



Aalborg Universitet

AALBORG UNIVERSITY
DENMARK

Study protocol for a multicentre, randomised, parallel group, sham-controlled clinical trial investigating the effect of transcutaneous vagal nerve stimulation on gastrointestinal symptoms in people with diabetes complicated with diabetic autonomic neuropathy

The DAN-VNS Study

Okdahl, Tina; Bertoli, Davide; Brock, Birgitte; Krogh, Klaus; Krag Knop, Filip; Brock, Christina; Drewes, Asbjørn M.

Published in:
BMJ Open

DOI (link to publication from Publisher):
[10.1136/bmjopen-2020-038677](https://doi.org/10.1136/bmjopen-2020-038677)

Creative Commons License
CC BY-NC 4.0

Publication date:
2021

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

[Link to publication from Aalborg University](#)

Citation for published version (APA):

Okdahl, T., Bertoli, D., Brock, B., Krogh, K., Krag Knop, F., Brock, C., & Drewes, A. M. (2021). Study protocol for a multicentre, randomised, parallel group, sham-controlled clinical trial investigating the effect of transcutaneous vagal nerve stimulation on gastrointestinal symptoms in people with diabetes complicated with diabetic autonomic neuropathy: The DAN-VNS Study. *BMJ Open*, 11(1), Article e038677. <https://doi.org/10.1136/bmjopen-2020-038677>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal -

BMJ Open Study protocol for a multicentre, randomised, parallel group, sham-controlled clinical trial investigating the effect of transcutaneous vagal nerve stimulation on gastrointestinal symptoms in people with diabetes complicated with diabetic autonomic neuropathy: the DAN-VNS Study

Tina Okdahl,¹ Davide Bertoli ,¹ Birgitte Brock,² Klaus Krogh,³ Filip Krag Knop,^{2,4,5,6} Christina Brock,^{1,7} Asbjørn M Drewes^{1,8}

To cite: Okdahl T, Bertoli D, Brock B, *et al*. Study protocol for a multicentre, randomised, parallel group, sham-controlled clinical trial investigating the effect of transcutaneous vagal nerve stimulation on gastrointestinal symptoms in people with diabetes complicated with diabetic autonomic neuropathy: the DAN-VNS Study. *BMJ Open* 2021;**11**:e038677. doi:10.1136/bmjopen-2020-038677

► Prepublication history and additional material for this paper is available online. To view these files, please visit the journal online (<http://dx.doi.org/10.1136/bmjopen-2020-038677>).

Received 19 March 2020
Revised 07 December 2020
Accepted 14 December 2020



© Author(s) (or their employer(s)) 2021. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

For numbered affiliations see end of article.

Correspondence to
Mrs Christina Brock;
christina.brock@rn.dk

ABSTRACT

Introduction A high proportion of people with diabetes experience gastrointestinal (GI) symptoms, which may be manifestations of diabetic autonomic neuropathy (DAN). The current treatment regime is ineffective and associated with major side effects. Transcutaneous vagal nerve stimulation (tVNS) is a new therapeutic option, which has been shown to increase GI motility and reduce inflammatory responses. As vagus is the main neuronal pathway for extrinsic coordination of GI secretion and motility, we hypothesise that tVNS will improve DAN-induced GI symptoms in subjects with diabetes.

Methods and analysis The DAN-VNS study is a randomised multicentre clinical trial investigating the effect of short-term, high intensity as well as long-term, medium-intensity tVNS on GI symptom alleviation in 120 subjects with diabetes. The primary outcome consists of changes from baseline in subjective ratings of symptom severity. Secondary outcomes include changes in gastric motility and GI transit time measured by MRI and wireless motility capsule. Moreover, cardiovascular and sudomotor function, glycaemic control, brain sensory processing and presence of low-grade inflammation will be investigated as secondary outcome measures. Lastly, 15 responders of tVNS treatment will be included in an explorative, randomised, cross-over study, in which the acute endocrine and metabolic response to short-term tVNS will be investigated.

Ethics and dissemination The study has been approved by the North Denmark Region Committee on Health Research Ethics (N-20190020). Results will be published in relevant international peer-reviewed journals.

Trial registration number NCT04143269.

Strengths and limitations of this study

- The prospective double-blinded, sham-controlled diabetic autonomic neuropathy (DAN)-vagal nerve stimulation (VNS) study was carefully designed by leading experts in the field of gastroenterology and DAN.
- The novel approach will provide needed clinical insight to the effect of transcutaneous VNS on gastrointestinal symptoms in diabetes via several experimental methods in various relevant organ systems.
- Patient-reported outcomes regarding symptom severity were chosen as the primary outcome in order to put the subjective view of patients in focus.
- In order to ensure optimal blinding, a parallel study design was chosen, which, in explorative studies, may be a weaker design than for example, cross-over trials.
- Self-administration may be another limitation of the study due to inability to verify correct tVNS in regard to anatomical stimulation site, stimulation intensity and duration.

INTRODUCTION

Clinical evidence of neuropathy can be demonstrated in 40%–50% of people with long-term diabetes.¹ The pathogenesis of diabetic neuropathy is multifactorial and includes microvascular dysfunction, metabolic and immune-mediated alterations, as well as chronic low-grade inflammation in response to long-term exposure to hyperglycaemia and hypoglycaemia.² Consequently,

progressive and seemingly irreversible damages to both peripheral, autonomic and central nerve systems may occur.³ Diabetic autonomic neuropathy (DAN) is a common, serious and yet under-recognised complication to diabetes.⁴ Up to 40% of people with diabetes experience gastrointestinal (GI) symptoms, which may be manifestations of DAN. The experienced cardinal symptoms are nausea, vomiting, early satiety and bloating. Moreover, abdominal pain, diarrhoea, constipation and faecal incontinence are also commonly reported.^{5,6}

Currently, management of DAN-related GI symptoms relies on dietary interventions, peroral antidiabetics, prokinetic agents, surgical procedures⁷ or gastric electrical stimulation.⁸ However, these interventions are often insufficient and sometimes associated with side effects.^{9,10} Consequently, the need for a novel, effective and safe treatment option is highly requested by patients as well as healthcare professionals.

