Children's Hospital and Health System™

INSTITUTIONAL REVIEW BOARD
SUMMARY
20-FEB-2019

STUDY TITLE: Does Improving Vagal Tone Increase Mitochondrial Bioenergetics?

PRINCIPAL INVESTIGATOR: Gisela Chelimsky, M.D.

COINVESTIGATORS: Thomas Chelimsky, MD
Lynn Rusy, MD
Michael Tracy Zundel, MD
Alfonso Martinez, MD

A. PURPOSE OF THE STUDY.

The main purpose of this pilot study is to understand the relationship of the vagal nerve to mitochondrial function. The relationship between mitochondrial bioenergetics and vagal modulation has been nicely summarized by He et al al (1) in the cardiovascular arena. Direct vagal stimulation provides the clearest evidence of this relationship. Vagal stimulation improves cardiac function in experimental congestive heart failure and after ischemic injury, reducing inflammation and apoptosis independent of heart rate change (2, 3) and is now being tested in humans (4). Current evidence supports a role for nicotinic cholinergic receptors in the cardio-protective and anti-inflammatory effects of the vagus (5, 6). In particular, alpha-7nACh receptors can prevent formation of the inflammasome complex by halting the release of mitochondrial DNA (7). The vagus also controls mitochondrial bioenergetic capacity through muscarinic cholinergic M2 receptors, and cardiovascular physiologic parameters through cholinergic M3 receptors.

We and others have documented that subjects with functional gastrointestinal disorders (FGID) have decreased vagal tone. A cross sectional meta-analysis of 11 articles of adult subjects with IBS (8) showed decreased vagal modulation with increased sympathetic tone. Low vagal modulatory activity occurs in other chronic pain conditions (9-13), and has been linked with poor adaptive behavior in a meta-analysis of 44 studies in 5000 children (14). In turn, poor adaptability and high pain severity in childhood more likely results in pain-related FGID, non-abdominal chronic pain, and co-morbid anxiety and depression in adulthood (15). We have also shown that the mitochondrial bioenergetics is decreased in children and adolescents with FGID and other comorbid conditions. Dr. Kovacic has also demonstrated in a recently accepted manuscript to The Lancet Gastroenterology & Hepatology that 4 weeks of auricular neuro-stimulation with an Electro Auricular Device (EAD) improves pain and functionality. We have also demonstrated that this is done through improvement in vagal tone by measuring heart rate variability (HRV) before and after EAD. In this proposal we would like to see if improving vagal modulation through a 4-week of neuro-stimulation with the EAD results in increase in mitochondrial bioenergetics and decrease in inflammatory markers.

B. SPECIFIC AIMS / HYPOTHESIS

Specific aim: determine if vagal stimulation through an electro auricular device (EAD) will improve mitochondrial bioenergetics and decrease inflammation.

Hypothesis: The vagal nerve modulates mitochondrial bioenergetics capacity through muscarinic cholinergic M2 receptors. Therefore, knowing that the mitochondrial bioenergetics is decreased in adolescents with FGID, we postulate that a 4 week neuro-stimulation with an EAD that has already shown to increase vagal tone will produce an increase in mitochondrial bioenergetics in this patient group. Furthermore, the vagus plays a critical role in the anti-inflammatory pathways, therefore increasing vagal tone should also decrease the inflammatory response.

C. BACKGROUND, SIGNIFICANCE, AND RATIONALE

Pediatric FGIDs (pFGIDs) are prevalent (16, 17) and disabling. For example IBS, just one of many pFGIDs, occurs in 6-15% of the pediatric population (18) and affects daily activity in 40% of the children (19). Chronic pain affects approximately 8.8 million children annually, with a cost of 11.8 billion, with about 1/3 of this burden

related to abdominal pain (20). Yet, studies rarely focus on children (16-18). Because pFGIDs are frequently co-morbid with one another and with other functional disorders (21-24), they likely share fundamental pathophysiologic elements that involve the brain (15, 25, 26). Defining brain-body connections that underlie pFGID evolution could significantly move the field to understand pathophysiology and treatment.

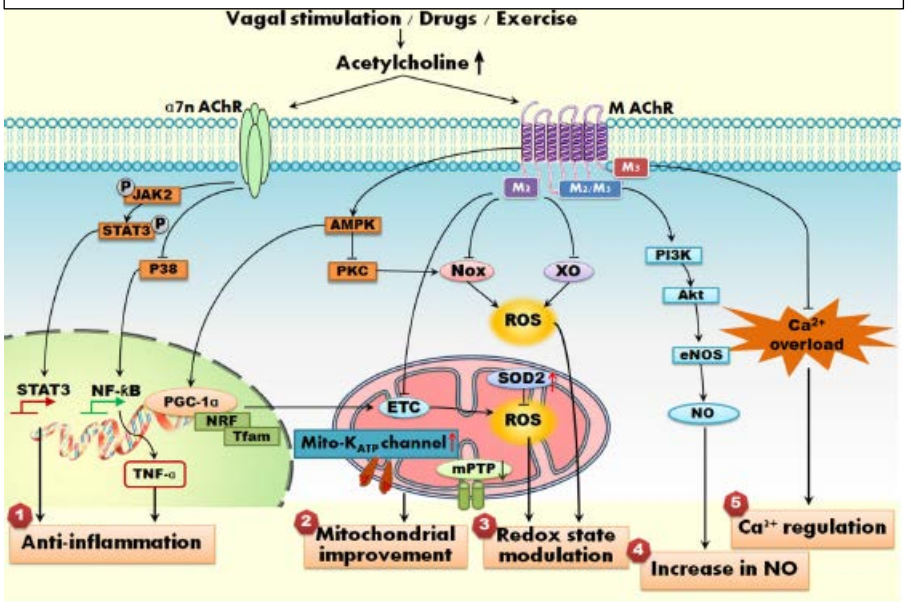
In our national referral center for pFGIDs, 2 important clinical observations emerged, confirmed by others (27-29). (1) Severe fatigue heralds symptom onset in many patients. (2) Those who implement a slowly progressive (ultimately rigorous) exercise program get better.

