RESEARCH PLAN
Strategic Research Agreement - Clinical

RESEARCH PLAN

*(Proposals in MS Word should be type-written, single-spaced and in typeface no smaller than **10-point font** and have no more than **six vertical lines per vertical inch**. Margins, in all directions, must be at least ½ inch. **For RFAs,** reference the official RFA document linked to the funding opportunity on JDRF.org for further guidelines, specific instructions, and page limits. **For SRAs not associated with an RFA**, the research plan cannot exceed 12 pages, including figures and tables. Citations and PI Assurance are an addition to this page limit. Additional information regarding SRAs can be found here.)*

Project title

Ketone Monitoring in T1D: Effect of SGLT2i during Usual Care and with Insulin Deficiency

Project description

Rationale

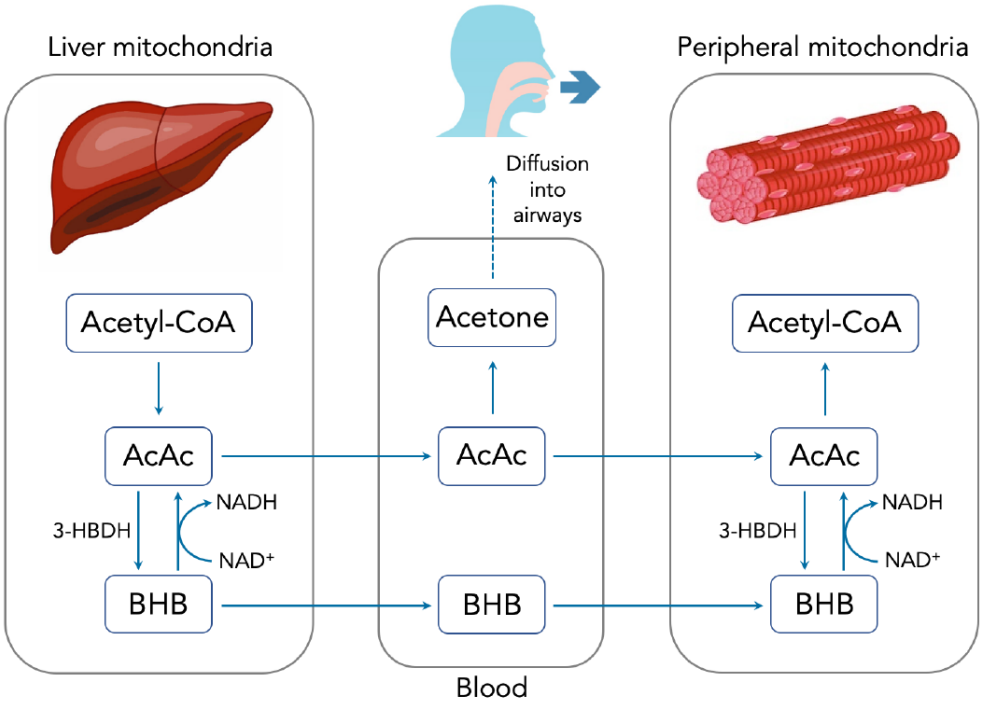
Type 1 diabetes (T1D) continues to increase in incidence and in prevalence due to improved acute and chronic disease management. Despite developments over the past 20 years in short and long-acting insulins, in insulin administration through pen devices and insulin pumps, and more recently the addition of continuous glucose monitoring systems, glucose control has not improved substantially in persons with T1D.[1] Consequently, the battle to reduce the incidence of long term complications such as diabetic nephropathy in persons with T1D has not been won. The risk of microvascular complications, particularly kidney disease, is strongly associated with the risk of cardiovascular disease in T1D.[2] Both need improved prevention and treatment strategies.

Sodium-glucose cotransport2 inhibitors (SGLT2i) have been irrefutably proven to reduce progression of chronic kidney disease (CKD), hospitalizations for heart failure and death due to cardiovascular causes in persons with type 2 diabetes.[3–6] Cardiovascular and kidney outcome trials have, in some cases, included persons without diabetes, and have shown equivalent benefits in these persons who are at high risk of serious health consequences from exacerbation of heart failure or from progression of CKD.[4] While the results of the cardiovascular or renal outcome clinical trials have varied depending on the drug studied, the population included and the specified outcomes of interest, the results show such significant benefits that SGLT2i are now recommended by major guidelines for the management of persons with (and in some cases without) type 2 diabetes and either heart failure or CKD. However, there is a group of individuals at high risk of adverse cardiovascular and kidney outcomes for whom SGLT2i are not indicated or approved, and those persons have T1D.

Clinical trials of each of the SGLT2i have been conducted in T1D for purposes of gaining regulatory approval for use in this population for glucose lowering and amelioration of weight gain and in some cases reduction of hypoglycemia.[7–14] The pivotal trials for glucose lowering are typically 24 – 26 weeks in length, with safety studies lasting 1 – 2 years. The trials have been conducted in adults with T1D, commonly with HbA1c levels greater than 7 – 7.5%. Most of the clinical programs in T1D have included two trials, so for each of the four SGLT2i (canagliflozin, empagliflozin, dapagliflozin and sotagliflozin), hundreds to more than a thousand persons with T1D have been included with more than half taking the active SGLT2i. With one exception of low dose empagliflozin (2.5 mg, a dose not otherwise available),[15] there has been a consistent signal of increased occurrence of ketosis-related adverse events, including diabetic ketoacidosis (DKA).[16] In general, the incidence of DKA in the SGLT2i groups is 2 – 3X the incidence in the placebo groups.[17] This increase in DKA occurred despite ketone action plans that included provision of ketone meters and clear directives when patients developed symptoms of hyperketonemia or when ketones were elevated, strategies currently proposed by national groups to mitigate DKA.[18] Since DKA can be a life-threatening event for persons with T1D, this increased risk is considered by regulatory agencies and most clinicians to be unacceptable, and thus persons with T1D are not able to benefit from the reduction in cardiovascular and kidney outcomes afforded by these agents.

