

## **COVER PAGE**

### **OFFICIAL TITLE**

Effect of Vestibular Nerve Stimulation on Fat Consumption and Energy Expenditure as assessed using indirect calorimetry.

NCT Number: NCT03138382

Date: 5/25/2017

## **STUDY PROTOCOL**

### **Study Design**

- Crossover design
- Double-blind
- Randomized
- Primary endpoint classification: Change in fat consumption from baseline
- Secondary endpoint classification: Change in energy expenditure from baseline

### **Pre-enrollment Screening**

In this visit the study coordinator, or designated Research Assistant, will review the study's purpose and procedures with prospective participants, answer any questions, obtain written consent from the participant and confirm that the participant meets study inclusion/exclusion criteria by asking them to complete a questionnaire about their medical history and medications to ensure that they fulfil the inclusion and exclusion criteria for the study. The completed questionnaires will be identified using an alphanumeric code and will not have any identifiable subject data on them. The questionnaires will be securely stored in a locked cabinet.

### **First Study Visit**

The first study visit for subjects who pass the screening process will require a visit to the EPARC laboratory for approximately two hours. Subjects should attend fasted from 7.30PM the previous evening, though they are allowed to continue drinking water. Subjects should not have engaged in any significant exercise for 24 hours prior to the test.

Testing will begin between 7AM and 8AM at the EPARC laboratory in the Qualcomm Institute (i.e Atkinson Hall) on the UCSD campus. A free designated parking space will be provided and laboratory staff will escort the subject into the lab where the testing takes place. Participants will have their age, height, weight and gender recorded, and undergo a 15-minute assessment of (sitting) resting energy expenditure (EE) using indirect calorimetry (COSMED, Italy) following a standardized protocol in which the subject is asked to sit quietly while watching a nature or science documentary from Netflix on a (provided) tablet computer. The subject will be fitted

with a mask that captures (and analyzes) all of their expired air to calculate oxygen uptake ( $\text{VO}_2$ ), carbon dioxide production ( $\text{VCO}_2$ ), energy expenditure (EE), and substrate (carbohydrate and fat) utilization.

The subject will then be randomized to treatment sequences via a randomized block procedure, with block sizes randomly chosen in the set  $\{2, 4, 6\}$ . Subjects will be assigned 1:1 between an initial active stimulation, and initial sham stimulation as a control. This will be done in a double-blind manner, and whichever group a subject is randomized to on their first visit they will crossover to the other group on their second study appointment. So if a subject is initially randomized to what happens to be the active stimulation protocol, then on their second study visit that subject will crossover and receive the sham protocol, and vice versa.

The subject will then undergo 45 minutes of either vestibular nerve stimulation or sham stimulation, as described below. The subject will continue to sit quietly watching a science or nature documentary. During this 45-minute stimulation period the subject will have their EE measured during the middle 15 minutes.

After the 45-minute stimulation period ends the subject will remain in the EPARC facility for one more hour. They will continue to sit quietly and watch a documentary. During this 1-hour post-stimulation period the subject will undergo two more 15-minute measurements of their EE. The first 15-minute measurement will take place during the first 15 minutes of this one-hour post-stimulation period, and the second 15-minute measurement will take place during the final 15 minutes of the one-hour post-stimulation period.

### **Second Study Visit**

The second study visit will take place at least two weeks after the first to allow a suitable washout period from the first study visit. Subjects should attend fasted from 7.30PM the previous evening, though they are allowed to continue drinking water. Subjects should not have engaged in any significant exercise for 24 hours prior to the test.

Testing will begin between 7AM and 8AM at the EPARC laboratory in the Qualcomm Institute (i.e. Atkinson Hall) on the UCSD campus. Again a free, designated parking space will be provided and subjects will be met at their car and escorted into the lab. The total visit will last about two hours. Participants will once again have their age, height and weight recorded, and undergo a 15-minute assessment of (sitting) resting energy expenditure (EE) using indirect calorimetry (COSMED, Italy) following a standardized protocol in which the subject is asked to sit quietly while watching a nature or science documentary from Netflix on a (provided) tablet computer. The subject will be fitted with a mask that captures (and analyzes) all of their expired air to calculate oxygen uptake ( $VO_2$ ), EE, and substrate (carbohydrate and fat) utilization.

