PROTOCOL

Background

1. Provide the scientific background, rationale and relevance of this project.

INSTRUCTIONS

- This should include a referenced systematic evidenced-based review when possible.
- If this study involves qualitative research explain the major constructs of your study.
- Do not state in this section what you plan to do in this study. This information should be entered later under "What will be done in this protocol?"
- Do not include the bibliography in this section.
- For studies submitted under the Expedited review criteria, this section need not be more than a few paragraphs.
- For those studies where data will be analyzed collaboratively by multiple sites doing a similar study for which there is no common protocol (Collaborative Site Analysis Study) include a description of the common scientific goals/ procedures/data points.
- If this is a FIVE YEAR UPDATE make sure the information throughout the protocol includes the most current information.

Type 1 diabetes (T1D) is a chronic metabolic condition characterized by the autoimmune destruction of insulin-producing pancreatic beta-cells [1]. As a consequence of this damage, individuals with T1D are not capable of endogenous insulin secretion and need to rely on exogenous insulin to control their blood glucose levels. Despite the improved accuracy of glucose monitoring devices [2],[3] and the growing development of decision support systems [4]-[5], achieving good glycemic control in T1D remains a challenge. In fact, insulin dosing is oftentimes complicated by a wide variety of factors influencing insulin demand (e.g., circadian rhythms [6], psychological stress [7], and physical activity [8]-[9]), which – if not properly accounted for – typically lead to increased glucose variability and worsened glycemic control. Among these, phases of the menstrual cycle have been shown to significantly impact insulin sensitivity (SI) and glycemic variability in females with T1D. Specifically, preliminary studies have documented that insulin requirements are significantly higher in the luteal phase of the cycle as compared to the early follicular, leading to increased exposure to hyperglycemia following ovulation [10]-[13]. With this project, we aim to further characterize SI fluctuations across the menstrual cycle in females with T1D and their impact on glycemic variability and quality of glycemic control; furthermore, we intend to investigate the impact of oral contraceptive therapy on the stabilization of glycemic control. The results from this study will be leveraged in future work to build ad hoc decision support systems to improve glycemic control across the menstrual cycle and help females with T1D in the management of their disease.

Objectives/Hypothesis

INSTRUCTIONS:
If this study involves biomedical research clearly state the objectives and hypotheses and clearly define the primary and any secondary outcome measures. If this study involves qualitative research clearly state your research hypothesis or question.

This section should not include information already included in other sections such as background information or information from the procedures section.

The primary aim of the study is to track and characterize fluctuations of insulin sensitivity and glycemic variability in females with T1D across the menstrual cycle, contrasting free-cycling females and females under oral contraceptive therapy.

Study Design: Biomedical

1. Will controls be used?

No

►IF YES, explain the kind of controls to be used.
Answer/Response:

2. What is the study design?

Example: case series, case control study, cohort study, randomized control study, single-blind, double-blind, met-analysis, systematic reviews, other. You may also view the IRB-HSR Learning Shot on this topic to help you answer this question. (http://www.virginia.edu/vpr/irb/learningshots/Writing_protocol_June09/player.html

This is an observational study. Forty females with type 1 diabetes (age ≥18 to ≤40 years) using a continuous glucose monitor and an insulin pump will be enrolled for a 3-month data collection. Study participants will include free-cycling females and females following oral contraceptive therapy. Study CGMs equipment and activity trackers will be provided for the entire data collection period. During data collection, participants will be asked to record meals consumed. The Clue App will be used to record dates of the participants' menses. In addition, free-cycling females will be asked to confirm ovulation by means of study-provided ovulation kits, and record ovulation dates. At the end of data collection, data will be analyzed to track insulin sensitivity and glycemic variability changes across the menstrual cycle, contrasting free-cycling females and females using oral contraceptives.

3. Does the study involve a placebo?

No

►IF YES, provide a justification for the use of a placebo

Answer/Response:

Human Participants

Ages: ≥18 to ≤40 years
Sex: F
Race: All

Subjects- see below

INSTRUCTIONS: For question 1-4 below insert an exact #. Ranges or OPEN is not allowed. This # should be the maximum # you expect to need to enroll (i.e. sign consent) If you are only collecting specimens the number of participants should equate to the # of specimens you need. If you are collecting only data from a chart review the number should designate the number of subjects whose medical records you plan to review. Age/ Sex/Race criteria should designate the demographics of participants from whom you will obtain the specimen/data.