Ketone generation, whether by fasting, insulin deficiency or other causes is the end result of complex metabolic pathways, and involves intermediates that have eluded clinical measurement, such as blood acetoacetate (AcAc) and acetone. Acetone is highly volatile and is excreted primarily through the lung, as shown below. The rapid breathing that occurs in T1D persons in DKA is an attempt to excrete acetone and reduce the level of circulating ketones.[19] Acetone diffusion and excretion can be measured in exhaled air, ideally from alveolar spaces at the end of exhalation. Prior work has shown that there is a linear relationship between breath acetone (BrACE) and plasma acetone[20] and between BrACE and beta hydroxybutyrate (BOHB) such that BrACE could be used in risk prediction model for diabetic ketoacidosis.[21] A study that compared BrAce with blood BOHB in non-diabetic persons showed a relatively weak correlation, R=0.57, however the ROC analysis confirmed that BrAce can identify levels of ketosis that would be actionable to a person with T1D.[22] BrACE is susceptible to interferences from foods, alcohol and environmental volatile organic compounds (VOC) such as hand sanitiizers, cleansers and nail polish remover, which needs consideration during home use.[23]

There is still quite a lot to learn about the relationship between BrAce and BOHB levels, particularly in persons with T1D, who are at risk of ketosis and DKA. Is there a correlation between BrAce and BOHB in persons with fluctuating glucose and insulin levels, characteristic of T1D? Can measuring BrAce identify actionable levels of ketones with or without a strong correlation with BOHB? Do elevated ketones always occur in the setting of elevated blood glucose, or is there a risk of ketogenesis with low glucose such as when fasting?[24] Monitoring of "ketone status" which is to say, identifying ketone levels that rise to actionable levels prior to the development of classic symptoms of ketosis, is now considered urgent.



Herring et al. propose that the increase in BOHB with the SGLT2i dapagliflozin is due to multiple factors, including increased lipolysis, reduced renal excretion, reduced uptake of BOHB by peripheral tissues and a metabolic switch to ketogenesis in the liver.[25] Ferrannini et al found that in persons with type 2 diabetes (T2D), the increase in BOHB secondary to SGLT2i was due to overproduction of ketones.[26] Diabetic ketoacidosis (DKA) with blood glucose that is lower than expected, known as euglycemic DKA, is now a recognized risk in persons with T2D who take SGLT2i.[27] A similar phenomenon can occur in T1D.

We propose a study that includes both real world testing at home, and testing during insulin deficiency that is patterned after the studies by Patel et al and Herring et al.[25,28] The study purpose, which is to

test whether measurement of BrAce, currently used to support persons on a ketogenic diet, can be re-purposed for ketone tracking in persons with T1D. The choice of the Biosense® (Readout) breath ketone analyzer (BKA) was predicated on its designation as a Class 1 device by FDA. In the prior clinical trial in non-diabetic persons, both BrAce and blood BOHB were found to vary widely throughout the day.[22] Multiple factors are known to create variability in ketone production and excretion in breath, including fasting, restriction in carbohydrate intake and exercise. Other factors at play may be cross contamination with other VOC such as alcohol, hand sanitizer, etc. The Biosense® BKA to be used in this study will have a filter to reduce the effects of competing substances during the on-site visits with insulin deficiency to mitigate this problem under controlled circumstances.

This proposed study of 4 distinct segments that include routine testing of ketones during usual care, during insulin deficiency, and while taking a SGLT2i in addition to usual care, then with insulin deficiency **after the SGLT2i has reached steady state** and ingested that morning. We will study 20 adults with T1D who are using Dexcom G6 continuous glucose monitor (CGM), plus any form of insulin delivery. Study participants will be randomized to Group A or Group B as shown below in Figure 1. Simultaneous ketone monitoring using a the Biosense® BKA and capillary blood BOHB measurement will be done 2-3X daily for 2 weeks during each of the outpatient segments. This will generate >800 data points comparing breath acetone, measured in units called ACEs, versus capillary blood BOHB measurements for each 2 week period. On days of insulin withdrawal, which will take place on the Washington University Intensive Research Unit (IRU), frequent monitoring with capillary blood measurements of BOHB (Precision Xtra®, Abbott) will be compared with breath BrAce measurements (Biosense®, Readout) and laboratory measurements of BOHB and acetoacetate. Electrolytes and glucose will also be measured.
The schematic is shown below:

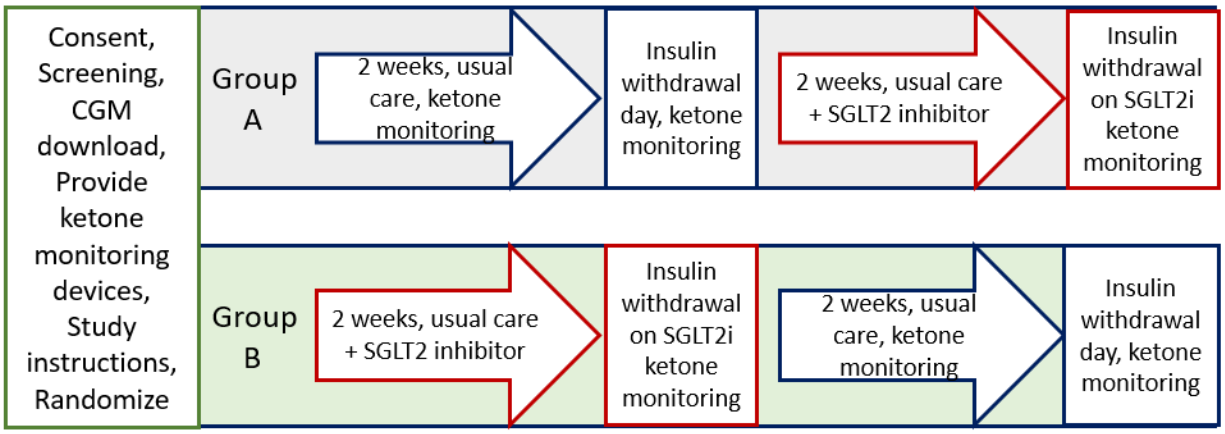


Figure 1. Study plan and study groups.