Projections from the central nervous system coordinate and regulate GI functions including motility, enzyme secretion and nutrient absorption. The primary extrinsic neuronal pathway is the parasympathetic vagus nerve. Therefore, DAN-induced vagal impairment, which shifts the sympatheticovagal balance, is likely to be involved in the development of GI symptoms in people with diabetes.⁴ Preclinical evidence has demonstrated an association between vagal input and basal gastric tone, fundic relaxation and gastric contractions, thus demonstrating its fundamental role in GI motility.^{11,12}

Transcutaneous vagus nerve stimulation (tVNS) is a non-invasive method for activation of the cervical branch of the vagus nerve (figure 1). Experimental studies on healthy volunteers have shown tVNS to increase frequency of antral contractions and gastroduodenal motility index.¹³ Furthermore, preclinical studies have suggested that vagal stimulation dampens inflammatory pathways

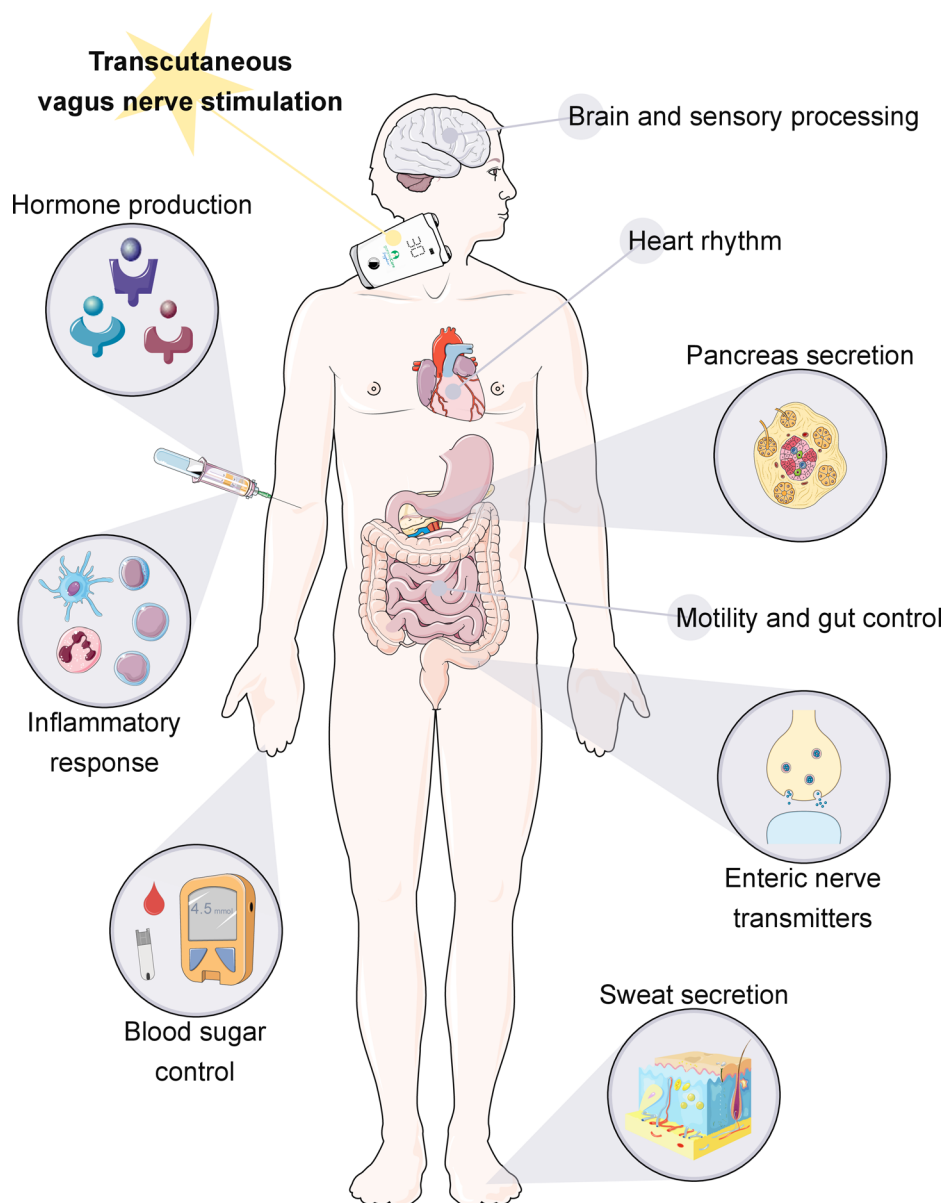


Figure 1 Possible physiological functions affected after transcutaneous vagus nerve stimulation

resulting in reduced levels of proinflammatory cytokines such as interleukin 6 (IL-6) and tumour necrosis factor (TNF)- α .¹⁴ These findings have been reproduced in a translational study on healthy volunteers.¹⁵ Additionally, preliminary clinical studies have demonstrated decreased disease activity and lowered levels of acute-phase reactant C reactive protein (CRP) in patients with rheumatoid arthritis, psoriasis arthritis, and ankylosing spondylitis in response to tVNS treatment.^{16,17} Similarly, a pilot study on vagal stimulation of patients with Crohn's disease showed reduced inflammation in response to the treatment.¹⁸ Currently, tVNS is associated with a high safety profile¹⁹ due to predominant stimulation of afferent A β -fibres within the vagal nerve. Hereby, potential adverse cardiac events are limited.²⁰

Taken together, we hypothesise that tVNS treatment will improve DAN-induced GI symptoms in people with diabetes. We aim to test this hypothesis in a multicentre, randomised, double-blinded, sham-controlled, parallel-group study; the DAN-VNS study.

METHODS AND ANALYSES

Study design and overview

The DAN-VNS study is composed of four work packages (WP) designed to explore the effects of tVNS on DAN-induced GI symptoms in people with diabetes (figure 2).

Work package I

In WP-I, the effect of short-term, high-intensity tVNS treatment will be investigated. Participants will be allocated to either active or sham treatment and will be instructed to self-administer the treatment four times a day for 7 days.

Work package II

Following a wash-out period of minimum 2 weeks, participants will continue in WP-II, in which the effect of long-term, medium-intensity treatment with tVNS will be investigated. Participants will follow the same treatment as allocated in WP-I. In WP-II, self-administration of the treatment will be performed twice a day for 8 weeks.

Follow-up

A follow-up visit will be conducted 7 days after end of WP-II including blood samples and ECG, which will be reviewed by a medical doctor in order to ensure participant safety after cessation of the intervention. Drop-outs will likewise be encouraged to participate in a follow-up visit after trial discontinuation.

Work package III

In WP-III, the acute endocrine and metabolic response to short-term tVNS will be investigated in a randomised, explorative, cross-over study. Enriched enrolment will include 15 responders from WP-II, defined as those having the greatest decrease in GI symptoms in response to active tVNS. Participants will be randomised to the order of active or sham treatment conducted at two study days separated by a wash-out period of minimum 2 weeks.

During each of the two study days, a semisolid test meal will be ingested, and tVNS will be administered by study personnel five times (at baseline and every hour thereafter) during a 4 hour session.

Work package IV

In order to provide a comparable baseline dataset, 40 age and gender-matched healthy controls will be recruited at the site in Aalborg, Denmark for a cross-sectional, descriptive study.

Study participants

Participants will be included at three different locations in Denmark: at Steno Diabetes Center North Jutland (Aalborg), at Steno Diabetes Center Copenhagen and at Steno Diabetes Center Aarhus. In total, 120 participants will be included for WP-I and WP-II with approximately 40 at each study site. In contrast, WP-III will be conducted in Copenhagen, and WP-IV in Aalborg.

Recruitment and inclusion

Potentially eligible participants will be identified at each study site after approval of the treating physician. The healthy controls will be recruited locally in Aalborg from an established volunteer database or advertisements. Eligible individuals will be provided with both oral and written information regarding the study. Before consenting to participation, subjects are encouraged to consider their decision for 24 hours. Furthermore, consent can be withdrawn at any point during the study period with no consequences for future treatment. Subjects confirm their willingness to participate by signing the informed consent form after which they are allowed to enter the trial. Screening and inclusion of candidates will be performed by a medical doctor.

