se is considered the most important factor, but prevention also includes the treatment of traditional cardiovascular risk factors.

Other factors predicting diabetic complications may include the recently suggested subgroups for diabetes, with SIDD showing increased risk of retinopathy, SIRD showing increased risk of nephropathy and MARD showing low risk of complications (37).

Diabetes complications are recommended assessed a minimum of once a year, both in international and Norwegian guidelines (38, 39). However, adherence to this is suboptimal, with only 12% of patients followed in Norwegian general practice having all microvascular complications assessed within one year (40).

Whether prediabetes is an individual risk factor for micro-and macrovascular disease is debated. An extensive review has found prediabetes to be positively associated with the risk of cardiovascular disease, heart failure, atrial fibrillation, chronic kidney disease, dementia, cancer, and all-cause mortality (41). The results are supported in another meta-analysis on cardiovascular disease and all-cause mortality (42). However, the increased risk of cardiovascular disease seems to be mainly driven by other associated cardiovascular risk factors, including obesity, and not hyperglycaemia per se (43, 44). An association has also been found with prediabetes and increased risk of retinopathy and neuropathy (45).

1.3 Diabetic neuropathy

1.3.1 Definition and classifications

Diabetic neuropathy is the most common microvascular complication, affecting at least 50% of patients with diabetes mellitus over time. It is defined as a neurodegenerative disorder of the peripheral nervous system, and is assumed to first target sensory axons and autonomic axons (small nerve fibres), and later also motor axons (large nerve fibres) (46).

Diabetic (sensorimotor) polyneuropathy (DPN) is the most prevalent manifestation of diabetic neuropathy, estimated to affect approximately one third of people with diabetes. DPN is a distal and length-dependent symmetric polyneuropathy that most often affects the feet and lower limbs. Symptoms can include different types of pain (burning, electrical, stabbing), hyper and hypoalgesia, numbness, paraesthesia, dysesthesia, or hypoesthesia. It can also include different sensations to temperature, touch and vibration, and even ataxia and cramps. Importantly, up to 50% do not report symptoms at the time of diagnosis. DPN is often classified as painful or

painless, and a feared complication related to DPN, in combination with other risk factors, is diabetic foot ulcers (47).

Diabetic neuropathy can also manifest in more atypical forms like radiculoplexopathy, mononeuropathy and treatment induced neuropathy, often different from DPN in terms of onset (often rapid), course, reversibility, asymmetry in symptoms and signs, and are less associated with duration of diabetes and other complications (48).

The prevalence of neuropathy increases with age and diabetes duration, found in up to 50% of people with type 2 diabetes after 10 years of disease duration (48). DPN can be present in earlier stages of diabetes, early data reporting a prevalence of 21% in those with diabetes duration less than five years, while a more recent study reported a prevalence of 35% in newly diagnosed type 2 diabetes (49, 50). Also, prediabetes has been reported in 25% to 62% of patients with idiopathic peripheral neuropathy, while peripheral neuropathy can be found in 11% to 25% of patients with prediabetes (51). Investigations using corneal confocal microscopy in people with impaired glucose tolerance test detected small-fibre neuropathy and showed a dynamic worsening or improvement in corneal and intraepidermal nerve morphology related to glucose tolerance status (52, 53).

Diabetic autonomic neuropathy is further described in Section 1.4.

1.3.2 Pathogenesis of diabetic neuropathy

The pathogenesis of diabetic neuropathy is multifactorial and include hyperglycaemia, dyslipidaemia, hyperinsulinaemia, inflammation, and advanced glycation end products. Neuronal glucose uptake is highly dependent on extracellular glucose concentration, primarily mediated via glucose transporter 3. The consequence of this is increased glucose uptake during hyperglycaemia, with glucose neurotoxicity (54). The multiple cellular pathways are shown in figure 3, with eventually activation of inflammation and stress with changes in upregulation of cytokines, apoptotic signalling, and changes in gene expression. Damage seems to target the entire neuron,

including cell bodies (in the dorsal root ganglia), degeneration of axons, reduced myelination, damage to Schwann cells, and reduction in neurofilament. Distal sensory nerves seem to be one of the most vulnerable, where of DPN is considered a length-dependent neuropathy. Deficiencies in blood supply to peripheral nerves is also considered a part of the pathogenesis of diabetic neuropathy (46).

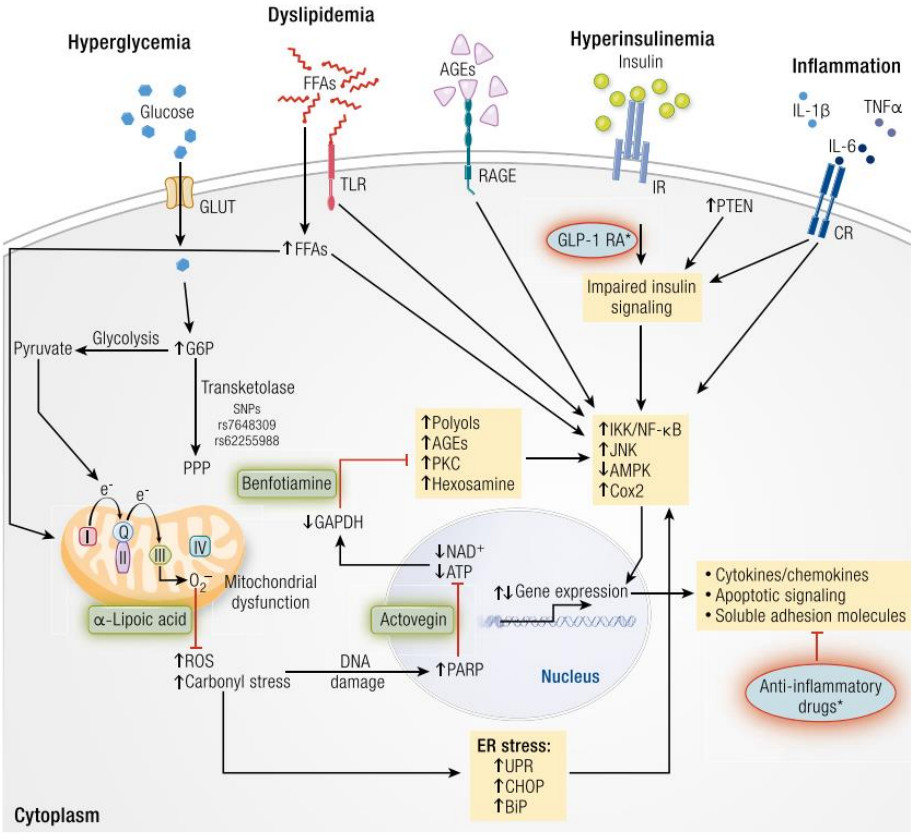


Figure 3: Risk factors and cellular pathways of diabetic neuropathy, including possible pathogenesis-derived treatment options. Abbreviations: FFA; free fatty acid, AGEs; advanced glycation end products, IL; interleukins, TNF; tumour necrosis factor, GLUT; glucose transporter, TLR; Toll-like receptor, RAGE; receptor of AGE, IR; insulin receptor, PTEN; phosphatase and tensin homolog deleted on chromosome 10, CR; cytokine receptor, GLP-1 RA; glucagon like peptide-1 receptor agonist, G6P; glucose-6-phosphate, PPP; pentose phosphate pathway, SNPs; single-

nucleotide polymorphisms, ROS; reactive oxygen species, ER; endoplasmic reticulum, UPR; unfolded protein response, CHOP; CCAAT/enhancer-binding protein homologous protein, BiP, binding immunoglobulin protein, PARP; poly(ADP-ribose) polymerase, ATP; adenosine triphosphate, NAD+; oxidized nicotinamide adenine dinucleotide, GAPDH; glyceraldehyde 3-phosphate dehydrogenase, PKC; protein kinase C, IKK; IkB kinase, NF; nuclear factor, JNK; c-Jun N-terminal kinase, AMPK, 5'adenosine monophosphate–activated protein kinase, Cox2; cyclooxygenase2. From Bönhof et al. (55), printed with permission (Licence nr. 5494270591354)

1.3.3 Diagnosis of diabetic peripheral neuropathy

The gold standard for the diagnosis of DPN is electrophysiological nerve conduction studies. However, these tests are time consuming, impractical to do in a clinical practice, and not available to everyone. In clinical practise, the evaluation of diabetic neuropathy is recommended at least annually in people with diabetes, including a history of symptoms and signs, and the monofilament test. Another test used is vibration sensation using a tuning fork (39, 56). Possible DPN is defined as the presence of symptoms or signs, probable DPN if symptoms or signs and at the same time presence of decreased distal sensation or ankle reflexes. Confirmed DPN is any of the above criteria with the presence of an abnormality on a nerve conduction test (57).

Several point-of-care devices have been developed for detection of DPN. NC-stat DPNCheck measures nerve conduction velocity and amplitude from the large sural nerve of the lower limb (58). Other tests include measurements of distal vibration sensation, skin autofluorescence, and sudomotor function, all showing some association for DPN detection (55). Emerging new screening tools include laser doppler imaging flare technique, corneal confocal microscopy, and perception threshold tracking (59-61).

Physicians should always consider differential diagnoses to DPN such as nerve damage due to alcohol abuse, vitamin B12 deficiency, hypothyroidism, uraemia, and

drug side effects. One should be especially alert for other causes if there is a predominant motor neuron deficit, asymmetric symptoms, rapid development, mononeuropathy, and cranial nerve involvement, and if symptoms occur or worsen despite glycaemic control and/or lack of other diabetic complications. In these circumstances, detailed neurophysiological assessment should be considered (47).

1.3.4 Treatment options

Prevention and management of diabetic neuropathy is based mainly on optimal glucose control and the treatment of other cardiovascular risk factors such as overweight, hypertension, and hyperlipidaemia. However, there is no convincing evidence that intensive glucose control prevents or is effective in treating diabetic neuropathy (46). Drugs for the treatment of neuropathy may be based on pathogenetic mechanisms, and are found in Figure 3. The best evidence is for the use of α-lipoic acid and benfotiamine, both licenced drugs and approved in several countries for use in DPN. α-lipoic acid is a naturally occurring antioxidant and has been found to be effective and safe in both meta-analyses and reviews in treating symptomatic DPN, also in the long term. Benfotiamine is a lipid-soluble thiamine, less studied than α-lipoic acid but also with evidence of improved neuropathic symptoms with few adverse events. Although some studies are underway, there remains a need for longer studies on benfotiamine. A third option is Actovegin, a poly (ADP-ribose) polymerase inhibitor, with some evidence alluding to improved neuropathic symptoms. Other novel agents include antimuscarinic drugs, vitamin E subtypes, glucagon like peptide (GLP)-1 analogues, anti-inflammatory drugs and agents related to growth factors (47, 55, 62). In Norway, α-lipoic acid and benfotiamine are found as dietary supplements.

A third major principle for treating diabetic neuropathy is symptomatic pain relief, but this is beyond the scope of this thesis to describe in detail.

1.4 Autonomic neuropathy

1.4.1 The autonomic nervous system

The autonomic nervous system innervates and controls almost all visceral organs, often referred to as the visceral nerve system. It consists of the sympathetic, parasympathetic, and enteric nervous system, the latter being described in more detail in Section 1.5.1.

The *efferent* preganglionic sympathetic nerves are derived from the levels of Th12 to L2 of the spinal cord, where most of them form synaptic links, called ganglions, in the sympathetic trunk. From here, the postganglionic nerves extend to their target organs. The main preganglionic neurotransmitter is acetylcholine, activating nicotinic receptors, while the postganglionic transmitter is norepinephrine activating α- and β-receptors. The parasympathetic efferent nerves derive from four cranial nerve nuclei, the largest portion contributing fibres to the vagus nerve, and a sacral portion at the level of S2-4. Parasympathetic nerves normally form synaptic links nearby their effector organs, and acetylcholine is the main neurotransmitter both pre- and postganglionic, activating nicotinic and muscarinic receptors respectively (63). A simple illustration of the *efferent* autonomic nervous system is shown in Figure 4.

Approximately 80-85% of nerve fibres in the vagal nerve are *afferent* (54). The afferent fibres of the autonomic nervous system are not necessarily classified as sympathetic or parasympathetic, but they often co-localise within sympathetic and parasympathetic nerves, running in parallel with the efferent nerves. They are supported by Schwann cells. The vagal nerves cluster bilaterally in the jugular and nodose ganglia, the sympathetic nerves in the dorsal root ganglia of the spinal cord, most of them terminating at the nucleus of the solitary tract (NTS) and other areas of the brainstem. Afferent fibres are activated mainly by mechanoreceptors, nociceptors and chemoreceptors. The nerve terminals respond to mechanical pressure or physical deformation, pain or pH, oxygen, carbon dioxide, and lactate, respectively, and show a large diversity with different morphology, size, molecular features, distribution, and partnering cells. Innervated organs and tissues include the heart, lungs, gut, arteries,

larynx, trachea, liver, pancreas, thyroid, and ear (64, 65). Vagal afferent signalling in the gut is thoroughly described in Section 1.5.2

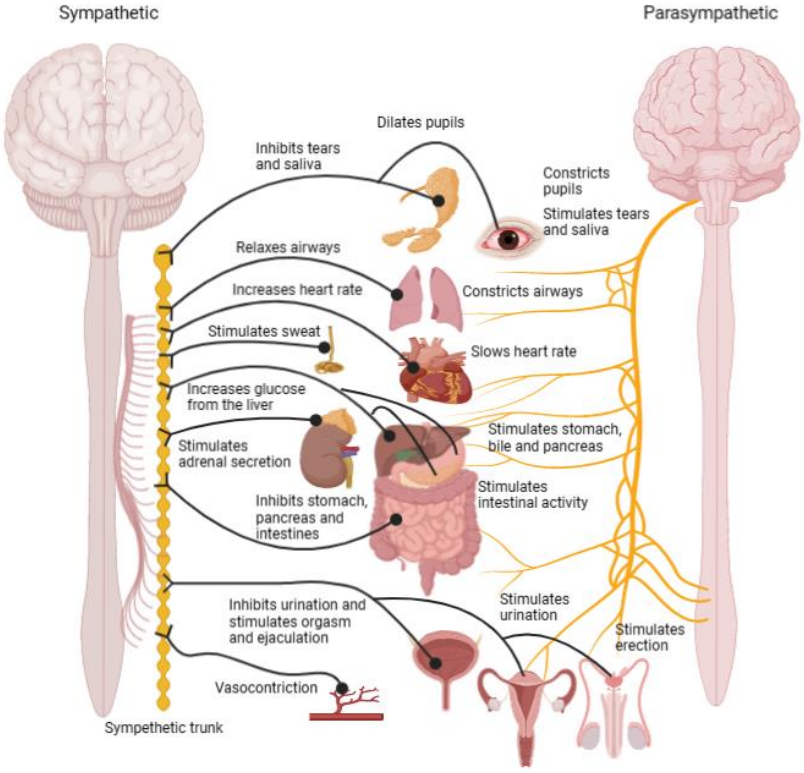


Figure 4: Functions of the autonomic nervous system. Figure created using Biorender.com.

1.4.2 Definition and manifestations

Diabetic autonomic neuropathy was in the Toronto consensus defined as a disorder of the autonomic nervous system in the setting of diabetes or metabolic derangements of prediabetes, after the exclusion of other causes. It may affect the cardiac, gastrointestinal (GI), genitourinary, and sudomotor systems (57). ADA has recommended assessing symptoms and signs of autonomic neuropathy at the time of diagnosis in type 2 diabetes, five years after diagnosis of type 1 diabetes, and thereafter annually. The recommendation has an evidence level E (expert consensus

or clinical experience) (56). Screening includes evaluating possible symptoms and additional tests depending on the affected organ.

The prevalence of autonomic neuropathies is best described for cardiovascular autonomic neuropathy (CAN). In unselected diabetes populations, CAN was found in 17 to 20% of patients with diabetes, increasing with age and diabetes duration. Other factors found to be related to predict CAN are glycaemic control, other cardiovascular risk factors and coinciding microvascular diabetes complications.

Although increased duration of diabetes is a risk factor, the prevalence of CAN in newly diagnosed type 2 diabetes is reported to be around 7% (66, 67). In fact, Ewing and his group in 1977 reported that autonomic nerve damage may be present even at the time of diagnosis of diabetes (68). In patients with prediabetes or metabolic syndrome, the prevalence of CAN is reported up to 11 and 24 %, respectively (69). Previous studies discovering neuropathy in prediabetes are described as heterogenic in terms of the category of glucose abnormality, CAN test modality and sample size, but still seem to indicate sympathovagal imbalance, with lower parasympathetic activity, in people with prediabetes. Correlates of autonomic indices in prediabetes are age, BMI, waist circumference, hypertension, fasting and two-hour glucose, most of them being part of the metabolic syndrome (66). A recent study found autonomic dysfunction, defined by changes in heart rate variability (HRV) to precede the development of type 2 diabetes, especially in younger individuals, but did not conclude on causality (70).

Diabetic gastroenteropathy is extensively described in Section 1.6.

The prevalence of bladder dysfunction is estimated to be around 25% in people with type 2 diabetes, and pathogenetic mechanisms may be due to neuronal dysfunction affecting smooth muscle detrusor and urothelial function (57).

The prevalence of erectile dysfunction among men varies in studies between 35 and 90%, with neuropathy thought to be one of several causes (71). Sexual dysfunction in women with diabetes has been associated with CAN, with an estimated prevalence

between 29 and 51%. A recent Norwegian study found a prevalence of sexual dysfunction in women with type 1 diabetes of 50% vs 35% for women without diabetes (72, 73).

Possible symptoms and manifestations of different autonomic neuropathies are found in Table 1.

Table 1: Symptoms and manifestations of diabetic autonomic neuropathy

Cardiovascular	Orthostatic hypotension, syncope Exercise intolerance Unexplained tachycardia Non-dipping QTi prolongation Impaired heart variability Sudden death, silent myocardial infarction
Urology	Diabetic cystopathy with possible dysuria, incontinence, urgency, hesitation, nocturia, and retention
Sexual	Men: Erectile dysfunction, decreased libido, abnormal ejaculation Women: Decreased libido, dyspareunia, reduced lubrication
Sudomotor	Distal hypohidrosis/anhidrosis Dry skin Hyperhidrosis Gustatory sweating (after food intake)
Others	Hypoglycaemia unawareness Abnormal pupillary function

Sources: (46, 48, 57, 66)

1.4.3 Diagnosing autonomic neuropathy

Cardiovascular autonomic reflex (CARTs) tests are considered the gold standard in the diagnostic work-up of diabetic autonomic neuropathy. They have good sensitivity, specificity, and reproducibility, are non-invasive, and are easy to perform in a clinical setting (57). The test derives from the Ewing tests first described in the late 1970s, and usually includes heart rate response from rest to postural change, during deep breathing, performing the Valsalva manoeuvre, and measuring orthostatic blood pressure (74). The response to postural change and deep breathing are considered predominantly parasympathetic tests, while the Valsalva manoeuvre is predominantly sympathetic. Response to deep breathing and Valsalva, along with orthostatic blood pressure are also considered measures of baroreflex sensitivity and capacity. However, both sympathetic and parasympathetic innervation play a role in all tests since the autonomic pathways involved are complex. Diagnostic criteria for

CAN are debated, but normally one abnormal test is considered as possible or early CAN, ≥ 2 abnormal tests are considered as definite or confirmed CAN, and if the latter includes orthostatic hypotension, the CAN is considered severe or advanced (57, 66). A drawback for the clinical testing of CAN is the lack of prospective studies that assess cost effectiveness.

Later years have seen the emergence of standardized HRV tests with both time- and frequency-domain indices being measured. HRV parameters are mainly used for research, but are also suggested for clinical use in addition to CARTs and for prognostic information (66).

Investigating bladder dysfunction should include a validated questionnaire for lower urinary tract symptoms and urodynamic tests. Evaluation of erectile dysfunction and sexual dysfunction includes a complete patient history to exclude other mechanisms, as well as using validated questionnaires (57).

Several tests exist for the assessment of sudomotor function. For research purposes sympathetic skin response, thermoregulatory sweat testing or quantitative sudomotor axon reflex sweat test are some options. For more clinical purposes, tests using iontophoresis can be used, e.g. the Sensitive Sweat Test or the Sudoscan (75). Sudoscan is reported to have high reproducibility and high to moderate sensitivity and specificity to predict peripheral neuropathy and CAN (76). However, studies on these devices have small sample sizes, have a large patient heterogeneity, and selection bias. There are no clinical studies on hard endpoints or exist any cost-effectiveness analysis implementing them in clinical practice (77). Few of the conducted studies on electrical skin conductance are defined as high quality (76).

The Composite Autonomic Symptom Score (COMPASS) 31 is a questionnaire derived from the original Autonomic Symptom Profile, which evaluates autonomic symptoms. COMPASS 31 assesses the six domains of orthostatic hypotension, vasomotor, secretomotor, GI, bladder, and pupillomotor functions. The questionnaire predicts both diabetic polyneuropathy and CAN with fair diagnostic accuracy and is also validated for evaluating treatment outcomes (78-80).

1.4.4 Clinical considerations

Cardiovascular autonomic neuropathy is associated with mortality and may be used for cardiovascular risk assessment. If detected, a more intense lifestyle intervention and focus on minimizing cardiovascular risk factors are recommended, including glycaemic control. Symptoms and clinical consequences such as tachycardia, non-dipping blood pressure, nocturnal hypertension, and orthostatic hypotension could be treated, and drugs that prolong QT prolongation or affect the autonomic nervous system should be avoided. Of interest, early impairment of baroreflex sensitivity appears to be reversible during slow breathing and is positively affected by physical activity in the long term (66).

For other diabetic autonomic neuropathies, treatment is mainly symptomatic.

1.5 The gut-brain axis

1.5.1 Anatomy of the gut and the enteric nervous system

Before considering diabetic gastroenteropathy in depth, an overview of the anatomy and physiology of the gut and its complex network of neurons and signalling will be provided, in conjunction called the gut-brain axis.

The nervous system of the gut is often divided into internal and external pathways. The internal pathway confers to the enteric nervous system, the ganglionic nerve plexus within the gut wall that can function independently, with reflex pathways controlling motility, blood flow, secretion, and absorption. The extrinsic system consists of the visceral *afferent* pathways, often referred to as the projections of the vagal and spinal (sympathetic) afferents. Extrinsic pathways convey information to higher reflexes involved in digestion and feeding regulation and give rise to painful and non-painful sensations. The enteric and extrinsic afferents also crosstalk extensively (54, 81-83).

An illustration of the gut wall with the enteric nervous system is found in Figure 5, with the caption explaining the main anatomy and physiology.

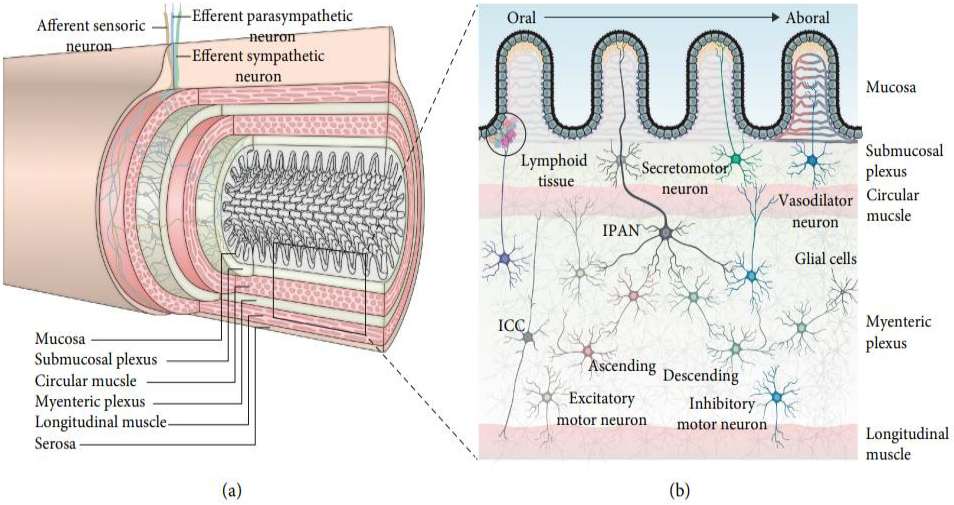


Figure 5: Anatomy of the GI tract and the enteric nervous system. The inner wall consists of the inner mucosa with an epithelial lining and the underlying lamina propria and muscularis. The underlying submucosal plexus is followed by a layer of circular muscle, the myenteric plexus, and a layer of longitudinal muscle. Outermost there is a mesothelial lining called the serosa. Secretomotor and vasodilator neurons are found in the submucosal plexus regulating fluid and molecular exchange between gut lumen, tissue, and vasculature. Peristaltic movements are facilitated by enteric neurons called intrinsic primary afferent neurons (IPANs), through mechanoreceptors or by acetylcholine secreted by enteroendocrine cells in the luminal epithelial cell layer upon luminal distension. IPANs activate ascending and descending interneurons, further activating upstream excitatory and downstream inhibitory motor neurons, respectively. Juxta-positioned networks of enteric glial cells (EGCs) and interstitial cells of Cajal (ICCs) are present in the myenteric plexus. EGCs provide neurotrophic support, mediating interactions between enteric neurons and other cell types (enteroendocrine cells, epithelial cells, immune effector cells and blood vessels). ICCs are not considered neurons but they generate and convey electrical impulses to smooth muscle cells, promoting peristaltic movement, and are often called "pacemakers". Note: The different thicknesses of the layers are not represented proportionally. From Meldgaard et al (54). Printed with permission under the Creative Commons Attribution Licence (CC BY 4.0).

1.5.2 Afferent signalling from the gut

Afferent sensory nerves follow either vagal or spinal routes to provide the central nervous system (CNS) with information needed to maintain GI homeostasis. The vagal and spinal afferents are often collectively called visceral afferents, although there exist several morphological, biochemical, and functional differences. Afferent terminals innervate the wall of the gut, detecting both mechanical stress and hormones related to digestion.

There are three basic *vagal nerve terminal endings* in the gut; Intramuscular arrays (*IMAs*) are located either in the circular- or the longitudinal muscle layer and detect distention of the layers. Intraganglionic laminar endings (*IGLEs*) are found in the myenteric plexus and are sensitive, detecting both distention and contraction of the gut wall, and also sensing changes in gut hormones. Lastly, *mucosal afferent* nerve terminals, in close contact with cells in the gut epithelium, detect changes in hormones related to the nutritional status, including possibly direct sensing of carbohydrates, fat and proteins, and respond to light mucosal stroking (54, 82-84). There also exist direct synapses from vagal afferents with some enteroendocrine cells (EECs), called neuropods, although the role of this pathway remains unclear (85, 86).

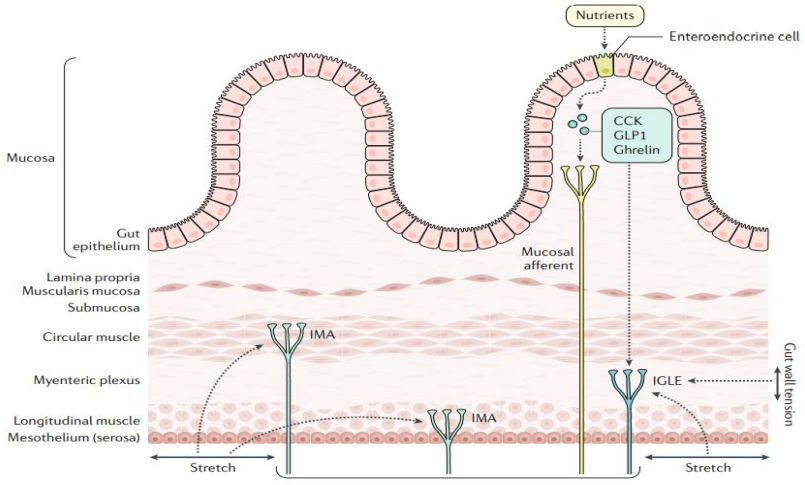


Figure 6: Terminals of the vagal afferent nerves. From Waise et al. (87), printed with permission (Licence nr. 5446450505231).

For *spinal afferent terminals*, the rectum will be delineated subsequently, where there are five classes of innervation: 1) *muscular*, responding to low distention pressures and low intensity stretch, 2) *mucosal*, responding to light stroking of the mucosa, 3) *muscular/mucosal*, with both the latter attributes 4) *serosal*, responding to noxious intensities of distention and 5) so-called *"silent" afferents*, lacking mechanosensitivity, but with the ability to respond to chemical stimuli. Several different nerve transmitters, ion channels and receptors are involved (83). The *muscular* wall is mainly innervated by C fibres, while the *mucosa* is innervated by Aδ fibres (88). The C fibres are unmyelinated with a conduction velocity of 0.5-2.0 m/s, while Aδ fibres are thinly myelinated with a conduction velocity of 3-30 m/s (in contrast to the fastest myelinated Aα fibres with a conduction velocity of 80-120 m/s) (89).

Of special importance to this thesis, the muscular afferents found in the rectum much resemble the IGLEs found in vagal afferents, in the rectum often called rIGLEs. rIGLEs are less complex in structure, smaller in size, are nonpeptidergic, and respond to physiological levels of distention important in the defecatory reflex-pathway. Also, the vagal and rectal mucosal afferents have similar attributes with the generation of brief bursts of action potential in response to light stroking of the mucosa. The response increase proportionally to increased stimuli and is probably important in the detection of stool consistency. Mucosal afferents in the rectum also communicate with enterochromaffin cells, which express serotonin, important for mechanical sensitivity (82, 83).

Vagal afferents follow the same path as efferent vagal nerves, with nerve terminals found in highest density in the upper part of the gut, however, also in the liver and pancreas, see Figure 7. The cell bodies of the vagal afferents are located in the nodose ganglion, with approximately 80% of the neurons being C-type (87). The ganglia are divided into the left and right and reside under the jugular foramen. Several receptors are synthesised in the nodose ganglion, including receptors for GLP-1, cholecystokinin (CCK), and ghrelin.

Different *spinal afferent* pathways include the thoracolumbar (splanchnic nerve), innervating the entire GI tract, and lumbosacral pathways (pelvic nerve) innervating the distal bowel, including the rectum. Spinal afferents have cell bodies in the dorsal root ganglia of the spinal cord (82, 87).

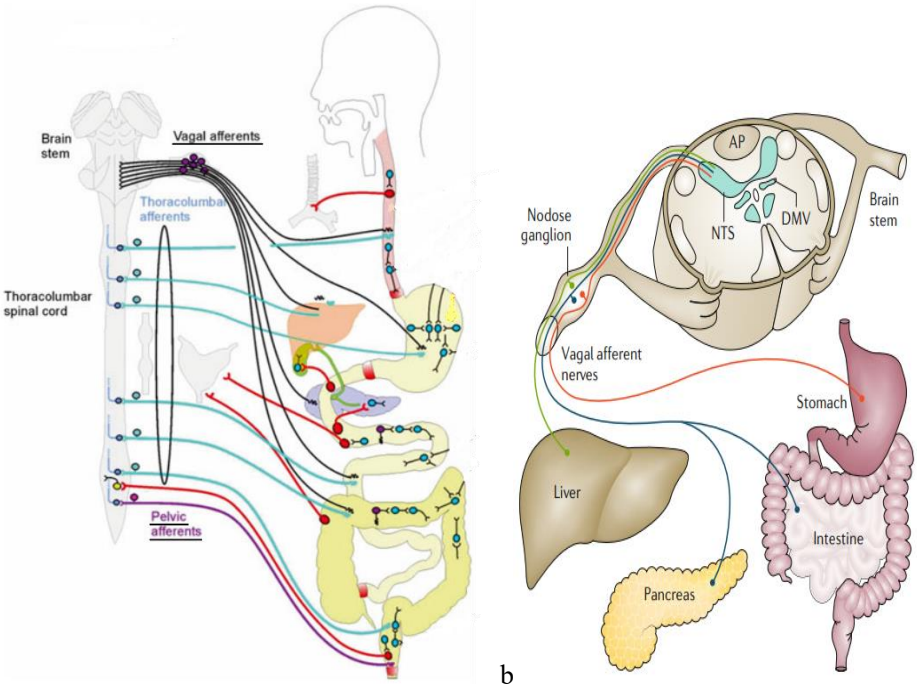


Figure 7: a) Gut afferent pathways. Modified from Payne et al. (90), printed with permission (Licence nr. 5518170231253). b) Vagal afferent pathways from the gut, liver, and pancreas, through the nodose ganglion to the dorsal vagal complex. NTS, nucleus tractus solitarius. AP, area postrema, DMV, dorsal motor nucleus of vagus. From Waise et al. (87), printed with permission (Licence nr. 5446450505231)

In the brainstem, all the nerve endings synapse with the dorsal vagal complex consisting of NTS, area postrema and dorsal motor nucleus of vagus. Its located just beneath the fourth ventricle, the main neurotransmitter being glutamate, and to some extent the neuropeptide Y (NPY). The NTS can also to some extent sense circulating factors directly, expressing receptors for, e.g., amylin, GLP-1, ghrelin, leptin, glucose, and amino acids. The NTS cells themselves express several peptides:

Neurotransmitters glutamate and gamma-aminobutyric acid (GABA), neuromodulators noradrenalin, nitric oxide and brain-derived neurotrophic factor, and neuropeptides GLP-1, NPY, proopiomelanocortin (POMC), cocaine- and amphetamine-regulated transcript, CCK, and prolactin-releasing peptide (91, 92).

From the brainstem, the signal is further transmitted to central brain areas, the most important convergence point being the hypothalamus. The arcuate nucleus of the hypothalamus consists of two main neuronal populations, the NPY/Agouti-related protein (AgPR) neurons and the POMC neurons. NPY/AgPR neurons constitute the main orexigenic (increase appetite) centre, active during negative energy balance, releasing the inhibitory neurotransmitters GABA and NPY. POMC neurons constitute the anorexigenic (decrease appetite) centre, active during positive energy balance, promoting satiety through its main transmitter α-melanocyte stimulating hormone.

From the arcuate nucleus, neurons further modulate the activity of neurons in the paraventricular nucleus. Also involved in the hypothalamus is the ventromedial nucleus expressing leptin, insulin, and brain-derived neurotrophic factor receptors. Both the arcuate nucleus and the ventromedial nucleus are important for glucose metabolism (93, 94). Another group of neurons probably related to increased appetite is the lateral hypothalamic area producing orexin (hypocretin) and melanin-concentrating cells (95). Signalling to these areas includes endocrine information, as the blood-brain barrier is permeable in this part of the brain (96).

Several other involved CNS areas are the prefrontal cortex, amygdala, the ventral tegmental area, anterior cingulate cortex, subfornical organ, striatum, nucleus accumbens, the hippocampus, and insula. These areas receive information from paraventricular areas, but are also directly affected by gut peptides, especially ghrelin. They involve food reward pathways, including food desire, and are capable of turning incoming signals into feeding behaviour (97, 98).

Together, the central brain areas connect to induce an efferent response to the signals received, mainly through the brainstem, vagal efferent, and endocrine signalling, including pituitary hormones.

1.5.3 The world of gut peptides

Gut peptides are produced by EECs spread throughout the GI tract, from the stomach to the rectum. EECs constitute approximately 1% of the endothelial cells in the gut, and are stimulated mainly by nutrients, bile acids and microbiota (98, 99).

The main gut-derived peptides in the gut-brain axis are ghrelin, CCK, GLP-1, glucose-dependent insulinotropic peptide (GIP), peptide YY (PYY), pancreatic peptide (PP), serotonin, neurotensin (NT) and oxyntomodulin (OXM). Gastrin, secretin and motilin are also mentioned to a minor degree affecting the gut-brain axis. Another emerging hormone with a possible key role in energy homeostasis is fibroblast growth factor 21. Primarily derived from the liver, but also expressed in the GI tract, pancreas, and adipose tissue, it increases after the consumption of carbohydrates and can act directly in the CNS with widely expressed receptors, its key target for metabolic effects probably in adipose tissue (100-104).

Traditional classifications of different EECs in the gut are to some extent outdated, as gene studies have shown to express transcripts for several peptides in the same cells. There is also overlap in where the different hormones are produced, e.g., glucagon, PP, and leptin are also produced in the gut, and GLP-1 in the pancreas (102, 105). Some peptides are also produced in the CNS, e.g., GLP-1, and PYY from the brainstem, and CCK, GLP-1 and NT from the central brain areas (92, 98, 102, 106).

Ghrelin has long been considered the only orexigenic peptide, stimulated by fasting and suppressed by nutrient ingestion. It increases appetite and promotes nutrient intake by stimulating the orexigenic centre (NPY/AgRP) and suppress the anorexigenic centre (POMC). Recently, a study has also shown that insulin-like 5 is a possible orexigenic peptide produced in colonic L cells due to caloric restriction (107). The rest of the gut peptides are considered anorexigenic, their main stimulus being exposure to nutrients. The mechanism of promoting satiety is mainly through inhibition of NPY/AgRP neurons or stimulation of POMC neurons. Low levels of leptin and insulin also stimulates NPY/AgRP (98, 99, 101-104).

For a simple overview of where the peptides are produced, and their main attributes, see Figure 8. The incretin hormones, especially GLP-1 will be further described in Section 1.8.

