RESEARCH PROTOCOL

Exploring the efficacy of Transcutaneous Auricular Vagus Nerve Stimulation (taVNS) in alleviating nausea in healthy adults

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PROTOCOL TITLE 'Exploring the efficacy of Transcutaneous Auricular Vagus Nerve Stimulation (taVNS) in alleviating nausea in healthy adults'

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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
BFI	The Big Five Inventory
CA	Competent Authority
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
CRF	Case report form
CRU	Clinical Research Unit
CTCM	Clinical Trial Centre Maastricht
CV	Curriculum Vitae
CVS	Cyclic vomiting syndrome
DMP	Data management plan
DMNV	Dorsal motor nucleus of the vagus
DSMB	Data Safety Monitoring Board
EEG	Electroencephalography
EU	European Union
EudraCT	European drug regulatory affairs Clinical Trials
GABA	Gamma-aminobutyric acid
GAD-7	Generalized Anxiety Disorder 7-Item Scale
GCP	Good Clinical Practice
GCSI	Gastroparesis Cardinal Symptom Index
GDPR	General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)
HRV	Heart Rate Variability
IB	Investigator's Brochure
IBS	Irritable bowel syndrome
IC	Informed Consent
IGJ	Inspectie van Gezondheidszorg en Jeugd
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier

METC	Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)
NTS	Nucleus of the solitary tract
PENFS	Percutaneous electrical nerve field stimulation
PHQ-9	Patient Health Questionnaire (PHQ-9)
(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
TaVNS	Transcutaneous Auricular Vagus Nerve Stimulation
UAVG	Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG
VAS	Visual Analogue Scale
WMO	Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen

SUMMARY

Rationale: Nausea is one of the most common digestive complaints, triggered by stimuli from both the central and peripheral nervous systems. Many patients report a clear relationship between food intake and the onset of nausea. Chemical agents in food can stimulate vagal neurocircuits, which are believed to play a pivotal role in the neurophysiological pathways involved in the nausea response. Transcutaneous auricular vagus nerve stimulation (taVNS) offers a non-invasive approach to modulate central nervous system activity, given the unique access point of the external ear to the vagus nerve. This modulation has the potential to influence various physiological processes related to information transfer between the brain and the body, including the nausea response. Therefore, patients with gastrointestinal conditions who experience nausea may particularly benefit from taVNS treatment. However, the precise physiological impact of taVNS on vagal and autonomic function remains unclear. A deeper understanding of the mechanisms of action for taVNS in healthy individuals is essential for effectively implementing taVNS-based treatment strategies in everyday practice.

Objective: The primary aim of this study is to assess the efficacy of taVNS in reducing nausea in healthy adults subjected to nausea induction through intragastric lipid infusion, compared to sham stimulation, as measured by 0-100 Visual Analogue Scale (VAS) scores. It is hypothesized that taVNS will significantly reduce nausea in terms of both intensity and duration compared to sham stimulation following nausea induction. A responder will be defined as a participant exhibiting a decrease of $\geq$ 25mm on the VAS.

The secondary objectives include evaluating the potential of taVNS to alleviate other gastrointestinal symptoms, such as abdominal pain, bloating, and fullness, as well as exploring its effects on the desire to eat, all measured using 0-100 VAS scores. Additionally, changes in autonomic parameters, plasma levels of ghrelin and motilin, and salivary cortisol will be evaluated. The relationship between the nausea response and affective symptoms, as well as personality traits, will also be explored.

Study design: This study concerns a single-centre, prospective, double-blind, randomized, placebo-controlled interventional trial with a (1:1) parallel design, with all measurements conducted at Maastricht University.

Study population: The study aims to enrol healthy volunteers aged 18-65 years until 26 responders to nausea induction have completed the study.

Intervention: Participants will be randomly assigned to either the taVNS or the sham stimulation group, with the intervention administered for 30 minutes immediately following nausea induction through intragastric lipid infusion.

Main study parameters/endpoints: The primary endpoint is a significant reduction in nausea in terms of intensity and duration induced by intragastric lipid infusion following taVNS or sham treatment, assessed through 0-100 Visual Analogue Scales at regular time intervals.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: This study, involving healthy volunteers aged 18-65 years, is a low-risk, single-center, double-blind, randomized, placebo-controlled trial conducted at Maastricht University. The study does not involve incapacitated or minority groups. Participants will undergo two visits: an initial visit for obtaining written informed consent and completing several (digital) questionnaires, followed by a test day lasting 3 hours. During the test day, participants will receive an intragastric lipid infusion to induce nausea, followed by 30 minutes of stimulation with either taVNS or sham. Participants will rate the severity of nausea, other gastrointestinal symptoms (e.g., abdominal pain), and the desire to eat using 0-100 Visual Analogue Scales (VASs). TaVNS is non-invasive, with no reported serious adverse events. Additional procedures include the placement of a nasogastric tube and the intragastric infusion of a 50% fat emulsion in water, which carry some minor risks (e.g., abdominal discomfort, bloating, vomiting), all of which will be closely monitored throughout the study. Furthermore, autonomic parameters, using a Shimmer3 GSR sensor and Fitbit smartwatch, will be measured. Blood samples will be collected to measure levels of ghrelin and motilin, and saliva samples will be collected to measure cortisol. While participants will not directly benefit, the risks involved are minimal and proportional to the scientific value of the research.

1. INTRODUCTION AND RATIONALE

1.1. Neurophysiological mechanisms of nausea

Nausea is one of the most prevalent digestive complaints (1). While significant progress has been made in understanding the physiological and neurophysiological pathways involved, the precise neurocircuitry responsible for nausea remains complex and a topic of debate (2, 3). Key structures involved in these neurocircuits, located within the medullary reticular formation of the hindbrain, include the area postrema (the emetic chemoreceptor trigger zone), the nucleus of the solitary tract (NTS), the dorsal motor nucleus of the vagus (DMNV), the reticular formation, and the ventrolateral medulla, with vagal neurocircuits playing a particularly critical role (2). Nausea may arise in response to stimuli in both the central and peripheral nervous systems, such as chemical agents found in food (4). Many patients report a clear temporal relationship between food intake and nausea (1). This pattern is also observed in individuals with functional dyspepsia, a condition defined by the absence of identifiable structural abnormalities in the gastrointestinal tract (1). In these patients, enhanced visceral sensitivity is believed to contribute to the dyspeptic symptoms, including nausea (5). Previous research has shown that dietary lipids can increase mechanosensitivity, which may explain why meals high in fat tend to worsen symptoms such as nausea in this population (5, 6). Additionally, gastroparesis is another condition associated with nausea. In this case, putative mechanism of nausea is primarily related to the decrease in gastric emptying.

1.2. Vagal neurocircuits involved in nausea

The vagal neurocircuits play a pivotal role in the neurophysiological pathways involved in nausea (2). The afferent vagus nerve transmits sensory information from the gastrointestinal tract to the central nervous system, with its neurons residing primarily within the nucleus of the solitary tract (7) and the area postrema (8). The NTS is crucial for modulating and regulating numerous autonomic reflexes associated with emesis (2). Neurons within the NTS have direct or indirect connections with several brain structures that coordinate the organ systems involved in the nausea response (9). Once the sensory information is processed, the efferent vagal nerve relays this integrated response to various peripheral organs involved in nausea (2). These vagal connections indicate that afferent input from the gastrointestinal tract to the brainstem plays a key role in the development of nausea, which may be triggered by the consumption of certain foods in susceptible individuals (1, 10).

In addition to its role in relaying sensory and motor signals, the vagus nerve is also believed to mediate hormonal responses to nausea (2). Gastrointestinal hormones such

as ghrelin, which regulates appetite, and motilin, which influences gastric motility, are both vagally mediated and are thought to play a role in modulating nausea (11, 12). Furthermore, acute nausea has been associated with activation of stress circuits, particularly the hypothalamic-pituitary-adrenal axis, leading to the release of the stress hormone cortisol (60). Measuring levels of these hormones could therefore serve as an indicator of vagal function and provide insight into the hormonal component of the nausea response. Given the central role of vagal pathways in both the neural and hormonal components of nausea, targeting vagal afferent and efferent nerve terminals offers a promising opportunity for the development of targeted treatment strategies to alleviate nausea (2).