The subject will then crossover to receive 45 minutes of either active stimulation or sham stimulation, as described below. As stated above, whichever stimulation arm the subject was randomized to during their first study visit, they will now receive the opposite. The subject will continue to sit quietly watching a science or nature documentary throughout. During this 45-minute stimulation period the subject will have their EE measured during the middle 15 minutes.

After the 45-minute stimulation period ends the subject will remain in the EPARC facility for one more hour. They will continue to sit quietly and watch a documentary. During this 1-hour post-stimulation period the subject will undergo two more 15-minute measurements of their EE. The first 15-minute measurement will take place during the first 15 minutes of this one-hour post-stimulation period, and the second 15-minute measurement will take place during the final 15 minutes of the one-hour post-stimulation period.

### **Stimulation Protocol for Both Active and Sham Devices**

Subjects will have the skin behind their ears cleaned with a disposable alcohol wipe before applying the device. The electrodes that will be used are 25mm in diameter hydrogel electrodes, which are hypo-allergenic. The devices will physically appear identical on the outside and both examiners and subjects will be blinded as to whether the device is active or "sham" control.

The devices will be brought in charged and no charging will take place during their usage in the study. Prior to the session subjects will be counseled that they may or may not experience a

rocking sensation during the stimulation and that not everyone can perceive the vestibular stimulation. This is true and also necessary to prevent subjects distinguishing between the active and sham devices.

There are buttons on the outside of the devices that allow their outputs to be tuned up or down. When initially powered up the devices do not deliver any current. The active device increases its output to the subject in 0.1mA increments. The sham device will discharge its current into an internal resistor rather than the subject.

The devices will be operated by the member of the research team, and over the course of minute or so they will turn it up to a level that the subjects say they are comfortable with for the 45-minute stimulation period. Afterwards subjects will have a little aloe vera gel applied to where the electrodes were, as this is thought to be particularly effective in preventing any skin irritation by VeNS.

### **Specific Measure**

The Cosmed CPET, or portable K4B2, metabolic assessment unit will be used for all measurements of indirect calorimetry. Subjects are fitted with a pliable facemask that covers their nose and mouth, and allows for normal breathing. However, the facemask allows us to measure all of the air the subject inspires as well as the fractional composition of inspired and expired gases. EE and substrate utilization are derived from the  $\text{VO}_2$  and  $\text{VCO}_2$  measurements.

The primary endpoint of the study will be change in fat consumption from the baseline measurement, and the secondary endpoint will be the change in energy expenditure from the baseline.

## **INCLUSION AND EXCLUSION CRITERIA**

### **Inclusion Criteria**

1. Signed informed consent
2. Body mass index (BMI)  $> 25 \text{ kg/m}^2$
3. 18-65 years of age inclusive on starting the study.
4. Successfully completed the screening questionnaire.

### **Exclusion Criteria**

1. History of vestibular dysfunction.
2. History of bariatric surgery, fundoplication, gastric resection or major upper-abdominal surgery (acceptable surgeries include cholecystectomy, hysterectomy).
3. History of skin breakdown, eczema or other dermatological condition (e.g. psoriasis) affecting the skin behind the ears, or of the head and neck.
4. History of weight loss device implantation (e.g. VBloc Maestro or Abiliti).
5. Positive response in Physical Activity Readiness Questionnaire.
6. Currently taking medication for asthma or other breathing conditions.
7. Untreated thyroid disorder (stable treatment for 3 months is acceptable).
8. Other endocrinological causes of weight gain (e.g. Cushing's disease, Cushing's syndrome or acromegaly)
9. Previous diagnosis of HIV infection or AIDS (HIV is known to cause a vestibular neuropathy which would prevent VeNS from working).
10. History of cirrhosis, or liver, kidney or heart failure.
11. Chronic pancreatitis.
12. Treatment with prescription weight-loss drug therapy in the year before starting the study.
13. Tobacco or marijuana smoking in the 6 months prior to study.
14. Known genetic cause of obesity (e.g., Prader-Willi Syndrome).
15. Diabetes mellitus (Types 1 & 2).
16. Diagnosis of epilepsy or use of anti-epileptic medication within six months of starting the study (e.g. for the treatment of peripheral neuropathy)