The goals of the study are to better understand ketone fluctuations in persons with T1D during usual care and when taking a SGLT2i in addition to usual care through frequent ketone monitoring. Additional information about usability of the breath ketone analyzer will be tested with simple questions using a Likert scale and a structured interview at the end of the study. The question is not whether the ketone measurements correlate perfectly with the gold standard BOHB measurements, but whether breath ketone measurements by the Biosense BKA are adequate for patients to understand their "ketone status" and identify states of rising ketones early enough for mitigation strategies to work.[16] The potential advantages of using a breath analyzer are that it is non-invasive; it is easy to use repeatedly without additional cost or pain from fingersticks, and it may be sufficiently informative to employ a ketone action plan before ketones rise to hazardous levels.

The long term goals of this study extend beyond the completion of the study to the development of DKA mitigation strategies that are manageable and accepted by patients. A ketone mitigation strategy must

identify excess ketones before symptoms of hyperketonemia or DKA occur.[16] A successful DKA mitigation strategy could be deployed in patients with recurrences of DKA and in those for whom a SGLT2i is indicated for reduction of cardiovascular or renal adverse outcomes. The mitigation strategies would need to be tested in a multi-center clinical trial conducted over longer periods of time in populations or with interventions of interest. This study offers a novel approach to ketone detection that has not been previously tested in persons with T1D.

Objective(s)

The objectives of this study are several, and the following hypotheses will be tested:

1. Ketone generation in persons with T1D can be identified by measuring capillary beta-hydroxybutyrate or breath acetone using a breath ketone analyzer during insulin deficiency, and the values will show a significant correlation.
2. Breath ketone measurements tested during usual care will correlate with capillary blood measurement of beta-hydroxybutyrate.
3. SGLT2i use will increase blood and breath ketones in persons with T1D during insulin deficiency, and simultaneous measurements of blood beta-hydroxybutyrate and breath acetone will show a significant correlation.
4. Breath ketone measurements tested during 2-weeks of SGLT2i therapy will correlate with capillary blood measurements of beta-hydroxybutyrate.
5. Patients will be able to identify episodes of ketogenesis during usual care and usual care plus SGLT2i therapy prospectively, before symptoms of ketosis occur, with the breath ketone analyzer.
6. Use of the breath ketone analyzer will be accepted by persons with T1D for routine and symptomatic monitoring of ketone levels such that mitigation strategies can be employed before elevations in ketones become severe.

Methodology

Research Design

This study is a single-center, investigator-designed and led clinical trial testing the efficacy of the breath ketone analyzer, Biosense, for the detection of ketogenesis during four distinct study periods, which will allow comparisons of the Biosense measured breath ketones with capillary BOHB and both of these point of care (POC) tests with laboratory measured BOHB and acetoacetate (AcAc). Ethics approval will be from the Washington University Human Studies Protection Office, and obtained prior to contact with any potential participant. Participants with T1D using a stable form of insulin delivery, either insulin pump or multiple daily injections (MDI) and will perform paired measurements of capillary BOHB and BrACE during usual care or usual care plus therapy with a SGLT2i. Paired testing will then be done during insulin withdrawal in usual care and insulin withdrawal while taking a SGLT2i that has reached steady state.

Participants will be randomized to Group A, in which the usual care segment and insulin withdrawal day is done first followed by the usual care plus SGLT2i and insulin withdrawal with SGLT2i or Group B, in which the SGLT2i will be taken during usual care for 2 weeks, then insulin withdrawal with SGLT2i followed by usual care for 2 weeks then insulin withdrawal during usual care. The study design is robust, and will allow more than 800 paired measurements of breath ketones with capillary BOHB during usual care, more than 800 paired measurements during usual care plus SGLT2i therapy. Additionally, due to concerns about ketogenesis during times of insulin deficiency, and the demonstrated increase in ketone generation with SGLT2i therapy, the pair-wise comparisons will be informative about the correlation of BrACE as measured with the Biosense device with POC and laboratory BOHB measurements and laboratory measured AcAc.

Dapagliflozin, 10 mg was selected over the other FDA approved SGLT2i drugs because this dose was used in the recently published study, DAPA-CKD, which showed remarkable reduction in progression of

kidney function decline in persons with and without T2D, HR 0.61 (95% CI, 0.51 – 0.72).[29] Reduction in the progression of diabetic kidney disease is a high priority for persons with T1D. In the study procedures, SGLT2i refers to dapagliflozin, 10 mg daily.

Research subjects will be persons with T1D who are using continuous glucose monitoring (CGM) with Dexcom G6 and either an insulin pump or multiple daily injections (MDI) with long-acting insulin (insulin glargine U100) administered in the morning. Recruitment will take place from our Washington University-Barnes Jewish Hospital Diabetes Center, which cares for over 1200 persons with T1D per year. Availability of medical records is considered key to appropriate patient selection and follow-up of any adverse events, thus persons outside of our system will not be recruited for the study. Participants must be able to use a smart phone with applications for their CGM device, insulin pump (if using) and the Biosense BKA device. The inclusion and exclusion criteria are listed below. Every effort will be made to recruit participants across the age range of the study, and to include persons of both genders and of different races and ethnicity, based on our local population.

Inclusion criteria:

- Type 1 diabetes for >1 year
- Age 18 – 75, any gender, race or ethnicity
- HbA1c $\leq$10%
- Stable insulin delivery method for the past 30 days
- Vision of 20/40 or better, including ability to read all device instructions and insulin pump settings
- Use of an insulin pump and ability to make adjustments to pump settings
- Insulin delivery by MDI with basal insulin (glargine U100) given in the morning **OR** willingness to transition to basal glargine given in the morning for at least 48 hours prior to insulin withdrawal visits
- Use of personal CGM, only Dexcom G6 will be permitted for data consistency
- Use of cellular phone (iOS11 and above or Android) with data capability with connectivity to Dexcom Clarity app and Biosense Ketone Monitoring app
- Willing to share CGM, capillary ketone results and BrACE data with the study group
- Able to understand the study requirements, risks and benefits and able to provide written informed consent
- Able to schedule the visits and perform study related tasks

Exclusion criteria:

