Collection of Breath and Sweat to Identify Indicators of Hypoglycemia

Principal Investigator:
Steven J. Russell, M.D., Ph.D.[1]

Co-Investigators:
Courtney Balliro BS, RN, CDE[1]
Rabab Jafri, MD[1]
Jason Sloane, MD[1]
Jordan Sherwood, MD[1]

[1]*Diabetes Research Center, Massachusetts General Hospital, Boston, Massachusetts.*

Address correspondence to Steven J. Russell, M.D., Ph.D., MGH Diabetes Center, 50 Staniford Street, Suite 301, Boston, MA 02114, email: sjrussell@mgh.partners.org, phone: 617-726-1848, fax: 643-0697, page: 617-726-2066.

NCT number: NCT03600116
IRB protocol number: 2018P000868

Version 2, May 10, 2018

TABLE OF CONTENTS

I. Background and Significance

1.a. Background

Hypoglycemia is a persistent and often unpredictable problem for patients with diabetes mellitus treated with insulin and/or oral hypoglycemic medications. Hypoglycemia can arise from simple miscalculations by the patient: ingestion of too few carbohydrates relative to the amount of medication taken, or too much medication taken with food or for correction of hyperglycemia. Another cause of hypoglycemia is exercise. Physical activity causes insulin-independent glucose uptake into the muscle both during exercise and for a long period thereafter, including overnight. When physical activity is coupled with too much medication or not enough carbohydrates, hypoglycemia often results. Identifying, treating and preventing hypoglycemia is critical to avoid both acute and chronic complications including slowed cognition, confusion, unconsciousness, seizures, and death. Recurrent hypoglycemia can lead to reduced or even absent symptoms prior to the onset of neuroglycopenia, which further inhibits the patient's ability to identify and treat their hypoglycemia before it reaches dangerous levels. Tragically, some patients experience irreversible brain damage or die as a consequence of a severe hypoglycemic event that was not detected in time. When this occurs overnight during sleep it is described, chillingly, as the "dead in bed" syndrome. Many patients develop a fear of hypoglycemia, and as a consequence, are not able to maintain tight enough glycemic control to avoid hyperglycemic complications later in life. The tools available to patients with diabetes for identification, treatment and prevention of hypoglycemia are inadequate for the risk they face.

Recently there has been interest in the use of "diabetes alert dogs" that are said to detect hypoglycemia by smell. If this is true, it suggests that there is an odor signature of hypoglycemia that is detected by dogs, and that this odor signature might also be detected by previously developed nose-like nanosensing technology. A hypoglycemia detecting nanosensor or nanosensor array could be built into a compact, non-invasive warning system that could be employed by diabetics to detect incipient hypoglycemia. Such a system might be particularly useful in the form of a bedside device or one that would be installed in an automobile.

I.b. Preliminary Studies

In preliminary studies we have sampled the breath, sweat, and blood of study participants with diabetes wearing the bionic pancreas while they exercised to see if it is possible to identify a volatile organic compounds (VOC) marker for hypoglycemia. These participants wore the bionic pancreas for 4 days as an outpatient run-in to their fasted exercise visit. Some of the subjects were tested multiple times during up to three rounds of testing. This provided sets of clinical samples from 79 exercise sessions. The breath samples have been analyzed by a research team at the MITRE Corporation (a non-profit research corporation) to identify and measure the VOCs present, as well as how their concentrations vary relative to the blood glucose of the participants. The variations in the concentrations of several VOCs in the breath of diabetics appeared to correlate with variations in the blood glucose concentration. However, in experiments performed thus far only two participants developed hypoglycemia due to the efficacy of the BP and the circumstances of the testing. Furthermore, since all subjects exercised it is possible that changes in VOCs could have been related to exercise rather than changes in blood glucose. Therefore, more data needs to be collected to allow demonstration of a statistically significant correlation between a VOC breath signature and hypoglycemia. Further clinical testing is necessary to examine the relationship between breath and sweat VOCs and hypoglycemia.

