RESEARCH PROTOCOL

The effect of non-invasive transauricular vagus nerve stimulation on upper gastrointestinal motility in healthy individuals

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PROTOCOL TITLE 'The effect of non-invasive transauricular vagus nerve stimulation on upper gastrointestinal motility in healthy individuals'.

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	General Assessment and Registration form (ABR form), the application form that is required for submission to the accredited Ethics Committee; in Dutch: Algemeen Beoordelings- en Registratieformulier (ABR-formulier)
AE	Adverse Event
AR	Adverse Reaction
CA	Competent Authority
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
CV	Curriculum Vitae
DSMB	Data Safety Monitoring Board
EU	European Union
EudraCT	European drug regulatory affairs Clinical Trials
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)
HR mapping	High Resolution mapping
IB	Investigator's Brochure
IC	Informed Consent
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
METC	Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)
MMC	Migrating motor complex
(S)AE	(Serious) Adverse Event
SPC	Summary of Product Characteristics; in Dutch: officiële productinformatie IB1-tekst
Sponsor	The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.
SUSAR	Suspected Unexpected Serious Adverse Reaction
tVNS	Transcutenous vagal nerve stimulation
UAVG	Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG
WMO	Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen

SUMMARY

Rationale: Although motor function in the GI tract is intrinsically regulated by pacemaker cells of the enteric nervous system (1), there is a strong extrinsic influence via the efferent vagus. Motor responses in the upper GI tract, which is innervated by the vagus nerve, are characterized by stereotypical motility patterns called the migrating motor complex (MMC). Gastrointestinal rumbling, which is mainly noticed during fasting, is associated with the MMC. The MMC is an aborally propagating contractility complex during the fasting state, characterized by three different phases: phase I is a period of motor quiescence, phase II displays progressively increasing motor activity and phase III has the highest contractile activity and can start in either the stomach or small intestine. The MMC moves from the stomach to the terminal ileum over a period of 1.5–2.0 hours and is interrupted by nutrient intake, after which the motor response switches to a less coordinated 'fed pattern,' which usually lasts at least 2 hours after meal ingestion before the MMC returns (2). The vagus nerve plays an important role in the initiation of MMC that originate from the stomach, as vagotomy was shown to block MMCs. In addition, acute stress has been shown to inhibit MMCs (3).
Whether a disturbance in upper GI tract motility has a role in the development of abdominal symptoms remains a subject of debate. In this study, we aim to investigate whether tVNS can induce phase III contractions as a result of stimulation of the vago-vagal intestinal reflex loop in healthy subjects.

Objective: *Primary objective:* To study the impact of tVNS on gut motor function, with regards to its potential to induce phase III contractions in the upper GI tract, in healthy subjects.

Hypothesis: We hypothesize that tVNS will induce on average an excess of 1 additional phase III contraction of antral origin in the fasted period compared to sham stimulation.

Secondary objective:

1. To study the effect of tVNS on other parameters characterizing upper GI motility such as the number of duodenal phase III contractions, gastric and duodenal phase MMCs, amplitude of antral contractions and motility index
2. To study the effect of tVNS on plasma levels of motilin and pancreatic polypeptide.
3. To evaluate the effect of tVNS on parameters related to autonomic outflow, using a pulse plethysmography for heart rate, and a Shimmer3 GSR Sensor for heart rate variability and skin conductance.
4. To compare the accuracy of the measurements of autonomic parameters performed by pulse plethysmography versus Shimmer3 GSR Sensor.

To compare the results of the measurements of antroduodenal manometry with measurements of Gastric Alimetry**Study design:** intervention study.

Study population: 12 healthy participants, aged 18-55 years old.

Intervention: transcutaneous vagal nerve stimulation to the cymba concha of the left ear, alternated with sham stimulation with a non-conducting electrode.

Main study parameters/endpoints: A clinically meaningful increase in the number of phase III contractions in the fasted period during the tVNS stimulation compared to the sham stimulation. Motor responses of the upper GI tract will be measured by antroduodenal manometry (current gold standard in clinical practice). Additionally, measuring GI hormones like motilin and pancreatic polypeptide (PP) provides insights into vagal efferent influence.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: Participants will not benefit from participating in this study.
Insertion of the antroduodenal manometry catheter can be uncomfortable, but in most patients this feeling disappears after a few minutes. After the manometry patients can have a slight irritation of the nose or throat. The radiation exposure associated with fluoroscopy during the positioning of the catheter is minimal, with a total radiation exposure of approximately 0.05-1 mSv.
The collection of blood samples can bring along some slight risks, i.e. hematoma, bleeding, pain and/or vasovagal reaction during or after vena punction. Subjects will be seated on an examination bed, to prevent side effects to occur.
Participation in this study will cost some time. Subjects must visit the hospital twice. The screening will take around 30-45 minutes. And the second visit will take around 9-10 hours.