Our group has been researching these disorders for many years in an attempt to understand the pathophysiology. This proposal stems from our previous findings:1) the mitochondria bioenergetics is decreased in subjects with FGID; 2) the vagal tone is decreased in subjects with FGID; 3) FGID subjects that underwent 4 weeks of neuromodulation with an EAD had significant improvement in the symptoms and increase in vagal tone.

We have found in 59 subjects (30 healthy controls -HC; 29 FGID) with no difference in age (HC: median 16.3, range 10.9-18.7 yrs; FGID median 15 range 13-18 yrs; p = 0.088), basal respiration (BCR), extracellular acidification rate (ECAR –non-mitochondrial respiration) and ATP production in patients with FGID were about half the values found in HC. These findings could explain the severe fatigue and multi-system involvement in FGID subjects. We have also found that vagal function is decreased. Real-time vagal activity can be indexed using heart rate variability (HRV) parameters, particularly high frequency (hfHRV), root mean square of successive differences (RMSSD) and heart rate recovery after exercise. These measures are well accepted as reflecting an individual's "vagal health" (1). A cross sectional meta-analysis of 11 articles of adult subjects with IBS (8) showed decreased vagal modulation with increased sympathetic tone. Low vagal modulatory activity occurs in other chronic pain conditions (9-13), and has been linked with poor adaptive behavior in a meta-analysis of 44 studies in 5000 children (14). In turn, poor adaptability and high pain severity in childhood more likely results in pain-related FGID, non-abdominal chronic pain, and co-morbid anxiety and depression in adulthood (15). We prospectively studied the changes in HRV in our clinic in 13 subjects who had 2 visit points (7 with improvement in the Functional Disability Inventory –FDI- and 5 with either no improvement or worsening). RMSSD from supine (33.14±11.06 ms) and from standing (11.39±3.37 ms) increased in the pFGID improved group (supine 59.11±25.04 ms, standing 38.40±21.69 ms). RMSSD from supine dropped in the group that did not improve. Comparison of RMSSD difference between 1st and 2nd visit supine showed a trend for a difference between the two groups (p=0.09).

The relationship between mitochondrial bioenergetics and vagal modulation in the cardiovascular arena is nicely summarized by He et al al (1) (FIG 1). Direct vagal stimulation provides the clearest evidence of this relationship. Vagal stimulation improves cardiac function in experimental congestive heart failure and after ischemic injury, reducing inflammation and apoptosis independent of heart rate change (2, 3) and is now being tested in humans (4). Current evidence supports a role for nicotinic cholinergic receptors in the cardio-protective and anti-inflammatory effects of the vagus (5, 6). In particular, alpha-7nACh receptors can prevent formation of the inflammasome complex by halting the release of mitochondrial DNA (7). The vagus also controls mitochondrial bioenergetic capacity through muscarinic cholinergic M2 receptors, and cardiovascular physiologic parameters through cholinergic M3 receptors.

Fig 1: Figure from He at al (1) showing relationship of the vagus to inflammation and mitochondrial bioenergetics



Our team has demonstrated in a randomized, sham-controlled trial of 115 adolescents (11–18 years) who had an FGID, who underwent a percutaneous,

electrical stimulation to cranial nerves in the external ear utilizing a non-invasive, FDA-cleared device EAD (NSS-2 BRIDGE®, Innovative Heath Solutions, IN, USA) had greater reduction in pain at all weeks vs. baseline ($p \leq 0{\cdot}001$) with a lower score vs. sham after 3 weeks: Median (IQR): 5 (4-7) vs 7 (5-9) ($p=0{\cdot}005$). Effects were sustained at long-term follow-up (Median 9·2 weeks) in treatment group: baseline to follow-up Median (IQR): 8·0 (7-9) to 6·0 (5-8) vs sham: 7·5 (6-9) to 7·0 (5–8) ($p \leq 0{\cdot}001$). PFSD composite scores decreased significantly in treatment group (24·5 to 8·4) vs sham (22·8 to 15·2) ($p=0{\cdot}005$) after 3 weeks and long-term: treatment (-8·4; -15·9 – 0·0) vs sham (0·0; - 9·0 – 9·1) ($p=0{\cdot}018$) (accepted to Lancet 2017). Furthermore, the vagal tone increased in the treatment group perhaps explaining the mechanistic action of the EAD.

Based on all these preliminary data and the association of the vagus and mitochondrial described in the cardiac literature, our hypothesis is that mitochondria bioenergetics is controlled by the vagus and subjects with FGID who undergo EAD stimulation will have clinical improvement, increase in mitochondrial bioenergetics and decrease in inflammatory markers.

D. DESIGN AND METHODS

Study Population

We will enroll 7 adolescents with a painful functional gastrointestinal disorder between ages of 12-18 years.

Inclusion Criteria:

- Children aged 12 years to 18 years who provide written assent and whose parents provide written permission for participation
- English-speaking and able to verbalize their condition and concerns about nausea, pain and other symptoms
- Subjects will meet Rome IV criteria for functional nausea, irritable bowel syndrome, dyspepsia or functional abdominal pain as determined by a pediatric gastroenterologist
- Patients must have an intact external ear that is free of infection or severe dermatological conditions, have stable vital signs for their respective age, no history of seizures and no currently implanted electrical device

Exclusion Criteria:

- Mental retardation or pervasive developmental disorder or epilepsy
- Psychosis
- Genetic or chromosomal disorders
- Pregnancy
- Subjects who admit to substance abuse during screening
- Patients with findings of peptic ulcer disease, H.pylori gastritis, celiac disease, inflammatory bowel disease, allergic disorders, or any chronic condition or medication that may cause nausea or pain
- Patients who are treated with opioids or who had any changes in their medical regimen in the past four weeks prior to study
- Patients with a history of allergy to adhesives

Study Design Overview

Subjects will undergo medical workup per standard of care. Subjects refractory to current medical therapy may be enrolled without changes to their current medical treatment. No new pharmacological agents will be added during the 4-week study period and no changes in dosing or scheduling of current therapy will be allowed during the study period. Subjects will be screened weekly for any side effects by registered nurses who are part of the Pediatric Translational Research Unit (pTRU). All subjects will be asked to notify the PI or research team with any worsening in symptoms or side effects of therapy. If symptoms worsen or fail to improve during the study or if their clinical presentation changes in any way, subjects will have the option to drop out any time, receive standard medical therapy and appropriate referrals. If per standard of care, a subject requires a change in one or several of their regular medications during the study, they will be asked to notify the research team. To monitor this, the subjects will be inquired about their medication list and doses during their weekly visits. If the medication change is determined to potentially affect the study results, the subject may be excluded from further study as determined by the PI.