17. Chronic (more than a month of daily use) treatment with opioid analgesic drugs within the last year.
18. Regular use (more than twice a month) of anti-histamine medication.
19. Use of oral or intravenous corticosteroid medication within two years of starting the study.
20. Use of the beta-blockers atenolol, metoprolol or propranolol within 3 months of starting the study.
21. Current use of tricyclic antidepressant medication for whatever reason.
22. Current alterations in treatment regimens of anti-depressant medication for whatever reason (other than tricyclic antidepressants) (Note: stable treatment regimen for prior six months acceptable).
23. An active diagnosis of cancer.
24. A previous myocardial infarction.
25. A history of stroke or severe head injury (as defined by a head injury that required intensive care). (In case this damaged the neurological pathways involved in vestibular stimulation).
26. Presence of permanently implanted battery powered medical device or stimulator (e.g., pacemaker, implanted defibrillator, deep brain stimulator, vagal nerve stimulator etc.).
27. Psychiatric disorders (including untreated severe depression, schizophrenia, substance abuse, bulimia nervosa etc.)
28. Current participant in a clinical trial for weight loss.

## **RISK ASSESSMENT**

### General Risks

1. Information of a personal nature will be collected in order to determine eligibility for the study, and therefore there is a risk of loss confidentiality.
2. Subjects may experience some degree of distress or anxiety due to confusion, the personal nature of the questions, the VeNS, or their disqualification based on the exclusion criteria.

### Risks related to VeNS

1. Skin irritation at the electrode sites.
2. That the stimulation sites may be uncomfortable at the time of stimulation – an electrical tingling sensation may occur and also VeNS may induce the sensation of being pushed towards the side of the cathode.
3. A sensation of disequilibrium, analogous to being on a boat, may occur during the test that some subjects find uncomfortable.
4. VeNS may induce nausea.
5. VeNS may occasionally induce vomiting.

### Risks related to Indirect Calorimetry

1. Risk of infection from the facemask used during indirect calorimetry.

It is possible some subjects may find the facemask to be claustrophobic.

## **RISK MANAGEMENT**

### Risks Related to Vestibular Nerve Stimulation (VeNS)

1. Any potential subjects a history of irritation to the skin behind the ear will be excluded
2. Hypoallergenic hydrogel electrodes of 25mm diameter will be used in order to minimize the likelihood of skin irritation.
3. Subjects will be asked to apply aloe vera gel to minimize any skin irritation after using the device.
4. Prior to the beginning of the study the possible discomforts of VeNS will be described to the participant in lay language, and the investigator will make sure that the subject understands what will happen when using the device.



5. The usage of the device is limited to 45 minutes and a member of the experimental team will oversee.
6. Usage is voluntary, and subjects will be informed at the beginning of the study that they may withdraw at any time, for any reason, with no negative repercussions.
7. Subjects will be warned about the sensation of disequilibrium which may occur and will only be using the device while seated.
8. The maximum current the device can deliver is 1.0mA but there is no requirement for the subjects to use it at this level. Instead they will be asked to indicate when the experimenter has reached a level that is comfortable. The minimum setting is 0.1mA and the current can be increased in 0.1mA increments.
9. If subjects find the sensation invoked at the level the current is set, to be uncomfortable (e.g. in terms of nausea or disequilibrium) then they can ask for a lower, and more comfortable, stimulation level.
10. The subjects can take off the devices at any point they wish.

#### Risks pertaining to indirect calorimetry

1. The risk of infection is minimized by cleaning the mask using liquid glutaraldehyde disinfectant (e.g. Cidex) which is consistent with CDC recommendations. We use EPA-registered sterilants for this purpose in our laboratory.
2. Subjects can remove the facemask at any time during the experiment if they feel that they no longer wish to continue.