1 INTRODUCTION AND RATIONALE

The gastrointestinal (GI) tract is a highly complex system responsible for the digestion, absorption, and propulsion of ingested food. Although the motor function in the GI tract is intrinsically regulated by pacemaker cells of the enteric nervous system (1), there is a strong extrinsic influence via the efferent vagus. Motor responses in the upper GI tract, which is innervated by the vagus nerve, are characterized by stereotypical motility patterns called the migrating motor complex (MMC). Gastrointestinal rumbling, which is mainly noticed during fasting, is associated with the MMC. The MMC is an aborally propagating contractility complex during the fasting state, characterized by three different phases: phase I is a period of motor quiescence, phase II displays progressively increasing motor activity and phase III has the highest contractile activity and can start in either the stomach or small intestine. The MMC moves from the stomach to the terminal ileum over a period of 1.5–2.0 hours and is interrupted by nutrient intake, after which the motor response switches to a less coordinated ‘fed pattern’, which usually lasts at least 2 hours after meal ingestion before the MMC returns.(2) Dysfunctions in the MMC have been associated with motility disorders like gastroparesis and functional dyspepsia, contributing to distressing symptoms such as abdominal pain, bloating, and nausea.(2)
MMCs can originate both from the stomach and the duodenum. The vagus nerve plays an important role in the initiation of MMC that originate from the stomach, as vagotomy was shown to block MMCs. In addition, acute stress has been shown to inhibit MMCs.(3) Whether a disturbance in upper GI tract motility has a role in the development of abdominal pain symptoms remains a subject of debate.

Given the significance of the MMC in maintaining healthy GI function and the role of the vagus nerve in its regulation, exploring the potential of transcutaneous vagal nerve stimulation (tVNS) to enhance or restore MMC activity becomes a compelling avenue for therapeutic intervention. Over the past few decades, techniques that modulate autonomic tone have been investigated as potential methods to modify autonomic nervous system tone in clinical disorders, so-called ‘autonomic neuromodulation’. One of these methods that has recently received increased attention is the non-invasive tVNS, consisting of small electrodes that interface with the concha of the outer ear. This area corresponds to the only place on the surface of the human body where there is afferent vagal innervation.(4) tVNS is safe, well-tolerated and user-friendly.(5) Studies have pointed towards the beneficial effects of tVNS in depression, epilepsy and tinnitus, among others, but firmly establishing its therapeutic efficacy remains warranted.(6) In addition, mechanistic evidence supporting the therapeutic potential of tVNS is still lacking. Therefore, tVNS is not yet ready for application in routine clinical practice for any of these conditions.

The question therefore arises whether tVNS can influence motor responses in the upper gastrointestinal tract and whether such changes can be detected. Motility of the upper gastrointestinal tract is routinely measured using an antroduodenal manometry. This diagnostic technique involves the insertion of a catheter to measure pressure changes in the antrum and duodenum, allowing us to monitor the patterns of muscular contraction.

Besides measuring motility responses, measurement of the GI hormones motilin and PP can be used as surrogate markers of vagal efferent influence on GI motor function. The release of PP is vagally mediated, and the principal regulatory mechanism is the vagal cholinergic stimulation of the antrum. It is of importance that the same efferent branches of the vagal nerve that mediate PP release also control antral and pyloric motility. In particular, an early response in PP release in the first 10-30 minutes following meal intake is vagally-mediated. This way, measuring the levels of PP can be indicative of vagal function and has been also applied by our group previously (7). This test is still infrequently used in clinical practice to test for vagus nerve integrity, which was more popular in the past when performing vagotomy was a customary way to treat peptic ulcer disease (8). Plasma motilin levels characteristically fluctuate in accordance with the occurrence of the MMC, with peak motilin levels observed at the start of phase III contractions. Plasma levels of motilin increase by 35% from phase I to phase III if phase III has a gastric origin (9). Therefore, motilin levels can be used as surrogate markers for gastrointestinal motor patterns (9). In addition, Electrical stimulation of the vagus results in a significant increase in the plasma motilin concentration in anesthetized dogs.(10)

This research seeks to investigate the efficacy, safety, and underlying mechanisms of tVNS in stimulating the MMC, in particular the phase III contractions. By shedding light on the potential benefits of tVNS in influencing MMC activity, this study endeavours to offer valuable insights into the development of innovative therapies for GI motility disorders, ultimately leading to improved quality of life for patients afflicted by such conditions.

Specifically, our primary interest is in gastric phase III contractions. Gastric phase III, but not intestinal phase III, is abolished by blockade of the cervical vago-sympathetic nerve trunk in conscious dogs in vivo. As sympathetic receptor blockers do not affect the inhibitory effect of vagal blockade, gastric phase III seems to be regulated by vagus nerve. Spontaneous phase III contractions after vagotomy are also less potent than that of before vagotomy. Therefore, it is generally accepted that motilin-induced gastric phase III is vagal dependent in a physiological condition.(10)

2 OBJECTIVES

Primary objective: To study the impact of tVNS on gut motor function, with regards to its potential to induce phase III contractions in the upper GI tract, in healthy subjects.
Primary hypothesis: we hypothesize that tVNS will induce on average an excess of 1 additional phase III contraction of antral origin in the fasted period compared to sham stimulation.