1.3 The presumed influence of taVNS on nausea

Transcutaneous auricular vagus nerve stimulation (taVNS) is a form of bioelectronic medicine that allows for non-invasive stimulation of the auricular branch of the vagus nerve (13, 14). Electrical stimulation of this solely afferent branch has a direct influence on the NTS, which projects to a range of higher brain regions, allowing for modulation of central nervous system activity. As a result, the auricular branch serves as a ‘gateway’ for modulating various physiological processes involved in information transfer between the brain and the body (13). Previous studies have shown promising results for taVNS in treating chronic pain disorders (13, 15) and gastrointestinal conditions, such as irritable bowel syndrome (IBS) and functional dyspepsia (16-18). Additionally, pilot and case studies have investigated the use of tVNS in treating nausea in both healthy (19) and diseased (17, 20, 21) subjects, yielding positive but preliminary findings. It is thought that stimulation of visceral vagal afferents transmits impulses to the NTS, which then projects to the thalami via the reticular formation, exerting an inhibitory influence on nausea control mechanisms (22). This may have implications for treating a subgroup of patients with gastrointestinal disorders that experience nausea, such as those with functional dyspepsia. However, despite the growing number of randomized controlled trials on taVNS, many studies remain preliminary. Furthermore, the underlying mechanisms by which taVNS exerts its therapeutic effects are not yet fully understood, highlighting the need for further research to ensure safe and effective implementation into clinical practice.

1.4 Aim of study

This research aims to investigate the efficacy of taVNS in alleviating nausea in healthy individuals. By exploring the potential benefits of taVNS in reducing nausea, this study

seeks to provide valuable insights into the development of innovative therapies for gastrointestinal disorders characterized primarily by nausea complaints, ultimately enhancing the quality of life for affected individuals.

2. OBJECTIVES

2.1 Primary Objective

To determine the efficacy of taVNS in alleviating nausea in healthy adults subjected to nausea induction through intragastric lipid infusion, compared to sham stimulation.
Hypothesis: We hypothesize that taVNS will significantly reduce nausea in terms of both intensity and duration compared to sham stimulation following nausea induction via intragastric lipid infusion, as measured by a 0-100 Visual Analogue Scale (VAS) score. A responder will be defined as a participant showing a decrease of ≥ 25 mm on the VAS (23).

2.2 Secondary Objectives

1. To assess the effect of taVNS on other gastrointestinal symptoms, such as bloating, abdominal pain, and fullness, following intragastric lipid infusion, compared to sham stimulation.
   Hypothesis: We hypothesize that taVNS will lead to a significant reduction in other gastrointestinal symptoms, such as bloating, abdominal pain, and fullness, compared to sham stimulation, as measured by a 0-100 Visual Analogue Scale (VAS) score at regular time intervals.

2. To explore the effects of taVNS on the desire to eat following nausea induction, compared to the sham stimulation group.
   Hypothesis: We hypothesize that taVNS will lead to an earlier increase in the desire to eat following nausea induction compared to sham stimulation, as measured by a 0-100 Visual Analogue Scale (VAS) at regular time intervals.

3. To investigate the effect of taVNS on plasma levels of ghrelin and motilin following nausea induction.
   Hypothesis: We hypothesize that nausea induction will result in a reduction in plasma levels of ghrelin and motilin, and that this reduction will be counteracted by taVNS.

4. To investigate the effects of taVNS on salivary cortisol levels following nausea induction.

Hypothesis: We hypothesize that nausea induction will result in an increase in salivary cortisol levels, and that this increase will be attenuated by taVNS.

5. To evaluate how affective symptoms and personality traits, utilizing the GAD-7, PHQ-9, and BFI, influence the nausea response.
   Hypothesis: We hypothesize that affective symptoms, such as anxiety, and personality traits will modify the nausea response.

6. To evaluate the effect of taVNS on parameters related to autonomic outflow, using a Fitbit smartwatch for heart rate variability (HRV), and a Shimmer3 GSR sensor for HRV and skin conductance.
   Hypothesis: We hypothesize that treatment with taVNS will lead to significant changes in the autonomic outflow reflective of an increase in parasympathetic activity.

3. STUDY DESIGN

The proposed project concerns a monocentric, prospective, double-blind, randomized, placebo-controlled interventional trial with a (1:1) parallel design. The study will be conducted over a total duration of 2 years, encompassing recruitment, intervention, and singular measurements, all carried out at the NUTRIM Clinical Research Unit (CRU) at Maastricht University.

Visit 1

Recruitment procedures are outlined in paragraph 11.2 and are in accordance with the study "taVNS and acute stress response" (NL87188.068.24/METC 24-029), which has received ethical approval. Interested study participants will receive a verbal explanation of the study over the phone and will be sent a comprehensive electronic information letter. At least 7 days later, they will be invited to attend an inclusion visit at Maastricht University. During this visit, participants will undergo eligibility screening and receive detailed instructions regarding the study procedures. Eligible participants will be asked to provide written informed consent before proceeding. Subsequently, eligible participants will be asked to complete several (digital) questionnaires, including a baseline characteristics questionnaire, the Generalized Anxiety Disorder 7-Item Scale (GAD-7), the Patient Health Questionnaire-9 (PHQ-9), and the Big Five Inventory (BFI). All information obtained during the screening visit, including participant data and study-related details, will be securely recorded using Castor, an electronic data capture system. After this screening visit participants will be randomized in a 1:1 parallel design by an investigator to receive either taVNS or sham stimulation on the second test day, with appropriate blinding procedures in place.

Visit 2

A second visit, which serves as the test day, will be scheduled. During this visit, participants will receive either taVNS or sham stimulation, administered immediately after nausea induction, based on their randomization.

Participants will arrive at the NUTRIM CRU at Maastricht University after an overnight fast. Compliance with test day regulations will be checked (e.g. fasting, no alcohol). Once compliance is confirmed, the coordinating investigator will properly set up the tVNS device to ensure it can be used immediately after nausea induction. This setup includes adjusting the stimulation current of the tVNS device according to each participant's sensitivity, ensuring it remains below the sensitivity threshold, with current intensities ranging from 0.25 to 10 mA. This is important because active stimulation will be programmed to deliver only subthreshold stimuli. Next, an intravenous cannula will be placed for blood collection. Thereafter, the

investigator (a medical doctor) will manually place a nasogastric tube to facilitate the intragastric infusion of a 50% fat emulsion in water. This method of infusion is selected to replicate the conditions under which dietary fat is ingested as part of a meal. The infusion will be administered at a rate of 5 ml/min, consistent with previous research in which nausea was induced through similar methodologies (1). Participants will be closely monitored throughout the infusion for the onset of nausea, with assessments every 5 minutes using a 0-100 Visual Analogue Scale (VAS) to quantify the nausea severity. Participants will receive clear instructions on how to rate their nausea and indicate its intensity. If a predetermined VAS score of 50/100 is reached, or should the participant start retching, the lipid infusion will be promptly halted to prevent excessive discomfort. Participants will be informed in advance about the 50/100 VAS threshold and provided with guidance on how to interpret and apply this rating. Following the cessation of the infusion, the nasogastric tube will be removed for patient comfort, after which either taVNS or sham stimulation will be administered for 30 minutes.

A duration of 30 minutes was selected based on the mechanism of action of taVNS, which is assumed to act via the activation of the nucleus of the solitary tract (NTS) in the brainstem. Furthermore, this choice is also consistent with other studies on taVNS conducted within our department (NL87188.068.24/METC 24-029). The NTS serves as the primary relay station for vagal input and plays a critical role in modulating autonomic functions. In a previous study by Frangos et al. (24), NTS activity gradually decreased during stimulation but peaked shortly after the cessation of a 7-min stimulation of the cymba conchae. Furthermore, other brain regions exhibited a gradual increase in activity during stimulation, reaching their peak during the post-stimulation phase, with this heightened activity persisting for up to 11 minutes. Hence, a stimulation period of 30 minutes is expected to provide an optimal timeframe for taVNS to exert its maximum effect and to counterbalance the effects of nausea.

Both the taVNS treatment and sham stimulation will be administered using a tVNS device that provides stimulation to the cymba conchae of the left ear. The left side is chosen due to the innervation pattern of the left vagus nerve, which predominantly innervates the stomach and the pyloric area (25, 26). This choice aligns with our ultimate clinical interest in conditions such as gastroparesis and functional dyspepsia. Additionally, it ensures comparability with other studies conducted within our department, including the “tVNS and GI motility” study (NL86446.068.24).

The stimulation parameters comprise a biphasic rectangular pulse wave with an impulse frequency of 25Hz, impulse duration of 30 seconds, impulse pause of 30 seconds, and pulse width of 450ms. These parameters have been identified as the most optimal treatment frequencies for taVNS based on systematic reviews (27, 28). Active stimulation will be programmed to deliver only subthreshold stimuli. The device will blink when stimulation is delivered. In the sham (control) group, the electrode will be non-conducting, but the device will still blink to maintain blinding (28). The electrodes used in the tVNS and sham groups differ slightly. Consequently, the investigator responsible for programming the devices will be aware of the treatment allocation, while participants will remain blinded. To ensure double blinding, the randomization code will be altered prior to data analysis, ensuring that the investigator conducting the analysis will be blinded to the treatment allocation. The randomization code will be kept confidential until after the modified intention-to-treat analysis has been reported.