#### **CONFIDENTIALITY**

1. Subject's information will be kept strictly confidential to the extent provided by law. An alphanumeric code will be assigned to each subject, and this code will be used in place of a name when reporting data. The hardcopy corresponding subjects with codes will be kept in a locked data cabinet in Mandler Hall, along with all results of all screening questionnaires. The computer to be used for data collection and analysis will require a password for access,

which only personnel directly involved in the study will have. Data in any format will only be available for those on the protocol. Information collected in the study will not be reused or disclosed for other purposes. Data collected in the study will be destroyed after seven years.

2. All aspects of the study will be explained to the subject in lay language, and the experimenter will make sure that the subject understands what will happen prior to his/her participation. The experimenters will inform the subject of the precautions that will be taken with regard to personal information collected during the course of the study. The study will be as short as possible. Participation is voluntary, and subjects will be informed at the beginning of the study that they may withdraw at any time, for any reason, with no negative repercussions. The research should in no way result in social stigmatization or any other long-term distress to the subjects.
3. In order to minimize the risk of coercing subjects to participate, and in keeping with the UCSD HRPP guidance in this area no attempts to recruit subjects during undergraduate classes will be made.
4. Similarly, due to the risk of coercion the following will be excluded from participating: graduate students in Psychology; undergraduate students working as research assistants at the CBC; and employees in Psychology.

## **References**

- Bikson M, Datta A, Elwassif M. Establishing safety limits for transcranial direct current stimulation. *Clin Neurophysiol* 2009; 120: 1033-1034.
- Brunoni AR, Amadera J, Berbel B, Volz MS, Rizzerio BG, Fregni F. A systematic review on reporting and assessment of adverse effects associated with transcranial direct current stimulation. *Int J Neuropsychopharmacol* 2011; 14: 1133-1145.
- Brunoni AR, Nitsche MA, Bolognini N, Bikson M, Wagner T, Merabet L, Edwards DJ, Valero-Cabre A, Rotenberg A, Pascual-Leone A, Ferrucci R, Priori A, Boggio PS, Fregni F.

- Clinical research with transcranial direct current stimulation (tDCS): challenges and future directions. *Brain Stimulat* 2012; 5: 175-195.
- Brunoni AR, Valiengo L, Baccaro A, Zanão TA, de Oliveira JF, Goulart A, Boggio PS, Lotufo PA, Bensenor, IM, Fregni F. The sertraline vs. electrical current therapy for treating depression clinical study: results from a factorial, randomized, controlled trial. *JAMA Psychiatry* 2013; 70: 383-391.
- Fitzpatrick RC, Day BL. Probing the human vestibular system with galvanic stimulation. *J Appl Physiol* 2004; 96: 2301-2316.
- Kessler SK, Turkeltaub PE, Benson JG, Hamilton RH. Differences in the experience of active and sham transcranial direct current stimulation. *Brain Stimulat* 2012; 5: 155-162.
- McIntire LK, McKinley RA, Goodyear C, Nelson J. A comparison of the effects of transcranial direct current stimulation and caffeine on vigilance and cognitive performance during extended wakefulness. *Brain Stimulat* 2014; 7: 499-507.
- Magis D, Sava S, d'Elia TS, Baschi R, Schoenen J. Safety and patients' satisfaction of transcutaneous supraorbital neurostimulation (tSNS) with the Cefaly® device in headache treatment: a survey of 2,313 headache sufferers in the general population. *J Headache Pain* 2013 Dec 1;14:95. doi: 10.1186/1129-2377-14-95.
- Morales-Quezada L, Cosmo C, Carvalho S, Leite J, Castillo-Saavedra L, Rozisky JR, Fregni F. Cognitive effects and autonomic responses to transcranial pulsed current stimulation. *Exp Brain Res* 2015; 233: 701-709.
- Nitsche MA, Liebetanz D, Lang N, Antal A, Tergau F, Paulus W. Safety criteria for transcranial direct current stimulation (tDCS) in humans. *Clin Neurophysiol* 2003; 114: 2220-2222.
- Paneri B, Khadka N, Patel V, Thomas C, Tyler W, Parra LC, Bikson M. (2015) The tolerability of transcranial electrical stimulation used across extended periods in a naturalistic context by healthy individuals. *PeerJ PrePrints* 3:e1574  
<https://doi.org/10.7287/peerj.preprints.1097v2>
- Poreisz C, Boros K, Antal A, Paulus W. Safety aspects of transcranial direct current stimulation concerning healthy subjects and patients. *Brain Res Bull* 2007; 72: 208-214.
- Raimundo RJS, Uribe CE, Brasil-Neto JP. Lack of clinically detectable acute changes on autonomic or thermoregulatory functions in healthy subjects after transcranial direct current stimulation (tDCS). *Brain Stimulat* 2012; 5: 196-200.