Protocol and Procedures

Pre (Baseline):

The primary investigator, a co-investigator or research coordinator will introduce the study to eligible patients seen in the CHW Autonomic and Gastroenterology Clinic during their regularly scheduled clinic visit or while

admitted to the hospital (at the end of admit). A member of the research team will explain the details and purpose of the study during an informed consent discussion. Once the patient and caregivers have had enough time to consider participation, understand all of the risks and benefits and would like to participate, they will complete the informed consent, assent and HIPAA documents.

Medical and psychological history will be obtained to verify eligibility and screen patients for alcohol, nicotine, and drug abuse following the completion of consent/assent documents. All eligible subjects will complete the questionnaires related to demographic and the Functional Disability Inventory (FDI). This will take about 5-10 minutes total to complete.

Visit 1 and 4: Subjects will undergo blood drawn for mitochondrial bioenergetics and have EAD placed. HRV will be measured before EAD placement at visit 1 and after EAD placement at visit 4. This visit will take about 60 min. They will complete the FDI again
Visit 2; 3: EAD will be changed and a new one will be placed.
Visit 5: This visit is about 2-3 month after the last EAD has been removed. At this visit they will undergo again blood drawn for mitochondrial bioenergetics, measurement of HRV and complete FDI.

The samples will be processed and stored in the laboratory of study research team members, Dr. Donald Basel in Health Research Center, H5720.

E. TOTAL NUMBER OF HUMAN RESEARCH PARTICIPANTS PROPOSED FOR THIS STUDY AT THIS SITE AND GLOBALLY. WHAT ARE THESE NUMBERS BASED ON?

7 patients aged 12-18 years, who are being evaluated in the Gastroenterology or FGID clinic are eligible to participate in the FGID group.

F. DRUGS OR PROCEDURES

Table 1.

	Pre	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5
Screening & Consent	x					
Heart Rate Variability		x			x	x
FDI	x				x	x
EAD		x	x	x	x	
Mitochondrial bioenergetics		x			x	x

All procedures will be performed in the Children's Hospital of Wisconsin (CHW) Pediatric Translational Research Unit (pTRU).

Measurements and Data Collection

At the time of each EAD placement, a member of the research team will inquire and document current medications and doses and if subjects experienced any side effects from the EAD during the previous week. Subjects will also be asked if they wore the device for all five days and it will be documented if the device was returned to the research team.

Demographic Information

Data will be collected as part of the clinic visit and at enrollment. This includes child's date of birth, age, sex, ethnicity, and caretaker marital status. The physician assessment will also include questions about prior medical diagnoses, co-morbid symptoms and the characteristics of pain and nausea such as timing and triggers. The FDI will be performed at visit 1; 4 and 5. This instrument has demonstrated reliability and validity and have been used for both clinical and research purposes in pediatric populations.

Medical Information

Information recorded for patients is part of clinical care and includes weight, height, vital signs, medications, gastrointestinal symptoms, early life events, family history, surgeries and other medical co-morbidities.

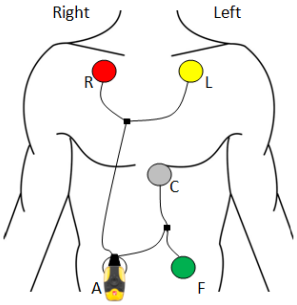
Heart Rate Variability

HRV reflects the variability of the R-R interval in the electrocardiogram and its frequency domains (30). HRV is an indirect measure of central autonomic control and an indicator of overall psychological well-being (31). Reductions in resting HRV reflect decreases in vagal output. The high frequency range of HRV is considered representative of vagal (parasympathetic) activity and studies show altered vagal tone as measured by HRV in subjects experiencing nausea (32). All subjects will undergo a 10 minute baseline HRV measurement (five minutes supine and 5 standing) after 10 minutes of rest. Recordings will be performed supine and standing to assess baroreflex response. HRV indices such as high and low frequency domains, low/high frequency ratio and the time domain RMSSD as a surrogate of vagal tone (30) will be analyzed using Kubious software. The RMSSD values will be the primary HRV outcome and compared to baseline (pre-EAD stimulation), post-stimulation and 2-3 month later at follow up.

We will use the following HRV collection methods; *eMotion* Faros Electrocardiography (ECG) Mobile 360 and the Actiwave by CamNTech. The *eMotion* Faros will primarily used, and in the event that the faros devices are not available, the Actiwave will be available as a backup device.

eMotion Faros ECG Mobile 360

The eMotion Faros ECG Mobile 360 is a remote electrocardiography monitoring system that is used for real-time HRV measurements. The ECG data will be transmitted from 3-5 electrodes (stickers) placed on the body (pictured below) to a mobile device through Bluetooth. The data can be retrieved in two ways: through a web browser for analyzing or with real time monitoring view on a computer. A password protected laptop will be used to import the data and then to import it into an HRV analysis software.



Actiwave by CamNTech

The Actiwave by CamNTech may be used to measure ECG. This device is a single channel recorder, where two electrodes (stickers) are placed on the patient's chest. A password protected laptop will be used to import the data and then to import it into an HRV analysis software.

Measurement of cytokines and mitochondrial bioenergetics: blood will be collected at visit 1; 4 and 5 and saved to perform cytokines (about 50ml). Dr. James Verbsky's Clinical Immunodiagnostic and Research Laboratory MFRC 5072, will be looking at "inflammatory markers'. Which means the blood will be tested to detect increase in protein which can be an indicator for inflammation.

