Diabetic Autonomic Neuropathy

Aspects of the Cardiovascular and Gastrointestinal Systems in Adults with Type 1 Diabetes

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BY
ANNE-MARIE LANGMACH WEGEBERG

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AALBORG UNIVERSITY
DENMARK

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Publications

1. Okdahl T, Brock C, Fløyel T, **Wegeberg AL**, Jakobsen PE, Ejskjaer N, Pociot F, Brock B, Størling J. Increased circulating levels of inflammatory factors is associated to presence of Diabetic symmetrical polyneuropathy in type 1 diabetes. Clinical Endocrinology 2020 (*in press*).

2. **Wegeberg AL**, Brock C, Ejskjaer N, Karmisholt JS, Jakobsen PE, Drewes AM, Brock B, Farmer AD. Gastrointestinal symptoms and cardiac vagal tone in type 1 diabetes correlates with gut transit times and motility index. Neurogastroenterology & Motility 2020 (*in press*).

3. **Wegeberg AL**, Hansen CS, Farmer AD, Karmisholt JS, Drewes AM, Jakobsen PE, Brock B, Brock C. Liraglutide accelerates colonic transit in people with type 1 diabetes and polyneuropathy: A randomised, double-blind, placebo-controlled trial. United European Gastroenterology Journal 2020.

4. **Wegeberg AL**, Meldgaard T, Hyldahl S, Jakobsen PE, Drewes AM, Brock B, Brock C. Quantities of comorbidities affects physical, but not mental health related quality of life in type 1 diabetes with confirmed polyneuropathy. World Journal Diabetes 2019.

5. **Wegeberg AL**, Brock C, Brock B, Farmer AD, Hobson AR, Semler JR, Scott SM. Regional gastrointestinal pH profile is altered in patients with type 1 diabetes and peripheral neuropathy. Neurogastroenterology & Motility 2018.

6. Farmer AD, **Wegeberg AL**, Brock B, Hobson AR, Mohammed AD, Scott, SM, Bruckner-Holt CE, Semler, JR, Hasler WL, Hellström, PM, Drewes AD, Brock C. Regional gastrointestinal contractility parameters using the wireless motility capsule: inter-observer reproducibility and influence of age, gender and study country. Alimentary Pharmacology & Therapeutics 2018.

LIST OF PAPERS

This thesis is based on the following papers

I) **Wegeberg AL**, Lunde ED, Riahi S, Ejskjaer N, Drewes AM, Brock B, Pop-Busui R, Brock C. Cardiac vagal tone as a novel screening tool to recognize asymptomatic cardiovascular autonomic neuropathy - Aspects of utility in type 1 diabetes (*Under preparation*)

II) **Wegeberg AL**, Okdahl T, Fløyel T, Brock C, Ejskjaer N, Riahi S, Pociot F, Størling J, Brock B. Circulating inflammatory markers are inversely associated with heart rate variability measures in type 1 diabetes. (*Submittet to Mediators of Inflammation May 2020*)

III) **Wegeberg AL**, Brock C, Ejskjaer N, Karmisholt JS, Jakobsen PE, Drewes AM, Brock B, Farmer AD. Gastrointestinal symptoms and cardiac vagal tone in type 1 diabetes correlates with gut transit times and motility index. Neurogastroenterology & Motility 2020 (*in press*)

IV) **Wegeberg AL**, Hansen CS, Farmer AD, Karmisholt JS, Drewes AM, Jakobsen PE, Brock B, Brock C. Liraglutide accelerates colonic transit in people with type 1 diabetes and polyneuropathy: A randomised, double-blind, placebo-controlled trial. United European Gastroenterology Journal 2020.

ABBREVIATIONS

CCL - C-C motif chemokine

CXCL - CXC motif chemokine

GLP-1 – Glucagon-like peptide-1

GM-CSF - granulocyte-macrophage colony-stimulating factor

HbA1c: haemoglobin A1c

HF: absolute power of the high-frequency band (0.15–0.4 Hz);

IFN - interferon

IL - interleukin

LVS – Linear vagal scale

LF: absolute power of the low-frequency band (0.04–0.15 Hz);

LF: HF: Ratio of LF-to-HF power.

RMSSD: root mean square of successive RR interval differences.

SDNN: standard deviation of normal-to-normal intervals;

SDANN: standard deviation of the average normal-to-normal intervals for each 5-minute segment the recording;

SDNNI: Mean of the standard deviation of all the normal-to-normal intervals for each 5-minute segment the recording;

TNF - tumour necrosis factor

VLF: absolute power of the very-low-frequency band (0.0033–0.04 Hz);

ENGLISH SUMMARY

Type 1 diabetes is an autoimmune metabolic disease characterized by insulinopaenia resulting in hyperglycaemia. It affects up to 50 million people worldwide and is accompanied by an array of debilitating complications. The most prevalent and burdensome are microvascular neuropathies, catalysed by diminished vascular supply and inadequate glycaemic control. These entities effect nerves throughout the body including peripheral nerves, sympathetic and parasympathetic branches of the autonomic system and nerves of the central nervous system.

This thesis focuses on aspects of diabetic autonomic neuropathy, which may develop early in the course of diabetes. However symptoms are pleomorphic or absent, and thus it is often unrecognized by patients and physicians. This PhD thesis aims to use existing methodological platform to assess diabetic autonomic neuropathies of the cardiovascular and gastrointestinal systems. The thesis is compiled of four original research papers, which investigates different aspects of autonomic neuropathy. The papers are based on data from I) a cross-sectional study investigating methods for assessment of autonomic neuropathy and II) a placebo-controlled trial investigating the neuroprotective effects of 26-weeks liraglutide treatment in adults with type 1 diabetes and severe polyneuropathy.

Paper I examined the utility of assessed cardiac vagal tone as an alternative, clinically applicable screening method to recognize cardiovascular autonomic neuropathy based on cardiovascular reflex testing performed with the handheld, portable, commercially available Vagus™, and compared its performance with heart rate variability parameters based on 24- and 120-hour electrocardiographically recordings and sudomotor function assessed with the commercially available SUDOSCAN®. Based on the results, we suggest a cardiac vagal tone cut-off value, clinically applicable in recognizing established and borderline cardiac autonomic neuropathy.

Paper II investigated the association between inflammatory markers and alterations in the neuro-cardiac regulation, as the pathogenesis of diabetic autonomic neuropathy is pleomorphic and includes an array of metabolic, inflammatory and immune-mediated factors. This novelty paper discovered that both pro- and anti-inflammatory cytokines as well as a marker of epithelial

dysfunction was associated with changes in heart rate variability but not cardiac vagal tone.

Paper III investigated the influence of autonomic (dys)function on gastrointestinal motility and symptoms, as autonomic neuropathies impact on the enteric nervous system leading to gastro-enteropathy, is an under-investigated aspect. The study concluded that pan-enteric changes are present and associated with symptoms of the gastrointestinal tract and presence of cardiac vagal tone.

Paper IV examined the effects of liraglutide treatment on gastrointestinal motility and symptoms in adults with peripheral and autonomic neuropathy. Liraglutide treatment has previously been associated with prolonged gastric emptying and severe gastrointestinal symptoms, such as nausea and vomiting. We showed that gastric stasis and nausea symptoms were transient. Instead, liraglutide improved large bowel function and symptoms, possibly through enhanced function of the enteric nervous system.

The knowledge obtained in this thesis contributes to an increased insight into the underlying mechanisms in diabetic autonomic neuropathy. By use of existing methodological platforms, we provide complementary information on clinically applicable tests for assessing cardiovascular autonomic neuropathy and diabetic gastro-enteropathy. We provide further evidence regarding the interaction between systemic low-grade inflammation and presence of cardiovascular autonomic neuropathy and suggest a beneficial role of the GLP-1 agonist liraglutide on colonic motility and function. Despite some limitations, the proposed methods may provide an alternative to existing methods, and may prospectively recognize diabetic autonomic neuropathy earlier, which ultimately lead to diminish mortality, improve individual's quality of life and reduce medical cost associated with type 1 diabetes.

DANSK RESUME

Type 1 diabetes er en kronisk autoimmun sygdom karakteriseret ved manglende produktion af insulin, hvilket resulterer i vedvarende forhøjede blodsukker niveauer. Op mod 50 millioner mennesker over hele verden har type 1 diabetes, hvilket kan ledsages af en række invaliderende komplikationer. Den mest udbredte og belastende komplikation er neuropati (nervebetændelse), hvis katalysatorer er nedsat blodforsyning og utilstrækkelig kontrol af blodsukkerniveauerne. Neuropati kan påvirke alle kroppens nerver inklusiv perifere nerver, sympatiske og parasympatiske grene af det autonome system og nerver i det centrale nervesystem.

Denne afhandling fokuserer på forskellige aspekter af diabetisk neuropati i det autonome nervesystem. Diabetisk autonom neuropati kan udvikle sig tidligt i diabetes forløbet, men da symptomerne ofte er alsidige eller helt fraværende, opdages det sent af både patienter og læger. Formålet med denne afhandling er at undersøge hvordan autonom neuropati påvirker hjerte-kar-systemet og mave-tarm-kanalen, ved brug af eksisterende metoder. Afhandlingen består af fire originale forskningsartikler, der undersøger forskellige aspekter af autonom neuropati. Artiklerne er baseret på data fra to studier: I) et tværsnits studie, der undersøger metoder til vurdering af autonom neuropati i voksne med type 1 diabetes; II) et placebokontrolleret forsøg, der undersøger den beskyttende effekt af 26 ugers liraglutid behandling mod forandringer i nervefunktionen hos voksne med type 1 diabetes og perifer neuropati.

I artikel I blev anvendeligheden af kardiel vagus tonus som et alternativt, klinisk screenings mål til at undersøge autonom neuropati i hjerte-kar systemet undersøgt. Undersøgelsen blev baseret på hjerte-refleksundersøgelser udført med den håndholdte, kommercielt tilgængelige Vagus™ og sammenlignet hjertefrekvensvariationer baseret på 24- og 120-timers elektrokardiografisk optagelse samt svedfunktion vurderet med den kommercielt tilgængelige SUDOSCAN®. Baseret på resultaterne, foreslår vi en grænseværdi for kardiel vagus tonus, der er klinisk anvendelig til undersøgelse af autonom neuropati.

I artikel II blev sammenhængen mellem inflammatoriske markører og ændringer i reguleringen mellem nervesystemet og hjertet, da diabetisk autonom neuropati's sygdomsproces inkluderer en række metaboliske, inflammatoriske og immunmedierende faktorer undersøgt. Resultatet blev at både pro- og antiinflammatoriske markører såvel som en markør for dysfunktionelt epitel var

forbundet med ændringer i hjertefrekvensvariabilitet, men ikke kardiel vagus tonus.

I artikel III blev indflydelsen af autonom dysfunktion på mave-tarm-kanalens bevægelighed og symptomer herfra undersøgt. Man ved at autonom neuropati kan involvere mave-tarm-kanalens nervesystem (det enteriske nervesystem), hvilket fører til neuropati symptomer fra maven og tarmene. Dette er dog ikke undersøgt tilstrækkeligt tidligere. I undersøgelserne fandt vi at forandringerne er til stede i hele mave-tarm-kanalens forløb og er forbundet med symptomer fra mave-tarm-systemet og nedsat kardiel vagus tonus.

I artikel IV blev effekten af liraglutid behandling på mave-tarm-kanalens bevægelighed og symptomer hos voksne med perifer og autonom neuropati undersøgt. Behandling med liraglutide har tidligere vist sig at give forlænget tømning af mavesækken og alvorlige symptomer fra mave-tarm-kanalen, såsom kvalme og opkast. Vi viste, at stase i maven og kvalme symptomer var kortvarige. I stedet forbedrede liraglutid tyktarmens funktion og de derfra kommende symptomer, muligvis ved at forbedre funktionen af det enteriske nervesystem.

Denne afhandling bidrager med øget viden om de underliggende mekanismer i diabetisk autonom neuropati. Ved hjælp af eksisterende metodologiske platforme, leverer vi komplementære oplysninger om klinisk anvendelige tests til vurdering af autonom neuropati i hjerte-kar-systemet og mave-tarm-kanalen. Vi leverer bevis for interaktionen mellem systemisk betændelse og tilstedeværelse af autonom neuropati i hjertet og vores studier finder tegn på at liraglutid har en gavnlig effekt på tyktarmens funktion. På trods af begrænsninger kan de foreslåede metoder eventuelt være et alternativ til eksisterende undersøgelsesmetoder og give mulighed for tidligere diagnosticering af diabetisk autonom neuropati. Dette kan i sidste ende føre til nedsat dødelighed, forbedring af livskvalitet og reduktion i medicinske omkostninger forbundet med type 1-diabetes.