During the stimulation phase, nausea severity will be assessed using a 0-100 VAS score, where 0 indicates “no nausea” and 100 represents the “worst nausea imaginable”. VAS scores will be recorded every 5 minutes, continuing for 30 minutes post-stimulation to evaluate the efficacy of taVNS in alleviating nausea. Additionally, other gastrointestinal symptoms and the desire to eat will be measured at predetermined time points using VAS scores.

Blood samples will be taken at four time points to assess changes in plasma ghrelin and motilin levels, selected for their presumed roles in the physiological response to nausea (3). Ghrelin is known to stimulate appetite, and since nausea is often accompanied by a decrease in appetite, it is likely that nausea leads to reduced ghrelin levels. Measuring ghrelin in response to taVNS will allow us to investigate whether this intervention influences the hormonal response and potentially uncover the mechanism by which taVNS alleviates nausea. Motilin is a gastrointestinal hormone primarily involved in regulating gastric motility and stimulating the migrating motor complex (MMC) during the fasting state (11). Although not previously investigated directly, it may play a role in the nausea response through its effects on gastric motility (29, 30). Levels of motilin, along with gastrointestinal motility, are likely to be altered in response to intragastric lipid infusion and the subsequent onset of nausea and a decrease in gastric emptying. TaVNS may modulate motilin levels, as it is expected to reduce nausea and influence the MMC (30). It is hypothesized that this reduction in nausea may correspond with an increase in motilin levels, facilitating the restoration of normal gastric motility (29). By measuring both hormones, we aim to gain deeper insights into the physiological effects of taVNS on the nausea response.

Saliva samples will be collected at the same four time points as the blood samples to assess changes in cortisol levels. Cortisol plays a crucial role in regulating the stress response. Nausea is considered a stressor, activating both the autonomic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis (60). As a result, cortisol could serve as an important marker for the degree of physiological dysregulation associated with nausea. By measuring cortisol, it can objectively be assessed to what extent nausea triggers a stress response and whether taVNS has a regulatory effect on this process. This provides valuable additional information regarding hormonal and autonomic changes, contributing to a deeper understanding of the potential physiological effects of taVNS.

Autonomic parameters, including heart rate variability and skin conductance, indicators of both parasympathetic and sympathetic activity, will be recorded continuously from the start of nausea induction until 30 minutes after either taVNS or sham stimulation. These measurements will be captured using a Shimmer3 GSR sensor and a Fitbit smartwatch.

After completing all measurements, participants will be asked to fill out the reimbursement form. Phone numbers of the investigators and the emergency department will be provided for any issues that arise within 24 hours after the test day. The investigators will ensure participants are in a stable condition before leaving Maastricht University.

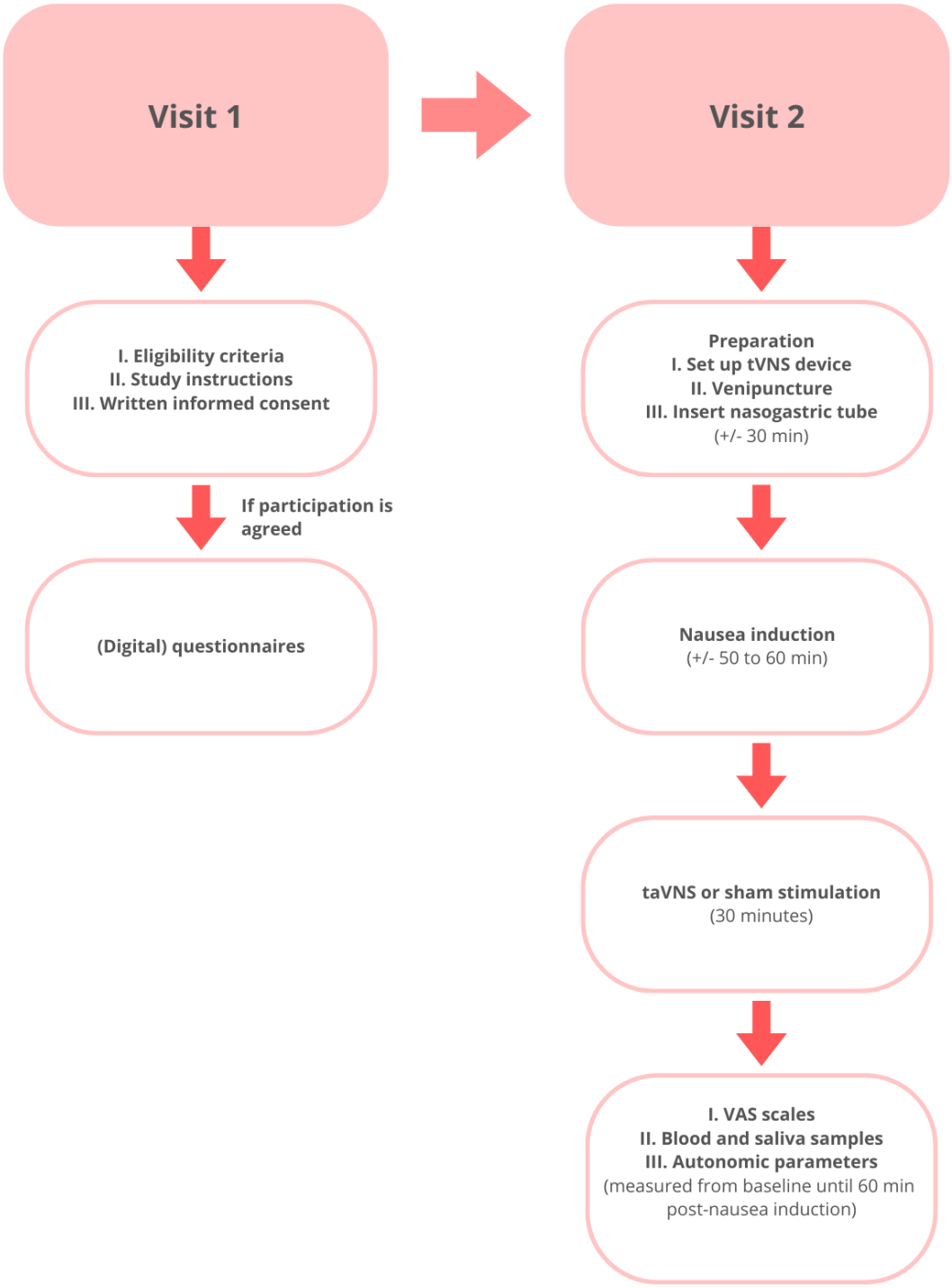

Figure 1: Schematic overview of the study design and the main procedures.

4. STUDY POPULATION

4.1 Population

To investigate whether taVNS is effective in counterbalancing the nausea response induced by intragastric lipid infusion, healthy volunteers will be enrolled until 26 responders to nausea induction have completed the study. Participants will be recruited through various channels, including public advertisements and local newspapers. Both males and females aged between 18 to 65 years are eligible for inclusion.

4.2 Inclusion criteria

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

- Healthy participants (defined as those without a pre-existing medical comorbidity).
- Aged between 18-65 years.
- Ability to understand and speak the Dutch language.
- BMI between 18 and 25 kg/m^2.

4.3 Exclusion criteria

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

- Medical history of chronic or severe diseases affecting the cardiovascular, respiratory, urogenital, gastrointestinal/hepatic, haematologic/immunologic, HEENT (head, ears, eyes, nose, throat), dermatological/connective tissue, musculoskeletal, metabolic/nutritional, endocrine, neurological/psychiatric systems.
- A history of major abdominal surgery.
- Gastrointestinal complaints.
- Any use of medication, especially those affecting gastric motility and nausea, apart from oral contraceptives.
- Current or lifetime psychopathology (including PHQ-9 and GAD-7 scores $\geq$ 10)
- Substance abuse, including excessive alcohol consumption (>20 alcoholic consumptions per week) and the use of recreational drugs.
- Smoking.
- Pregnancy, lactation, or intention to become pregnant during the study period
- Use of devices (e.g., cochlear implants) or other conditions (e.g. wounds, permanent ear-piercing) complicating the use of the tVNS device.

- Administration of investigational drugs or participation in any scientific intervention study that might interfere with this study (to be determined by the principal investigator) within 180 days preceding the commencement of the study.
- 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.