- History of DKA in the past 6 months, or more than 1 episode of DKA in the past 2 years
- Use of insulin degludec, insulin glargine U300 or insulin detemir as basal insulin and unwilling to transition to glargine given in the morning for at least 48 hours before the insulin withdrawal visits
- Use of an SGLT2i in the past 30 days or intolerance to SGLT2i use in the past for any reason
- Unstable heart disease, including hospitalization for any cardiac or vascular event in the past 6 months.
- eGFR <30 ml/min/1.73m2 or hemoglobin <11.0 g/dL measured in the past year
- Cancer that has been under treatment in the past 6 months, or treatment is likely in the 3 months after signing the consent, not including skin cancer or cancers under long-term hormonal reduction treatment (breast or prostate, no other active treatment)
- Psychiatric condition that interferes with daily activities or diabetes self-care, including substance abuse
- Any illness or condition that may interfere with study measurements, or contraindications for use of SGLT2i, based on investigator discretion (hemoglobinopathy, orthostatic hypotension)
- Unwilling to avoid alcohol during the active study periods, specifically to avoid alcohol for 4 hours prior to any breath ketone measurement

- Currently following or planning to adopt a diet that is low in carbohydrates (defined as <90 grams of carbohydrate per day) or is expected to be ketogenic
- Use of oral or injected corticosteroids within the past 30 days or planned during the study period
- Taking disulfiram (due to interference with breath ketone measurements)
- History of vomiting episodes for any reason in the past 30 days, or hospitalization for cyclic vomiting in the past year
- History of urinary tract infection in the past 3 months
- Pregnancy, breast-feeding or intention of becoming pregnant during the study time period and up to 30 days after study completion
- Pre-menopausal women not using acceptable birth control defined as abstinence, surgical sterilization, hysterectomy, hormonal contraception, IUD or proven effective barrier methods

The five study data-collection periods are as follows:

1. Visit 1 will collect demographic, medical history, insulin administration and insulin dose data; CGM download, lab draw if needed, and training on capillary ketone measurements using the Precision Xtra meter and breath acetone using the Biosense breath ketone analyzer. Dapagliflozin tablets will be provided to participants randomized to Group B. Recommendations for change in insulin regimen or insulin doses will be made at this visit.
2. Segment 1 will consist of 2 weeks of usual care with participants measuring fasting and random breath ketones paired with capillary BOHB (Group A) or usual care plus dapagliflozin with participants measuring fasting and random breath ketones paired with Capillary BOHB (Group B). Participants will be instructed to do paired ketone testing anytime their glucose is >250 mg/dL, or if they develop symptoms of feeling unwell for any reason.
3. Visit 2 will be a single day of on-site close monitoring during insulin withdrawal. Morning insulin doses will be withheld, and the participants will have hourly checks of glucose, capillary BOHB, breath ketones, blood BOHB and acetoacetate. CBC will be done at baseline, and BMP to check electrolytes will be done at baseline and every 2 hours until the study end. Instructions for Segment 2 will be provided and recommendations for change in insulin regimen or insulin doses will be made at this visit.
4. Segment 2 will consist of 2 weeks of usual care (Group B) or usual care plus therapy with dapagliflozin SGLT2i (Group A). Participants will be instructed to do paired ketone testing fasting and randomly during the day, and if their CGM glucose exceeds 250 mg/dl or if they feel unwell.
5. Visit 3 will be a single day of on-site close monitoring during insulin withdrawal (Group B), but after taking an SGLT2i tablet (Group A). Morning insulin doses will be withheld, and the participants will have hourly checks of glucose, capillary BOHB, breath ketones, blood BOHB and acetoacetate. CBC will be done at baseline, and BMP to check electrolytes will be done at baseline and every 2 hours until the study end.

The study design and schematics are depicted below:

GROUP A	Phone or clinic visit	Visit 1	Gap [d]	Usual Care + Ketone Monitoring	Visit 2 IRU Insulin Withdrawal	Gap[e]	Optional Visit	Usual Care + SGLT2i + Ketone Monitoring	Visit 3 /EOS IRU Insulin Withdrawal
		On-site		HOME- Segment 1 14 +/-2 days	On-site		Phone or on-site	HOME - Segment 2 14 consecutive	On-site
Phone screening	X								
Informed Consent discussion	X	X							
Informed Consent	X	X							
Demographics, medical, medication and device use history		X							
Vital signs, height, weight		X							
Inclusion/exclusion review	X	X							
Physician H&P		X							
Near Vision Accuity Screening		X							
CGM check for connectivity to clinic		X							
Dispense capillary ketone meter and supplies, breath ketone analyzer		X							
Training on the capillary ketone meter		X							
Training on the breath ketone analyzer, ensure app download and connectivity		X							
Training on the study requirements, diary		X							
Randomization		X							
CGM download	X				X		X		X
Ketone testing 3X daily, capillary and breath				X				X	
Home Pregnancy test WOCBP[a]								X[a]	
Vital signs q4 hours and prn					X				X
Urine Pregnancy test as needed		X			X		X		X
HbA1c		X							
BMP and CBC[b]		X[b]							
CBC, BMP, BOHB, AcAc[c] baseline					X				X
BMP q 2 hours					X				X
Laboratory BOHB , AcAc[c] q 1-2 hours					X				X
Future research stored blood samples					X				X
Capillary BOHB q 1 hour					X				X
Capillary glucose q 1 hour					X				X
Breath ketone measurement q 1 hour					X				X
Urine sample for ketones					X				X
Standard meal at the end					X				X
Physician oversight, MDI user if basal insulin adjustment required		X							
Physician oversight, safety assessment, correction insulin dose +/- fluids administered					X				X
Dispense SGLT2i tablets					X				
Adverse events and concomitant medication review		X			X		X		X
Return and count SGLT2i tablets									X
Ketone Use Survey [f]					X				X

a. Home pregnancy test for WOCBP before starting SGLT2i if 4 weeks or more since on-site Visit 2

b. If lab results within 12 months prior to screening are not available

c. May be performed

d. May be Gap between Visit 1 & Start of Usual care segment of up to 2-3 weeks

e. May be Gap between Visit 2 & start of SGLT2i segment of up to 2-3 weeks if scheduling issues arise. Subject should NOT start dapagliflozin sooner than 14 days before Visit 3. Subject must have taken dapagliflozin for 14 consecutive days prior to the day of Visit 3.