ACKNOWLEDGEMENTS

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CHAPTER 1. BACKGROUND

1.1. TYPE 1 DIABETES MELLITUS

Diabetes mellitus constitutes a heterogeneous group of metabolic diseases characterised by hyperglycaemia, of which type 1 diabetes accounts for 5-10% [1]. Worldwide, up to 50 million people have type 1 diabetes, with increased prevalence in Scandinavian countries [2] and current trends being indicative of continuous increases which may be attributed to improved earlier diagnosis, improved management and survival rates [3].

Type 1 diabetes is cellular-mediated autoimmune destruction of insulin producing pancreatic β-cells, resulting in chronic insulinopaenia. It typically presents during childhood and classical onset is accompanied by polyuria, polydipsia, polyphagia or ketoacidosis, though this is more variable in adult onset. Diagnosis is based on either oral glucose tolerance test, increased fasting plasma glucose concentration or the glycated haemoglobin A1C criteria (≥6.5% or 48 mmol/mol)[1,4]. Current treatment focuses on maintaining an euglycemic state using insulin therapy, while avoiding severe hypoglycaemia, hyperglycaemia and ketoacidosis [1]. Future treatment prospects focus on pancreas transplantation or the "artificial pancreas" system, an integrated dual hormone (insulin and glucagon) closed-loop system incorporating pumps and continuous glucose monitors [4].

Despite advances in care, type 1 diabetes is still accompanied by a marked physical, psychological and financial burden [4]. People with diabetes are known to have a diminution in quality of life attributed to frequent self-monitoring and anxiety of acute glycaemic events, long-term complications and morbidity [5]. Although the economic burden of diabetes may vary across world regions, both the direct (i.e. medication, hospitalization, and complication treatments) and indirect (i.e. absenteeism, presenteeism) cost of type 1 diabetes are in general increased compared to type 2 diabetes [6]. This increased cost is largely attributed to longer disease durations, higher medicine cost and elevated medical cost associated with the increased risk of comorbidities [7].

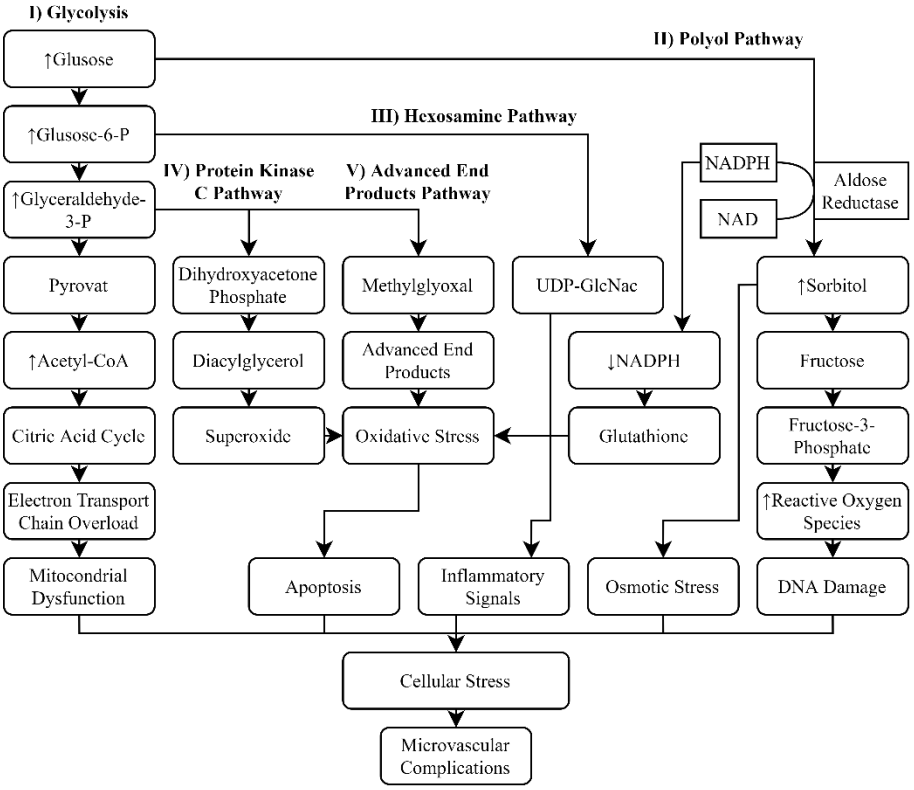

Figure 1.1: The series of metabolic events that result in microvascular complications. *Through different pathways (I-V), increased glycose levels associated with diabetes wreak havoc in the human neural cells. The cytotoxic effects results in mitochondrial dysfunction, osmotic and oxidative stress, DNA damage, pro-inflammatory gene expression and apoptosis, resulting in microvascular complication of the eyes, kidneys and nerves. AGE – advanced glycation end products; DNA - deoxyribonucleic acid; NAD - nicotinamide adenine dinucleotide; NADPH - nicotinamide adenine dinucleotide phosphate; ROS – reactive oxygen species.*

1.2. COMPLICATIONS OF TYPE 1 DIABETES

Complications of type 1 diabetes can be subdivided into macrovascular and microvascular complications. Macrovascular complications encompass the process of atherosclerosis leading to cardiovascular diseases like coronary heart disease, cerebrovascular disease and peripheral vascular disease, which are non-specific to diabetes. Conversely, microvascular complications encom-

passes diabetes specific nephropathy, retinopathy and neuropathy [8]. A common denominator of the cells of the renal glomeruli, retina, and nerves, are their shared inability to downregulate glucose uptake in the presence of elevated extracellular glucose levels. Though the exact mechanisms are incompletely understood, evidence suggests that long-standing or severe hyperglycaemia activates a plethora of destructive metabolic and structural alterations [9,10], outlined in Figure 1.1. Thus microvascular complications can be prevented, to a degree, by intensive glycaemic treatment [11].

The most common microvascular complication is neuropathy, which affects up to 50% of people with type 1 diabetes [12,13]. Neuropathy may manifest in various forms and display considerable heterogeneity in their symptoms, cause, pathological alterations and underlying mechanisms, seen in Figure 1.2. The most commonly encountered and studied forms are distal symmetrical polyneuropathy and autonomic neuropathy [14], of which the latter will be the focus in this thesis. Distal symmetrical polyneuropathy accounts for the majority of recognized neuropathies [15]. It is a length-dependent sensorimotor neuropathy, that can involve both small-fibre and large-fibre nerve dysfunction but initially affects longer nerves [10,15]. Classically it presents as stocking and glove distribution [13,16]. Loss of protective sensation is prominent and thus, this form of neuropathy is the primary cause of foot ulceration and in the worst case scenario can necessitate amputation [8].

1.3. DIABETIC AUTONOMIC NEUROPATHY

Diabetic autonomic neuropathy is defined by the Toronto consensus as "*a disorder of the autonomic nervous system in the setting of diabetes after the exclusion of other causes*" [15]. It involves both sympathetic and parasympathetic branches of every autonomic system in the body, including the cardiovascular and gastrointestinal systems, presenting with site-specific dysfunction and symptoms [14]. The primarily neural substrate of the parasympathetic nervous system is the vagus nerve, which innervates the majority of the visceral organs [17,18]. As the longest cranial nerve in the human body, the vagus nerve is particularly vulnerable long-term or severe hyperglycaemia [18]. Thus, early manifestation of autonomic neuropathy tends to be expressed as parasympathetic denervation in vagal innervated organs [19]. The effects of this are highly visible after surgical vagotomy (previously used as a treatment of peptic ulceration)

underlining the important influence of the vagus nerve. However, these symptoms are often silent or diverse in nature, and autonomic neuropathy often goes unnoticed by the patient and physician [20]. Autonomic dysfunction was classically thought to develop over time, especially after long-standing periods of hypo- or hyperglycaemia, however resent evidence suggest that autonomic alteration can be present as early as the time of diagnosis in some cases of type 1 diabetes [15,18]. This is troublesome and problematic as autonomic neuropathy is associated with increased morbidity and mortality [8].

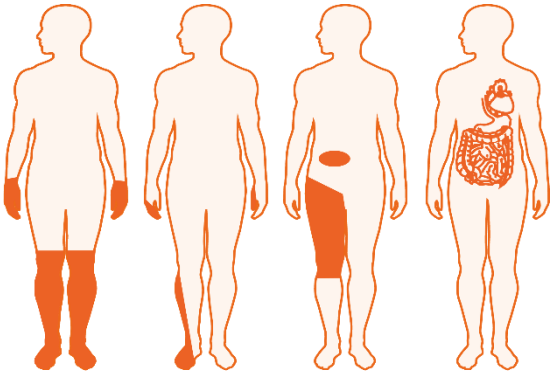

Figure 1.2: Schematic diagram of neuropathy types. *From left to right: Distal symmetrical polyneuropathy, mononeuropathy, radiculopathy, and autonomic neuropathy.*

1.3.1. CARDIOVASCULAR AUTONOMIC NEUROPATHY

Cardiovascular autonomic neuropathy is the most frequently studied and severe autonomic neuropathy [14], defined by the Toronto consensus as "*the impairment of autonomic control of the cardiovascular system*" [15]. The pathogenesis is a complex interaction of disrupted coordination of heart control influenced by alterations of sympathetic and parasympathetic input, coupled with hyperglycaemia induced metabolic, oxidative and inflammatory processes [21,22] (Figure 1.1). Vagal denervation is expressed as tachycardia, changes in circadian blood pressure, increased contractility and peripheral vasoconstriction, all contribution to increased myocardial stress [19], and present with symptoms of light-headedness, weakness, palpitations, faintness, and ultimately syncope [14,23]. However, early stages can be completely asymptomatic (silent), and as routine testing is not recommended until five years after diabetes diagnosis [22,24], it is plausible that a large number of unrecognised cases are present. Timely diagnosis can be difficult and preventive initiatives are interim retained, while mortality increases [14,25]. Up to 7% of newly diagnosed

people with type 1 diabetes have measureable cardiovascular autonomic neuropathy and the prevalence increases yearly with 2% hereafter [18].

1.3.2. INFLUENCE OF INFLAMMATION ON NEUROPATHY

The inflammatory reflex is a highly regulated interaction between the immune and nervous system. It responds to inflammatory or infectious stimuli by activating the brain or vagal afferents through cytokines release, which in turn induces a cholinergic anti-inflammatory response, deactivating macrophages, and thus, effectively inhibiting the synthesis of pro-inflammatory cytokines [17,26,27]. The autonomic system, especially the vagus nerve, is a central health-preserving component in this neuro-immuno-regulatory reflex, providing a fast and subconscious anti-inflammatory response [17,26]. Additionally, inflammation induced neurotoxicity is known to be part of the underlying pathogenesis of peripheral neuropathy, a pathomechanism undoubtedly also at play in the autonomic system [28–30]. Thus, a link between the inflammatory reflex and autonomic dysfunction, like cardiovascular autonomic neuropathy, is a plausible concept. It has been supported by studies demonstrating that endotoxin-induced inflammation reduces heart rate variability, while increased parasympathetic function is known to decrease production of inflammatory cytokines [27,31]. In type 1 diabetes, the relationship between heart rate variability and inflammation is not found in newly diagnosed, but more likely arises as a long-term dysfunction [32,33 34,35].

1.3.3. GASTROINTESTINAL ENTEROPATHY

Homeostasis of the enteric nervous system, an independent network within the autonomic nervous system, is governed by three interconnected pan-enteric networks; I) intrinsic neurons, influencing motility, secretion and absorption, II) interstitial cells of Cajal, facilitating slow-wave peristaltic movement of the gut walls, and III) enteric glial cells, mediating interactions between neurons and intestinal cells [36]. Normal gastrointestinal motility is initiated by physiological stimulation of smooth muscle cells by ingested nutrient, which activate neuro-hormonal reflexes and neurotransmitter release, which regulates and coordinates the gut-brain-axis in a bidirectional manner. Disruption of these networks, either locally by reduced number and structural changes of intrinsic neurons or centrally by altered brain-gut communication, can result in gastrointestinal neuropathy. Classically, this has be thought of as multiple neuropathies, defined by their accompanying disabling symptoms e.g. nausea,

bloating, diarrhoea and constipation, rather than the underlying motility disturbances [37,38]. However, recent evidence, backed by anatomical function, suggest that pan-enteric dysfunction represents one coherent disease [39].