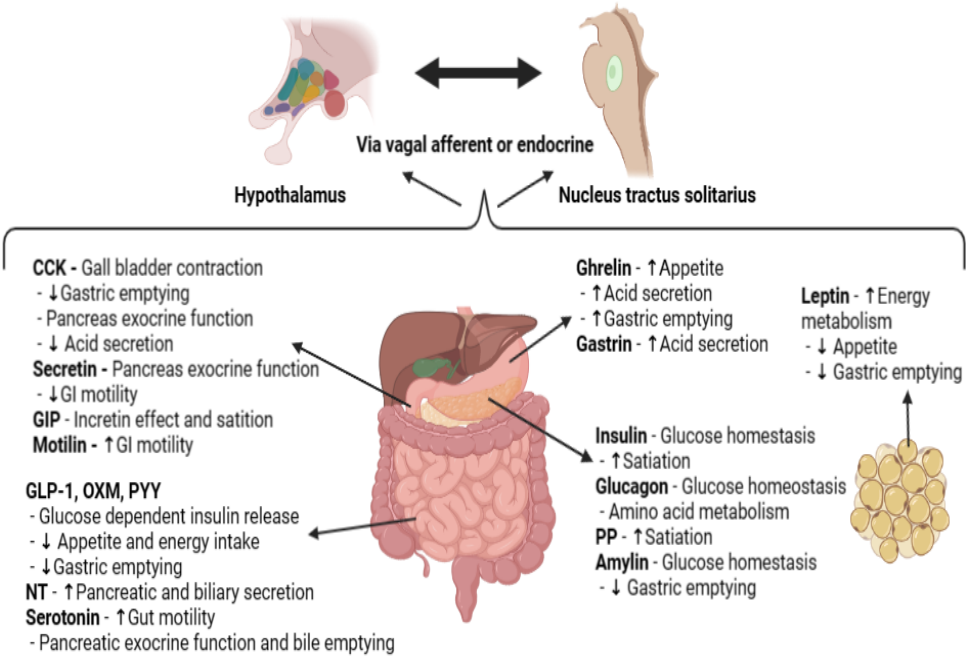


Figure 8: Gut peptides, where and what. Based on (98, 99, 101-104, 108). Figure created using Biorender.com. Abbreviations are found throughout the main text.

Receptors for several peptides are found both on vagal afferents in the gut (ghrelin, GLP-1, PYY, CCK, gastric leptin), in the NTS (GLP-1, CCK) and the hypothalamus (ghrelin, GLP-1, PYY, NT). Peptides are also able to work in an endocrine way, as the mentioned areas do not have a blood-brain barrier, or have fenestrated capillaries allowing the transport of peripheral signals (87, 94).

Concerning the rectum, there exist enterochromaffin cells, D cells and L cells, secreting serotonin, somatostatin, and PYY and GLP-1, respectively (109).

1.6 Diabetic gastroenteropathy

1.6.1 Pathogenesis

The pathogenesis of diabetic gastroenteropathy is complex, with autonomic neuropathy presumed to be a main mechanism, involving several of the same factors and pathways as for diabetic neuropathy in general, described in Section 1.3.2. Particularly enteric glial cells and enteric neurons are found to be vulnerable to hyperglycaemia. The resulting changes include a reduced number of enteric nerve cells, reduced sensory ganglia, structural neuronal changes, intraneuronal biochemical changes, change in neurotransmitter secretion, and altered function of enteric glial cells and interstitial cells of Cajal. Changes also include altered gut-brain communication, with modification in afferent autonomic signalling, loss of neurons in the ganglion, altered neurotransmitters and central processing, with a possible central reorganisation of responses to visceral stimuli. Also contributing to the pathogenesis are angiopathy, small muscle myopathy, and possibly changes in the gut microbiota and lower levels of short-chain fatty acids (anti-inflammatory, promotes GLP-1). Clinical consequences are mainly changes in secretory function and motility (54, 62, 110). When evaluating GI motility, it is important to remember that changes in glycaemia per se also can induce dysmotility, with hyperglycaemia causing delayed gastric emptying, while hypoglycaemia can accelerate gastric emptying (111, 112).

1.6.2 Definition and symptoms

Diabetic gastroenteropathy refers to any complication induced by diabetes mellitus that affects any part of the GI tract, where other causes are excluded. Different subtypes have traditionally included oesophageal dysmotility, gastroparesis, and different manifestations of enteropathy in the distal gut. Therefore, symptoms can arise from different parts of the GI tract and there are a multitude of differential diagnoses. GI symptoms are common in the general population but are still considered more common in people with diabetes. The prevalence of GI symptoms in

unselected patients with diabetes varies for the different symptoms between 1% and 40% (113).

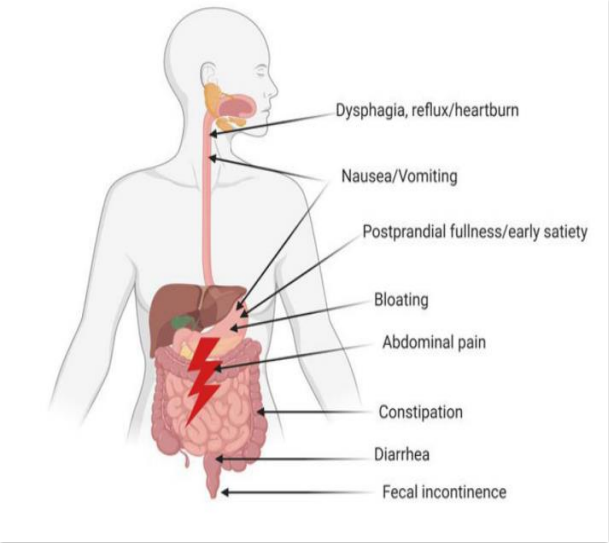


Figure 9: Symptoms in diabetic gastroenteropathy. Figure created using Biorender.com

1.6.3 Diagnosis of diabetic gastroenteropathy

Diabetic gastroenteropathy is regarded an exclusion diagnosis. There are several modalities to assess gastric emptying and gastroenteric motility, but they do not always correspond with symptoms or severity (114, 115). Diagnostics should include a complete history, especially with regard to alarm symptoms and signs, searching for other causes, and the use of medication. Different questionnaires are developed for scoring symptoms, e.g., the patient assessment of upper GI symptom severity index (PAGI-SYM) or gastroparesis cardinal symptom index (GCSI). Basic examinations often include upper endoscopy, colonoscopy, and/or ultrasound of the liver/gallbladder/pancreas. If symptoms of gastroparesis, assessment of gastric emptying is required, the gold standard being scintigraphy. Other validated options are the wireless motility capsule or paracetamol absorption studies. In cases with mild symptoms, an initial treatment trial can be justified (62).

1.6.4 Clinical considerations

Established treatments for diabetic gastroenteropathy are nutritional and self-care advice and symptomatic medical treatment. Symptomatic treatment for gastroparesis includes metoclopramide, ondansetron, erythromycin, rifaximin and prucalopride for gastroparesis, linaclotide for chronic constipation, and clonidine for diarrhoea. An option in the early stages of gastroenteropathy could be medications targeting pathophysiology, described in Section 1.3.4. For more extensive description of both established and novel treatment options I refer to a previous review article from our group (62).

1.7 Evoked potentials following gut stimuli

There is a lack of methods directly investigating the nervous system of the gut and its brain axis. Recent years have seen emerging possibilities for examining how the brain processes sensory signals. Functional magnetic resonance imaging (fMRI) is based on regional changes in blood flow during specific activity compared to control activity. It is non-invasive with good spatial resolution, but with poor temporal resolution and limitations regarding deeper structures. Positron emission tomography also measures cerebral blood flow and can provide information on neurotransmitters and receptors. Disadvantages with positron emission tomography are poor temporal solution, invasive with radiation exposure, and high expense. A non-invasive brain imaging tool, magnetoencephalography, measures cortical neuromagnetic activity. It allows both good temporal and spatial resolution, but is not sensitive to deep areas of the brain, and not widely available (81).

Investigating how central areas of the brain react to different sensory stimuli requires good time resolution. A non-invasive, harmless investigation with good time resolution is the electroencephalogram (EEG) measuring electrical brain activity. How to exploit this for investigation of the gut-brain axis is further described in the following sections.

1.7.1 The basics

An EEG measures electrical signals created from synchronised synaptic activity in populations of similarly orientated cortical neurons, the summation of graded post-synaptic potentials (dipoles between the soma and the apical dendrites of the neurons) of large cell ensembles. Potentials are measured using electrodes, placed on the scalp at specific locations (116). Advantages of measuring EEG is good time resolution, measuring neuronal function directly, it is non-invasive, portable, easily tolerable and little time consuming. Disadvantages include the presence of artefacts and low spatial resolution, with difficulties in determining which brain areas are involved (81, 116, 117)

EEG can be measured continuously in resting state, or as an evoked/event-related potential, in response to a specific sensory, cognitive or motoric event. Evoked/event-related potentials are time- and phase-locked, measuring synchronised neuronal firing as a response to a stimulus, resulting in negative and positive peaks. Peaks are typically quantified by latency and peak-to-peak amplitudes, designated by their polarity, negative (N) or positive (P), and order of their appearance, N1, P1, etc. They are sometimes termed also after mean latency, e.g., P300. The terms evoked and event-related potentials are often used synonymously, but evoked potentials are most often used in the case of short latencies (<100 ms) and small amplitudes (<1 μV), and event-related potentials if longer latencies (100-600 ms) and higher amplitudes (10-100 μV). Early potentials involve mainly sensory processes, while late potentials involve higher cognitive processes (118, 119). For the sake of simplicity, we have decided to use the terms early or late evoked potentials.

1.7.2 Evoked potentials investigating gut visceral sensitivity

Investigating cerebral responses to GI tract stimulations was developed in the 1980s, to study gut visceral nervous connections to the brain. The first observation of reproducible cerebral evoked potentials elicited by rectosigmoid electrical stimulation was published in 1989 (120). The method has later repeatedly been used to evaluate the afferent pathways of visceral sensation, with stimuli including electricity, barostat

pressure, thermal and different types of rapid balloon distention (121-124). Varying techniques and different stimulation devices have been a challenge, and until recently, electrical stimuli were considered the most robust and well-controlled visceral stimulus. However, electrical stimuli have the drawback of bypassing peripheral mechanoreceptors and depolarising axons directly. Hence, mechanical stimulation is considered a more physiological stimuli, e.g., mimicking the passage of stool. Evoked potentials to balloon distention has on the other hand been hampered by low signal to noise-ratio, probably because of long inflation-deflation time, and a lack of standard approach (125, 126).

Attempts to establish more accurate and reproducible models have also been limited by insufficient mechanical pumps and recording techniques, until Nissen et al. in 2013 showed that combining mechanical rectal distention and recording evoked brain potential was translational between rats and humans. The results showed good reproducibility, both within and between days (126). The method has been further validated and found reproducible, and has later been used in studying patients with idiopathic faecal incontinence and patients treated with primary radiation therapy for anal cancer (123, 127-129). Rectal mucosa is innervated by visceral afferents consisting of thinly myelinated Aδ and unmyelinated C-fibres, with slow conduction speeds compared to somatic afferents. Hence, cerebral responses induced by visceral mechanical activation occur *after* 40-50 ms. and should allow a reasonable separation between somatic Aβ and visceral signalling (124). Studies on selective activation of C-fibre afferents with laser have even shown evoked potential latencies above 800 ms (130, 131). Results from earlier studies using the method therefore makes it reasonable to believe that rapid balloon distention mainly recruits low-threshold Aδ-fibres in the mucosa (123, 126-129). Consequently, the method is intriguing for investigating physiological afferent signalling from the GI tract, the stimulus not necessarily needing to be noxious.

1.7.3 Earlier studies in diabetes

Our group and collaborators have previously used the evoked potential test in several studies on diabetes and gastroenteropathy. Studies in patients with type 1 diabetes, definite autonomic neuropathy and GI symptoms have shown an overall gut hyposensitivity in multimodal sensitivity testing, indicating peripheral and central neuronal changes, and altered central processing (122, 132, 133). In response to electrical rectal stimulation, patients with GI symptoms and autonomic neuropathy have shown hyposensitivity with prolonged latency and reduced amplitude. In the same category of patients, changes have been found in the organisation of the brain network, reported to correlate with GI symptoms, indicating involvement of the CNS (121, 134).

1.8 The incretin system

"***I worried that I had lost my chance to work on exciting research***"

Daniel Drucker, incretin pioneer, after missing the chance to work with thyroid hormone research, having to settle with gut hormones…

1.8.1 Incretins in normal physiology

The term incretin was first known to be used by La Barre et al. in 1929, derived from the stimulation of *in*ternal se*creti*o*n* of the pancreas. At that point, Claude Bernard had already in the second half of the 19th century tried to explain the fact that significantly larger amounts of glucose could be administered orally than intravenously without glucosuria (135). The two main incretin hormones are GLP-1 and GIP. It was the latter, which was first described as a gastric acid secretion inhibitor in 1969, that some years later was also found to stimulate glucose-dependent insulin secretion. The discovery of GLP-1 followed some years later, in 1987 (136, 137).

GLP-1 originates from proglucagon, which in the gut is cleaved by prohormone convertase 1/3 into glicentin, GLP-1 and GLP-2. Glicentin is again cleaved into

OXM and glicentin-related pancreatic polypeptide. Proglucagon expressed in the pancreatic α-cells are cleaved by prohormone convertase 2 into glucagon, glicentin-related pancreatic polypeptide and the major proglucagon fragment (138). GLP-1 is also produced by preproglucagon neurons in the NTS.

In the gut, GLP-1 and GIP are secreted from L-cells and K-cells respectively, mainly in response to food ingestion. L-cells are sparsely distributed in the duodenum, increasing in the jejunum, and peaking in the ileum and colon. The highest density and number of L-cells, using signal markers for GLP-1 and PYY, are actually found in the rectum, where they contribute to approximately 14% of the total EECs of the rectum (109, 139, 140).

The incretin effect refers to the amplified insulin secretion when glucose is administered orally compared to given intravenously (132, 141). It plays an important role in maintaining normal postprandial glucose, and is believed to be responsible for between 50 and 80% of postprandial insulin secretion in healthy subjects (105, 136, 142). The contribution of individual components has recently been quantified to approximately GIP 48%, GLP-1 27%, and glucose alone accounting for 25% of the insulin response (136). This indicates that GIP could be the main incretin hormone, particularly with regard to insulin secretion, while glucagon suppression is more important for GLP-1, although the effects of the two hormones have been found to be additive (143).

The hormone response depends on the size of the meal, the composition and correlates with gastric emptying rate. A study in humans have shown a peak circulating GLP-1 after an oral glucose load after 20-30 minutes, being elevated for two hours, while another study reported a peak 90 minutes after a mixed meal, being elevated for three hours. A third study showed a peak of GLP-1 after 30 min after carbohydrate intake and 150 min. after a fat load. GIP has been found to peak after 60 minutes following a mixed meal and 90 minutes after an oral glucose load, then gradually decreasing (144-146). In rodents, studies have supported biphasic secretion,

with a first peak around 10 minutes and a second phase 30-60 minutes after food intake (86). There is also evidence for a basal secretion of GLP-1 (138).

The secretion of GLP-1 has also been shown to be partially dependent on bile acids, the composition of the microbiota and other hormones, with ghrelin, leptin, GIP and gastrin releasing peptide shown to increase GLP-1, while somatostatin and insulin decrease GLP-1. Also, inflammatory cytokines and lipids from the blood stream are shown to promote GLP-1 release. Finally, GLP-1 secretion has also been shown to be stimulated by efferent nerve signalling, including enteric nervous signalling and parasympathetic involvement, involving a large number of neuropeptides (86, 138, 147, 148). However, a study performing ganglionic blockade in humans did not affect the secretion of GLP-1 or GIP, and the concept of neural-induced hormone secretion is still not firmly established (149). Furthermore, a GLP-1 response in the blood is not noticeable until around 10 minutes after a meal, i.e., after the cephalic phase, suggestive of a low influence of neuronal signals on incretin secretion (150). On the other hand, sympathetic innervation of the gut is confirmed to play an inhibitory role in GLP-1 secretion (138, 151).

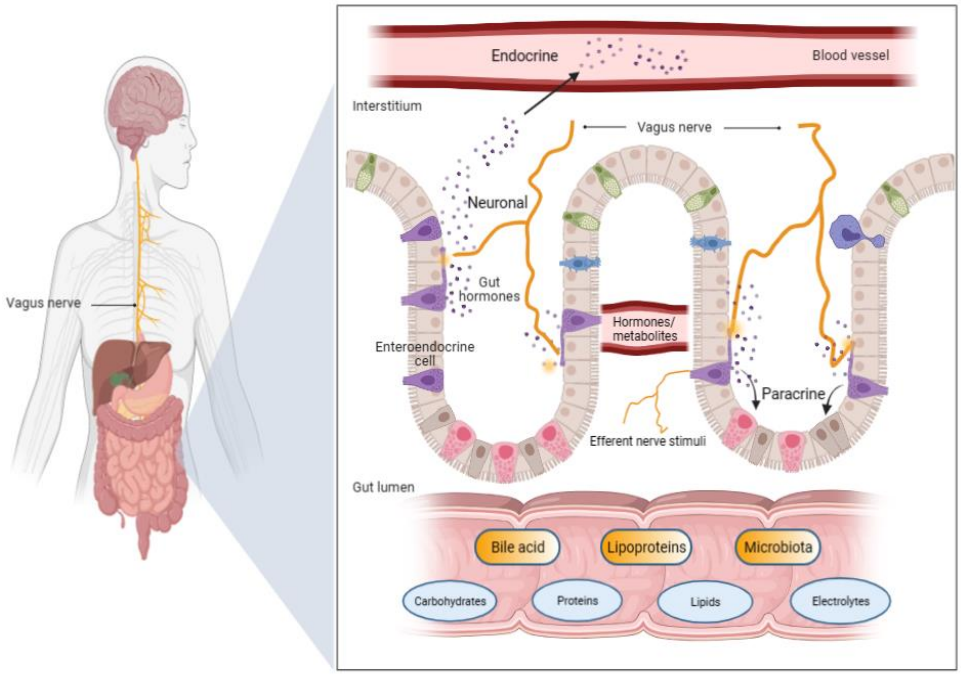


Figure 10: Stimulation of L-cells, secretion and transmission of GLP-1. Figure created using Biorender.com

Target organs and tissues throughout the body express receptors for GLP-1 (pancreas, brain, heart, kidney, gut) and GIP (pancreas, adipose tissue, gut, heart, bone, brain) (138, 152). The main physiological attributes of GLP-1 and GIP have been discovered both through studies using *antagonists*, blocking the normal effect, and in studies using *agonists,* in supraphysiological amounts. The main attributes are summarized in table 2.

Table 2: The main effects of GLP-1 a) and GIP b). Based on (136, 152-154).

a)

Organ	**GLP-1**	
	Physiological	*Supraphysiological*
Pancreas	Glucose stimulated insulin secretion Glucose stimulated glucagon inhibition ↓ Pancreatic exocrine secretion	↑ Insulin biosynthesis and secretion ↑ Somatostatin secretion ↑ β-bell proliferation ↓ Apoptosis
Gut	↓ Gastric emptying ↓ Acid secretion	↑ Intestinal growth ↓ Lipoprotein secretion ↓ Intestinal motility and gastric emptying
Brain	↑ Satiation	↓ Food intake ↑ Neuroprotection and neurogenesis
Cardio-vascular		↑ Cardio/Vaso-protection ↑ Glucose utilisation/↓ Fatty acid metabolism ↓ Inflammation
Kidney		↑ Sodium excretion
Immune cells		↓ Inflammation
Liver†		↓ Glucose and lipid output ↓ Steatosis
Adipose tissue†		↓ Lipogenesis (white adipocytes) ↑ Thermogenesis (brown adipocytes)
Muscle†		↑ Insulin resistance ↓ Glucose utilisation

b)

Organ	**GIP**	
	Physiological	*Supraphysiological*
Pancreas	↑ Insulin in response to nutrients ↑ Glucagon secretion during fasting	↑ Insulin biosynthesis and secretion ↓ Apoptosis ↑ Glucagon secretion
Gut	Inhibitory control of postprandial gallbladder ejection	
Brain		↑ Neuroprotection and neurogenesis ↓ Food intake

Cardio-vascular		↑ Triglyceride metabolism ↑ Endothelial function/capillary recruitment ↓ Blood pressure, ↑ Heart rate
Adipose tissue	↑ Blood flow ↑ Triacylglycerol (TGA) uptake	↑ Lipogenesis ↑ Adipokine secretion ↑ TGA uptake
Bone	↑ Bone formation ↓ Bone resorption	↑ Bone formation ↓ Bone resorption

† Probably indirect effects due to uncertain presence of GLP-1 receptors

1.8.2 GLP-1 signalling, with vagus in the lead role

GLP-1 is rapidly degraded to its inactive form by the enzyme dipeptidyl peptidase (DPP) 4. Approximately 25% of active GLP-1 reaches the liver, while only 10-15% reaches the pancreas (154-157). Even smaller amounts are expected to reach the brain, with studies reporting progressively decrease of GLP-1 concentration with increasing distance from site of secretion (158). Although peak concentration of GLP-1 is reached between 20-90 minutes after a meal, GLP-1 can be detected increasing within 10 minutes after ingestion of food. Hence, this seems to be even before the nutrients reach the location of the presumed GLP-1 producing L-cells, located primarily in the distal ileum and colon (138, 146). GIP-producing K-cells are located more proximally in the small intestine, and GIP has, in rats, been shown to stimulate GLP-1 via vagal nerve pathways (159). Another explanation for the rapid rise in GLP-1 is a possible co-secretion, with GLP-1 producing cells often co-localising with GIP. In EECs of the mid small intestine 55-75 % of cells staining for GLP-1 or GIP, also express the other hormone (138). Still, both the early rise in GLP-1 and the rapid degradation support the hypothesis of other ways of transmitting incretin signals than through circulation.

Already in the 70s, it was suggested that the effects of gut hormones were dependent on intact vagal innervation, following experiments with atropine blunting insulin secretion after oral glucose intake, but not intravenously (160). Studies supporting the importance of an intact vagal transmission for the incretins have shown that the ability of GLP-1 to inhibit gastric acid secretion effect was lost in people who had formerly undergone vagotomy (161). In another population of vagotomised subjects,

GLP-1 was found to be accentuated, however, the disposal of GI glucose was reduced (162). Further, the effect of exogenous GLP-1 in previously vagotomised people had no effect on ad libitum eating, with increased gastric emptying, but with the same reduction in postprandial glucose level as controls, the latter probably because of simultaneously reduced insulinotropic and glucagonostatic effects (163). Another approach to investigate this was through the use of capsaicin, which creates neuron necrosis. When targeting the vagus nerve it was shown to increase meal size, postprandial glycaemia, and prolong gastric emptying. Both vagotomy and capsaicin have been shown to abolish peripheral GLP-1 induced satiation, but neither of these methods are able to differentiate between afferent or efferent disturbances (86). A recent study, using a neurotoxin called saporin (conjugated to CCK), injected into the nodose ganglion confirmed that peripheral GLP-1 induced satiation was dependent on vagal sensory signalling (164). Several animal models have shown that selective removal or knockdown of GLP-1 receptors block all physiological effects of GLP-1 (86, 165). Finally, using new investigation modalities like optogenetics or chemogenetics, activation of GLP-1 receptor positive vagal afferents has shown to robustly reduce food intake in mice (166).

Sensory neurons expressing the GLP-1 receptor are found throughout the gut. In the stomach these are primarily neurons that detect stretch and gastric distention (resembling IGLEs), and a smaller number of these are also found in the intestines, colon, and rectum. In the mucosa of the intestines, GLP-1 receptor positive chemo sensing neurons, directly binding GLP-1, have been detected (86, 167). So, there seems to be two different GLP-1 receptor positive vagal afferents in the gut, with one important for detecting mechanical distention, and one for intestinal nutrient detection.

The hepatoportal region is exposed to higher concentrations of GLP-1 than the systemic circulation, and GLP-1 receptors were first found expressed at the nerve terminals of the portal vein (168). Further, it was demonstrated that a bolus injection of GLP-1 into the portal vein of the rat activated hepatic vagal afferents and pancreatic vagal efferents (169). The findings have later been supported by

transcriptomic data (167). Therefore, both the GLP-1 receptor afferents in the intestinal tract and the portal vein afferents can activate the CNS pathways (138).

Signals are further transduced via the nodose ganglion, the place for vagal afferent neuronal cell bodies, where GLP-1 receptor mRNA has been found in vagal afferent nerves (168). Via the nodose ganglion, the signals pass further on to the brainstem, mainly the NTS and area postrema.

The NTS has its own preproglucagon neurons producing GLP-1, for a long time assumed to be the link between peripheral and central GLP-1 systems. Recent evidence indicates that these neurons, independently of peripheral GLP-1, induce satiation, with vagal signals predominantly received from oxytocin-expressing mechanoreceptor neurons. The recent hypothesis is therefore that there exists two parallel, independently systems; one peripheral with GLP-1 receptor expressing vagal neurons projecting to the NTS, and one system of vagal mechanosensitive oxytocin-expressing neurons driving the CNS GLP-1 system (86). The importance of peripheral vs. central GLP-1 is not fully understood; still, the systems seem independent, but with potentially additive effects, one study showing that the increase of preproglucagon neurons in NTS enhanced the effect of semaglutide-induced eating suppression (170). A recent review highlighted endogenous GLP-1 from the intestines inducing satiation via peripheral vagal transmission, rather than central (167).

Further projections centrally mirror the central distribution of GLP-1 receptors. The main central brain area being the hypothalamus, especially the arcuate and paraventricular nuclei, the parabrachial nucleus, and the subfornical organ, all of where the proglucagon gene has been found expressed (138, 167).

Figure 11 shows a simplified illustration of vagal pathways for GLP-1 transmission.

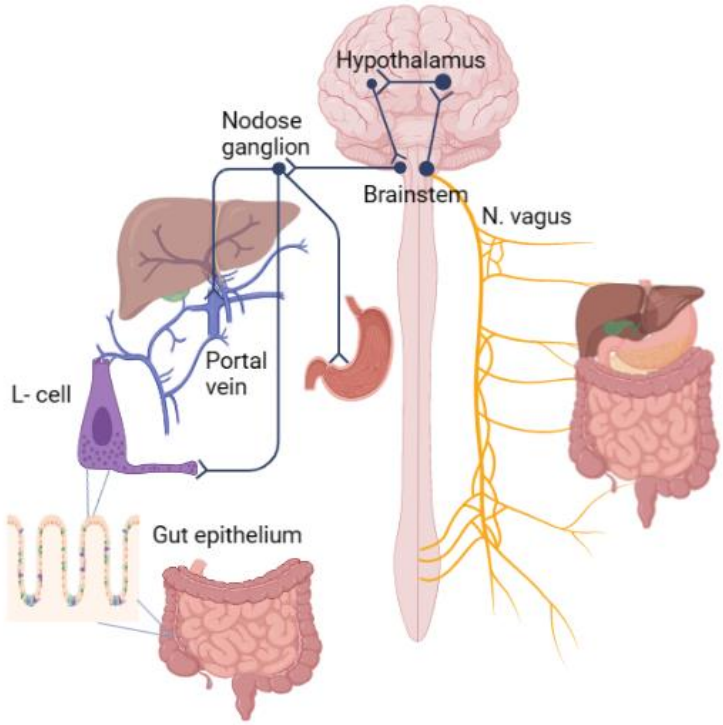


Figure 11: Vagal transmission of the GLP-1 signal. Figure created using Biorender.com

Although GIP is also degraded by DPP-4, 50% is found circulating in its active form after a meal. This is also in line with the fact that GIP is produced more proximally in the gut. There is not sufficient evidence that GIP has the same neuronal transmission route as GLP-1, and GIP receptors have not yet been found in the nodose ganglia (87, 154). However, GIP receptors have been found expressed in the hypothalamus of mouse models, suppressing food intake, supporting a central role also for GIP in the regulation of energy balance (171).

1.8.3 The incretin effect – why is it reduced in type 2 diabetes?

Evidence for a reduced incretin effect in type 2 diabetes was established in 1986, see Figure 12 (142). It has later been firmly described that there is a clearly reduced ability to dispose of orally ingested glucose, confirming an inability of incretin

hormones to elicit a proper insulin secretion (172, 173). For GLP-1, it seems that it retains some of its stimulatory activity in the pancreas, but this is completely lost for GIP, a so far unexplained phenomenon. It does not appear to involve down-regulation of GIP receptors (143).

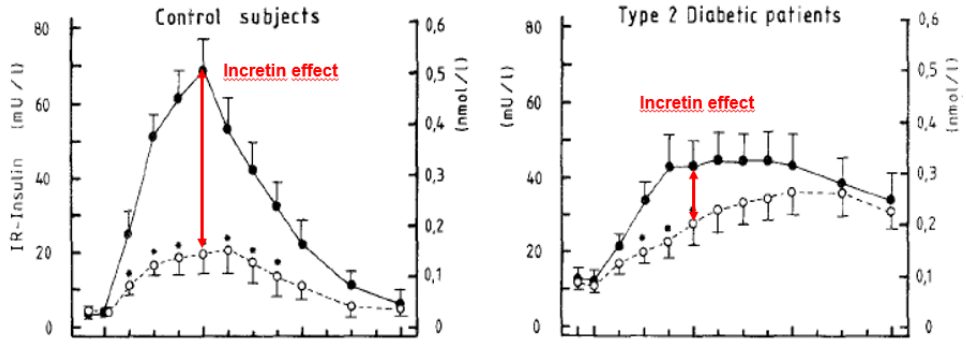


Figure 12: From Nauck et al. (142). Printed with permission (Licence number 5526391013838)

There is also uncertainty whether the reduced incretin response in type 2 diabetes is a cause or a consequence of the condition. Several studies point to the reduced incretin effect as a consequence of mild hyperglycaemia or glucose intolerance, with the phenomenon shown to be reduced in people with diabetes secondary to chronic pancreatitis, in people with early-stage type 1 diabetes, LADA and MODY (174-176). Other studies supporting the theory of consequence point to the impaired incretin effect found in healthy subjects after a short period of physical inactivity, eating a high-calorie diet, and the use of corticosteroids (177). In a similar study, healthy glucose-tolerant first-degree relatives of patients with type 2 diabetes was given dexamethasone, to increase insulin resistance, finding a reduced incretin effect when insulin resistance increase, even while retaining normal glucose tolerance. The incretin effect was further reduced if coinciding with glucose intolerance, suggesting that both factors contribute independently (178). The incretin effect has also been shown to be reduced during gestational diabetes, but reversibly, with a normalised effect after birth, suggesting that increased transient insulin resistance can cause a

transient impaired incretin effect (179). To support the possibility of reversing a reduced incretin effect, a study in people with type 2 diabetes showed improved β-cell sensitivity to GLP-1 after four weeks of strict glucose control with insulin (180).

There is also an uncertainty regarding why the effect is diminished, if it is reduced secretion, a failure in transmission transduction or a failure at receptor level. Although some studies have shown a reduced GLP-1 response to mixed meals, systematic reviews and meta-analysis have concluded that patients with type 2 diabetes do not exhibit reduced total GLP-1 responses during oral glucose or liquid meal tests (156, 181-183). Hence, there should be some other mechanisms explaining the reduced incretin effect. On the other side, even a small decrease in secretion could play a role given that the GLP-1 sensitivity of β-cells are reduced (172). Several studies trying to explain the reduced incretin effect have shown an involvement of generalised β-cell impairment and a defective response to incretins, with the resulting decreased insulinotropic action. Other possible explanations have been impaired glucose sensing by the enteroendocrine cells, or a defect in activation of stimulatory neural signals (174, 184).

The incretin effect, also depending on gastric emptying, indicates a role for perception of the food bolus (visceral sensitivity), starting the gut digestive reflex, and that timed gut motility are of importance. Another aspect is the adequate and timely secretion of pancreatic enzymes and bile acids, with the microbiota also believed to be of relevance (86, 108, 138, 147, 185). Finally, intact signalling in the autonomic nervous system appear to be of importance for both feeding and glucose homeostasis (87).

Regardless of whether it is a cause or a consequence of glucose intolerance, the reduced incretin effect increases postprandial glucose levels due to its lack of insulinotropic effects, worsening glycaemic control in people with type 2 diabetes.

1.8.4 At what stage is the incretin effect diminshed?

The loss of the incretin effect is described as an early characteristic in type 2 diabetes and has also found to be reduced in prediabetes (172). Studies reporting a reduced incretin effect include groups with impaired glucose tolerance, insulin resistance, obesity and end-stage renal disease, before signs of β-cell dysfunction (178, 182, 186-188). Especially obesity appears as a significant negative determinant for the GLP-1 response, and a study has shown that glucose tolerance and obesity affect the incretin effect independently of each other (172, 189). A longer duration of diabetes and diminished glycaemic control are associated with a poor GLP-1 response, which support the notion that the reduced effect follows a continuum, starting from early insulin resistance, with gradual worsening with reduced glycaemic control and long duration (172, 182, 190).

1.8.5 Measuring the incretin effect – some considerations

The incretin effect reported in studies also depend on whether the effect is estimated using serum insulin, c-peptide or insulin secretion rate. Using c-peptide when calculating the incretin effect eliminates the influence of the varying hepatic insulin extraction, since c-peptide is not affected by this. Insulin secretion rate is derived from deconvolution of circulating c-peptide values, put into an equation (191). Estimates based on insulin secretion rate or insulin tend to be lower than those based on c-peptide, mainly due to the hepatic insulin extraction. Whether calculations should be based on c-peptide or insulin depends on the focus of interest, peripheral glucose disposal or insulin regulation of hepatic glucose production (136).

The term gastrointestinal-mediated glucose disposal (GIGD) was introduced in 2010, in order to better understand how oral and iv. glucose affect glucose disposal differently (192). To estimate GIGD you need to know the amount of glucose ingested orally and amount of intravenous glucose needed to replicate the oral plasma excursion. Colloquially called “the poor man’s incretin estimate” it actually could be the most important physiologically parameter telling you how good the body really is to cope with an oral carbohydrate load (143). Most of the GIGD is probably

attributed to the incretin effect, but it also includes differences in porta and venous blood concentrations, tissue glucose uptake, inhibition of hepatic first-pass glucose uptake, a change in glucagon secretion, changes in hepatic uptake of insulin, gastric emptying/gut motility, and gut-brain or liver-brain axis. There may also still be unknown factors in the equation (105, 162, 172). The GIGD in healthy subjects is reported to be around 60%, while in people with type 2 diabetes between 10 and 30% (105).

1.8.6 Incretins in other conditions

In addition to type 2 diabetes, incretin hormones have a suggested role in the development of obesity and in postprandial reactive hypoglycaemia. There is substantial evidence for a reduced meal-induced GLP-1 secretion in obese people. The support for a role in the development of obesity is based on the regulation of food and appetite, but data suggest that if there is even reduced GLP-1 secretion, as a consequence, possibly related to insulin resistance, rather than a cause of obesity. However, decreased secretion has been confirmed in many studies, often combined with reduced levels of PYY, indicating an impairment of L-cell function, which may result in decreased post meal satiation and a positive energy balance. In line with this role in obesity development GLP-1 secretion is markedly enhanced after bariatric surgery, believed to be important for both weight loss, due to reduced appetite and food intake, and improved glucose tolerance (138, 193).

Regarding postprandial reactive hypoglycaemia, all conditions with accelerated gastric emptying, especially bariatric surgery, are associated with increased GLP-1 secretion. Insulin hyperstimulation, plausibly together with improved insulin sensitivity, may explain reactive hypoglycaemia (138). Of interest, the use of GLP-1 analogues has in clinical practice shown to reduce reactive hypoglycaemia, proposedly because of reduced gastric emptying rate, reduced cravings for sweets or mechanisms related to glucagon and insulin secretion when in a hypoglycaemic state.

1.8.7 Incretin-based therapy in type 2 diabetes and overweight

The discovery of a reduced incretin effect has dramatically changed the treatment possibilities for type 2 diabetes and, later, for the treatment of overweight. In 1993 it was definitively shown that a GLP-1 infusion in people with diabetes could normalise fasting glucose, while in 1998 it was also shown to inhibit appetite and food intake in humans (194, 195). For the treatment of type 2 diabetes, there exists several GLP-1 agonists, mostly subcutaneous injections, and also DPP-4 inhibitors, which reduce endogenous incretin degradation. With the latest development of GLP-1/GIP co-agonists it was possible to achieve the most potent HbA1c reduction for people with type 2 diabetes from 8 % to 5.9 %, and a weight loss of 11 % (196). In overweight people, but without type 2 diabetes, the most potent dose reduced weight with 22.5% (197). The addition of a GIP agonist to a GLP-1 agonist seems to potentiate the GLP-1 effect with both potentially activating the GLP-1 receptor (154). Recent years have also provided data on cardiovascular outcomes, and GLP-1 analogues demonstrate reduced composite outcomes of cardiovascular mortality, myocardial infarction, and stroke (198). Ongoing studies investigate incretins for kidney outcomes, for the treatment of non-alcoholic hepatic cirrhosis and steatosis, osteoporosis, and neurodegenerative diseases, including Parkinson's and Alzheimer’s disease.