Inclusion criteria include either a verified diagnosis of type 1 diabetes for a minimum of 1 year and stable hyperglycaemic medication ensuring that participants as a minimum have received the given treatment (long acting and fast acting insulin or insulin pump with dosing adjustments according to regimens) for at least 3 months prior to study entrance, or a verified diagnosis of type 2 diabetes for a minimum of 1 year, for which the patient receives no or stable (at least 3 months prior to study entrance) hyperglycaemic medication. Additionally, GI symptoms will be evaluated using the Gastroparesis Cardinal Symptom Index (GCSI) (see online supplemental material 1) and the Gastrointestinal Symptom Rating Scale (GSRS) (see online supplemental material 2). Inclusion in the study require a combined weighted GCSI and GSRS score of minimum 2.3. The justification of the cut-off value was based on the calculated weighted mean in several cohorts of healthy subjects plus one (GCSI) and two (GSRS) SDs.^{21–26} Furthermore, to confirm the diagnosis of DAN, participants must have either (1) minimum one abnormal cardiac autonomic reflex test (heart rate variability (HRV) in rest, in response to expiration:inspiration, lying to standing position and Valsalva manoeuvre)

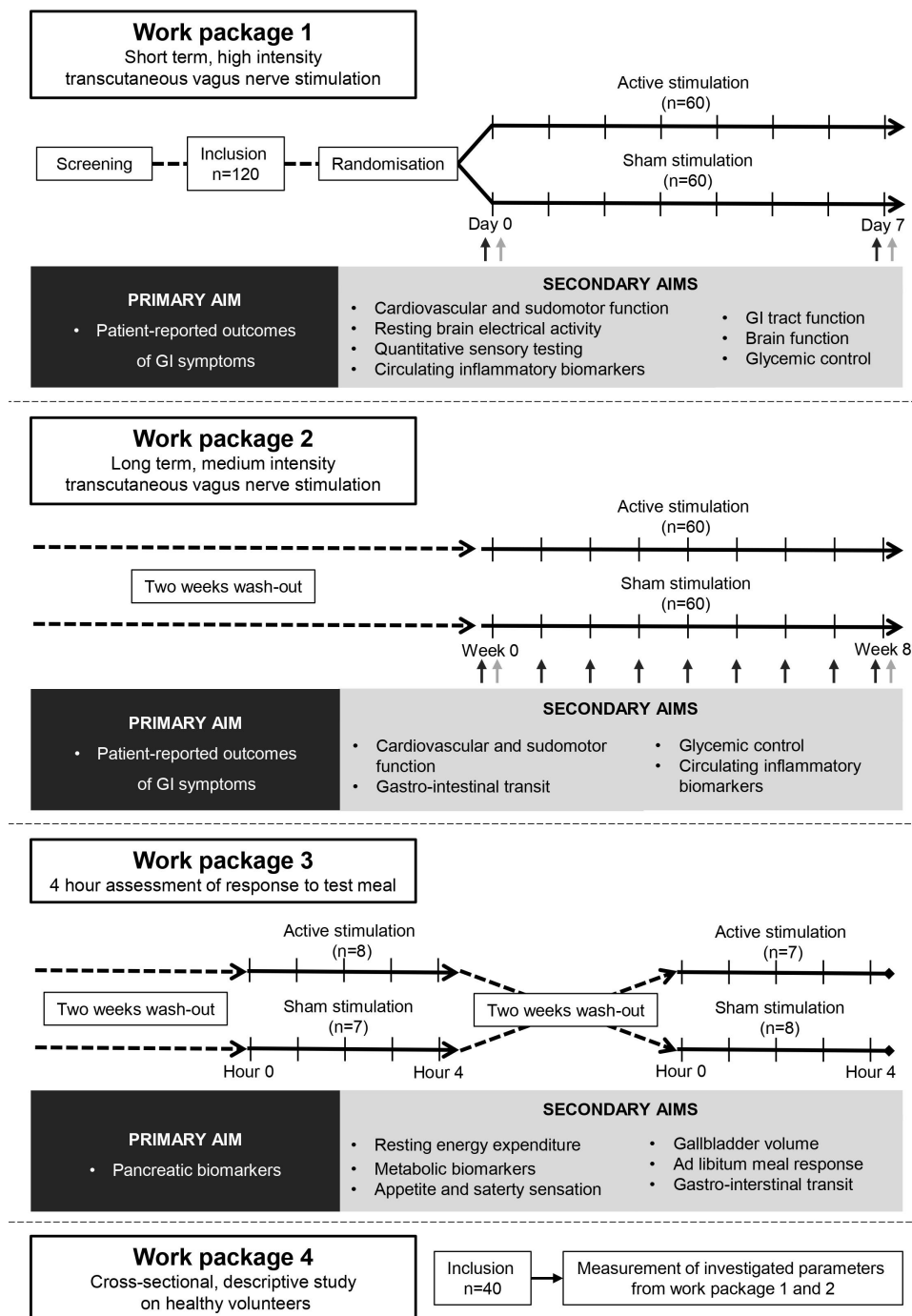


Figure 2 Overview of the diabetic autonomic neuropathy (DAN)-vagus nerve stimulation (VNS) study. GI, gastrointestinal.

assessed with the validated VAGUS device,^{27 28} or (2) decreased electrochemical resistance assessed in both hands and feet with the SUDOSCAN device, using a cut-off value $<50 \mu\text{S}$ at hand and $<70 \mu\text{S}$ in the feet,²⁹ or (3) a autonomic symptom score >16 assessed by the validated Composite Autonomic Symptom Score (COMPASS-31) questionnaire²⁹⁻³¹ (see online supplemental material 3). A complete list of inclusion and exclusion criteria can be found in table 1. Lists of screened and included subjects will be kept at a secure location in order to ensure confidentiality.

Participants can be discontinued from the trial based on medical judgement or if they are considered non-compliant. Drop-outs will be mirror randomised by new participants in order to ensure sufficient power of the study.

Intervention

The study intervention consists of tVNS, which will be applied by a battery-powered handheld tVNS device (GammaCore, ElectroCore LLC, Basking Ridge, New Jersey, USA). The GammaCore is registered as a class

Table 1 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Age ≥ 18 years	Significant GI diseases not related to diabetes
Verified diagnosis of either type 1 or type 2 diabetes for a minimum of 1 year and with stable medication	Significant cardiovascular diseases
Weighted composite score of GCSI and GSRS ≥ 2.3	GI surgery within 3 months prior to study inclusion
One or more of the following: (1) CAN-score ≥ 1*, (2) electrochemical resistance < 50 μS in hands or < 70 μS in feet†, (3) COMPASS-31 score ≥ 16‡	Swallowing disorders
Ability to read and understand Danish	Blood pressure < 100/60 or > 160/105
Personally signed and dated informed consent documents	Clinically significant bradycardia or tachycardia
Willingness and ability to comply with scheduled visits, treatment plan, laboratory tests and other trial procedures	Implanted portable electromechanical medical devices including pacemaker, defibrillator, cochlear implant and infusion pump
	Previous surgery of the vagus nerve
	Active laser treatment for proliferative retinopathy
	Contraindications for MRI§
	Any clinical abnormalities, that in the opinion of the investigator may increase the risk associated with trial participation or may interfere with the interpretation of the trial results
	Pregnancy or intention to become pregnant or father a child during the course of the study
	Participation in other clinical trials less than 3 months prior to inclusion, unless such a participation is judged to have no influence on the recordings

*Cardiovascular Autonomic Neuropathy measured by VAGUS (procedure elaborated under the sections Outcomes and Experimental Procedure; Cardiovascular and Sudomotor Function).