1. Provide target # of subjects (at all sites) needed to complete protocol.

INSTRUCTIONS: If this is NOT a database protocol, this number should be the same as the number of subjects needed to obtain statistically significant results.

Up to forty (40), free-cycling females and females under oral contraceptive therapy, are needed to complete the study. Study team will attempt to recruit an even number of females into each group.

2. Describe expected rate of screen failure/ dropouts/withdrawals from all sites.

The screen failure/dropout rate is anticipated to be 15%.

3. How many subjects will be enrolled at all sites?

INSTRUCTIONS: This number must be the same or higher than the # from question # 1 in order to account for the # of screen failures, dropouts, withdrawals described in question # 2.

Up to 55 females will be enrolled at UVA.

4. How many subjects will sign a consent form under this UVa protocol?

INSTRUCTIONS: If the protocol does not have a consent form- the number listed here should reflect such things as the number of subjects from whom specimens will be obtained, the number of charts to be reviewed etc.

Up to 55 females will sign consent at UVA.

Inclusion/Exclusion Criteria

INSTRUCTIONS:

- The inclusion and exclusion criteria should be written in bullet format.
- *This item applicable if the study will require consent (verbal or written).* Unless there is a scientific reason for not recruiting a certain type of vulnerable population(e.g. not enrolling fetuses, neonates or children in a study regarding Alzheimer's) list the following vulnerable populations under either Inclusion or Exclusion criteria below: pregnant women, fetuses, neonates, children, prisoners, cognitively impaired, educational or economically disadvantage, non- English speaking subjects .
- If you will not enroll subjects who do not speak English because certain procedures cannot be carried out if the subject does not speak English (e.g. a survey is not validated in other languages) insert the following as an Inclusion Criteria: Willingness and ability to comply with scheduled visits and study procedures.
- If this is a collection of only retrospective* specimens or data, the inclusion criteria must include a start and stop date for when specimens/data will be collected.
- The stop date must be prior to the version date of this protocol.
- *Retrospective: all specimens are in a lab at the time this protocol is approved by the IRB. All data exists in medical records or records from previous studies at the time this protocol is approved by the IRB.

1. List the criteria for inclusion

- Type 1 diabetes for at least 12 months
- Use of glucose sensor in the last 6 months
- Use of insulin pump in the last 6 months
- Age ≥18 to ≤40 years
- HbA1c ≤8.5% if measured at screening or available from historical medical report performed within the last 6 months; in absence of a valid HbA1c measurement, average blood glucose estimated from CGM data to be approximately 200 mg/dL or less
- Absence of perimenopausal/menopausal symptoms
- Willingness to keep track of beginning of menstrual cycle
- Willingness to keep track of ingested carbohydrates
- Willingness to not become pregnant during study participation
- Regularly menstruating (at least every month with no missed cycles)
- Only free-cycling participants: willingness to use ovulation kits to confirm ovulation
- Only participants under oral contraceptive therapy: use of monophasic pill, with 3 weeks of active pill and 1 week of placebo
- Willingness to use the study Dexcom G6 during the study
- Participants must have Internet access and computer system that meets the requirements for uploading the study equipment

2. List the criteria for exclusion

- Pregnancy
- Hormonal birth control therapy except monophasic pill contraceptive
- Polycystic ovary syndrome (PCOS) diagnosis
- Current use of steroids
- Concurrent use of any non-insulin glucose-lowering agent (including GLP-1 agonists, pramlintide, DPP-4 inhibitors, SGLT-2 inhibitors, sulfonylureas, metformin)
- Uncontrolled thyroid disease
- Active fertility treatment
- Concomitant disease or condition that may compromise patient safety or ability to follow the protocol including: planned or current dialysis treatment; moderate to advanced nephropathy; known or suspected allergy to medical grade adhesives.
- Severe hypoglycemia or diabetes ketoacidosis (DKA) in the previous 6 months

3. List any restrictions on use of other drugs or treatments.

INSTRUCTIONS: List only those drugs or treatments that are prohibited while on study, not those listed as an exclusion criteria.