It is probable that endogenous and exogenous GLP-1 exerts their effects using different mechanisms, the latter predominantly working directly on GLP-1 receptors in the pancreas, brainstem, hypothalamus and subfornical organ, where dense receptor expression is found, accessing leaks in the blood-brain barrier. The doses of GLP-1 in analogues are supraphysiological, up to four-five times the physiological postprandial secretion (86, 199). The fact that supraphysiological doses increase insulinotropic activity and not physiological levels, support the hypothesis of a preserved β-cell capacity, but that it needs a stronger stimuli to respond properly (172). The effect of exogenous GLP-1 is most prominent for GI motility, gastric emptying and insulin secretion.

There are ongoing trials both with increasing doses of existing GLP-1 agonists, and with dual and triple agonism, especially including amylin and glucagon, in addition to either GLP-1 and/or GIP (200, 201). There is no doubt that we are entering a new era for the treatment of type 2 diabetes and overweight. Still, with possible side effects, inter-individual differences in treatment responses, the risk of tachyphylaxis, and uncertain long-term outcomes, research should keep focusing on revealing the true mechanisms behind the reduced incretin effect. Hopefully we can someday prevent the failure of the incretin system and maintain a functional gut-brain axis.

2. AIMS OF THE STUDY

The overall aim for the study was to explore different factors involved in the reduced incretin effect in type 2 diabetes, especially regarding the relationship with the autonomic nervous system, both visceral sensation and central transduction.

- Concordantly, our main hypothesis was that the incretin effect correlates with autonomic nerve function.

To do this we planned a thorough neuronal phenotyping performing established tests for both sensory and autonomic neuropathy, as well as a novel questionnaire on autonomic symptoms. To investigate GI sensitivity and nerve function, we employed the method of measuring evoked potentials following rapid balloon distention in the rectum.

- A secondary hypothesis was that the novel visceral sensitivity test and evoked potential correlate with other neuropathy tests, including self-reported symptoms.

We also aimed to delineate at what stage of diabetes the reduced incretin effect and/or autonomic neuropathy could be discovered. Therefore, we planned to recruit people with different duration of diabetes and a control group matched for age, sex, and BMI.

- Thus, another secondary hypothesis was that autonomic dysfunction can occur in early stages of diabetes and that a reduced incretin effect is found in early stages of diabetes and is related to the degree of dysglycemia.

The overall project, named PanGut, also include measuring gall bladder emptying following a mixed meal, with several other secondary aims and hypothesis. This is further mentioned in Section 7.

3. MATERIALS AND METHODS

3.1 Participants

3.1.1 Recruitment, inclusion- and exclusion criteria, study days

We designed a case-control study and recruited participants into three groups; one group of people with type 2 diabetes for more than ten years (*longstanding diabetes group*), one group with newly diagnosed, untreated type 2 diabetes diagnosis within one year (*early diabetes group*), and controls matched in age, sex, and BMI. Participants were recruited mainly through regional newspaper advertisements. All investigations were performed at a single centre (Bergen, Norway).

Exclusion criteria were major abdominal surgery, rectosigmoid disease that interferes with sensitivity (e.g., any history of proctitis, ongoing malignancy, previous surgery), chronic pancreatitis, uremic condition (eGFR<30ml/min), retinopathy, atrial fibrillation or other major dysrhythmia, cardiac pacemaker, or current use of GLP-1 agonist or insulin. Neuropathy was not an exclusion diagnosis, but we did not actively search for people with diabetic neuropathy.

3.1.2 Baseline characteristics

We recruited a total of 66 participants with a mean age of 69 years, all of Caucasian origin, 52% women. There were significant different values for both fasting glucose, 2-h glucose and HbA1c between the groups. Baseline characteristics are found in Table 3.

Clinical characteristics	Longstanding N=21	Early N=15	Controls N=30	p-value
Age (years at recruitment)	68.9±7.8	69.3±5.5	69.5±6.2	0.950
Gender (women/men)	10/11	8/7	16/14	0.911
BMI (kg/m^2)	26.5±4.4	25.7±4.1	25.5±3.8	0.680
Diabetes duration (years)	16.8±4.9	0	0	n/a
Fasting glucose (OGTT), mmol/L	9.4 (2.1)	7.2 (1.0)	6.0 (0.6)	<0.01
2-hour glucose (OGTT), mmol/L	18.7 (3.9)	13.1 (4.2)	7.9 (1.5)	<0.01
HbA1c, mmol/mol	53.5 (11.2)	43.3 (4.9)	37.1 (3.0)	<0.01
Total cholesterol, mmol/L	4.2±0.8 [a]	4.5±1.2 [a]	5.5±1.0	<0.001
HDL, mmol/L	1.3±0.3 [a]	1.4±0.4 [a]	1.9±0.5	<0.001
LDL, mmol/L	2.4±0.6 [a]	2.8±1.1	3.3±0.8	0.001
Triglycerides, mmol/L	1.7±1.3 [a]	1.3±0.5	1.0±0.4	0.009
eGFR, ml/min per 1.73m^2	84.9±13.5	82.3±11.7	80.3±12.3	0.458
Systolic blood pressure rest, mmHg	135±15[b]	152±14	139±20 [b]	0.015
Diastolic blood pressure, rest, mmHg	80±6 [b]	86±7	81±7	0.023
Comorbidity (N)				
Nephropathy	0	0	0	n/a
Distal neuropathy, %	4.8	6.7	0	0.400
Hypertension, %	52	47	17	0.017
Cardiovascular disease, %	4.8	13	3.3	0.401
Drugs (N)				
Metformin, %	81	0	0	n/a
Sulphonylurea, %	19	0	0	n/a
DPP-4 inhibitor, %	48	0	0	n/a
SGLT2 inhibitor, %	38	0	0	n/a
Another antidiabetic medication, %	9.5	0	0	n/a
Diet treated diabetes, %	9.5	100	0	n/a
Betablocker, %	4.8	20	13	0.370
ACE-inhibitor/ARB, %	48	40	10	<0.001
Other antihypertensive medication, %	19	13	7	0.410
Lipid modifying treatment, %	67	47	13	<0.001
Smoking status,% (present/past/never)	10/38/52	7/13/80	3/43/54	0.300

Table 3: Diabetes duration, comorbidity, smoking status and drugs are self-reported. 80% of early diabetes was newly discovered and diabetes duration therefore set to zero. Data are mean±SD unless otherwise indicated. p-values using one-way ANOVA or Pearson Chi Square test. Post hoc test for continuous data between groups using Bonferroni: [a]=significant compared to controls, [b] =significant compared to early diabetes, all other significant for all groups. Abbreviations not explained elsewhere: HDL=high density lipoprotein, LDL=low density lipoprotein, SGLT=sodium glucose transporter, ACE=angiotensin converting enzyme, ARB=angiotensin II receptor blocker.

3.2 Investigations and procedures

The PanGut project included five days of examination (Table 4).

Day	Investigation/Procedure	Preparations
1	Information, inclusion, and neuronal phenotyping	- Avoid alcohol, sedative or stimulant drugs 24 hours prior to day 1 - Avoid food, coffee, tea and nicotine products 3 hours prior to examinations
2	Evoked potential following rapid rectal balloon distention	- Same as for day 1
3	Oral glucose tolerance test (OGTT)	- Minimum of 10 hours fasting, including medications and nicotine products - Antidiabetic medication paused 3 days prior to examinations - Otherwise, the same as for day 1 and day 2.
4	Isoglycaemic intravenous glucose infusion (IIGI)	- Same as for day 3
5	Ultrasound measured gall bladder emptying following a mixed meal	- Same as for day 3

Table 4 Days of examinations

Additionally, the COMPASS 31 questionnaire was answered at home, for most participants between day 3 and 4.

3.2.1 Neuronal phenotyping

HRV and CARTs were measured using the Vagus^{TM} Device (Medicus Engineering, Aarhus, Denmark), first for 5 minutes lying in a semi-reclined position (at rest), then shortly after standing up (RS-ratio), during deep breathing (EI-ratio) and while performing the Valsalva manoeuvre (VM-ratio). The test defines the stages of CAN as borderline if one ratio is abnormal and as definite or confirmed if two or three ratios are abnormal. If the latter is combined with orthostatic hypotension, CAN is defined as severe or advanced, according to international consensus (57). Orthostatic blood pressure test was conducted using the WelchAllyn Connex ProBP 3400^{TM} (EMEAI, Leiden, Netherlands), after resting recline for 5 minutes, then upon standing up, and after 1- and 3-minutes standing. We defined orthostatic hypotension as a

decrease in systolic blood pressure of > 20 mmHg or diastolic > 10 mmHg within three minutes of standing (202)

For the measurement of electrical skin conductance, we used the Sudoscan™ Device (Impeto Medical, Paris, France). The subjects' hands and feet were placed on steel electrodes. The test calculates electrical skin conductance by measuring the flow of chloride ions produced by sweat glands in hand and feet, using reverse iontophoresis, following low voltage electrical stimulation. It is thought to assess mainly small C fibres. The results are given as continuous conductance parameters and categorical as no sudomotor dysfunction, intermediate dysfunction, or high dysfunction. The test is regarded as reliable, objective and quantitative for detecting DPN, with a moderate sensitivity and specificity to suspect CAN (203).

The monofilament test was performed with a 10 g monofilament, bilaterally pinpricking the dorsum of the foot four times. The subject was placed in a supine position with eyes closed. We defined feeling 7-8 of the 8 sensations as no suspected DPN, feeling 4-6 as possible DPN, and feeling 3 or less, as probable DPN (204).

The sural nerve conduction velocity test was performed using the NC-stat DPN Check™ (NEUROMetrix, Boston, USA). This point-of-care device measures the nerve conduction velocity of the sural nerve by stimulating the nerve at the level of the ankle and recording the resulting response on the calf. The device provides absolute values as well as categorized results based on normative values dividing results into normal nerve conduction, mild, moderate, or severe neuropathy. The device has demonstrated excellent reliability and acceptable accuracy compared to standard nerve conduction examination (58).

3.2.2 Rectal sensitivity and evoked potentials

The equipment and protocol are based on recent studies and are described in more detail in reference studies (123, 126-129). Our project group was instructed in how to prepare and perform the examination by collaborators who developed the method in Aalborg, Denmark. In summary, a balloon catheter was made using a CH 16

duodenal tube (Levin X-ray, Unomedical, UK), with a 5 cm index finger of a nitrite examination glove (Klinion Protection, Medeco, The Netherlands) attached to the tube, wrapped and secured with a fast absorbable Novosyn Quick Polyglactin 910 suture (B.Braun, Germany). The tube was attached to an extension catheter, with a length of 2.1 m. and connected to a custom-designed inflation device (Mech-Sense, Aalborg University Hospital, Denmark).

Forty-five minutes before testing, a bisacodyl enema (Toilax, Orion pharma, Espoo, Finland) was administered to optimize conditions in the rectum. The EEG was recorded using a standard 64-channel surface cap, with Ag/AgCl electrodes in an extended 10-20 system, with Fz as reference. Electrode gel was applied to reduce impedance. A resting EEG was recorded (two minutes), with eyes opened. Subjects were instructed to use a 0-10 visual analogue scale (VAS). The rectal balloon was then placed 15 cm above the anal verge. The balloon was inflated to 0.01 bar, resembling 1 psi used in the reference studies, approximately the same as 1 kPa. The pressure was increased by 0.01 bar increments until the earliest sensation, VAS 1. The procedure was repeated two times, finding the most probable pressure for VAS 1, averaging the pressure of the three attempts. We then performed a stimulation period with 30 single stimulations, while recording EEG. Subsequently, we increased the pressure with increments of 0.1 bar until discomfort or an urge to defecate (VAS 5). The procedure was repeated, finding the most probable pressure causing VAS 5. Another 30 single stimulations were then performed while recording the EEG.

The balloon inflator used a digitised trigger signal to synchronise the EEG recording. The inflation time was 150 ms, followed by an instant deflation facilitated by a vacuum pump, providing a rapid, distinct and short stimulus. A random interval between stimuli of 8 ± 2 seconds was used. To minimise environmental noise, all unnecessary electrical equipment was turned off. For safety reasons, a pressure of 2.07 bar, which corresponds to 30 psi from the reference studies, was used as maximum pressure. The evoked potential latency was calculated as the time in milliseconds after the onset of the external trigger of the balloon distension, the peak-to-peak amplitude was the absolute difference in amplitude between two consecutive

peaks. All data were analysed in cooperation with our collaborators in Aalborg, Denmark. Data were cleaned and analyzed using MATLAB (R2020b Mathworks, Inc, Natick, MA, USA) and EEGLAB toolbox (version 14.1.2; Schwartz Center for Computational Neuroscience, Institute for Neural Computation, University of California, San Diego, CA, USA). The clean_artifacts toolbox was used with default settings and Window Criterion off. A one Hz filter was applied to remove direct current drift. Channels containing noise were interpolated using spherical interpolation and referenced to a common reference. All data were visually inspected prior to independent component analysis (ICA). Data were down sampled to 250 Hz for ICA to visually distinguish independent components containing biological or outside noise. After selecting "bad" components, the analysed data were added to the complete data set (1000 Hz) and the bad components were pruned from the data set. Evoked potentials were generated by averaging the EEG signals recorded in each session. The data were not corrected for any delays in balloon inflation (205, 206).

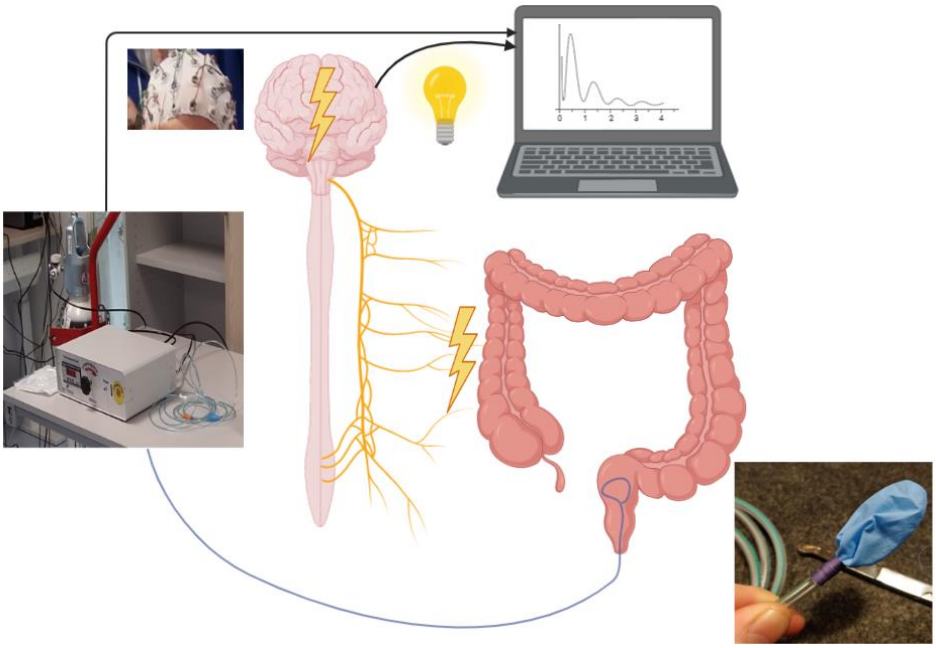


Figure 13: Illustration of the evoked potential test following rapid rectal balloon distention. Figure created using Biorender.com. Photographs by S. Meling.

3.2.3 Oral glucose tolerance test

On the day of examination, a cannula was placed in a cubital vein, and the forearm on the same side was placed in a heating cuff to ensure arterialised blood. Fasting blood samples were taken at -30, -15 and 0 minutes. The participant ingested a 250 ml solution of 75 g anhydrate glucose (Finnomedical, Finland) with an added 1.5 grams of paracetamol (Karo Pharma Aktiebolag, Stockholm, Sweden) between 0 and 5 minutes. Blood samples (serum and EDTA blood) were then collected at 10, 20, 30, 50, 70, 90, 120, 150, 180 and 240 minutes. Glucose was measured at the same time and additionally at 5, 15, 25, 40, 60, 80, 105 and 135 minutes, using the HemoCue Glucose 201 DM RT (HemoCue, Angelholm, Sweden).

Baseline blood tests were taken at 0 minutes. Blood was sampled using Vacuette EDTA tubes for HbA1c and other baseline blood tests, analysed consecutively. For planned gut hormone measurement we used Vacuette EDTA/Aprotinin with an added 10 uL DPP-4 inhibitor (AH Diagnostics AS, Denmark) per ml blood, cooled on ice and immediately centrifuged cold, with plasma kept frozen until analysing. Vacuette serum tubes with clot activator were used for the analysis of c-peptide and paracetamol, also kept frozen until the analysis. The total amount of blood collected from a participant for each test was estimated at 175 ml.

3.2.4 Intravenous isoglycaemic glucose infusion

On the day of examination, two cannulas were placed in cubital veins, one in each arm. Blood was sampled and glucose was measured at the same intervals as OGTT. At 0 minutes, a 200 mg/ml Glucose infusion (B.Braun, Melsungen AG, Germany) was started. Infusion rates were adjusted at each time point to ensure duplication of the plasma glucose profile from OGTT, and the total amount of glucose given was noted.

3.2.5 Calculating incretin parameters

From the two tests we calculated GIGD from the following formula: ((glucose OGTT-glucose IIGI)/glucose =GTT) x 100%. Incretin effect was estimated using c-

peptide: ((AUC c peptide-OGTT-AUC c peptide IIGI)/AUC c-peptide OGTT) x 100%. The gastric emptying rate was calculated from paracetamol area under the curve (AUC) after 70 minutes. For a valid duplication of glucose values, we used a "rule of thumb" accepting a maximum difference of glucose AUC of 20% between OGTT and IIGI.

3.2.6 The Composite Autonomic Symptom Score 31

The linguistically validated Danish version of COMPASS 31 was translated into Norwegian using a forward/backward translation method (207). The questionnaire was distributed using the EasyTrial.net programme (EasyTrial ApS, Aalborg, Denmark) and answered online at the discretion of the participants, except for one participant who responded by letter. Maximum domain-specific weighted scores in the orthostatic, vasomotor, secretomotor, GI, bladder and pupillomotor domains were 40, 5, 15, 25, 10 and 5 points, respectively. Maximum total weighted score was 100 points. The recommended threshold supporting borderline CAN is 16 points (79).

3.3 Ethics

All participants signed a written consent in accordance with the Declaration of Helsinki, following both oral and written information, with consent obtained prior to study-related procedures. The PanGut study was approved by the Western Norway Regional Ethics Committee for Medical and Health Research Ethics, REK Vest 2018/#1790. The approval is found in the Appendix.

3.4 Statistics

A formal power calculation was not feasible, due to the nature of an explorative pilot project, and due to an unknown effect size for the rapid rectal balloon test. However, using effect sizes from previous studies on visceral sensitivity, the lowest number of participants needed to achieve a power of 0.8 would be 15 participants in each group (122, 127, 208). Results are given as means with standard deviation for data with

normal distribution, and median with interquartile range for skewed data. We used one-way ANOVA comparing data where normal distributed, and Tukey or Bonferroni test for post hoc testing, assuming equal variance. Where assumed unequal variance we used the Welch test, with Games-Howell for post hoc testing. For skewed data, *p*-values were found using Kruskal Wallis test for several independent samples and Mann Whitney U test for two independent samples. For categorical data we used the Chi Square test. For correlation analyses, we used Spearman's rank-order correlation test. For diagnostic accuracy we calculated the area under the receiver operating characteristic curve, as well as sensitivity and specificity. For glucose, c-peptide and paracetamol, the AUC was calculated using the trapezoidal rule. Missing values for c-peptide and paracetamol were imputed from the average values before and after. For other missing data, subjects were removed from that analysis. The evoked potential data sets were pooled and analysed blindly. Statistical significance was defined as *p*-value ≤ 0.05 for all analyses. Statistical analyses were performed using SPSS Version: 28.0.1.0.

4. RESULTS

4.1 Paper I

The first paper aimed to compare visceral sensitivity and evoked potentials following rapid balloon distention in the rectum, between groups, and with established tests for neuropathy. We hypothesized that reduced rectal sensitivity and changes in evoked potentials were more prevalent in both longstanding and early diabetes, and that the novel test was correlated with other tests for neuropathy. The main finding was that significantly higher pressure was needed to induce the first rectal sensation in longstanding diabetes (0.037 bar) and early diabetes (0.040 bar), compared to controls (0.030 bar), indicating rectal hyposensitivity. There was also a trend towards longer evoked potential latencies for the group of longstanding diabetes, but not significant. Rectal hyposensitivity was also found in people with possible or probable neuropathy measured by the monofilament test.

Altogether we detected only two cases of definite CAN, one in the early diabetes group and one in the control group. Although there were more people in the longstanding group of diabetes with probable CAN, however, this was not significantly different from the other two groups. There were generally few between-group differences regarding both sensory and autonomic tests. People with longstanding diabetes were more likely to have sensory neuropathy when performing the monofilament test, compared to the control group, and had significantly higher resting heart rate than controls, 69 bpm vs. 63 bpm. Correlations were found between various amplitudes and latencies, and parameters for HRV (SDNN and RMSDD), CARTs (EI- and RS ratio), sural nerve test, and the monofilament test, but the results should be interpreted with caution, as detailed in Section 5, Discussion.

4.2 Paper II

In the second paper we wanted to explore the COMPASS 31 questionnaire, both for feasibility in digital and remote capturing of the data, but at the same time

hypothesising that both reported symptoms and objective findings were more prevalent in people with longstanding and early diabetes, and that the total score was correlated with the results of the established tests for neuropathy.

We found the questionnaire easy to distribute digitally and to interpret the results. We found the median total score to be significantly higher in people with longstanding diabetes (14.9 points), than early diabetes (7.3 points) and controls (8.6 points). Women also scored significantly higher than men. The subdomains that contributed the most to the differences were the secretomotor and GI domains. People with longstanding diabetes who used a DPP-4 inhibitor scored higher in the GI domain than those who did not use this. A correlation was found for the total COMPASS 31 score with definite or borderline CAN, with a cut-off at 10 points providing a fair sensitivity (0.83), acceptable specificity (0.55) and, in our population, a good negative predictive value (0.92) for the evaluation of CAN.

4.3 Paper III

In the last paper, our aim was to investigate the association between the degree of autonomic nerve dysfunction and the incretin effect. We hypothesised that GIGD and the incretin effect was correlated with autonomic nerve dysfunction, especially degree of rectal sensitivity, and also aimed to confirm that the incretin effect was correlated with the degree of dysglycaemia and duration of diabetes. The results show a significant correlation between rectal hyposensitivity and GIGD (rho -0.341, p=0.005), but not with the incretin effect. This was confirmed with a borderline significant higher value for GIGD for those with a rectal pressure for first sensation $\leq$0.03 bar (47$\pm$25%) vs. those >0.03 bar (34$\pm$24%), p=0.051, but not for the incretin effect. GIGD was significantly different between all groups, while the incretin effect was significantly different for both diabetes groups compared to controls. There were significantly correlations between all glucose values and HbA1c with both GIGD and the incretin effect. The gastric emptying rate, based on paracetamol absorption, was accelerated in both diabetes groups, compared to controls, significantly only for the longstanding group.

5. GENERAL DISCUSSION

5.1 The main findings

5.1.1 Rectal hyposensitivity in early stage diabetes

To our knowledge, this is the first time that rectal hyposensitivity has been reported in early stages of diabetes in humans, previously reported in rats, with visceral afferents in the rectum affected in early stages of diabetes (209). Changes in sensitive functions of the GI tract, such as hyposensitivity, observed in diabetes mellitus, strongly supports the presence of peripheral neuropathy (210). Rectal hyposensitivity to multimodal stimulations has previously been reported in people with diabetes, including type 2 diabetes, and prior sensorimotor neuropathy, autonomic neuropathy, and/or gastroparesis symptoms (121, 211, 212). In contrast to this, the present population was people with both early and longstanding diabetes, exclusively type 2 diabetes, no former autonomic neuropathy and not necessarily with GI symptoms.

Whether the rectal hyposensitivity reflects changes in the peripheral or central neurons is difficult to decipher, as we did not find any differences in the evoked potential latencies or amplitudes and did not perform inverse modelling of the brain signals. Imaging studies in people with diabetes and GI symptoms using MRI have shown microstructural changes, especially in areas involved in visceral sensory processing, and the existing literature on patients with diabetes and GI symptoms indicates the presence of both functional and structural changes in the brain (213). A decrease in conduction velocity was reported already in 1992 to be associated with diabetic autonomic neuropathy, with an increase in evoked potential latency found in patients with gastroparesis (214). Also, recent studies have shown that people with diabetes, severe GI symptoms, and clinical suspicion of autonomic neuropathy are less sensitive to painful rectal stimulation, with prolonged latency and diminished amplitude and change of dipole sources in the brain (121). Other studies with different oesophageal stimulation modalities, in similar cohorts, report the same,

indicating both peripheral visceral changes, as well as changes in the CNS (122, 132, 215).

The trend towards longer latencies in the group of longstanding diabetes was not significant and we found no consistency in the results for amplitudes. However, these results could prove significant in a larger population and support changes in the CNS, as found in several of the recently mentioned studies. The lack of pathological findings on evoked potentials could also be explained by our low incidence of neuropathy, one possible hypothesis being that central changes occur only after long-lasting damage, with manifestations of symptoms and other signs.

An alternative interpretation of the lack of difference in latency and amplitude could be that peripheral mechanisms are dominant. Other support of a peripheral mechanism is the nature of applying mechanical pressure, not electrical stimuli, which is more likely to recruit mucosal afferents and/or mechanoreceptors, without bypassing these receptors. Rectal hyposensitivity has also previously been found to be more pronounced for mechanical distention than for electrical stimulation (212). The question whether peripheral, central or dual mechanisms are involved in the rectal hyposensitivity remains to be answered in larger follow-up studies.

5.1.2 Concurrent diabetic neuropathy?

A recurring – and clinically important - question regarding diabetic neuropathy is whether peripheral (large nerve fibres) and autonomic (small nerve fibres) neuropathy occur concurrently and also if autonomic neuropathy occurs concomitantly in different organs? Drawing conclusions based on our findings is difficult, as data showed meaningful clinical correlations between evoked potentials and other neuropathy tests, but was not able to detect statistically significant differences, especially given multiple testing.

Peripheral and autonomic nerve damage: Rectal hyposensitivity was correlated with reduced peripheral sensitivity found using the monofilament test, but not nerve conduction parameters using the point-of-care device. An association between

sensorimotor neuropathy and rectal hyposensitivity has previously been reported in people with diabetes (211). Several studies have assessed the possible co-existence of diabetic sensory and autonomic neuropathies with studies reporting divergent results (216-219). However, it is increasingly recognised that small nerve fibres are involved early in the course of peripheral sensory neuropathy and therefore the assessment of early small nerve fibres should be included in diabetes care, to prevent both autonomic and peripheral nerve damage (89). A recent study supports the theory that CAN is predominantly existing with concomitant somatosensory nerve damage (220). Although there is no strong support from our present study, there still appears to be ample reasons for clinicians to consider autonomic complications in patients presenting with peripheral neuropathy, or symptoms suspicious of other autonomic neuropathies (211).

Concomitant autonomic damage: Previous studies have provided evidence for a correlation between visceral hyposensitivity and the degree of CAN (121, 211). However, we were unable to detect any correlations between rectal sensitivity, evoked potentials, CAN and sudomotor function. The results may be explained by our low prevalence of definite CAN, but they can also be interpreted as disturbances in the nerves of the GI tract before damage to other autonomic nerves. Possible explanations for this could be neurons of the GI tract being more vulnerable to mild hyperglycaemia, their different anatomical positions, length and other physiological differences (54, 221). Other possible explanations could be that impaired function of other autonomic nerves is better counterbalanced or even overruled by other mechanisms, e.g., the cardiovascular system with different neurons both in the heart and vessels, largely affected by other hormones and renal involvement. Also, there might be different mechanisms in different organs involving neuroplastic changes, including supraspinal and spinal reorganization (122). As my colleague and co-author Christina Brock so elegantly writes in a recent editorial: "*Obviously, the heart rate variability (HRV) measures represent the autonomic regulation of the heart and not the autonomic regulation of the GI tract, and thus we seek—and still fail to find—an association between autonomic regulations of the two*" (221). Our results support the suggestion by Frokjaer in 2009 that visceral evoked potentials may be a useful

biomarker of neuropathic changes in the gut induced by diabetes that are not evaluated by cardiovascular tests (122). Therefore, and importantly, our study supports the diagnosis of diabetic gastroenteropathy without necessarily performing CARTs or HRV.

5.1.3 Use of COMPASS 31 in research and in the clinic

We found the questionnaire easy to distribute and evaluate; therefore, there is a potential for distribution to larger and possibly more representative cohorts. Also, in the daily clinic it could be of value to detect symptoms of possible autonomic origin, both for treatment reasons and risk prediction. Although recommendations exist for assessment of autonomic neuropathy in people with diabetes, our experience is that this is a neglected area in the diabetes care, hence a simple questionnaire could be one mean to rectify the situation.

Symptoms related to autonomic neuropathy were significantly higher in the group of longstanding diabetes; and it follows that we did not find increased symptoms in early diabetes as hypothesised. This is in support of the notion of *symptomatic* autonomic neuropathy being a relatively late diabetes complication (57, 222). Rectal sensitivity was reduced in both longstanding and early diabetes, but the symptom burden was only increased in the longstanding group, which could be interpreted as early autonomic neuropathy existing without symptoms. A possible explanation for this is that impaired peripheral and autonomic afferent function can be counterbalanced or overruled by increased central neuronal excitability, and we must always keep in mind the complexity of the nervous system (210). It could also be that symptoms only occur after a certain degree of neuropathic changes, and perhaps behind some kind of “point of no return”? For the cause of prevention, this highlights the need for tests, preferably small fibre tests, that detect damage before symptoms occur.

If detecting a condition is dependent on invasive, time-consuming, or less available tests, there is often a desire for an easier and more accessible way of screening for it, such as a questionnaire. A correlation was found between total COMPASS 31 score

and definite or borderline CAN, and we suggest using a cut-off at 10 points, with a good negative predictive value, to possibly rule out CAN. To support this, a cut-off point has previously been proposed at 10 points for small fibre neuropathy confirmed by epidermal nerve fibre density (223). Other studies recommend a cut-off point of around 16 points for diagnosing CAN, but in our population, this cut-off point yielded poor values for sensitivity, specificity, positive, and negative prediction (79, 224). One study even recommends a cut-off score of 28.7 points for definite CAN (225). The different results and recommendations certainly reflect the disparity and prevalence of autonomic neuropathy in the different cohorts.

Regarding people with longstanding diabetes who report more GI symptoms, this is in line with previous studies in people with diabetes (113). We could neither detect an association between the rectal sensitivity nor evoked potentials and symptoms regarding the GI domain of the questionnaire. This is also in line with earlier studies in people with diabetes and GI symptoms where symptoms correlate poorly with GI motility tests (114). However, a study has reported that reduced oesophageal sensitivity is related to GI symptoms, in longstanding diabetes, being a possible biomarker of GI dysfunction in diabetes (226).

5.1.4 Rectal hyposensitivity correlates with GIGD, but not the incretin effect

A correlation was detected between rectal hyposensitivity and GIGD, but not with the incretin effect. This opens for the possibility of autonomic nerve dysfunction being more important with regards to non-incretin factors of GIGD.

Studies in vagotomised humans support the notion of reduced GIGD, but preserved incretin effect, possibly related to higher GIP and GLP-1, lack of glucagon suppression, and accelerated gastric emptying. Accelerated gastric emptying was not associated with rectal hyposensitivity in our current cohort. We eagerly await the results of GIP, GLP-1 and glucagon from our study subjects, with possible hypotheses being increased GIP and GLP-1 because of a reduced effect of neuronally transmitted signals, or hyperglucagonaemia, as found in early stages of diabetes, and

also reported in people with diminished GIGD, but preserved incretin effect (227, 228). A role for GIP was also proposed in a study that explored the association between autonomic neuropathy and the incretin effect, also with a proposed role for hepatic insulin extraction (229).

First-pass glucose uptake affects GIGD, and reduced uptake has been associated with parasympathetic neuropathy (230). Interestingly, early impairment of the autonomic nerves in type 2 diabetes has shown a mainly reduced parasympathetic activity, with an abnormal sympathetic predominance (66, 231). It is also possible that neuronal transmission is more important for the central mechanisms of the incretin hormones, especially regarding satiation and gastric emptying. This is supported by effects of exogenous GLP-1 being diminished in regard to ad libitum eating and gastric emptying in vagotomised subjects, but with the same postprandial glucose values as their controls (163).

Other possible explanations for the phenomenon of a reduced GIGD, but a preserved incretin effect, could be the effects of GIP, which do not exhibit the same degree of dependence on neuronal transmission as GLP-1, or that the endocrine effect of GLP-1, although found in low concentrations in systemic circulation, is enough to elicit an adequate incretin effect (136, 143). The latter is supported by evidence of a preserved incretin effect after surgical denervation of the pancreas (232).

The findings of an early rectal hyposensitivity in diabetes, strengthen its possible association with a reduced GIGD, as GIGD, including the incretin effect, are also found reduced at the same stage of the condition. There is an increased awareness that peripheral and autonomic neuropathy may be present at the time of diabetes diagnosis and may also be present in prediabetes (50, 51, 66). Autonomic dysfunction, in combination with poor physical fitness, has even been suggested as a mechanism associated with early glucose intolerance and the subsequent development of diabetes, and one study recently found that autonomic neuropathy precedes the development of type 2 diabetes, especially in younger individuals, although it was not possible to claim causality (70, 233). The reduced incretin effect

is also an early manifestation in type 2 diabetes, even found in prediabetes and obesity (172). From a clinical perspective, overall, this highlights the importance of early intervention, both to prevent neuropathy and to prevent deterioration of the incretin effect and other factors of GIGD.

5.1.5 Should we consider changing the diagnostic criteria?

This is obviously a huge and controversial question, but I find it interesting to touch upon this topic, as our results show a gradual progress of reduced GIGD and incretin effect starting well within normal HbA1c levels. There is a strong association between HbA1c $\geq$ 6.5% and the development of retinopathy, while for diabetic nephropathy and neuropathy, the association with HbA1c is reported to be linear, but without a distinct threshold increased risk (20, 234). Knowing that the incidence of diabetes seems to stagnate since the introduction of HbA1c as diagnostic criteria, while cardiovascular disease may seem to increase, perhaps we should lower the HbA1c criteria or fall back on the glucose tolerance test (8)? Given that incretins may play a protective role in cardiovascular disease; could we measure it in some way, predicting who will benefit the most from converting from prediabetes to normoglycaemia (235) ?

Further, what is the glucose threshold (if any exists) for the primary prevention of neuropathy ? The susceptibility for early nerve damage, caused by even mild hyperglycaemia, before reaching the current criteria for diagnosis, may support a lower threshold for HbA1c, or performing an OGTT in individuals at risk.

Lastly, the earlier treatment is induced, the better the diabetes remission rates, as shown for bariatric surgery and for dietary weight loss (236, 237). Also, weight reducing medications seem to be more effective in earlier stages of diabetes, at least before developing hyperglycaemia (238, 239). Several large studies, especially the UK Prospective Diabetes Study have shown that it is beneficial in the long term, regarding most complications, to reach normoglycaemia as soon as possible, often termed “the metabolic memory” (29).

So, from several perspectives it seems relevant to focus on earlier prevention and treatment. Preventing diabetes, or aiming at remission if diagnosed, could plausibly increase the effect of incretins and decrease the risk of developing diabetic neuropathy and vice versa.

5.2 Methodological considerations and limitations

5.2.1 An interrupting virus

"Lightning struck" in March 2020, with the Covid-19 pandemic. Performing a clinical diabetes study in the Covid-19 era turned out to be challenging. One challenge was recruitment, most people trying to avoid contact with others, and colleagues in general practice being occupied with other things than to help including people to a clinical study. Another challenge was that several participants who had undergone the OGGT had to wait longer before performing the IIGI. We tried to compensate this by prioritising the group with early diabetes for IIGI after the pandemic, but with such a transitional and heterogenous condition, we cannot exclude that the delayed testing affected the results in either directions.

5.2.2 Testing visceral sensitivity

Different testing modalities regarding the CNS are mentioned in section 1.7. Both because of extensive experience in our group, the development of a valid, rapid rectal balloon distention test, and the importance of time-resolution rather than spatial resolution made us choose the evoked potential test for our study. The test probably includes testing the peripheral part of the visceral afferents, using mechanical stimulation, being better at mimicking normal physiology than electrical stimulation. Still, to even mimic physiologically stimuli more, the balloon distention could optimally be slower, but that would probably not create a proper evoked potential (127).

Looking at the conduction velocity, it seems likely that the nerves stimulated are predominantly Aδ-fibres, hence mainly the mucosal afferents responsible for

detecting light mucosal stroking and in the rectum communicating with EECs (126). The mucosal afferents closely resemble the vagal mucosal afferents found in close contact with the intestinal epithelium and detect changes in hormones related to nutritional status. This supports a plausible physiological correlation between the probable nerve endings we stimulated in the rectum, and the nerve endings responsible for transmission of peripheral peptide signals. Still, we cannot exclude stimulation of mechanoreceptors, including more C-fibres, especially with this type of rapid stimulation, and with increasing pressure.