f. Survey may be provided on paper or electronically

GROUP B	Phone or clinic visit	Visit 1	Optional Visit	Gap [d]	Usual Care + SGLT2i + Ketone Monitoring	Visit 2 IRU Insulin Withdrawal	Gap [e]	Usual Care + Ketone Monitoring	Visit 3/EOS IRU Insulin Withdrawal
		On-site	Phone or on-site		HOME - 14 consecutive days	on-site		HOME - 14 +/-2 days	On-site
Phone screening	X								
Informed Consent discussion	X	X							
Informed Consent	X	X							
Demographics, medical, medication and device use history		X							
Vital signs, height, weight		X							
Inclusion/exclusion review	X	X							
Physician H&P		X							
Near Vision Accuity Screening		X							
CGM check for connectivity to clinic		X							
Dispense capillary ketone meter and supplies, breath ketone analyzer		X							
Training on the capillary ketone meter		X							
Training on the breath ketone analyzer, ensure app download and connectivity		X							
Training on the study requirements, diary		X							
Randomization		X							
CGM download	X		X			X			X
Ketone testing 3X daily, capillary and breath					X			X	
Home Pregnancy test WOCBP[a]					X[a]				
Vital signs q4 hours and prn						X			X
Urine Pregnancy test as needed		X	X			X			X
HbA1c		X							
BMP and CBC[b]		X[b]							
CBC, BMP, BOHB, AcAc[c] baseline						X			X
BMP q 2 hours						X			X
Laboratory BOHB , AcAc[c] q 1-2 hours						X			X
Future research stored blood samples						X			X
Capillary BOHB q 1 hour						X			X
Capillary glucose q 1 hour						X			X
Breath ketone measurement q 1 hour						X			X
Urine sample for ketones						X			X
Standard meal at the end						X			X
Physician oversight, MDI user if basal insulin adjustment required		X							
Physician oversight, safety assessment, correction insulin dose +/- fluids administered						X			X
Dispense SGLT2i tablets		X							
Adverse events and concomitant medication review		X	X			X			X
Return and count SGLT2i tablets						X			
Ketone Use Survey[f]					X				X

a. Home pregnancy test for WOCBP before starting SGLT2i if 4 weeks or more since on-site visit 1

b. If lab results within 12 months prior to screening are not available

c. May be performed

d. May be Gap between Visit 2 and start of SGLT2i segment of up to 2-3 weeks if scheduling issues arise. Subject should NOT start dapagliflozin sooner than 14 days before Visit 2. Subject must have taken dapagliflozin for 14 consecutive days prior to the day of Visit 2.

e. May be Gap between Visit 2 & usual care of up to 2-3 weeks

f. Survey may be provided on paper or electronically

Figure 2: Study visits

The study visits will include the following activities and measurements:

PRESCREENING
The prescreening visit may be conducted by phone after the clinic chart of the potential participant has been reviewed and a discussion with the treating endocrinologist has taken place. The prescreening study staff will explain the study purpose, study activities, study expectations and the risks and benefits of participation. The informed consent will sent via email with the patient's permission to use email communication. A second discussion may take place by phone or in person at the screening visit to answer questions about information provided in the informed consent document, study procedures and risks and benefits.

VISIT 1
This visit will take place on-site in the Diabetes Center. After all questions have been answered and the patient provides written informed consent, which will be co-signed by an investigator, study activities will commence. Health information collected will include basic demographics, history of diabetes onset, current therapy, episodes of severe hypoglycemia or DKA and diabetes complications. Medications will be reviewed and recorded, including mean insulin doses over the past 3 days. If participant is on MDI therapy taking basal insulin in the evening and/or uses other than glargine as basal insulin, study physician will adjust delivery time to morning and/or make changes to basal insulin glargine for at minimum 48 hours prior to visits 2 and 3. History of alcohol use, smoking (including marijuana) and any CNS depressants (sleeping pills, pain medications) will be reviewed, and may be a cause for investigator recommended non-participation if paired testing cannot be completed without interferences. Women with child-bearing potential must be using acceptable birth control (barrier, hormonal, IUD, confirmed abstinence or surgical) during the study and must consent to pregnancy testing at specified intervals. Breast-feeding is a cause for exclusion from the study. Vital signs, height, weight and HbA1c will be obtained. An optional BMP and/or CBC may be done if evaluation of kidney function or red blood cell count is determined to be important to meet eligibility criteria. All data will be entered into a dedicated Redcap database.

After the investigator or sub-investigator determines that the participant meets all of the inclusion and none of the exclusion criteria, the study team will proceed with interrogation of the participant's CGM to ensure appropriate connectivity with the study team. Participants will be asked to demonstrate competency in making changes to insulin pump settings. The study team members will train the participant in the use of the Precision Xtra ketone meter and the Biosense BKA. Participants will be instructed to wait 60 minutes after eating, smoking/vaping, drinking sodas or flavored waters or consuming items such as toothpaste, mouthwash, cough drops, breath mints, chewing gum, lip balm and artificial sweeteners before doing a BrACE measurement. If using alcohol, participants should wait 4 hours before testing with the BKA. Participants will be instructed in avoidance of environmental contaminants also. Participants will be able to test capillary BOHB even when conditions are not optimal for BrACE.

The participant will upload the Biosense app onto his or her smart phone and test a test run will be conducted. A diary will be provided for entry of BOHB ketone measurements and BrAce with space for comments in case of known interference with BrAce or other problems with measurements. A written ketone action plan will be provided to all participants in the study. Participants will be informed that they may stop participating in the study at any time without loss of usual care of their diabetes.

A ketone action plan will be provided to each participant that will include the following elements: ingestion of carbohydrates, fluid intake and insulin administration with doses of insulin adjusted according to individual requirements.[16,17] Participants will be asked to employ the ketone action plan if BOHB reading is >1.5 mmol/L. BrACE levels of >15 in the absence of corresponding BOHB level >1.5 mmol will be considered due to contamination and the participant will be asked to repeat the levels in 1 – 2 hours.