Secondary objective:

1. To study the effect of tVNS on other parameters characterizing upper GI motility such as the number of duodenal phase III contractions, gastric and duodenal phase MMCs, amplitude of antral contractions and motility index

Hypothesis: we hypothesize that tVNS will increase the number of duodenal phase III contractions, the number of antral and duodenal MMCs, the amplitude of antral contractions and the motility index.

2. To study the effect of tVNS on plasma levels of motilin and PP

Hypothesis: we hypothesize that plasma motilin will increase parallel with the occurrence of a MMC and PP will increase when tVNS is applied.

3. To evaluate the effect of tVNS on parameters related to autonomic outflow, using a pulse plethysmography for heart rate, and a Shimmer3 GSR Sensor for heart rate variability and skin conductance.

Hypothesis: We hypothesize that treatment with tVNS will lead to significant changes in the autonomic outflow reflective of an increase in parasympathetic activity.

4. To compare the accuracy of the measurements of autonomic parameters performed by pulse plethysmography versus Shimmer3 GSR Sensor

Hypothesis: we hypothesize the accuracy of measurements is comparable.

5. To compare the results of the measurements of antroduodenal manometry with measurements of Gastric Alimetry

Hypothesis: we hypothesize that Gastric Alimetry can detect phase III contractions on the same level as an antroduodenal manometry.

3 STUDY DESIGN

This is an intervention study with 12 healthy volunteers, aged 18-55 years old.

Visit 1 (screening visit)

Recruitment procedures are described in paragraph 11.2. Following written informed consent, the medical history of each subject will be checked during a structured interview. A pregnancy will be excluded by a urine test (due to the use or fluoroscopy during insertion of the manometry catheter) and a 12-lead ECG will be performed at the end of the screening visit. The rationale for this is to ensure no evidence of cardiac conduction disorders and an additional safety step to ensure no cardiac dysrhythmic affect with tVNS use. All information obtained during the screening visit will be recorded in using an electronic CRF provided by Castor EDC, provided by the Clinical Trial Centre Maastricht (CTCM).

Visit 2

Participants will arrive after an overnight fast at the GI physiology unit of the Maastricht University Medical Center, where a trained and experienced nurse will insert an antroduodenal manometry catheter through the nose into the duodenum under fluoroscopy control and supervision of a gastroenterologist (as per routine clinical procedure). Using this catheter, stomach and duodenum motility patterns will be recorded for 8 hours. After the manometry is inserted, the tVNS device will be connected with an electrode which delivers stimulation transcutaneous in the outer ear. Participants will receive two types of stimulation (i.e. tVNS vs sham) in 2 blocks of 4 hours during the 8-hour registration period. The order of stimulation applied is randomized. Gastric phase III contractions generally occur every 90 minutes, so the 4-hour period is considered sufficient to detect two phase III contractions considered sufficient to detect the two phase III contractions.(10)

An iv catheter will be inserted to draw blood during the 8-hour registration period. Blood samples, each witch a maximum volume of 10 mL, will be taken every 30 minutes to measure motilin and PP as surrogate markers of vagal efferent influence on GI motor function. In addition, autonomic parameters will be registered using pulse plethysmography and a wearable (Shimmer3 GSR sensor, see under 8.3.1) during the entire test period. After the 8-hour test period, the manometry catheter, the electrodes of the tVNS device and the iv cannula are removed.

Additionally, participants may opt to take part in a body surface gastric mapping assessment. This non-invasive, electrophysiological measurement is performed using the Gastric Alimetry system, which involves placing a large adhesive patch with multiple electrodes on the upper abdomen. The system records gastric myoelectrical activity over time, providing insight into gastric motility patterns.The measurement is conducted concurrently with the manometry registration period. This device is already being used in non-WMO study METC 2022-3541 and METC 23-048.

4 STUDY POPULATION

4.1 Population

Healthy volunteers (n=12) will be recruited, both male and female, aged aged 18-55 (to eliminate potential effects of ageing on GI motility).

4.2 Inclusion criteria

To be eligible to participate in this study, a subject must meet all the following criteria:

- Healthy participants (defined as those without a pre-existing medical comorbidity, including those without a history of cardiac arrhythmia).
- Age between 18 and 55 years.
- Ability to understand and speak the Dutch language.

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Pregnancy or lactation.
- Use of any substances (such as medication or recreational drugs) influencing gastrointestinal motility
- Not meeting the inclusion criteria above.
- Students and employees of Maastricht University are not precluded from participation, unless they have a direct personal, professional of hierarchical position with regards to any of the study team members or their department.