Russo R, Wallace D, Fitzgerald PB, Cooper NR. Perception of comfort during active and sham transcranial direct current stimulation: a double blind study. *Brain Stimulat* 2013; 6: 946-951.

Tadini L, El-Nazer R, Brunoni AR, Williams J, Carvas M, Boggio P, Priori A, Pascual-Leone A, Fregni F. Cognitive, mood, and electroencephalographic effects of noninvasive cortical stimulation with weak electrical currents. *J ECT* 2011; 27: 134-140.

Utz KS, Korluss K, Schmidt L, Rosenthal A, Oppenländer K, Keller I, Kerkhoff G. Minor adverse effects of galvanic vestibular stimulation in persons with stroke and healthy individuals. *Brain Inj* 2011; 25: 1058-1069.

Wilkinson DT, Zubko O, Sakel M. Safety of repeated sessions of galvanic vestibular stimulation following stroke: A single-case study. *Brain Injury* 2009; 23: 841-845.

Zink R, Bucher SF, Weiss A, Brandt T, Dieterich M. Effects of galvanic vestibular stimulation on otolithic and semicircular canal eye movements and perceived vertical. *Electroencephalogr Clin Neurophysiol* 1998; 107: 200-205.

## **Appendix 1: Study Devices and Risk Assessment**

### **Background**

The active vestibular nerve stimulation (VeNS) device is being made bespoke by i4 Product Design and a Risk Assessment is detailed below. It will administer a 0.5Hz square wave stimulation up to a maximum current of 1mA. A binaural placement of the electrodes will be used, which means an electrode will be placed on the skin overlying each mastoid process. The battery (4.25V) powered control unit which connects to deliver the stimulation will rest in front of the subject and be connected to the electrodes via cables.

### **Risk Assessment**

In terms of this amendment the following risk assessment was also carried out for vestibular nerve stimulation devices that can deliver up to 1mA. Under the guidance for IRBs from the FDA a device will be determined to be non-significant risk (NSR) if it does not meet the definition of a significant risk (SR) device (see <https://irb.ucsd.edu/device.pdf>). A SR device is

one which meets at least one of the following criteria:

- a. The device is intended as an implant.
- b. The device supports or sustains human life.
- c. The use of the device is of substantial importance in diagnosing, curing, mitigating, or treating disease, or preventing impairment of health.
- d. The device could cause significant harm to any subjects.
- e. The subject must undergo a procedure as part of the device study.
- f. The device appears on the FDA list of significant risk devices.
- g. The study or any of the study procedures could cause harm to the subjects which:
  - i. could be life threatening;
  - ii. could cause permanent impairment of a body function;
  - iii. could cause permanent damage to body structure;
  - iv. or could necessitate medical or surgical intervention to preclude permanent impairment of a body function or preclude permanent damage to body structure.

As the device is not implanted, no procedure is required to use it, it does not support or sustain life and it is not on the FDA list of significant risk devices, criteria a, b, e and f clearly do not apply. Regarding criterion c, the aim of this study is to investigate whether vestibular stimulation can, in conjunction with lifestyle interventions, assist with fat loss. It may be the case that VeNS has such a role, but it is unlikely that this will be of “substantial importance in ... curing, mitigating, or treating disease, or preventing impairment of health.” Thus criterion c does not apply.

This then leaves criteria d and g, which both pertain to users suffering significant harm from the device. When the device is turned on it will start at 0mA and the current can then be increased in 0.1mA increments up to 1.0mA. The waveform is a bipolar rectangular shape with 50% duty cycle and – i.e. half the time no current is being delivered. There is also a hardware interlock to ensure the device cannot be switched on when the charging cable is being used. Please note the device is battery, and not mains, powered.