Neuro-stimulator

Electro Auricular Device (EAD), (EAD) treatment protocol

Subjects will enter a 4-week, prospective treatment trial with the FDA-approved and commercially available EAD manufactured by Key Electronics (Jeffersonville, IN, USA) and distributed by Innovative Health Solutions (Versailles, IN, USA). The device is cleared under the PZR Class II (substantially equivalent, generic device) classification, with intended as a percutaneous nerve stimulator for substance use disorders determined by the states. EAD is an ambulatory, neurological device which consists of a battery powered, externally affixed generator with 4 wire leads attached to 3 electrode/ needle arrays and one single point needle. The arrays are designed to produce a field effect similar to surgically implanted peripheral neurostimulators. The electrodes will be placed percutanesously in the external ear with the help of a transilluminator to visualize the neurovascular bundles. Three electrodes will be placed on the ventral and one on the dorsal aspect of the ear. The electrodes will be taped and secured behind the ear next to the generator itself which is secured to the skin with adhesive. The entire device may be covered by longer hair. The placement of the devices is within standard of care by properly trained medical doctors or pTRU nurses. Training of the MDs and pTRU nurses were performed by representatives of Innovative Health Solutions and certificates were issued upon completion. Research coordinators within the division of gastroenterology and neurology will store the devices as pre-packaged by manufacturer in boxes of 4 devices and label the devices with a serial number and patient ID number. A labeled folder for each patient with all clinical information and demographics needed for the entire study will also be kept with each subject package. The devices and folders will be stored in a locked office in

the gastroenterology division, only accessible by research coordinators. A research coordinator will bring each subject's numbered package to the research area (Translational Research Unit) when patient arrives. The package for the subject will be stored locked in the TRU until the subject finishes the 4 week therapy. The coordinator will ask the subject demographic questions and hand the device (numbered with serial # and patient ID only) to one of the certified MDs or pTRU nurses for placement. The devices will be "made to order" when requested by the PI and will be shipped packaged as above.

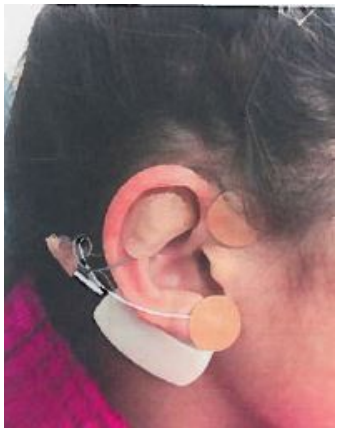
Patients will be told that they may or may not feel a slight flushing or tingling sensation after device placement. The electrode/needle arrays are implanted according to the individual's arterial and cranial nerve anatomy. The exact location of the implantation may vary slightly from person to person but is determined by both knowledge of auricular neuro-anatomy and visualization of the neurovascular bundles by transillumination (IHS, Versailles, IN, USA). The points will be targeted by four-point electrical stimulation using EAD after carefully disinfecting the ear. The small device will be positioned and secured behind the ear similar to a hearing aid, which may be covered by hair. Neurostimulation will be delivered below sensation threshold continuously for 5 consecutive days. The EAD will be applied by a trained MD or pTRU nurse. Subjects will remove the device independently at home as they are non-functioning after 5 days (5 days battery life). The subject will be asked to return the used device for proper disposal when they return for their follow-up appointment. If a device is lost or broken, the research team will attempt to schedule placement of a new EAD within 1 day if you wish. The devices are easily removed by removing the tapes and adhesives.

The EAD surgical kit consists of :

- an alcohol swab
- prep and stay swab
- round fixation plasters
- fixation plasters to fasten the generator
- Steri-strip adhesive vial
- Sterile wire harness pack
- Generator
- Tweezers
- Surgical marker
- Transilluminator

EAD placement details:

1. The EAD placement will be performed as directed and per EAD training protocol instructions identically to prior CHW IRB approved trial.
2. Before EAD placement, the subject should be advised that some discomfort is normal at first but should report if the discomfort persists or gets worse after a few minutes. The patients should be advised that they may feel a slight pulsing sensation and perhaps a warming sensation in the ear to which the electrodes are affixed. The pulsing and warming sensation may disappear after approximately 5 minutes. If the discomfort level increases the offending electrode can be slightly repositioned until the patient's discomfort level decreases to an acceptable level.
3. Device placement will take approximately 5 minutes. The patient will remain at rest for the next 5 minutes.
4. The subject will be advised not to immerse the device in water as the device is water resistant but not water proof. If showering or washing their hair, subjects will be instructed to place a dry wash cloth or plastic such as Press n' seal over the area to help protect the device. Subjects will be given a contact person (primary investigator and research coordinator) to call if they are having any problems with the device or if it falls off.
5. The patient will be instructed on removing the device after 5 days. If not comfortable doing so or unable to carry out the instructions, the patient may be seen that day for removal by one of the investigators.
6. The patient will be asked to return the used device for proper disposal when they return for their follow-up appointment.
7. The subject will be given a handout with information (attached) on device handling, exact date and time of device removal and date and time to return.
8. The subject will be advised to refrain from using any new or as needed anti-emetic or pain medications.
9. The subject is dismissed.



G. RISK CATEGORY

45 CFR 46.405- Research involving greater than minimal risk but presenting the prospect of direct benefit to the individual child subjects involved in the research.

H. RISKS AND THE PRECAUTIONS WHICH WILL BE TAKEN TO MINIMIZE RISK EXPOSURE

Safety Data

All safety variables (e.g., adverse events will be summarized for each assessment time (including follow-up) using descriptive statistics. Incidence of adverse events will be summarized by treatment.

Heart Rate Variability:

Participants will be asked in advance if they have a history of skin allergy to adhesives to avoid provoking any skin irritation or rash. However, some redness from the adhesive on the ECG surface electrodes may temporarily appear.

Neuro-stimulation:

Overall risks/ discomforts involved are very minimal – Rare (event rate 1% - < 5%)

Possible risks/discomforts may involve:

a. Discomfort upon insertion of the electrodes for < 5 minutes - Rare (event rate 1% - < 5%)
b. Discomfort at the lead placement site > 5 minutes – Rare (1 % - < 5%)
c. Bleeding at the electrode site if the neurovascular bundle is penetrated - Rare (event rate 1% - < 5%)
d. Localized discomfort if the electrodes should become dislodged during the wearing of the device - Rare (event rate 1% - < 5%)
e. Localized dermatitis - Rare (event rate 1% - < 5%)
f. Drop in blood pressure - Rare (event rate 1% - < 5%)
g. Syncope (fainting) - Rare (event rate 1% - < 5%)

Adverse effects to supporting personnel

a. Skin piercing with percutaneous needles - Rare (event rate 1 % - < 5%)

**In our recently completed randomized trial of auricular neuro-stimulation for functional abdominal pain there were no significant or serious adverse events noted. Of 115 enrolled subjects, 10 reported side effects for following reasons, none of which were serious: ear discomfort n=6 (three active/three placebo device); adhesive allergy n=3 (one active/two placebo); syncope due to needle phobia n=1 (placebo).