I. c. Rationale and Potential Benefits

In this study, we will again measure volatile organic compounds in breath and sweat of participants with type 1 diabetes with normoglycemia and induced hypoglycemia to determine if there is a relationship between VOC signature and hypoglycemia. We will administer an insulin injection to provoke mild hypoglycemia and thereby increase the number of breath and sweat samples collected under hypoglycemic conditions. The subjects will not exercise to avoid the risk of spurious correlations hypoglycemia and VOCs that are produced during exercise.

II. Hypothesis and Specific Aims

We hypothesize that by collecting the breath and sweat of type 1 diabetics while they are in normoglycemic and hypoglycemic states, and comparing the profile of volatile organic compounds in both between these two states, we will be able to determine a relationship between volatile organic compounds in breath and sweat and hypoglycemia.

Aim 1. To collect samples of breath and sweat during normoglycemia (both in the early and late post-prandial periods) and hypoglycemia in 20 adult (≥ 18 years of age) subjects with type 1 diabetes.

Breath and sweat samples will be collected at intervals throughout the visit, with increased frequency during hypoglycemia. Collaborators with the MITRE Corporation will perform analyses on these samples to identify any relationships between volatile organic compounds in breath and sweat and hypoglycemia.

III. Subject Selection

III. a. Inclusion Criteria

- Informed consent by the subject documented prior to any study procedures
- Age ≥ 18 years
- Have had clinical type 1 diabetes for at least one year
- Willing and able to avoid deodorant, scented lotions, and scented laundry detergent on your clothes on the day of the visit

No subjects will be excluded on the basis of gender or race. The requirement that subjects manage their diabetes using subcutaneous insulin infusion pump therapy is imposed because multiple daily injection therapy involves the use of long-acting basal insulin that would require an extended washout period.

III. b. Exclusion Criteria

- Unable to provide informed consent (e.g. impaired cognition or judgment)
- Unable to safely comply with study procedures and reporting requirements (e.g. impaired memory or unable to speak and read English)
- Current participation in another diabetes-related clinical trial that, in the judgment of the principal investigator, will compromise the results of this study or the safety of the subject
- Pregnancy (positive urine HCG), breast feeding, plan to become pregnant in the immediate future, or sexually active without use of contraception
    - Subjects must use acceptable contraception for the two weeks prior to the study, throughout the study and for the two weeks following the study.
    - Acceptable contraception methods include:
        - Oral contraceptive pill (OCP)
        - Intrauterine Device (IUD, hormonal or copper)
        - Male condoms
        - Female condoms
        - Diaphragm or cervical cap with spermicide
        - Contraceptive patch (such as OrthoEvra)
        - Contraceptive implant (such as Implanon, Nexplanon)
        - Vaginal ring (such as NuvaRing)
        - Progestin shot (such as Depo-Provera)
        - Male partner with a vasectomy proven to be effective by semen analysis
- Abnormal EKG consistent with coronary artery disease or increased risk of malignant arrhythmia including, but not limited to, evidence of active ischemia, prior myocardial infarction, proximal LAD critical stenosis (Wellen's sign), prolonged QT interval (> 440 ms). Non-specific ST segment and T wave

changes are not grounds for exclusion in the absence of symptoms or history of heart disease. A reassuring evaluation by a cardiologist after an abnormal EKG finding may allow participation.
- History of hypoglycemic seizures (grand mal) or coma in the last year
- History of poor venous access or inadequate venous access as determined by trial nurse or physician at time of screening
- Hemoglobin < 12 g/dl for men, < 11 g/dl for women
- Any factors that, in the opinion of the principal investigator would interfere with the safe completion of the study

III. c. Source of Subjects

Volunteers who fit the selection criteria will be considered as candidates for this study. We will contact individuals who have previously inquired about participation in our studies and have asked us to have their contact information kept on file. In addition, advertisements for the study may be posted at the MGH Diabetes Center and other places, and may be distributed in the weekly broadcast email of research studies seeking volunteers. A letter may be sent to adult endocrinologists in the Boston metropolitan as well as selected nearby endocrinologists informing them of the study and asking them to refer any eligible patients who might be interested. We will post information about the trial along with contact information on our website www.bionicpancreas.org and on www.clinicaltrials.gov.