Patients will be randomized by card draw in lots of 4 to Group A or Group B

USUAL CARE (Group A will do this first, Group B will do this segment after the SGLT2i)
The participant will manage his or her diabetes in the usual way, with no effort to modulate glucose levels for the study. Participants will check breath ketones with the Biosense BKA and capillary BOHB with the Precision Xtra ketone meter when fasting and at one or two other times during the day, plus any optional measurements. See instructions above for avoidance of biologic or environmental contaminants. There is no mandated contact with the participant, but participants can contact the study team at any time for questions, to report device issues or discuss glucose or ketone levels.

On-site Visit
The on-site will be conducted at the Intensive Research Unit at Washington University. Patients will be asked to arrive fasting, to stop their basal insulin delivery 1 hour before arrival at the center if on an insulin pump or to hold injectable insulin doses. Prior to leaving home, the participant's blood glucose should be 90 – 250 mg/dL, and BOHB <1.5 mmol/L, or the test day will be rescheduled. Time of the last insulin dose will be recorded. Vital signs and baseline labs including a CBC, BMP and pregnancy test in women with child-bearing potential will be done. Measurements of capillary BOHB using the Precision Xtra meter and BrACE using the Biosense BKA will be done hourly X7 or 8 if the patient remains relatively asymptomatic, and the capillary BOHB level does not exceed 4 mmol/L. If the capillary BOHB reaches 2.5 mmol/L or capillary glucose reaches 250 mg/dL, the capillary finger sticks and breath measurements may be collected at 30 minute intervals. The venous blood samples will continue to be collected on the hour intervals. Venous blood samples for BMP, BOHB and AcAc* will be drawn at baseline and every 2 hours X3 plus the end of the study.

The insulin withdrawal portion of the study will end (stopping rules) upon patient request for any reason, if the patient becomes symptomatic with nausea or vomiting (even if he or she does not request that the study be stopped), after 7 hours of fasting without insulin, or if either the capillary BOHB is $\geq$4 mmol/L or the CGM blood glucose is $\geq$400 mg/dL. BMP will be done every 2 hours and at the end of the study. At the termination of the ketogenic fast, participants will be given a correction dose of rapid acting insulin subcutaneously or regular insulin IV, per investigator discretion. They will then restart insulin via insulin pump, or administer both basal and mealtime insulin via subcutaneous injection and be given a standard meal. Participants will be observed for a minimum of 1 hour after the study end for return to near normal metabolic status. An additional contact by phone or other means in the evening will be made to confirm safety and no adverse consequences of the study day.

At the end of this visit, the next part of the study will be scheduled. The participant will be provided with dapagliflozin, 10 mg, 14 tablets. The participant will be asked to take the pills daily for 14 days prior to Visit 3 which is conducted on-site. If a gap in scheduling occurs such that visits 2 and 3 are more than 4 weeks apart, women of child-bearing potential will be asked to complete a home pregnancy test, with photographic proof that it is negative before starting dapagliflozin.

The ketone action plan will be discussed again with each participant. Elements of the plan will during PERIOD 2 will include: stopping the SGLT2i medication, ingestion of carbohydrates, fluid intake and insulin administration with doses of insulin adjusted according to individual requirements.[16,17] Participants will be asked to employ the ketone action plan if BOHB reading is >1.5 mmol/L. BrACE levels of >15 in the absence of corresponding BOHB level >1.5 mmol will be considered due to contamination and the participant will be asked to repeat the levels in 1 – 2 hours. Participants will be given numbers of study personnel to call for ongoing advice about management of ketosis.

USUAL CARE + SGLT2i (Group B will do this segment first, followed by usual care without SGLT2i)
The participant will commence taking dapagliflozin, 10 mg daily, for 14 days before Visit 3. Insulin doses will be reduced by 10% basal and 15% bolus. Insulin dose adjustments will be determined by an investigator at the end of Visit 2. Participants will check BrACE with the Biosense BKA and capillary BOHB with the Precision Xtra ketone test strips when fasting and at two other times during the day. Each time point should be at least 4 hours after ingesting alcohol. Participants will be instructed in avoidance of contamination with other alcohol sources such as swabs, nail polish remover, hand sanitizer and other

solvents during the testing period. The study team will remotely monitor the participant's glucose within a few days of starting the SGLT2i and again prior to the on-site visit to ensure safety. There is no mandated contact with the participant, but participants can contact the study team at any time for questions.

On-site visit, SGLT2i
The on-site visit with SGLT2i will be conducted at the Intensive Research Unit at Washington University, the same format as described for usual care. Patients will be asked to arrive fasting, to stop their basal insulin delivery 1 hour before arrival at the center if on an insulin pump or to hold injectable insulin doses. Prior to leaving home, the participant's blood glucose should be 90 – 250 mg/dL, and BOHB <1.5 mmol/L, or the test day will be rescheduled. Time of the last insulin dose will be recorded. Patients will be given a dose of dapagliflozin, 10 mg upon arrival at the IRU. Vital signs and baseline labs including a CBC, BMP and pregnancy test in women with child-bearing potential will be done. Measurements of capillary BOHB using the Precision Xtra meter and breath ketones using the Biosense BKA will be done hourly X7 if the patient remains relatively asymptomatic, and the capillary BOHB level does not exceed 4 mmol/L. If the capillary BOHB reaches 2.5 mmol/L or capillary glucose reaches 250 mg/dL, the capillary finger sticks and breath measurements may be collected at 30 minute intervals. The venous blood samples will continue to be collected on the hour intervals. Venous blood samples for BMP, BOHB and AcAc* will be drawn at baseline and every 2 hours X3 plus the end of the study.

The insulin withdrawal portion of the study will end (stopping rules) upon patient request for any reason, if the patient becomes symptomatic with nausea or vomiting (even if he or she does not request that the study be stopped), after 7 hours of fasting without insulin, or if either the capillary BOHB is $\geq$4 mmol/L or the CGM blood glucose is $\geq$400 mg/dL. BMP will be done every 2 hours and at the end of the study. At the termination of the ketogenic fast, participants will be given a correction bolus dose of insulin by IV or subcutaneous injection, restart insulin via insulin pump, and or administer an injection of pre-meal insulin and be given a standard meal. Participants will be observed for 1 hour or more if needed after the study end for reduction in ketones and glucose into safe ranges. Glucose and ketone monitoring will continue into the evening with a phone call or other contact made with the patient for safety.