Safety Monitoring Plan

All procedures will be performed by trained professionals within the standard of care under continuous medical supervision in the hospital setting (research unit). Side effects will be inquired about and documented every week during the study and at the post-study follow-up visit. If any serious harm or discomfort is identified by the subjects or the study personnel, the treatment will be discontinued. Any patient with worsening in symptoms

during the study will be able to drop out at any time and receive standard of care medical therapy. The skin of the external ear will be carefully disinfected to avoid any infection risks. Subjects will undergo vital sign screening before enrollment and vital sign assessment before EAD placement.

Safety Analysis Plan

There are no dangerous interventions related to this study, and the short study timeline allows for discontinuation of treatment when it is proven to be ineffective.

I. PROVISION FOR THE PROTECTION OF PRIVACY OF SUBJECTS AND TO MAINTAIN THE CONFIDENTIALITY OF DATA

All research projects that collect electronic data must use appropriate security measures to ensure that data is protected from theft or loss in order to prevent breaches of confidentiality. You must indicate what encryption tools (or why they are not necessary) from the options below. The IRB will not review this protocol unless you indicate the encryption tools being used to secure your research data. If you do not have encryption in place on your systems, please contact your Information Systems support to arrange for one of the encryptions options listed below.

The following encryption products employ cryptographic modules that the National Institute of Standards and Technology has certified as meeting FIPS 140-2 requirements. Children's Hospital and Health System endorsed the use of these products made to encrypt hard drives and removable media. All electronic research data must be encrypted using one or more of these products.

Please indicate which encryption tools you are using to secure your research data.

___Credent Mobile Guardian (RS, PD)
___GuardianEdge Hard Disk and GuardianEdge Removable Storage Encryption (HD, RS, PD)
___IronKey encrypted flash drives (RS)
___McAfee Endpoint Encryption (HD, RS)
___Microsoft Bitlocker (HD, RS when used with Windows 7 and FIPS compliant algorithms are enabled)
___PGP Whole Disk Encryption and PGP Portable (HD, RS)
___SafeNet Protect Disk and SafeNet Protect File (HD, RS)
___Seagate Secure Self-Encrypting Drives (HD when encryption option is enabled)
___Symantec Endpoint Encryption (HD, RS, PD)
___WinMagic SecureDoc encryption (HD) (for MCW owned computers)
___Other (*add description*)

Does not apply because:

___ Data is de-identified – no PHI collected
___ Data is stored on paper only
___ Data is stored on CHW secured shared drives.
_X_ Data is stored on MCW secured shared drives.

Key

HD = Hard Drive
RS = Removable Storage (USB flash drive, CD, etc.)
PD = Portable Device (iPod; iPhone; PDA, etc.)

Each subject will be assigned a unique identifying number. The only identifiers listed on the questionnaires, HRV, cytokines and EAD kits and data collection forms will be date of service and the subjects unique identifying number. Each subject's identifying number and related electronic data will be kept on a secured, MCW password-protected database that provides access only to the PI and research staff. Only authorized research personnel will have access to the database. A separate secure database will have the subject identification number linked to the patient's name and info. The EAD devices will be shipped directly from the manufacturer (Key Electronics) to the GI office research coordinator who will label each package of 4 EAD devices with a patient ID and serial number with a prepared sticker label. A subject folder will also be prepared

and labeled with the same ID and kept together with the device package. The devices will be stored in a locked room in the division of gastroenterology. The kits will be transported by a research coordinator to the TRU on the day needed. The remaining kits will be stored in the a locked room in the GI clinic, labeled with the patient's ID. Only research coordinators within the division of gastroenterology will have access to the storage room. To track use and proper disposal of the devices, subjects will be asked to return the used device the following week (at time of the next stimulator placement or follow-up visit 5 if week 4). The doctor or research coordinator will record that device was returned and dispose it properly. The research coordinator will log this data every week on each subject as devices are returned.

Data Acquisition and Analysis

Data acquisition: we will collect EKG tracing to analyze HRV, which will include heart rate, high frequency HRV, low frequency HRV and RMSSD.

For the mitochondrial bioenergetics, we will collect basal respiration, ATP production, maximal respiration, spare respiratory capacity and extracellular respiration.

We will collect cytokines at baseline, week 4 and at visit 5

Statistical Analysis

Data Management

The data gathered will be entered into REDCap data system with each ID having a unique identifier for each participant. REDCap is a secure, web-based application for building and managing online databases. All data will be de-identified and samples will only have a unique identifier that will link the unique identifier with the patient's name.

The REDCap clinical database will be maintained by the Department of Quantitative Health Sciences, at the Children's Research Institute under the direction of Pippa Simpson, PhD.

EKG Data, questionnaire responses, and demographic data collection forms will be coded by a member of the research team so that all protected health information is removed. This coded data will not contain any visit dates. This coded data will be analyzed by collaborators Julian Thayer, Ph.D. (Ohio State University) and Dewayne Williams Ph.D. (University of California, Irvine) who will not be privy to any PHI. Coded data will be shared via Onedrive. Each subject's identifying number and related electronic data will be kept on a secured, MCW password-protected database that provides access only to the PI and research staff.

Missing Data Handling

Missing Data: Every effort will be made to avoid missing data. Using logistic regression, the pattern of missing data will be explored. Assuming the data are missing at random (MAR) multiple imputations for items will be used. Data analysis overview: Summary statistics, such as mean, median, standard deviation, range and correlation will be used as a first step to examine data. To satisfy parametric assumptions, we may perform transformations with justifications if possible and otherwise use non-parametric tests. We will use CART and biological and physiological information to identify interactions when formulating regression models. Statistical software employed for data analysis will be: Cytel StatXact, SAS version 9.4, Salford System CART for trees, SPSS Version 24.The power calculation was made using PASS 13.