4.4 Sample size calculation

Our primary outcome of interest is a reduction in nausea, as measured by visual analogue scales (VAS). Currently, no directly comparable studies are available for an accurate sample size calculation, as previous research has not examined the effect of tVNS on nausea severity in healthy participants using VAS scores. However, a randomized controlled trial in patients with chronic nausea and vomiting due to gastroparesis or gastroparesis-like syndrome evaluated nausea improvement after either aprepitant or placebo treatment (23). This study indicated that a reduction in the mean 28-day VAS score from baseline of at least 25 mm was considered a responder. A standard deviation of 21.5 was noted in the treatment group.

The sample size calculation was based on an unpaired-sample t-test (taVNS vs. sham stimulation) with a mean difference of 25 mm and a standard deviation of 21.5. The following parameters were applied: a significance level (alpha) of 0.05 (two-sided) and a power of 0.80. Based on this calculation, we determined that 26 participants are needed. Because non-responders to nausea induction can occur, we will continue enrolment until 26 participants who meet the responder definition for nausea induction (VAS = 50 during induction) have completed the protocol. The calculation can be found at the following web address:
https://homepage.univie.ac.at/robin.ristl/samplesize.php?test=ttest

5. TREATMENT OF SUBJECTS

This study aims to investigate the efficacy of taVNS in mitigating nausea caused by intragastric lipid infusion.

5.1 Investigational product/treatment

The intervention of interest is taVNS, with sham stimulation employed as the appropriate control.

5.1.1 taVNS

Electrical stimulation will be administered to the auricular branch of the vagus nerve, specifically at the cymba conchae of the left ear, using a custom-made device called tVNS R (tVNS Health GmbH, Grünwald, Germany). This device has previously been used in the RESILIENCE study (NL84720.068.23/METC 23-032) and comprises a stimulation unit and an ear electrode. This electrode is connected as an anode to the stimulation device by a cable and is worn similarly to an earphone. The stimulation unit sends electrical impulses through the electrode, which stimulates the auricular branch of the vagus nerve. On the test day, subjects assigned to the taVNS group will receive 30 minutes of active stimulation after the nausea induction. According to recent systematic analyses determining the most optimal treatment frequencies for taVNS, the stimulation parameters will consist of biphasic rectangular pulse trains with an impulse frequency of 25Hz, impulse duration of 30 seconds, followed by an impulse pause of 30 seconds, and an impulse width of 450ms (27, 28). Each participant will undergo sensory testing prior to the commencement of the treatment period during which the current will be adjusted to suprathreshold stimulation intensity, with current intensities ranging between 0.25 and 10 mA. The device will indicate stimulation delivery by blinking.

5.1.2 Sham stimulation

In the control group, sham stimulation will be administered to the same electrode location (left cymba conchae) using an identical device as that used for taVNS. The electrode used for sham stimulation will be non-conducting, ensuring that there is no stimulation of the vagus nerve. Although the devices for tVNS and sham stimulation appear identical, the electrodes for each group are slightly different. Consequently, the investigator responsible for programming the devices will be aware of the treatment allocation. Participants will not be aware of the treatment received since stimulation is provided to the same location, and the device will still blink during both types of stimulation to maintain blinding (28). The randomization code will be altered

before analysis, ensuring that the investigator conducting the data analysis remains unaware of the treatment allocation. The randomization code will be kept confidential until after the modified intention-to-treat analysis has been reported.

5.2 Use of co-intervention

5.2.1 Intragastric lipid infusion

As a co-intervention, an intragastric infusion of a 50% fat emulsion in water will be administered to induce nausea. A 10-F gauge single-lumen polyurethane nasogastric tube (Nutricair Enteral-Enfit, Charrière 10) will be manually inserted by the coordinating investigator, positioning the catheter in the stomach and connecting it to a nutrition enteral delivery pump (Applix Smart pomp). A 20/60ml Nutricair Enteral-Enfit syringe will be used to administer the fat emulsion through the feeding tube. Standard clinical protocols for nasogastric tube placement will be followed, including a pH measurement to verify the tube's position, ensuring participant's safety and comfort.

The fat emulsion (Calogen, NUTRICIA, Netherlands) used is comparable to that employed in previous research on intragastric nausea induction (1). It is a medical nutritional product typically used to address an 'energy deficit' in patients who are unable to meet their recommended energy intake through regular food and/or oral nutritional supplements. The emulsion is suitable for enteral use and can be administered via a nasogastric tube (31). See D6.1_SPC_Calogen_Versie1.0_dd 16-10-2024 & D6.2_Calogen_Voedingstabel_Versie1.0_dd 15-10-2024 for additional information.

The fat emulsion consists of 50% long-chain triglyceride and 50% water (osmolarity: 0 mOsm/L, Calogen, NUTRICIA, Netherlands). Previous research has shown that long-chain triglycerides elicit a stronger nausea response than medium-chain triglycerides, which is why long-chain triglycerides were selected as the primary component (1). The infusion rate will be set at 5 ml/min (22.5 kcal/min) and will continue until the participant experiences nausea, indicated by a VAS score of 50/100 (1). Based on previous studies, it is expected that approximately 258 + 32 ml of the fat emulsion is required to induce nausea through gastric relaxation, with the process taking approximately 52 $\pm$ 6 minutes (1). If the nausea score does not reach the VAS of 50/100 after 1.5 hours of infusion, the infusion will be discontinued, and the test day will be terminated. In this case, the participant will be considered a drop-out due to non-response to the nausea-inducing stimulus.

Participants will be closely monitored throughout the procedure to ensure their safety, and nausea severity will be assessed using validated scales. For a detailed description of the procedure, please see F4.1_Nasogastric_Tube_Placement_SOP_Versie1.0_dd 14-10-2024 and paragraph 8.3.

5.3 Escape medication

To ensure participant safety, an ondansetron dissolvable tablet will be administered as escape medication when nausea reaches a severity level of 80 or higher out of 100 on the VAS scale, indicating severe discomfort and an imminent vomiting sensation. Following the immediate termination of the lipid infusion, a single dose of 4 mg will be given, with a maximum of four doses (16mg total) allowed per day if necessary. Based on previous research using more intensive nausea induction methods, including intraduodenal lipid infusion and gastric distension, in which 2 out of 12 healthy participants experienced vomiting, we do not expect frequent vomiting in our study (10).

Ondansetron is a well-established anti-emetic that exerts its effects primarily through antagonism of 5HT3 (serotonin) receptors. It is commonly used for the management of nausea in clinical settings (32). See D6.4_Ondansetron_SPC_Versie1.0_dd 22-07-2024 for additional information.

Given that participants will be healthy volunteers, it is anticipated that no contraindications, such as relevant medication use, will be present. However, if ondansetron is administered, participants will be closely monitored for both relief from nausea and potential side effects, including headache, dizziness, and other known reactions, for at least 1 hour following administration.

The decision to administer ondansetron will be documented, including the time of administration, the participant’s reported nausea level, and any subsequent symptoms.

6. INVESTIGATIONAL PRODUCT

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_Versie1.0_dd 31-05-2021' and D4_Confirmation_of_equivalence_tVNS_Versie1.0_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. This exact same device was previously used by our study group in the "RESILIENCE" study (NL84720.068.23/METC23-032).

6.1 Name and description of investigational product(s)

The tVNS R is a vagus nerve stimulation device comprising a stimulation unit and an ear electrode that is worn like an earphone. The stimulation unit delivers electrical impulses through the electrode, stimulating the auricular branch of the vagus nerve transcutaneously (through the skin). Further details are provided in paragraph 5.1.

6.2 Summary of findings from non-clinical studies

Animal studies have demonstrated that transcutaneous vagus nerve stimulation in rats experiencing transient focal ischemia leads to activation of the locus coeruleus and a reduction in the volume of the infarct zone (33). Furthermore, another study revealed that transcutaneous vagus nerve stimulation can decrease systemic tumor necrosis factor levels and enhance survival in mice with lethal endotoxemia (sepsis) (34). In healthy humans, transcutaneous vagus nerve stimulation has been established as a safe and well-tolerated procedure, particularly in patients without a history of cardiac disease (28, 35, 36). Notably, heart rate and blood pressure remain unaffected during the stimulation. Transcutaneous vagus nerve stimulation likely operates through a mechanism similar to invasive VNS, involving the activation of the locus coeruleus (and subsequent release of noradrenaline) and elevation of GABA levels (37). Healthy participants undergoing transcutaneous vagus nerve stimulation have reported reduced pain levels compared to those receiving sham stimulation (38). Furthermore, a pilot study showed the potential of taVNS to mitigate visually induced motion sickness and nausea in healthy individuals compared to sham stimulation (19).