†Measured by the SUDOSCAN device (procedure elaborated under the sections Outcomes and Experimental Procedure; Cardiovascular and Sudomotor Function).

‡Composite Autonomic Symptom Score.

§Only relevant for participants included at SDCN.

GCSI, gastroparesis cardinal symptom score; GI, gastrointestinal; GSRS, gastrointestinal symptom rating scale.

IIa medical device, and has been CE marketed (CE 571753) for the following indications in adults: primary headache (migraines, cluster headache, and hemicranias continua) and medication overuse headache.³² The GammaCore device provides electrical stimulation of the cervical region of the vagal nerve by producing a proprietary low-voltage electrical signal comprising a 5-kHz sine wave burst lasting for 1 millisecond (five sine waves, each lasting 200 microseconds). Bursts are repeated once every 40 ms (25 Hz), generating a 24 V peak voltage and 60 mA peak output current. On stimulation, the device is positioned at the cervical course of the vagal nerve, situated anterior to the sternocleidomastoid muscle and lateral to the carotid artery. Stimulation is provided through two steel contact electrodes, which, prior to use, are covered with conductive gel (Sigma gel, Parker Laboratories, New Jersey, USA). Based on the manufacturer's recommendations, each stimulation in the DAN-VNS study will consist of two consecutive bilateral tVNS doses, which corresponds to 2 × 120 s. Intensity of the stimulation can be adjusted by the user in the range of 1–40 (arbitrary units) via the digital user interface. The stimulation has

been optimised, in regard to intensity (24 V peak voltage and 60 mA peak output current), pulse duration (1 ms), and frequency (5 kHz), to predominantly target afferent Aβ-fibres. Hereby, interference with cardiovascular innervating efferent C-fibres is limited and potential adverse effects such as bradycardia and bronchoconstriction are limited.²⁰

Participants will be instructed to use the highest tolerable intensity setting in order to obtain maximum vagal stimulation. Placebo intervention will be applied by a sham GammaCore device (ElectroCore LLC, Basking Ridge, New Jersey, USA), which is identical to the active device in regard to appearance and application. In contrast to the electrical stimulation provided by the active device, the sham device provides a stimulation sound in order to mimic the active treatment. However, to eliminate the possibility of participants being able to distinguish between the active and sham stimulation, a parallel study design, in which all subjects remain in their allocated treatment group throughout the entire study, was chosen for WP-I and WP-II.

During the instruction session, correct placement of the device will be marked on the cervical region with a permanent marker. Participants are encouraged to refresh these marks at regular intervals in order to ensure tVNS/sham compliance. Furthermore, before the GammaCore is handed out, a thorough instruction of the use of the device and of compliance and daily doses will be given to ensure that the participants acknowledge the importance of receiving sufficient self-administered stimulation. In addition, this is emphasised in the handed-out Danish version of the user manual, which encourages stimulation within certain time windows, for example, morning between 06:00 and 07:00.

tVNS will be applied as an add-on treatment, in the sense that any habitual medication of participants will be continued without any changes. Habitual medication must be stable prior to inclusion in the study. During the study period, no new medication must be introduced to participants.

In WP-I and II, tVNS/sham treatment will be self-administered as two bilateral doses conducted four or two times a day, respectively, with approximately six- or 12 hours intervals. Participants will note time and intensity level of each stimulation or lack of such in a provided diary. In WP-III, tVNS/sham treatment will be applied by study personnel.

Outcomes and experimental procedures

An overview of outcomes and methods of assessment is presented in [table 2](#) and [tables 3–5](#). Experimental procedures are described below.

Patient-reported outcomes

Upper GI symptoms will be evaluated by the Patient Assessment of Upper GI Symptom Severity Index, which is a validated tool developed for measurement of symptom severity in patients with gastroparesis and includes the GCSI covering three subgroups: nausea/vomiting, bloating and postprandial fullness³³ (see online supplemental material 1). Predominantly lower GI symptoms will be evaluated by the GSRS questionnaire, which was developed and validated for patients with luminal GI symptoms, covering five subgroups: reflux, abdominal pain, indigestion, diarrhoea, constipation³⁴ (see online supplemental material 2). Additionally, the COMPASS-31³⁰ will be applied for quantitative self-assessment of autonomic dysfunction symptoms (see online supplemental material 3).

Cardiovascular and sudomotor function

Several aspects of cardiovascular function will be assessed in order to investigate the presence of cardiac autonomic neuropathy in participants. (1) Cardiac vagal tone will be measured during 5 min of rest using a non-invasive ECG monitor (eMotion Faros, Mega Electronics, Kuopio, Finland). (2) Cardiovascular reflexes will be evaluated in response to three standardised tests: (a) posture change (supine to standing), (b) deep breathing respiratory cycle

and (c) the Valsalva manoeuvre using the Vagus device (Medicus Engineering, Aarhus, Denmark). Based on age-dependent cut-off values, the device provides a cardiovascular autonomic neuropathy (CAN) score ranging from 0 to 3, indicating the level of CAN as being either absent, borderline or definitive.³⁵ (3) Five days continuous Holter monitoring will be applied for extraction of HRV measures (time and frequency domain) of study participants using the ePatch device (BioTelemetry Technology Aps, Hørsholm, Denmark). The HRV measurements will be performed in accordance with the standards defined by The Task Force of the European Society of Cardiology and The North American Society of pacing and Electrophysiology. Additionally, sudomotor function has been suggested as an early marker of small fibre neuropathy.³⁶ The electrochemical reaction mediated by chloride ions after stimulation of sweat glands will be assessed in both hands and feet by a non-invasive device (SUDOSCAN, Impeto Medical, California, San Diego, USA).³⁷

Resting brain electrical activity

Electroencephalography (EEG) will be recorded in resting state for 5 min during which the subject is instructed to keep the eyes closed. The recording will be conducted with 40 electrodes by a direct current amplifier (NuAmp, Neuroscan, El Paso, USA). As previously reported by this group, resting EEG will be used to model connectivity between brain centres as well as to identify dominating centres of brain activity.³⁸ The data will be preprocessed offline. First, notch filtered from 49 to 51 Hz, followed by a bandpass filter from 1 to 70 Hz. Afterwards, time intervals with clear artefacts in the majority of channels due to heavy muscle activity, and so on, will be removed along with interpolation of channels with abnormal traces or high amplitudes. The signal will lastly be re-referenced to the average reference before further analysis. The spectral incidencies of the data will be analysed using a continuous wavelet analysis using a Morlet wavelet function with a bandwidth parameter of 10 Hz and a wavelet centre frequency of 1 Hz. Source localisation will be performed by the standardised low-resolution brain electromagnetic tomography.