The devices are placed on the head in a manner analogous to headphones, and the VeNS devices will deliver a small electrical current (1.0mA or less) to the skin behind the ears over the mastoid processes. The device can only be used for up to one hour a day, and after being used for this time period the device will automatically lock out until the next day. Delivery of electrical current to the skin over the mastoid region is known to activate all five components of the vestibular apparatus, but lower level currents (below 3mA) are thought to particularly activate the two otolith organs responsible for detecting linear acceleration and gravity (Zink et al., 1998). It is these otolith organs that, from the animal studies, are particularly associated with a reduction in body fat. This technique – known as bilateral bipolar vestibular stimulation – has been known about since the 19<sup>th</sup> century and is known to be safe, though skin irritation behind the ears can occasionally occur (Fitzpatrick & Day, 2004).

More recently Paneri et al. (2015) reviewed the safety of repeated sessions of transcranial electrical stimulation (tES), a term that they use to encompass both the transdermal electrical modulation of cranial nerves (i.e. as in VeNS) and transcranial direct current stimulation (tDCS) of the cerebral cortex. Based on studies of tES on patient groups with depression (Brunoni et al., 2013) and migraine (Magis et al., 2013), and an array of studies of tES on normal volunteers (Brunoni et al., 2011; Kessler et al., 2012; McIntire et al., 2014; Morales-Quezada et al., 2015; Nitsche et al., 2003; Poreisz et al., 2007; Raimundo et al., 2012; Russo et al., 2013; Tadini et al., 2011), together with theoretical assessments of tES (Bikson et al., 2009; Brunoni et al., 2012), they commented that the “safety and tolerability profile previously accumulated regarding extended use of tES in clinical populations is compelling and supports a low-risk or non-significant risk designation” (Paneri et al., 2015).

Paneri et al. (2015) also assessed the safety and tolerability of repeated tES across time in a group of 100 volunteers who were split into three groups. The first group received sham stimulation, the second tES at 2mA, and the third pulses of tES at between 5 to 7 mA (this is notably higher than the 1mA maximum we are proposing to use). A total of 1905 treatment sessions were carried out in total across the three groups. The authors report no serious adverse events in any treatment condition, and that the common side effects were restricted to skin tingling, itching, and mild burning. Moreover, the incidence of these events were not statistically

higher in the tES groups (Paneri et al., 2015). These mild skin sensations were not associated with withdrawal from the study and the authors report they became less salient after the first few sessions. Other adverse events, such as headache, were rare (<5%) and statistically indistinguishable across the three groups. Paneri et al. (2015) concluded that the “repeated use of limited output tES across extended periods, is well tolerated and poses no significant risks to healthy subjects, as previously observed in clinical studies”.

This conclusion is in keeping with the findings of Wilkinson et al. (2009), who reported on a stroke patient who received repeated VeNS sessions as part of his rehabilitation therapy. The authors observed no adverse events during stimulation over 5 consecutive daily sessions of VeNS at 1mA for 30 minutes per day. Utz et al. (2011) studied the adverse effects of 255 VeNS sessions at 1.5mA (again higher than the 1mA we propose to use) in 55 stroke patients and 30 healthy controls. They found only a few mild adverse effects, with the most common being slight itching (mean 10.2%) and tingling (mean 10.7%) underneath the electrodes. They concluded that VeNS induces “very few and mild adverse effects in healthy and persons with stroke and [is] safe” (Utz et al., 2011). There is thus a body of literature supporting the safe usage of both tES and specifically VeNS devices delivering at least 1mA in stimulation.

Similarly, in February 2016, Halo Neuroscience, a company based in San Francisco, released additional data on the safety of tES; on this occasion for a 2mA tDCS device used on 1010 subjects.<sup>1</sup> They state that “there were zero reports of burn or seizure activity across all 1010 subjects, zero serious or unexpected adverse effects and zero withdrawals due to adverse events other than unpleasant sensation”. They conclude that their tES study showed “a favorable safety profile and [was] associated with a very low incidence of adverse events, with no unexpected or serious adverse events”.