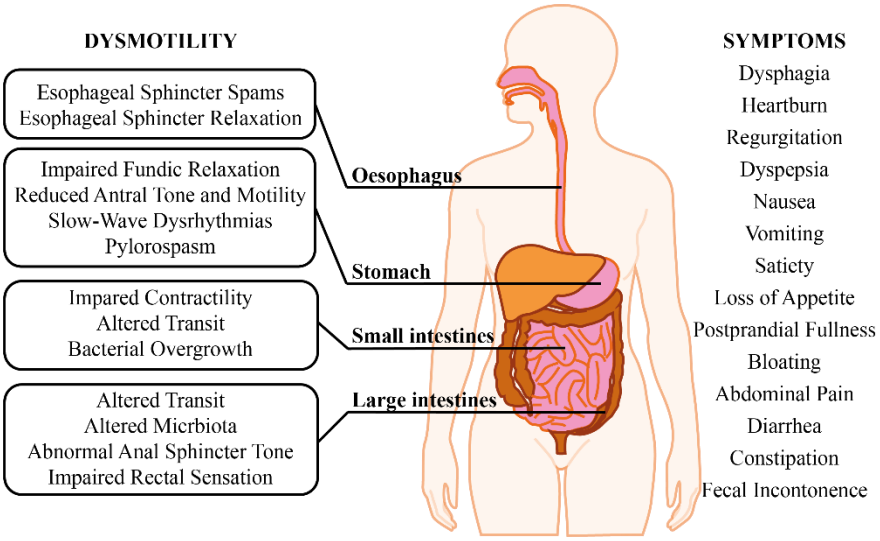

Figure 1.3: Regional dysmotility and symptoms of the gastrointestinal tract.

Both gastrointestinal dysfunction, and the resulting symptoms, can be regarded as an outcome of a dyscoordination and dysregulation within the gut-brain axis. The majority of the gastrointestinal tract is innervated by the vagus nerve, and autonomic neuropathy may manifest as impaired coordination within the enteric nervous systems [40]. Consequences are disruption of this delicately balanced regulation, resulting in altered wall tone, sphincter function, secretion, dysmotility, all contributing to interrupted digestion and plausibly symptom generation. However, classical patient-reported gastrointestinal symptoms e.g. nausea, abdominal pain, bloating, postprandial fullness, diarrhoea and constipation, are often unspecific and inaccurately categorized in terms related to the anatomical origin [41,42]. This may be connected with evident localised hyposensitivity, as well as pronounced alterations in specific visceral processing areas the brain, including insula and cingula cortex have been revealed to be involved in gastrointestinal function and especially symptom generation and maintenance [43–45]. Dysmotility and perceived symptoms may in worst case affect nutritional and pharmacological efficacy [46]. Effectively this

manifest as gastroparesis which retards movement of chyme, diminishes motility and hinders absorption in the intestines, which may be complicated by symptoms such as nausea and vomiting. Such symptoms have the potential to impact on calorific intake which can complicate insulin administration.

1.4. LIRAGLUTIDE

Glucagon-like peptide 1 (GLP-1) is an incretin hormone, homologous to glucagon, which is released from pancreatic α-cells and intestinal enteroendocrine L-cells, in response to ingestion of nutrients [47]. Among its effects are glucose-dependent enhanced insulin, and reduced glucagon, secretion resulting in stabilisation of glycaemia, slowing of gastric emptying and increased satiety, which consistently have shown to lead to weight loss [47,48]. The first GLP-1 receptor agonist was approved in 2005 by the FDA for regulation of dysglycaemia in type 2 diabetes and many analogues, which mimic the native GLP-1 function, have been synthesised and marketed since then. Liraglutide is a long-acting synthetic human GLP-1 analogue attached to a carbon fatty acid molecule, which effectively increases its half-life compared to the natural hormone. Compared to short-acting agonist, which leaves intermittent periods without measurable drug concentrations, liraglutide serum concentrations are permanently elevated, leading to continuous stimulation of receptor, and therefore it triggers tachyphylaxis of the gastric response leading to adverse effects [49]. The most frequents adverse effects, and a major withdrawal reason of participants within clinical trials investigating liraglutide and GLP-1 agonist, are gastrointestinal side effects of which nausea and vomiting are the most common [47–49]. These are not thought to originate directly from alterations in gastrointestinal secretomotor function, but rather from the central nervous system as both pancreatic and extra-pancreatic effects of GLP-1 and agonist are believed to be mediated via the vagus nerve. In type 1 diabetes, where insulin is depleted and provide exogenously, it is especially these extra-pancreatic effects that are of interest in liraglutide's regulation of glycaemic control [47].

1.5. ASSESSING AUTONOMIC NEUORPATHY

Direct assessment of the autonomic system is challenging due to the invasiveness of testing, and thus only indirect, or proxy, methods are currently used. Since no universal assessment of autonomic neuropathy exist, current assessment is end organ effect based, most commonly though the cardiovascular and gastrointestinal systems.

1.5.1. CARDIOVASCULAR AUTONOMIC TESTS

Cardiovascular autonomic reflex tests has been defined as the gold standard for diagnosis of cardiovascular autonomic neuropathy by the Toronto consensus[15] and supported by learned neurological societies [50]. The tests were first suggested by Ewing in the 1970's [51] and the recommended test paradigm consists of heart rate response to deep breathing, Valsalva manoeuvre, and postural change, as well as blood pressure response to orthostatic changes and previously sustained handgrip [14,15,52]. Based on the Toronto consensus[15] cardiovascular autonomic neuropathy are categorically divided into early (one abnormal test), confirmed (two or more abnormal test) or severe (orthostatic hypotension), based on the numbers and types of abnormal cardiovascular autonomic reflex tests [15]. Different version of these tests have been developed, with a recent addition being the handheld Vagus™, able to perform the three recommended heart rate test procedure [53,54].

Apart from cardiovascular autonomic reflex test, the only other clinically suggested method is heart rate variability, which, using power spectral analysis time- and frequency-domains parameters can be derived. These are, when applied properly, the earliest way to detect asymptomatic cardiovascular autonomic neuropathy [14,17,21,55]. Depending on the computed domains, heart rate variability provides insight to both sympathetic and parasympathetic neural activity, and long-term recording allow investigation of the influence of normal daily activities and routines. For easy clinical applicability short-term derived heart rate variability measures have been proposed, however, they are less sensitive and reproducible than long-term recording for autonomic neuropathy purposes [55].

Table 1.1: Overview of diagnostic test of cardiovascular autonomic neuropathy.

Author	Cohort	CAN (%)	Reference method	Test method	Sn (%)	Sp (%)
Cardiovascular autonomic reflex tests				Clinical use – 30 minutes		
Bellavere 2019[56]	334 T2	NR	OH >15mmHg	DB	66.7	65.4
				PC	70.4	72.6
				VM	84.6	48.2
				VM+PC	42.0	82.8
				DB+PC	33.1	88.1
				VM+DB	28.4	89.5
Lai 2019[57]	238 T2	37%	CASS	DB	80.2	72.6
				VM	72.3	64.5
				OH	63.4	62.9
Körei 2017[58]	64 T1 195 T2	37%	Toronto	SHG	24.6	79.4
Razanskaite-Virbickiene 2017[59]	349 T1	60%	Ewing	DB	97.3	96.2
				Suspire	94.3	91.5
				Standing	96.2	93.0
Pafili 2015[60]	152 T2	48%	Toronto	DB	19	98
				PC	96	65
				VM	62	92
				DB+PC	81	62
				DB+VM	69	79
				PC+VM	62	70
Vinik 2003[18]	3516DM 205 HV	NR	>5th PCTL of age cut-offs	DB	93	93
				VM	98	91
				PC	93	93
Heart rate variability (HRV) (long-term)				Clinical use – 1-5 days		
Lin 2017[61]	7 T1 83 T2 20 HC	44%	Ewing	HRV	72	55
				HRT	75	65
				HRT+SDNN	98	63
Heart rate variability (HRV) (short-term)				Research use – 10-20 minutes		
Bhati 2019[62]	42 T2	64%	Ewing	Rest mean NN	81.4	60
				Rest SDNN	77.7	60
				Rest RMSSD	70.3	66.6
				Rest pNN50	92.5	73.2
				Rest TP	92.5	60
				Rest LF	85.1	73.3
				P-E mean NN	96.3	66.6
				P-E SDNN	92.5	40
				P-E RMSSD	88.8	66.6
				P-E pNN50	100	86.6
				P-E TP	100	66.6
				P-E LF	100	73.3
Jelinek 2017[63]	140 HC	49%	Ewing	HRV	100	29.5
Chen 2015[64]	56 DM	61%	Ewing	HRV	83.7	83.7
Tang 2014[65]	446 DM	49%-53%	Ewing	HRV (uniform)	85.0	85.2
				HRV (age)	85.4	84.7
Howorka 1998[66]	107 T1 11 T2	58%	Ewing	Frequency HRV	82	89

Continued

Cardiac vagal tone (CVT)				**Research use – 5 minutes**		
Wegeberg 2020 (Paper I)	56 T1	32%	Toronto (- OH)	CVT (bCAN) CVT (eCAN)	67 88	87 63
Ambulatory blood pressure measurement (ABPM)				Research use – 24 hours		
Spallone 2009[67]	84 T1 80 T2	32%	Toronto (- OH)	20 mmHg 30 mmHg	50.0 30.8	94.6 98.2
Spallone 2007[68]	87 T1	57%	Toronto	ΔBP(sys) 0% ΔBP(sys) 5% ΔBP(sys) 10% ΔBP(dia) 0% ΔBP(dia) 5% ΔBP(dia) 10%	26.0 42.0 70.0 14.0 26.0 40.0	95.0 86.0 51.0 95.0 92.0 78.0
QT-interval				Research use – 1 minute		
Pappachan 2008[69]	42 T1	60%	Ewing	QTi > 440ms	77.0	76.5
Whitsel 2000[70]	4115 T1 469 T2	26%	Toronto	QTc >441	28.0	86.0
Sudoscan®				Research use – 5 minutes		
D'Amato 2019[71]	44 T1 81 T2	41%	Toronto	ESC (CAN) ESC (eCAN)	78 83	64 57
Yuan 2018[72]	9 T1 94 T2	NR	Toronto	CANRS (ESC, age, BMI)	90	30
He 2017[73]	75 T2 45 HC	40%	Toronto	ESC (feet) ESC (hands)	80.0 76.7	60.0 75.6
Selvarajah [74]2015	45 T1 25 HV	44%	Toronto	ESC (feet) ESC (hands) CAN-RS	60.0 45.0 65.0	76.0 96.0 80.0
Yajnik 2013[75]	232 T2	NR	Toronto (- VM)	ESC risk score	92	49
Neuropad®				Research use 10-20 minutes		
Mendivil 2015[76]	154 T2	68%	Toronto	CAN DB PC VM	70.1 77.9 67.1 75.7	37.0 40.5 29.1 34.5
Spallone 2009[77]	51 DM	22%	Toronto	10 min 15 min 18 min	82.0 82.0 73.0	27.0 52.5 75.0
Bilen 2007[78]	105 T2	38%	QTi >0.440	Neuropad	88	43
Liatis 2007[79]	117 DM	38%	Toronto	CAN sCAN	59.1 80.9	46.5 50.0
Corneal Confocal Microscopy				Research use – 2 minutes		
Tavakoli 2015[80]	34 DM	59%	CASS >2	Fibre density Branch density Fibre length	86.0 100 86.0	78.0 56.0 78.0

Continued

COMPASS 31				**Research use – 15 minutes**		
D'Amato 2019[71]	44 T1 81 T2	41%	Toronto	CAN eCAN	58 75	56 56
Greco 2016[81]	73 DM	36%	Toronto	bCAN sCAN	75.0 70.0	64.9 66.7
Singh 2011[82]	60 T2		Ewing	COMPASS-31 Orthostatic Gastrointestinal	77.8 77.8 77.8	71.1 57.4 60.0
Survey of Autonomic Symptoms (SAS)				Research use – 15 minutes		
Zilliox [83]	30 HC 62 DM		Symptom Profile	Symptoms score Total impact	95.0 80.0	50.0 50.0
Clinical characteristics				Research use		
Riguetto 2019[84]	222 T1	35%	Toronto	CAN formula	90	82
Xue 2017[85]	455 DM	29%	Toronto	CAN formula	80.6	63.7
Ge 2014[86]	2092 HC	19%	Toronto	CAN formula	72.9	67.5
Neumann 1995[87]	38 T1 94 T2	51%	Ewing	≥2 symptoms	93	83
Peripheral measurements				Research use		
Pafili 2020[88]	152 T2	NR	Toronto	NC-stat Monofilament VPT Ipswich touch test NDS small NDS large NDS	50 46 62 39 89 65 54	76 69 75 85 73 41 70
Lai 2019[57]	238 T2	37%	CASS	Sural nerve SNAP	71.3	69.4

bCAN: borderline cardiovascular autonomic neuropathy, BP: blood pressure, CAN: cardiovascular autonomic neuropathy, CASS: composite autonomic severity score, DB: deep breathing (E:I), dia: diastolic, DM: diabetes mellitus (unknown T1/T2 ratio), eCAN: established cardiovascular autonomic neuropathy, ESC: electrochemical skin conductance, HRT: heart rate turbulence, NDS: neuropathy disability score, OH: orthostatic hypotension (blood pressure response to standing, P-E: post exercise, PC: postural change (lying to standing or 30:15), QTi: QT interval, sCAN: severe cardiovascular autonomic neuropathy, SHG: sustained handgrip, sys: systolic, T1: type 1 diabetes, T2: type 2 diabetes, VM: Valsalva manoeuvre (Valsalva ratio), VPT: vibration perception threshold. Ewing – DB, PC, VM, SHG, OH. Toronto – DB, PC, VM, OH.