IV. Subject Enrollment

IV. a. Number of Subjects

It is expected that we will have 12 subjects complete the study and reach a plasma glucose of <60 mg/dl for 3 measurements at 5 minute intervals or a plasma glucose of <50 mg/dl for 1 measurement. We expect that the experiments and analysis can be accomplished over a period of 6-12 months. Up to 24 subjects will be enrolled. The upper bound is based on the expectation that some volunteers will be excluded after the screening visit, the possibility that some experiments may have to be discontinued before completion, and the likelihood that some subjects will not reach the hypoglycemic threshold during the experiment.

IV. b. Enrollment and Consent Procedures

The study will be approved by the Partners IRB before any subjects are enrolled. Prospective participants will be briefed by a study staff member by phone or e-mail regarding the study procedures and the inclusion and exclusion criteria. Potential subjects will be sent an informed consent document by mail, fax, or e-mail.

Once potential subjects have had time to review the consent document, they will meet with a study provider (MD or NP) that will explain the study, answer any questions, and administer informed consent. In the event that a volunteer is a patient of one of the study MDs or NPs, another staff MD or NP will answer questions and administer consent. A licensed physician investigator will be available to speak with the subjects during the consent process in the event of an NP administering consent.

Study staff will answer any questions that the subjects may have during their participation. They will share any new information in a timely manner that may be relevant to the subject's willingness to continue participating in the trial. The subjects may choose to discontinue their participation at any time.

V. Study Procedures

V. a. Screening Data

- Age
- Sex
- Race and ethnicity
- Date of last menstrual period in female volunteers

- Date of diabetes diagnosis
- Medical, surgical, and social history, allergies, and review of systems relevant to inclusion and exclusion criteria
- Medications (prescription and non-prescription) and date of last change in medication regimen
- Insulin regimen
- Average total daily dose of insulin in the last 30 days
- Assessment of impaired awareness of hypoglycemia
- Weight
- Height
- Blood pressure
- EKG (if > 50 years old or duration of diabetes > 20 years)
- Urine HCG (pre-menopausal females)
- Hemoglobin
- Hemoglobin A1C

V. b. Drugs

The study involves subcutaneous administration of insulin lispro (Humalog, Lilly), which is commercially available by prescription and is indicated for lowering blood glucose in patients with diabetes.

V. c. Devices

Ascensia Diabetes Care Contour Next One Glucose Meter: The Contour Next One glucometer is FDA approved and commercially available. This meter is Bluetooth enabled, allowing it to connect with the iLet and automatically log BG measurements. Blood glucose measurements for Dexcom CGM calibrations and other required BG measurements will be obtained via finger stick with the Contour Next One in all study arms.

Yellow Springs Instrument (YSI) 2300 STAT PLUS: The YSI model 2300 STAT PLUS Glucose and Lactate Analyzer is a laboratory instrument that is intended for use in clinical care. It provides quick measurements of glucose in whole blood, plasma or serum and will be used to measure plasma glucose during the study visit. This device will be stored at the Diabetes Research Center when not in use, and study staff will follow proper maintenance and quality assurance procedures.