4.4 Sample size calculation

Our primary outcome is an increase of 1 phase III contraction in the tVNS group compared to the sham group. Previous research in healthy adults showed a mean of 2.03 phase III contractions with a standard deviation of 1.1 (11). The sample size calculation was based on a paired-sample t-test (tVNS vs sham in the fasted condition) with a mean difference of 1 and a standard deviation of 1.1. The following values were applied: significance level (α two sided) = 0.05; power = 0.80. Based on this calculation, 12 participants are needed. We will enroll participants until we reach a total of 12 who successfully complete the study. The calculation can be found at the following web address:
https://homepage.univie.ac.at/robin.ristl/samplesize.php?test=pairedttest

5 TREATMENT OF SUBJECTS

In this study, the impact of tVNS on gut motor function is compared to sham stimulation.

5.1 Investigational product

tVNS

A custom-adjusted device named tVNS R (tVNS Health GmbH, Grünwald, Germany) will deliver electric signals to the cymba conchae of the left ear. The left side is based on the fact that the stomach and the pyloric area is innervated by the left vagus (12, 13). This device has previously been used in the RESILIENCE study (NL84720.068.23/METC 23-032). The device consists of a stimulation unit and an ear electrode that is worn like an earphone. The stimulation unit sends electrical impulses through the electrode, which stimulates a branch of the vagus nerve transcutaneously in the auricle. Subjects will use the device during the 8-hour registration period, in 4-hour blocks. Stimulation parameters will be rectangular pulse wave in biphasic signal form, impulse duration 30 s, impulse pause 30 s, impulse frequency 25 Hz, based on the most-optimal treatment frequencies for tVNS as per recent systematic analyses.(14, 15) Each participant will undergo sensory testing prior to the commencement of the treatment period and the threshold for perception will be determined in each individual. The stimulation amplitude (current intensities) can be varied between 0.25 and 10 mA and will be set to a perception level in which the stimulation intensity is barely subliminal (this is to ensure proper blinding). The device will blink when the stimulation is delivered. During sham stimulation, the same location of the electrodes will be used, but the electrode will be non-conducting so there will be no stimulation of the vagus nerve. The device will still blink to assure blinding. (15) An independent investigator will label the electrodes as either 'A' or 'B' for the intervention and sham electrodes randomly. This procedure ensures that the investigator conducting the analysis remains blinded. The randomization result will indicate which electrode, 'A' or 'B', to begin with.

5.2 Use of co-intervention

Subjects arrive after an overnight-fast and are maintained in a fasting state during the intervention.

5.3 Escape medication

Not applicable.

6 INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational product(s)

In this clinical investigation we will use tVNS R. tVNS L already has a declaration of conformity. However, tVNS R and tVNS L are completely equivalent concerning all electrical as well as mechanical aspects. The only difference is the software of the products. Where in the tVNS L device the stimulation parameters are fixed, these can be changed in the tVNS R for research purposes. See annex 'D2_Declaration_of_conformity_tVNS_L_signed, versie 1.0 dd 31-05-2021' and 'D4_Confirmation_ of_equivalence_tVNS, versie 1.0 dd 22-10-2021'. Because the tVNS device will be used outside the intended use of the declaration of conformity this investigation falls under article 82 of the MDR.

The tVNS R is a vagus nerve stimulation device. The device consists of a stimulation unit and an ear electrode that is worn like an earphone. The stimulation unit sends electrical impulses through the electrode, which stimulates a branch of the vagus nerve transcutaenously (through the skin) in the auricle. See 5.1 for further description. The device also records the amount of time it was used to delivered electrical stimulation. This parameter will be used to assess compliance to use. This exact same device was previously used by our study group in RESILIENCE (NL84720.068.23/METC23-032).

6.2 Summary of findings from non-clinical studies

Animal studies have shown that transcutaneous vagus nerve stimulation can reduce systemic tumor necrosis factor levels and increase survival in mice with lethal endotoxemia (sepsis).(16) Furthermore, transcutaneous vagus nerve stimulation in rats with transient focal ischemia revealed activation in the locus coeruleus and reduction of the volume of the infarct zone.(17) In healthy humans, transcutaneous vagus nerve stimulation has been shown to be a safe and well-tolerated procedure, specifically in patients without a history of cardiac disease.(18-20) Heart rate and blood pressure are not affected. Most likely, transcutaneous vagus nerve stimulation has a similar mechanism as vagus nerve stimulation, activating the locus coeruleus (and noradrenalin) and increasing GABA levels.(20) Healthy participants undergoing transcutaneous vagus nerve stimulation reported reduced pain levels compared to the sham condition.(21) Sukasem et al. demonstrated the effects of tVNS on the gastric slow waves in the pig stomach, evaluated by applying high-resolution (HR) mapping from the serosa of the stomach. The main finding was that the characteristics of the slow waves changed significantly in the subjects that responded to the stimulation protocols, but the effects were not consistently observed in all the subjects studied (22). Calder et al. proved that BSGM, when analysed with the specific algorithms, is a suitable method for noninvasively measuring the frequency and propagation of gastric slow waves (23).