Power

This is not applicable due to this being an exploratory pilot study. This study will be supported by a $12000 from CTSI (if approved), and number of subject was based on budget.

We will similarly analyze the change in vagal tone at visit 1, visit 4 and visit 5 and the different variables of mitochondrial bioenergetics including basal respiration, ATP production, maximal respiration, spare respiratory capacity and extracellular respiration.

We will also compare cytokines at visit 1; 4 and 5 and if possible correlate the change sin cytokines to changes in hfHRV and RMSSD.

J. PROVISIONS FOR MONITORING DATA TO ENSURE THE SAFETY OF SUBJECTS; AND ADDITIONAL SAFEGUARDS TO PROTECT THE RIGHTS AND WELFARE OF SUBJECTS WHO ARE LIKELY TO BE VULNERABLE

The PI will monitor the health of all patients in the study per standard clinical practice. The PI will monitor protocol adherence and supervise data collection, entry, and analysis.

K. ANTICIPATED BENEFITS ASSOCIATED WITH THE PROTOCOL TO HUMAN RESEARCH PARTICIPANTS AND SOCIETY

There are numerous, possible direct and long-term benefits to the subjects in this study. Our recently completed study of EAD in children with various functional abdominal pain disorders demonstrated improved pain, global well-being and functioning after only 3 weeks of therapy with effects sustained at 2 months follow-up (Accepted for publication: Kovacic, K Lacet GAstroenetrology and Hepatology 2017). Since it is well documented that functional disability and quality of life is worse in adolescents with functional pain disorders with concurrent nausea than in those without nausea,(33, 34) and as many subjects in the above trial were noted to have improvement in concurrent nausea, we postulate that some of the improvement in well being and functioning may be due to reduction in nausea along with pain. There is now also mechanistic data through heart rate variability studies before and after therapy (unpublished data), suggesting the EAD acts through peripheral vagal nerve stimulation effects. Given the known vagal pathways involved in nausea and vomiting, we anticipate a reduction in nausea in subjects suffering from chronic functional nausea. Our prior studies on functional nausea show a high prevalence of anxiety and co-morbid GI symptoms including bloating, fullness and discomfort.(33, 35). Based on our prior data, we therefore also anticipate that concurrent symptoms such as bloating, post-prandial fullness, and anxiety may improve. Of 104 subjects analyzed, there were no significant side effects reported other than mild ear discomfort (n=6; n=3 in active and n=3 in placebo arm), adhesive allergy (n=3), and one subject with syncope due to needle phobia (placebo arm). Adolescents suffering from functional GI disorders thus have the opportunity to benefit from a non-pharmacological and non-invasive therapy without the adverse effects of pharmacological agents. The results of this study may provide important insights to the medical field regarding a poorly characterized group of patients and the underlying mechanisms.

L. STOPPING POINTS THAT WOULD NOT ALLOW THE STUDY TO CONTINUE AS PROPOSED

Stopping points for the study include unanticipated adverse events, and inability to obtain enough data or patients/caregivers electing to discontinue the study.

Reporting Adverse Events and Unanticipated Problems

Expected adverse events that are not serious will be reported on the Continuing Review Progress Report. Continuing Review will be performed on a 12-month cycle, starting at time of protocol's initial approval. More frequent progress reports will be submitted at the request of the IRB.

Serious Adverse Events: The PI, within 24 hours, will report all serious adverse events occurring in any enrolled subjects to the IRB. Unexpected (but not serious) adverse events occurring in enrolled subjects which, in the opinion of the PI, are possibly related to participation in the protocol will be reported by the PI within 5 working days to the IRB.

Unanticipated problems involving risks to subjects or others will be reported to the IRB within 24 hours.

M. IS THERE A DATA SAFETY MONITORING BOARD IN PLACE? WHO ARE IT'S MEMBERS? HOW OFTEN DO THEY MEET?

As this is a low risk protocol, no data safety monitoring board will be appointed unless requested.

N. DESCRIBE HOW THE CONSENT PROCESS WILL TAKE PLACE. INCLUDE A LIST OF APPROPRIATELY TRAINED PERSONNEL WHO WILL BE INVOLVED.

Written informed consent for participation will be obtained from the parents and children for their child's participation. Written assent will be obtained from youth 12 and 13 years of age using the CHW assent form. Patients ages 14 and above will sign the assent line on the consent form per CHW policy. Consent will be obtained by a study investigator or member of the research team at the patients' appointment in the GI Clinic. Volunteers' consent will allow for accessing information collected for program evaluation/clinical purposes. Participating children and parents will have the option of having the consent/assent document read aloud to them to facilitate understanding. Copies of signed consent/assent documents will be given to participants. When consent is given for a minor child to participate in a research study by one or both parents, and the child reaches the age of majority (18 years) the child will be re-consented as an adult to continue participation in the study. If age of majority is reached while the participant is active and continues to receive study interventions per the study protocol, he/she will be re-consented using the most current approved IRB informed consent document and HIPAA form. If age of majority is reached after all study interventions have been completed and

the participant remains in the study for purposes of data collection for outcomes only a separate "Age of Majority" informed consent will be obtained.

O. PROCEDURES TO BE EMPLOYED IN ANALYZING DATA AND THE ANTICIPATED SIGNIFICANCE OF THE PROPOSED STUDY

Analyses will be conducted with SPSS and SAS software programs. Probability levels of < .05 will be used as cut offs for statistical significance.

The key significance of this study is the identification of a reduction in inflammatory cytokines, and increase in hfHRV. With this study, we hope to lay the foundation for the application of AED for pediatric functional GI disorders by demonstrating improvement in mitochondrial bioenergetics and reduction in inflammation as part of the pathophysiology. This is an innovative and novel understanding of the pathophysiology of FGID, which will enhance our knowledge on the brain-gut neural connectivity underlying functional disorders and ultimately improve patient care.