Quantitative sensory testing

Sensory function will be assessed by quantitative sensory testing. First, pain tolerance threshold to bone and muscle pressure will be performed on the tibia bone and quadriceps muscle. A handheld pressure algometer (Type 2, Somedic Production AB, Sweden), applied at a rate of 30 kPa/s, will be used. Second, a cold pressor test will be applied. Participants will be instructed to immerse the left hand in circulating ice-chilled water (2°C) for 120 s or until pain becomes intolerable. During the immersion, pain intensity will be rated regularly on a visual analogue scale. Furthermore, immediately after the hand is withdrawn from the ice water, a pain test stimulus (quadriceps muscle pressure) will be applied, allowing for quantification of the conditioned pain modulation capacity, which

Table 2 Outcomes and methods of assessment

Work package I	
Primary outcome	Methods of assessment
Patient-reported outcomes of gastrointestinal (GI) symptoms	PAGI-SYM and GSRS questionnaire
Secondary outcomes	Methods of assessment
Cardiovascular and sudomotor function	i) Cardiac vagal tone, ii) Cardiovascular reflexes, iii) Holter monitoring, iv) Stimulation of sweat glands in hands and feet
Resting brain electrical activity	Electroencephalogram
Quantitative sensory testing	i) Bone- and muscle pressure ii) Cold pressor test with conditioned pain modulation
Brain function	MRI
GI tract function	MRI
Glycaemic control	Continuous glucose monitoring
Circulating inflammatory biomarkers	Multiplex analysis of serum samples
Work package II	
Primary outcome	Methods of assessment
Patient-reported outcomes of GI symptoms	PAGI-SYM and GSRS questionnaire
Secondary outcomes	Methods of assessment
Cardiovascular and sudomotor function	i) Cardiac vagal tone, ii) Cardiovascular reflexes, iii) Holter monitoring, iv) Stimulation of sweat glands in hands and feet
GI transit	Wireless motility capsule
Glycaemic control	Continuous glucose monitoring
Circulating inflammatory biomarkers	Multiplex analysis of serum samples
PRO of subjective autonomic symptoms	COMPASS-31 questionnaire
Work package III	
Primary outcome	Methods of assessment
Postprandial levels of pancreatic polypeptides	Sequential blood sampling during test meal and multiplex analysis of serum samples
Secondary outcomes	Methods of assessment
Postprandial levels of metabolic biomarkers	Sequential blood sampling during test meal and multiplex analysis of serum samples
Appetite and satiety sensation	Subjective rating on Visual Analogue Scale
GI transit	Wireless motility capsule
Gallbladder volume	Ultrasound
Resting energy expenditure	Indirect calorimetry
Ad libitum meal response	Registration of ingested amount of food
Work package IV	
Primary outcome	Methods of assessment
Patient-reported outcomes of GI symptoms	PAGI-SYM and GSRS questionnaire
Secondary outcomes	Methods of assessment
Cardiovascular and sudomotor function	i) Cardiac vagal tone, ii) Cardiovascular reflexes, iii) Holter monitoring, iv) Stimulation of sweat glands in hands and feet
Resting brain electrical activity	Electroencephalogram
Quantitative sensory testing	i) Bone- and muscle pressure, ii) Cold pressor test with conditioned pain modulation
Brain function	MRI

Continued

Table 2 Continued

GI tract function	MRI
Glycaemic control	Continuous glucose monitoring
Circulating inflammatory biomarkers	Multiplex analysis of serum samples
GI transit	Wireless motility capsule

GCSI, gastroparesis cardinal symptom index; GSRS, gastrointestinal symptom rating scale; PAGI-SYM, patient assessment of upper GI symptom severity index.

provides insights to the function of descending inhibitory pain pathways.³⁹

Brain imaging and GI tract function

Structural and functional assessments of brain and GI tract will be explored by 3 T and 1.5 magnetic resonance imaging (Signa HDxt, General Electrics, Milwaukee, Wisconsin, USA). Brain networks during rest and concentrations of brain metabolites will be investigated by functional MRI and magnetic resonance spectroscopy, respectively. Stomach volume, adaptive capacity and motility, small bowel motility and colonic volume will be assessed at regular time points after intake of a 400 mL standardised liquid meal (1.5 kcal per mL).

Glycaemic control

Continuous glucose monitoring will be applied in order to be able to measure real-time fluctuations in blood glucose and estimate 'Time in Range'. Participants will be equipped with the FreeStyle Libre device (Abbot Diabetes Care Denmark, Copenhagen Ø, Denmark), which allows for continuous glucose monitoring via a portable system for 14 days before changing of needle and patch.⁴⁰

Circulating inflammatory biomarkers

Serum collected from fasting blood samples will be aliquoted in appropriate volumes and stored in a biobank at -80°C . The complete set of serum samples will be analysed after study end for levels of inflammatory biomarkers such as TNF- α , IL-2, IL-4, IL-6 and IL-10 by multiplexing technology.

Gastrointestinal transit

A wireless motility capsule (SmartPill, Given Imaging, Yokneam Illit, Israel) will be used to evaluate the whole gut transit time as well as segmental for example, stomach, small bowel and large bowel transit times. After consumption, the indigestible capsule traverses through the GI tract while continuously measuring temperature, pH and pressure in the gut lumen.⁴¹ Such parameters can be used as proxies for the motor function of the enteric nervous system.

Postprandial levels of endocrine and metabolic biomarkers

Following an overnight fast, blood samples from participants in WP-III will be collected at regular intervals during the study session. In order to ensure similar glycogen storage status and macronutrient balance, participants

will be instructed to avoid strenuous physical exercise and alcohol consumption 48 hours prior to WP-III study days. Levels of pancreatic polypeptides and gut hormones (gastrin, cholecystokinin, glucose-dependent insulinotropic polypeptide, glucagon-like peptide 1 and 2, oxyntomodulin, neurotensin, fibroblast growth factor 19) will be assessed after study completion.

Appetite and satiety sensation

Hunger, satiety, fullness and prospective food consumption will be reported by WP-III participants on a Visual Analogue Scale at regular time intervals.

Gallbladder volume

Gallbladder emptying will be investigated by volumetric measurements using ultrasonography (LOGIQ E9, GE Healthcare, Waukesha, Wisconsin, USA).

Resting energy expenditure

Following 30 min of rest, resting energy expenditure will be measured for 15 min by resting gas exchange monitoring (CCM Express, Indirect Calorimeter, Medgraphics, St. Poul, Minnesota, USA).

Ad libitum meal response

In order to assess voluntary food intake, participants in WP-III will be provided with an ad libitum pasta Bolognese meal and the ingested amount will be registered.