6.3 Summary of findings from clinical studies

Transcutaneous vagus nerve stimulation has been effectively utilized in a controlled trial with patients diagnosed with epilepsy, resulting in a reduction in seizure frequency and improvements in EEG measures, depressive symptoms, anxiety, and quality of life (39-41). In addition, a pilot study indicated that taVNS enhances mood and reduces handicap scores in patients with tinnitus (42). A recent systematic review and meta- analysis further demonstrated the potential of taVNS in reducing both the frequence of migraine days and the intensity of headaches (43). Furthermore, an open-label proof-of-concept study on non-invasive VNS in patients with severe drug-refractory gastroparesis revealed a response rate (defined as a $\geq$1 point decrease from baseline in the Gastroparesis Cardinal Symptom Index [GCSI] score) of 35% at 3 weeks and 43% for the overall duration of therapy (3-6 weeks) (17). The most notable improvements were observed in symptoms of nausea and stomach fullness. Another study demonstrated promising results for auricular percutaneous electrical nerve field stimulation (PENFS), a method comparable to non-invasive VNS (44), in treating cyclic vomiting syndrome (CVS), showing significant reductions in nausea complaints (21).

6.4 Summary of known and potential risks and benefits

Transcutaneous vagus nerve stimulation is a non-invasive treatment that is generally well-tolerated and safe in patients without pre-existing cardiac pathology (45, 46). Thus far, no serious risks have been mentioned in the literature. Some studies have reported occasional side effects, such as dizziness or daytime drowsiness during prolonged stimulation, which alleviated upon reducing the stimulation intensity (40). In a pilot study, taVNS exhibited no serious or long-lasting side effects, and no significant heart rate changes were observed during stimulation. Some participants experienced temporary fatigue, concentration difficulties, or a tingling sensation at the stimulation site, which subsided within 90-minutes (47). In a randomized controlled trial involving adolescents, using a more invasive method percutaneous electrical nerve field stimulation, eight individuals discontinued treatment due to aesthetic reasons, discomfort from the device's fit, needle phobia, or anxiety, as well as adhesive allergy (16). However, these are concerns are less relevant in the current study, as it employs ear electrodes that fit comfortably without the need for plasters and stimulate the vagus nerve in a non-invasive, transcutaneous manner. In addition, patients may experience slight hearing impairment on the side where the electrode is placed. A recent systematic review and meta-analysis reported no serious adverse effects with taVNS. Mild symptoms of dizziness, headache, and slight redness around the stimulation area were noted, all of which resolved shortly

after discontinuing the intervention (48). Furthermore, taVNS can be provided safely both left and right-sided, as was illustrated by a review article (49).

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 significant reduction in nausea in terms of intensity and duration induced by intragastric lipid infusion following taVNS or sham treatment, assessed through 0-100 Visual Analogue Scales at regular time intervals.

8.1.2 Secondary study parameters/endpoints

- Gastrointestinal symptoms, including bloating, abdominal pain, and fullness following taVNS or sham stimulation after intragastric lipid infusion, assessed through 0-100 VAS scales.
- Changes in the desire to eat following nausea induction after taVNS or sham stimulation, assessed through 0-100 VAS scores.
- Changes in plasma ghrelin and motilin levels following taVNS or sham stimulation after nausea induction.
- Changes in salivary cortisol levels following taVNS or sham stimulation after nausea induction.
- Nausea response in relation to affective symptoms and personality traits, assessed using the GAD-7, PHQ-9, and BFI questionnaires.
- Autonomic response to nausea induction following taVNS or sham stimulation, measured using a Fitbit for HRV and a Shimmer3 GSR sensor for HRV and skin conductance.

8.1.3 Other study parameters

- Patient characteristics: sex, age, BMI, lifestyle factors, and medical history.

8.2 Randomisation, blinding and treatment allocation

After confirming suitability for inclusion and obtaining written informed consent during the initial visit, participants will be scheduled for a second visit (referred to as the test day). Prior to this test day, an investigator will randomise the subjects in a 1:1 parallel fashion to receive either taVNS or sham stimulation. The randomisation of participants will be conducted using Castor, an electronic data capture system. The concealment list will be generated and provided to a co-worker in the same department as the coordinating investigator. For emergencies, drs. Hawinkels will be provided with the concealment list and will be able to de-blind. The concealment list provides information on which participant will get active taVNS and who will get

sham stimulation. This information will remain confidential, and the concealment list will be sealed until after the initial analysis.

In this study, blinding procedures will be implemented. The tVNS device used will be identical for both the active and sham groups, and the ear electrodes will be placed at the same anatomical location. Furthermore, the device will blink in both groups to ensure that participants remain blinded to their treatment allocation. The key differences are that the sham group will receive a device with a non-conducting electrode, and that the electrodes for the taVNS and sham groups will be slightly different. As a result, the investigator responsible for programming the devices on the test day will be aware of treatment allocation. To maintain proper blinding for the initial analysis, the randomization code will be changed prior to the data analysis using Castor. The randomization code will be kept confidential until after the modified intention-to-treat analysis has been reported.

8.3 Study procedures

8.3.1 Visit 1: Screening visit and informed consent

The screening visit will take place at the NUTRIM Clinical Research Unit (CRU) of Maastricht University. At this first visit, all participants will be asked to confirm that they have read the information letter, received oral information, and have no further questions regarding the study. The investigator will ensure the participant understands all the information through an interview. Afterward, the eligibility criteria will be checked, and if suitable, the participant will be asked to complete written informed consent prior to any study procedures. Following inclusion, the medical history of each participant will be checked during a structured interview and participants will be requested to complete several questionnaires, including a baseline characteristics questionnaire (F1.1_Baseline_Characteristics_Questionnaire_Versie2.0_dd 26-03-2025), GAD-7 (F1.2_GAD7_Versie1.0_dd 10-10-2024), PHQ-9 (F1.3_PHQ9_Versie1.0_dd 10-10-2024)), and BFI (F1.4_BFI_Versie1.0_dd 10-10-2024), with the support of Castor EDC. Given that current or lifetime psychopathology serves as an exclusion criterion and the GAD-7 and PHQ-9 assess for the presence of anxiety and depression disorders, respectively, participants scoring 10 or above on either questionnaire will be excluded from the study (50).

8.3.2 Visit 2: Test day

Participants will arrive at the NUTRIM CRU at Maastricht University after an overnight fast. To account for the distinct circadian rhythms of the hormones being measured, the test day is scheduled between 9:00 a.m. and 12:00 p.m. This timing ensures that measurements are taken before the natural ghrelin peak, which typically occurs around noon, optimizing sensitivity to detect any effects of the intervention (61, 62). Although cortisol levels peak around 8:00 a.m. and are still in decline during this window, they are not yet at their most stable (62). However, this is deemed acceptable since cortisol is a secondary outcome. Additionally, this time frame helps minimize the required fasting duration for participants, enhancing both feasibility and comfort.

Upon arrival, all participants will complete a checklist to verify compliance with the test day regulations. These regulations include not eating for at least 6 hours before the test day and not consuming extremely spicy or fatty foods, as well as alcohol, on the day prior to the test day. On the day of the test, participants are only permitted to drink small amounts of water. Additionally, due to the cortisol sampling, participants must refrain from consuming caffeine or engaging in physical exertion on the day of the test day. Participants should also avoid brushing their teeth, chewing gum, or smoking for at least 1 hour before the test session begins.

Once compliance is confirmed, the coordinating investigator will prepare the tVNS device to ensure it is ready for immediate use after nausea induction. This preparation involves adjusting the stimulation current based on each participant's sensitivity, keeping it below the sensory threshold, with current intensities ranging from 0.25 to 10mA.

Following this, the investigator will place an intravenous cannula for blood collection to measure plasma levels of ghrelin and motilin at four different time points. Thereafter, the investigator will manually insert a nasogastric tube (Nutricair Enteral-Enfit, Charrière 10) to facilitate intragastric infusion of a 50% fat emulsion (Calogen, NUTRICIA, Netherlands) in water (see D6.1_SPC_Calogen_Versie1.0_dd 16-10-2024 & F4.1_Nasogastric_Tube_Placement_SOP_Versie1.0_dd 14-10-2024. The fat emulsion consists of 50% long-chain triglycerides and 50% water. The infusion will be administered at a rate of 5 ml/min (22.5 kcal/min) using an Applix Smart pump and a Nutricair Enteral-Enfit syringe, following methodologies used in previous research that successfully induced nausea (1). Based on a prior study, we expect that a mean

volume of 258 ± 32 ml will be necessary to induce nausea (1). Participants will be closely monitored throughout the infusion for the onset of nausea, with assessments every 5 minutes using a 0-100 Visual Analogue Scale (VAS) to record their symptoms. If a predetermined VAS score of 50/100 is reached, or when the participant starts retching, the lipid infusion will be promptly halted to prevent excessive discomfort. Participants will explicitly be informed about the predetermined 50/100 threshold on the VAS scale, but also the maximum time limit for the infusion, and the possibility of being considered a drop-out if the nausea score does not reach 50/100.