6.3 Summary of findings from clinical studies

Transcutaneous vagus nerve stimulation has been successfully applied in a controlled trial involving patients with epilepsy, where a decrease in seizure frequency and an improvement

in EEG measures, depressive symptoms, anxiety and quality of life have been observed (24-26). A pilot study showed that transcutaneous vagus nerve stimulation improves mood and decreased handicap scores in patients with tinnitus. (27) A recent systematic review and meta-analysis showed that tVNS is a promising method on reducing the number of migraine days and headache intensity.(28) tVNS was proven effective in normalizing gastric dysrhythmias by a 5-minute water-load test in healthy subjects, evaluated by BSGM (29).

6.4 Summary of known and potential risks and benefits

Since tVNS is a non-invasive method, there are no serious risks mentioned in the literature. In a randomized controlled trial done with adolescents using a more invasive method with percutaneous electrical nerve field stimulation, eight individuals discontinued treatment because of aesthetic reasons, an uncomfortable fit of the device, needle phobia or anxiety, and adhesive allergy.(30) Since in our current study ear electrodes are used which fit perfectly without any plasters and can stimulate the vagus nerve in a non-invasive transcutaneous manner, the last two reasons mentioned in the previous study are less relevant for this study. Furthermore, patients' hearing might be slightly impaired on the side on which the electrode is worn. A recent systematic review and meta-analysis showed no severe side effects. Mild dizziness, headache and slight redness around the stimulation area which disappeared in a short period after ceasing intervention were reported.(31)
The potential and most important, beneficial effect for the patient can be a decrease in abdominal pain after using tVNS.

6.5 Description and justification of route of administration and dosage

Not applicable

6.6 Dosages, dosage modifications and method of administration

Not applicable

6.7 Preparation and labelling of Investigational Medicinal Product

Not applicable

6.8 Drug accountability

Not applicable

7 NON-INVESTIGATIONAL PRODUCT

Not applicable

8 METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameter/endpoint

A clinically meaningful increase in the number of phase III contractions of antral origin in the fasted period during the tVNS stimulation compared to the sham stimulation.

8.1.2 Secondary study parameters/endpoints

- Plasma levels of motilin and pancreatic polypeptide
- Parameters determined by antroduodenal manometry and BSGM as previously described by our group.(32)
    - Antral and duodenal contraction frequency,
    - Contraction amplitude,
    - Motility index: MI=ln((contractionfrequency∗amplitude)+1),
    - Number of duodenal phase II contractions
    - Number of MMCs.
- The association between autonomic parameters and motility patterns
- Number and severity of adverse events
- Autonomic parameters measured by pulse plethysmography and Shimmer3 GSR Sensor: heart rate variability as determined by RMSSD (Root mean square of the successive differences) and skin conductance

8.1.3 Other study parameters

- Patient characteristics: sex, age, lifestyle factors

8.2 Randomisation, blinding and treatment allocation

After written informed consent has been given and the participant has successfully completed the screening visit, the coordinating investigator will then randomise the participant in Castor EDC. Randomisation will be used to decide the order in which stimulation or sham stimulation will be given. The ear electrodes of the active group and the sham group will be placed anatomically at the same location, so neither the participant nor the investigators will know who is getting active or sham stimulation. In both groups, the device will also blink to assure blinding. The only difference is that the sham group receives devices with a non-conducting electrode. Two concealment lists will be generated by the CTCM and provided to a co-worker of the same department as the coordinating investigators. For emergencies, drs. Sweerts has been provided with the concealment list and will be able to de-blind. The concealment list provides information on the order in which participants receive sham stimulation or tVNS. This information will not be given to the investigators.

8.3 Study procedures

8.3.1 Visit 1: screening visit and informed consent

The screening will take place at the “Metabolic Research Unit” of Maastricht University. At the first visit, all participants will be asked to conform that they have read the information letter, have received oral information and whether they have any additional queries regarding the study. The investigator makes sure the participant understood all the information with an interview. Afterwards, the participant is requested to complete written informed consent prior to any study procedure. Following inclusion, the medical history of each subject will be checked during a structured interview and subjects will be screened for any disease related symptoms by completing general questionnaire ‘F1.1’, with the support of Castor EDC. A pregnancy will be excluded by a urine test and a 12-lead ECG will be performed.

8.3.2 Visit 2: test day

Participants will arrive after an overnight fast at the GI physiology unit (‘functiekamer’) of the Maastricht University Medical Center, where they will be inserted an antroduodenal manometry catheter through the nose into the duodenum under fluoroscopy control. The high-resolution catheter contains 36 transducers spaced at 1-cm intervals (Unisensor AG, Attikon, Switzerland). Using this catheter, stomach and duodenum motility patterns will be recorded for 8 hours.

Participants will undergo tVNS (cymba concha of the left ear) alternated with sham (a non-conducting electrode) in a blinded pre-randomized fashion using 2 blocks of 4 hours during the 8-hour registration period. In addition, blood samples will be taken at baseline and at intervals of 30 minutes during the 8-hour test period. Levels of the GI hormones motilin and PP will be measured as surrogate markers of vagal efferent influence on GI motor function.