Randomisation and blinding

Included participants will be allocated a unique anonymised identification number kept throughout the entire study. Even though path-dependency may exist, we judged that a centrally generated block-randomisation list for WP-I and WP-II, generated by use of the website www.randomization.com was preferable in order to control seasonal and geographical bias. The randomisation list will be devised by the sponsor and kept in a sealed envelope until last participant last visit in WP-II. Furthermore, sealed envelopes, containing treatment allocation for each participant, will be kept in a secure location to allow for individual unblinding in case of medical emergencies. To ensure blinding of the study personnel, sponsor will be responsible for assigning active/sham tVNS medical devices to study sites. Nevertheless, blinding of participants and study personnel may be complicated by the fact that the sham device is unable to mimic the active stimulation in regard to associated fascial and cervical muscle

Table 3 Time and events of work package I and II

	Screening	Work package I		Washout (14 days)		Work package II		Follow-up			
		Day -5	Day 0	Day 7	Day -5	Day 0	Day 7	Day 14, 21, 28, 35, 42, 49	Day 51	Day 56	Day 56+7
Enrolment											
Informed consent	X										
In/exclusion criteria	X										
Objective examination	X										
Safety outcomes											
Standard blood samples	X	X	X	X	X	X	X	X	X	X	X
Adverse events											
ECG											X
Primary outcome											
GI symptom questionnaires	X	X	X	X	X	X	X	X	X	X	X
Secondary outcomes											
MRI scan of brain and GI tract		X									
Continuous glucose monitoring											↔
Holter monitoring											↔
Sudomotor function											X
Cardiovascular reflexes											X
Inflammation markers											X
Cardiac vagal tone											X
Resting EEG											X
QST											X
Gastrointestinal transit											↔
Autonomic function questionnaire		X									X

GI, gastrointestinal.



Table 4 Time and events of work package III

Work Package III Study Day I and II separated by a 14 days washout period

	-20min	-15min	-5min	0min	30min	45min	60min	75min	90min	105min	120min	150min	180min	210min	225min	240min
Interventions		X	X													
Test meal				X												
tVNS stimulation (active or sham)		X			X								X			X
Safety outcomes																
Adverse events																
Primary outcome				X	X	X	X	X	X	X	X	X	X	X	X	X
Pancreatic biomarkers				X	X	X	X	X	X	X	X	X	X	X	X	X
Secondary outcomes																
Resting energy expenditure				X	X	X	X	X	X	X	X	X	X	X	X	X
Metabolic biomarkers				X	X	X	X	X	X	X	X	X	X	X	X	X
Appetite and satiety sensation				X	X	X	X	X	X	X	X	X	X	X	X	X
Gallbladder volume				X	X	X	X	X	X	X	X	X	X	X	X	X
Ad libitum meal response				X	X	X	X	X	X	X	X	X	X	X	X	X
Gastrointestinal transit				X	X	X	X	X	X	X	X	X	X	X	X	X

tVNS, transcutaneous vagus nerve stimulation.

contractions. Therefore, a parallel-group design was chosen for WP-I and II. Additionally, to optimise blinding of study personnel during WP-I and II, an unblinded and otherwise non-involved person at each study site will be responsible for the training of participants in the use of the tVNS GammaCore device. Furthermore, participants will be instructed to restrain from sharing any information regarding stimulation and associated physical sensations to other study personnel.

After completion of WP-I and WP-II, the unblinding of treatment allocation will be conducted by the sponsor. Likewise, sponsor will prepare a blinded data set divided in ‘Treatment A’ and ‘Treatment B’, in order to allow for blinding of data analysts. Additionally, the sponsor will identify responders of long-term active treatment of tVNS (WP-II) for identification of those subjects that are eligible for WP-III.

For participants entering WP-III, a new centrally generated randomisation code for the order of active/sham treatment in the cross-over design of WP-III will be assigned. Again, study personnel and participants will be blinded to the randomisation of treatment order, and even though blinding may be complicated, this aspect is regarded as acceptable given the fact that the primary outcome of WP-III consists of objective measures.

Harms and adverse events

Based on evidence of safety data regarding tVNS from previous studies,^{19 42} we do not expect the intervention to cause harm or substantial discomfort for participants. Transient side effects during stimulation including shortness of breath, hoarseness and twitching of facial muscles, are, however, listed as potential adverse reactions to GammaCore use (GammaCore User Manual). Furthermore, rare cases of syncope have been reported.⁴³ Participants experiencing unacceptable levels of side effects will be instructed to reduce the level of stimulation intensity.

Adverse events, regardless of severity, will be collected regularly during trial conduct and recorded in the case report form. Investigators are responsible for immediate (within 24 hours) reporting of any serious adverse events to the sponsor. Furthermore, on a yearly basis, sponsor will notify relevant authorities of all registered serious adverse events. Causality of serious adverse events with the intervention will be assessed by a physician, and all serious adverse device effects will be reported to the manufacturer. Based on interim analyses of serious adverse device effects, sponsor will be able to terminate the trial if the safety of study participation is considered to be insufficient. During participation in the trial, subjects are covered by the patient insurance of the respective study site.

Sample size calculation

The study is powered to detect a minimal difference of 30% between the active and the sham tVNS group on the GCSI score 1 week (WP-I) or 2 months (WP-II) after study initiation. Based on a baseline score of 2.0±1.0

Table 5 Time and events of work package IV

		Screening	Work package IV			
			Day -5	Day 0	Day 7	
Enrolment	Informed consent	X				
	In/exclusion criteria	X				
	Objective examination	X				
Safety outcomes	Standard blood samples			X		
Primary outcome	GI symptom questionnaires	X		X		
Secondary outcomes	MRI scan of brain and GI tract		X			
	Continuous glucose monitoring					
	Holter monitoring					
	Sudomotor function			X		
	Cardiovascular reflexes			X		
	Inflammation markers			X		
	Cardiac vagal tone			X		
	Resting EEG			X		
	QST			X		
	GI transit					
	Autonomic function questionnaire				X	

EEG, electroencephalography; GI, gastrointestinal; QST, quantitative sensory testing.

(mean±SD),³³ 60 subjects in each arm are required to provide a statistical power of 90% with the use of a two-sided significance level of 0.05. A power of 90% was chosen in order to account for secondary endpoints. A drop-out rate of up to 30% is estimated in the patient group. In order to achieve the required sample size, new subjects will be mirror randomised to account for participants withdrawing from the study.

Data collection and data management

Data will be collected by highly experienced research personnel, all of which have been trained in the principles of good clinical practice (GCP). Experimental data will be recorded directly and managed on a secure electronic platform (REDCap Electronic Data Capture Tool, hosted at Aalborg University Hospital, Aarhus University, and The Capital Region of Denmark). Filled-out questionnaires will be transferred to REDCap by two individual entry persons in order to limit the risk of errors in the process. Digitalised data are stored for up to 5 years after trial end. For each participant, a physical Case Report Form, containing all physical source material, will be kept in a secure and locked location for 5 years.

Serum samples will be stored at a research biobank for a maximum of 10 years. Samples will be anonymised and labelled with protocol number, subject randomisation ID and date of sample collection.

Monitoring

Monitoring of the trial will be conducted prior to, during and after study conduction by the GCP Unit (GCP Unit,

Aalborg, Aarhus, and Copenhagen, Denmark) in order to ensure compliance with study protocol and national standards. The monitoring will include complete inspection of signed informed consent forms and adverse events logs. Likewise, investigators and study sites will allow for any quality assurance audits by relevant regulatory authorities.