A novel alternative to heart rate variability is cardiac vagal tone. Based on a 5-minute resting electrocardiogram, this measure is a validated cardio-metrically derived index of parasympathetic efferent tone. Alterations in heart rate is rapidly adjusted though collaboration of baroreceptor stretching, parasympathetic vagal activation and sinoatrial depolarisation, in reflex of brainstem efferent modulation of the heart. At rest this relationship resembles linearity and thus is quantified as cardiac vagal tone on a linear vagal scale (LVS), where zero represents atropinisation [89,90]. An important advantage of cardiac

vagal tone is that is has been validated for time epochs of less than 5 minutes and does not require the subject to follow complicated instructions or active participation to perform. Normal data for this measure exist and it is known to be decreased in people with type 1 diabetes and has been shown to be reproducible over time [89,91].

Due to the prevalent and increased risk of mortality associated with cardiovascular autonomic neuropathy, implementation of technically uncomplicated methods for early clinical detection is pivotal [21,92]. A comprehensive overview of different diagnostic tests sensitivity and specificity validated for recognizing cardiovascular autonomic reflex test (primarily) can be found in Table 1.1. Although many different approaches have been proposed, the majority lacks the prerequisite receiver operator characteristics required for routine clinical use.

1.5.2. GASTROINTESTINAL TESTS

The gastrointestinal tract is a complex organ that is relatively difficult to assess readily. Different approaches have been applied to indirectly measure the vagal influence on the gastrointestinal tract including proxies such as serum pancreatic polypeptide response to sham feeding or cardiac measures like heart rate variability [93–95]. However, in most cases, direct evaluation of the gastrointestinal tract is preferred, primarily by evaluation of regional transit times, with gastric emptying the most applied. Several different methods have been applied to asses either transit or motility in people with diabetes and autonomic neuropathy. To get an overview over the different methods used to investigate autonomic neuropathy in people with type 1 diabetes, a PubMed search of Mesh terms “Diabetes Mellitus, Type 1”, “Gastrointestinal Transit”, “Gastrointestinal Motility” and “Diabetic Neuropathies” was performed and yielded a result of 90 articles. Of these, 17 were non-original articles (the majority of reviews concerned the stomach and gastroparesis), three were animal studies, four were not in type 1 diabetes, and ten concerned the oesophagus or gallbladder, eight did not concern intestinal transit or motility (e.g. gastric volume), while six only depicted peripheral neuropathy. The rest are presented categorized by type of method in Table 1.2. From these it is clear that gastric emptying has received the most attention. Gastric emptying can be measured using nuclear medicine scintigraphy, which is currently considered the gold standard [96]. Delayed gastric emptying is a defined feature of gastroparesis.

However, many of the current and historical techniques to objectively quantifying gastrointestinal transit and motility have marked disadvantages, including invasiveness, exposure to radiation or just plainly being expensive and difficult to interpret, seen Table 1.3. Thus, in recent years new methods have been developed to overcome these obstacles.

Though several different methods have been applied to assess either transit or motility, only one method has so far been able to combine region gastrointestinal evaluation of combined transit time and motility measures into one investigation – the wireless motility capsule. The wireless motility capsule is a single use, ingestible capsule, which, when ingested, concomitantly measures temperature, pH and pressure as it transverses the gastrointestinal tract.- Based on stereotypical patterns of the aforementioned measures, transit and motility measures for segmental (i.e. gastric, small bowl and large bowel) and the whole gut can be derived [42], see Figure 1.4. Ingested together with a standardized meal and following the natural intestinal movement, the wireless motility is a safe, minimally invasive, standardized, ambulatory measure.

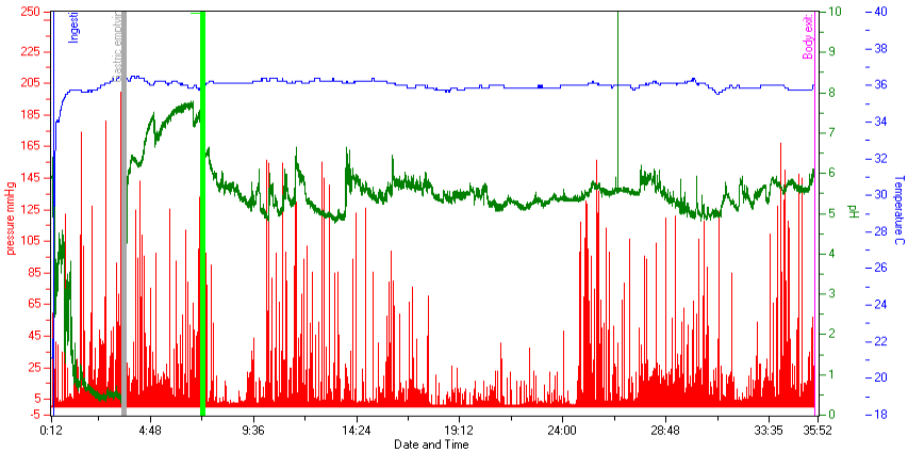

Figure 1.4: Wireless motility capsule trace from an adult male with type 1 diabetes. *On the x-axis is time in hour: minutes. On the red y-axis is pressure (mmHg), with pressure patterns noted in red. On the green y-axis is pH (pH units), with pH data point noted in green. On the blue x-axis is temperature (^{o}C), with temperature points noted in blue. The blue vertical line is ingestion of the capsule, the grey vertical line passage across the gastric pyloric sphincter, the light green vertical line passage across the ileocecal sphincter and the pink vertical line expulsion of the capsule.*

Table 1.2: Transit, motility and autonomic neuropathy studies in type 1 diabetes

Author (year)	Aim	Neuro pathy	Results
Scintigraphy			
Parkman (2019)[97]	Determine prevalence of complications in pt with Gp symptoms and altered GE	DAN 52% PN 45%	Delayed GE associated with number of complications. Gp occur without complications.
Punkkinen (2008)[98]	Examine relationship between upper abdominal symptoms to DAN and GE	CAN 62%	GE and symptoms were not correlated. 1 increased CAN score, lowered GE was 20%.
Vazeous [99] (2004) (Add: carmine red)	Define etiological role of DAN and motility impairment with GI symptoms.	CAN UK	CAN is not a factor of GI symptoms. Hyperglycaemia does not affect GI motility.
Stacher (2003)[100]	Evaluate underlying factors of GE and intergastric meal distribution.	CAN 62% PN 56%	Delayed GE and increased retention of meal are mainly attributed to CAN
Kockar (2002)[101]	Determine simple screening parameters to diagnose Gp	CAN UK	CAN testing (QTi) may be useful in screening for Gp
De Luis (2001)[102]	Elucidate effect Hp eradication on gastric motor function and glycaemic control	DAN 15% PN 46%	DAN and PN did not alter GE. Hp eradicate delayed GE.
Huszno (2001)*[103]	Assess concordance of CAN to GE	CAN 74%	CAN has no influence on GE
Stacher (1999)[104]	Determine effects cisapride on GE and glycaemic control.	CAN 57%	Cisapride had no effect on GE or glycaemic control. GE was slower with DAN.
Loba (1997)[105]	Evaluate association of GE and PP secretion and effect of CAN	CAN 50%	Impaired PP secretion was found with delayed GE and CAN.
Merio (1997)[106]	Elucidate relationship between GE and CAN or glycaemic control.	CAN 47%	CAN, but not glucose levels affected GE
Okano (1996)[107]	Evaluate effects of EM523L (motilin agonist) GE and plasma concentration of PP	DAN 63%	EM523L delayed GE and augmented postprandial PP secretion.
Rosa-e-Silva (1996)[108]	Evaluate relations between GI transit, DAN and occurrence of diarrhoea.	DAN 35%	DAN accelerated small bowel transit, possibly affecting diarrhoea.
Fraser (1990) [109]	Evaluate GE relation to glycaemic control	DAN100 %	Glycaemic alterations affect GE
Janssens (1990)[110]	Examine effect of erythromycin on GE.	CAN 90% PN 80%	Erythromycin improved GE
Keshaverzian (1987)*[111]	Evaluate relationship between Gp, symptoms, glycaemic control and DAN.	DAN 33%	Delayed GE is common with DAN though often asymptomatic.
Keshavazian (1987) [112]	Evaluate frequency and extent of GI involvement in symptoms	DAN 33% PN 100%	Symptoms were more severe and extensive with GI involvement and DAN
Horowitz (1985)[113]	Evaluate effects of domperidone on GE, symptoms and glycaemic control	DAN100 %	Domperidone increased GE, decreased symptoms and lowered glycose levels

Continued

Ultrasound			
Darwiche (2001)[114]	Evaluate relationship between GE and CAN	CAN 50% PN 80%	GE is lower in pt with CAN, unrelated to symptoms Gp
Undeland (1998)[115]	Study meal accommodation and relationship to vagal tone	DAN UK	Low vagal tone was associated with a small proximal stomach and a wide antrum.
Vaisman (1998)[116]	Study prevalence of asymptomatic delayed GE.	DAN UK	Pt suffer from prolonged GE despite lack of symptoms
Melga (1997)[117]	Elucidate the effects of levosulpiride on GE and glycaemic control	DAN100%	Levosulpiride shortened GE and improved glycaemic control.
Weck (1997)*[118]	Investigate relation between GE and mesenteric blood flow in pt with or without CAN	CAN 53%	Pt with CAN showed delayed gastric emptying and diminished mesenteric blood flow
Vogelberg (1986)*[119]	Study antral contraction in pt with or without DAN	DAN 50%	Antral contractions were reduced even without DAN.
Manometry			
Rosztóczy (2004)[120]	Establish prevalence of GI symptoms, and relation to GI motor abnormalities	CAN 63%	GI symptoms and motility disorders are frequent and the latter underestimated.
Samsom (1996)[121]	Examine relationship between gastric motility, CAN, glycaemic control and symptom	CAN100%	Motor abnormalities are common. Abnormal motility patterns is related to the composition meal.
Jebbink (1993)[122]	Examine prevalence of gastric and small intestinal motility abnormalities and relation to symptoms and CAN	CAN100%	CAN is associated with motor abnormalities, which correlate with symptoms.
Wehrmann (1991)[123]	Examine influence of cisapride on antroduodenal motility.	DAN100% PN 27%	Cisapride increases antroduodenal motility.
Camilleri (1984) [124]	Investigate pan-enteric motor abnormalities in Gp	36% DAN 64% PN	Gastric and small intestine is frequently affected with Gp
Breath test			
Chang (2013)[125]	Prognosis and mortality of Gp after 25 years	DAN UK	Delayed GE is not associated with mortality
Zahn (2003)[126] (Add: scintigraphy)	Compare breath test to gold standard scintigraphy for GE	CAN 29% PN 46%	Breath test is a valid, sensitive and easy alternative to scintigraphy.
Braden (2002)[127]	Investigate influence of cisapride treatment on GE in Gp, symptoms and glycaemic control	DAN100% PN 100%	Cisapride accelerate GE, reduces dyspeptic symptoms, but has no effect on glycaemic control
Keshavarzian (1986)*[128]	Assess relations between GI transit and complications	DAN 28% PN 64%	Abnormal GI transit is common, but not prerequisite of diarrhoea.