Participants will also be informed in advance about how to use the VAS scale and how to indicate their nausea score. Concrete examples will be discussed, such as: "If you don't feel nauseous at all, mark 0 on the scale," "If you feel slightly nauseous but can still function normally, give a score of 30 or 40," "If you feel clearly nauseous and uncomfortable, but can still function normally, give a score of 50," "If you feel very nauseous, for example, if you are about to vomit, give a score of 80 or higher." This is based on progressive intensity scores that were defined as follows: 0, absent sensation; 10 and 20, faint sensation; 30 and 40, mild sensation; 50 moderate sensation; 60 and 70, strong sensation, and 80, imminent vomiting sensation (1).

Throughout the procedure, participants will be closely monitored for the severity of nausea. To ensure safety, the VAS will be administered at regular 5-minute intervals. Furthermore, participants will be asked to notify the research team if a VAS score of 50/100 (or higher) is reached between scheduled assessments, allowing the infusion to be stopped earlier if necessary.

The lipid infusion will be immediately halted if the participant’s nausea score reaches the 50/100 threshold or if they begin retching. This is to prevent excessive discomfort. Any signs of severe nausea, such as vomiting or a participant’s request to stop the procedure, will also lead to immediate cessation of the infusion.

After stopping the infusion, the nasogastric tube will be promptly removed, and participants will then receive either taVNS or sham stimulation for 30 minutes. During this stimulation phase, the severity of nausea will be assessed using a 0-100 VAS score, where 0 signifies “no nausea” and 100 represents “the worst nausea imaginable”. VAS scores will be recorded every 5 minutes, continuing for 30 minutes post-stimulation to evaluate the efficacy of taVNS in alleviating nausea. VAS scores

for other gastrointestinal symptoms will also be recorded at the same time points as nausea severity. Additionally, VAS scores for the desire to eat will be recorded at four specific time intervals.

Plasma levels of ghrelin and motilin and salivary cortisol will be measured on four different time points: baseline, at the end of nausea induction, immediately after the taVNS vs. sham stimulation, and 60-minutes post-nausea induction.

Autonomic parameters, including heart rate variability and skin conductance, will be continuously recorded from the onset of nausea induction until 30 minutes after either taVNS or sham stimulation. These measurements will be captured using a Shimmer3 GSR sensor and Fitbit smartwatch, as was previously utilized in other studies within our department (NL87188.068.24/METC 24-029). The Shimmer3 GSR sensor, a CE-certified device (see K6.6_Shimmer_CE_Certificate_of_conformity_Versie1.0_dd 04-09-2014) will be used to collect data on heart rate variability and skin conductance. The Fitbit wearable device will be used to collect participant data concerning HRV. The device will be connected to the coordinating investigator's smartphone via Bluetooth and the Fitbit app. Heart rate data will then be automatically collected by the Fitbit.

Once all measurements are completed, the tVNS device, intravenous cannula, Shimmer, and Fitbit will be removed.

The investigators will ensure that participants are in stable condition before they leave Maastricht University. Since nausea is influenced by gastric distension and chemical signals, its resolution likely follows the reduction of these factors. After stopping the fat infusion, gradual gastric emptying reduces mechanical stimulation. Due to slow fat digestion, nausea is expected to subside gradually, with acute distension resolving first, though mild residual symptoms may persist. If the VAS score falls below 50/100 after stimulation (expected within 30-60 minutes), participants are assessed for fitness to leave based on subjective well-being and symptom resolution. They must function independently without severe nausea (e.g., vomiting, disorientation). If still above 50/100, monitoring continues every 5 minutes for safety until nausea sufficiently declines.

Finally, participants will be asked to fill out a reimbursement form. Contact numbers of the investigators and the emergency department will be provided for any issues that arise within 24 hours after the test.

8.3.3 Biological samples

Plasma ghrelin and arginine vasopressin: On the test day, plasma levels of ghrelin and motilin will be measured in response to nausea induction, as these hormones are expected to be influenced by the nausea response (3). Blood samples will be drawn at four predetermined time points: baseline, at the end of nausea induction, immediately after the taVNS vs. sham stimulation, and 60-minutes post-nausea induction. Plasma will be collected in EDTA tubes kept on ice, followed by centrifugation. The samples will then be stored at -80 °C until analysis. For a detailed description of the sample collection and processing procedures, see F4.2_Blood_Samples_SOP_Versie1.0_dd 14-10-2024.

Salivary cortisol: Salivary cortisol levels will be measured in response to nausea induction, as this hormone is expected to elevate due to the stress response triggered by nausea (60). Saliva samples will be collected at four predetermined time points: baseline, at the end of nausea induction, immediately after the taVNS vs. sham stimulation, and 60-minutes post-nausea induction. Saliva samples will be collected using synthetic Salivette devices. The samples will be processed as soon as possible and will be promptly stored at -80 ◦C until further analysis. The analysis of the samples will be conducted at the end of the study period in the laboratory of Maastricht University using immunoassay (ELISA SLV-2930). See F4.4_Cortisol_Samples_SOP_Versie1.0_dd 2-4-2025 for a detailed description of the saliva cortisol sample collection process and subsequent processing.

8.3.4 Questionnaires

Participants will be asked to complete several questionnaires during the screening visit and the test day. These questionnaires will be completed in a secure electronic environment, named Castor, accessible via the Internet through a telephone or computer. Castor was developed by the Maastricht Center and Information and Data Management (MEMIC), affiliated with Maastricht University. Castor utilizes a NEN7510 and ISO 27001:2005 certified server called “True”. Our research group

has previously used Castor in the “FORTITUTE” study (NL67607.068.18/METC 18-037) and the ”RESILIENCE” study (NL84720.068.23/METC 23-032).

Baseline characteristics questionnaire

‘F1.1_Baseline_Characteristics_Questionnaire_Versie2.0_dd 26-03-2025’

At visit 1, participants will complete this questionnaire concerning baseline characteristics, demographics, lifestyle, and comorbidities.

Generalized Anxiety Disorder 7-Item Scale (GAD-7)

‘F1.2_GAD7_Versie1.0_dd 10-10-2024’

The GAD-7 is a validated 7-item questionnaire, which has been demonstrated to be an efficient tool for screening generalized anxiety disorder and assessing its severity in clinical practice and research (51). It includes seven anxiety symptoms, with cut-off levels for mild, moderate, and severe anxiety. Participants will be asked to complete the GAD-7 during the first visit to screen for anxiety.

Patient Health Questionnaire (PHQ-9)

‘F1.3_PHQ9_Versie1.0_dd 10-10-2024’

The PHQ-9 is a validated instrument utilized for screening, diagnosis, and measuring the severity of depression. It evaluates each of the nine DSM-IV criteria for depression along with key symptoms of major depressive disorder, condensing them into a concise self-report questionnaire. The questionnaire establishes cut-off points for mild, moderate, moderately severe, and severe depression (52). During the initial visit, participants will complete the PHQ-9 to assess for the presence of depression.

The Big Five Inventory (BFI)

‘F1.4_BFI_Versie1.0_dd 10-10-2024’

The BFI is an instrument designed to assess the personality of individuals aged 18 and above. It comprises 44 items divided into five sub-scales, each representing one of the five personality factors: extraversion (8 items), neuroticism (8 items), conscientiousness (9 items), agreeableness (9 items), and openness to experience (10 items). Participants are required to rate the extent to which they agree with the statement using a 5-point Likert scale ranging from 1 (Disagree strongly) to 5 (Agree strongly) (53). During the initial visit, participants will complete the BFI to evaluate their personality traits.

0-10 Visual Analogue Scale (VAS) – Nausea
'F1.5_VAS_Scales_Versie1.0_dd 10-10-2024'

Participants will be requested to assess the severity of nausea at regular time intervals following nausea induction and either taVNS or sham stimulation. The score will range from 0 to 100, where 0 indicates 'no nausea' and 100 indicates 'the worst nausea imaginable'. In total, 14 questionnaires will be completed: one at baseline (pre-infusion), one immediately after stopping the lipid infusion (when the predetermined nausea score of 50/100 is reached), at six time points during stimulation at 5-minute intervals, and at six time points in the post-stimulation phase at 5-minute intervals.