Data analysis

The initial analysis of primary outcomes will be by intention to treat regardless of adherence to study protocol. Secondary outcomes will be analysed per protocol, in the sense that only subjects, who have completed the specific experimental setup, will be included. In WP-I and WP-II and WP-III, both primary and secondary outcomes will be evaluated as changes from baseline by appropriate statistical tests including paired samples t-test and equivalent non-parametric tests. Analyses of WP-III data will include a linear mixed effects model with fixed effects for period and treatment, and random effects for sequence and subject nested in sequence. To account for multiple testing in the analysis of secondary outcomes, the Bonferroni Correction or other applicable corrections will be applied. Missing data will be handled by applicable statistical methods including multiple imputation. Data collected from drop-outs will be included in the data analysis if appropriate (eg, data from completed WP-I).

After primary analysis of study specific outcomes, the complete anonymised data set will be available for



additional analyses by the respective research groups at each study site.

Patient and public involvement

The original idea behind the DAN-VNS study was based on reports from patients with DAN-induced GI symptoms, who requested a simple, safe, and effective treatment option for their condition. The primary objective of the study was chosen to be patient-reported outcomes in order to put the subjective effect of tVNS in focus rather than objective measures, which may not correlate with patient evaluation and satisfaction with the treatment. After study completion, participants will receive information regarding own study arm allocation (active/sham) as well as overall findings in the form of a written report. Additionally, study results will be disseminated to relevant patient associations. No public involvement was included in the design phase of the study.

Ethics and dissemination

The study protocol (version 1.4) has been approved by the Danish Health and Medicines Authority (CIV-19-07-029105) and the North Denmark Region Committee on Health Research Ethics (N-20190020). Any amendments to the protocol will be implemented after approval of the mentioned authorities. Sponsor is responsible for informing all study sites of any such changes.

The conduction of the study will be in accordance with the protocol, the standard “Clinical investigation of medical devices for human subjects – Good Clinical Practise” (DS/EN ISO 14155:2012), designated Standard Operating Procedures, any applicable local regulatory requirements and laws, as well as the principles of the World Medical Association, Declaration of Helsinki amended by the 52nd General Assembly, Edinburgh, Scotland, October 2000, clarified by the General Assembly in Washington 2002, Tokyo 2004, Seoul 2008, and Fortaleza 2013.

Trial results, positive, negative or inconclusive, will be published in relevant international peer-reviewed journals and presented at national and international conferences. Authorship of published material will be decided by the steering committee. Additional dissemination of results will be initiated after approval of the principal investigator at each study site. Likewise, sponsor may grant access to the final data set on request.

DISCUSSION

The strengths of the DAN-VNS study is, that it is a multi-centre study designed to investigate the hypothesis that vagal stimulation will improve GI function in people with diabetes with DAN, assessed with sophisticated MRI methods to complement the existing knowledge on scintigraphic evaluation.

Limitations

Limitations of the study include the inability to verify correct stimulation of the vagal nerve during

self-administration in WP-I and II. As mentioned above, measures such as markings on the neck for correct device placement, as well as registration of applied doses in patient diaries, have been incorporated in order to limit this risk. Another limitation of the study is the lack of tVNS treatment in the cohort of healthy controls, which could provide novel insights to the effect of vagal stimulation on a healthy and functioning GI tract. However, this aspect was decided as being out of the scope of this study, but relevant for future studies.

CONCLUSION

With the DAN-VNS study, we expect to provide novel clinical evidence regarding the effect of tVNS for treatment of DAN-induced GI symptoms in people with diabetes. Furthermore, is it our hope that this knowledge will contribute to the introduction of a safe and effective non-pharmacological treatment option for this patient group.

Author affiliations

¹Mech-Sense, Department of Gastroenterology and Hepatology, Aalborg University Hospital, Aalborg, Denmark

²Steno Diabetes Center Copenhagen, The Capital Region of Denmark, Gentofte, Denmark

³Steno Diabetes Center Aarhus, Central Denmark Region, Aarhus, Denmark

⁴Center for Clinical Metabolic Research, Gentofte Hospital, University of Copenhagen, Hellerup, Denmark

⁵Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

⁶Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

⁷Clinical Institute, Aalborg University, Aalborg, Denmark

⁸Steno Diabetes Center North Jutland, Aalborg University Hospital, Aalborg, Denmark

Contributors AMD, CB, KK, FKK and BB conceived and designed the study. All authors participated in the logistical planning of the study. All authors made significant contributions to the development and conceptualisation of the protocol. TO drafted the initial version of the manuscript. All authors reviewed the draft versions of the manuscript and have read and approved the final manuscript.

Funding This work was supported by the Novo Nordic Foundation, grant number NNF180C0052045.

Expected time frame and trial status The study is expected to run from ultimo 2019 to ultimo 2022 including data analyses. First participant first visit is expected to take place in November 2019, and last participant last visit in June 2021. As of September 2019, the screening and recruitment process has been initiated.

Competing interests None declared.

Patient consent for publication Not required.