Autonomic parameters will be registered using pulse plethysmography. A wearable device (Shimmer3 GSR Sensor) will also be used to measure heart rate variability and skin conductance. Heart rate variability is regarded as a parameter reflecting cardiac parasympathetic activity.(33, 34) The root mean square of successive differences (RMSSD) is a commonly utilized and thoroughly validated measure of heart (and pulse) rate variability. (35, 36).

After the 8-hour registration period, all measurement equipment and the iv. cannula is removed. Participants are provided a meal voucher to be consumed at the hospital visitor’s restaurant, whereafter the test day is finished.

Optionally, participants may also undergo body surface gastric mapping using the Gastric Alimetry system (Alimetry Ltd, New Zealand). This non-invasive, electrophysiological technique involves applying a large adhesive array with multiple electrodes to the upper abdomen to record gastric myoelectrical activity. The measurement runs in parallel with the manometry procedure. Participants are instructed to remain still during the recording to ensure signal quality. Participation in this component is voluntary and does not impact inclusion in the primary study protocol.

8.3.3 Questionnaires

Subjects will be asked to complete one questionnaire during the screening visit.

See ‘F1.1_Algemene_Vragenlijst, versie 1.0 dd 2024-03-11’2024-03-11’
A questionnaire regarding baseline characteristics, demographics, life-style and comorbidities.

8.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

8.5 Replacement of individual subjects after withdrawal

We anticipated for dropouts within our sample size calculation.

8.6 Follow-up of subjects withdrawn from treatment

Subjects withdrawn from the study will not be followed up, unless it seems necessary in case of any urgent medical reason.

8.7 Premature termination of the study

We do not expect that this study will be terminated prematurely. However, if certain urgent medical problems occur during the study the investigator (if necessary, together with METC or CCMO) can decide to halt the study to check whether it is safe to proceed or that termination is preferred.

9 SAFETY REPORTING

9.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs

9.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether considered related to the investigational product or trial procedure. All adverse events reported spontaneously by the subject or observed by the investigator, or his staff will be recorded during the test day.

9.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

The investigator will report all SAEs to the sponsor without undue delay after obtaining knowledge of the events. The sponsor will report the SAEs through the web portal ToetsingOnline to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

9.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Not applicable

9.3 Annual safety report

Not applicable

9.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported till end of study within the Netherlands, as defined in the protocol.

9.5 [Data Safety Monitoring Board (DSMB) / Safety Committee]

Not applicable.

10 STATISTICAL ANALYSIS

Demographic data and patient-reported data will be with the help of Castor EDC. Database cleaning will be carried out by internal consistency checks and identification of database entries outside expected ranges by the study coordinator and the local research nurses. Primary analysis will be both intention-to-treat (ITT) and per-protocol (PP). SPSS, R, Python, and Excel will be used to perform the statistical analysis.

10.1 Primary study parameter(s)

The analysis will begin by assessing the baseline characteristics of the participants, including demographic variables. Dichotomous variables will be analysed using the Chi-square test. To determine the impact of tVNS on phase III contractions, the occurrence and frequency of phase III contactions during active tVNS and sham stimulation phases will be compared. If the continuous variables are normally distributed an independent sample t-test will be done. A p-value of <0.05 will be considered statistically significant. Furthermore, logistic regression analysis will be used to evaluate potential predictors of phase III contactions occurrence during tVNS.

10.2 Secondary study parameter(s)

The effect of tVNS vs sham on other motility parameters will be analyzed in identical manner to the primary outcome.

To study the effect of tVNS on plasma levels of motilin and PP, the change in hormone levels during active tVNS and sham stimulation will be analyzed. A linear mixed model will be used to assess temporal associations between motor and autonomic responses, and gut hormones. The same methods will be used to compare the autonomic responses as obtained using pulse plethysmography vs Shimmer3 GSR sensor.

Considering the exploratory nature of the secondary outcomes, no formal correction for multiple testing will be applied.

10.3 Other study parameters

Not applicable.

10.4 Interim analysis

Not applicable.

11 ETHICAL CONSIDERATIONS

11.1 Regulation statement

The study will be conducted according to the Declaration of Helsinski (last version October 2013, Brazil), the Medical Research Involving Human Subjects Act (WMO), the FDA guidelines for the conduct of clinical trials and article 82 of the Medical Device Regulation (MDR).