*Acetoacetate (AcAc) has been rarely measured in studies of ketone metabolism due to lack of a reliable assay, stability of the analyte and high cost. A plate-based nonenzymatic spectrophotometric assay is now available, but it is new to the WU Core Laboratory.[30] There are several unknowns about the assay. Given the wide range of AcAc levels during ketogenesis, dilution of specimens is recommended, however, the number and extent of dilution is unclear. Therefore, we will plan to draw and store samples for AcAc every 2 hours for a total of 4-5 samples during each of the insulin withdrawal visits. Samples will be batched and run after 4 patients have insulin withdrawal visits, providing 16-20 samples. Once we better understand the assay, the number of dilutions, the stability of the samples and the approximate correlation with BOHB and acetone, we will develop further plans for measurement of AcAc, whether on all participants or a sub-group of participants. Measuring AcAc is of significant interest, but it will be considered an exploratory aim of the study due to technical uncertainties and cost of the assay. This will be reflected in the informed consent.

Sample Size

The sample size is based on the study by Suntrup et al., which documented wide variability in ketone measurements during the day in persons undergoing dietary modification to enhance ketogenesis, also in the control group. In that study of 21 participants, over 1000 paired measurements were generated to determine the correlation between BrAce with BOHB using the Precision Xtra ketone meter, providing R^2 =0.57. Further analysis demonstrated receiver operating characteristic curves with AUC $\geq$0.80 for values of BOHB at the 0.5 mM and 1.0 mM thresholds. This study will generate ~1400 paired measurements, which are expected to be in the same range during usual care and usual care plus SGLT2i. Since the main objective of the study is to determine whether the Biosense BKA provides sufficient information for the patient to consider action steps to avoid worsening ketosis, the ROC analysis is crucial and the

number of observations needed is expected to be about 1000. For the insulin withdrawal studies, a difference of BOHB levels of 1.0 mmol/L between usual care and usual care plus dapagliflozin, a sample size of 16 completing both study days can be detected with 80% power and two sided significance level of 5%.

Data Management and Analysis

Participants will be assigned a randomly generated patient ID number, not in order of participation. All data will be entered into a dedicated Redcap database without patient identifiers for analysis. Baseline cohort information will include demographic information, diabetes duration, history of last DKA episode, complications, eGFR and insulin doses during usual care and at the end of the usual care + SGLT2i therapy. Glucose parameters, including time in range, time above and below range will be recorded at the end of each 2-week segments from CGM downloads during the usual care and usual care + SGLT2i study segments. Capillary BOHB measurements and BrAce levels will be paired, and only those taken within a 1 hour window will be entered as data during the usual care and usual care + SGLT2i (study segments 1 and 2). Data entry for on-site insulin-withdrawal visits will include patient vitals and weight, all laboratory values, hourly paired capillary BOHB and BrAce levels with laboratory BOHB and AcAc. Adverse events during the study will be manually tabulated.

The study will be monitored for data integerity by an uninvolved study coordinator or fellow within the division to check for errors, omissions and to generate a report to the DSMB.

Data analysis will be done using SAS through either the WU CTSA Statistics center or a faculty member in the division with SAS capability.

Assessment of Efficacy

The main efficacy parameter will be the correlation between BrAce levels and capillary blood BOHB levels during segments 1 and 2, usual care and usual care + SGLT2i of the study using linear regression. Efficacy will be established with R^2 >0.6. Separate linear regressions will determine the correlation of BrAce levels with capillary blood BOHB, of BrAce levels and laboratory BOHB, and of BrAce levels and AcAc. Efficacy of BrAce for correctly indicating actionable ketosis will be established with R^2 >0.6. Additionally, a receiver operating characteristic (ROC) analysis will be performed to clarify the diagnostic ability of BrAce compared to capillary blood BOHB to correctly or incorrectly identify ketone levels that would be considered actionable for a person with T1D, or >1.5 mmol/L.

Qualitative analysis will be undertaken based on a simple Likert scale questionnaire with the following questions rated from 1 to 5:

- Did testing breath ketones help you to understand your "ketone status"?
- Were you able to identify episodes of mild ketosis with the Biosense breath ketone analyzer?
- Are you likely to continue to use the Biosense breath ketone analyzer for this purpose?

The main efficacy parameter will be assessed with an interim analysis after completion of 12 subjects in the trial, with results reported to the DSMB and to the funding partner, JDRF. Adverse events will be manually tabulated and reported.

Assessment of Safety

Safety parameters will be analyzed separately for the usual care segments and for the insulin withdrawal days. For the usual care segment, the main safety concern is a level of capillary blood BOHB >1.5 mmol/L at any time. Participants will be asked to call the study coordinator or investigator for advice if this happens, and to start a ketone action plan. The participant can call for concerns about any level of capillary BOHB during the study, but in particular while taking an SGLT2i. The participant will be

provided with a ketone-action plan that will include ingestion of carbohydrates, fluid and insulin administration and frequent follow-up testing. The study investigator will monitor the participant with contact every 1 – 2 hours until the episode resolves. Any capillary blood BOHB level >1.5 mmol/L will be reported as an event of special interest, with follow-up ketone action plan results noted. BrACE levels of >15 without corresponding BOHB level >1.0 mmol/L will be considered likely due to contamination and repeated in 1 hour. Likewise, any severe hypoglycemia event will be reported as an adverse event. Patients with level 2 hypoglycemia, glucoses <54 mg/dL for more then 1% of the day on weekly CGM reviews will have insulin doses adjusted and a discussion with the investigator.