During an examination with 30 stimuli, there is the possibility of adaptation to distention (Aδ-fibres being adaptive). However, the evoked potentials in our study, at both VAS1 and VAS5, had significant peaks with clearly eliciting central signals. Activation of mechanoreceptors can also cause reflex relaxation of the bowel. Either way, the pressure found to elicit both first sensation and unpleasant threshold was found prior to the 30 stimuli, and were therefore not subject to adaptation.

Using the VAS scale to determine level of sensitivity has advantages and disadvantages, as it is clearly being subjective. Still, if we were to use the same pressure for everyone, the stimulus might be harmful for someone, while not eliciting evoked potential for others. Also, considering the putative heterogeneous anatomy of the rectum in different people, it seems plausible to use the subjective scale. This also constitutes a limitation regarding several of the participants, as they did not reach the threshold of VAS 5, a total of 17 participants, though a similar amount in all groups of approximately 25%.

5.2.3 Test battery for neuronal phenotyping

The choice of test battery for neuronal phenotyping was mainly based on availability, economical aspects, and former familiarity in our group.

For CARTs, the Vagus test was available, is user-friendly, includes HRV parameters, and is gaining widespread use around the world.

The monofilament test is well implemented in clinical practise and is easy to perform and to interpret. On the other hand, its subjective, has limitations regarding specificity for prediction of future neuropathy, and is only considered appropriate as a simple screening instrument in a clinical setting (204). Furthermore, there is still no definitive role for the sudomotor or point-of-care sural nerve conduction test, the studies being small with heterogeneous populations and prone to selection bias. Still, we chose to incorporate them both for more objective measurements (sural nerve) and for investigating autonomic neuropathy from different aspects (sudomotor function). We consider it more robust to perform several neuropathy tests, strengthening the diagnosis if the test results are in agreement.

A possible limitation is the lack of any validated screening instrument for pain, sensory neuropathy, or GI symptoms, although the latter was to some extent assessed using COMPASS 31.

5.2.4 Continuation or discontinuation, and for how long?

We acknowledge that before performing CARTs, it is recommended to discontinue several medications (75). This was up for discussion in our group and decided to be avoided, because of the risk of also short-term discontinuation impacting the results, like rebound tachycardia when discontinuing betablockers. Sub-analyses between those with and without betablockers did not show any difference in heartrate, SDNN or RMSSD (p-values of 0.38, 0.73 and 0.64 respectively).

We also acknowledge an uncertainty regarding how the use of metformin and DPP-4 inhibitors affect GIGD and the incretin effect. Although these were discontinued a total of three days ahead of OGTT and IIGI, thus, according to plasma half-life should not affect glucose levels, studies have shown that metformin can induce islet incretin receptor gene expression and that metformin increases total GLP-1 following administration (240). Similar studies as ours have discontinued medications between two and seven days (241, 242). In our consideration, discontinuation for more than three days could have led to an unacceptable hyperglycaemia situation.

5.2.5 Diagnostic considerations and a selective population

Section 1.1.3 reviews the diagnostic approaches in diabetes. We originally planned to use the ADA criteria for HbA1c values, with regard to diabetes and prediabetes. However, after performing an OGTT, several of the participants displayed a fasting or 2-hour glucose value above the diagnosis threshold, and therefore we needed to recategorize several of the participants. The decision was based on the ADA recommendation of using fasting and/or 2-hour glucose value if discordance between HbA1c and glucose values. The 2-hour value is reported to diagnose more people with diabetes and prediabetes than the other two (9). Performing the OGTT, it also turned out that several people in the control group had glucose values consistent with prediabetes.

A limitation regarding diagnosis is that we did not confirm the early diagnose with retesting, and we did not measure relevant antibodies to exclude people with LADA, although glucose stimulated c-peptide values point towards type 2 diabetes in all participants. Neither did we investigate for secondary diabetes, but also in these, we would suspect a lower value of c-peptide.

The longstanding diabetes group was recruited based on self-reported type 2 diabetes and duration. We consider it a strength that all participants in this group underwent an OGTT to confirm the diagnosis, as many of them were treated to a normal value for HbA1c.

We acknowledge the fact and limitation regarding our selective cohort. We first aimed to recruit people with longstanding diabetes, then to match the other groups accordingly. We ended up with a group of longstanding diabetes with a fairly normal average BMI, good glycaemic control, and few complications. This is probably a consequence of excluding people using insulin, GLP-1 analogues or those with retinopathy. This may raise the question if our study population is too selective and if the results are applicable to other populations. On the other hand, the group of longstanding diabetes is to a certain degree representable, with the average debut of type 2 diabetes in Norway being around 60 years, our participants at the time of study

being around 70 years. The average BMI with the onset of type 2 diabetes is 29 kg/m^2, with an average BMI in our population of 25 (40). Obviously, our group of early diagnosed people is on average diagnosed at a later stage, with a lower BMI, and would probably fit into the new proposed category of MARD (moderate age-related diabetes). The nature of our cohort is certainly also a reason for the low detection of diabetic neuropathy.

On the contrary, we were able to shed light on an age-group formerly not studied, which exhibit an increasing prevalence of type 2 diabetes, mainly due to longer life expectancy. With regards to the impact of low BMI on external validity, this may also be seen as a strength, more certainly attributing the reduced incretin effect to diabetes, as obesity itself has also been shown to deteriorate the incretin effect (227).

In conclusion, we ended up with a group of well-controlled people with long-term diabetes, a group of recently diagnosed diabetes, and a control group where several would be classified as prediabetes from the OGTT. The composition might be more homogenous than planned for, and this probably contributes to the few differences found between our groups in several of the tests. Still, we do have significantly different HbA1c, fasting, and two-hour values between all groups.

The inclusion of people with both longstanding and early diabetes is either way a strength that made us able to examine the time aspect of both GIGD, the incretin effect and neuropathy and to be able to indicate a continuum in the processes.

5.2.6 Other possible limitations

Regarding evoked potentials and rectal sensitivity, previous studies have shown that acute hyperglycaemia in people without diabetes increases rectal sensitivity, probably mediated by central mechanisms (243, 244). In people with diabetes, acute hyperglycaemia has been shown to inhibit the external anal sphincter and decrease rectal compliance, suggesting that investigation of rectal sensitivity should be performed euglycaemic (245). We did not perform rapid rectal balloon distention during a euglycaemic clamp, and therefore cannot completely exclude that this may

have affected our results. On the other hand, no association was found between normo- and hyperglycaemia in sensitivity to electrical oesophagus or median nerve stimuli in diabetes (208).

5.2.7 Statistical aspects

Lastly, we must acknowledge the limitation of being a pilot study and without known effect size for the rapid balloon distention test, making it difficult to calculate statistical power. Consequently, several of our secondary aims and hypothesis neither have power calculations. For some of our correlation analysis, we performed multiple testing. We decided not to adjust for multiple tests regarding correlation analyses between the rapid rectal balloon distention test and the other tests, mostly because our aim was not to draw firm conclusions, but to generate new hypotheses. With respect to this, we need to interpret the correlations with caution, a fact that we acknowledge.

6. CONCLUSIONS

In this thesis, we may draw the following conclusions:

- Rectal hyposensitivity was present in both people with longstanding and early diabetes, indicating an early visceral afferent impairment in the development of diabetes.
- Rectal hyposensitivity was present in people with reduced peripheral sensation, judged by the monofilament test.
- Based on the nature of the mechanosensitive stimuli used, the mechanism for the hyposensitivity appears to be of peripheral origin, although correlations between evoked potentials and other neuropathy tests exist, and we therefore cannot rule out simultaneous central neuronal affection.
- Measurement of evoked potentials after rapid rectal balloon distention may be a promising tool to investigate the autonomic nervous system of the gut, independently of other neuropathy tests.
- We found the Norwegian version of the COMPASS 31 questionnaire easy to use and feasible for digital distribution and interpretation in larger cohorts.
- The highest burden of autonomic symptoms was found in people with longstanding diabetes and women.
- Using COMPASS 31 in a population similar to ours, a score $\leq$10 points seem suitable for the exclusion of cardiac autonomic neuropathy, with a good negative predictive value.
- Although there were signs of visceral afferent dysfunction, people with newly diagnosed diabetes did not appear to have symptoms of autonomic neuropathy, supporting the development of neuropathic changes before symptoms develop.
- Rectal hyposensitivity was associated with reduced GIGD, but not with the incretin effect, suggesting a possible role for autonomic neuropathy in other factors of GIGD than the incretin effect.
- The incretin effect was associated with glucose values at all time-points of the OGTT, HbA1c and duration of diabetes.

7. FUTURE PERSPECTIVES

7.1 Further exploration of the PanGut material

- We are in the process of examining GLP-1, GIP and glucagon, to see whether levels of hormones differ in our participant groups, can predict incretin effect or GIGD, or correlate with other findings, such as rectal hyposensitivity.
- Subject to availability and affordability we will investigate the possibility of measuring other related gut-peptides, including novel emerging peptides and possibly relevant metabolomic parameters.
- The PanGut study includes examination of gall bladder emptying during a mixed meal test, to explore the role of gall bladder emptying and bile acids, on the incretin effect. These analyses and disseminations are well underway, and subject to another PhD work by my colleague Tæraneh Jouleh.
- We have the possibility of investigating and comparing differences in central brain areas activated, using the "inverse modelling" technique.
- It would be interesting to go more in depth sorting out what characterizes those with low and high GIGD, and incretin effect, or those which require low or high pressure for first rectal sensation.
- Furthermore, it would be exciting from a clinical perspective to focus on the reduced GIGD and incretin effect despite normal values for HbA1c, and further discuss diagnostic criteria, and/or importance of detecting prediabetes. New hypotheses could include exploring who is more likely to develop diabetes and if there are biomarkers for this.
- Further subject for discussion is whether we should also perform gut motility examinations and if we should include examining microbiota, but neither of these were prespecified in the project protocol.

7.2 Future studies – a plethora of possibilities

So, were should we go from here, with so many possibilities?

1) My first objective would be to perform a study to confirm our findings of early rectal hyposensitivity in a more representative cohort of newly diagnosed type 2 diabetes, with younger age and higher BMI, and consider the following:

- To exclude the evoked potential part, only investigating sensitivity, making the study more feasible. The test should be performed blindly, so that the investigator does not expect what pressure to anticipate. If feasible, the test should be performed euglycemic, but as a minimum, with a measure of glucose values before or during the test.
- To recruit from the hospitals' "beginner-course" for newly diagnosed type 2 diabetes, and include a matching control group in age, sex and BMI.
- Investigations should include an OGTT and IIGI, to estimate GIGD and the incretin effect, and perform investigations before the subject need antidiabetic medication, to avoid any limitations regarding former use of metformin, GLP-analogues or DPP-4.
- A new study should include investigations of all possible factors involved in GIGD, e.g., gastric emptying and gut motility performing a wireless motility capsule, possibly including tracing techniques to assess endogenous glucose production and glucose uptake of liver and other tissue.
- Measures should include GLP-1, GIP, and glucagon, and other related gut peptides or metabolites that the PanGut study should find interesting.

2) A similar study as mentioned above could also be performed in people with obesity, possibly splitting them into groups of prediabetes and controls. It would be interesting to investigate baseline rectal sensitivity, GIGD and the incretin effect and to follow the participants, e.g., yearly, to see who develops manifest diabetes or not, and to search for different baseline markers to predict outcomes.

3) A different angle could be a further validation of the rapid rectal balloon distention test as a test for diabetic gastroenteropathy. This kind of study should include a group with known autonomic neuropathy, to see if the test correlates with other tests, alternatively, in a group with known gastroenteropathy, with GI symptoms, and

investigate if the test is related to GI symptoms and signs. A future study should probably include a more accurate test regarding small fibre neuropathy, with agreement that small nerve fibre tests are necessary for early detection of neuropathy. For this purpose, it would be easier if the diabetic neuropathy society could unite on which test would be best suited in research and/or clinical setting.

4) Finally, studies on a larger population should be performed using the COMPASS 31 questionnaire. Optimally we should validate the questionnaire in Norwegian, in a group with autonomic neuropathy compared to controls. Given proper validation, I would very much like to distribute the questionnaire to a larger population of our patients at the clinic, as my hypothesis is that both symptoms and signs of autonomic neuropathy are neglected by both patients and healthcare professionals in the clinical setting.

5) Although far from my field of expertise, further exploring of the connection between neuropathy and GIGD, should include basic science research. This could include new evolving techniques for disruption of autonomic nerve receptors and neurotransmitters after a meal, performing vagal afferent branch point signal gating to increase our understanding on how, where and when hormonal signals are integrated and possibly disturbed, further exploration of central mechanisms, perhaps using MRI techniques, and better morphological visualization of vagal nerves in the gut. Other possibilities may be transcriptomic and genetic studies to better delineate the difference and role of neurons expressing the GLP-1 receptor in the stomach (mechanosensitive) and intestinal mucosa (chemo sensitive). There also exist emerging tests called optogenetics, in vivo ganglion imaging, in mouse models shown to be a promising modality for further investigation of the intricate gut nervous system (86, 87, 246). Hopefully, clinical and basic research will go hand in hand to increase knowledge in the field.

7.3 Possible clinical consequences

"Superior doctors prevent disease. Mediocre doctors treat the disease before it is evident. Inferior doctors treat the full-blown disease." Huang Dee Nai-Chang, from the first known Chinese medical text, 2600 BCE

From a clinical approach, prevention of diabetes and remission of both diabetes and prediabetes should be the first line measure to prevent further deterioration of GIGD, the incretin effect, and the development of neuropathy. This involves lifestyle interventions and measures both at individual and society levels, and requires strategies for early detection. Treatment possibilities include the use of incretin mimetics with an increasingly stronger weight-reducing effect, potent glucose lowering, and even - for GLP-1 analogues - a possibility of neuroprotection (196, 247). Recent studies in bariatric surgery have also shown a high degree of diabetes remission, and an ability to reverse peripheral neuropathy and stabilize CAN (236, 248, 249). Other neuroprotective interventions have shown little to modest effect trying to prevent neuropathy in type 2 diabetes, but trials have mostly used large nerve fibre function as outcome, with regeneration of small nerve fibre capacity being better documented (250). This supports early detection of small nerve fibre damage and performing studies on neuroprotective treatment with outcomes on small nerve fibres (251).

Another possible treatment approach in the future is to induce better conditions for the gut-brain axis based on modulation of neuroplastic changes with afferent nerve stimulation. Vagal nerve stimulation has, in animal studies, been shown to supress food intake and prevent weight gain, and humans with depression treated with vagal stimulation have experienced weight reduction (252, 253). The method is believed to mimic the actions of gastric mechanoreceptors and jejunal chemoreceptors, but is largely limited by its high cost and invasiveness. However, recently studies that performed transcutaneous auricular vagal nerve stimulation have shown promising results, activating NTS and other vagal projections in the brainstem and forebrain, suggesting the possibility of restoring insulin resistance and secretion, and

counteracting autonomic dysfunction (254). Ongoing studies include investigation on whether performing vagal nerve stimulation can reduce GI symptoms induced by autonomic neuropathy in people with diabetes.

In a larger, future clinical perspective we should try to answer the following:

- Could there be an early biomarker for neuronal damage as a consequence of hyperglycaemia, and to pinpoint at what level of hyperglycaemia nerves are damaged?
- Can we find more mechanisms to treat, or even prevent neuronal damage, including neurons in the gut?
- Could we decipher what part of GIGD that is possibly affected by nerve damage, and detect potential markers for this?
- Since GIGD and the incretin effect seem to follow a continuum; Is there a biomarker for whom with prediabetes are most susceptible of developing diabetes or related complications, and could the degree of remaining GIGD or incretin effect, or some derivate thereof, help in this prediction?

Although exogenous GLP-1, GIP, and other gut hormones are shown to be powerful in treating type 2 diabetes and obesity, few medications are without side effects, some people are non-responders and we know that they activate the sympathetic nervous system, which is probably undesirable for chronic therapy (106). This means that there is still a window of opportunity in finding ways to improve the function of the endogenous incretin hormones based on increased understanding of the pathomechanisms. There is still a lot to learn from the gut!

8. SOURCE OF DATA

1. Federation ID. IDF Diabetes Atlas, 10th edition. 2021 [Available from: https://diabetesatlas.org/.
2. WHO. Fact sheet 2023 [Available from: https://www.who.int/news-room/fact-sheets/detail/diabetes.
3. Hostalek U. Global epidemiology of prediabetes - present and future perspectives. Clin Diabetes Endocrinol. 2019;5:5.
4. Stene LC, Ruiz PL, Åsvold BO, Bjarkø VV, Sørgjerd EP, Njølstad I, et al. How many people have diabetes in Norway in 2020? Tidsskr Nor Laegeforen. 2020;140(17).
5. Magliano DJ, Chen L, Islam RM, Carstensen B, Gregg EW, Pavkov ME, et al. Trends in the incidence of diagnosed diabetes: a multicountry analysis of aggregate data from 22 million diagnoses in high-income and middle-income settings. The Lancet Diabetes & Endocrinology. 2021;9(4):203-11.
6. Fang M, Wang D, Coresh J, Selvin E. Undiagnosed Diabetes in U.S. Adults: Prevalence and Trends. Diabetes Care. 2022:dc220242.
7. Jørgensen ME, Ellervik C, Ekholm O, Johansen NB, Carstensen B. Estimates of prediabetes and undiagnosed type 2 diabetes in Denmark: The end of an epidemic or a diagnostic artefact? Scandinavian Journal of Public Health. 2018;48(1):106-12.
8. Knudsen JS, Knudsen SS, Hulman A, Witte DR, Gregg EW, Lauritzen T, et al. Changes in type 2 diabetes incidence and mortality associated with introduction of HbA1c as diagnostic option: A Danish 24-year population-based study. The Lancet Regional Health - Europe. 2022;14:100291.
9. ElSayed NA, Aleppo G, Aroda VR, Bannuru RR, Brown FM, Bruemmer D, et al. 2. Classification and Diagnosis of Diabetes: Standards of Care in Diabetes—2023. Diabetes Care. 2022;46(Supplement_1):S19-S40.
10. Chung WK, Erion K, Florez JC, Hattersley AT, Hivert MF, Lee CG, et al. Precision Medicine in Diabetes: A Consensus Report From the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetes Care. 2020;43(7):1617-35.
11. Defronzo RA. Banting Lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. Diabetes. 2009;58(4):773-95.
12. Taylor R, Al-Mrabeh A, Sattar N. Understanding the mechanisms of reversal of type 2 diabetes. Lancet Diabetes Endocrinol. 2019;7(9):726-36.
13. Tabák AG, Herder C, Rathmann W, Brunner EJ, Kivimäki M. Prediabetes: a high-risk state for diabetes development. The Lancet. 2012;379(9833):2279-90.
14. Rooney MR, Rawlings AM, Pankow JS, Echouffo Tcheugui JB, Coresh J, Sharrett AR, et al. Risk of Progression to Diabetes Among Older Adults With Prediabetes. JAMA Internal Medicine. 2021.
15. Forouhi NG, Luan J, Hennings S, Wareham NJ. Incidence of Type 2 diabetes in England and its association with baseline impaired fasting glucose: The Ely study 1990–2000. Diabetic Medicine. 2007;24(2):200-7.

16. Kendall DM, Cuddihy RM, Bergenstal RM. Clinical application of incretin-based therapy: therapeutic potential, patient selection and clinical use. Am J Med. 2009;122(6 Suppl):S37-50.
17. Zaharia OP, Strassburger K, Strom A, Bonhof GJ, Karusheva Y, Antoniou S, et al. Risk of diabetes-associated diseases in subgroups of patients with recent-onset diabetes: a 5-year follow-up study. Lancet Diabetes Endocrinol. 2019;7(9):684-94.
18. Li X, Yang S, Cao C, Yan X, Zheng L, Zheng L, et al. Validation of the Swedish Diabetes Re-Grouping Scheme in Adult-Onset Diabetes in China. J Clin Endocrinol Metab. 2020;105(10).
19. Pigeyre M, Hess S, Gomez MF, Asplund O, Groop L, Paré G, et al. Validation of the classification for type 2 diabetes into five subgroups: a report from the ORIGIN trial. Diabetologia. 2021.
20. Gillett MJ. International Expert Committee report on the role of the A1c assay in the diagnosis of diabetes: Diabetes Care 2009; 32(7): 1327-1334. Clin Biochem Rev. 2009;30(4):197-200.
21. Cowie CC, Rust KF, Byrd-Holt DD, Gregg EW, Ford ES, Geiss LS, et al. Prevalence of diabetes and high risk for diabetes using A1C criteria in the U.S. population in 1988-2006. Diabetes Care. 2010;33(3):562-8.
22. Meijnikman AS, De Block CEM, Dirinck E, Verrijken A, Mertens I, Corthouts B, et al. Not performing an OGTT results in significant underdiagnosis of (pre)diabetes in a high risk adult Caucasian population. Int J Obes (Lond). 2017;41(11):1615-20.
23. Gonzalez A, Deng Y, Lane AN, Benkeser D, Cui X, Staimez LR, et al. Impact of mismatches in HbA(1c) vs glucose values on the diagnostic classification of diabetes and prediabetes. Diabet Med. 2020;37(4):689-96.
24. Bansal N. Prediabetes diagnosis and treatment: A review. World journal of diabetes. 2015;6(2):296-303.
25. Young KG, McGovern AP, Barroso I, Hattersley AT, Jones AG, Shields BM, et al. The impact of population-level HbA1c screening on reducing diabetes diagnostic delay in middle-aged adults: a UK Biobank analysis. Diabetologia. 2022.
26. Yahyavi SK, Snorgaard O, Knop FK, Schou M, Lee C, Selmer C, et al. Prediabetes Defined by First Measured HbA1c Predicts Higher Cardiovascular Risk Compared With HbA1c in the Diabetes Range: A Cohort Study of Nationwide Registries. Diabetes Care. 2021.
27. Ruiz PLD, Chen L, Morton JI, Salim A, Carstensen B, Gregg EW, et al. Mortality trends in type 1 diabetes: a multicountry analysis of six population-based cohorts. Diabetologia. 2022.
28. Ali MK, Pearson-Stuttard J, Selvin E, Gregg EW. Interpreting global trends in type 2 diabetes complications and mortality. Diabetologia. 2021.
29. Lind M, Imberg H, Coleman RL, Nerman O, Holman RR. Historical HbA1c Values May Explain the Type 2 Diabetes Legacy Effect: UKPDS 88. Diabetes Care. 2021:dc202439.
30. Visseren FLJ, Mach F, Smulders YM, Carballo D, Koskinas KC, Bäck M, et al. 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice: Developed by the Task Force for cardiovascular disease prevention in clinical practice with representatives of the European Society of Cardiology and 12 medical

societies With the special contribution of the European Association of Preventive Cardiology (EAPC). European Heart Journal. 2021;42(34):3227-337.
31. Birkeland KI, Bodegard J, Eriksson JW, Norhammar A, Haller H, Linssen GCM, et al. Heart failure and chronic kidney disease manifestation and mortality risk associations in type 2 diabetes: A large multinational cohort study. Diabetes Obes Metab. 2020;22(9):1607-18.
32. Davies MJ, Aroda VR, Collins BS, Gabbay RA, Green J, Maruthur NM, et al. Management of hyperglycaemia in type 2 diabetes, 2022. A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetologia. 2022.
33. Vision Loss Expert Group of the Global Burden of Disease Study; Causes of blindness and vision impairment in 2020 and trends over 30 years, and prevalence of avoidable blindness in relation to VISION 2020: the Right to Sight: an analysis for the Global Burden of Disease Study. Lancet Glob Health. 2021;9(2):e144-e60.
34. Purola PKM, Ojamo MUI, Gissler M, Uusitalo HMT. Changes in Visual Impairment due to Diabetic Retinopathy During 1980–2019 Based on Nationwide Register Data. Diabetes Care. 2022:dc212369.
35. Afkarian M, Zelnick LR, Hall YN, Heagerty PJ, Tuttle K, Weiss NS, et al. Clinical Manifestations of Kidney Disease Among US Adults With Diabetes, 1988-2014. Jama. 2016;316(6):602-10.
36. Levin A, Tonelli M, Bonventre J, Coresh J, Donner JA, Fogo AB, et al. Global kidney health 2017 and beyond: a roadmap for closing gaps in care, research, and policy. Lancet. 2017;390(10105):1888-917.
37. Ahlqvist E, Storm P, Karajamaki A, Martinell M, Dorkhan M, Carlsson A, et al. Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables. Lancet Diabetes Endocrinol. 2018;6(5):361-9.
38. ElSayed NA, Aleppo G, Aroda VR, Bannuru RR, Brown FM, Bruemmer D, et al. 4. Comprehensive Medical Evaluation and Assessment of Comorbidities: Standards of Care in Diabetes—2023. Diabetes Care. 2022;46(Supplement_1):S49-S67.
39. National Guidelines for Diabetes: The Norwegian Directorate of Health; 2019 [Available from: https://www.helsedirektoratet.no/retningslinjer/diabetes.
40. Bakke A, Cooper JG, Thue G, Skeie S, Carlsen S, Dalen I, et al. Type 2 diabetes in general practice in Norway 2005-2014: moderate improvements in risk factor control but still major gaps in complication screening. BMJ Open Diabetes Res Care. 2017;5(1):e000459.
41. Schlesinger S, Neuenschwander M, Barbaresko J, Lang A, Maalmi H, Rathmann W, et al. Prediabetes and risk of mortality, diabetes-related complications and comorbidities: umbrella review of meta-analyses of prospective studies. Diabetologia. 2021.
42. Cai X, Zhang Y, Li M, Wu JH, Mai L, Li J, et al. Association between prediabetes and risk of all cause mortality and cardiovascular disease: updated meta-analysis. BMJ. 2020;370:m2297.

43. Welsh C, Welsh P, Celis-Morales CA, Mark PB, Mackay D, Ghouri N, et al. Glycated Hemoglobin, Prediabetes, and the Links to Cardiovascular Disease: Data From UK Biobank. Diabetes Care. 2020;43(2):440.
44. Vistisen D, Witte DR, Brunner EJ, Kivimaki M, Tabak A, Jorgensen ME, et al. Risk of Cardiovascular Disease and Death in Individuals With Prediabetes Defined by Different Criteria: The Whitehall II Study. Diabetes Care. 2018;41(4):899-906.
45. Diabetes Prevention Program Research Group; The prevalence of retinopathy in impaired glucose tolerance and recent-onset diabetes in the Diabetes Prevention Program. Diabet Med. 2007;24(2):137-44.
46. Feldman EL, Callaghan BC, Pop-Busui R, Zochodne DW, Wright DE, Bennett DL, et al. Diabetic neuropathy. Nature Reviews Disease Primers. 2019;5(1):41.
47. Ziegler D, Tesfaye S, Spallone V, Gurieva I, Al Kaabi J, Mankovsky B, et al. Screening, diagnosis and management of diabetic sensorimotor polyneuropathy in clinical practice: International expert consensus recommendations. Diabetes Research and Clinical Practice. 2021:109063.
48. Pop-Busui R, Boulton AJM, Feldman EL, Bril V, Freeman R, Malik RA, et al. Diabetic Neuropathy: A Position Statement by the American Diabetes Association. 2017;40(1):136-54.
49. Young MJ, Boulton AJ, MacLeod AF, Williams DR, Sonksen PH. A multicentre study of the prevalence of diabetic peripheral neuropathy in the United Kingdom hospital clinic population. Diabetologia. 1993;36(2):150-4.
50. Pfannkuche A, Alhajjar A, Ming A, Walter I, Piehler C, Mertens PR. Prevalence and risk factors of diabetic peripheral neuropathy in a diabetics cohort: Register initiative “diabetes and nerves”. Endocrine and Metabolic Science. 2020;1(1):100053.
51. Papanas N, Vinik AI, Ziegler D. Neuropathy in prediabetes: does the clock start ticking early? Nature Reviews Endocrinology. 2011;7:682.
52. Azmi S, Ferdousi M, Petropoulos IN, Ponirakis G, Alam U, Fadavi H, et al. Corneal Confocal Microscopy Identifies Small-Fiber Neuropathy in Subjects With Impaired Glucose Tolerance Who Develop Type 2 Diabetes. Diabetes Care. 2015;38(8):1502-8.
53. Asghar O, Petropoulos IN, Alam U, Jones W, Jeziorska M, Marshall A, et al. Corneal confocal microscopy detects neuropathy in subjects with impaired glucose tolerance. Diabetes Care. 2014;37(9):2643-6.
54. Meldgaard T, Olesen SS, Farmer AD, Krogh K, Wendel AA, Brock B, et al. Diabetic Enteropathy: From Molecule to Mechanism-Based Treatment. J Diabetes Res. 2018;2018:3827301.
55. Bonhof GJ, Herder C, Strom A, Papanas N, Roden M, Ziegler D. Emerging Biomarkers, Tools, and Treatments for Diabetic Polyneuropathy. Endocr Rev. 2019;40(1):153-92.
56. ElSayed NA, Aleppo G, Aroda VR, Bannuru RR, Brown FM, Bruemmer D, et al. 12. Retinopathy, Neuropathy, and Foot Care: Standards of Care in Diabetes—2023. Diabetes Care. 2022;46(Supplement_1):S203-S15.
57. Tesfaye S, Boulton AJ, Dyck PJ, Freeman R, Horowitz M, Kempler P, et al. Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. Diabetes Care. 2010;33(10):2285-93.

58. Lee JA, Halpern EM, Lovblom LE, Yeung E, Bril V, Perkins BA. Reliability and validity of a point-of-care sural nerve conduction device for identification of diabetic neuropathy. PLoS One. 2014;9(1):e86515.
59. Krishnan ST, Quattrini C, Jeziorska M, Malik RA, Rayman G. Abnormal LDIflare but normal quantitative sensory testing and dermal nerve fiber density in patients with painful diabetic neuropathy. Diabetes Care. 2009;32(3):451-5.
60. Perkins BA, Lovblom LE, Bril V, Scarr D, Ostrovski I, Orszag A, et al. Corneal confocal microscopy for identification of diabetic sensorimotor polyneuropathy: a pooled multinational consortium study. Diabetologia. 2018;61(8):1856-61.
61. Roikjer J, Croosu SS, Frokjaer JB, Hansen TM, Arendt-Nielsen L, Ejskjaer N, et al. Perception threshold tracking: validating a novel method for assessing function of large and small sensory nerve fibers in diabetic peripheral neuropathy with and without pain. Pain. 2022.
62. Meling S, Bertoli D, Sangnes DA, Brock C, Drewes A, Ejskjaer N, et al. Diabetic Gastroenteropathy, Soothe the Symptoms or Unravel a Cure? Curr Diabetes Rev. 2021.
63. Jansen JKS, Glover J. Det autonome nervesystemet i Store medisinske leksikon på snl.no. (The autonomic nervous system in the Norwegian Great medical encyclopedia at snl.no). Retrieved 16. march 2023 from https://sml.snl.no/det_autonome_nervesystemet.
64. Sanvictores T, Tadi P. Neuroanatomy, Autonomic Nervous System Visceral Afferent Fibers and Pain. [Updated 2022 Oct 3]. In: StatPearls Treasure Island (FL): Available from: https://www.ncbi.nlm.nih.gov/books/NBK560843/. 2022.
65. Prescott SL, Liberles SD. Internal senses of the vagus nerve. Neuron. 2022;110(4):579-99.
66. 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.
67. Spallone V, Ziegler D, Freeman R, Bernardi L, Frontoni S, Pop-Busui R, et al. Cardiovascular autonomic neuropathy in diabetes: clinical impact, assessment, diagnosis, and management. Diabetes Metab Res Rev. 2011;27(7):639-53.
68. Fraser DM, Campbell IW, Ewing DJ, Murray A, Neilson JM, Clarke BF. Peripheral and autonomic nerve function in newly diagnosed diabetes mellitus. Diabetes. 1977;26(6):546-50.
69. Williams SM, Eleftheriadou A, Alam U, Cuthbertson DJ, Wilding JPH. Cardiac Autonomic Neuropathy in Obesity, the Metabolic Syndrome and Prediabetes: A Narrative Review. Diabetes Ther. 2019;10(6):1995-2021.
70. Wang K, Ahmadizar F, Geurts S, Arshi B, Kors JA, Rizopoulos D, et al. Heart rate variability and incident type 2 diabetes in general population. The Journal of Clinical Endocrinology & Metabolism. 2023:dgad200.
71. Malavige LS, Levy JC. Erectile dysfunction in diabetes mellitus. J Sex Med. 2009;6(5):1232-47.
72. Haugstvedt A, Jorgensen J, Strandberg RB, Nilsen RM, Haugstvedt JF, Pop-Busui R, et al. Sexual dysfunction in women with type 1 diabetes in Norway: A

cross-sectional study on the prevalence and associations with physical and psychosocial complications. Diabet Med. 2021:e14704.
73. Hotaling JM, Sarma AV, Patel DP, Braffett BH, Cleary PA, Feldman E, et al. Cardiovascular Autonomic Neuropathy, Sexual Dysfunction, and Urinary Incontinence in Women With Type 1 Diabetes. Diabetes Care. 2016;39(9):1587-93.
74. Ewing DJ. Cardiovascular reflexes and autonomic neuropathy. Clin Sci Mol Med. 1978;55(4):321-7.
75. Ziemssen T, Siepmann T. The Investigation of the Cardiovascular and Sudomotor Autonomic Nervous System-A Review. Front Neurol. 2019;10:53.
76. Novak P. Electrochemical skin conductance: a systematic review. Clin Auton Res. 2019;29(1):17-29.
77. Selvarajah D, Kar D, Khunti K, Davies MJ, Scott AR, Walker J, et al. Diabetic peripheral neuropathy: advances in diagnosis and strategies for screening and early intervention. Lancet Diabetes Endocrinol. 2019;7(12):938-48.
78. Sletten DM, Suarez GA, Low PA, Mandrekar J, Singer W. COMPASS 31: a refined and abbreviated Composite Autonomic Symptom Score. Mayo Clin Proc. 2012;87(12):1196-201.
79. Greco C, Di Gennaro F, D'Amato C, Morganti R, Corradini D, Sun A, et al. Validation of the Composite Autonomic Symptom Score 31 (COMPASS 31) for the assessment of symptoms of autonomic neuropathy in people with diabetes. Diabet Med. 2017;34(6):834-8.
80. Peng Y, Liu YS, Wu MY, Chen CN, Li CQ, Jiang AQ, et al. Evaluation of the Degree of Agreement of Four Methods for Diagnosing Diabetic Autonomic Neuropathy. Front Neurol. 2021;12:637099.
81. Sharma A, Lelic D, Brock C, Paine P, Aziz Q. New technologies to investigate the brain-gut axis. World J Gastroenterol. 2009;15(2):182-91.
82. Blackshaw LA, Brookes SJ, Grundy D, Schemann M. Sensory transmission in the gastrointestinal tract. Neurogastroenterol Motil. 2007;19(1 Suppl):1-19.
83. Brierley SM, Hibberd TJ, Spencer NJ. Spinal Afferent Innervation of the Colon and Rectum. Front Cell Neurosci. 2018;12:467.
84. de Lartigue G, Diepenbroek C. Novel developments in vagal afferent nutrient sensing and its role in energy homeostasis. Current Opinion in Pharmacology. 2016;31:38-43.
85. Yu CD, Xu QJ, Chang RB. Vagal sensory neurons and gut-brain signaling. Current Opinion in Neurobiology. 2020;62:133-40.
86. Brierley DI, de Lartigue G. Reappraising the role of the vagus nerve in GLP-1-mediated regulation of eating. Br J Pharmacol. 2022;179(4):584-99.
87. Waise TMZ, Dranse HJ, Lam TKT. The metabolic role of vagal afferent innervation. Nat Rev Gastroenterol Hepatol. 2018;15(10):625-36.
88. Hobday DI, Hobson A, Furlong PL, Thompson DG, Aziz Q. Comparison of cortical potentials evoked by mechanical and electrical stimulation of the rectum. Neurogastroenterology & Motility. 2000;12(6):547-54.
89. Sharma S, Vas P, Rayman G. Small Fiber Neuropathy in Diabetes Polyneuropathy: Is It Time to Change? J Diabetes Sci Technol. 2021:1932296821996434.