V. d. Experimental Procedures and Data Collection

V. d. i. Screening Visit

- All subjects will have a screening visit to confirm eligibility. Informed consent will be obtained and documented with a signed informed consent document before any trial-related procedures are conducted.
- The subject will be interviewed and the case report form will be completed by study staff to establish whether the subject is eligible to continue with the screening.
- A urine pregnancy test will be performed in female volunteers. If the test is positive the volunteer will be informed of the result and the visit will be ended.
- Height, weight and blood pressure will be measured.
- If the volunteer is not excluded based on historical criteria, blood pressure, or urine pregnancy test, blood will be drawn for hemoglobin and hemoglobin A1c.
- Questionnaires will be administered to assess impaired awareness of hypoglycemia.
- Once all of the laboratory results have been returned, a study MD or NP will review the case report form to determine subject eligibility. If subjects are not eligible to continue in the study the results of abnormal tests will be reported to the subjects and to a health care provider of their choosing.
- Subjects who have been screened and are eligible can participate without having to be re-screened for a period of one year. The study staff should verbally confirm that there have been no health events that

would make them ineligible if the interval between screening and participation is longer than 3 months.

V. d. ii. Visit Procedures

- Subjects will arrive to the visit having fasted since 12:00 AM the night before. Treatment with simple carbohydrates of up to 30 grams for a low blood sugar is allowed. Subjects are also allowed to treat any hyperglycemia as they normally would. If the patient is not fasted, the visit will be rescheduled.
- A urine pregnancy test will be performed in female volunteers upon arrival to the study visit. If the test is positive the volunteer will be informed of the result and the visit will be ended. The date of the last menstrual period will also be documented, along with usual cycle length, for female subjects.
- A plasma glucose will be checked via fingerstick using the Contour Next One meter upon arrival. If PG is ≥250 mg/dL the visit will be rescheduled. If PG is <50 mg/dL, the patient will be treated with simple carbohydrates according to the 15/15 rule (15 grams of ≥carbohydrates, repeated in 15 minutes if necessary). If PG is <70 mg/dL and the patient has symptoms of hypoglycemia they may elect to be treated with simple carbohydrates according to the 15/15 rule (15 grams of ≥carbohydrates, repeated in 15 minutes if necessary).
- We will ask subjects to avoid certain skin care products that will interfere with sweat sample collection on the day of the visit.
- We will ask subjects to bring a breakfast of their choosing to the visit.
- A 20 gauge or smaller peripheral I.V. will be placed.
- Subjects will calculate their insulin dose based on their insulin to carbohydrate ratio for the breakfast meal they brought to the visit. A correction bolus of insulin will also be added to the meal correction dose.
    - The dose of insulin will be calculated using their own correction factor, correcting them down to a target PG of 40 mg/dl using their current PG.
    - Both doses of insulin will be combined into one syringe and injected into the subcutaneous tissue.
- Subjects will then eat breakfast.
- BG measurements using the YSI will be obtained per the following protocol:
    - If PG is ≥ 120 mg/dl, BG measurements will be obtained off the IV line every 30 minutes.
    - If PG is 100-119 mg/dl, BG measurements will be obtained off the IV line every 20 minutes.
    - If PG is 80-99 mg/dl, BG measurements will be obtained off the IV line every 10 minutes.
    - If PG is < 80 mg/dl, BG measurements will be obtained off the IV line every 5 minutes.
    - Study staff will collect samples of sweat from the subject's underarm and will ask subjects to exhale into a breath collection device throughout the visit, with increased frequency during any episodes of hypoglycemia. These samples will be collected, de-identified and shipped out to collaborators at the MITRE Corporation for analysis of the relationship between volatile organic compounds in breath and sweat and hypoglycemia.
- Subjects will be required to treat with 15g of rapid acting carbohydrates:
    - If PG <50 mg/dl for two consecutive measurements
    - If PG is <40 mg/dl for one measurement
- Subjects will be given the option to treat with 15g of rapid acting carbohydrates:
    - If PG is <60 for three consecutive measurements
    - If PG is <50 mg/dl for one measurement
    - Repeat treatments will be given at 15 minute intervals if hypoglycemia continues
    - If the YSI fails, the Contour Next One meter will be used for glucose measurements.
- If the subject reaches a hypoglycemia threshold they will be treated with carbohydrates as above and they will be discharged from the DRC when they have maintained a PG ≥ 70 mg/dl for 1 hour. They will be allowed to treat with further oral carbohydrates at their discretion during this time to maintain their blood glucose.