11.2 Recruitment and consent

Healthy volunteers will be recruited via advertisements in local and faculty newspapers, social media and public spaces using document 'E3_wervingsadvertentie, versie 1.0 dd 2024-03-19'. No vulnerable individuals will be included. Students and employees of Maastricht University are not precluded from participation, unless they have a direct personal, professional or hierarchical position with regards to any of the study team members or their department. When a volunteer contacts the study team by telephone, detailed explanation of the study will be provided first. In addition, the written information brochure will be sent by regular mail or email. In the e-mail containing the information brochure, the volunteer is asked to contact the study team when he or she wishes to participate, after a minimum time of consideration of 7 days. If a volunteer contacts the study team by e-mail without providing their telephone number, the written information brochure will be provided without requesting the telephone number. In case the volunteer has not received any oral information first, this will be given, provided the volunteer discloses her telephone number, and the consideration time of 7 days will commence anew, i.e. after the potential participant has received both written and oral information. If the participant does not reply to the email with the information brochure, a maximum of 1 reminder will be sent. The volunteer can call drs. Hawinkels, or send a confirmation e-mail (providing telephone number, after which she will be contacted by drs. Hawinkels). If the volunteer is interested, an appointment will be made for visit 1 to sign the informed consent and to carry out a medical screening.

Prior to the commencement of the screening visit, the researcher will verify that the potential participant has received and understood all information regarding the study. Any outstanding queries will be answered. To ensure that the participant has fully understood the information, the researcher will ask the participant to explain in their own words what the study involves and what is expected of them. After this explanation, all participants will have to sign an informed consent before screening can commence.

11.3 Objection by minors or incapacitated subjects (if applicable)

Participants under the age of 18 and legally incompetent adults are excluded from the trial.

11.4 Benefits and risks assessment, group relatedness

Although participants will not benefit directly from participating in this study, the risks associated with participation are minor and in proportion to the scientific value of this study. See 6,4 'Summary of known and potential risks and benefits' for previously reported risk of

transcutaneous nerve stimulation. This study can give us more insight in the role of nerve stimulation in the gastric peristalsis. Future studies can investigate this effect in clinical populations.

Insertion of the antroduodenal manometry catheter can be uncomfortable, but in most patients this feeling disappears after a few minutes. After the manometry patients can have a slight irritation of the nose or throat.

The collection of blood samples can bring along some slight risks, i.e. hematoma, bleeding, pain and/or vasovagal reaction during or after vena punction. Subjects will be seated on an examination bed, to prevent side effects to occur.
The application of other measurement instruments Shimmer3 GSR sensor and pulse plethysmography pose no risks.
Participation in this study will cost some time. Subjects must visit the hospital twice. The screening will take around 30-45 minutes. And the second visit will take around 9-10 hours.

11.5 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO. The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study.
The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

11.6 Incentives

Subjects will be compensated for their travel expenses, reimbursing €0,23 per kilometer if travelled by car or a full reimbursement of the costs of public transport. Subjects included in the study will receive €200 euros in addition to a €25 euro voucher for the hospital's restaurant as compensation for their participation. Subjects who withdraw or are deemed non-suitable during the screening visit will be compensated for their travel expenses. Subjects who withdraw during the test day will receive compensation for their travel expenses and a €25 euro voucher for the hospital's restaurant.

12 ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

All obtained data will be handled confidentially and coded to protect the privacy of the participants, in accordance with EU General Data Protection Regulation (Algemene Verordening Gegevensbescherming, 2016) and the Dutch Act on Implementation of the General Data Protection Regulation (Uitvoeringswet Algemene Verordening Gegevensbescherming, 2020).

Data will be handled confidentially and coded and the privacy of the participants will be guaranteed. All data will be coded ("ADM01," "ADM02" and the number allocated to each subject will be based on the order of entrance in the study, 'ADM' standing for 'Antroduodenal Manometry') in such a way that no personal information about the participants is available. The coordinating and principal investigator will keep the code in a locked cabinet, to which only the coordinating and principal investigator have access. In case of inspection, the Dutch Health Care Inspectorate (Inspectie Gezondheidszorg en Jeugd) and the appointed monitor will have access as well.

A data management plan (DMP) will be created in collaboration with an expert from MEMIC. All (coded) data will be stored and analysed at Maastricht University, using a certified database.

The data collection framework will consist of the following elements:

1. Castor EDC for case report forms (CRF): Castor EDC is a web-based tool for collecting (clinical) data. Within the electronic CRF, various information will be recorded, including the inclusion and exclusion criteria, the general questionnaire, the findings of the researcher during the visits, and any adverse events. CRFs will be coded and will not contain personal details of subjects. Castor EDC uses "True", a NEN7510 and ISO 27001:2005 certified server, as per previous studies performed by our group.

2. Shimmer3 GSR sensor: For the Consenys software, an anonymized test subject ID will be used to ensure no traceable data is recorded. All data storage is managed by end users, with Shimmer having no visibility or access to the data. Shimmer adheres to all EU guidelines and directives, ensuring data handling complies with European data protection legislation, including the General Data Protection Regulation (GDPR).

3. Datahub: All data will eventually be stored in Datahub in accordance with the FAIR principles, the EU General Data Protection Regulation (in Dutch: AVG) and the Dutch Act on Implementation of the General Data Protection Regulation (in Dutch: UAVG). Datahub is a MUMC+ initiative designed to support researchers from MUMC+ and Maastricht University in the field of research data management for both clinical and non-clinical studies. Datahub provides a central infrastructure including an institutional repository for storing metadata and research data. Datasets will be archived in Datahub Maastricht. The

datahub infrastructure will ensure that only individuals with the appropriate authorization can upload new data and access existing data.