P. FINANCIAL RELATIONSHIPS

The EAD devices will be supplied by the distributing company (IHS) at manufacturing rate. A grant from Advancing a Healthier Wisconsin (AHW) will partially support this proposal and a CTIS Start Up Pilot award ($12000) funding decision is pending. Patients will receive a stipend of $20 at each of the following visits: Visit 1, Visit 4 and Visit 5. (After each blood draw is completed)

Q. ADVERTISEMENTS / FLIERS

Not applicable.

R. BIBLIOGRAPHY

1. He X, Zhao M, Bi X, Sun L, Yu X, Zhao M, Zang W. Novel strategies and underlying protective mechanisms of modulation of vagal activity in cardiovascular diseases. Br J Pharmacol. 2015;172(23):5489-500. Epub 2014/11/08. doi: 10.1111/bph.13010. PubMed PMID: 25378088; PMCID: 4667861.

2. Kong SS, Liu JJ, Yu XJ, Lu Y, Zang WJ. Protection against ischemia-induced oxidative stress conferred by vagal stimulation in the rat heart: involvement of the AMPK-PKC pathway. Int J Mol Sci. 2012;13(11):14311-25. Epub 2012/12/04. doi: 10.3390/ijms131114311. PubMed PMID: 23203066; PMCID: 3509582.

3. Zhao M, He X, Bi XY, Yu XJ, Gil Wier W, Zang WJ. Vagal stimulation triggers peripheral vascular protection through the cholinergic anti-inflammatory pathway in a rat model of myocardial ischemia/reperfusion. Basic Res Cardiol. 2013;108(3):345. Epub 2013/03/23. doi: 10.1007/s00395-013-0345-1. PubMed PMID: 23519622.

4. Schwartz PJ, De Ferrari GM, Sanzo A, Landolina M, Rordorf R, Raineri C, Campana C, Revera M, Ajmone-Marsan N, Tavazzi L, Odero A. Long term vagal stimulation in patients with advanced heart failure: first experience in man. Eur J Heart Fail. 2008;10(9):884-91. Epub 2008/09/02. doi: 10.1016/j.ejheart.2008.07.016. PubMed PMID: 18760668.

5. Calvillo L, Vanoli E, Andreoli E, Besana A, Omodeo E, Gnecchi M, Zerbi P, Vago G, Busca G, Schwartz PJ. Vagal stimulation, through its nicotinic action, limits infarct size and the inflammatory response to myocardial ischemia and reperfusion. J Cardiovasc Pharmacol. 2011;58(5):500-7. Epub 2011/07/19. doi: 10.1097/FJC.0b013e31822b7204. PubMed PMID: 21765369.

6. Xiong J, Yuan YJ, Xue FS, Wang Q, Cheng Y, Li RP, Liao X, Liu JH. Postconditioning with alpha7nAChR agonist attenuates systemic inflammatory response to myocardial ischemia--reperfusion injury in rats. Inflammation. 2012;35(4):1357-64. Epub 2012/03/07. doi: 10.1007/s10753-012-9449-2. PubMed PMID: 22391744.

7. Lu B, Kwan K, Levine YA, Olofsson PS, Yang H, Li J, Joshi S, Wang H, Andersson U, Chavan SS, Tracey KJ. alpha7 nicotinic acetylcholine receptor signaling inhibits inflammasome activation by preventing

mitochondrial DNA release. Mol Med. 2014;20:350-8. Epub 2014/05/23. doi: 10.2119/molmed.2013.00117. PubMed PMID: 24849809; PMCID: 4153835.

8. Liu Q, Wang EM, Yan XJ, Chen SL. Autonomic functioning in irritable bowel syndrome measured by heart rate variability: A meta-analysis. Journal of Digestive Diseases. 2013;14(12):638-46. doi: Doi 10.1111/1751-2980.12092. PubMed PMID: WOS:000326707400003.

9. Kang JH, Chen HS, Chen SC, Jaw FS. Disability in patients with chronic neck pain: heart rate variability analysis and cluster analysis. Clin J Pain. 2012;28(9):797-803. doi: 10.1097/AJP.0b013e3182442afd. PubMed PMID: 22751023.

10. Mazurak N, Seredyuk N, Sauer H, Teufel M, Enck P. Heart rate variability in the irritable bowel syndrome: a review of the literature. Neurogastroenterol Motil. 2012;24(3):206-16. doi: 10.1111/j.1365-2982.2011.01866.x. PubMed PMID: 22256893.

11. Micieli G, Cavallini A, Bosone D, Tassorelli C, Barzizza F, Rossi F, Nappi G. Imbalance of heart rate regulation in cluster headache as based on continuous 24-h recordings. Clin Auton Res. 1993;3(5):291-8. PubMed PMID: 8124060.

12. Kristiansen J, Ektor-Andersen J, Bondesson E, Orbaek P, Persson R, Garde AH, Hansen AM. Low heart rate variability is associated with extended pain-related sick leave among employed care-seekers. J Rehabil Med. 2011;43(11):976-82. doi: 10.2340/16501977-0882. PubMed PMID: 22031342.

13. Lerma C, Martinez A, Ruiz N, Vargas A, Infante O, Martinez-Lavin M. Nocturnal heart rate variability parameters as potential fibromyalgia biomarker: correlation with symptoms severity. Arthritis Res Ther. 2011;13(6):R185. doi: 10.1186/ar3513. PubMed PMID: 22087605; PMCID: PMC3334634.

14. Graziano P, Derefinko K. Cardiac vagal control and children's adaptive functioning: a meta-analysis. Biol Psychol. 2013;94(1):22-37. doi: 10.1016/j.biopsycho.2013.04.011. PubMed PMID: 23648264; PMCID: PMC4074920.

15. Walker LS, Sherman AL, Bruehl S, Garber J, Smith CA. Functional abdominal pain patient subtypes in childhood predict functional gastrointestinal disorders with chronic pain and psychiatric comorbidities in adolescence and adulthood. Pain. 2012;153(9):1798-806. doi: DOI 10.1016/j.pain.2012.03.026. PubMed PMID: WOS:000307299500009.

16. Boey C, Yap S, Goh KL. The prevalence of recurrent abdominal pain in 11- to 16-year-old Malaysian schoolchildren. J Paediatr Child Health. 2000;36(2):114-6. Epub 2000/04/12. PubMed PMID: 10760006.