- If the subject does not reach the hypoglycemia threshold 3 hours after the insulin injection, another correction bolus will be given via syringe per the same protocol:
    - The dose of insulin will be calculated using their own correction factor, correcting them down to a target PG of 40 mg/dl using their current PG.
    - If the subject does not reach the hypoglycemia threshold after an additional two hours, they will be permitted to eat breakfast and be discharged.
- If the subject reaches a hypoglycemia threshold a hypoglycemia threshold they will be treated with carbohydrates as above and they will be discharged from the DRC when they have maintained a PG ≥ 70 mg/dl for 1 hour. They will be allowed to treat with further oral carbohydrates at their discretion during this time to maintain their blood glucose.

VI. Bio Statistical Analysis

VI. a. Data Collected

VI. a. 1. Prior to start of experiment:

- Age
- Sex
- Race and ethnicity
- Date of last menstrual period in female subjects
- Date of diabetes diagnosis
- Medical, surgical, and social history, allergies, and review of systems relevant to inclusion and exclusion criteria
- Medications (prescription and non-prescription) and date of last change in medication regimen
- Insulin regimen
- Average total daily dose of insulin in the last 30 days as available
- Height and weight
- Blood pressure
- EKG if applicable
- Hemoglobin A1c
- Hemoglobin
- Urine HCG (pre-menopausal females)

VI. a. 2. During the experiment:

- Plasma glucose
- Breath and sweat VOCs
- Carbohydrate intake

VI. b. Study Endpoints

VI. b. 1. Primary endpoint analyses

VOC outcomes

- Identification of volatile and organic compound or compounds in breath and sweat that correlate(s) with hypoglycemia
- Identification of volatile organic compounds in breath and sweat that correlate with normoglycemia and/or hyperglycemia

VI. c. Power Analysis

Power Calculation for Aim 1

No formal power analysis has been completed for the determination of a relationship between VOCs and hypoglycemia. We expect to capture breath and sweat in at least 12 visits during which hypoglycemia (BG < 60 mg/dl) occurs. During visits in which hypoglycemia occurs, we will capture at least one set, and likely 2 or more

sets, of samples of breath and sweat during hypoglycemia, and at least 4 during normoglycemia in the same participants on the same day. This is anticipated to provide samples from 12 hypoglycemic episodes and paired samples during normoglycemia (both before and after hypoglycemia) from the same participants. If there is a robust correlation between a VOC marker and hypoglycemia, this should provide sufficient power to identify VOC markers of hypoglycemia.

VII. Risks and Discomforts

The protocol is designed to cause mild hypoglycemia. Given frequent PG monitoring, protocols for treating hypoglycemia, and direct supervision by an NP, RN, or MD at all times, the risk of a hypoglycemic episode leading to significant harm to volunteers is expected to be very low, but the symptoms of hypoglycemia may be unpleasant. All study NPs, RNs, and MDs have experience in managing hypoglycemic episodes.

Subjects may experience discomfort with insertion of the peripheral intravenous line and the injection of insulin into the subcutaneous tissues.

There is a risk of risk of dizziness or lightheadedness from blood loss. However, typical blood loss will be limited to no more than 170 ml. Exclusion of subjects with anemia will help mitigate this risk.

VIII. Potential Benefits

The data derived from this study will allow us to evaluate whether there is a relationship between volatile organic compounds in the breath and sweat and hypoglycemia. This information could be useful in developing noninvasive technologies to identify impending and actual hypoglycemia.

Subjects will be financially compensated for participating in the study.

IX. Data and Safety Monitoring
IX. a. Monitoring of Source Data

An audit of procedures, regulatory documentation, and a sample of subject files will be performed by a member of the Diabetes Research Center at least biannually. The audit will be conducted by a staff member who is not directly involved in the conduct of the study. This audit will include a review of regulatory documentation, such as IRB and FDA correspondence, and a review of subject files, including a review of consents, case report forms, and other data from study visits.