0-10 Visual Analogue Scale (VAS) – Other gastrointestinal symptoms
'F1.5_VAS_Scales_Versie1.0_dd 10-10-2024'

Participants will be asked to assess the severity of additional gastrointestinal symptoms, including abdominal pain, bloating, and fullness. They will provide a score ranging from 0 to 100, where 0 indicates 'not at all' and 100 indicates 'extremely severe'. These symptoms will be evaluated at the same 14 time points as for nausea severity.

0-10 Visual Analogue Scale (VAS) – The desire to eat
'F1.5_VAS_Scales_Versie1.0_dd 10-10-2024'

Participants will be asked to assess their desire to eat by providing a score on a scale from 0 to 100, where 0 indicates 'no desire to eat at all' and 100 indicates 'extremely strong desire to eat '. This will be evaluated at four time points: at baseline (pre-infusion), immediately after stopping the lipid infusion, after taVNS or sham stimulation, and at the end of the post-stimulation period.

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 accounted for potential dropouts in our sample size calculation.
If a subject decides to discontinue participation before completing all study procedures, this subject will be considered a dropout and will be excluded from analyses. In case this happens during the recruitment period of the study,

researchers may choose to replace the subject. However, subject IDs will not be reused to prevent data confusion; instead, a new subject ID will be assigned to the “replacing” participant.

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.

8.1 Premature termination of the study

There are no expected reasons for premature termination of the study. However, in the event of urgent medical issues arising during the study (for example SAEs that result in death or are life threatening) the investigator (if necessary, together with the medical committee) can decide to halt the study to check whether it is safe to proceed or if 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 or not considered related to transcutaneous auricular vagal nerve stimulation or the nausea induction by intragastric lipid infusion. All adverse events reported spontaneously by the subject or observed by the investigator, or his staff will be recorded. Subjects are promptly asked to report any changes in their health in the 24 hours following the test day via phone or email to the coordinating investigator. These adverse events will then be recorded in Castor.

9.2.2 Serious adverse events (SAEs)

A serious adverse event is any adverse event that led to any of the following:

- Death,
- Serious deterioration in the health of the subject, that resulted in any of the following:
    - Life-threatening illness or injury,
    - Permanent impairment of a body structure or a body function,
    - Hospitalisation or prolongation of patient hospitalisation,
    - Medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function,
    - Chronic disease,
- Foetal distress, foetal death or a congenital physical or mental impairment or birth defect.

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 *Research Portal* to the review committee 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 collected and saved in 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 and secondary outcomes will be analyzed based on a modified intention-to-treat (ITT) approach. SPSS, R, Python, and Excel will be used to perform the statistical analysis.

Subject characteristics will be presented and analysed using descriptive statistics. Continuous parameters will be presented as means and standard deviations, and assessed between the two groups (taVNS vs. sham) with a two-sample unpaired t-test (for normally distributed data) or a nonparametric Mann-Whitney U test (for non-normally distributed data). Categorical variables will be represented as frequencies and analyzed using a Chi-square test or a Fisher's exact test A p-value < 0.05 will be considered statistically significant. No missing data are expected; however, if present, missing data will be handled using appropriate statistical methods.

The modified intention-to-treat population will be defined as all randomized participants who successfully complete the nausea induction procedure and in whom the intervention (taVNS or placebo) is initiated. All primary and secondary outcome analyses will be conducted based on this analysis set.

10.1 Primary study parameter(s)

To assess the effect of taVNS on nausea severity, Visual Analogue Scale (VAS) scores will be compared at various timepoints following nausea induction between the taVNS and sham stimulation groups. A linear mixed-effects model (LMM) will be utilized to evaluate both intergroup differences and within-group changes in VAS scores over time. LMM offers flexibility by adjusting for baseline nausea severity (if needed), handling missing data, and accounting for individual variability through random effects. LMM uses all available data points for each individual in likelihood estimation, rather than excluding participants with missing data. If LMM assumptions are violated, a nonparametric alternative may be considered. Potential confounding factors will be included as covariates in the LMM to adjust for their effects. Differences in VAS scores will be reported along with their corresponding 95% confidence intervals (CIs) and two-sided p-values, using a significance level (type I error rate) of 0.05.

10.2 Secondary study parameter(s)

The effects of taVNS compared to sham stimulation on other gastrointestinal complaints, the desire to eat, plasma levels of ghrelin and motilin, and salivary cortisol levels following nausea induction, will be analyzed using a linear mixed-effects model, consistent with the approach used for the primary outcome.

To assess the relationship between the nausea response and scores on the questionnaires measuring affective symptoms and personality treats (GAD-7, PHQ-9, and BFI), LMM with questionnaire scores as covariates will be used.

To evaluate the effect of taVNS vs. sham on autonomic parameters, including heart rate variability and skin conductance, a linear mixed-effects model will be employed. This model allows for the evaluation of the impact of taVNS on each autonomic parameter while accounting for within-subject correlations and missing data.

10.3 Other study parameters

Not applicable.

10.4 Interim analysis

Not applicable.

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

This study will be conducted according to the most recent version of the Declaration of Helsinki (75th WMA General Assembly, Helsinki, Finland, October 2024), the Medical Research Involving Human Subjects Act (WMO), and adheres to article 82 of the Medical Device Regulation (MDR, EU no 2017/745).

11.2 Recruitment and consent

Healthy volunteers will be recruited through a variety of channels, including advertisements in local and faculty newspapers, flyers/posters in public spaces, social media, and by reaching out to individuals who have previously participated in research and consented to be contacted for future studies, utilizing advertisement document E3_Wervingsmateriaal_Versie2.0_dd 02-04-2025. This document will outline the study's objectives, inclusion criteria and the timeline of this study. The contact details of the coordinating investigator will be provided at the bottom of the advertisement document, including a link/QR-code, that automatically generates an email, for easy access by potential participants. Upon contacting the study team via telephone, volunteers will receive a detailed oral explanation of the study from a researcher, and the written information brochure and informed consent form (see E1-2b_Informatiebrief_en_toestemmingsformulier_Versie2.0_dd 25-03-2025) will be sent via regular mail or email. For volunteers who contact the study team via email, the written information brochure and informed consent form will be promptly provided. Additionally, a verbal explanation of the study will be given over the phone. After sending the written information brochure and providing the verbal explanation, a minimum period of one week is given for the participant to decide whether they would like to participate. The participant is encouraged to contact the investigator by telephone to ask additional questions and to discuss their decision to participate. If the participant does not initiate contact with the researcher within this timeframe, the researcher will reach out to them once, at the earliest 7 days after sending the written information brochure and the verbal explanation, and only if the participant has given permission to do so during the initial conversation or in the initial email. During this contact, the researcher ensures that the participant understands the provided information and addresses any remaining questions. If the participant expresses a positive decision to participate, the first visit will be scheduled at Maastricht University. During this visit, participants will be explicitly asked to state in their own words what the study involves, including the associated risks and burdens. After this, the written informed consent form will be signed, (digital) questionnaires will be answered,

and a second visit for the measurements will be planned. No study procedures will be performed until the informed consent form is signed.

11.3 Objection by minors or incapacitated subjects

Not applicable.

11.4 Benefits and risks assessment, group relatedness

This study does not involve any incapacitated or minority groups and is considered a low-risk study. Although volunteers will not benefit directly from participating in this study, the risks associated with participation are minor and proportional to the scientific value of the research. This study can contribute to understanding the putative mechanism of action for taVNS, providing new insights and perspectives for further research into the application of taVNS in pathophysiological states, and potentially leading to new treatment options for various indications.

Subjects will be informed about the risks and burdens associated with the measurements beforehand. However, the burdens and risks of the current study are considered to be minimal to the benefits. The study protocol involves two visits, with the test day having a maximum expected duration of 3 hours. These visits take some time for participants but are not expected to interfere with their regular daily activities.

TaVNS stimulation is a non-invasive treatment approach, and to date, no serious adverse events have been reported in literature. For a detailed overview of previously reported risks of taVNS, see paragraph 6.4 ‘Summary of known and potential risks and benefits.

Insertion of a nasogastric tube will be performed manually by a doctor, the coordinating investigator, which is considered a safe, routine medical procedure with a low risk of complications. The entire procedure will take only a few minutes and is not expected to cause significant negative effects beyond mild discomfort in the nose or throat, which typically subsides after a short time. While potential complications include aspiration, bleeding, or soreness in the nose or throat, the risk in this study is anticipated to be much smaller, as the procedure will be conducted in young, healthy volunteers without gastrointestinal disorders or other clinical indications, as opposed to patients requiring a nasogastric tube for medical reasons (54). The infusion of a 50% fat emulsion in water is expected to induce nausea. Participants may also experience bloating, abdominal discomfort, cramps, or diarrhea, as fat emulsions can alter gastric emptying and motility. Vomiting may occur with larger fat volumes. Participants will be carefully monitored to manage and reduce any excessive discomfort.