Continued

Electrogastrography (EGG)			
Toporowska-Kowalska (2006)[129]	Evaluate gastric myoelectrical activity in relation glycaemic control.	CAN 2%	Glucose levels influence gastric myoelectrical activity, without influence of CAN
Kawagishi (1997) [130]	Investigate glycaemic control and DAN on gastric motility.	DAN 72%	Gastric myoelectric activity is associated with DAN.
Jebbink (1994)[131]	Examine prevalence gastric myoelectrical abnormalities and relation to CAN and symptoms.	CAN100%	Gastric myoelectrical activity is nor disturbed under euglycaemia except when symptomatic.
Jebbink (1994)[132]	Prevalence of gastric myoelectric abnormalities, and effect of glycaemic control	CAN100%	Hyperglycaemia is a factor in the generation of gastric myoelectrical disturbances.
Radio-opaque markers			
Lehman (2003)[133] (Add: scintigraphy)	Effect of accelerated GE (Cisapride) on glucose-control in Gp	CAN UK PN 100%	Accelerated GE had no effect on glycaemic control or hypoglycaemic episodes in Gp
Kawagishi (1993)*[134]	Investigate motilins role in Gp.	DAN100%	GE is delayed despite elevated motilin levels.
Werth (1992) [135] (Add: Breath test)	Evaluate GE, orocecal and colonic transit time with or without CAN	CAN 42%	Colonic transit time was decreased with CAN. Other parameter not affected.
MRI			
Lehmann (1996)[133] (Add: ROM)	Validate MRI technique in pt with and without CAN against ROM	CAN 53% PN 60%	MRI for GE has high specificity but lower sensitivity compared to ROM.
Paracetamol absorption			
Lydon (2000)[136]	Association between GE rate and DAN	DAN 69%	DAN is not predictive of delayed GE in Gp

UK – autonomic testing performed but prevalence not noted. * - based on abstract alone
Add: additional method used. CAN – cardiac autonomic neuropathy, DAN – diabetic autonomic neuropathy, GI – gastrointestinal, PN – peripheral (sensory) neuropathy, Pt – patients (T1DM), Gp – gastroparesis, GE – gastric emptying, Hp – Helicobacter pylori, PP – pancreatic polypeptide

Table 1.3: Advantages and disadvantages of gastrointestinal investigation methods

Methods	Measures				Advantages/Disadvantages						
	Transit times	Contractility	Motor patterns	pH	Non or minimally invasive	Standardised	Ambulatory	Time efficient	Inexpensive	No radiation exposure	Easy data interpretation
Scintigraphy	✓	×	×	×	✓	×	×	×	×	×	×
Ultrasound	✓	×	×	×	✓	×	×	×	×	✓	×
Manometry	×	✓	✓	×		×	×	×	×	✓	×
Breath test	✓	×	×	×	✓	×	×	✓	✓	✓	✓
Electrogastrography	✓	✓	×	×	✓	×	×	×	×	✓	×
Radio-opaque markers	✓	✓	×	×	✓	✓	✓	✓	✓	×	✓
3D- transit	✓	✓	✓	×	✓	×	✓	×	×	✓	×
Wireless motility capsule	✓	✓	×	✓	✓	✓	✓	✓	×	✓	✓

CHAPTER 2. RATIONALE AND AIMS

The overall aim of this PhD thesis was to use existing methodological platforms to assess diabetic autonomic neuropathies of the cardiovascular and gastrointestinal systems. We investigated: I) a novel method for recognizing cardiovascular autonomic neuropathy; II) the association between the presence of cardiovascular autonomic neuropathy and systemic levels of inflammatory markers; III) enteropathy in the gastrointestinal systems in terms of symptoms and regional motility; IV) the clinical effect of liraglutide treatment on gastrointestinal motility and symptoms.

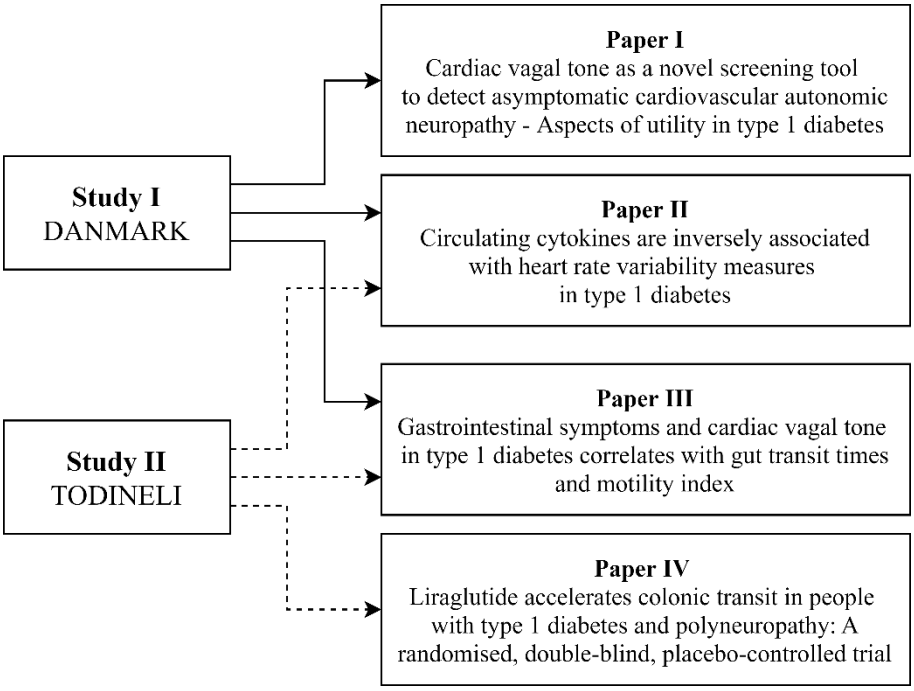

Figure 2.1: Overview of studies and papers.

The thesis is based on two peer-reviewed original papers, a paper submitted to a peer-review journal and a paper under preparation. The four papers report data from two studies. Study I is a cross-sectional study, which prospectively assessed the autonomic function in adults with type 1 diabetes, by

investigating cardiac response, cytokine profile and the gastrointestinal motility. Study II is a randomized, double-blinded, parallel-group, placebo-controlled trial, investigated the neuroprotective effect of liraglutide for treatment of diabetic distal symmetrical polyneuropathy in adults with type 1 diabetes. An overview of studies and papers are illustrated in Figure 2.1.

In detail, the rationale and hypothesis for each paper is as follows:

Paper I: Cardiovascular autonomic neuropathy is associated with increased mortality but is often underappreciated as it is frequently asymptomatic. Currently available testing is time-consuming and is not suitable for widespread screening in a standard clinical setting.

Thus, we hypothesized that a short cardiac vagal tone measure could serve as a clinical applicable screening method for cardiovascular autonomic neuropathy in adults with type 1 diabetes.

Paper II: Adequate vagal function is essential in the anti-inflammatory reflex, providing a fast and subconscious anti-inflammatory response by inhibiting pro-inflammatory pathways. Additionally, neurotoxicity, neuro-inflammation and inflammation *per se* have been suggested as contributing factors in the pathogenesis of neuropathy.

Thus, we hypothesised that increased low-grade systemic inflammatory would be associated with altered neuro-cardiac function in adults with type 1 diabetes.

Paper III: Gastro-enteropathy is pleomorphic in its symptoms and due to the relative inaccessibility of portions of the gastrointestinal tract, transit times and patient reported outcome measures have hitherto been part of the standard evaluation. However, these may represent an oversimplification of a complex system, calling for in depth investigations of contractility and connection to autonomic neuropathy.

Thus, we hypothesised that patient-reported gastrointestinal symptoms in diabetes are correlated with changes in gastrointestinal motility, and that neuronal impairment is involved in the severity of gastrointestinal dysfunction and symptoms.

Paper IV: Though liraglutide's effect on upper gastrointestinal motility and symptoms are known to induce tachyphylaxis of gastric emptying, and transient nausea, the pan-enteric effects are less studied.

Thus, we hypothesised that liraglutide would exert an effect on all gut segments and promote gastrointestinal symptoms in people with type 1 diabetes and distal symmetrical polyneuropathy (and concomitant autonomic neuropathy).

Based on the hypothesis above, the aims of this thesis are as follows:

I. To test the clinical applicability of cardiac vagal tone in terms of sensitivity and specificity for recognize cardiovascular autonomic neuropathy based on cardiovascular reflex test. Furthermore, to test the performance of cardiac vagal tone compare with heart rate variability and sudomotor function.

II. To investigate a broad profile of inflammatory cytokines, adhesion molecules and chemokines and their association to cardiovascular autonomic function assessed with heart rate variability and cardiac vagal tone.

III. To investigate if gastrointestinal symptoms are correlated with transit times and motility index and if altered cardiac vagal tone and the presence of established distal symmetrical polyneuropathy influences segmental transit times, motility index and patient-reported symptoms in a cohort of people with type 1 diabetes.

IV. To investigate whether liraglutide administration induced changes in regional gastrointestinal transit time and motility, self-reported gastrointestinal symptoms, and how changes in these measures correlate.

CHAPTER 3. MATERIALS AND METHODS

Data for this thesis was based on two studies conducted at Mech-Sense, Department of Gastroenterology and Hepatology at Aalborg University Hospital in cooperation with Department of Endocrinology and Department of Cardiology. Both studies were approved by The North Jutland Denmark Region Committee on Health Research Ethics (Study I: N-20170045; Study II: N-20130077). Study II was furthermore approved by the Danish Medicines Agency with information about the study available on trial registration websites (EUDRACT: 2013-004375-12; clinicaltrials.gov: NCT02138045). The studies conform to the Declaration of Helsinki and International Conference on Harmonization Good Clinical Practice guidelines. All participants gave their written informed consent prior to entry into the studies.

3.1. STUDY I

Study I was a prospective cross-sectional study were a total of 56 adults with type 1 diabetes were included. All participants underwent evaluation of:

I. Cardiovascular system in terms of cardiovascular autonomic reflex test performed with Vagus^{TM}, cardiac vagal tone, heart rate variability and sudomotor function.
II. Inflammatory profile evaluated by pro- and anti-inflammatory cytokines, chemokines, and adhesion molecules from serum samples.
III. Gastrointestinal system in terms of regional transit times and motility assessed with the wireless motility capsule and evaluation of patient reported gastrointestinal symptoms.
IV. Regional gastrointestinal transit times and motility indices and evaluation of gastrointestinal symptom before and after treatment

3.2. STUDY II

Study II was a randomised placebo-controlled trial including a total of 48 adults with type 1 diabetes with concomitant distal symmetrical polyneuropathy evaluated by classical neurophysiological testing. Hereof 9 participants discontinued treatment before 26 weeks due to gastrointestinal symptoms. Participants were evaluated at baseline and after 26 weeks of treatment with either liraglutide or placebo. Secondary analysis on data from both baseline

and after 26 weeks are included in this thesis. All participants underwent evaluation of:

I. Regional gastrointestinal transit times and motility indices and evaluation of gastrointestinal symptom before and after treatment

3.3. ASSESSMENT OF NEURO-CARDIAC FUNCTION

3.3.1. CARDIOVASCULAR AUTONOMIC REFLEX TESTS

Cardiovascular autonomic reflex heart rate test of deep breathing, Valsalva manoeuvre and postural changes were performed using the Vagus™ (Medicus Engineering ApS, Aarhus, Denmark), preceded by a resting period. The Vagus™ device has a good reproducibility, strong correlation and high diagnostic agreement against stationary equipment [54,137]. A cardiovascular autonomic neuropathy score for each individual was calculated if two or more tests were satisfactory performed. Based on normal range of specific age-dependent cut-off values [52], three cardiovascular autonomic neuropathy categories were defined as follows: I) *established CAN* was defined as two or more abnormal tests, II) *borderline CAN* by one abnormal test and III) *no CAN* by none abnormal test.

3.3.2. CARDIAC VAGAL TONE

Cardiac vagal tone was based on a 5-minute, 3-lead electrocardiography using eMotion Faros 180 (Bittium, Oulu, Finland), following a resting period. Cardiac vagal tone was computed using the automated online app ProBioMetrics (version 1.0, ProBioMetrics, Kent, UK). From these data, recording artefacts, defined as changes exceeding 15 beats per minute in two succeeding QRS complexes indicative of coughing or movements, were removed by standardized processes. Files where the editing procedure exceeded 20% of the recording were discarded.

3.3.3. HEART RATE VARIBILITY

Heart rate variability index the fluctuations between subsequent heartbeats, indicative neuro-cardiac function, and can be quantified by validated time and frequency domains [138]. Using recommendations from the Task Force of the European Society of Cardiology and the North American Society of Pacing

and Electrophysiology [55], the following parameters were computed: heart rate, SDNN, SDANN, SDNNI, RMSSD, VLF, LF, HF, and LF:HF.

Study I: Heart rate variability was based on a 24- or 120-hour electrocardiographic recording using ePatch® (BioTelemetry Technology, Hørsholm, Denmark). Heart rate variability time and frequency domains were computed using the automatic editing software the CardiScope™ (HASIBA Medical GmbH, Graz, Austria).

Study II: Heart rate variability was based on a 24-hour electrocardiographic recording Lifecard CF Holter monitor (Del Mar Reynolds, Spacelabs Healthcare, Snoqualmie, WA, USA). Heart rate variability time and frequency domains were computed using the Pathfinder software (revision B code; Spacelabs Healthcare).

3.3.4. SUDOMOTOR FUNCTION

Sudomotor function was based on the 3-minute measurement of electrochemical skin conductance using SUDOSCAN® (Impeto Medical, Paris, France). The machine measures the electrochemical reaction between the chloride ions in the sweat glands and the steel electrodes [139], and provides a electrochemical skin conductance readout for all four extremities.

3.3.5. NEUROPHYSIOLOGICAL EVALUATION

Distal symmetrical polyneuropathy was evaluated as part of Study II using standardized nerve testing, including conduction velocity, amplitudes, and F-waves of the median, ulnar, sural, radial, tibia and peroneal nerves. All recordings were evaluated by a neurophysiological specialist, and a composite score of these was used to assess severity of distal symmetrical polyneuropathy [140].