Specific measures taken to reduce the incidence of skin irritation are the 27mm diameter of the electrodes, which are themselves hypo-allergenic, and the 50% duty cycle (i.e. half the time no current at all is being delivered), and the maximum current of 1.0mA. This means that the

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<sup>1</sup> <https://halo-website-static-assets.s3.amazonaws.com/whitepapers/safety.pdf>

maximum phase current that can be delivered is only 500 $\mu$ C. In conclusion, there is not a significant risk of harm from using the device, and as such criteria d and g do not apply. Thus as the device does not meet the criteria of a SR device it is a NSR device.

Based upon the statistical power calculations, the aim is to enroll a total of 20 subjects. There will be no exclusion from the study on the basis of race, socioeconomic status, language spoken, or ethnicity.



## **STATISTICAL ANALYSIS PLAN**

## 1.1 Specific Aims

The aim of this study is to evaluate whether non-invasive electrical vestibular nerve stimulation (VeNS) causes a change in fat consumption in humans as measured using indirect calorimetry.

## 1.2 Analysis Plan and Sample Size Calculation

To account for potential period effects, a cross-over design will be used to assess the effect of VeNS on fat consumption. Subjects will be randomized to either the Sham VeNS sequence or VeNS Sham-sequence. To eliminate the effect of period and sequence effects, standard cross-over design analysis will be performed with an independent samples t-test of differences between the first and second measurement by sequence (Sham → VeNS or VeNS → Sham).

When  $n = 10$  subjects data have been collected, we will perform an interim analysis of the primary aim. Our only considerations will be whether to stop the study to reject the null hypothesis or to continue to full recruitment. As such, we will apply the Pocock alpha spending function to account for the effect of this interim look on the type I error of the study. This amounts to using a significance level of 0.0294 at both the interim analysis and the final analysis, in order to maintain an overall type I error rate of 0.05.

With a total sample size of  $n = 20$  subjects, we will have 80% power at the  $\alpha = 0.05$  significance level (adjusted for two analyses with the Pocock alpha spending function) to detect a medium effect size of  $d = 0.72$ . Assuming a moderate within-subject correlation of  $\rho = 0.50$  and a standard deviation of mean fat consumption over the 60 minute treatment period of  $\sigma = 5.44$ , this translates to a detectable difference in fat consumption of 3.9%.

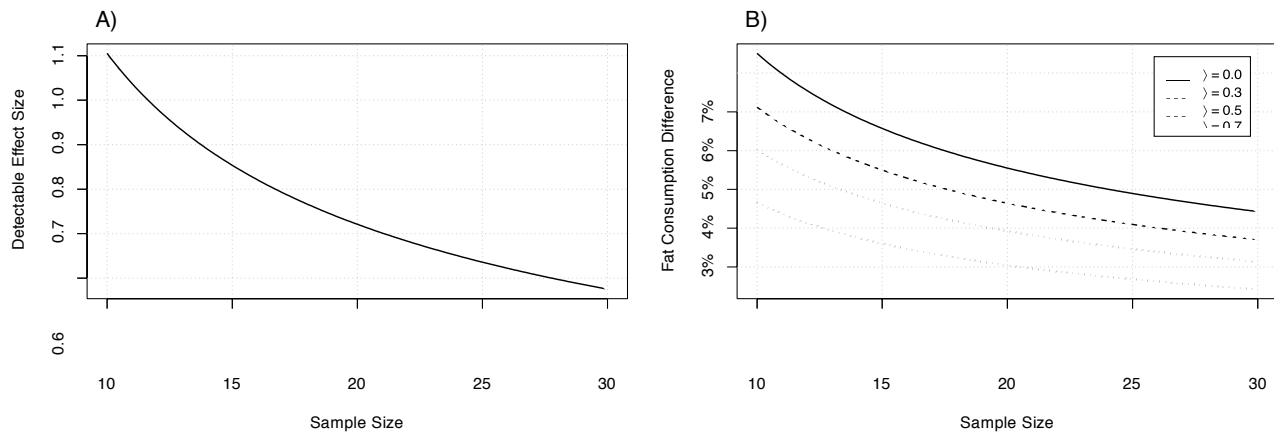


Figure 1: Detectable effect sizes by total sample size (A), and detectable effect sizes in terms of fat consumption assuming certain within-subject correlations (B).