Supplemental material This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

ORCID iD

 Davide Bertoli <http://orcid.org/0000-0003-1598-9053>

REFERENCES

- Sandireddy R, Yerra VG, Areti A, *et al.* Neuroinflammation and oxidative stress in diabetic neuropathy: futuristic strategies based on these targets. *Int J Endocrinol* 2014;2014:1–10.
- Vinik AI, Park TS, Stansberry KB, *et al.* Diabetic neuropathies. *Diabetologia* 2000;43:957–73.
- Freeman R. Diabetic autonomic neuropathy. *Handb Clin Neurol* 2014;126:63–79.
- Vinik AI, Freeman R, Erbas T. Diabetic autonomic neuropathy. *Semin Neurol* 2003;23:365–72.
- Bytzer P, Talley NJ, Leemon M, *et al.* Prevalence of gastrointestinal symptoms associated with diabetes mellitus: a population-based survey of 15,000 adults. *Arch Intern Med* 2001;161:1989–96.
- Keller J, Bassotti G, Clarke J, *et al.* Expert consensus document: advances in the diagnosis and classification of gastric and intestinal motility disorders. *Nat Rev Gastroenterol Hepatol* 2018;15:291–308.
- Boland BS, Edelman SV, Wolosin JD. Gastrointestinal complications of diabetes. *Endocrinol Metab Clin North Am* 2013;42:809–32.
- Wo JM, Nowak TV, Waseem S, *et al.* Gastric electrical stimulation for gastroparesis and chronic unexplained nausea and vomiting. *Curr Treat Options Gastroenterol* 2016;14:386–400.
- Giudicessi JR, Ackerman MJ, Camilleri M. Cardiovascular safety of prokinetic agents: a focus on drug-induced arrhythmias. *Neurogastroenterol Motil* 2018;30:e13302.
- Drewes AM, Munkholm P, Simrén M, *et al.* Definition, diagnosis and treatment strategies for opioid-induced bowel dysfunction—Recommendations of the Nordic Working group. *Scand J Pain* 2016;11:111–22.
- Azpiroz F, Malagelada JR. Importance of vagal input in maintaining gastric tone in the dog. *J Physiol* 1987;384:511–24.
- Berthoud H-R, Hennig G, Campbell M, *et al.* Video-based spatio-temporal maps for analysis of gastric motility in vitro: effects of vagal stimulation in guinea-pigs. *Neurogastroenterol Motil* 2002;14:677–88.
- Frøkjær JB, Bergmann S, Brock C, *et al.* Modulation of vagal tone enhances gastrooduodenal motility and reduces somatic pain sensitivity. *Neurogastroenterol Motil* 2016;28:592–8.
- Olofsson PS, Rosas-Ballina M, Levine YA, *et al.* Rethinking inflammation: neural circuits in the regulation of immunity. *Immunol Rev* 2012;248:188–204.
- Brock C, Brock B, Aziz Q, *et al.* Transcutaneous cervical vagal nerve stimulation modulates cardiac vagal tone and tumor necrosis factor- α . *Neurogastroenterol Motil* 2017;29:e12999.
- Rasmussen SE, Pfeiffer-Jensen M, Drewes AM, *et al.* Vagal influences in rheumatoid arthritis. *Scand J Rheumatol* 2018;47:1–11.
- Koopman FA, van Maanen MA, Vervoordeldonk MJ, *et al.* Balancing the autonomic nervous system to reduce inflammation in rheumatoid arthritis. *J Intern Med* 2017;282:64–75.
- Bonaz B, Sinniger V, Hoffmann D, *et al.* Chronic vagus nerve stimulation in Crohn's disease: a 6-month follow-up pilot study. *Neurogastroenterol Motil* 2016;28:948–53.
- Kinfe TM, Pinteá B, Muhammad S, *et al.* Cervical non-invasive vagus nerve stimulation (nVNS) for preventive and acute treatment of episodic and chronic migraine and migraine-associated sleep disturbance: a prospective observational cohort study. *J Headache Pain* 2015;16:101.
- ElectroCore. Instructions for use for gammaCore sapphire, 2018. Available: <https://www.gammacore.com/wp-content/themes/gammacore-p2/pdf/gammacore-IFU.pdf> [Accessed 4 April 2021].
- Nilsson M, Poulsen JL, Brock C, *et al.* Opioid-Induced bowel dysfunction in healthy volunteers assessed with questionnaires and MRI. *Eur J Gastroenterol Hepatol* 2016;28:514–24.
- Lelic D, Brock C, Simrén M, *et al.* The brain networks encoding visceral sensation in patients with gastrointestinal symptoms due to diabetic neuropathy. *Neurogastroenterol Motil* 2014;26:46–58.
- Kabadi A, Saadi M, Schey R, *et al.* Taste and smell disturbances in patients with gastroparesis and gastroesophageal reflux disease. *J Neurogastroenterol Motil* 2017;23:370–7.
- Verrengia M, Sachdeva P, Gaughan J, *et al.* Variation of symptoms during the menstrual cycle in female patients with gastroparesis. *Neurogastroenterol Motil* 2011;23:625–e254.
- Thijssen AY, Mujagic Z, Jonkers DMAE, *et al.* Alterations in serotonin metabolism in the irritable bowel syndrome. *Aliment Pharmacol Ther* 2016;43:272–82.
- Jones KL, Russo A, Stevens JE, *et al.* Predictors of delayed gastric emptying in diabetes. *Diabetes Care* 2001;24:1264–9.
- Ejskjaer N, Fleischer J, Fleischer J, *et al.* A pocket-size device to detect autonomic neuropathy. *J Diabetes Sci Technol* 2008;2:692–6.
- Gulichsen E, Fleischer J, Ejskjaer N, *et al.* Screening for diabetic cardiac autonomic neuropathy using a new handheld device. *J Diabetes Sci Technol* 2012;6:965–72.
- D'Amato C, Greco C, Lombardo G, *et al.* The diagnostic usefulness of the combined COMPASS 31 questionnaire and electrochemical skin conductance for diabetic cardiovascular autonomic neuropathy and diabetic polyneuropathy. *J Peripher Nerv Syst* 2020;25:44–53.
- Sletten DM, Suarez GA, Low PA, *et al.* Compass 31: a refined and abbreviated composite autonomic symptom score. *Mayo Clin Proc* 2012;87:1196–201.
- Greco C, Di Gennaro F, D'Amato C, D'Amato C, *et al.* Validation of the composite autonomic symptom score 31 (COMPASS 31) for the assessment of symptoms of autonomic neuropathy in people with diabetes. *Diabet Med* 2017;34:834–8.
- NICE. Interventional procedure overview of vagus nerve stimulation for treatment-resistant depression, 2009. Available: <https://www.nice.org.uk/guidance/ipg330/documents/vagus-nerve-stimulation-for-severe-depression-interventional-procedures-overview-2> [Accessed 4 April 2021].
- Revicki DA, Camilleri M, Kuo B, *et al.* Development and content validity of a gastroparesis cardinal symptom index daily diary. *Aliment Pharmacol Ther* 2009;30:670–80.
- Hutchings HA, Cheung W-Y, Russell IT, *et al.* Psychometric development of the gastrointestinal symptom rating questionnaire (GSRQ) demonstrated good validity. *J Clin Epidemiol* 2015;68:1176–83.
- Charles M, Fleischer J, Witte DR, *et al.* Impact of early detection and treatment of diabetes on the 6-year prevalence of cardiac autonomic neuropathy in people with screen-detected diabetes: ADDITION-Denmark, a cluster-randomised study. *Diabetologia* 2013;56:101–8.
- Kempler P, Amarenco G, Freeman R, *et al.* Management strategies for gastrointestinal, erectile, bladder, and sudomotor dysfunction in patients with diabetes. *Diabetes Metab Res Rev* 2011;27:665–77.
- Casellini CM, Parson HK, Richardson MS, *et al.* Sudoscan, a noninvasive tool for detecting diabetic small fiber neuropathy and autonomic dysfunction. *Diabetes Technol Ther* 2013;15:948–53.
- Frøkjær JB, Graversen C, Brock C, *et al.* Integrity of central nervous function in diabetes mellitus assessed by resting state EEG frequency analysis and source localization. *J Diabetes Complications* 2017;31:400–6.
- Arendt-Nielsen L, Andresen T, Malver LP, *et al.* A double-blind, placebo-controlled study on the effect of buprenorphine and fentanyl on descending pain modulation: a human experimental study. *Clin J Pain* 2012;28:623–7.
- Leelarathna L, Wilmot EG. Flash forward: a review of flash glucose monitoring. *Diabet Med* 2018;35:472–82.
- Farmer AD, Wegeberg A-ML, Brock B, *et al.* Regional gastrointestinal contractility parameters using the wireless motility capsule: inter-observer reproducibility and influence of age, gender and study country. *Aliment Pharmacol Ther* 2018;47:391–400.
- Busch V, Zeman F, Heckel A, *et al.* The effect of transcutaneous vagus nerve stimulation on pain perception – an experimental study. *Brain Stimul* 2013;6:202–9.
- Ellrich J, Professor of Medical Physiology, Department of Health Science and Technology, Medical Faculty, Aalborg University and Chief Medical Officer, Medical Department, cerbomed GmbH. Transcutaneous vagus nerve stimulation. *Eur Neurol Rev* 2011;6:254–6.