3.4. ASSESSMENT OF INFLAMMATORY LEVELS

Concentrations of systemic inflammatory marker were evaluated from a cubital vein blood sample. Inflammation 20-Plex Human ProcartaPlex™ Panel (Thermo Fischer Scientific, MA, USA) was used to analyse duplicate concentrations of cytokines (interleukin (IL)-1α, IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-17A, interferon (IFN)-α, IFN-γ, tumour necrosis factor (TNF)-α and granulocyte-macrophage colony-stimulating factor (GM-CSF)), adhesion molecules (E-selectin, P-selectin and intracellular CAM (ICAM)-1)

and chemokines (C-C motif chemokine (CCL)2, CCL3, CCL4 and CXC motif chemokine(CXCL)10).

3.5. ASSESSMENT OF GASTROINTESTINAL FUNCTION

3.5.1. WIRELESS MOTILITY CAPSULE

Regional gastrointestinal motility was investigated in both studies by use of the non-invasive, ambulatory wireless motility capsule system (SmartPill®, Medtronic, Minneapolis, USA), consisting of a single-use capsule, a portable data receiver, a docking station, and a computer containing the software program MotiliGI. The capsule measures temperature (range: 25-49^{o}C, accuracy: 1^{o}C), pH (range: 0.5- 9.0 pH units, accuracy: +/- 0.5 pH units) and pressure (range: 0-350mmHg, accuracy: 5mmHg below or 10mHg above 100 mmHg). The capsule was ingested following an overnight fast together with a standardized meal consisting of a known nutritional content (SmartBar®, Medtronic, Minneapolis, USA, 260-kcal, composed of 18% protein, 16% carbohydrates, 3% fat, and 8% fibre) and 200mL of water. Subsequently, food and water were restricted for 6 hours, as well as strenuous physical activity. A patient diary was used to record bowel movement, meal-times, sleeping and gastrointestinal symptoms for the duration of the test e.g. until the capsule was expelled.

Analysis was based on temperature rise and fall, plus rapidly occurring pH changes at anatomical landmarks, a methods described in details by Sarosiek *et al.* [141]. Data from the wireless motility capsule provides parameters describing regional transit times and motility indices. While transit times are self-explanatory, motility index is a composite measure incorporating both contraction frequency and amplitude, which was calculated as suggested by Camilleri et al. [124]. Data was supported by a vast normative material aiding analysis [142,143]. Though critics pinpoint that the solid capsule is unable to empty from the stomach until migrating motor complexes commence in response to fasting state, a number of studies have validated the system against standard measures of from scintigraphy and radio-labelled markers in healthy [144–147].

3.5.2. SELF-ASSESSED GASTROINTESTINAL SYMPTOMS

In both studies, two questionnaires were consistently used to characterize upper and lower gastrointestinal symptoms: the Gastroparesis Cardinal Symptom Index (GCSI) and Gastrointestinal Symptom Rating Scale (GSRS).

The 9-item GCSI is a gastroparesis focused sub-questionnaire of the Patient Assessment of Upper Gastrointestinal Symptom Severity Index. It was rated on a 6-point-Likert response scale ranging from 0 (none) to 5 (very severe). Three subscales can be calculated evaluating the severity of nausea/vomiting, post-prandial fullness/early satiety, and bloating [148]. In Study I the GCSI daily diary version of the questionnaire was used while the original version of GCSI was used in Study II. In the original version symptoms are recalled for the last 14 days. In contrast, the Daily Diary version, established by the American Neurogastroenterology and Motility Society, was developed to reduce the risk of recall bias, by encouraging participants to complete the GCSI every day over a period of 14 days [149].

The 15-item GSRS questionnaires was rated on a 7-point-Likert scale ranging from 1 (no discomfort) to 7 (very severe discomfort). Five sub-scales can be calculated thereof, evaluating the severity of abdominal pain, reflux syndrome, diarrhoea syndrome, indigestion syndrome, and constipation syndrome. GSRS was evaluated in all participants, however, in Study II GSRS was only answered after intervention [150].

CHAPTER 4. KEY RESULTS

4.1. AIM I

To test the clinical applicability of cardiac vagal tone in terms of sensitivity and specificity for recognize cardiovascular autonomic neuropathy based on cardiovascular reflex test. Furthermore, to test the performance of cardiac vagal tone compare with heart rate variability and sudomotor function.

Key results:

- The prevalence of cardiac autonomic neuropathy was 32% (16 of 48 participants)
- The cardiac vagal tone cut-off value of 3.2 LVS for recognition of established CAN, showed 67% sensitivity and 87% specificity, at an AUC of 0.80 (p=0.01), and performed worse than heart rate variability, but better than electrochemical skin conductance.
- A suggested cardiac vagal tone cut-off value of 5.2 LVS for recognition of borderline CAN, indicated 88% sensitivity and 63% specificity, at an AUC of 0.72 (p=0.07) and performed better than heart rate variability and electrochemical skin conductance.

Interpretation: Implementation of cardiac vagal tone using a clinically applicable cut-off value may meet the unmet need for cardiovascular autonomic neuropathy screening tests. Using this quick screening toll, screening may more regularly and easily be performed in clinical settings, decreasing numbers of asymptomatic, undiagnosed cardiovascular autonomic reflex testing cases and initiate early prevention initiatives prospect increasing quality of life and survival time.

4.2. AIM II

To investigate a broad profile of inflammatory cytokines, adhesion molecules and chemokines and their association to cardiovascular autonomic function assessed with heart rate variability and cardiac vagal tone.

Key results:

- Both pro-inflammatory (TNF-α, IL-1α) and anti-inflammatory (IL-4 and IL-12p70) cytokines inversely associated with sympathetic and parasympathetic heart rate variability measures.
- E-selectin suggestive of epithelial dysfunction inversely associated with heart rate variability measures.
- None of the cytokines, chemokines and adhesion molecules were associated with cardio-vagal dysfunction assessed with cardiac vagal tone

Interpretation: A greater emphasis should be placed on the complex immunoregulatory system in type 1 diabetes, as inflammatory mediators involved in nerve- and epithelial damage may contribute to the pathogenesis of cardiovascular autonomic neuropathy.

4.3. AIM III

To investigate if gastrointestinal symptoms were correlated with transit times and motility index and if altered cardiac vagal tone and the presence of established distal symmetrical polyneuropathy influences segmental transit times, motility index and patient-reported symptoms in a cohort of people with type 1 diabetes.

Key results:

- Colonic transit time and motility index positively correlated with both classically upper and lower gastrointestinal symptoms
- Presence of cardio-vagal dysfunction increased colonic transit time and gastric motility index
- Both presence of cardio-vagal dysfunction and polyneuropathy was associated to decreased perception of abdominal pain (hyposensivity).

Interpretation: An increased focus should be placed on whole gut motility investigations as gastrointestinal dysmotility is not confined to the stomach in adults with type 1 diabetes. Neural impairment is involved in gastrointestinal symptoms and dysmotility. An apparent hyposensitivity appears to play a role.

4.4. AIM IV

To investigate whether liraglutide administration induced changes in regional gastrointestinal transit time and motility, self-reported gastrointestinal symptoms, and how changes in these measures correlate.

Key results:

- 26 weeks of liraglutide treatment shortened the colonic transit time with 32% and decreased motility index with 6% in adults with type 1 diabetes.
- 26 weeks of liraglutide treatment increased the sensation of postprandial fullness with 29%, while nausea subsided.

Interpretation: liraglutide increases gastrointestinal motility, inducing persistent satiety, but transient nausea and gastric stasis

CHAPTER 5. DISCUSSION

The overall aim of this thesis was to use existing methodological platforms to assess diabetic autonomic neuropathies of the cardiovascular system with the influence of inflammatory markers, as well as the enteropathy of the gastrointestinal tract and the effect of liraglutide therein. This discussion is divided into three parts focusing on investigation of cardiovascular system, investigation of the gastrointestinal tract and methodological considerations.

5.1. INVESTIGATING THE CARDIOVASCULAR SYSTEM

5.1.1. CARDIOVASCULAR AUTONOMIC NEUROPATHY

A large body of evidence exist applying different methods for investigating cardiovascular autonomic neuropathy (see Table 1.1). Most generally accepted, but also time-consuming, are the performance of cardiovascular autonomic reflex tests. Though this paradigm has been accepted by the Toronto consensus[15] as the gold standard, other consortiums still apply different standards, and consequently no universal consensus has been reached within the field [21]. One of the obvious challenges with different methods suggested over the years, is the lack of diagnostic agreement between the test results [56]. Hence, an abnormal result from one test may appear normal using another. This discrepancy may lie in the investigation of different nerves and functions of autonomic system. Thus, when it comes to clarification of which test is the most reliable in diagnosing cardiovascular autonomic neuropathy, large prospective longitudinal trials are needed, where the diagnosis and prognosis of cardiovascular autonomic neuropathy is investigated with complementary methods.

Another hindrance is, that the recommendations for testing for autonomic neuropathy five years after diagnosis and every year thereafter in people with type 1 diabetes, are rarely carried out, as it is both time and resource demanding. Physicians are generally hesitant to test for autonomic neuropathy, possibly because they find it time-consuming, but also in an attempt not to inflict unnecessary worry in the patients, as the general idea is that nothing can be done from a treatment perspective. Consequently, little attention has hitherto been paid to silent occurrence of autonomic neuropathy, or neuropathy in general, until clinical symptoms are evident and intervention initiatives are less

efficacious. However, while no cure is currently available, results from large clinical outcome studies (i.a. Diabetes Control and Complication Trial & Epidemiology of Diabetes Interventions and Complications) have showed, that intensified glycaemic control, early in the disease, can significantly reduce prevalence and severity of neuropathy later in life [11]. Consequently, treatment guidelines were changed in the 1990's to ensure stable hyperglycaemic medication, e.g. that patients as a minimum had received the given treatment: long acting and fast acting insulin or recently, insulin pump with dosing adjustments according to regimens. However, clinical experience show that some patients have unforeseen challenges in keeping with the recommended tight glycaemic target, resulting in glycaemic fluctuations and hypoglycaemic events not measurable with haemoglobin A1c (HbA1c). HbA1c have long been the standard indicator of long-term (8-12 weeks) blood glucose control, however, it does not account for glycaemic variability and hypoglycaemia. Contrary, continuous glucose monitoring provides comprehensive information about glycaemic variability including time in hypo- and hyperglycaemia, linked to daily events e.g. eating, sleeping and exercising [151]. While inadequate glycaemic control may be sequel to already established neuropathy and enteropathy, emerging evidence suggest that glycaemic variability may play a bigger role in cardiac autonomic neuropathy, and possibly other neuropathies due to hypoglycaemia, than previously thought [37]. Arguably, until we decide to focus even more on these difficult patients, we cannot provide evidence of a delay in progression, however implementation of regular screening for the entity will enable monitoring of the progression.

We proposed a relatively inexpensive, easily applicable 5-minute measure, the cardiac vagal tone, as a screening tool for cardiovascular autonomic neuropathy. We established a cut-off value that balance sensitivity (ability to correctly identify people with cardiovascular autonomic neuropathy) and specificity (ability to correctly identify people without cardiovascular autonomic neuropathy)[152] to recognize established or borderline cardiovascular autonomic neuropathy (see Figure 5.1). We found that cardiac vagal tone performs good based on the more time-consuming gold standard of cardiovascular autonomic reflex test performed with Vagus™. Specifically, we found that cardiac vagal tone had a good discriminatory power. While cardiac vagal tone could correctly detect 87% of those without established cardiovascular autonomic neuropathy, it detected 88% of those with borderline cardiovascular autonomic neuropathy, at low cut-points, making it just as (or more) accurate

than most of the test presented in Table 1.1. Thus we confirmed our hypothesis that, a short cardiac vagal tone measure could serve as a clinical applicable screening method for recognition of degrees of cardiovascular autonomic neuropathy in adults with type 1 diabetes. However, it should still be noted that the majority of those patients who tested positive for cardiovascular autonomic neuropathy, had cardiac vagal tone values within the normative 95% confidence interval [153]. Consequently, cardiac vagal tone does not provide a "sharp" cut-off between normal and abnormal. The overall accuracy of the test was depicted using a receiver operating characteristics curve, and furthermore, compared with heart rate variability and sudomotor function. In this comparison, the performance of cardiac vagal tone was superior and thus showed increased accuracy against heart rate variability and sudomotor function in most instances, though heart rate variability exerted in recognizing established cardiovascular autonomic neuropathy.