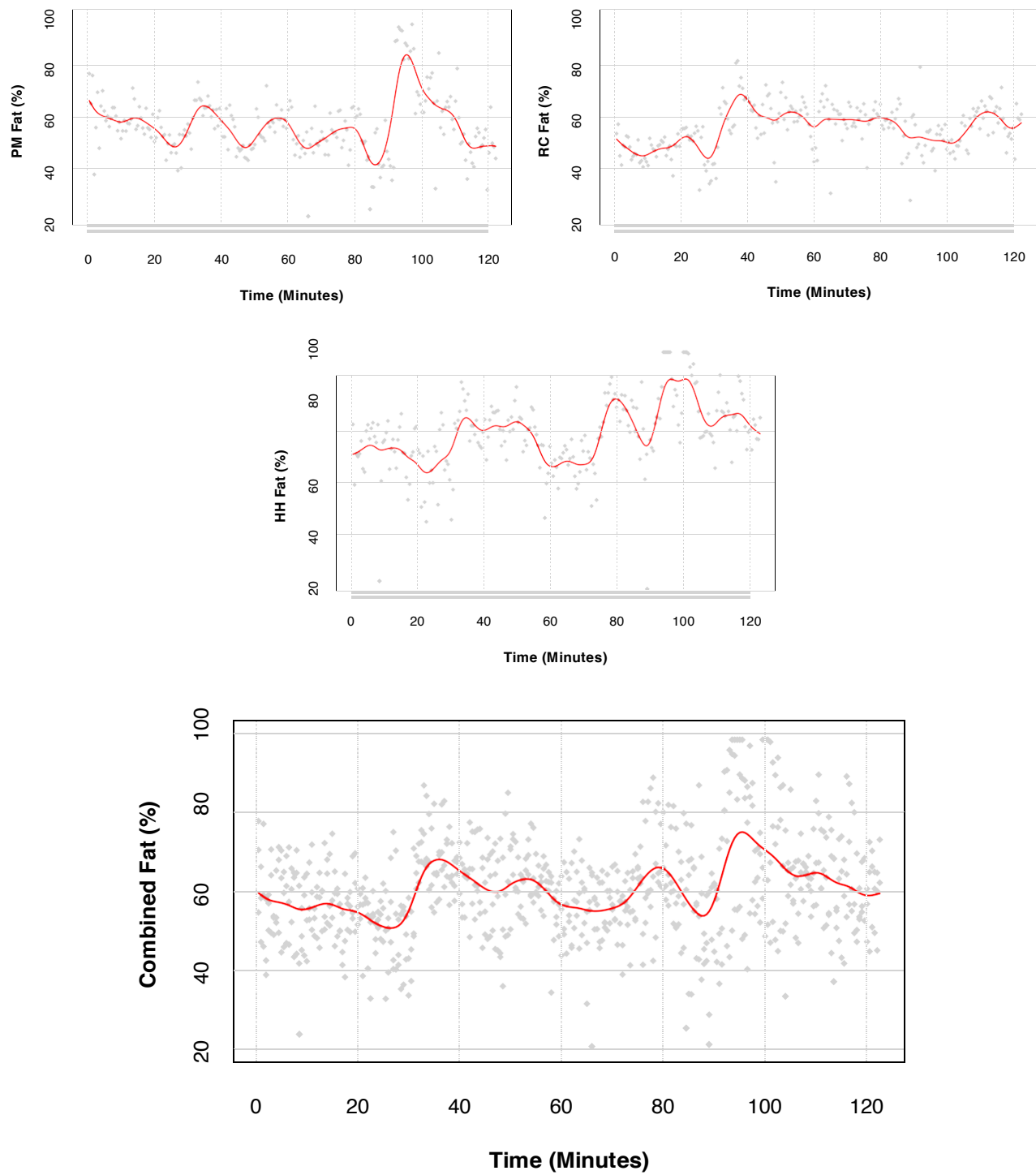


Figure 2: Pilot data of energy expenditure over a two hour period for  $n = 3$  subjects.