17. N AJN. Recurrent Abdominal Pains: A Field Survey of 1,000 School Children. Arch Dis Child. 1958;33(168):165-70.

18. Hyams JS, Davis P, Sylvester FA, Zeiter DK, Justinich CJ, Lerer T. Dyspepsia in children and adolescents: a prospective study. J Pediatr Gastroenterol Nutr. 2000;30(4):413-8. Epub 2000/04/25. PubMed PMID: 10776953.

19. Saps M, Adams P, Bonilla S, Chogle A, Nichols-Vinueza D. Parental report of abdominal pain and abdominal pain-related functional gastrointestinal disorders from a community survey. J Pediatr Gastroenterol Nutr. 2012;55(6):707-10. doi: 10.1097/MPG.0b013e3182662401. PubMed PMID: 22744191.

20. Groenewald CB, Wright DR, Palermo TM. Health care expenditures associated with pediatric pain-related conditions in the United States. Pain. 2015;156(5):951-7. doi: 10.1097/j.pain.0000000000000137. PubMed PMID: 25734992; PMCID: PMC4607282.

21. Chelimsky G, Safder S, Chelimsky T. FGIDs in Children Are Associated With Many Nonpsychiatric Comorbidities: The Tip of an Iceberg? Journal of Pediatric Gastroenterology and Nutrition. 2012;54(5):690-1. doi: Doi 10.1097/Mpg.0b013e3182496b1f. PubMed PMID: WOS:000302868000023.

22. Johansson PA, Farup PG, Bracco A, Vandvik PO. How does comorbidity affect cost of health care in patients with irritable bowel syndrome? A cohort study in general practice. Bmc Gastroenterology. 2010;10. doi: Artn 31
Doi 10.1186/1471-230x-10-31. PubMed PMID: WOS:000276342500001.

23. Lackner JM, Ma CX, Keefer L, Brenner DM, Gudleski GD, Satchidanand N, Firth R, Sitrin MD, Katz L, Krasner SS, Ballou SK, Naliboff BD, Mayer EA. Type, Rather Than Number, of Mental and Physical Comorbidities Increases the Severity of Symptoms in Patients With Irritable Bowel Syndrome. Clinical Gastroenterology and Hepatology. 2013;11(9):1147-57. doi: DOI 10.1016/j.cgh.2013.03.011. PubMed PMID: WOS:000323816100021.

24. Whitehead WE, Palsson OS, Levy RR, Feld AD, Turner M, Von Korff M. Comorbidity in irritable bowel syndrome. American Journal of Gastroenterology. 2007;102(12):2767-76. doi: DOI 10.1111/j.1572-0241.2007.01540.x. PubMed PMID: WOS:000251249300025.

25. Chang FY, Lu CL. Irritable Bowel Syndrome and Migraine: Bystanders or Partners? Journal of Neurogastroenterology and Motility. 2013;19(3):301-11. doi: DOI 10.5056/jnm.2013.19.3.301. PubMed PMID: WOS:000325005100005.

26. Lee YC, Nassikas NJ, Clauw DJ. The role of the central nervous system in the generation and maintenance of chronic pain in rheumatoid arthritis, osteoarthritis and fibromyalgia. Arthritis Research & Therapy. 2011;13(2). doi: Artn 211
Doi 10.1186/Ar3306. PubMed PMID: WOS:000292449700047.

27. Frandemark A, Jakobsson Ung E, Tornblom H, Simren M, Jakobsson S. Fatigue: a distressing symptom for patients with irritable bowel syndrome. Neurogastroenterol Motil. 2016. Epub 2016/07/13. doi: 10.1111/nmo.12898. PubMed PMID: 27401139.

28. Simren M, Svedlund J, Posserud I, Bjornsson ES, Abrahamsson H. Predictors of subjective fatigue in chronic gastrointestinal disease. Aliment Pharmacol Ther. 2008;28(5):638-47. Epub 2008/06/20. doi: 10.1111/j.1365-2036.2008.03770.x. PubMed PMID: 18564325.

29. Han CJ, Yang GS. Fatigue in Irritable Bowel Syndrome: A Systematic Review and Meta-analysis of Pooled Frequency and Severity of Fatigue. Asian Nurs Res (Korean Soc Nurs Sci). 2016;10(1):1-10. Epub 2016/03/30. doi: 10.1016/j.anr.2016.01.003. PubMed PMID: 27021828.

30. Billman GE, Huikuri HV, Sacha J, Trimmel K. An introduction to heart rate variability: methodological considerations and clinical applications. Frontiers in Physiology. 2015;6:55. doi: 10.3389/fphys.2015.00055. PubMed PMID: PMC4340167.

31. Chalmers JA, Quintana DS, Abbott MJA, Kemp AH. Anxiety Disorders are Associated with Reduced Heart Rate Variability: A Meta-Analysis. Frontiers in Psychiatry. 2014;5:80. doi: 10.3389/fpsyt.2014.00080. PubMed PMID: PMC4092363.

32. Lacount L, Napadow V, Kuo B, Park K, Kim J, Brown E, Barbieri R. Dynamic Cardiovagal Response to Motion Sickness: A Point-Process Heart Rate Variability Study. Computers in cardiology. 2009;36:49-52. Epub 2009/01/01. PubMed PMID: 20445767; PMCID: PMC2863354.

33. Kovacic K, Williams S, Li BU, Chelimsky G, Miranda A. High prevalence of nausea in children with pain-associated functional gastrointestinal disorders: are Rome criteria applicable? J Pediatr Gastroenterol Nutr. 2013;57(3):311-5. doi: 10.1097/MPG.0b013e3182964203. PubMed PMID: 23591912.

34. Youssef NN, Murphy TG, Langseder AL, Rosh JR. Quality of life for children with functional abdominal pain: a comparison study of patients' and parents' perceptions. Pediatrics. 2006;117(1):54-9. Epub 2006/01/07. doi: 10.1542/peds.2005-0114. PubMed PMID: 16396860.

35. Kovacic K, Miranda A, Chelimsky G, Williams S, Simpson P, Li BU. Chronic idiopathic nausea of childhood. J Pediatr. 2014;164(5):1104-9. doi: 10.1016/j.jpeds.2014.01.046. PubMed PMID: 24607239.