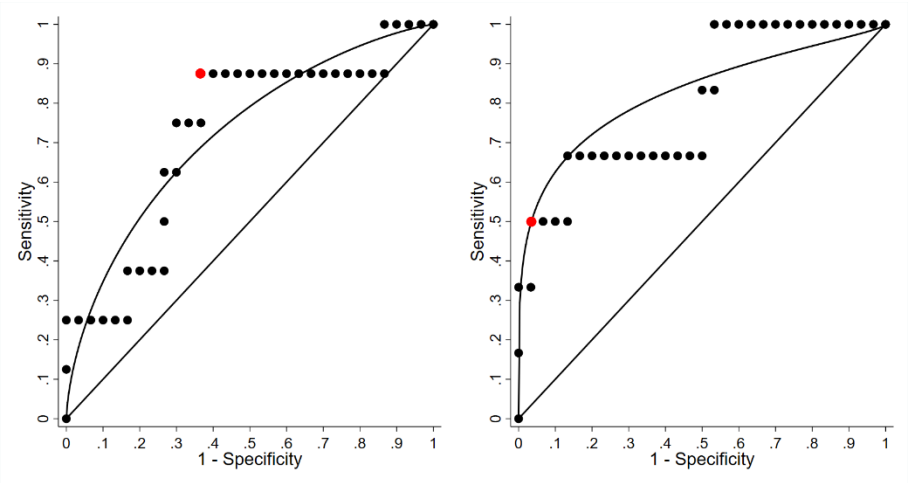

Figure 5.1: Cardiac vagal tone ROC curves for borderline and established cardiovascular autonomic neuropathy*. Graphs show parametric (line) and non-parametric (dots) receiver operator curves for cardiac vagal tone recognizing borderline (left) and established (right) cardiovascular autonomic neuropathy. The red dot marks the estimated non-parametric optimal cut-pint (Youden's index), which was 5.2 (AUC 0.7) and 3.2 LVS (AUC 0.8), respectively.*

Heart rate variability has been used for years for assessing cardiovascular autonomic regulation, and low variability is an acknowledged predictor of mortality [154]. In clinical practice, heart rate variability provides additional

prognostic information to the cardiovascular autonomic reflex testing, though the two methods are notorious for disagreeing when applied as diagnostic tools. Additionally, heart rate variability recordings are subject to noise and artefacts, a trade-off for the increased recording time and influence of normal daily activities and routines. Thus, noise and artefacts could be a course of bias if not correctly filtered out. We used an automated software to avoid subjectivity bias from manual editing. However, multiple software programs exist, dealing with ectopic beats and artefacts by different methods and with little to no standardization these could produce difference result [154]. Our own experiences with different analysis programs (unpublished data), applying more or less autonomous editing, highlighted the fact that, especially noise traces, showed the biggest differences in the results. Thus, this should be carried forward when interpreting heart rate variability data, especially between research centres.

Sudomotor function have been suggested by experienced researches as a possible screening tool for autonomic neuropathy as it is a quick (3-minute test time) and easy test of the sympathetic regulated sudomotor function [74]. However, a recent review suggested that many studies investigating sudomotor function tested with SUDOSCAN® were at high risk of bias due to the involvement of the device manufacture (Impeto Medical), as they supplied parts of the data sets [155]. Though moderate sensitivity (45-92%) and specificity (30-69%) have been found for detecting cardiovascular autonomic neuropathy in previous studies (see Table 1.1), we found that it underperformed compared to cardiac vagal tone and heart rate variability. This discrepancy may be due to the difference in measured nerves between the methods. The electrochemical skin conductance of SUDOSCAN® allegedly evaluates sympathetic unmyelinated thin-type C nerve fibres, as it has a higher association with test reflective of sympathetic function like orthostatic hypotension and low frequency power components of heart rate variability than those reflective of parasympathetic function [75,156]. Thus, we would not suggest this measure to diagnose cardiac autonomic neuropathy in future studies, despite the easy uncomplicated applicability.

Thirty-two percent (16 participants) of our cohort had cardiovascular autonomic neuropathy to some degree and though this is comparable with the proportion in other studies, the cohort is relatively small and therefore, data should be interpreted cautiously. Additionally, the exclusion of participants with severe cardiovascular problem may have curbed the true proportion of

participants with cardiovascular autonomic neuropathy as these are closely related. If included, these would possibly have provided more participants in the established cardiovascular autonomic neuropathy group, increasing the prognostic value of cardiac vagal tone for recognizing cardiovascular autonomic neuropathy.

However, implementing new bedside methods for investigation of cardiovascular autonomic neuropathy does not automatically improve the clinical health of people with diabetes. Consequently, a number of expert believe that assessment of sensitivity and specificity should only be applied in the search for screening methods, as they fear introduction of these methods may lead to over-investigation, over-diagnosis and over-treatment [157,158]. Nevertheless, the complexity and cost associated with performance of cardiovascular autonomic reflex test and heart rate variability in the clinical setting, as well as the under-recognition of cardiovascular autonomic neuropathy, provides an incentive to develop and explore inexpensive, simple screening tools. These could ideally aid healthcare professionals with screening of a larger proportions of adults (or adolescents) with diabetes, ensuring that the right people are referred for more extensive and time-consuming testing. Additionally, introduction of new validated methods could provide a better understanding of the pathophysiology and acts as a much-needed endpoint in prospective, longitudinal clinical trials testing the efficacy of novel treatment approaches.

5.1.2. INFLAMMATION

Though inflammatory contributions to traditional neuropathies widely has been neglected, accumulating evidence suggest that hyperglycaemia induced neurotoxicity is associated to systemic levels of pro-inflammatory pathways [28–30]. Indeed, neuropoietic cytokines are involved in phagocytosis and demyelination of neurons, fostering nerve damage. Similarly, they may also activate the vagal branch of the inflammatory reflex as pro-inflammatory cytokines have been linked to cardiovascular autonomic neuropathy in long-term type 1 diabetes [34,35]. However, the exploration of this field have been limited by the definite markers investigated, including acute C-reactive protein, interleukin (IL)-6 and tumour necrosis factor (TNF)-α [32,33].

Using a multiplex immunoassay, we were able to quantify multiple inflammation markers simultaneously, providing a larger picture of the ongoing processes at hand. However, the multiplex assays are not without limitations,

which was seen as seven of the twenty investigated inflammatory biomarkers had detection rates below limit and therefore to mitigate increased rate of type 1 error, we lowered the inferred p-value for statistical significance. In the future, a wider evaluation may be beneficial in the search for identifying viable biomarkers, which ultimately could aid the identification of underlying pathomechanism, disease progression and targeted treatment strategies [159].

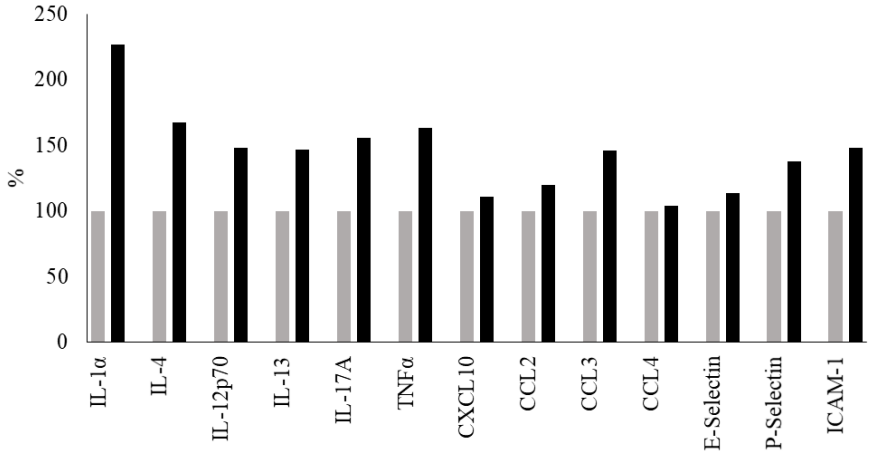

Figure 5.2: Percentage change of inflammatory markers in participants with or without cardiovascular autonomic neuropathy. *For each inflammatory marker, the percentage change from participants without cardiovascular autonomic neuropathy (Grey standardized 100% lines) to participants with cardiovascular autonomic neuropathy (Black lines) are shown. Overall participants with cardiovascular autonomic neuropathy had increased serum concentrations of inflammatory markers.*

The systemic inflammatory mechanism, which affects both peripheral neuropathy and autonomic neuropathy are likely similar, however, the autonomic compartment is less investigated. We found that both pro- and anti-inflammatory cytokines as well as E-selectin (a marker of epithelial dysfunction) were associated with heart rate variability measures. The finding of both pro- and anti-inflammatory cytokines suggest compensating neuro-immune mechanism are at play indicating normal active immunoregulation. Thus, we confirmed our hypothesis that increased low-grade systemic inflammatory would be associated with altered neuro-cardiac function in adults with type 1 diabetes. Interestingly, in a complementary study of the same cohort [160], we inves-

tigated the association between inflammatory markers and the presence of distal symmetrical polyneuropathy and found that IL-13, IL-17A and the chemokine CCL2 were additionally associated. Upregulation of CCL2 has furthermore been observed in directly injured peripheral nerves [161]. Additionally, using another multiplex assay (multiplex cytokine assay, Meso-Scale Discovery), IL-6 have previously been quantify in the Study II cohort, where pro-inflammatory and neuroregenerative cytokine were found to be reduced after 26 weeks of liraglutide. This suggest liraglutide have an anti-inflammatory effect by modulating the inflammatory reflex, possibly deactivating macrophages and inhibition of pro-inflammatory pathways. However, as none of other investigated markers (IL-8, Il-10, IFN-γ and TNF-α) were affected, the effect may purely have been an effect of weight loss as IL-6 sources are prevalent in adipose tissue [162].

Contrary to our hypothesis, it is worth noting that cardiac vagal tone was not associated with the levels of systemic inflammatory markers. Though the concrete reason is unknown, one could speculate that cardiac stressor such as changing workloads and circadian processes found in long-term heart rate variability parameters, could be lacking [55,138]. Additionally, heart rate variability directly measures differences in consecutive heart rates, while cardiac vagal tone applies a linear vagal scale for quantification of neuro-cardiac modulation and these may associate different with inflammatory markers. Though cardiac vagal tone had a good performance as a suggested screening tool for cardiovascular autonomic neuropathy, the lack of association with inflammatory markers either devaluates its strength in type 1 diabetes or provides us with additionally information on the interactions between inflammations, changes in heart rate and neuro-cardiac modulation.

5.2. INVESTIGATING THE GASTROINTESTINAL TRACT

5.2.1. GASTROINTESTINAL AUTONOMIC NEUROPATHY

The burdensome occurrence of gastrointestinal autonomic neuropathy is one of the least investigated neuropathies by the scientific community. This may be because of the relatively difficult accessibility of the gastrointestinal tract, or an underappreciation of the influence it has on quality of life. Even though, these symptoms are not associated with increased mortality, such as the presence of cardiovascular autonomic neuropathy, or exhibit of amputation fol-

lowing peripheral neuropathy, the daily experience of burdensome gastrointestinal troubles and symptoms in diabetes deserve more clinical attention. Enteropathy can induces troublesome symptoms like nausea, bloating, diarrhoea and constipations [163]. Though it seems contradictory, these symptoms are associated with an evident lack of sensation from the gut, so-called hyposensitivity, reflective of altered sensation and pain processing. These central neuropathic-like changes mimic the loss of protective sensation found in peripheral neuropathies [45,164].While gastroparesis causes delayed absorption of nutrients, that when dyscoordinated with insulin-treatment leads to hypoglycaemia [38], abnormal motility throughout the intestines can affect absorption and thus bioavailability of pharmaceutical. Recognition of these sequelae are especially needed when considering the role of digestion and absorption of nutrients have on achieved glycaemic response.

A relatively new method, the wireless motility capsule, has become popular due to its minimally invasive nature, safety and applicability in ambulatory settings. The analysis is relative effortless, applying an analysis "wizard" and comprehensive information making it applicable for physicians compared to other capsule system like the 3D-transit [165]. Additionally, large normative data materials exist on both transit, motility and pH, aiding analysis [142,143], though they also highlight the large variability of the measures, reflective of "normal" gastrointestinal physiology. As it provides more facets than other methods, measuring both transit, contractility, temperature and pH in a single investigation, the wireless motility capsules aides countless investigative possibilities, though sadly they are relatively unused. Primary focus has so far been on transit times, as this is an easily comprehended measure, however, transit time in itself may be a crude measure, as it is not explicitly linked to motility.

We found that gastrointestinal symptoms were correlated with prolonged gastric emptying and colonic transit time, while colonic motility was decreased in presence of symptoms. Additionally, motility and symptoms were altered in participants with low cardiac vagal tone. These finding support our hypothesis that patient-reported gastrointestinal symptoms in diabetes are correlated with changes in gastrointestinal motility, and that neuronal impairment is involved in the severity of gastrointestinal dysfunction and symptoms. We have previously shown that motility index is a particularly useful summery measures as it encompasses both contraction frequency and amplitudes [143] . In both paper III & IV, this was shown to associate to symptoms and increase with administration of liraglutide, suggesting an alteration in the contractile

element of digestion. One large limitation of the wireless motility capsule and it contractility measures is that it cannot detect propulsive or retrograde peristaltic movement, compared to methods like manometry or 3D-transit (see Table 1.3).