90. Payne SC, Furness JB, Stebbing MJ. Bioelectric neuromodulation for gastrointestinal disorders: effectiveness and mechanisms. Nature Reviews Gastroenterology & Hepatology. 2019;16(2):89-105.
91. D'Agostino G, Luckman SM. Brainstem peptides and peptidergic neurons in the regulation of appetite. Current Opinion in Endocrine and Metabolic Research. 2022;24:100339.
92. Holzer P, Reichmann F, Farzi A. Neuropeptide Y, peptide YY and pancreatic polypeptide in the gut–brain axis. Neuropeptides. 2012;46(6):261-74.
93. Yoon NA, Diano S. Hypothalamic glucose-sensing mechanisms. Diabetologia. 2021.
94. Jais A, Brüning JC. Arcuate Nucleus-Dependent Regulation of Metabolism—Pathways to Obesity and Diabetes Mellitus. Endocrine Reviews. 2021.
95. Subramanian KS, Lauer LT, Hayes AMR, Décarie-Spain L, McBurnett K, Nourbash AC, et al. Hypothalamic melanin-concentrating hormone neurons integrate food-motivated appetitive and consummatory processes in rats. Nature Communications. 2023;14(1):1755.
96. Broberger C, Hökfelt T. Hypothalamic and vagal neuropeptide circuitries regulating food intake. Physiology & Behavior. 2001;74(4):669-82.
97. Zanchi D, Depoorter A, Egloff L, Haller S, Mählmann L, Lang UE, et al. The impact of gut hormones on the neural circuit of appetite and satiety: A systematic review. Neuroscience & Biobehavioral Reviews. 2017;80:457-75.
98. Cifuentes L, Acosta A. Homeostatic regulation of food intake. Clinics and Research in Hepatology and Gastroenterology. 2022;46(2):101794.
99. Furness JB. Integrated Neural and Endocrine Control of Gastrointestinal Function. Adv Exp Med Biol. 2016;891:159-73.
100. Lewis JE, Ebling FJP, Samms RJ, Tsintzas K. Going Back to the Biology of FGF21: New Insights. Trends Endocrinol Metab. 2019;30(8):491-504.
101. Suzuki K, Jayasena CN, Bloom SR. The gut hormones in appetite regulation. J Obes. 2011;2011:528401.
102. Makaronidis JM, Batterham RL. The role of gut hormones in the pathogenesis and management of obesity. Current Opinion in Physiology. 2019;12:1-11.
103. Murphy KG, Bloom SR. Gut hormones and the regulation of energy homeostasis. Nature. 2006;444(7121):854-9.
104. Gilliam-Vigh H, Jorsal T, Nielsen S, Forman J, Pedersen J, Poulsen S, et al. Expression of Neurotensin and its Receptors Along the Intestinal Tract in Type 2 Diabetes Patients and Healthy Controls. The Journal of clinical endocrinology and metabolism. 2023.
105. Knop FK. EJE PRIZE 2018: A gut feeling about glucagon. Eur J Endocrinol. 2018;178(6):R267-R80.
106. Drucker DJ. Evolving Concepts and Translational Relevance of Enteroendocrine Cell Biology. J Clin Endocrinol Metab. 2016;101(3):778-86.
107. Grosse J, Heffron H, Burling K, Akhter Hossain M, Habib AM, Rogers GJ, et al. Insulin-like peptide 5 is an orexigenic gastrointestinal hormone. Proc Natl Acad Sci U S A. 2014;111(30):11133-8.
108. Holst JJ, Gribble F, Horowitz M, Rayner CK. Roles of the Gut in Glucose Homeostasis. Diabetes Care. 2016;39(6):884.

109. Gunawardene AR, Corfe BM, Staton CA. Classification and functions of enteroendocrine cells of the lower gastrointestinal tract. Int J Exp Pathol. 2011;92(4):219-31.
110. Meldgaard T, Keller J, Olesen AE, Olesen SS, Krogh K, Borre M, et al. Pathophysiology and management of diabetic gastroenteropathy. Therap Adv Gastroenterol. 2019;12:1756284819852047.
111. Schvarcz E, Palmer M, Aman J, Horowitz M, Stridsberg M, Berne C. Physiological hyperglycemia slows gastric emptying in normal subjects and patients with insulin-dependent diabetes mellitus. Gastroenterology. 1997;113(1):60-6.
112. Schvarcz E, Palmer M, Aman J, Lindkvist B, Beckman KW. Hypoglycaemia increases the gastric emptying rate in patients with type 1 diabetes mellitus. Diabet Med. 1993;10(7):660-3.
113. Du YT, Rayner CK, Jones KL, Talley NJ, Horowitz M. Gastrointestinal Symptoms in Diabetes: Prevalence, Assessment, Pathogenesis, and Management. Diabetes Care. 2018;41(3):627.
114. Sangnes DA, Softeland E, Bekkelund M, Frey J, Biermann M, Gilja OH, et al. Wireless motility capsule compared with scintigraphy in the assessment of diabetic gastroparesis. Neurogastroenterol Motil. 2020;32(4):e13771.
115. Talley NJ, Verlinden M, Jones M. Can symptoms discriminate among those with delayed or normal gastric emptying in dysmotility-like dyspepsia? Am J Gastroenterol. 2001;96(5):1422-8.
116. Jackson AF, Bolger DJ. The neurophysiological bases of EEG and EEG measurement: a review for the rest of us. Psychophysiology. 2014;51(11):1061-71.
117. Lelic D, Olesen SS, Valeriani M, Drewes AM. Brain source connectivity reveals the visceral pain network. Neuroimage. 2012;60(1):37-46.
118. Kappenman ES, Luck SJ. Best Practices for Event-Related Potential Research in Clinical Populations. Biol Psychiatry Cogn Neurosci Neuroimaging. 2016;1(2):110-5.
119. Zani A. Evoked and Event-Related Potentials. In: Runehov ALC, Oviedo L, editors. Encyclopedia of Sciences and Religions. Dordrecht: Springer Netherlands; 2013. p. 787-92.
120. Frieling T, Enck P, Wienbeck M. Cerebral responses evoked by electrical stimulation of rectosigmoid in normal subjects. Dig Dis Sci. 1989;34(2):202-5.
121. Brock C, Softeland E, Gunterberg V, Frokjaer JB, Lelic D, Brock B, et al. Diabetic autonomic neuropathy affects symptom generation and brain-gut axis. Diabetes Care. 2013;36(11):3698-705.
122. Frokjaer JB, Softeland E, Graversen C, Dimcevski G, Egsgaard LL, Arendt-Nielsen L, et al. Central processing of gut pain in diabetic patients with gastrointestinal symptoms. Diabetes Care. 2009;32(7):1274-7.
123. Haas S, Brock C, Krogh K, Gram M, Nissen TD, Lundby L, et al. Cortical evoked potentials in response to rapid balloon distension of the rectum and anal canal. Neurogastroenterol Motil. 2014;26(6):862-73.
124. Harris ML, Hobson AR, Hamdy S, Thompson DG, Akkermans LM, Aziz Q. Neurophysiological evaluation of healthy human anorectal sensation. Am J Physiol Gastrointest Liver Physiol. 2006;291(5):G950-8.

125. Hollerbach S, Kamath MV, Lock G, Scholmerich J, Upton AR, Tougas G. Assessment of afferent gut--brain function using cerebral evoked responses to esophageal stimulation. Z Gastroenterol. 1998;36(4):313-24.
126. Nissen TD, Brock C, Graversen C, Coen SJ, Hultin L, Aziz Q, et al. Translational aspects of rectal evoked potentials: a comparative study in rats and humans. Am J Physiol Gastrointest Liver Physiol. 2013;305(2):G119-28.
127. Lelic D, Nissen TD, Brock C, Aziz Q, Drewes AM. Rapid balloon distension as a tool to study cortical processing of visceral sensations and pain. Neurogastroenterol Motil. 2015;27(6):832-40.
128. Haas S, Brock C, Krogh K, Gram M, Lundby L, Drewes AM, et al. Abnormal neuronal response to rectal and anal stimuli in patients with idiopathic fecal incontinence. Neurogastroenterol Motil. 2015;27(7):954-62.
129. Haas S, Faaborg P, Gram M, Lundby L, Brock C, Drewes AM, et al. Abnormal neuronal response to rectal and anal stimuli in patients treated with primary radiotherapy for anal cancer. Radiother Oncol. 2018;128(2):369-74.
130. Domnick C, Hauck M, Casey KL, Engel AK, Lorenz J. C-fiber-related EEG-oscillations induced by laser radiant heat stimulation of capsaicin-treated skin. J Pain Res. 2009;2:49-56.
131. Tzabazis AZ, Klukinov M, Crottaz-Herbette S, Nemenov MI, Angst MS, Yeomans DC. Selective nociceptor activation in volunteers by infrared diode laser. Mol Pain. 2011;7:18.
132. Frokjaer JB, Andersen SD, Ejskaer N, Funch-Jensen P, Arendt-Nielsen L, Gregersen H, et al. Gut sensations in diabetic autonomic neuropathy. Pain. 2007;131(3):320-9.
133. Frokjaer JB, Egsgaard LL, Graversen C, Softeland E, Dimcevski G, Blauenfeldt RA, et al. Gastrointestinal symptoms in type-1 diabetes: is it all about brain plasticity? Eur J Pain. 2011;15(3):249-57.
134. Lelic D, Brock C, Softeland E, Frokjaer JB, Andresen T, Simren M, et al. Brain networks encoding rectal sensation in type 1 diabetes. Neuroscience. 2013;237:96-105.
135. Rehfeld JF. The Origin and Understanding of the Incretin Concept. Front Endocrinol (Lausanne). 2018;9:387.
136. Gasbjerg LS, Bergmann NC, Stensen S, Christensen MB, Rosenkilde MM, Holst JJ, et al. Evaluation of the incretin effect in humans using GIP and GLP-1 receptor antagonists. Peptides. 2020;125:170183.
137. Holst JJ, Orskov C, Nielsen OV, Schwartz TW. Truncated glucagon-like peptide I, an insulin-releasing hormone from the distal gut. FEBS Lett. 1987;211(2):169-74.
138. Holst JJ. The physiology of glucagon-like peptide 1. Physiol Rev. 2007;87(4):1409-39.
139. Gribble FM, Reimann F. Metabolic Messengers: glucagon-like peptide 1. Nature Metabolism. 2021;3(2):142-8.
140. Jorsal T, Rhee NA, Pedersen J, Wahlgren CD, Mortensen B, Jepsen SL, et al. Enteroendocrine K and L cells in healthy and type 2 diabetic individuals. Diabetologia. 2018;61(2):284-94.

141. 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):E199-206.
142. Nauck M, Stockmann F, Ebert R, Creutzfeldt W. Reduced incretin effect in type 2 (non-insulin-dependent) diabetes. Diabetologia. 1986;29(1):46-52.
143. Holst JJ. The incretin system in healthy humans: The role of GIP and GLP-1. Metabolism. 2019;96:46-55.
144. Kim BJ, Carlson OD, Jang HJ, Elahi D, Berry C, Egan JM. Peptide YY is secreted after oral glucose administration in a gender-specific manner. J Clin Endocrinol Metab. 2005;90(12):6665-71.
145. Vollmer K, Holst JJ, Baller B, Ellrichmann M, Nauck MA, Schmidt WE, et al. Predictors of Incretin Concentrations in Subjects With Normal, Impaired, and Diabetic Glucose Tolerance. 2008;57(3):678-87.
146. Elliott RM, Morgan LM, Tredger JA, Deacon S, Wright J, Marks V. Glucagon-like peptide-1 (7-36)amide and glucose-dependent insulinotropic polypeptide secretion in response to nutrient ingestion in man: acute post-prandial and 24-h secretion patterns. J Endocrinol. 1993;138(1):159-66.
147. Psichas A, Reimann F, Gribble FM. Gut chemosensing mechanisms. J Clin Invest. 2015;125(3):908-17.
148. Gagnon J, Baggio LL, Drucker DJ, Brubaker PL. Ghrelin Is a Novel Regulator of GLP-1 Secretion. Diabetes. 2014;64(5):1513-21.
149. Ahren B. The neuro-incretin concept. Regul Pept. 2014;194-195:3-5.
150. Ahren B, Holst JJ. The cephalic insulin response to meal ingestion in humans is dependent on both cholinergic and noncholinergic mechanisms and is important for postprandial glycemia. Diabetes. 2001;50(5):1030-8.
151. Hansen L, Lampert S, Mineo H, Holst JJ. Neural regulation of glucagon-like peptide-1 secretion in pigs. American Journal of Physiology-Endocrinology and Metabolism. 2004;287(5):E939-E47.
152. Holst JJ, Rosenkilde MM. Recent advances of GIP and future horizons. Peptides. 2020;125:170230.
153. Campbell Jonathan E, Drucker Daniel J. Pharmacology, Physiology, and Mechanisms of Incretin Hormone Action. Cell Metabolism. 2013;17(6):819-37.
154. Holst JJ. Treatment of Type 2 Diabetes and Obesity on the Basis of the Incretin System: The 2021 Banting Medal for Scientific Achievement Award Lecture. Diabetes. 2021;70(11):2468.
155. Hansen L, Deacon CF, Orskov C, Holst JJ. Glucagon-like peptide-1-(7-36)amide is transformed to glucagon-like peptide-1-(9-36)amide by dipeptidyl peptidase IV in the capillaries supplying the L cells of the porcine intestine. Endocrinology. 1999;140(11):5356-63.
156. Vilsboll T, Krarup T, Deacon CF, Madsbad S, Holst JJ. Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients. Diabetes. 2001;50(3):609-13.
157. Smits MM, van Raalte DH, Tonneijck L, Muskiet MH, Kramer MH, Cahen DL. GLP-1 based therapies: clinical implications for gastroenterologists. Gut. 2016;65(4):702-11.

158. Müller TD, Finan B, Bloom SR, D'Alessio D, Drucker DJ, Flatt PR, et al. Glucagon-like peptide 1 (GLP-1). Molecular Metabolism. 2019;30:72-130.
159. Rocca AS, Brubaker PL. Role of the vagus nerve in mediating proximal nutrient-induced glucagon-like peptide-1 secretion. Endocrinology. 1999;140(4):1687-94.
160. Henderson JR, Jefferys DB, Jones RH, Stanley D. The effect of atropine on the insulin release caused by oral and intravenous glucose in human subjects. Acta Endocrinol (Copenh). 1976;83(4):772-80.
161. Wettergren A, Wøjdemann M, Meisner S, Stadil F, Holst JJ. The inhibitory effect of glucagon-like peptide-1 (GLP-1) 7-36 amide on gastric acid secretion in humans depends on an intact vagal innervation. Gut. 1997;40(5):597-601.
162. Plamboeck A, Veedfald S, Deacon CF, Hartmann B, Wettergren A, Svendsen LB, et al. Characterisation of oral and i.v. glucose handling in truncally vagotomised subjects with pyloroplasty. Eur J Endocrinol. 2013;169(2):187-201.
163. Plamboeck A, Veedfald S, Deacon CF, Hartmann B, Wettergren A, Svendsen LB, et al. The effect of exogenous GLP-1 on food intake is lost in male truncally vagotomized subjects with pyloroplasty. Am J Physiol Gastrointest Liver Physiol. 2013;304(12):G1117-27.
164. Diepenbroek C, Quinn D, Stephens R, Zollinger B, Anderson S, Pan A, et al. Validation and characterization of a novel method for selective vagal deafferentation of the gut. Am J Physiol Gastrointest Liver Physiol. 2017;313(4):G342-g52.
165. Krieger JP, Arnold M, Pettersen KG, Lossel P, Langhans W, Lee SJ. Knockdown of GLP-1 Receptors in Vagal Afferents Affects Normal Food Intake and Glycemia. Diabetes. 2016;65(1):34-43.
166. Bai L, Mesgarzadeh S, Ramesh KS, Huey EL, Liu Y, Gray LA, et al. Genetic Identification of Vagal Sensory Neurons That Control Feeding. Cell. 2019;179(5):1129-43.e23.
167. Krieger JP. Intestinal glucagon-like peptide-1 effects on food intake: Physiological relevance and emerging mechanisms. Peptides. 2020;131:170342.
168. Vahl TP, Tauchi M, Durler TS, Elfers EE, Fernandes TM, Bitner RD, et al. Glucagon-like peptide-1 (GLP-1) receptors expressed on nerve terminals in the portal vein mediate the effects of endogenous GLP-1 on glucose tolerance in rats. Endocrinology. 2007;148(10):4965-73.
169. Nishizawa M, Nakabayashi H, Uehara K, Nakagawa A, Uchida K, Koya D. Intraportal GLP-1 stimulates insulin secretion predominantly through the hepatoportal-pancreatic vagal reflex pathways. American Journal of Physiology-Endocrinology and Metabolism. 2013;305(3):E376-E87.
170. Brierley DI, Holt MK, Singh A, de Araujo A, McDougle M, Vergara M, et al. Central and peripheral GLP-1 systems independently suppress eating. Nature Metabolism. 2021.
171. Adriaenssens AE, Gribble FM, Reimann F. The glucose-dependent insulinotropic polypeptide signaling axis in the central nervous system. Peptides. 2020;125:170194.
172. Holst JJ, Knop FK, Vilsboll T, Krarup T, Madsbad S. Loss of incretin effect is a specific, important, and early characteristic of type 2 diabetes. Diabetes Care. 2011;34 Suppl 2:S251-7.

173. Bagger JI, Knop FK, Lund A, Vestergaard H, Holst JJ, Vilsboll T. Impaired regulation of the incretin effect in patients with type 2 diabetes. J Clin Endocrinol Metab. 2011;96(3):737-45.
174. Knop FK, Vilsboll T, Hojberg PV, Larsen S, Madsbad S, Volund A, et al. Reduced incretin effect in type 2 diabetes: cause or consequence of the diabetic state? Diabetes. 2007;56(8):1951-9.
175. Greenbaum CJ, Prigeon RL, D'Alessio DA. Impaired beta-cell function, incretin effect, and glucagon suppression in patients with type 1 diabetes who have normal fasting glucose. Diabetes. 2002;51(4):951-7.
176. Vilsboll T, Knop FK, Krarup T, Johansen A, Madsbad S, Larsen S, et al. The pathophysiology of diabetes involves a defective amplification of the late-phase insulin response to glucose by glucose-dependent insulinotropic polypeptide-regardless of etiology and phenotype. J Clin Endocrinol Metab. 2003;88(10):4897-903.
177. Hansen KB, Vilsbøll T, Bagger JI, Holst JJ, Knop FK. Reduced Glucose Tolerance and Insulin Resistance Induced by Steroid Treatment, Relative Physical Inactivity, and High-Calorie Diet Impairs the Incretin Effect in Healthy Subjects. The Journal of Clinical Endocrinology & Metabolism. 2010;95(7):3309-17.
178. Jensen DH, Aaboe K, Henriksen JE, Vølund A, Holst JJ, Madsbad S, et al. Steroid-induced insulin resistance and impaired glucose tolerance are both associated with a progressive decline of incretin effect in first-degree relatives of patients with type 2 diabetes. Diabetologia. 2012;55(5):1406-16.
179. Kosinski M, Knop FK, Vedtofte L, Grycewiczv J, Swierzewska P, Cypryk K, et al. Postpartum reversibility of impaired incretin effect in gestational diabetes mellitus. Regul Pept. 2013;186:104-7.
180. Hojberg PV, Vilsboll T, Zander M, Knop FK, Krarup T, Volund A, et al. Four weeks of near-normalization of blood glucose has no effect on postprandial GLP-1 and GIP secretion, but augments pancreatic B-cell responsiveness to a meal in patients with Type 2 diabetes. Diabet Med. 2008;25(11):1268-75.
181. Toft-Nielsen M-B, Damholt MB, Madsbad S, Hilsted LM, Hughes TE, Michelsen BK, et al. Determinants of the Impaired Secretion of Glucagon-Like Peptide-1 in Type 2 Diabetic Patients. The Journal of Clinical Endocrinology & Metabolism. 2001;86(8):3717-23.
182. Calanna S, Christensen M, Holst JJ, Laferrere B, Gluud LL, Vilsboll T, et al. Secretion of glucagon-like peptide-1 in patients with type 2 diabetes mellitus: systematic review and meta-analyses of clinical studies. Diabetologia. 2013;56(5):965-72.
183. Nauck MA, Vardarli I, Deacon CF, Holst JJ, Meier JJ. Secretion of glucagon-like peptide-1 (GLP-1) in type 2 diabetes: what is up, what is down? Diabetologia. 2011;54(1):10-8.
184. Lee J, Cummings BP, Martin E, Sharp JW, Graham JL, Stanhope KL, et al. Glucose sensing by gut endocrine cells and activation of the vagal afferent pathway is impaired in a rodent model of type 2 diabetes mellitus. Am J Physiol Regul Integr Comp Physiol. 2012;302(6):R657-66.

185. Holst JJ, Jepsen SL, Modvig I. GLP-1 – Incretin and pleiotropic hormone with pharmacotherapy potential. Increasing secretion of endogenous GLP-1 for diabetes and obesity therapy. Current Opinion in Pharmacology. 2022;63:102189.
186. Muscelli E, Mari A, Natali A, Astiarraga BD, Camastra S, Frascerra S, et al. Impact of incretin hormones on beta-cell function in subjects with normal or impaired glucose tolerance. Am J Physiol Endocrinol Metab. 2006;291(6):E1144-50.
187. Hansen KB, Vilsboll T, Bagger JI, Holst JJ, Knop FK. Impaired incretin-induced amplification of insulin secretion after glucose homeostatic dysregulation in healthy subjects. J Clin Endocrinol Metab. 2012;97(4):1363-70.
188. Idorn T, Knop FK, Jørgensen M, Holst JJ, Hornum M, Feldt-Rasmussen B. Gastrointestinal factors contribute to glucometabolic disturbances in nondiabetic patients with end-stage renal disease. Kidney International. 2013;83(5):915-23.
189. Muscelli E, Mari A, Casolaro A, Camastra S, Seghieri G, Gastaldelli A, et al. Separate impact of obesity and glucose tolerance on the incretin effect in normal subjects and type 2 diabetic patients. Diabetes. 2008;57(5):1340-8.
190. Færch K, Torekov SS, Vistisen D, Johansen NB, Witte DR, Jonsson A, et al. GLP-1 Response to Oral Glucose Is Reduced in Prediabetes, Screen-Detected Type 2 Diabetes, and Obesity and Influenced by Sex: The ADDITION-PRO Study. Diabetes. 2015;64(7):2513-25.
191. Hovorka R, Soons PA, Young MA. ISEC: a program to calculate insulin secretion. Computer Methods and Programs in Biomedicine. 1996;50(3):253-64.
192. Hare KJ, Vilsboll T, Holst JJ, Knop FK. Inappropriate glucagon response after oral compared with isoglycemic intravenous glucose administration in patients with type 1 diabetes. Am J Physiol Endocrinol Metab. 2010;298(4):E832-7.
193. Holst JJ. Glucagon and other proglucagon-derived peptides in the pathogenesis of obesity. Front Nutr. 2022;9:964406.
194. Nauck MA, Kleine N, Orskov C, Holst JJ, Willms B, Creutzfeldt W. Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7-36 amide) in type 2 (non-insulin-dependent) diabetic patients. Diabetologia. 1993;36(8):741-4.
195. Flint A, Raben A, Astrup A, Holst JJ. Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. The Journal of Clinical Investigation. 1998;101(3):515-20.
196. Rosenstock J, Wysham C, Frías JP, Kaneko S, Lee CJ, Fernández Landó L, et al. Efficacy and safety of a novel dual GIP and GLP-1 receptor agonist tirzepatide in patients with type 2 diabetes (SURPASS-1): a double-blind, randomised, phase 3 trial. The Lancet. 2021;398(10295):143-55.
197. Jastreboff AM, Aronne LJ, Ahmad NN, Wharton S, Connery L, Alves B, et al. Tirzepatide Once Weekly for the Treatment of Obesity. New England Journal of Medicine. 2022;387(3):205-16.
198. Nauck MA, Quast DR, Wefers J, Meier JJ. GLP-1 receptor agonists in the treatment of type 2 diabetes – state-of-the-art. Molecular Metabolism. 2021;46:101102.
199. Gabery S, Salinas CG, Paulsen SJ, Ahnfelt-Rønne J, Alanentalo T, Baquero AF, et al. Semaglutide lowers body weight in rodents via distributed neural pathways. JCI Insight. 2020;5(6).

200. Enebo LB, Berthelsen KK, Kankam M, Lund MT, Rubino DM, Satylganova A, et al. Safety, tolerability, pharmacokinetics, and pharmacodynamics of concomitant administration of multiple doses of cagrilintide with semaglutide 2.4 mg for weight management: a randomised, controlled, phase 1b trial. Lancet. 2021;397(10286):1736-48.
201. Knerr PJ, Mowery SA, Douros JD, Premdjee B, Hjøllund KR, He Y, et al. Next generation GLP-1/GIP/glucagon triple agonists normalize body weight in obese mice. Molecular Metabolism. 2022;63:101533.
202. Freeman R, Wieling W, Axelrod FB, Benditt DG, Benarroch E, Biaggioni I, et al. Consensus statement on the definition of orthostatic hypotension, neurally mediated syncope and the postural tachycardia syndrome. Clinical Autonomic Research. 2011;21(2):69-72.
203. Selvarajah D, Cash T, Davies J, Sankar A, Rao G, Grieg M, et al. SUDOSCAN: A Simple, Rapid, and Objective Method with Potential for Screening for Diabetic Peripheral Neuropathy. PLoS One. 2015;10(10):e0138224.
204. Perkins BA, Orszag A, Ngo M, Ng E, New P, Bril V. Prediction of incident diabetic neuropathy using the monofilament examination: a 4-year prospective study. Diabetes Care. 2010;33(7):1549-54.
205. Navid MS, Niazi IK, Lelic D, Drewes AM, Haavik H. The Effects of Filter's Class, Cutoff Frequencies, and Independent Component Analysis on the Amplitude of Somatosensory Evoked Potentials Recorded from Healthy Volunteers. Sensors (Basel). 2019;19(11).
206. Chaumon M, Bishop DV, Busch NA. A practical guide to the selection of independent components of the electroencephalogram for artifact correction. J Neurosci Methods. 2015;250:47-63.
207. Brinth L, Pors K, Mehlsn J, Sletten DM, Terkelsen AJ, Singer W. Translation and linguistic validation of the Composite Autonomic Symptom Score COMPASS 31 in Danish. Dan Med J. 2021;69(3).
208. Frokjaer JB, Softeland E, Graversen C, Dimcevski G, Drewes AM. Effect of acute hyperglycaemia on sensory processing in diabetic autonomic neuropathy. Eur J Clin Invest. 2010;40(10):883-6.
209. Beyak MJ, Bulmer DC, Sellers D, Grundy D. Impairment of rectal afferent mechanosensitivity in experimental diabetes in the rat. Neurogastroenterol Motil. 2009;21(6):678-81.
210. Gregersen H, Liao D, Drewes AM, Drewes AM, Zhao J. Ravages of Diabetes on Gastrointestinal Sensory-Motor Function: Implications for Pathophysiology and Treatment. Curr Gastroenterol Rep. 2016;18(2):6.
211. Softeland E, Brock C, Frokjaer JB, Brogger J, Madacsy L, Gilja OH, et al. Association between visceral, cardiac and sensorimotor polyneuropathies in diabetes mellitus. J Diabetes Complications. 2014;28(3):370-7.
212. Softeland E, Brock C, Frokjaer JB, Simren M, Drewes AM, Dimcevski G. Rectal sensitivity in diabetes patients with symptoms of gastroparesis. J Diabetes Res. 2014;2014:784841.
213. Drewes AM, Softeland E, Dimcevski G, Farmer AD, Brock C, Frokjaer JB, et al. Brain changes in diabetes mellitus patients with gastrointestinal symptoms. World journal of diabetes. 2016;7(2):14-26.

214. Tougas G, Hunt RH, Fitzpatrick D, Upton AR. Evidence of impaired afferent vagal function in patients with diabetes gastroparesis. Pacing Clin Electrophysiol. 1992;15(10 Pt 2):1597-602.
215. Brock C, Graversen C, Frokjaer JB, Softeland E, Valeriani M, Drewes AM. Peripheral and central nervous contribution to gastrointestinal symptoms in diabetic patients with autonomic neuropathy. Eur J Pain. 2013;17(6):820-31.
216. Töyry JP, Partanen JVS, Niskanen LK, Länsimies EA, Uusitupa MIJ. Divergent development of autonomic and peripheral somatic neuropathies in NIDDM. Diabetologia. 1997;40(8):953-8.
217. Tentolouris N, Pagoni S, Tzonou A, Katsilambros N. Peripheral neuropathy does not invariably coexist with autonomic neuropathy in diabetes mellitus. European Journal of Internal Medicine. 2001;12(1):20-7.
218. Moţăţăianu A, Maier S, Bajko Z, Voidazan S, Bălaşa R, Stoian A. Cardiac autonomic neuropathy in type 1 and type 2 diabetes patients. BMC Neurology. 2018;18(1):126.
219. Pafili K, Trypsianis G, Papazoglou D, Maltezos E, Papanas N. Correlation of cardiac autonomic neuropathy with small and large peripheral nerve function in type 2 diabetes mellitus. Diabetes Res Clin Pract. 2019;156:107844.
220. Røikjer J, Croosu SS, Hansen TM, Frøkjær JB, Brock C, Mørch CD, et al. The co-existence of sensory and autonomic neuropathy in type 1 diabetes with and without pain. Acta diabetologica. 2023.
221. Brock C. Are measures of enteric and autonomic nervous system associated? J Intern Med. 2021.
222. Low PA, Benrud-Larson LM, Sletten DM, Opfer-Gehrking TL, Weigand SD, O'Brien PC, et al. Autonomic symptoms and diabetic neuropathy: a population-based study. Diabetes Care. 2004;27(12):2942-7.
223. Treister R, O'Neil K, Downs HM, Oaklander AL. Validation of the composite autonomic symptom scale 31 (COMPASS-31) in patients with and without small fiber polyneuropathy. Eur J Neurol. 2015;22(7):1124-30.
224. D'Amato C, Greco C, Lombardo G, Frattina V, Campo M, Cefalo CMA, et al. The diagnostic usefulness of the combined COMPASS 31 questionnaire and electrochemical skin conductance for diabetic cardiovascular autonomic neuropathy and diabetic polyneuropathy. J Peripher Nerv Syst. 2020;25(1):44-53.
225. Singh R, Arbaz M, Rai NK, Joshi R. Diagnostic accuracy of composite autonomic symptom scale 31 (COMPASS-31) in early detection of autonomic dysfunction in type 2 diabetes mellitus. Diabetes, metabolic syndrome and obesity : targets and therapy. 2019;12:1735-42.
226. Frokjaer JB, Brock C, Brun J, Simren M, Dimcevski G, Funch-Jensen P, et al. Esophageal distension parameters as potential biomarkers of impaired gastrointestinal function in diabetes patients. Neurogastroenterol Motil. 2012;24(11):1016-e544.
227. Knop FK, Aaboe K, Vilsbøll T, Vølund A, Holst JJ, Krarup T, et al. Impaired incretin effect and fasting hyperglucagonaemia characterizing type 2 diabetic subjects are early signs of dysmetabolism in obesity. Diabetes Obes Metab. 2012;14(6):500-10.

228. Oh TJ, Kim MY, Shin JY, Lee JC, Kim S, Park KS, et al. The incretin effect in Korean subjects with normal glucose tolerance or type 2 diabetes. Clinical Endocrinology. 2014;80(2):221-7.
229. Kazakos KA, Sarafidis PA, Yovos JG. The impact of diabetic autonomic neuropathy on the incretin effect. Med Sci Monit. 2008;14(4):CR213-20.
230. Junker AE, Gluud LL, Holst JJ, Knop FK, Vilsboll T. Influence of gastrointestinal factors on glucose metabolism in patients with cirrhosis. J Gastroenterol Hepatol. 2015;30(10):1522-8.
231. Rasmussen TK, Finnerup NB, Singer W, Jensen TS, Hansen J, Terkelsen AJ. Preferential impairment of parasympathetic autonomic function in type 2 diabetes. Autonomic Neuroscience: Basic and Clinical. 2022;243.
232. Nauck MA, Busing M, Orskov C, Siegel EG, Talartschik J, Baartz A, et al. Preserved incretin effect in type 1 diabetic patients with end-stage nephropathy treated by combined heterotopic pancreas and kidney transplantation. Acta diabetologica. 1993;30(1):39-45.
233. Carnethon MR, Jacobs DR, Sidney S, Liu K. Influence of Autonomic Nervous System Dysfunction on the Development of Type 2 Diabetes. Diabetes Care. 2003;26(11):3035.
234. Fonseca V, Inzucchi SE, Ferrannini E. Redefining the diagnosis of diabetes using glycated hemoglobin. Diabetes Care. 2009;32(7):1344-5.
235. Vistisen D, Kivimaki M, Perreault L, Hulman A, Witte DR, Brunner EJ, et al. Reversion from prediabetes to normoglycaemia and risk of cardiovascular disease and mortality: the Whitehall II cohort study. Diabetologia. 2019;62(8):1385-90.
236. Sjostrom L, Peltonen M, Jacobson P, Ahlin S, Andersson-Assarsson J, Anveden A, et al. Association of bariatric surgery with long-term remission of type 2 diabetes and with microvascular and macrovascular complications. JAMA. 2014;311(22):2297-304.
237. Lean MEJ, Leslie WS, Barnes AC, Brosnahan N, Thom G, McCombie L, et al. Primary care-led weight management for remission of type 2 diabetes (DiRECT): an open-label, cluster-randomised trial. The Lancet. 2018;391(10120):541-51.
238. Davies M, Færch L, Jeppesen OK, Pakseresht A, Pedersen SD, Perreault L, et al. Semaglutide 2.4 mg once a week in adults with overweight or obesity, and type 2 diabetes (STEP 2): a randomised, double-blind, double-dummy, placebo-controlled, phase 3 trial. The Lancet. 2021;397(10278):971-84.
239. Wilding JPH, Batterham RL, Calanna S, Davies M, Van Gaal LF, Lingvay I, et al. Once-Weekly Semaglutide in Adults with Overweight or Obesity. N Engl J Med. 2021.
240. Maida A, Lamont BJ, Cao X, Drucker DJ. Metformin regulates the incretin receptor axis via a pathway dependent on peroxisome proliferator-activated receptor-α in mice. Diabetologia. 2011;54(2):339-49.
241. Shah A, Holter MM, Rimawi F, Mark V, Dutia R, McGinty J, et al. Insulin Clearance After Oral and Intravenous Glucose Following Gastric Bypass and Gastric Banding Weight Loss. Diabetes Care. 2019;42(2):311-7.
242. Lund A, Bagger JI, Christensen M, Grondahl M, van Hall G, Holst JJ, et al. Higher Endogenous Glucose Production During OGTT vs Isoglycemic Intravenous Glucose Infusion. J Clin Endocrinol Metab. 2016;101(11):4377-84.

243. Russo A, Smout AJ, Kositchaiwat C, Rayner C, Sattawatthamrong Y, Semmler J, et al. The effect of hyperglycaemia on cerebral potentials evoked by rapid rectal distension in healthy humans. Eur J Clin Invest. 1999;29(6):512-8.
244. Russo A, Sun WM, Sattawatthamrong Y, Fraser R, Horowitz M, Andrews JM, et al. Acute hyperglycaemia affects anorectal motor and sensory function in normal subjects. Gut. 1997;41(4):494-9.
245. Russo A, Botten R, Kong MF, Chapman IM, Fraser RJ, Horowitz M, et al. Effects of acute hyperglycaemia on anorectal motor and sensory function in diabetes mellitus. Diabet Med. 2004;21(2):176-82.
246. Williams EK, Chang RB, Strochlic DE, Umans BD, Lowell BB, Liberles SD. Sensory Neurons that Detect Stretch and Nutrients in the Digestive System. Cell. 2016;166(1):209-21.
247. Erbil D, Eren CY, Demirel C, Kucuker MU, Solaroglu I, Eser HY. GLP-1's role in neuroprotection: a systematic review. Brain Inj. 2019;33(6):734-819.
248. Reynolds EL, Watanabe M, Banerjee M, Chant E, Villegas-Umana E, Elafros MA, et al. The effect of surgical weight loss on diabetes complications in individuals with class II/III obesity. Diabetologia. 2023.
249. Fatima F, Hjelmesaeth J, Birkeland KI, Gulseth HL, Hertel JK, Svanevik M, et al. Gastrointestinal Hormones and beta-Cell Function After Gastric Bypass and Sleeve Gastrectomy: A Randomized Controlled Trial (Oseberg). J Clin Endocrinol Metab. 2022;107(2):e756-e66.
250. Mehra S, Tavakoli M, Kallinikos PA, Efron N, Boulton AJ, Augustine T, et al. Corneal confocal microscopy detects early nerve regeneration after pancreas transplantation in patients with type 1 diabetes. Diabetes Care. 2007;30(10):2608-12.
251. Sharma S, Vas P, Rayman G. Small Fiber Neuropathy in Diabetes Polyneuropathy: Is It Time to Change? Journal of Diabetes Science and Technology. 2021;16(2):321-31.
252. Bugajski AJ, Gil K, Ziomber A, Zurowski D, Zaraska W, Thor PJ. Effect of long-term vagal stimulation on food intake and body weight during diet induced obesity in rats. J Physiol Pharmacol. 2007;58 Suppl 1:5-12.
253. Pardo JV, Sheikh SA, Kuskowski MA, Surerus-Johnson C, Hagen MC, Lee JT, et al. Weight loss during chronic, cervical vagus nerve stimulation in depressed patients with obesity: an observation. Int J Obes (Lond). 2007;31(11):1756-9.
254. Guarino D, Nannipieri M, Iervasi G, Taddei S, Bruno RM. The Role of the Autonomic Nervous System in the Pathophysiology of Obesity. Front Physiol. 2017;8:665.