The most important safety parameter of the insulin withdrawal test days will be capillary blood BOHB levels >3 mmol/L, or symptoms of nausea or vomiting at any level of BOHB. Capillary BOHB level of >3 mmol/L constitutes a stopping point for the insulin withdrawal study, and immediate treatment with fluids, insulin and carbohydrates will be commenced, and the patient followed on-site until the episode resolves. Resolution will be defined as cessation of symptoms and capillary BOHB level <1.0 mmol/L, with blood glucose <250 mg/dL. Note that in a similar study of insulin withdrawal before and after treatment with the SGLT2i, canagliflozin, it took about 6 hours for glucose to increase from 100 to 300 mg/dl (pre-treatment) and from 100 to 220 mg/dL with canagliflozin.[28] During the six hours, BOHB increased from 0.2 to 1.2 mmol/L (pre-treatment) and from 0.4 to 1.5 mmol/L with canagliflozin.[28] This proposal uses a similar time sequence, and thus we anticipate similar results from the insulin withdrawal.

Other adverse events, such as genital mycotic infections, urinary tract infections, orthostatic dizziness or any other acute or worsening illness will be reported. Study fatigue resulting in termination of the study will also be reported.

Statistics

The statistical methods to be employed include simple descriptive statistics of baseline cohort parameters, laboratory values and CGM glucose metrics. Means with standard deviations will be reported. The primary outcome of the study is the correlation coefficient relating capillary blood BOHB concentration and BrACE level during the 2-week usual care period of the study. The data will first be tested for normality using the D'Agostino-Pearson omnibus K2 test. If both capillary blood BOHB data and BrACE data are normally distributed, then the Pearson r will be calculated. Otherwise, the Spearman ρ will be calculated. A correlation coefficient > 0.7 will be interpreted as strong. Similar correlation procedures will be carried out to analyze the relationships between: 1) capillary blood BOHB concentration and BrACE level during the 2-week SGLT2i-treatment period of the study; 2) capillary blood BOHB and BrACE level during insulin withdrawal; 3) venous blood BOHB and BrACE level during insulin withdrawal; 4) venous blood AcAc and BrACE level during insulin withdrawal; 5) venous blood BOHB and venous blood AcAc during insulin withdrawal.

The relationship between time from insulin withdrawal and: 1) capillary blood BOHB, 2) BrACE, and 3) venous blood BOHB will be modeled by least-squares linear regression. The slopes of these 3 best-fit lines will be compared using the extra sum-of-squares F test. A receiver-operating characteristic (ROC) curve will be generated to describe the ability of BrACE to identify clinically actionable ketonemia, defined as capillary blood BOHB > 1.5 mmol/L, by pooling all paired BrACE and capillary blood BOHB measurements collecting during usual care and insulin withdrawal. Separate curves will be generated during the segments with and without SGLT2i treatment.

The effect of SGLT2i on random BrACE and random capillary blood BOHB during the 2-week free-living period will be assessed using unpaired two-tailed Student's t-tests. The effect of SGLT2i on 1) frequency of random capillary blood BOHB > 1.5 mmol/L during the 2-week free-living period, and 2) frequency of venous blood BOHB > 1.5 mmol/L during the insulin withdrawal study, will be assessed using Fisher's exact test. The effect of SGLT2i on the correlation between BrACE and BOHB during the 2-week free-living period, and the effect of SGLT2i on the slope and y-intercept of the best-fit line relating time from

insulin withdrawal to capillary blood BOHB, will be assessed using the extra sum-of-squares F test to compare linear regression models.

Baseline participant characteristics, laboratory results, CGM glucose metrics, and Likert scale values regarding breath ketone meter usage will be reported as mean ± SD.
Statistical analyses will be performed using GraphPad Prism 9. Interim analyses of these outcomes will be performed after 5 and 10 participants have completed the study protocol.

Ethical considerations

This study will be submitted to the Washington University Human Research Protection Office (HRPO), which is the institutional review board for ethics review and approval. The informed consent will carefully detail the previously described risks to persons with T1D taking a SGLT2i. The risks of insulin withdrawal including hyperglycemia and the development of mild ketosis will be described, along with the stopping rules and treatment plans for resolution of the event. A written informed consent, approved by the WU HRPO, will be used in the study, and consent obtained prior to any study procedure being performed.
Ethics concerns for this study include:

- Loss of confidentiality is a risk with this clinical study. This risk will be handled by careful attention to HIPAA guidelines and de-identification of data. All research personnel have been trained in confidentiality measures and good clinical practice.
- Loss of time while performing study-related procedures, both over 4 weeks of ketone monitoring while at home, and on-site study visits. There is no mitigation strategy for this risk, but communication about time commitments and scheduling concerns will be handled both verbally and in writing in the informed consent.
- Hyperglycemia and development of ketones will occur during on-site study visit days due to insulin withdrawal. This could result in symptoms of feeling unwell, nausea or vomiting. This is a clearly identifiable risk. Stopping rules and mitigation strategies of frequent monitoring and treatment to the point of recovery from the event will be described. An investigator will either be present at the end of the visit or be in close proximity for consultation.
- Adverse effects of dapagliflozin will be described. Mitigation strategies will be offered (such as treatment of genital infections), and stopping the drug will be an option.
- Hyperglycemia from insulin dose reduction while starting dapagliflozin or hypoglycemia due to insufficient insulin dose reduction can occur. Both of these risks will be explained at the beginning of the study and at the start of the SGLT2i treatment. Mitigation strategies include daily assessment of glucoses by CGM and insulin dose adjustments to accommodate the added anti-hyperglylcemic treatment.

A local data safety and monitoring committee composed of two faculty clinicians familiar with SGLT2i therapy plus a statistician or three clinicians if one has a background in statistics will be convened. Stopping rules for futility or safety concerns will be established at the onset of the trial. An interim analysis will be conducted after completion of 12 subjects to review efficacy and safety. Adverse events will be collated and reported. The DSMB report will be made available to the HRPO and to JDRF.

Intellectual Property (IP)

No intellectual property or commercial efforts will be pursued as a result of this study.

Principal investigator assurance

The principal investigator, Janet B. McGill, MD, agrees to accept responsibility for the scientific and technical conduct of the research project and agrees to all terms and conditions of the award.

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Summary of protocol changes

10Jan2023 – Updated stopping rules for on-site visits: blood ketone ≥ 4mmol/L or glucose ≥ 400 mg/dL. This does not present an increase in risk/benefit for participants. Finger sticks for glucose/ketone monitoring may be collected every 30 minutes to 1 hour for the duration of the study.