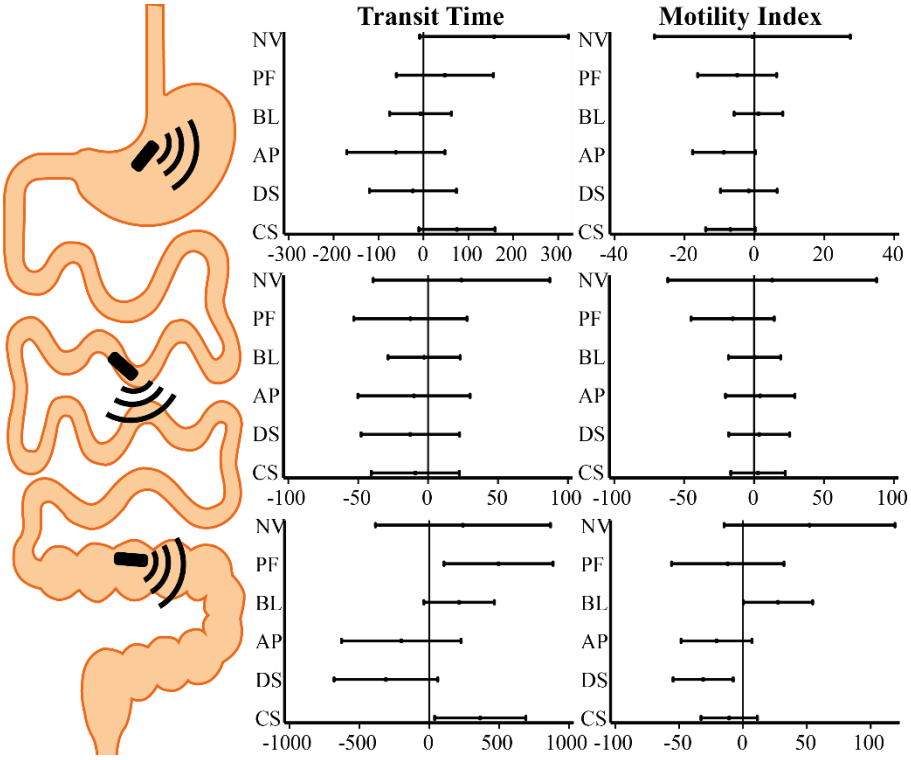

Figure 5.3: Association between symptoms and gastrointestinal transit time and motility. *Forest plots depicting the correlation coefficient and 95% confidence intervals for associations between gastric (upper), small intestinal (middle) and colonic (lower) transit time (left) or motility indices (right) and symptoms representative of nociceptive, upper and lower gastrointestinal troubles. NV: nausea/vomiting, PF: postprandial fullness, BL: bloating, AP: abdominal pain, DS: diarrhoea, CS: constipation.*

However, diabetes related gastrointestinal dysfunctions are not entirely localized to the enteric nervous system. Many gastrointestinal functions and symptoms have a central component, and thus, alterations in the brain, particularly in descending inhibitory/facilitating control mechanisms from the central nervous system to the gut, knowns as the brain-gut axis, may be affected

by autonomic neuropathy [166,167]. An altered central sensory processing is present in people with diabetes and concomitant gastrointestinal symptoms. This is evident as alterations of viscerally elicited evoked brain potentials e.g. in the oesophagus or rectal electrical stimulation, have shown enhanced neural activity of the insula, a brain region known to integrate visceral sensation, which is associated with severity of symptoms [168]. Moreover, malfunction of the cross-communication between brain region, and microstructural neuronal changes in areas involved in sensory processing, suggests that autonomous neuropathy is an accomplice in the symptom pathogenesis [43,166,167]. Additionally, the altered central processing could inhibit or facilitate sensory input experienced from the gastrointestinal tract e.g. hyposensitivity or hypersensitivity, of which the latter in a chronic state may develop to central sensitization, and thereby aggregate the perception of stimuli. We have investigated this phenomenon and have shown that the participants in Study I had a 2.3 times increased risk of being central sensitized compared to a normative cohort (unpublished data), with 11% having widespread central sensitization.

5.2.2. EFFECTS OF LIRAGLUTIDE

Apart from regulating glycaemic levels and affecting gastrointestinal motility, liraglutide and other GLP-1 receptor agonist have been suggested to possess a neuroprotective effect, based on data from animal models [169]. This protective effect has primarily been measured on brain neurons, but could as well be the case for the autonomic, peripheral and enteric branches of the nervous system [162]. In a previous study, the authors show a reduction in IL-6 and concomitant improvement of renal function, however, the study fails to show neuronal repair, plausible as a consequence of relatively short (26 weeks) intervention in the cohort with severe polyneuropathy [162].

Liraglutide induces temporary prolonged gastric emptying and decreasing degrees of nausea possibly linked to tachyphylaxis occurring with liraglutide administration. Nevertheless, we showed an average reduction of 10-hour in colonic transit times in response to liraglutide treatment and furthermore, we showed decreased contraction patterns (see Figure 5.4). Hence, we did not confirmed our hypothesised that liraglutide would exert an effect on all gut segments and promote gastrointestinal symptoms, as only the colonic segment was affected, and only postprandial fullness persisted. This suggest a compart-

mentalisation of gastrointestinal tract, possibly due to different neural innervations, as the upper gastrointestinal tract is thought to be primarily innervated by the vagus nerve, and evidence suggest a splanchnic effect on the lower gastrointestinal tract as well. Thus, vagally mediated inhibitory signals of liraglutide on motility could be diminished by neuropathy leaving only a possible splanchnic effect, resulting in the changes found in colon transit and motility. As 90% of the cohort in Study II had severe cardiovascular autonomic neuropathy, shown as orthostatic hypotension, concomitant to peripheral neuropathy, this support the speculation of diminished vagal mediation on the isolated colonic alterations.

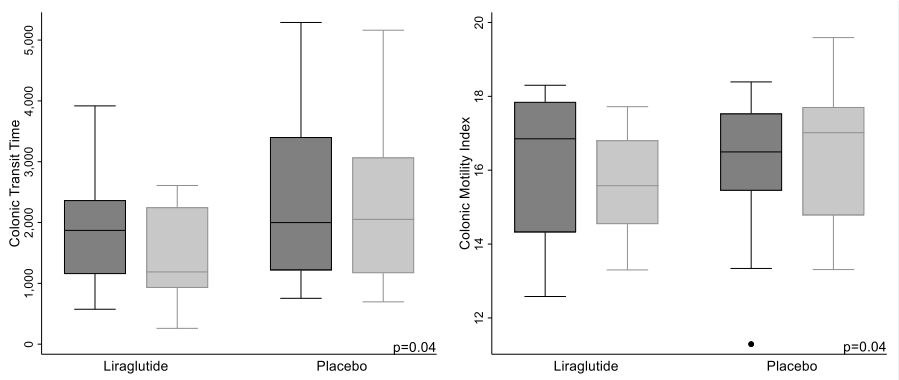

Figure 5.4: Changes in colonic transit time and motility index between treatments of liraglutide and placebo. *Box plots show colonic transit time (left) and motility indices (right) before (dark) and after (light) 26 weeks of liraglutide or placebo treatment. Both colonic transit time and motility indices were decreased in the liraglutide group.*

As rigorous glycaemic control is the therapeutic goal in type 1 diabetes, addition of liraglutide to the treatment regime may offer an opportunity even though the indication at first sight seems contradictory. The results of Paper IV confirm the notions by other studies that gastrointestinal symptoms like nausea are transient, satiety persists and gastric delay of nutrients subside, while other sections of the gastrointestinal tract increases in function. Thus, the negative aspects of this add-on treatment are limited.

5.3. METHODOLOGICAL CONSIDERATIONS

The results of this thesis were based on data from two studies, a prospective cross-sectional study and secondary analysis on data derived from a randomized controlled trial. Participants entered into the studies based on recorded diagnose of type 1 diabetes, however, no additional testing for autoimmune antibodies were performed. Data from both studies were pooled in Paper II-III, which increases the cohort and thereby the power of the analysis. However, there are some drawbacks in combining cohorts from different studies, as not all aspects are comparable. The largest difference here was in the notion that participants in Study II were included based on a confirmed diagnosis of distal symmetrical polyneuropathy, assessed by nerve conduction velocity testing. This is not the case in Study I, and thus the diagnostic criteria of participants with peripheral neuropathy may have been underestimated and thereby possibly skewing the results. However, the presence of participants with neuropathy in Study I would only strengthen our results. Thus, in Paper III, though no significance was found in gastrointestinal motility between those with and without polyneuropathy, a difference cannot be dismissed complete.

Papers I-III were based on cross-sectional data (as only baseline data from Study II was included in Paper II-III) in an analytical and hypothesis generating fashion [170]. Though strong associations were found, such design does not allow for interpretation of causal inferences. To pose an example, we cannot infer from Paper II, if autonomic neuropathy (as measured with heart rate variability) is caused by increased levels of cytokines and epithelial dysfunction, or if this is an effect a disrupted inflammatory reflex caused by autonomic neuropathy.

Paper IV was based on the randomized controlled trial investigation the effects 26 weeks of liraglutide vs. placebo treatment. This study experiences nine drop-outs primarily due to gastrointestinal complications [162]. Thus, the estimated changes in symptoms found in this study may be biased by the drop-out of participants with high severity symptoms. Thus, it could be expected that the incidence of nausea and postprandial fullness were in fact higher in the liraglutide group than reported.

Based on the inclusion exclusion criteria of both studies, these may have been subject to selection bias. Both studies had an exclusion criteria of psy-

chiatric disease, though it is well known that major depressive disorder, generalized anxiety disorder, schizophrenia, and eating disorders are more prevalent in the diabetic population [171]. It is therefore expected that, this reduces the external validity and thereby decreases the generalizability of the results. Additionally, due to the methods used, exclusion criteria like coeliac disease and symptomatic ischaemic heart disease or cardiac heart failure, may inadvertently affect the overall results of other test methods. However, the exclusion criteria also contained a ban against present or previous chemotherapy or use of drugs that affect the nervous system, thus supporting the investigation of nerve damage due to diabetic neuropathy and no other causes.

Both studies were conducted in the same laboratory taking place in the morning as to avoid diurnal influences on the results. Accordingly, wireless motility capsule procedures were conducted as the initial investigation (after fasting blood samples), as to avoid the occurrence of hypoglycaemia and accompanying distraction though out the subsequent testing.

CHAPTER 6. CONCLUSION

This PhD thesis had an overall aim to use existing methodological platforms to assess diabetic autonomic neuropathy of the cardiovascular and gastrointestinal system. Based on the four aims, we concluded the following in adults with type 1 diabetes:

I: Using clinically applicable cut-off values, implementation of the simple 5-minute cardiac vagal tone measure is sensitive and specific for recognising degrees of cardiovascular autonomic neuropathy and, in most cases, more so than more established measures of heart rate variability and sudomotor function, confirming our first hypothesis.

II: Pro- and anti-inflammatory mediators involved in neurodegenerative processes, like cytokines and e-selectin, are inversely associated with heart rate variability measures and may therefore contribute to the pathogenesis of cardiovascular autonomic neuropathy, confirming our second hypothesis.

III: Both upper and lower gastrointestinal symptoms positive correlated with colonic transit and motility measures influenced by cardio-vagal dysfunction, but not peripheral neuropathy, confirming our third hypothesis.

IV: 26 weeks of liraglutide treatment accelerated colonic transit and decreased motility measures, whilst gastrointestinal symptoms, except sensation of postprandial fullness, were transient, disproving our fourth hypothesis that liraglutide would exert an effect on all gut segments and promote gastrointestinal symptoms in people with type 1 diabetes and distal symmetrical polyneuropathy (and concomitant autonomic neuropathy).

6.1. CLINICAL IMPLICATIONS AND FUTURE PERSPECTIVES

The contributing framework of this thesis has not only improved our understanding of underlying mechanisms of diabetic autonomic neuropathy, but the methods proposed may provide easily applicable and highly informative alternatives to existing standards. Thus, our proposals may prospectively:

I: provide an inexpensive, practical and reliable method, to easily screen asymptomatic patient who potentially have underlying autonomic neuropathy.

This has the potential to increase the rates of early diagnosis which will facilitate the earlier introduction of preventative measures. This may ultimately, in the long term, beneficially impact morbidity and mortality.

II: attract attention to the complex immunoregulation present in diabetes and the possible neurodegenerative actions of inflammatory mediators on the development of autonomic neuropathy.

III: increase the awareness of the availability of technologies that allow evaluation of whole gut motility, not limiting the symptoms and disorders to the upper gastrointestinal tract and possible acknowledge the role of neural impairment and a hyposensitive state as part of the pathogenesis.

IV: provide increased evidence of the beneficial role of GLP-1 agonists, such as liraglutide, in improving colonic motility, normalising autonomic neuropathy induced prolongation of colonic transit times, and improving glycaemic control.

Future prospective clinical studies should consider incorporating some of the methods utilised in this body of work as they would ease testing practices, improve the granularity of the data and provide salient and reliable clinical endpoint in the investigation of novel treatment approaches.

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