9. Appendix

9.1 REK approval

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK vest	Ingvild Haaland	55978498	14.02.2019	2018/1790/REK vest
			Deres dato:	Deres referanse:
			14.01.2019	

Vår referanse må oppgis ved alle henvendelser

Eirik Søfteland
Medisinsk avdeling & Hormonlaboratoriet

2018/1790 Autonom nevropati – forbindelsen mellom redusert inkretineffekt og type 2 diabetes?

Forskningsansvarlig: Helse Bergen HF - Haukeland universitetssykehus
Prosjektleder: Eirik Søfteland

Vi viser til søknad om forhåndsgodkjenning av ovennevnte forskningsprosjekt og tilbakemelding innsendt 14.01.2019. Tilbakemeldingen er behandlet av leder ved REK vest på fullmakt. Vurderingen er gjort med hjemmel i helseforskningsloven (hforskn) § 10.

Prosjektomtale
Dette er en studie der vi ønsker å sammenligne personer med langvarig type 2 diabetes, personer med begynnende type 2 diabetes og friske kontroller. Vi vil undersøke autonom nervefunksjon ved gjennom en sonde å stimulere sensoriske nerver i endetarmen med et signal og avlese hastighet (tiden) til dette signalet kan registreres over hjernen med elektroencefalografi. Vi ønsker videre å undersøke inkretinhormonnivåer og inkretinhormonrespons ved hjelp av glukosebelastninger, der det måles hormon- og glukoseprofiler. Videre vil vi å måle bukspyttproduksjon ved å samle opp prøver av bukspytt etter stimulering av bukspyttkjertelen med sekretin, og sekretinstimulert MR. Vi vil også estimere ustimulert bukspyttkjertelfunksjon ved å måle elastase i avføring. Hypotesen er at diabetesutløst skade i det autonome nervesystemet kan korreleres til redusert inkretinhormonrespons og redusert ustimulert bukspyttkjertelfunksjon, mens inkretinhormonnivåer og stimulert bukspyttkjertelfunksjon er normale.

Vurdering
Komiteen spurte tidligere om tilbakemelding på følgende:

Et informasjonsskriv og samtykkeskjema til den generelle forskningsbiobanken skal legges ved i en tilbakemelding til REK vest.

Prosjektleder har sendt inn informasjonsskriv med bredt samtykke til den generelle forskningsbiobanken. Under informasjon om fremtidige prosjekter er det beskrevet: "Dersom materiale skulle benyttes til andre formål enn beskrevet i samtykkeskrivet vil du bli informert om det via vårt nyhetsbrev." Komiteen gjør oppmerksom på at man ikke kan benytte materialet i den generelle biobanken til andre formål enn det som er beskrevet under bredt samtykke for den generelle biobanken. Det må da innhentes nytt samtykke fra deltakerne. Komiteen ber derfor om innsending av revidert informasjonsskriv og samtykkeskjema for den generelle biobanken der dette omformuleres. I tillegg er det under kontaktperson oppgitt to personer. Det bes om at det her oppføres kontaktinfo for ansvarshavende for biobanken.

Revidert informasjonsskriv bes sendes på e-post til REK vest.

Besøksadresse:
Armauer Hansens Hus (AHH), Tverrfløy Nord, 2 etasje. Rom 261. Haukelandsveien 28

Telefon: 55975000
E-post: post@helseforskning.etikkom.no
Web: http://helseforskning.etikkom.no/

All post og e-post som inngår i saksbehandlingen, bes adressert til REK vest og ikke til enkelte personer

Kindly address all mail and e-mails to the Regional Ethics Committee, REK vest, not to individual staff

Vedtak

REK vest godkjenner prosjektet på betingelse av at ovennevnte vilkår tas til følge.

Sluttmelding og søknad om prosjektendring
Prosjektleder skal sende sluttmelding til REK vest på eget skjema senest 30.06.2024, jf. hfl. § 12. Prosjektleder skal sende søknad om prosjektendring til REK vest dersom det skal gjøres vesentlige endringer i forhold til de opplysninger som er gitt i søknaden, jf. hfl. § 11.

Klageadgang
Du kan klage på komiteens vedtak, jf. forvaltningsloven § 28 flg. Klagen sendes til REK vest. Klagefristen er tre uker fra du mottar dette brevet. Dersom vedtaket opprettholdes av REK vest, sendes klagen videre til Den nasjonale forskningsetiske komité for medisin og helsefag for endelig vurdering.

Med vennlig hilsen

Marit Grønning
dr.med, professor
Komiteleder

Ingvild Haaland
rådgiver

Kopi til:postmottak@helse-bergen.no

10. Papers I-III

Journal of Diabetes and Its Complications 37 (2023) 108452

Contents lists available at ScienceDirect

Journal of Diabetes and Its Complications

journal homepage: www.elsevier.com/locate/jdiacomp

The PanGut-study: Evoked potentials following rectal balloon distention, a way of evaluating diabetic autonomic neuropathy in the gut?☆

Sondre Meling [a,b,*], Erling Tjora [b,c], Heike Eichele [d,e], Rasmus Bach Nedergaard [f], Niels Ejskjaer [g,h], Christina Brock [f,g,h], Eirik Søfteland [b,i]

[a] Department of Medicine, Stavanger University Hospital, Stavanger, Norway
[b] Department of Clinical Science, University of Bergen, Bergen, Norway
[c] Department of Pediatrics and Adolescent Medicine, Haukeland University Hospital, Bergen, Norway
[d] Department of Biological and Medical Psychology, Faculty of Psychology, University of Bergen, Bergen, Norway
[e] Regional Resource Centre for Autism, ADHD and Tourette Syndrome Western Norway, Division of Psychiatry, Haukeland University Hospital, Bergen, Norway
[f] Mech-Sense, Department of Gastroenterology and Hepatology, Aalborg University Hospital, Aalborg, Denmark
[g] Department of Clinical Medicine, Aalborg University, Aalborg, Denmark
[h] Steno Diabetes Center North Jutland and Department of Endocrinology, Aalborg, Denmark
[i] Department of Medicine, Haukeland University Hospital, Bergen, Norway

ARTICLE INFO

Keywords:
Evoked potentials
Rectal balloon distention
Rectal hyposensitivity
Diabetic autonomic neuropathy
Type 2 diabetes

ABSTRACT

Aim: There is a lack of methods for investigating the autonomic nerves of the gastrointestinal tract. Our aim was to explore a novel test measuring visceral sensory evoked potentials (EPs) in response to rapid balloon distention in the rectum and compare it to established tests for diabetic neuropathy.
Method: Participants with longstanding type 2 diabetes, newly onset, untreated diabetes <1 year, and matched controls, were included. Tests included cardiovascular reflex tests, orthostatic blood pressure, electrical skin conductance assessment, sural nerve testing and monofilament test. The rectal balloon distention pressure at earliest sensation and threshold of unpleasantness were identified and used to elicit mechanical EPs.
Results: The pressure at earliest sensation was higher in people with diabetes, 0.038 (0.012) bar vs. controls 0.030 (0.009) bar, $p = 0.002$, and in people with signs of peripheral neuropathy, 0.045 (0.014) bar, $p < 0.01$. Clinical correlations between EP amplitude and latency, and other tests were found.
Conclusions: Rectal hyposensitivity was associated with both longstanding and early diabetes, indicating enteric sensory dysfunction already in early stages of diabetes. Correlation analyses may indicate that central afferent processing is affected in parallel with peripheral neuronal function.

1. Introduction

Diabetic autonomic neuropathy was in the Toronto consensus defined as a disorder of the autonomic nervous system in diabetes or metabolic derangements of prediabetes, after the exclusion of other causes. It may affect the cardiac, gastrointestinal (GI), genitourinary systems, and sudomotor function.[1] The prevalence of autonomic neuropathies are best described for cardiovascular autonomic neuropathy (CAN). In unselected diabetes populations, CAN was found in 17 % to 20 % of patients with diabetes, increasing with age and diabetes duration. In newly diagnosed diabetes, the prevalence was reported to be around 7 %.[2]

Cardiovascular autonomic reflex tests (CARTs) are considered gold standard in the diagnosis of diabetic autonomic neuropathy. They have good sensitivity, specificity, and reproducibility. Furthermore, they are non-invasive and easy to perform in a clinical setting.[1] Emerging tests, measuring sudomotor function through electrical skin conductance, corneal small fiber structure by confocal microscopy, or the axon reflex-mediated neurogenic vasodilatation are increasingly available, but there is a lack of consensus on their role in the diagnostic armamentarium.[3]

Gastrointestinal symptoms are frequent in the general population, but even more so in people with diabetes. The prevalence of GI symptoms in unselected people with diabetes varies between 1 % and 40 % for the different symptoms.[4] There are several modalities to assess

☆ Conflict of interest: No authors declare conflict of financial or competing interest.
* Corresponding author at: Department of Medicine, Stavanger University Hospital, Gerd-Ragna Bloch Thorsens gate 8, 4011 Stavanger, Norway.
E-mail address: sondre.vatne.meling@sus.no (S. Meling).

https://doi.org/10.1016/j.jdiacomp.2023.108452
Received 21 November 2022; Received in revised form 26 January 2023; Accepted 6 March 2023
Available online 9 March 2023

gastric emptying and gastroenteric motility, but they do not always correspond with symptoms, symptom severity or CARTs.[5] We have previously shown, that both gastrointestinal autonomic neuropathy as well as central neuroplastic changes are involved in the pathomechanism.[6,7] Accordingly, in addition to GI motility studies, there is a need for tools to examine both the GI autonomic nervous system and its interplay with the brain.

Investigating cerebral responses to GI tract stimulations was developed in the 1980s and has been extensively employed by our and other study groups.[6,8,9] Rectal mucosa is innervated by visceral afferents running in both sympathetic and parasympathetic nerves. Visceral afferents consist mainly of small myelinated Aδ and unmyelinated C-fibers, with slow conduction speeds compared to somatic afferents. Hence, cerebral responses induced by visceral mechanical activation occur after 40–50 ms and should enable a reasonable separation between somatic Aβ and visceral signaling.[10] Consequently, this could be an interesting method for investigating the afferent signaling from the GI tract, with the potential to better the understanding of the differences between cardiac and gastric autonomic nerve function.

In the current study, we wanted to explore a novel test measuring cortical evoked potentials (EPs) in response to rapid balloon distention in the rectum and compare it to established tests for diabetic neuropathy. As a primary outcome, we hypothesized that reduced rectal sensitivity and pathological findings in EPs were more prevalent in both longstanding as well as early diabetes, compared to controls. Secondarily, we hypothesized that different manifestations of diabetic neuropathy run in parallel, and thus, that rectal sensitivity and EPs correlate with measures from other neuropathy tests.

2. Material and methods

We aimed to recruit three groups; one group of people with type 2 diabetes for more than ten years (*longstanding diabetes group*), one group with newly diagnosed, untreated type 2 diabetes diagnosis within one year (*early diabetes group*), and controls matched in age, gender, and body mass index (BMI). Participants were recruited mostly through regional newspaper advertisement. All investigations were performed at a single center (Bergen, Norway). Exclusion criteria were major abdominal surgery, rectosigmoid disease interfering with sensitivity (e.g., any history of proctitis, ongoing malignancy, previous surgery), chronic pancreatitis, uremic condition (eGFR<30 mL/min), atrial fibrillation or other major dysrhythmia, cardiac pacemaker, or present use of glucagon like peptide (GLP)-1 agonist or insulin.

The study was part of a larger project, the PanGut study, and was approved by the Western Norway Regional Ethics Committee for Medical and Health Research Ethics (REK Vest #1790). All participants signed an informed consent following oral and written information.

The study included three days of examination. Information, inclusion, and neuronal phenotyping were performed on day one, evoked potentials following rapid rectal balloon distention on day two, and oral glucose tolerance test (OGTT) on day three. The three days of examination were not necessarily consecutively. Prior to examinations, the participants were instructed to avoid alcohol 24 h before, and coffee, tea, or nicotine 2 h before. They were allowed to take their normal medications and to eat a small meal a minimum of 3 h prior, except for the day of the OGTT.

2.1. Oral glucose tolerance test

Participants in the early diabetes and control groups were assigned to group based on the results of an OGTT, according to the American Diabetes Association criteria.[11] The OGTT was performed after an overnight (10 h) fast. One cannula was placed in a cubital vein, with the forearm on the same side placed in a heating cuff to ensure arterialized blood. The participant ingested a 2–300 mL solution of 75 g anhydrate glucose. Blood glucose was measured before and 2 h after glucose ingestion, using the HemoCue Glucose 201 DM RT (HemoCue, Angelholm, Sweden).

2.2. Visceral sensitivity: evoked potentials following rapid balloon distention in the rectum

The equipment and protocol are based on recent studies and described in greater length previously.[11–15] A balloon catheter was made using a CH 16 duodenal tube (Levin X-ray, Unomedical, UK), with an index finger from a nitrite examination glove (Klinion Protection, Medeco, The Netherlands) attached to the tip, wrapped and secured with Novosyn Quick Polyglactin 910 fast absorbable suture (B.Braun, Germany). The tube was attached to an extension catheter, with a length of 2.1 m and connected to a custom-designed inflation device (MechSense, Aalborg University Hospital, Denmark).

Forty-five minutes prior to testing, a bisacodyl enema (Toilax, Orion pharma, Espoo, Finland) was administered. Electroencephalogram (EEG) was recorded using a standard 61-channel surface cap, with Ag/AgCl electrodes in an extended 10–20 system, with Fz as reference. Electrode gel was applied to reduce impedance. A resting EEG was recorded (2 min), with eyes opened. The subjects were instructed in using a 0–10 visual analogue scale (VAS). The rectal balloon was then placed 15 cm above the anal verge (Fig. 1). The balloon was inflated starting at 0.01 bar, resembling 1 psi used in the reference studies, approximately the same as 1 kPa. The pressure was increased by 0.01 bar increments until the earliest sensation, VAS 1. The procedure was repeated two times, finding the most probable pressure for VAS 1, by averaging the pressure of the three attempts. We then performed a stimulation period with 30 single stimulations, while recording EEG. Subsequently, the pressure was increased with 0.1 bar increments until discomfort or an urge to defecate (VAS 5). The procedure was repeated, finding the most probable pressure causing VAS 5. Another 30 single

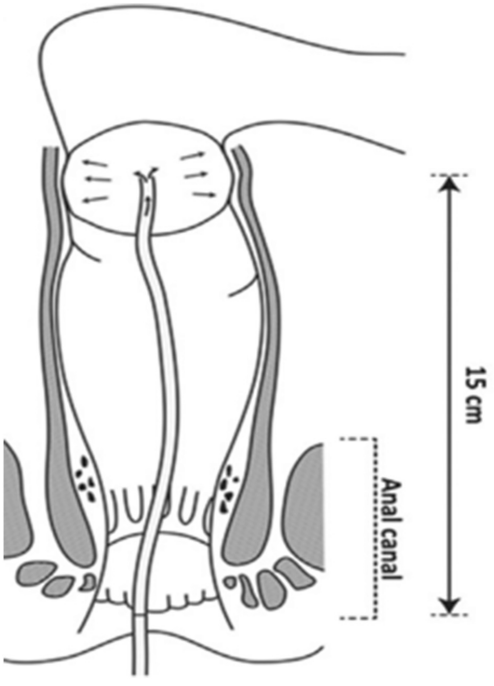


Fig. 1. Schematic drawing of the balloon placement in the rectum.
Att. Copyright Haas, Elsevier, license number for reuse: 5306391124493.

stimulations while recording EEG were performed (Fig. 2).

The balloon inflator used a digitized trigger signal to synchronize the EEG recording. The inflation time was 150 ms, followed by an instant deflation facilitated by a vacuum pump, providing a rapid, distinct, and short stimulus. A random inter-stimulus interval of 8±2 s was used. To minimize environmental noise, all unnecessary electrical equipment was turned off. For safety reasons, a pressure of 2.07 bar, which corresponds to 30 psi from the reference studies, was used as maximum pressure. The EP latency was calculated as time in milliseconds after the onset of the external trigger of the balloon distension, the peak-to-peak amplitude was the absolute difference in amplitude between two consecutive peaks.

Data were cleaned and analyzed using MATLAB (R2020b Mathworks, Inc., Natick, MA, USA) and EEGLAB toolbox (version 14.1.2; Schwartz Center for Computational Neuroscience, Institute for Neural Computation, University of California, San Diego, CA, USA). The clean_artifacts toolbox using default settings and Window Criterion off. A 1 Hz filter was applied to remove DC drift. Channels containing noise were interpolated using spherical interpolation and referenced to a common reference. All data were visually inspected prior to independent component analysis (ICA). The data was down sampled to 250 Hz for ICA to visually distinguish independent components containing biological or outside noise. After selecting "bad" components, the analyzed data were added to the full dataset (1000 Hz) and the bad components were pruned from the dataset. Evoked potentials were generated from averaging EEG signals recorded in each session. The data were not corrected for any delays in the inflation of the balloon.

2.3. Neuronal phenotyping

2.3.1. Cardiovascular reflex tests, heart rate variability, and orthostatic blood pressure test

Heart rate variability (HRV) and CARTs were measured using the Vagus™ Device (Medicus Engineering, Aarhus, Denmark), first for 5 min lying in a semi-reclined position (at rest), then shortly after standing up (RS-ratio), during deep breathing (EI-ratio) and while doing the Valsalva maneuver (VM-ratio). The test defines stages of CAN as borderline if one ratio is abnormal and as definite or confirmed if two or three ratios are abnormal. If the latter is combined with orthostatic hypotension, CAN is defined as severe or advanced, in line with international consensus.[1] Orthostatic blood pressure test was conducted using the WelchAllyn Connex ProBP 3400™ (EMEAI, Leiden, Netherlands), after sitting down for 5 min, then upon standing up, and after 1 and 3 min standing. We defined orthostatic hypotension as a decline in systolic blood pressure of >20 mm Hg or diastolic > 10 mm Hg within 3 min of standing.[16]

2.3.2. Sudomotor function

For measurement of electrical skin conductance, we used the Sudoscan™ Device (Impeto Medical, Paris, France). The subject's hands and feet were placed on steel electrodes. The test calculates electrical skin conductance by measuring the flow of chloride ions produced by sweat glands in hand and feet, using reverse iontophoresis, following low voltage electrical stimulation. It is thought to assess mainly small C fibers. The results are given as continuous parameters for conductance and interpreted by the device categorical as no sudomotor dysfunction, intermediate dysfunction, or high dysfunction.[17]

2.3.3. Monofilament test and sural nerve conduction

The subject was placed in a supine position with eyes closed. The monofilament test was performed with a 10 g monofilament, bilaterally pinpricking the dorsum of the foot four times. We defined feeling 7–8 of 8 sensations as no suspected distal polyneuropathy (DPN), feeling 4–6 as possible DPN, and feeling 3 or less, as probable DPN.[18]

The sural nerve conduction velocity test was performed using the NC-stat DPN Check™ (NEUROMetrix, Boston, USA). This point of care device measures nerve conduction velocity of the sural nerve by stimulating the nerve at the level of the ankle and recording the resulting response on the calf. The device provides absolute values as well as categorized results based on normative values dividing results into normal nerve conduction, mild, moderate, or severe neuropathy.

2.4. Data analysis and statistics

Being part of a pilot project, a formal power analysis was unfeasible. However, using effect sizes from previous studies on visceral sensitivity, the lowest number of participants needed to achieve a power of 0.8 would be 15 participants in each group.[9,12,19] Results are given as means with standard deviation, and median with interquartile range for non-parametric data. For the primary outcome we used one-way ANOVA comparing data where normal distributed, and Tukey test for post hoc testing, assuming equal variance. For non-parametric data, *p*-values were found using Kruskal Wallis test for several independent samples and Mann Whitney *U* test for two independent samples. For the secondary outcome of correlations between tests, we used the Spearman's rank-order correlation test. Correlation analyses between the EPs and other neuronal phenotyping were done for all three groups of participants combined. Statistical significance was defined as p-value ≤ 0.05 for all analyses. Evoked potential data sets were pooled and analyzed blindly. Statistical analyses were performed using SPSS Version: 28.0.1.0.

3. Results

3.1. Subjects

We recruited a total of 66 participants with a mean age of 69 years, including 51.5 % women. There were significant different values for fasting glucose, 2-h glucose and HbA1c between the groups. No participants used opioid medications or had self-reported pain conditions. For clinical characteristics see Table 1.

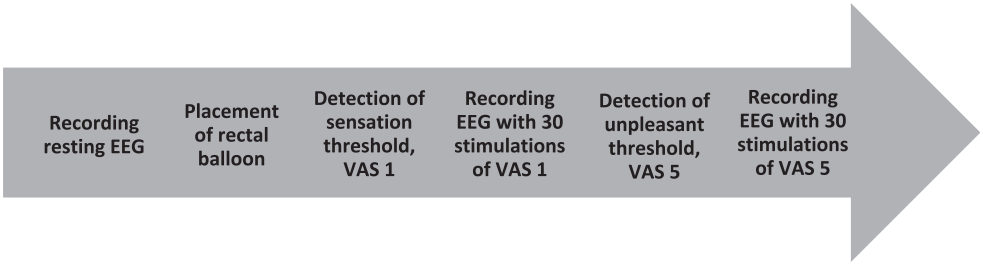


Fig. 2. Timeline of EEG recordings.

Table 1
Clinical characteristics.

Clinical characteristics	Longstanding diabetes N = 21	Early diabetes N = 15	Controls N = 30	*p*-Value
Age (years at recruitment)	68.9 (7.8)	69.3 (5.5)	69.5 (6.2)	0.95
Gender (female/male), N	10/11	8/7	16/14	0.91
BMI (kg/m^2)	26.5 (4.4)	25.7 (4.1)	25.5 (3.8)	0.68
Diabetes duration (years)	16.8 (4.9)	–	–	n/a
Fasting glucose (OGTT), mmol/L	9.4 (2.1)	7.2 (1.0)	6.0 (0.6)	<0.01
2-h glucose (OGTT), mmol/L	18.7 (3.9)	13.1 (4.2)	7.9 (1.5)	<0.01
HbA1c, mmol/mol	53.5 (11.2)	43.3 (4.9)	37.1 (3.0)	<0.01
Total cholesterol, mmol/L	4.2 (0.8)	4.5 (1.2)	5.5 (1.0)	<0.01
HDL, mmol/L	1.3 (0.3)	1.4 (0.4)	1.9 (0.5)	<0.01
LDL, mmol/L	2.4 (0.6)	2.8 (1.1)	3.3 (0.8)	<0.01
Triglycerides, mmol/L	1.7 (1.3)	1.3 (0.5)	1.0 (0.4)	<0.01
eGFR, mL/min/1.73 m^2	84.9 (13.5)	82.3 (11.7)	80.3 (12.3)	0.46
Blood pressure rest, mm/Hg	135/80 (15/6)	152/86 (14/7)	139/81 (20/7)	0.02[a]
Comorbidity				
Nephropathy	–	–	–	n/a
Distal neuropathy, %	4.8	6.7	–	0.40
Hypertension, %	52.4	46.7	16.7	0.02
Cardiovascular disease, %	4.8	13.3	3.3	0.40
Drugs				
Metformin, %	81	–	–	n/a
Sulphonylurea, %	19	–	–	n/a
DPP-4 inhibitor, %	47.7	–	–	n/a
SGLT-2 inhibitor, %	38.1	–	–	n/a
Other antidiabetic medication, %	9.5	–	–	n/a
Diet treated diabetes, %	9.5	–	–	n/a
Betablocker, %	4.8	20	13.3	0.37
ACE-I/angiotensin receptor blocker, %	47.6	40	10	<0.01
Other antihypertensive medication, %	19	13.3	6.7	0.41
Statin use, %	66.7	46.7	13.3	<0.01
Smoking status, % (present/past/never)	10/38/52	7/13/80	3/43/54	0.30

Data are given in means with (SD) unless otherwise indicated, *p*-values from ANOVA and Pearson Chi-Square test. Diabetes duration, comorbidity, drugs and smoking status are self-reported.

[a] Value from systolic pressure.

3.2. Evoked potentials following rapid balloon distention in the rectum, between-group differences

The pressure needed to reach VAS 1 was higher in the groups with longstanding diabetes and in early diabetes compared to the control group. There was no difference in pressure needed to reach VAS 5 (Table 2).

There were no significant differences between groups in terms of EP latencies nor amplitudes performing the test (all $p > 0.07$), see Fig. 3a) and b). There was a non-significant trend towards longer latencies in all complexes, in the group of longstanding diabetes compared to the others combined. For amplitudes, the results were more inconsistent. Fig. 3c) shows the mean for all observations for VAS 1 and 5. Specific values for all latencies and amplitudes can be found in Supplemental material (S1).

3.3. Neuronal phenotyping

Overall, few between-group differences were detected. In terms of CARTs, definite CAN was detected in two participants, one in the early diabetes group, and one in the control group. People with longstanding

Table 2
Neuronal phenotyping.

Parameters	Longstanding diabetes	Early diabetes	Controls	p-Value
Rectal sensation				
Pressure VAS 1, bar	0.037 (0.011)*	0.040 (0.013)*	0.030 (0.009)	<0.01
Pressure VAS 5, bar	1.45 (0.50)	1.47 (0.58)	1.50 (0.50)	0.94
CARTS and HRV				
HR, bpm	68.5 (8.3)*	64.0 (8.0)	63.1 (11.6)	0.15
SDNN, ms	29.5 (19.6–46.7)	32.7 (17.0–38.3)	29.1 (21.5–37.5)	0.94
RMSSD, ms	18.1 (10.8–45.6)	19.5 (11.0–26.2)	21.2 (9.6–31.8)	0.96
LF, ms^2	58.5 (33.7–145.4)	93.9 (25.7–126.8)	77.9 (34.5–155.5)	0.97
HF, ms^2	69.7 (13.6–152.0)	63.4 (15.5–104.1)	44.1 (13.0–99.4)	0.99
Total, ms^2	247.2 (121.9–600.6)	306.3 (134.9–469.5)	324.2 (161.9–586.8)	0.72
R/S ratio	1.08 (1.03–1.12)	1.09 (1.04–1.15)	1.08 (1.06–1.15)	0.38
Abnormal R/S ratio, %	6.3	6.7	6.7	0.96
E/I ratio	1.18 (1.06–1.31)	1.14 (1.07–1.23)	1.13 (1.11–1.23)	0.73
Abnormal E/I ratio, %	25	20	6.7	0.34
VM ratio	1.41 (1.33–1.65)	1.41 (1.32–1.48)	1.47 (1.33–1.62)	0.33
Abnormal VM ratio, %	0	6.7	3.3	0.55
CAN				
No/borderline/definite, %	69/31/0	77/15/8	83/13/4	0.54
Orthostatic hypotension, %	14.3	20	17.2	0.90
Sural nerve check				
DPN, Velocity, m/s	46.5 (43–50)	47 (40.2–51)	48 (43.5–52)	0.56
DPN, Amplitude, μV	6.5 (5–9)	5 (4–9)	7 (5–9)	0.54
Peripheral neuropathy: No/mild/mod./serious, %	76/10/10/0	80/13/7/0	87/0/7/3	0.68
Monofilament test				
Felt pinpricks (n)	8 (6–8)*	8 (8–8)	8 (8–8)	0.08
Peripheral neuropathy: Unlikely/possibly/likely, %	71/24/5	87/13/0	90/0/10	0.06
Sudomotor function				
Hands, μSiemens	65.8 (14.4)	67.2 (14.1)	71.3 (15.2)	0.39
Normal/mod. reduced /severely reduced, %	70/25/5	67/33/0	77/20/3	0.81
Feet, μSiemens	73.6 (12.4)	77.7 (6.7)	75.3 (13.5)	0.60
Normal/mod. reduced /severely reduced, %	71/19/10	80/20/0	87/7/7	0.45

Results of neuronal phenotyping given as mean (SD) or median (IQR). Asterisk (*) indicates statistically significant differences compared to controls. HR = mean heart rate, bpm = beats per minute, during 5 min. rest, SDNN = standard deviation from the mean heartbeat interval value (net effect of the autonomic regulation), RMSSD = root mean square of the standard deviation (activity level of the parasympathetic regulation). LF = low frequency activity (represents sympathetic tone), HF = high frequency activity (represents parasympathetic tone), TP = total power (power spectrum of RR intervals throughout the frequency ranges – net autonomic function). R/S ratio: 30:15 Ratio = ratio between maximum HR within the first 15 s after standing up and

minimum HR within the first 30 s after standing up (predominantly parasympathetic test). E/I-ratio = mean ratio between the longest and shortest RR-interval during deep respiration (measures of baroreflex sensitivity and capacity — predominantly parasympathetic tests). VM ratio: ratio between maximum heart rate at the end of forced expiration and minimal heart rate during inspiration-expiration in rest after appr. 30 s after releasing pressure (predominantly sympathetic and baroreflex mediated test). Reference CART ratios are age adjusted. CAN is predicted as borderline if one abnormal CART ratio, and definite if two or more abnormal ratios. Orthostatic hypotension is defined as a decline in systolic blood pressure of > 20 mmHg or diastolic > 10 mmHg within three minutes of standing. Risk of peripheral neuropathy performing the sural nerve check is defined by the software. For the monofilament test feeling 7–8 of 8 sensations is defined as unlikely distal polyneuropathy, feeling 4–6 as possible DPN, and feeling 3 or less, as likely DPN. Stages of sudomotor function are also defined by the software. P-values from categorical outcomes from Chi Square test.

diabetes had significantly higher resting heart rate compared to people with early diabetes and controls. For the monofilament test there was a difference between people with longstanding diabetes and the control group. There were no between-group differences in orthostatic blood pressure, and neither continuous nor categorical values for sudomotor function test or sural nerve testing. All results are found in Table 2.

3.4. Clinical correlations between different neuropathy measures and EPs

There were no correlations between EPs and resting heart rate. For HRV, there were non-significant trends towards those with lower SDNN and RMSSD, showing longer EP latencies and smaller amplitudes. There were no correlations between EPs and other HRV parameters. For the CARTs, there were significant correlations between decreased RS- and EI-ratios, and longer latencies and smaller amplitudes of EPs, but this was not found for VM-ratio or orthostatic hypotension. Correlation for all EP latencies and amplitudes to SDNN, RMSSD, RS-, EI-, VM-ratio, sural nerve parameters and monofilament test are found in Supplemental material, S2.

There were no correlations between EPs and electrical skin conductance. Sural nerve amplitude and VAS 5 EP amplitudes tended to correlate, while reduced sensation on the monofilament test correlated with EP amplitudes and pressure needed to induce VAS 1, the latter with rho = −0.287, $p = 0.02$. For the group with no suspected DPN in the monofilament test, the pressure needed for VAS 1 was 0.033 (0.01) bar, compared to those with possible or probable DPN, who needed 0.045 (0.01) bar, $p < 0.01$. We found no correlation between rectal hyposensitivity and other neuropathy measures.

4. Discussion

In this study we demonstrated that people with both longstanding and early diabetes had rectal hyposensitivity to mechanical pressure, compared to matched controls. Furthermore, in all groups combined, we found that reduced rectal sensitivity correlated with reduced peripheral sensitivity on the monofilament test. The overall incidence, for both suspected autonomic and peripheral neuropathy in all three groups was surprisingly low, probably reflected by our study population and few phenotypical differences between the groups. There were biologically plausible clinical correlations between EPs following rapid balloon distention in the rectum and other tests for autonomic and peripheral neuropathy, indicating that neuronal degeneration in different nerves could progress in parallel.

4.1. Clinical considerations

A higher mean rectal balloon pressure was needed to induce the earliest sensation in people with diabetes, regardless of disease duration, compared to healthy controls. The results indicate early onset of visceral hyposensitivity of the mechanoreceptors in the gut wall. Previous studies from our group and others have shown hyposensitivity to somatic median nerve stimulation and visceral electrically elicited EPs in the rectum and esophagus, which is characterized by bypassing the peripheral receptors and depolarizing the nerves directly.[9] Furthermore, prolonged latencies and diminished amplitudes to rectal elicited EPs were found, and the EP dipolar localization in the brain (representing the center of gravity) have even shown associations to clinical symptoms, indicating involvement of the brain-gut-axis.[6] However, in contrast to these previous results, which included people with longstanding type 1 diabetes, severe GI symptoms, and clinical suspicion of autonomic neuropathy, our cohort consisted of people with newly diagnosed and longstanding diabetes, but not necessarily GI symptoms. Reduced rectal sensitivity has also been found in people with diabetes, including type 2 diabetes, with known sensorimotor neuropathy, using multimodal rectal stimulation, including electrical, thermal and mechanical stimulation.[20] Finally, rectal hyposensitivity has also been found in people with diabetes and symptoms of gastroparesis.[7] Our results indicate that this hyposensitivity occurs early on as diabetes develops. This finding is interesting for several reasons. Firstly, to our knowledge, this is the first time an early involvement of the visceral and GI autonomic nerves in diabetes have been demonstrated in humans, supporting earlier studies in streptozotocin-induced diabetic rats indicating that the function of rectal visceral afferents deteriorated at an early stage of diabetes.[21] Secondly, although hyposensitivity was mild to moderate in our study, this could still implicate a reduced sensitivity to stretch as a factor in the development of rectal incontinence and the sense of incomplete evacuation, which is a dreaded late complication of diabetes. Further studies are prompted to investigate this possible association.