The collection of blood samples can bring along some slight risk, i.e. hematoma, bleeding, pain and/or vasovagal reaction during or after vena punction. Participants will be seated on an examination bed, to prevent side effects to occur.

Further study procedures involve measurements of salivary cortisol levels and autonomic functions, using the Shimmer3 GSR sensor and Fitbit smartwatch, which carry no risks.

In the event of incidental findings during the study period, the general practitioner will be contacted. If a subject does not wish to be informed of incidental findings, they will not be allowed to participate in the study.

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 who complete the entire study, including the screening and test day, will receive a compensation of 150 euros. Furthermore, travel expenses will be compensated, reimbursing €0.23 per kilometre if travelled by car or full reimbursement for public transportation costs. Participants who choose to withdraw before the test day will be compensated for travel expenses but will not receive any additional compensation. In case the participant does not have a nausea response to the lipid infusion and the test day is terminated for this reason, a compensation of 150 euros will be provided.

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).

At study entrance, all subjects are assigned an individual study ID that is not directly traceable to their identity. The code starts with “VNSN” (standing for ‘Vagus Nerve Stimulation Nausea’) to indicate the study, followed by a three-digit participant number in chronological order, starting with “001” for the first subject, and so on (for example: “VNSN001”). The coordinating investigator will keep the key to the code in a locked cabinet, to which only the coordinating and principal investigator have access. In case of inspection, the Inspectie van Gezondheidszorg en Jeugd (IGJ) 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) and questionnaires

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 findings of the researcher during the visits, and any adverse events. CRFs will be coded and will not contain personal details of the subjects. Completed validated questionnaires (i.e. baseline characteristics questionnaire, GAD-7, PHQ-9, BFI, and VAS scores) will be saved within Castor EDC. Castor Electronic Data Capture, Ciwit BV, http://castoredc.com, Amsterdam, uses “True”, a NEN7510 and ISO 27001:2005 certified server, in line with previous studies performed by our group (PERSUADE and TENDER, NL56000.068.16/METC 162009 and NL62932.068.17/METC 173051 resp.).

2.Shimmer3 GSR sensor

For the Consenys software, an anonymized test subject ID will be used to ensure no traceable data is recorded. The key to this code will be stored securely in a locket

cabinet, accessible only to a select part of the research team, specifically the principal investigator (Prof. Dr. D. Keszthelyi) and the coordinating investigator (F. Veldman). 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.FitBit wearable

For the FitBit application, an anonymized test subject ID will be used to ensure no traceable data is recorded. The key to this code will be stored securely in a locket cabinet, accessible only to a select part of the research team, specifically the principal investigator (Prof. Dr. D. Keszthelyi) and the coordinating investigator (Drs. F. Veldman). All data on phones, on the server and in-transit are encrypted and pseudo-anonymized using industry-standard encryption techniques. Encrypted data from Fitbit will be stored via servers of Amazon Web Services (United States). As a result, data exchange of the encrypted 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 and the fact that the level of protection for data transfer to countries outside the European Economic Area is not exactly the same as the level of protection for data transfer to countries inside the European Economic Area. 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. Our group previously used the Fitbit applications for similar purposes (DISCOvERIE, NL75159.068.20/METC 20-076 & RESILIENCE, METC23-032 / NL84720.068.23 & tVNS and autonomic responses, METC24-029 / NL87188.068.24).

4.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 authorisation can upload new data and access existing data.

All primary documents and data will be stored for 15 years after the end of the study at Maastricht University and will be accessible to the principal investigators (Prof. Dr. D. Keszthelyi), coordinating investigator (F. Veldman), the Dutch Health Care Inspectorate (Inspectie Gezondheidszorg en Jeugd), and monitors assigned by the Clinical Trial Center Maastricht (CTCM). Samples taken from the subjects 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 primary investigator (Prof Dr. D. Keszthelyi) and the coordinating investigator (F. Veldman) 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 the current investigation. If subjects deny this consent, personal data and biological samples will only be used for the analysis as described in this protocol. By any means, personal data and biological samples will be securely stored for 15 years within Maastricht University.

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 patient’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

The tVNS R is a transcutaneous vagus nerve stimulation device that will be investigated outside the intended use of declaration of conformity (D2_Declaration_of_conformity_tVNS_L_signed_Versie1.0_31-05-2021).

a. Level of knowledge about mechanism of action

The therapeutic potential of taVNS is hypothesized to result from its ability to influence sensory feedback from the body to the brain. The auricular vagus nerve serves as the target of taVNS, providing a unique access point to the ANS since the external ear is the only location where the vagus nerve sends its only peripheral branch. Modulation of this solely vagal afferent leads to activation of the nucleus of the solitary tract (NTS), which serves as the primary relay station for sensory vagal afferents in the brainstem. The NTS has direct or indirect projections to the nuclei that provide noradrenergic, endorphinergic, and serotonergic fibers to various brain regions (13). Efferent outflow is then generated either via the efferent vagus through the vago–vagal reflex loop or via splanchnic nerves to various organs. Brain imaging studies have shown that taVNS can activate the NTS (55). However, the physiological impact of this activation on vagal or autonomic function in humans remains uncertain and is merely based on assumptions derived from animal studies or invasive VNS (13). Hypotheses suggests that taVNS stimulation may restore reduced vagal tone, which is commonly seen in various diseases, and subsequently restore homeostasis. Nevertheless, there is no conclusive evidence explaining why taVNS elicits therapeutic effects (35).

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

Studies have demonstrated the clinical efficacy of VNS in patients with depression (48), migraine (43), epilepsy (39-41), and tinnitus (42), among others. For a more detailed description of these studies, see paragraph 6.3. In addition, a previous study in adolescents with functional abdominal pain has also shown promising results of vagal neuromodulation (16).

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

TaVNS has been investigated in mice (34) and rats (33). See paragraph 6.2 for further details regarding these studies.

d. Selectivity of the mechanism to target tissue in animals and/or human beings
Not applicable.

e. Analysis of potential effect
The tVNS device is considered safe. Previous studies have shown no serious or long-lasting adverse events (40, 45-47). See paragraph 6.4 ‘summary of known potential risks and benefits’ for previously reported risks.

f. Pharmacokinetic considerations
Not applicable.

g. Study population
All participants are healthy volunteers aged 18-65 years. No risks have been identified for this population (see also paragraph 6.2). Vagus nerve stimulation has been applied to both healthy controls and patient populations for several years (56-59).

h. Interaction with other products
Not applicable.

i. Predictability of effect
Not applicable.

j. Can effects be managed?
In healthy humans, taVNS has been demonstrated to be a safe and well-tolerated procedure, particularly in individuals without a history of cardiac disease (37, 45, 46). Subjects with cardiac pathology or pacemakers will be excluded from the study. However, autonomic parameters will be monitored continuously throughout the taVNS stimulation and nausea induction period. No serious complications have been reported in literature. A few subjects have reported dizziness, fatigue, concentration problems and a tingling sensation at the ear, which are typically associated with longer stimulation durations or with higher stimulation intensities (47). Therefore, prior to the experiment, the stimulation current will be adjusted according to each subject’s sensitivity to ensure it remains below the pain threshold. Participants are allowed to withdraw from the study at any time.

13.1.2 Potential concerns related to the co-intervention
The introduction of a nasogastric tube poses no significant risks, although it may cause temporary discomfort in the nose or throat. The infusion of a 50% fat emulsion in water is expected to induce nausea. Participants may also experience bloating, abdominal discomfort, cramps, or diarrhea, as fat emulsions can influence gastric emptying and

motility. With higher fat volumes, vomiting may occur. Participants will be closely monitored to manage and minimize excessive discomfort. Rescue medication will be used in case nausea symptoms are too severe.

13.2 Synthesis

TaVNS is a non-invasive and a safe approach to use in research. The risks associated with participation in this study are minor and proportional to the scientific value of the research. Serious adverse events have not been reported in studies examining the efficacy of the tVNS device. Mild to moderate adverse events, including dizziness, fatigue, concentration problems, and a tingling sensation at the ear, have been reported. However, these symptoms typically and quickly disappear after removing the tVNS device. It is anticipated that this study will reveal important insights into the physiological impact of taVNS on vagal and autonomic function in healthy individuals, with an expected reduction in nausea severity.

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