A numeric code will be substituted for the subjects personal identifying information in the study database, which will be password protected. The key linking the medical record number of the subject with the numeric code, along with case report forms, and all information that is personally identifiable, will be kept in a locked filing cabinet in an investigator's locked office. All electronic records will be kept in a password protected computer database. All printed computer data will be disposed of confidentially when no longer needed. Only the study staff will have access to the study database. Subjects may not withdraw from the de-identified database, but they may elect to have the key linking their medical record to the de-identified database destroyed.

The study data may be shared with collaborators at the MITRE Corporation (a non-profit research corporation), but only in a form in which all personally identifiable information has been removed. Shared data will be in the form of a database in which only a number identifies subjects.

Subjects may not withdraw their data, as it will be stored in non-personally identifiable form.

IX. b. Safety Monitoring

This study is considered moderate risk. An external Data and Safety Monitoring Board will oversee the conduct of the study and review its results on a regular basis. Additionally, the DSMB will be informed in the event of any severe or unexpected adverse events. The DSMB will be informed if there are any changes to the study protocol that could significantly impact the safety or scientific validity of the study. A final DSMB meeting will convene after the completion of the study. All serious and unexpected events will be reported to the DSMB within 72 hours.
Note that subjects may discontinue participation at any time and subjects may be removed from the trial for other reasons, for instance failure to comply with study procedures or concurrent illness. Discontinuation of participation for these reasons will not contribute to a decision to discontinue the trial.

IX. c. Adverse Event Reporting Guidelines

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered/using product and which does not necessarily have to have a causal relationship with this treatment. An Adverse Event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a product, whether considered related to the product or not.

A serious adverse event (SAE) is an experience that at any dose results in any of the following: (death, life-threatening experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity or is a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require Hospitalization may be considered a serious adverse event when, based upon appropriate medical judgement, they may jeopardize the patient or subject or require medical or surgical intervention to prevent one of the outcomes listed in this definition. Suspicion of transmission of infectious agents must always be considered an SAE.

Serious adverse reaction (SAR) is an adverse event that fulfils both the criteria for a serious adverse event and the criteria for an adverse reaction.

Whether adverse events are expected or unexpected will be based on product labeling for the insulin and the potential risks described in the consent document.

The causality of each AE should be assessed by the Investigator per the following classification:

- Probable: Good reason and sufficient documentation to assume a causal relationship
- Possible: A causal relationship is conceivable and cannot be dismissed
- Unlikely: The event is most likely related to etiology other than the trial product

The PI and co-investigators will review any adverse events after each experiment. Any serious or unexpected but possibly related adverse events will be communicated to the PI as soon as possible and within 48 hours of the time they are detected. Adverse events will be reported promptly to the Partner's IRB.

AE information should include the following:

- Study name
- Patient identification (i.e. subject number, initials, sex, age)
- Event (diagnosis)
- Trial drug
- Reporter
- Causality

- Outcome

X. Subject Compensation

Financial compensation will be provided to all subjects who complete the screening visit. Subjects will be paid $25 for completing the screening visit whether they are eligible to participate in the study or not. Study participants will be compensated $100 for completing the study visit. Thus, the total compensation for a subject who completed the study could be up to $125. Parking expenses will be paid for up to $30 per subject. Subjects who are unable to complete the study or chose to stop participation will receive prorated compensation for the portion of the study visits that they complete.

XI. Publication Plans

The data derived from this study will be published in a peer-reviewed journal. Candidate journals include: Diabetes Care; Diabetes; Diabetologia; Diabetes, Obesity and Metabolism; and Journal of Clinical Endocrinology and Metabolism.

XII. References

1. The Diabetes Control and Complications Trial research Group (DCCT) (1993) The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin dependent diabetes mellitus. N. Engl. J. Med. 329, 977–986.
2. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group (2005) Intensive Diabetes Treatment and Cardiovascular Disease in Patients with Type 1 Diabetes. N. Engl. J. Med. 353, 2643–2653.