In the present study, we cannot decipher whether the rectal hyposensitivity was a result of decreased visceral sensation/GI autonomic neuropathy or central nervous system changes. Previously, others have argued that both of these mechanisms are involved, because the nerves are tightly connected in the intrinsic and extrinsic nerve supply of the enteric nervous system.[22] On the other hand, we have shown in an earlier study, that rectal hyposensitivity was more pronounced for distention than electrical stimulation, indicating a role for the mechanoreceptors, which favors involvement of peripheral receptors and afferent nerves.[7]

The pressure needed for earliest sensation was higher in those with reduced sensation on the monofilament test, independent of diabetes status. As rectal hyposensitivity has previously been associated with reduced HRV and sensorimotor neuropathy, in populations different from ours, the findings support the notion of concurrent damage to small and large nerve fibers.[7,20]

There was a non-significant trend towards longer latencies in EPs in participants with longstanding diabetes. Prolonged latency is reported, using the same method for rapid rectal balloon distention, in conditions like idiopathic fecal incontinence and former treatment with primary radiotherapy for anal cancer and thus, is suggestive of afferent dysfunction.[13,14] Prolonged latencies have also been reported in patients with constipation and rectal hyposensitivity, and, combined with diminished amplitude, in people with diabetes and suspected autonomic neuropathy, and in diabetes with gastrointestinal symptoms.[6,23,24] A decrease in conduction velocity of both peripheral and central Aδ-fibers has been found to be associated with diabetic autonomic neuropathy, creating longer latencies.[22]

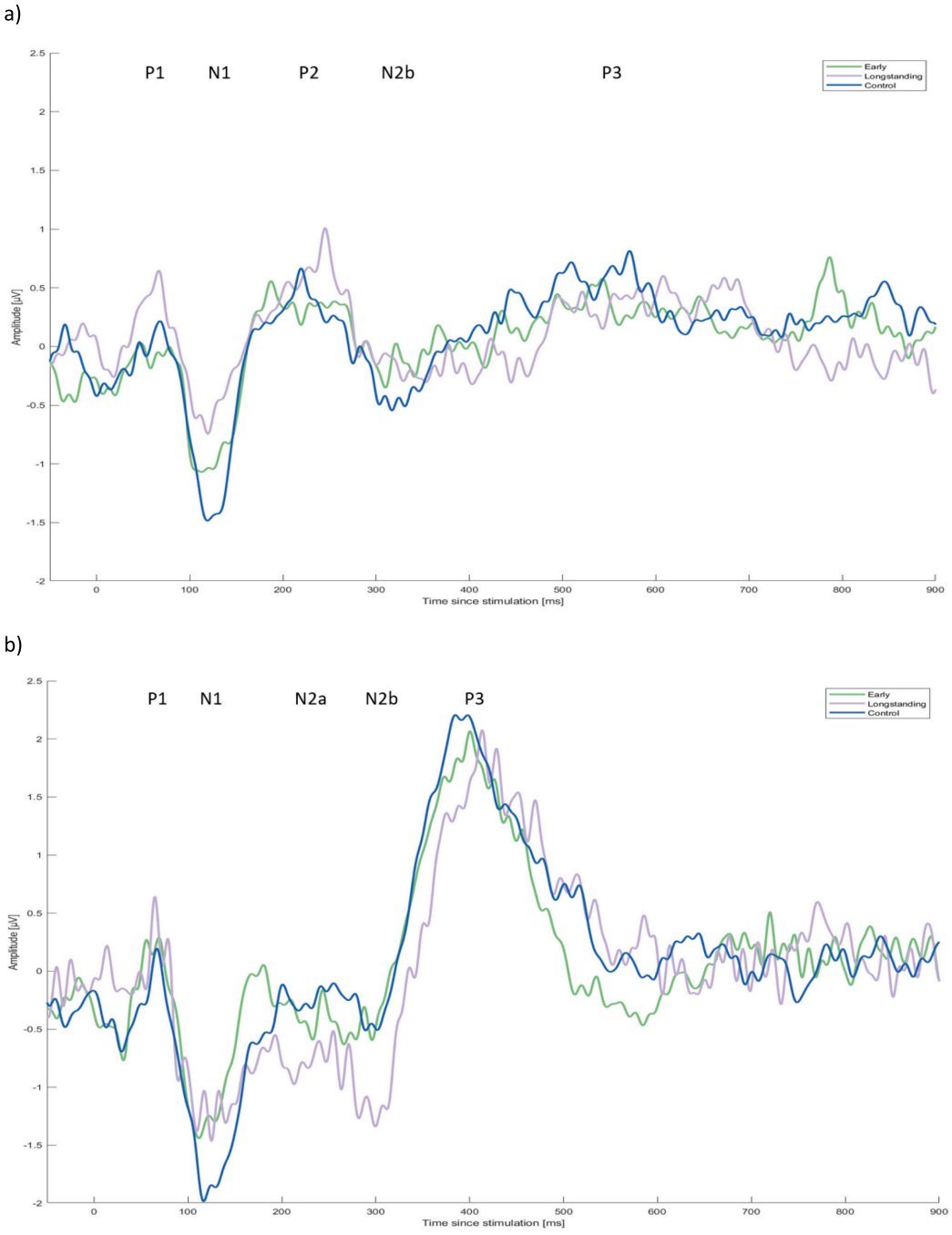


Fig. 3. Evoked potentials following rapid rectal balloon distention.
Amplitude and latency for VAS 1 and VAS 5 respectively, for the three groups (a and b) and mean for all observations for VAS 1 and 5 (c). P1 indicates the first positive peak, N1 the first negative peak, etc. Signals occurring <250 ms (P1–2, N1–2a) represent stimulus-specific processing, providing information on the sensitivity of the visceral afferent pathways, while >250 ms (N2b and P3) are regarded as also involving cognitive processes. As expected, N2b and P3 in VAS 5 have shorter latency and larger amplitude due to the increased stimuli compared to VAS 1.[10]

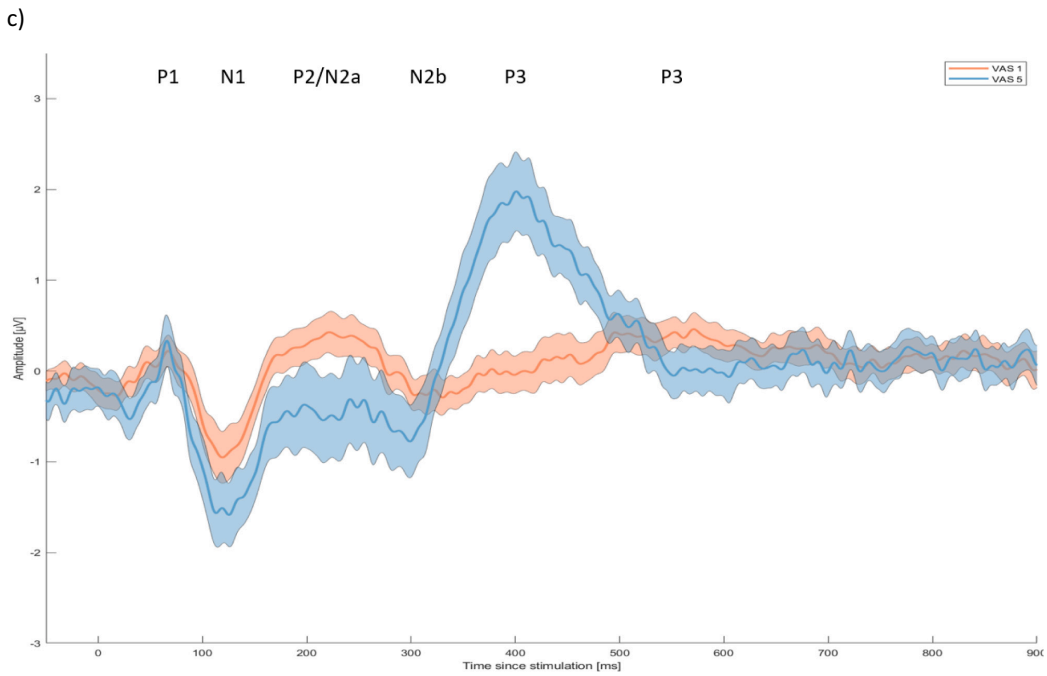


Fig. 3. (*continued*).

4.2. Autonomic neuropathy and lack of between-group differences

We hypothesized that neuropathy could be detected to a larger degree in people with longstanding diabetes and early diabetes compared to matched controls. The prevalence in our population turned out to be low for both suspected autonomic neuropathy and peripheral neuropathy, although there was a non-significant trend towards more borderline CAN (by CARTs), in the diabetes groups. One possible reason could be the uneven use of beta-blockers in the three groups (higher in early diabetes and controls). Also, the lack of differences between our groups could be explained by our study population composition. For reasons relevant for another PanGut sub study, we excluded people with longstanding diabetes who were using GLP-1 analogues or insulin. Our participants with longstanding diabetes had a near normal average BMI, an acceptable HbA1c and very few microvascular complications. Hence, we may have recruited a well-controlled diabetes population with a milder phenotype, that despite long duration and relatively high age, resulted in surprisingly few cases of neuropathy. Also, in line with the results from the OGTT, some healthy volunteers were re-classified as early diabetes, and some controls satisfied the criteria for prediabetes. Consequently, our groups were more homogenous than we would have expected, which may explain the few between-group differences. The specific characteristics of our study population are of importance with regards to external validity. At the same time, and of interest, this highlights the gradual process of onset of diabetic as well as pre-diabetic hyperglycemia in this age group. We also did not include people with known autonomic neuropathy, mainly because one of our hypothesis in a larger perspective was that neuropathy could be detected early on, in an unselected population of early diabetes. Interestingly, although prevalence of CAN was low, the reduced rectal sensation could be detected in both early and late diabetes, indicating early alteration in enteric sensory function. This may further affect GI hormonal signaling and will be elucidated in other parts of the PanGut study.

4.3. Evoked potentials, a way of detecting autonomic neuropathy in the gut?

Correlations were found between amplitudes and latencies of EPs and other more established tests for diagnosing neuropathy. There was a trend towards smaller amplitudes and longer latencies in EPs in people with reduced SDNN, RMDSS, RS and EI-ratio. The two latter ratios mainly represent parasympathetic activity, indicating that parasympathetic withdrawal may cause sensory alterations in the gut. The results are in line with a recent study, finding that early autonomic dysfunction in type 2 diabetes is predominantly a parasympathetic impairment.[25] We have also earlier supplied evidence of a generalized nature of diabetic autonomic neuropathy, showing reduced rectal sensitivity and impaired heart rate variability in patients with upper GI symptoms.[7] The findings may support a hypothesis of concurrent affection of autonomic nerves and intracerebral signal processing, and strengthen the idea that rectally evoked brain potentials could be an interesting marker of GI autonomic function.

There were also correlations between EPs and suspected distal polyneuropathy, in both sural nerve test and the monofilament test. Reduced rectal sensitivity is previously found to correlate with sensorimotor neuropathy in diabetes patients with upper GI symptoms.[20] Another explanation for the association with the peripheral polyneuropathy could be that the VAS 5 stimulations unintentionally also recruited nearby somatic sensory nerves, which may have given input to the pudendal nerve.

4.4. Limitations

The study-related procedures were performed between autumn 2019 and 2022, including a period of one year with no activities due to Covid-19 regulations. We cannot exclude that the length between individual procedures may have affected the patient's condition and results. To

limit the impact, we prioritized the early diabetes group – as these could be regarded as being in a translational period in terms of their health conditions.

We did not enforce discontinuation of any medications during cardiac autonomic function tests. A number of drugs could impact HRV and CARTs, however, short term discontinuation would also impact results, e.g. rebound-tachycardia with discontinuation of betablockers.[26] As seen in clinical characteristics, the use of beta-blockers (which likely has the most pronounced impact) was prevalent – although not identical – in all three groups. Sub-analyses showed that this did not seem to have any impact on heartrate, not being significantly lower in those using beta-blocker (62 vs 66 bpm, $p = 0.38$), and without significant differences in SDNN or RMSSD ($p = 0.73$ and 0.64 respectively).

In this study, we did not use validated screening instruments or questionnaires for DPN. However, participants were asked about the presence of known neuropathy. Further, a foot examination, including monofilament and a point-of-care sural nerve electrophysiology were performed.

We did not measure glucose immediately prior or during neuronal phenotyping or rectal balloon distention tests, and no examinations were done while performing a euglycemic clamp. There is conflicting evidence for the effect of hyperglycemia on rectal sensitivity, both in healthy subjects and in diabetes.[27–29] We therefore cannot exclude that glycemic status, on the day of examinations, may have affected our results.

While tests investigating the cardiovascular autonomic reflexes are well proven and standardized, and the monofilament test well established in clinical practice, there is still no definite role for the sudomotor or sural nerve conduction test. Studies on these devices are of small sizes, have large patient heterogeneity, and are prone to selection bias.[30] Other methods to examine diabetic autonomic neuropathy were not available in our study.

Due to the unknown effect-size for the rapid rectal balloon distention test, our power calculations were uncertain. With many non-significant tendencies in this study, it is possible that a larger sample size would have avoided type II errors. Finally, we acknowledge that not adjusting the correlation analyses between EPs and other nerve tests for multiple testing increase the risk of making type 1 errors, falsely rejecting a null hypothesis. Still, we have decided not to adjust for multiple testing due to the nature of the correlation analysis being a secondary outcome, with the intent not to conclude, but to generate further, and more sharpened hypotheses to be followed up in later studies. Also, we note that the detected correlations were in line with biologically plausible hypotheses and relatively consistent. Overall, on these grounds, we recognize that these analyses must be interpreted with caution.

5. Conclusion

Rectal hyposensitivity was present in both longstanding as well as early diabetes and indicate that visceral hyposensitivity of the mechanoreceptors in the gut wall occurs early in the development of diabetes. Rectal hyposensitivity was also associated with distal polyneuropathy, indicating concurrent small and large nerve fiber damage.

Based on correlations between brain evoked potentials in response to rapid rectal balloon distention and other tests for both autonomic and peripheral neuropathy, we suggest that central neuronal signal processing seems to be affected in parallel with peripheral neuronal function, which should be further elucidated. Consequently, this method may be useful as research tool when evaluating gut autonomic neuropathy, potentially exploring the whole aspect of gastroenteropathy, from newly diagnosed diabetes in young adults to people with longstanding diabetes and GI symptoms.

Abbreviations

EP	Evoked potential
CAN	Cardiovascular autonomic neuropathy
GI	Gastrointestinal
CARTs	Cardiovascular reflex tests
BMI	Body mass index
eGFR	Estimated glomerular filtration rate
GLP-1	Glucagon like peptide-1
OGTT	Oral glucose tolerance test
EEG	Electroencephalography
VAS	Visual analogue scale
psi	Pound-force per square inch
DC	Direct current
ICA	Individual component analysis
HRV	Heart rate variability
DPN	Distal polyneuropathy
ANOVA	Analysis of variance

Funding

The study was supported by grants from Helse Vest, Johan Selmer Kvanes Legat and the Department of Laboratory Medicine and Pathology, Haukeland University Hospital.

CRediT authorship contribution statement

All authors have a substantial contribution in either planning the study, conducting examinations, analyzing, and interpreting data, and/or writing and editing the article.

Acknowledgements

Thanks to the biostatisticians at Stavanger University Hospital for valuable inputs. Thanks to the invaluable support conducting examinations from people working at the Research Unit for Health Surveys and Center for Diabetes Research, University of Bergen, and all other members of the PanGut study group at Haukeland University Hospital. Last, but not least, thanks to all people with diabetes and healthy controls participating in this study.

References

1. Tesfaye S, Boulton AJ, Dyck PJ, et al. Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. *Diabetes Care*. Oct 2010; 33:2285–2293. https://doi.org/10.2337/dc10-1303.
2. Spallone V, Bellavere F, Scionti L, et al. Recommendations for the use of cardiovascular tests in diagnosing diabetic autonomic neuropathy. *Nutr Metab Cardiovasc Dis*. Jan 2011;21:69–78. https://doi.org/10.1016/j.numecd.2010.07.005.
3. Sharma S, Vas P, Rayman G. Small fiber neuropathy in diabetes polyneuropathy: is it time to change? *J Diabetes Sci Technol*. 2021;16:321–331. https://doi.org/10.1177/1932296821996434, 2022/03/01.
4. Du YT, Rayner CK, Jones KL, Talley NJ, Horowitz M. Gastrointestinal symptoms in diabetes: prevalence, assessment, pathogenesis, and management. *Diabetes Care*. 2018;41:627. https://doi.org/10.2337/dc17-1536.
5. Meling S, Bertoli D, Sangnes DA. Diabetic gastroenteropathy, soothe the symptoms or unravel a cure? *Curr Diabetes Rev*. 2021. https://doi.org/10.2174/1573399817666210322154618. Mar 22.
6. Brock C, Softeland E, Gunterberg V, et al. Diabetic autonomic neuropathy affects symptom generation and brain-gut axis. *Diabetes Care*. Nov 2013;36:3698–3705. https://doi.org/10.2337/dc13-0347.
7. Softeland E, Brock C, Frokjaer JB, Simren M, Drewes AM, Dimcevski G. Rectal sensitivity in diabetes patients with symptoms of gastroparesis. *J Diabetes Res*. 2014; 2014, 784841. https://doi.org/10.1155/2014/784841.
8. Frieling T, Enck P, Wienbeck M. Cerebral responses evoked by electrical stimulation of rectosigmoid in normal subjects. *Dig Dis Sci*. Feb 1989;34:202–205. https://doi.org/10.1007/bf01536051.
9. Frokjaer JB, Softeland E, Graversen C, et al. Central processing of gut pain in diabetic patients with gastrointestinal symptoms. *Diabetes Care*. Jul 2009;32: 1274–1277. https://doi.org/10.2337/dc09-0324.
10. Harris ML, Hobson AR, Hamdy S, Thompson DG, Akkermans LM, Aziz Q. Neurophysiological evaluation of healthy human anorectal sensation. *Am J Physiol Gastrointest Liver Physiol*. Nov 2006;291:G950–G958. https://doi.org/10.1152/ajpgi.00010.2006.

11. Haas S, Brock C, Krogh K, et al. Cortical evoked potentials in response to rapid balloon distension of the rectum and anal canal. *Neurogastroenterol Motil.* Jun 2014; 26:862–873. https://doi.org/10.1111/nmo.12341.
12. Lelic D, Nissen TD, Brock C, Aziz Q, Drewes AM. Rapid balloon distension as a tool to study cortical processing of visceral sensations and pain. *Neurogastroenterol Motil.* Jun 2015;27:832–840. https://doi.org/10.1111/nmo.12557.
13. Haas S, Brock C, Krogh K, et al. Abnormal neuronal response to rectal and anal stimuli in patients with idiopathic fecal incontinence. *Neurogastroenterol Motil.* Jul 2015;27:954–962. https://doi.org/10.1111/nmo.12567.
14. Haas S, Faaborg P, Gram M, et al. Abnormal neuronal response to rectal and anal stimuli in patients treated with primary radiotherapy for anal cancer. *Radiother Oncol.* Aug 2018;128:369–374. https://doi.org/10.1016/j.radonc.2018.04.012.
15. Nissen TD, Brock C, Graversen C, et al. Translational aspects of rectal evoked potentials: a comparative study in rats and humans. *Am J Physiol Gastrointest Liver Physiol.* 2013;305:G119–G128. https://doi.org/10.1152/ajpgi.00403.2012.
16. Freeman R, Wieling W, Axelrod FB, et al. Consensus statement on the definition of orthostatic hypotension, neurally mediated syncope and the postural tachycardia syndrome. *Clin Auton Res.* 2011;21:69–72. https://doi.org/10.1007/s10286-011-0119-5, 2011/04/01.
17. Selvarajah D, Cash T, Davies J, et al. SUDOSCAN: a simple, rapid, and objective method with potential for screening for diabetic peripheral neuropathy. *PLoS One.* 2015;10, e0138224. https://doi.org/10.1371/journal.pone.0138224.
18. Perkins BA, Orszag A, Ngo M, Ng E, New P, Bril V. Prediction of incident diabetic neuropathy using the monofilament examination: a 4-year prospective study. *Diabetes Care.* Jul 2010;33:1549–1554. https://doi.org/10.2337/dc09-1835.
19. Frokjaer JB, Softeland E, Graversen C, Dimcevski G, Drewes AM. Effect of acute hyperglycaemia on sensory processing in diabetic autonomic neuropathy. *Eur J Clin Invest.* Oct 2010;40:883–886. https://doi.org/10.1111/j.1365-2362.2010.02335.x.
20. Softeland E, Brock C, Frokjaer JB, et al. Association between visceral, cardiac and sensorimotor polyneuropathies in diabetes mellitus. *J Diabetes Complicat.* 2014;28: 370–377. https://doi.org/10.1016/j.jdiacomp.2013.10.009. May-Jun.
21. Beyak MJ, Bulmer DC, Sellers D, Grundy D. Impairment of rectal afferent mechanosensitivity in experimental diabetes in the rat. *Neurogastroenterol Motil.* Jun 2009;21:678–681. https://doi.org/10.1111/j.1365-2982.2009.01266.x.
22. Kucera P, Goldenberg Z, Varsik P, Buranova D, Traubner P. Spinal cord lesions in diabetes mellitus. Somatosensory and motor evoked potentials and spinal conduction time in diabetes mellitus. *Neuro Endocrinol Lett.* Apr 2005;26:143–147.
23. Burgell RE, Lelic D, Carrington EV. Assessment of rectal afferent neuronal function and brain activity in patients with constipation and rectal hyposensitivity. *Neurogastroenterol Motil.* Mar 2013;25:260–267. https://doi.org/10.1111/nmo.12047. e167-8.
24. Lelic D, Brock C, Softeland E, et al. Brain networks encoding rectal sensation in type 1 diabetes. *Neuroscience.* 2013;237:96–105. https://doi.org/10.1016/j.neuroscience.2013.01.049.
25. Rasmussen TK, Finnerup NB, Singer W, Jensen TS, Hansen J, Terkelsen AJ. Preferential impairment of parasympathetic autonomic function in type 2 diabetes. *Auton Neurosci.* 2022:243. https://doi.org/10.1016/j.autneu.2022.103026.
26. Ziemssen T, Siepmann T. The investigation of the cardiovascular and sudomotor autonomic nervous system-a review. *Front Neurol.* 2019;10:53. https://doi.org/10.3389/fneur.2019.00053.
27. Chey WD, Kim M, Hasler WL, Owyang C. Hyperglycemia alters perception of rectal distention and blunts the rectoanal inhibitory reflex in healthy volunteers. *Gastroenterology.* Jun 1995;108:1700–1708. https://doi.org/10.1016/0016-5085(95)90131-0.
28. Hernando-Harder AC, Singer MV, Harder H. Effect of duodenal glucose and acute hyperglycemia on rectal perception and compliance in response to tension-controlled rectal distension in healthy humans. *Dig Dis Sci.* Jun 2008;53:1624–1631. https://doi.org/10.1007/s10620-007-0032-x.
29. Russo A, Botten R, Kong MF, et al. Effects of acute hyperglycaemia on anorectal motor and sensory function in diabetes mellitus. *Diabet Med.* Feb 2004;21:176–182. https://doi.org/10.1111/j.1464-5491.2004.01106.x.
30. Selvarajah D, Kar D, Khunti K, et al. Diabetic peripheral neuropathy: advances in diagnosis and strategies for screening and early intervention. *Lancet Diabetes Endocrinol.* Dec 2019;7:938–948. https://doi.org/10.1016/S2213-8587(19)30081-6.

The Composite Autonomic Symptom Score 31 Questionnaire, a sensitive test to detect risk for diabetic autonomic neuropathy

Sondre Meling[1,2](ORCID: 0000-0002-9045-1408), Erling Tjora[2,3](ORCID: 0000-0002-8878-8669), Heike Eichele[4,5](ORCID: 0000-0001-8916-2484), Niels Ejskjaer[6,7,8](ORCID: 0000-0003-3749-3403), Siri Carlsen[1](ORCID 0009-0002-7484-6036), Pål Rasmus Njølstad[2,3,9](ORCID: 0000-0003-0304-6728), Christina Brock[6,7,10](ORCID: 0000-0002-3381-1884), Eirik Søfteland[2,11,12](ORCID: 0000-0002-7221-1013)

1. Department of Medicine, Stavanger University Hospital, Stavanger, Norway.
2. Department of Clinical Science, University of Bergen, Bergen, Norway
3. Children and Youth Clinic, Haukeland University Hospital, Bergen, Norway.
4. Department of Biological and Medical Psychology, Faculty of Psychology, University of Bergen, Bergen, Norway
5. Regional resource centre for autism, ADHD and Tourette syndrome Western Norway, Division of Psychiatry, Haukeland University Hospital, Bergen, Norway
6. Department of Clinical Medicine, Faculty of Medicine, Aalborg University Hospital, Aalborg, Denmark.
7. Steno Diabetes Center North Denmark, Aalborg University Hospital, Aalborg, Denmark
8. Department of Endocrinology, Aalborg University Hospital, Aalborg, Denmark
9. Mohn Center for Diabetes Precision Medicine, Department of Clinical Science, University of Bergen, Bergen, Norway
10. Mech-Sense, Department of Gastroenterology and Hepatology, Aalborg University Hospital, Aalborg, Denmark.
11. Department of Medicine, Haukeland University Hospital, Bergen, Norway
12. Hormone Laboratory, Haukeland University Hospital, Bergen, Norway

Corresponding author: Sondre Meling, sondre.vatne.meling@sus.no, Department of Medicine, Stavanger University Hospital, Gerd-Ragna Bloch Thorsens gate 8, 4011 Stavanger, Norway.

Abstract:

Background and aims: Autonomic neuropathy is a common, but often neglected complication of diabetes, with signs and symptoms recommended assessed at time of diagnosis for type 2 diabetes, and then regularly. The Composite Autonomic Symptom Score (COMPASS) 31 is a validated and easy to use questionnaire regarding autonomic symptoms. We aimed to use a Norwegian version of the COMPASS 31 in people with different duration of diabetes and healthy controls, to consider feasibility, and to investigate if scores could discriminate between positive and negative outcomes for established tests for diabetic neuropathy, including cardiovascular autonomic neuropathy (CAN) and a novel method of examining the gastrointestinal visceral sensitivity. *Method:* We included 21 participants with longstanding type 2 diabetes, 15 with early type 2 diabetes, and 30 healthy, matched controls. Participants were phenotyped by cardiovascular autonomic reflex tests, electrical skin conductance, sural nerve electrophysiology and the monofilament test. As a proxy for gastrointestinal visceral and autonomic nerve function, evoked potentials were measured following rapid rectal balloon distention. *Results:* Participants with longstanding diabetes scored a median (IQR) of

14.9 (10.8-28.7) points, early diabetes 7.3 (1.6-15.2), and matched controls, 8.6 (4.1-21.6), p=0.04. Women and men scored 14.4 (5.5-28.7) and 7.8 (3.6-14.6) points respectively, p=0.01. Participants with definite or borderline CAN scored 14.3 (10.4-31.9) points, compared to participants with no CAN, 8.3 (3.2-21.5), p=0.04. Lowering the diagnostic cut-off from 16 to 10 points increased the sensitivity from 0.33 to 0.83, with a decreased specificity from 0.68 to 0.55. *Conclusion:* We successfully used COMPASS 31 in Norwegian. Thus, in accordance with guidelines, we suggest clinical implementation for assessment of autonomic neuropathy. Participants with longstanding diabetes had increased likelihood of symptoms and signs of diabetic autonomic neuropathy. For screening purposes, the sensitivity was improved by lowering the cut-off to 10 points, suggesting further testing if scoring above.

1. Introduction:

Autonomic neuropathy is a common complication in diabetes mellitus, associated with a wide range of symptoms, varying from mild to severe [1]. The condition is defined as a disorder of the autonomic nervous system in the setting of diabetes or metabolic derangements of prediabetes, after the exclusion of other causes, and may affect the cardiovascular, gastrointestinal and genitourinary systems, as well as sudomotor function [2].

Guidelines from the American Diabetes Association recommend assessing symptoms and signs of autonomic neuropathy in type 2 diabetes, starting at the time of diagnosis [3]. Further, a 2010 expert consensus recommended screening for cardiovascular autonomic neuropathy (CAN) at the onset of type 2 diabetes, particularly if a history of poor glycaemic control or other known complications are present [2]. Assessment for CAN is also recommended before major surgery [4]. However, the lack of feasible tests, as well as their demands in terms of time, resources, operator training and patient preparations strongly limit the implementation of these guidelines. Hence, symptom-based questionnaires could represent a promising surrogate for the gold standard CAN tests. Detecting early autonomic dysfunction would have implications for recommended treatment targets, interventions and to aid symptom management.

The Composite Autonomic Symptom Score (COMPASS) 31 is a revised version of the 169-item Autonomic Symptom Profile assessing 11 domains of autonomic function, to the 31-item COMPASS, now assessing six domains: orthostatic hypotension, vasomotor, secretomotor, gastrointestinal, bladder and pupillomotor functions. It is a validated, easy to use self-assessment instrument designed for clinical autonomic research and practice, which predicts CAN and diabetic polyneuropathy with a fair diagnostic accuracy. It is also valid for evaluating treatment outcomes [5-7].

The aim of this study was to explore the use of a Norwegian, digitally distributed version of the COMPASS 31 in a present cohort with different diabetes duration, and matched controls. We hypothesized that symptoms of autonomic neuropathy, and objective findings of neuropathy, were more prevalent in both longstanding diabetes and early diabetes, compared to controls. We also hypothesized that the COMPASS 31 score correlated with established tests of diabetic neuropathy, and plausibly with a novel method investigating rectal sensitivity and autonomic nerve conduction, assessed with rectally elicited evoked potentials.

2. Material and methods:

We conducted an observational case-control study, recruiting three groups; one group of people with type 2 diabetes for more than ten years (*longstanding diabetes group*), one with newly diagnosed type 2 diabetes diagnosis within one year without using antidiabetic medication (*early diabetes group*), and controls matched for age, gender, and body mass index (BMI). Diagnosis was confirmed performing an oral glucose tolerance test, using criteria for the American Diabetes Association [8]. Exclusion criteria were major abdominal surgery, rectosigmoid disease interfering with sensitivity, chronic pancreatitis, uremic condition, atrial fibrillation or other major dysrhythmia, cardiac pacemaker, diabetic retinopathy or present use of glucagon-like peptide 1 (GLP-1) receptor agonist or insulin.

The study was part of a larger project, the PanGut-study, approved by the Regional Ethics Committee. (REK Vest 2018#1790). Written consent in accordance with the Declaration of Helsinki was obtained from all participants prior to any study-related procedures. The relevant part of this study included three study days, one day of information, consent and neuronal phenotyping, one day of oral glucose tolerance test and basic blood samples, and one day performing rapid rectal balloon distention. Lastly, the COMPASS 31 was answered digitally at home. Recruitment and investigation were performed between September 2019 and December 2022, all investigations performed at a single centre (Bergen, Norway). Most of the participants were recruited through local newspaper advertising.

2.2 Examinations, measures, and variables

2.2.1 Oral glucose tolerance test

The test was performed after an overnight (10 h) fast, with antidiabetic medications withdrawn a total of three days, including the day of examination. One cannula was placed in a cubital vein, with the forearm on the same side placed in a heating cuff to ensure arterialized blood. The participant ingested a 2–300 mL solution of 75 g anhydrate glucose. Blood glucose was measured before and 2 h after glucose ingestion, using the HemoCue Glucose 201 DM RT (HemoCue, Angelholm, Sweden).

2.2.2 COMPASS 31

The linguistically validated Danish version of COMPASS 31 was translated into Norwegian using a forward/backward translation method.[9] The questionnaire was distributed using the EasyTrial.net program (EasyTrial ApS, Aalborg, Denmark) and answered online upon participants' discretion. The maximum domain-specific weighted scores in the domains orthostatic, vasomotor, secretomotor, gastrointestinal, bladder and pupillomotor were 40, 5, 15, 25, 10 and 5 points, respectively. Maximum total weighted score was 100 points. The recommended threshold supporting borderline CAN is 16 points [6].

2.2.3 Neuronal phenotyping

Alcohol consumption was not allowed 24 hours prior to testing. Participants were instructed not to eat, use nicotine products, or drink caffeinated beverage within three hours prior to examinations. Medications were taken as normal, except for the use of stimulants or sedatives prior to the rapid rectal balloon distention test.

For cardiovascular reflex tests (CARTs) and short recording of heart rate variability, we used the Vagus™ Device (Medicus Engineering, Aarhus, Denmark). CARTs were performed at rest, shortly after standing up, during deep breathing and Valsalva manoeuvre. Blood pressure was measured following five minutes sitting down at rest, upon standing, and after one- and three-minutes standing, using the WelchAllyn Connex ProBP 3400™ (EMEAI, Leiden, Netherlands). Orthostatic hypotension was defined as a decline in systolic blood pressure > 20 mmHg or in diastolic > 10 mmHg within three minutes of standing.[10] Stages of CAN were defined as borderline if one CART was abnormal, as definite if two or more CARTs were abnormal, and as severe if the latter was combined with orthostatic hypotension [2].

The Sudoscan™ Device (Impeto Medical, Paris, France) was used to test electrical skin conductance by measuring the chloride-ion flow produced by sweat glands in hands and feet, following low voltage electrical stimulation [11].

We used a 10 g monofilament, to bilaterally pinprick the dorsum of each foot four times, with participant's eyes closed. Feeling 7-8 of 8 sensations was defined as no suspected diabetic peripheral neuropathy, 4-6 as possible, and ≤ 3 as likely [12].

Sural nerve conduction was tested using the point-of-care device NC-stat DPN Check™ (NEUROMetrix, Boston, USA). The device stimulates the sural nerve at the level of the ankle, recording the resulting responses on the calf [13].

2.2.4 Visceral sensitivity: Evoked potentials following rapid balloon distention in the rectum

The equipment and protocol are described in detail elsewhere [14-17].
Electroencephalography (EEG) was recorded using a 64-channels extended 10-20 montage, with reference electrode Fz. A rectal balloon was placed 15 cm above the anal verge. We recorded EEG continuously during two task conditions: elicitation of earliest sensation, and unpleasant threshold/feeling urge to defecate, with 30 balloon pressure stimuli in each recording respectively. A distinct and short stimulus was used with 150 ms inflation, followed by an instant deflation. A random inter-stimulus interval of 8±2 seconds was enforced. EEG pre-processing and artefact reduction were done using independent component analysis in MatLab (Mathworks, Natick, MA, USA). Data from the sensory evoked potentials were pooled and analysed blindly.

2.2.5 Data analysis and statistics

As the data represents secondary analyses, a formal power calculation was not feasible. Means ± standard deviations are used for data with normal distribution, and medians with interquartile ranges for skewed data. Missing data were removed from the analysis. For parametric data one-way ANOVA was used, for non-parametric data we used Kruskal-Wallis test for several independent samples and Mann-Whitney U test for two samples. We used the Spearman's rank order test for correlation analysis. For diagnostic accuracy we calculated the area under the receiver operating characteristic curve (AUC), as well as sensitivity and specificity. Statistical significance was defined as p-value ≤0.05 for all analyses. Statistical analyses were performed using SPSS Version: 28.0.1.0 (IBM, US).

3. Results:

3.1 Subjects

We recruited a total of 66 participants (34 women), of whom 21 had longstanding type 2 diabetes, 15 had early type 2 diabetes (80% newly detected in the project) and 30 healthy controls (table 1).

Table 1: Clinical characteristics at baseline

Clinical characteristics	Longstanding diabetes N=21	Early diabetes N=15	Controls N=30	p-value
Age (Years at recruitment)	68.9±7.8	69.3±5.5	69.5±6.2	0.950
Gender (Women/Men)	10/11	8/7	16/14	0.911
BMI (kg/m^2)	26.5±4.4	25.7±4.1	25.5±3.8	0.680
Diabetes duration (Years)	16.8±4.9	0	0	n/a
Fasting glucose (OGTT), mmol/L	9.4±2.1	7.2±1.0	6.0±0.6	<0.001
2-hour glucose (OGTT), mmol/L	18.7±3.9	13.1±4.2	7.9±1.5	<0.001
HbA1c, mmol/mol (%)	54±11.2 (7.1)	43±4.9 (6.1)	37±3.0 (5.5)	<0.001
Total cholesterol, mmol/L	4.2±0.8†	4.5±1.2†	5.5±1.0	<0.001
HDL, mmol/L	1.3±0.3†	1.4±0.4†	1.9±0.5	<0.001
LDL, mmol/L	2.4±0.6†	2.8±1.1	3.3±0.8	0.001
Triglycerides, mmol/L	1.7±1.3†	1.3±0.5	1.0±0.4	0.009
eGFR, ml/min/1.73m^2	84.9±13.5	82.3±11.7	80.3±12.3	0.458
Systolic blood pressure rest, mmHg	135±15††	152±14	139±20††	0.015
Diastolic blood pressure, rest, mmHg	80±6††	86±7	81±7	0.023
Comorbidity (N)				
Nephropathy	0	0	0	n/a
Distal neuropathy, %	4.8	6.7	0	0.400
Hypertension, %	52	47	17	0.017
Cardiovascular disease, %	4.8	13	3.3	0.401
Drugs (N)				
Metformin, %	81	0	0	n/a
Sulphonylurea, %	19	0	0	n/a
DPP-4 inhibitor, %	48	0	0	n/a
SGLT2 inhibitor, %	38	0	0	n/a
Another antidiabetic medication, %	9.5	0	0	n/a
Diet treated diabetes, %	9.5	0	0	n/a
Betablocker, %	4.8	20	13	0.370
ACE-I/ARB, %	48	40	10	<0.001
Other antihypertensive medication, %	19	13	7	0.410
Lipid modifying treatment, %	67	47	13	<0.001
Smoking status,% (present/past/never)	10/38/52	7/13/80	3/43/54	0.300

Data are means ±SD unless otherwise indicated. p-values using one-way ANOVA or Pearson Chi Square test. Post hoc test for continuous data between groups using Bonferroni: †=significant compared to controls, ††=significant compared to early diabetes, all other groups where <0.001 with significant difference to each other. Diabetes duration, comorbidity, smoking status and drugs are self-reported. Abbreviations: BMI: body mass index, OGTT: oral glucose tolerance test, HDL: high-density lipoprotein, LDL: low-density lipoprotein, eGFR: estimated glomerular filtration rate, DPP-4: dipeptidyl peptidase-4, SGLT-2: sodium-glucose co-transporter 2, ACE-I: angiotensin converting enzyme inhibitor, ARB: angiotensin receptor blocker

3.2 COMPASS 31 scores and clinical correlations

3.2.1 Between-group differences

Participants with longstanding diabetes had a significantly higher COMPASS 31 score than both the group of early diabetes and the control group (p=0.01, table 2). The most contributing domains were the secretomotor and gastrointestinal.

Table 2: COMPASS 31 score for groups

	Group score				
	Longstanding diabetes	Early diabetes	Control	p-value	All groups
Orthostatic	0.0 (0.0-14.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.23	0.0 (0.0-12.0)
Vasomotor	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.76	0.0 (0.0-0.0)
Secretomotor	4.3 (0.0-6.4) †	0.0 (0.0-0.0)	2.1 (0.0-4.3)	**0.03**	1.1 (0.0-4.8)
Gastrointestinal	5.4 (2.7-8.9)	1.8 (0.0-6.3)	2.7 (0.0-7.1)	0.06	3.6 (0.9-7.1)
Bladder	1.1 (0.0-2.8)	1.1 (0.0-2.2)	1.1 (0.0-2.2)	0.81	1.1 (0.0-2.2)
Pupillomotor	1.0 (0.3-2.0)	1.0 (0.0-2.0)	1.0 (0.0-1.7)	0.49	1.0 (0.0-1.7)
Total weighted	**14.9 (10.8-28.7) ††**	**7.3 (1.6-15.2)**	**8.6 (4.1-21.6)**	**0.04**	**11.9 (4.5-21.6)**

Data are medians with interquartile range, p-values comparing all three groups using Kruskal-Wallis test. †=Significant in pairwise comparison to early diabetes. ††Significant in pairwise comparison to both other groups. For pairwise comparison the Mann-Whitney test was used.

3.2.2 Scores influenced by medications

There was an association between those with longstanding diabetes using dipeptidyl peptidase 4 (DPP-4) inhibitors, and a higher total score (rho -0.319, p=<0.01), score in the secretomotor domain (rho -0.248, p=0.05) and score in the gastrointestinal domain (rho -0.333, p=<0.01), with significantly different score in the gastrointestinal domain (table 3). Other medications with known gastrointestinal side effects or known to affect the autonomic nervous system had no significant influence on COMPASS 31 scores.

Table 3: COMPASS 31 score for the group with *longstanding* diabetes, with and without DPP-4 inhibitors.