4. When the BSGM measurement is completed, the data will be sent from the Alimetry reader to the Gastric Alimetry app. The BSGM data will be in the Alimetry Cloud. This data does not contain any personal information besides sex and date of birth. The database is located on a secure server in the UK. As a result, data exchange of the coded data will take place outside the European Economic Area. However, all parties concerned state that the data exchange is in accordance with European data protection legislation, including the General Data Protection Regulation (GDPR). In addition, we will inform the subjects about the data exchange outside the European Economic Area (EEA) what occurs based on the 2 adequacy decisions for the UK, ensuring that the level of data protection is considered equivalent to that of the EAA. We will ask for explicit consent for the data exchange to a country not part of the European Economic Area in the informed Consent form.

All primary documents and data shall be stored for 15 years after the end of the study and are accessible to the principal investigators (Prof. Dr. D. Keszthelyi), coordinating investigator (Drs. Hawinkels), the Dutch Health Care Inspectorate (Inspectie Gezondheidszorg en Jeugd) and monitors assigned by the Clinical Trial Center Maastricht (CTCM). Samples taken from the subject during the study will also be kept for 15 years after the end of the study for possible additional analysis for this study. The label of each sample will contain the individual study ID and the date of collection. The principal investigator and coordinating investigator have access to the stored samples. In the informed consent form, subjects indicate whether they give consent for storing and keeping personal data and biological samples, which may be used for additional analysis in the line of current investigation. If subject deny this consent, personal data and biological samples will only be used for the analysis as described in this protocol and stored for 15 years.

12.2 Monitoring and Quality Assurance

A qualified monitor of the CTCM will monitor the conduct of the study. A monitoring plan will be drafted after the first application to the METC.

12.3 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.

12.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject,

numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.5 Temporary halt and (prematurely) end of study report

The investigator/sponsor will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last participant's last visit. The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action. In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination. Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

12.6 Public disclosure and publication policy

Publication will occur in accordance with the CCMO-statement on publication policy (CCMO-statement publicatiebeleid, 2002).

13 STRUCTURED RISK ANALYSIS

13.1 Potential issues of concern

13.1.1 Concerns related to tVNS

a. Level of knowledge about mechanism of action

The therapeutic potential of tVNS is assumed to be the result of the fact that tVNS can impact the sensory feedback from the body to the brain. tVNS is purported to work through vagal afferents terminating in the nucleus of the solitary tract (NTS), the primary relay station for sensory vagal afferents in the brainstem. NTS in turn has direct or indirect projections to the nuclei providing noradrenergic, endorphinergic, and serotonergic fibers to different parts of the brain.(4) Then, efferent outflow is generated either via efferent vagus via the vago–vagal reflex loop or from the brain to the spinal cord and then the spinal cord via splanchnic nerves towards the intestine and other organs. tVNS has been postulated to be able to restore the missing or altered signalling of intestinal afferents.(37)

b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism

Studies have shown clinical efficacy in depression, migraine, epilepsy and tinnitus, among others. A previous study in adolescents with functional abdominal pain has also shown promising results of vagal neuromodulation.(30)

c. Can the primary or secondary mechanism be induced in animals and/or in *ex-vivo* human cell material?

In dogs, bilateral transthoracic vagotomy was found to selectively block the migrating motor complex (MMC) pattern of the stomach, while leaving the MMC pattern in the small intestine unaffected.(38) Other studies with dogs showed vagal blockade administered either during or before food intake resulted in the abolishment of the typical fed pattern and instead induced motor quiescence in the stomach, accompanied by a vagally induced contraction in the upper small bowel. (39, 40)

d. Selectivity of the mechanism to target tissue in animals and/or human beings

Not applicable

e. Analysis of potential effect

tVNS device is considered safe. Previous studies showed no serious adverse events. See 6.4 ‘summary of known potential risks and benefits’ for previously reported risks.

f. Pharmacokinetic considerations

Not applicable

g. Study population

Healthy volunteers, aged 18-55 years.

h. Interaction with other products
Not applicable

i. Predictability of effect
Not applicable

j. Can effects be managed?
Not applicable

13.1.2 Concerns related to insertion of the antroduodenal manometry catheter (co-intervention)

The introduction of an antroduodenal manometry catheter under fluoroscopy control poses no significant risks, although it may cause discomfort in the nose or throat. Administering 10% lidocaine spray to the nasal mucosa can help minimize these symptoms. The radiation exposure associated with fluoroscopy during the positioning of the catheter is minimal, with a total radiation exposure of approximately 0.05-1 mSv.

13.2 Synthesis

tVNS is considered safe. Serious adverse events have not been reported in studies examining the efficacy of the tVNS device. Mild to moderate adverse events are reported, however they disappear quickly after removing the tVNS device. It is expected that the tVNS will increase the number of MMCs.

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