	With DPP-4 inhib. N=10	Without DPP-4 inhib. N=11	p-value
Secretomotor	4.3 (0.0-7.0)	2.1 (0.0-6.4)	0.65
Gastrointestinal	6.7 (5.8-10.5)	3.6 (1.8-5.4)	**0.02**
Total weighted	17.7 (11.4-35.8)	14.9 (7.3-20.8)	0.43

Data are medians with interquartile range, p-values using Mann-Whitney test.

3.2.3 Sex differences

Women scored higher than men on total COMPASS 31 score, and in all domains except for bladder function. The domains contributing the most were secretomotor and gastrointestinal (table 4). Women with longstanding diabetes had the highest median score of 24.3 points.

Table 4: COMPASS 31 score for different sex

	Sex		
	Women	Men	p-value
Orthostatic	0.0 (0.0-13.0)	0.0 (0.0-3.0)	0.40
Vasomotor	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.14
Secretomotor	4.3 (0.0-6.4)	0.0 (0.0-2.1)	**<0.01**
Gastrointestinal	5.8 (1.5-9.8)	1.8 (0.0-4.5)	**<0.01**
Bladder	1.1 (0.0-2.2)	1.1 (0.0-2.2)	0.87
Pupillomotor	1.0 (0.0-2.1)	1.0 (0.0-1.7)	0.86
Total weighted	**14.4 (5.5-28.7)**	**7.8 (3.6-14.6)**	**0.01**

Data are medians with interquartile range, p-values using Mann-Whitney test.

3.3 Neuronal phenotyping and COMPASS 31 score

Two participants had definite CAN based on CARTs, one in the early diabetes group, and one in the control group. Definite or borderline CAN were detected in 31% of participants with longstanding diabetes, in 23 % with early diabetes and in 17 % of controls, p=0.54. Based on the monofilament test the prevalence of possible or likely peripheral neuropathy was 29% (longstanding diabetes), 13% (early diabetes) and 6.7% (controls), p=0.04 for longstanding diabetes compared to controls.

No associations were found within the respective groups when comparing CARTs and heart rate variability with COMPASS 31 scores, but *independently* of groups, scores correlated with definite or borderline CAN, rho=0.283, p 0.04. The score difference was also significant (table 5). No significant associations were detected between neither total score nor different domain scores and sudomotor, monofilament, sural nerve function, rectal sensitivity or evoked potentials following rapid rectal balloon distention.

All results from CARTs, heart rate variability, sudomotor function, sural nerve check, monofilament test and rapid rectal balloon distention tests can be found in supplemental files (S1-3) or in a previous publication from the present study [17].

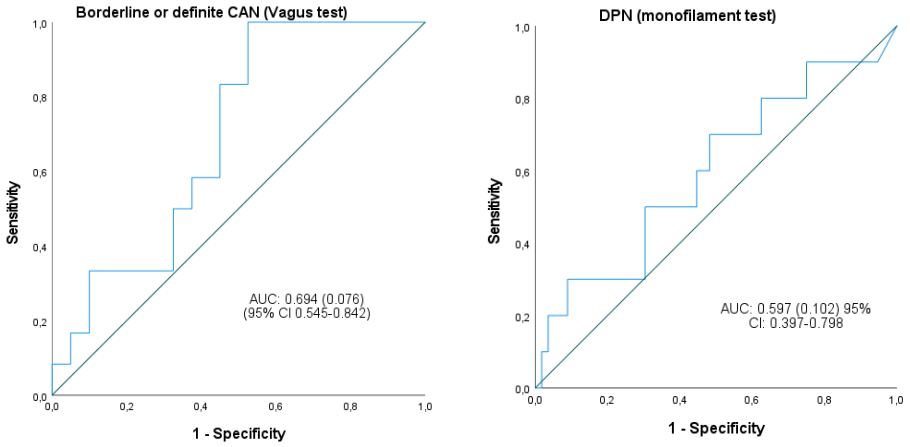
Using a cut off for total COMPASS 31 score at 16 points for CAN in our population, the sensitivity was 0.33, and specificity 0.68. Changing the cut off to 10 points increased the sensitivity to 0.83, with a specificity of 0.55 (table 5 and figure 1).

Table 5: COMPASS 31 score for definite/borderline CAN, no CAN, and results from monofilament test, for the entire population.

	COMPASS 31 score	AUC (95% CI)	Cut off: 16 p. Sensitivity PPV	Specificity NPV	Cut off: 10 p. Sensitivity PPV	Specificity NPV
CARTs						
Definite/Borderline CAN (n=12)	14.3 (10.4-31.9)	0.69 (0.55-0.84)	0.33 0.24	0.68 0.78	0.83 0.36	0.55 0.92
No CAN (n=40)	8.3 (3.2-21.5)					
p-value	0.04					
Monofilament test						
Possible/likely DPN (n=10)	15.5 (4.4-32.3)	0.60 (0.40-0.80)	0.40 0.23	0.70 0.87	0.70 0.29	0.50 0.83
No DPN (n=56)	11.0 (4.3-21.8)					
p-value	0.33					

Data are medians with interquartile range. p-values using Mann-Whitney test. Area under the curve (AUC) estimated for predicting diagnostic accuracy. PPV=Positive predictive value, NPV=Negative predictive value. CARTs=cardiovascular reflex tests, CAN=cardiovascular autonomic neuropathy, DPN=diabetic peripheral neuropathy. Definite/borderline CAN=one or more pathological CARTs. Possible/probably DPN with <six sensations on monofilament test.

Figure 1: ROC curves for CAN (Vagus test) and DPN (monofilament test)



4. Discussion:

We successfully used a Norwegian version of the COMPASS 31. More symptoms and signs associated with autonomic neuropathy were reported in those with longstanding diabetes, than in people with early diabetes and controls. The results were partly influenced by DPP-4

inhibitors, mostly in the gastrointestinal domain. Women reported more symptoms than men. Independently of diabetes status, there were clinical correlations between increased symptom burden and definite or borderline CAN. No significant correlations were detected with other established neuropathy tests or with the novel test of evoked potential following rapid rectal balloon distention or rectal sensitivity.

4.1 COMPASS 31, ease of use

Symptoms and signs of autonomic neuropathy should lead to further testing, but until recently, questionnaires regarding symptoms and signs have not been validated [2,4]. Since being revised to COMPASS 31 in 2012, the questionnaire has been validated in different languages and used in several research trials, including diabetic neuropathies [5-7,9,18-21]. In our experience, the questionnaire was easy to use, and digital distribution and answering was feasible, despite the rather high average age of our participants. We conclude that the questionnaire is suitable for clinical studies, with larger real-life studies feasible, and needed to address this in detail. In our opinion, symptoms of diabetic autonomic neuropathy are a neglected area in the care for people with diabetes, and we did not experience issues that would limit the questionnaires application to an individual clinical settings.

4.2 Symptoms of autonomic neuropathy, higher burden in longstanding diabetes and women

People with longstanding diabetes had a higher COMPASS 31 score than the groups of early diabetes and controls. This is in line with other studies on autonomic symptoms in diabetes, indicating that duration of diabetes is a risk factor [2,22]. We hypothesized that symptoms and signs of autonomic neuropathy were more often present also in people with early diabetes, but could not detect this neither in the questionnaire nor objective tests. The hypothesis was based on the knowledge of autonomic dysfunction possibly being present already at pre-diabetic stages [4]. However, a challenge when screening for small fibre neuropathy at early stages of diabetes is that it can often be asymptomatic [2,22]. To our knowledge, no other studies on COMPASS 31 included a group of early diabetes. A different questionnaire, The Survey of Autonomic Symptoms, has been validated for detecting autonomic symptoms in early diabetic neuropathy, but with inclusion criteria that makes the study incomparable to ours [23].

The gastrointestinal domain contributes the most to the higher total score in longstanding diabetes. This supports former knowledge that patients with diabetes experience more gastrointestinal symptoms than people without diabetes [24]. However, we did not uncover any impact on COMPASS 31 scores by medications to have known gastrointestinal side-effects. Surprisingly, there was a slightly higher total score in people using DPP-4 inhibitors, driven by the gastrointestinal domain. The DPP-4 inhibitor mostly used was sitagliptin (80%). Earlier studies in people with diabetes using sitagliptin have reported a marginally elevated risk of gastrointestinal adverse events vs. placebo (5.0% vs. 4.6 and 1.8% vs. 1.4%) [25,26]. Our study was not powered to detect such differences, and the results may be due to other causes, such as confounding by indication (i.e. more people with diabetes who had gastrointestinal symptoms due to other causes, may fail on metformin and/or GLP-1 receptor agonists, and hence receive DPP-4 inhibitors). It might also be a spurious finding.

Women scored higher in total, and in all domains, except for bladder symptoms. The most significant sex differences were found in the secretomotor and gastrointestinal domains. The

secretomotor domain contains a question regarding the degree of sweating, which could be explained by remaining symptoms of menopause. Women did not use more medications that are related to an increase in any of the symptoms reported. Regarding the higher score for women in the gastrointestinal domain, this has also been previously reported in a population-based study, with one of the reasons proposed for this related to a higher prevalence of functional disorders in women [27]. The same study also suggested that the negative effect diabetes exerts on daily life is more pronounced in women, as a possible explanation. Epidemiologic studies have reported a higher prevalence of gastrointestinal symptoms in women, regardless of having diabetes or not [27,28]. The results could also reflect that men may generally underreport symptoms, which have been proposed for other conditions, such as self-reporting in depression [29].

4.3 Correlation between COMPASS 31 score and other tests

We did not uncover any correlations between continuous scores in COMPASS 31 and CARTs, sudomotor function testing, sural nerve function or the monofilament test. Other studies have reported correlations between COMPASS 31 and CARTs, especially deep breathing and lying to standing, and for some parameters of heart rate variability [18,20]. One possible explanation could be the limited cases of definite CAN in the present population. Despite an average of 17 years since diagnosis, our longstanding diabetes population had few microvascular complications, acceptable values for HbA1c and a near normal average BMI. The reasons for this may partly be explained by excluding people using GLP-1 analogue and/or insulin, and people with retinopathy, the first mentioned because of other aspects of the PanGut study investigating the incretin effect. Both obesity in type 2 diabetes and retinopathy have been correlated to CAN [2,30]. The specific characteristics of our study population is of importance with regards to external validity.

We could also not detect any correlation between COMPASS 31 scores, including the gastrointestinal domain, and rectal sensitivity or evoked potentials following rapid balloon distention in the rectum. This might support other studies reporting a lack of correlation between symptoms, especially regarding diabetic gastroenteropathies, and objective findings such as motility disturbances, in the gastrointestinal tract [31].

4.4 Comparison to studies validating COMPASS 31

Our reported prevalence of 31% borderline CAN in the group with longstanding diabetes is comparable to other studies validating the questionnaire reporting a prevalence of 29-36% (although these included 13-14% with definite CAN as well) [6,18]. The mentioned studies display higher scores than in our study, the differences probably reflecting the different populations and few participants in our group of longstanding diabetes. The mentioned studies also included people with type 1 diabetes, who are reported to display autonomic symptoms more often than people with type 2 diabetes [22].

4.5 COMPASS 31 as a screening tool

COMPASS 31 is considered a well validated screening tool for autonomic dysfunction and small nerve fibre neuropathy, both independently and in combination with other tests [2,18,19,21]. In our population, using the recommended threshold score of 16 points, we found a particular poor sensitivity (0.33) for borderline/definite CAN. However, by reducing the cut off to 10 points, sensitivity increased markedly (0.83) with only a slight decrease in

specificity (0.68 to 0.55), and a high negative predictive value (0.92). Of interest in this regard, Treister et al also reported a cut-off of 10 being the optimal for screening purposes, with a sensitivity of 93% and specificity of 38% for small fibre polyneuropathy, confirmed by epidermal nerve fibre density [19]. In contrast to this, another study, which reported a prevalence of 17 % confirmed CAN, recommended a cut off score of 28.7 points for definite CAN. Though, this study had a population of 89 % with borderline or definite CAN, and compared to ours, had higher values for HbA1c and BMI, and higher total score mainly because of higher score in the orthostatic domain [32].

4.6 Methodological considerations:

COMPASS 31 is yet to be formally validated in Norwegian language. Still, with the high similarity between written Danish and Norwegian languages, as well as similar cultures and demographics, we argue that the risk of biases in the Norwegian version is low [9]. A forward-backward translation was performed, and no discrepancies were detected. Internal validity for subjects with diabetes and CAN or peripheral neuropathy are reported as acceptable in a comparable country (Italy) [6,18].

When performing the tests for neuronal phenotyping, we did not enforce discontinuation of any medications. Several drugs could impact autonomic function tests, however, short term discontinuation could also influence results, e.g. rebound tachycardia discontinuing betablockers, so we opted to leave them unchanged [33].

We acknowledge a limitation regarding our selected nature of participants, with a mean age of 70 years and near normal weighted. Still, especially regarding age, we find the present cohort less investigated, but with an increasing prevalence of type 2 diabetes, probably due to generally increased life expectancy.

Finally, we acknowledge the few participants in every group, thus a lack of statistical power. Comparable studies validating COMPASS 31 has found a sample size between 60 and 90 participants adequate [6,18].

5. Conclusion

We found COMPASS 31 easy to use, and to evaluate, and believe it is feasible for both research in larger groups and clinical practice. In the present cohort, higher COMPASS 31 scores were associated with definite or borderline CAN, with longstanding diabetes, and female sex, but not with results from other tests for diabetic neuropathy or the novel test investigating evoked potential after rectal balloon distention or with rectal sensitivity. Screening for people with early autonomic dysfunction, we propose a cut-off at 10 points, considering further CAN diagnostics if the patient scores above this level.

Data availability: The participants of this study did not give written consent for their data to be shared publicly, so due to the sensitive nature of the research supporting data is not available.

Conflicts of interest: None of the authors declare conflict of financial or competing interests.

Funding Statement: The study was supported by grants from Helse Vest, Johan Selmer Kvanes Legat and the Dep. Of Laboratory Medicine and Pathology, Haukeland University Hospital.

Acknowledgments: Thanks to the biostatisticians of the Stavanger University Hospital for valuable inputs. Thanks to the Research Unit for Health Surveys and Centre for Diabetes Research, University of Bergen, and all other members of the PanGut study group at Haukeland University Hospital.

Supplementary Materials: S1: Neuronal phenotyping, **S2:** Amplitude and latency (mean average) from evoked potentials following rapid balloon distention in the rectum.**S3**: Values for latencies and amplitudes for evoked potentials

References:

1. Spallone V, Bellavere F, Scionti L, et al. Recommendations for the use of cardiovascular tests in diagnosing diabetic autonomic neuropathy. *Nutr Metab Cardiovasc Dis*. Jan 2011;21(1):69-78. doi:10.1016/j.numecd.2010.07.005
2. Tesfaye S, Boulton AJ, Dyck PJ, et al. Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. *Diabetes Care*. Oct 2010;33(10):2285-93. doi:10.2337/dc10-1303
3. ElSayed NA, Aleppo G, Aroda VR, et al. 12. Retinopathy, Neuropathy, and Foot Care: Standards of Care in Diabetes—2023. *Diabetes Care*. 2022;46(Supplement_1):S203-S215. doi:10.2337/dc23-S012
4. Spallone V, Ziegler D, Freeman R, et al. Cardiovascular autonomic neuropathy in diabetes: clinical impact, assessment, diagnosis, and management. *Diabetes Metab Res Rev*. Oct 2011;27(7):639-53. doi:10.1002/dmrr.1239
5. Sletten DM, Suarez GA, Low PA, Mandrekar J, Singer W. COMPASS 31: a refined and abbreviated Composite Autonomic Symptom Score. *Mayo Clin Proc*. 2012;87(12):1196-1201. doi:10.1016/j.mayocp.2012.10.013
6. Greco C, Di Gennaro F, D'Amato C, et al. Validation of the Composite Autonomic Symptom Score 31 (COMPASS 31) for the assessment of symptoms of autonomic neuropathy in people with diabetes. *Diabet Med*. Jun 2017;34(6):834-838. doi:10.1111/dme.13310
7. Peng Y, Liu YS, Wu MY, et al. Evaluation of the Degree of Agreement of Four Methods for Diagnosing Diabetic Autonomic Neuropathy. *Front Neurol*. 2021;12:637099. doi:10.3389/fneur.2021.637099
8. ElSayed NA, Aleppo G, Aroda VR, et al. 2. Classification and Diagnosis of Diabetes: Standards of Care in Diabetes—2023. *Diabetes Care*. 2022;46(Supplement_1):S19-S40. doi:10.2337/dc23-S002
9. Brinth L, Pors K, Mehlsn J, Sletten DM, Terkelsen AJ, Singer W. Translation and linguistic validation of the Composite Autonomic Symptom Score COMPASS 31 in Danish. *Dan Med J*. Feb 9 2021;69(3)
10. Freeman R, Wieling W, Axelrod FB, et al. Consensus statement on the definition of orthostatic hypotension, neurally mediated syncope and the postural tachycardia syndrome. *Clinical Autonomic Research*. 2011/04/01 2011;21(2):69-72. doi:10.1007/s10286-011-0119-5
11. Selvarajah D, Cash T, Davies J, et al. SUDOSCAN: A Simple, Rapid, and Objective Method with Potential for Screening for Diabetic Peripheral Neuropathy. *PLoS One*. 2015;10(10):e0138224. doi:10.1371/journal.pone.0138224
12. Perkins BA, Orszag A, Ngo M, Ng E, New P, Bril V. Prediction of incident diabetic neuropathy using the monofilament examination: a 4-year prospective study. *Diabetes Care*. Jul 2010;33(7):1549-54. doi:10.2337/dc09-1835
13. Lee JA, Halpern EM, Lovblom LE, Yeung E, Bril V, Perkins BA. Reliability and validity of a point-of-care sural nerve conduction device for identification of diabetic neuropathy. *PLoS One*. 2014;9(1):e86515. doi:10.1371/journal.pone.0086515
14. Haas S, Brock C, Krogh K, et al. Cortical evoked potentials in response to rapid balloon distension of the rectum and anal canal. *Neurogastroenterol Motil*. Jun 2014;26(6):862-73. doi:10.1111/nmo.12341

15. Lelic D, Nissen TD, Brock C, Aziz Q, Drewes AM. Rapid balloon distension as a tool to study cortical processing of visceral sensations and pain. *Neurogastroenterol Motil*. Jun 2015;27(6):832-40. doi:10.1111/nmo.12557
16. Nissen TD, Brock C, Graversen C, et al. Translational aspects of rectal evoked potentials: a comparative study in rats and humans. *Am J Physiol Gastrointest Liver Physiol*. Jul 15 2013;305(2):G119-28. doi:10.1152/ajpgi.00403.2012
17. Meling S, Tjora E, Eichele H, et al. The PanGut-study: Evoked potentials following rectal balloon distention, a way of evaluating diabetic autonomic neuropathy in the gut? *Journal of Diabetes and its Complications*. 2023/05/01/ 2023;37(5):108452. doi:https://doi.org/10.1016/j.jdiacomp.2023.108452
18. D'Amato C, Greco C, Lombardo G, et al. The diagnostic usefulness of the combined COMPASS 31 questionnaire and electrochemical skin conductance for diabetic cardiovascular autonomic neuropathy and diabetic polyneuropathy. *J Peripher Nerv Syst*. Mar 2020;25(1):44-53. doi:10.1111/jns.12366
19. Treister R, O'Neil K, Downs HM, Oaklander AL. Validation of the composite autonomic symptom scale 31 (COMPASS-31) in patients with and without small fiber polyneuropathy. *Eur J Neurol*. 2015;22(7):1124-1130. doi:10.1111/ene.12717
20. Zhang Z, Ma Y, Fu L, et al. Combination of Composite Autonomic Symptom Score 31 and Heart Rate Variability for Diagnosis of Cardiovascular Autonomic Neuropathy in People with Type 2 Diabetes. *J Diabetes Res*. 2020;2020:5316769. doi:10.1155/2020/5316769
21. Ruska B, Pavicic T, Pavlovic I, et al. Performance of the COMPASS-31 questionnaire with regard to autonomic nervous system testing results and medication use: a prospective study in a real-life setting. *Neurol Sci*. Dec 2018;39(12):2079-2084. doi:10.1007/s10072-018-3542-8
22. Low PA, Benrud-Larson LM, Sletten DM, et al. Autonomic symptoms and diabetic neuropathy: a population-based study. *Diabetes Care*. Dec 2004;27(12):2942-7. doi:10.2337/diacare.27.12.2942
23. Zilliox L, Peltier AC, Wren PA, et al. Assessing autonomic dysfunction in early diabetic neuropathy: the Survey of Autonomic Symptoms. *Neurology*. Mar 22 2011;76(12):1099-105. doi:10.1212/WNL.0b013e3182120147
24. Du YT, Rayner CK, Jones KL, Talley NJ, Horowitz M. Gastrointestinal Symptoms in Diabetes: Prevalence, Assessment, Pathogenesis, and Management. *Diabetes Care*. 2018;41(3):627. doi:10.2337/dc17-1536
25. Moses RG, Round E, Shentu Y, et al. A randomized clinical trial evaluating the safety and efficacy of sitagliptin added to the combination of sulfonylurea and metformin in patients with type 2 diabetes mellitus and inadequate glycemic control. *J Diabetes*. Sep 2016;8(5):701-11. doi:10.1111/1753-0407.12351
26. Green JB, Bethel MA, Armstrong PW, et al. Effect of Sitagliptin on Cardiovascular Outcomes in Type 2 Diabetes. *New England Journal of Medicine*. 2015/07/16 2015;373(3):232-242. doi:10.1056/NEJMoa1501352
27. Bytzer P, Talley NJ, Leemon M, Young LJ, Jones MP, Horowitz M. Prevalence of gastrointestinal symptoms associated with diabetes mellitus: a population-based survey of 15,000 adults. *Arch Intern Med*. Sep 10 2001;161(16):1989-96. doi:10.1001/archinte.161.16.1989
28. Talley NJ, Zinsmeister AR, Melton LJ, 3rd. Irritable bowel syndrome in a community: symptom subgroups, risk factors, and health care utilization. *Am J Epidemiol*. Jul 1 1995;142(1):76-83. doi:10.1093/oxfordjournals.aje.a117548
29. Shi P, Yang A, Zhao Q, Chen Z, Ren X, Dai Q. A Hypothesis of Gender Differences in Self-Reporting Symptom of Depression: Implications to Solve Under-Diagnosis and Under-Treatment of Depression in Males. Review. *Frontiers in Psychiatry*. 2021-October-25 2021;12doi:10.3389/fpsyt.2021.589687
30. Valensi P, Paries J, Attali JR, French Group for R, Study of Diabetic N. Cardiac autonomic neuropathy in diabetic patients: influence of diabetes duration, obesity, and microangiopathic

complications--the French multicenter study. *Metabolism*. Jul 2003;52(7):815-20. doi:10.1016/s0026-0495(03)00095-7
31. Sangnes DA, Softeland E, Bekkelund M, et al. Wireless motility capsule compared with scintigraphy in the assessment of diabetic gastroparesis. *Neurogastroenterol Motil*. Apr 2020;32(4):e13771. doi:10.1111/nmo.13771
32. Singh R, Arbaz M, Rai NK, Joshi R. Diagnostic accuracy of composite autonomic symptom scale 31 (COMPASS-31) in early detection of autonomic dysfunction in type 2 diabetes mellitus. *Diabetes, metabolic syndrome and obesity : targets and therapy*. 2019;12:1735-1742. doi:10.2147/DMSO.S214085
33. Ziemssen T, Siepmann T. The Investigation of the Cardiovascular and Sudomotor Autonomic Nervous System-A Review. *Front Neurol*. 2019;10:53. doi:10.3389/fneur.2019.00053

.

Supplementary Materials: S1: Neuronal phenotyping

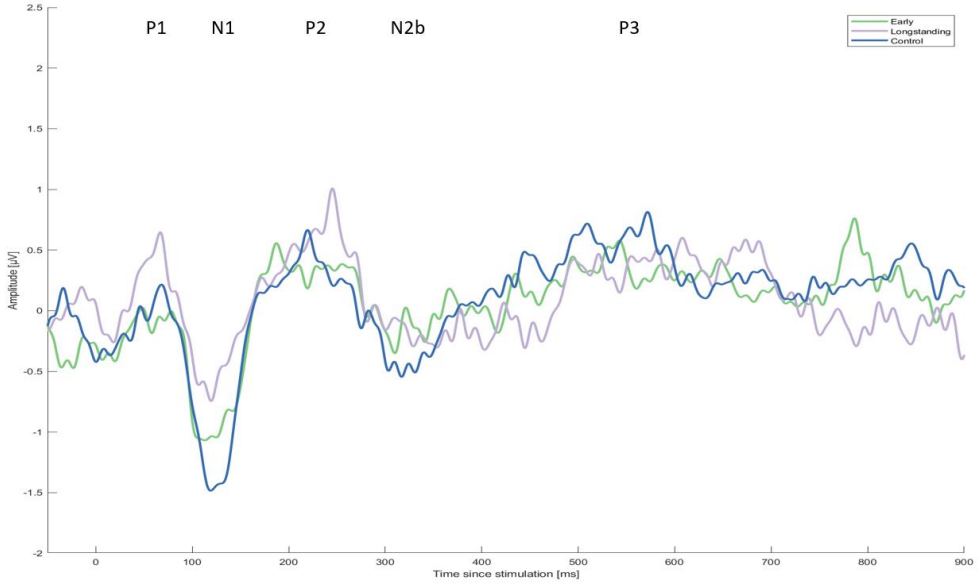
Parameters	Longstanding diabetes	Early diabetes	Controls	p-value
Rectal sensation				
Pressure VAS 1, bar	0.037 (0.011)*	0.040 (0.013)*	0.030 (0.009)	<0.01
Pressure VAS 5, bar	1.45 (0.50)	1.47 (0.58)	1.50 (0.50)	0.94
CARTS and HRV				
HR, bpm	68.5 (8.3)*	64.0 (8.0)	63.1 (11.6)	0.15
SDNN, ms	29.5 (19.6-46.7)	32.7 (17.0-38.3)	29.1 (21.5-37.5)	0.94
RMSSD, ms	18.1 (10.8-45.6)	19.5 (11.0-26.2)	21.2 (9.6-31.8)	0.96
LF, ms^2	58.5 (33.7-145.4)	93.9 (25.7-126.8)	77.9 (34.5-155.5)	0.97
HF, ms^2	69.7 (13.6-152.0)	63.4 (15.5-104.1)	44.1 (13.0-99.4)	0.99
Total, ms^2	247.2 (121.9-600.6)	306.3 (134.9-469.5)	324.2 (161.9-586.8)	0.72
R/S ratio	1.08 (1.03-1.12)	1.09 (1.04-1.15)	1.08 (1.06-1.15)	0.38
Abnormal R/S ratio, %	6.3	6.7	6.7	0.96
E/I ratio	1.18 (1.06-1.31)	1.14 (1.07-1.23)	1.13 (1.11-1.23)	0.73
Abnormal E/I ratio, %	25	20	6.7	0.34
VM ratio	1.41 (1.33-1.65)	1.41 (1.32-1.48)	1.47 (1.33-1.62)	0.33
Abnormal VM ratio, %	0	6.7	3.3	0.55
CAN				
No/borderline/definite, %	69/31/0	77/15/8	83/13/4	0.54
Orthostatic hypotension, %	14.3	20	17.2	0.90
Sural nerve check				
DPN, Velocity, m/s	46.5 (43-50)	47 (40.2-51)	48 (43.5-52)	0.56
DPN, Amplitude, µV	6.5 (5-9)	5 (4-9)	7 (5-9)	0.54
Peripheral neuropathy:				
No/mild/mod./serious, %	76/10/10/0	80/13/7/0	87/0/7/3	0.68
Monofilament test				
Felt pinpricks (n)	8 (6-8)*	8 (8-8)	8 (8-8)	0.08
Peripheral neuropathy:				
Unlikely/possibly/likely, %	71/24/5	87/13/0	90/0/10	0.06
Sudomotor function				
Hands, µSiemens	65.8 (14.4)	67.2 (14.1)	71.3 (15.2)	0.39
Normal/mod. reduced				
/severely reduced, %	70/25/5	67/33/0	77/20/3	0.81
Feet, µSiemens	73.6 (12.4)	77.7 (6.7)	75.3 (13.5)	0.60
Normal/mod. reduced				
/severely reduced, %	71/19/10	80/20/0	87/7/7	0.45

Results of neuronal phenotyping given as mean (SD) or median (IQR). Asterisk (*) indicates statistically significant differences compared to controls. HR = mean heart rate, bpm = beats per minute, during 5 min. rest, SDNN = standard deviation from the mean heartbeat interval value (net effect of the autonomic regulation), RMSSD = root mean square of the standard sensitivity and capacity — predominantly parasympathetic tests). VM ratio: ratio between maximum heart rate at the end of forced expiration and minimal heart rate during inspiration-expiration in rest after appr. 30 seconds after releasing pressure (predominantly sympathetic and baroreflex mediated test). Reference CART ratios are age adjusted. CAN is predicted as borderline if one abnormal CART ratio,

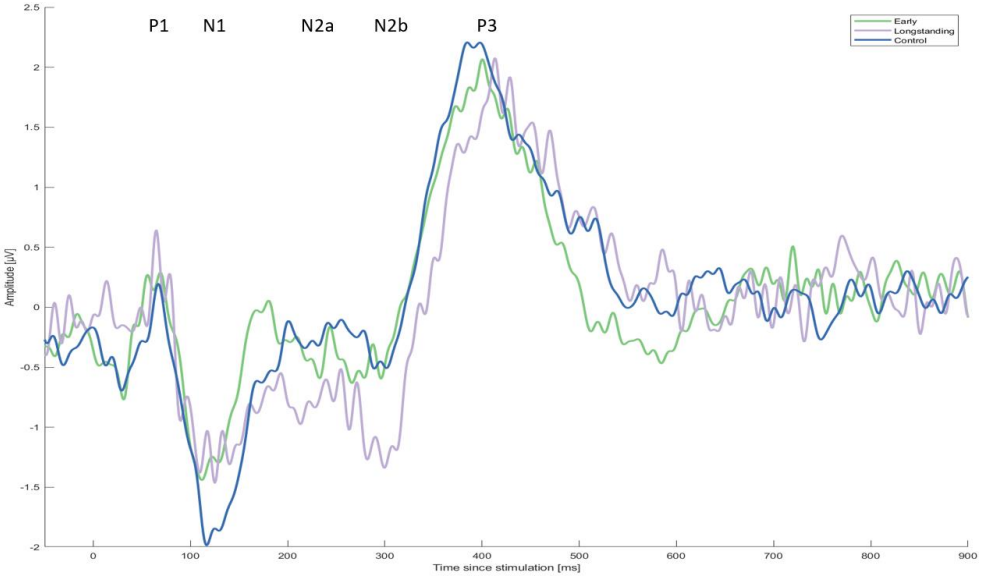
and definite if two or more abnormal ratios. Orthostatic hypotension is defined as a decline in systolic blood pressure of > 20 mmHg or diastolic > 10 mmHg within three minutes of standing. Risk of peripheral neuropathy performing the sural nerve check is defined by the software. For the monofilament test feeling 7-8 of 8 sensations is defined as unlikely distal polyneuropathy, feeling 4-6 as possible DPN, and feeling 3 or less, as likely DPN. Stages of sudomotor function are also defined by the software. P-values from categorical outcomes from Chi Square test.deviation (activity level of the parasympathetic regulation). LF = low frequency activity (represents sympathetic tone), HF = high frequency activity (represents parasympathetic tone), TP = total power (power spectrum of RR intervals throughout the frequency ranges − net autonomic function). R/S ratio: 30:15 Ratio = ratio between maximum HR within the first 15 s after standing up and minimum HR within the first 30 s after standing up (predominantly parasympathetic test). E/I-ratio = mean ratio between the longest and shortest RR-interval during deep respiration (measures of baroreflex

S2: Amplitude and latency (mean average) from evoked potentials following rapid balloon distention in the rectum.

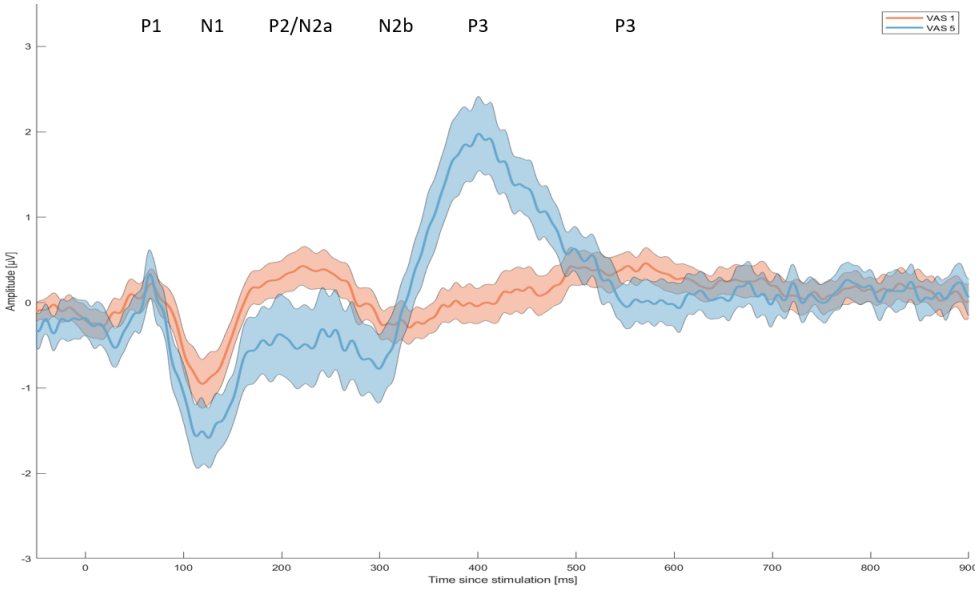
a)



b)



c)



Amplitude and latency for VAS 1 and VAS 5 respectively, for the three groups (a and b) and mean for all observations for VAS 1 and 5 (c). P1 indicates the first positive peak, N1 the first negative peak, etc. Signals occurring <250 ms (P1-2, N1-2a) represent stimulus-specific processing, providing information on the sensitivity of the visceral afferent pathways, while >250 ms (N2b and P3) are regarded as also involving cognitive processes. As expected, N2b and P3 in VAS 5 have shorter latency and larger amplitude due to the increased stimuli compared to VAS 1.

S3: Values for latencies and amplitudes for evoked potentials

	Longstanding diabetes	Early diabetes	Controls	p-values
VAS 1-amplitude				
P1	1.6 (1.4)	0.7 (0.6)	1.1 (1.0)	0.08
N1	-2.0 (1.2)	-1.9 (1.0)	-2.3 (1.0)	0.42
P2	1.8 (1.1)	1.3 (1.5)	1.6 (1.1)	0.40
N2b	-1.8 (0.8)	-1.3 (0.8)	-1.5 (1.1)	0.39
P3	1.6 (1.0)	1.5 (1.5)	1.7 (0.9)	0.76
VAS 1- latency				
P1	64.1 (22.0)	56.7 (25.3)	52.4 (24.4)	0.32
N1	125.2 (31.9)	115.3 (26.7)	114.8 (17.2)	0.36
P2	221.5 (58.6)	210.4 (54.2)	220.0 (61.4)	0.84
N2b	353.8 (87.3)	308.3 (61.6)	327.0 (56.8)	0.17
P3	518.2 (55.6)	487.9 (85.9)	513.8 (62.5)	0.36
VAS 5 -amplitude				
P1	1.4 (1.4)	0.8 (0.8)	0.7 (0.9)	0.08
N1	-2.9 (2.1)	-2.4 (1.4)	-2.8 (1.3)	0.57
N2b	-2.6 (2.2)	-1.8 (1.6)	-1.4 (1.6)	0.07
N2a	0.25 (2.6)	1.2 (1.6)	1.0 (1.4)	0.30
P3	3.1 (1.7)	2.7 (1.9)	3.0 (1.5)	0.73
VAS 5-latency				
P1	64.1 (29.7)	59.6 (14.1)	54,7 (14.4)	0.76
N1	131.8 (36.6)	113.5 (21.4)	122.5 (24.9	0.20
N2a	230.9 (50.6)	193.4 (54.9)	217.1 (46.6)	0.12
N2b	297.3 (42.7)	285.3 (50.5)	283.5 (36.3)	0.52
P3	417.7 (43.1)	405.8 (22.3)	398.5 (29.8)	0.16

Data given in mean with (SD), means compared with one-way ANOVA, between all groups. Latencies are in milliseconds, amplitude in microvolt.

Errata for
Diabetes mellitus type 2

The incretin effect and interaction with the autonomic nervous system

Sondre Vatne Meling

Thesis for the degree philosophiae doctor (PhD)
at the University of Bergen

30.08.23 Sondre Vatne Meling
(date and sign. of candidate)

31.08.23
(date and sign. of faculty)

Errata

Page 13 Addendum under II.: "Paper published 9. Aug 2023.
DOI: 10.1155/2023/4441"

Page 18 Missing headline: "**10. PAPERS I-III.....113**"

Page 33, line 12 Incorrect words: "in between 11 and 24 %" corrected to "up to 11 and 24 %"

Graphic design: Communication Division, UiB / Print: Skipnes Kommunikasjon AS

uib.no

ISBN: 9788230845615 (print)
9788230865040 (PDF)