None

Statistical Considerations

1. Is stratification/randomization involved?

No

►IF YES, describe the stratification/ randomization scheme.

INSTRUCTIONS:
The stratification factors and/or the randomization plan should be identified. If there is no randomization component or important patient characteristics that will be used in treatment allocation or data analysis, a statement to this effect should be included.

Stratification factors: These are pretreatment patient characteristics which could be balanced across treatment arms by design or may be used to determine starting dose or treatment allocation.

If randomization is going to be used, the details of the randomization plan should be described.

The description should include:
--the method and timing of randomization
--the type of randomization scheme that will be used in the study

--whether or not the randomization masked/blinded/if so, then to whom is it masked/blinded
--who has access to the randomization scheme

Answer/Response:

►**IF YES, who will generate the randomization scheme?**

_____ Sponsor
_____ UVa Statistician. Insert name Answer/Response:
_____ UVa Investigational Drug Service (IDS)
_____ Other*:* Specify Answer/Response:

2. What are the statistical considerations for the protocol?

The objectives section and the statistical section should correspond, and any objective for which analysis is unfeasible should be deleted. Also, the estimates and non-statistical assumptions of the statistical section should be supported by discussion in the background section.

The answer to this question should include:
--Study Design/Endpoints
--Recap of study objectives and endpoint definitions. An assessment of how study objectives will be assessed by identifying & defining which endpoints will be used to assess each component of the study objectives.
--The study design should include contingencies for early stopping, interim analyses, stratification factors (If applicable), and any characteristics to be incorporated in analyses.
--The power/precision of the study to address the major study endpoint(s), the assumptions involved in the determination of power/precision.
--If statistical hypothesis testing is included then specify the null and alternative hypotheses, the test statistic, and the type I and II error rates
--If precision of an estimate, then provide a definition for precision
--If other, then specify

This observational trial is not powered to a specific outcome. A sample size of 40 females has been chosen with the purpose of being able to compute the Cohen's *d* metric once the trial is completed. Cohen's *d* is defined as the difference between two means divided by a standard deviation for the data (standardized difference between two means), and will serve as effect size to appropriately power the subsequent main trial.

3. Provide a justification for the sample size used in this protocol.

Include sample size calculations or statistical power estimation. If not applicable, please provide explanation.

Also include the anticipated accrual rate, the accrual goal for the study, including accrual goals by strata if appropriate, adjustments for drop-outs etc. and study duration.

The sample size is determined based on the fact that this study is a pilot trial including two groups (i.e., free-cycling females and females using oral contraceptives).

4. What is your plan for primary variable analysis?

Include primary outcome(s)/predictor variable(s), statistical methods/models/tests to be employed, or descriptive summaries as appropriate. If not applicable, please provide explanation.

The primary variable will be insulin sensitivity (SI) computed using a Kalman filter-based algorithm embedding a model of glucose-insulin dynamics; SI will be tracked over the duration of the menstrual cycle and compared between phases.

5. What is your plan for secondary variable analysis?

Include the following:
--Secondary outcome(s)/predictor variables, statistical methods/models/tests to be employed, or descriptive summaries as appropriate. If not applicable, please provide explanation.
--For phase III studies, the power/precision of the study to address the secondary objective(s).

Secondary variables will include metrics to quantify glycemic variability (e.g., percent time spent in different glycemic ranges, low/high blood glucose indices, coefficient of variation).

6. Have you been working with a statistician in designing this protocol?

Consultation with a professional statistician is highly recommended to ensure good science of the study and facilitate the review process.

No

IF YES, what is their name?
Answer/Response:

7. Will data from multiple sites be combined during analysis?

No

INSTRUCTIONS: IF YES, answer the following questions

7(a). Does the study involve randomization?
Answer/Response:
IF YES, will randomization be done at each site or among sites?
Answer/Response:

7(b). Has the sample size calculation considered the variation among sites?
Answer/Response:

7(c). When combining the data from multiple sites to assess the study results, is the effect of the treatment to be tested (or the association to be tested) assumed to be the same across sites or vary among sites? What is the modelling strategy?

Answer/Response:

7(d). Is there a common protocol used in all sites?

Answer/Response:

IF NO, how will differences among sites, such as those related to the implementation, inclusion criteria, patient characteristics, or other sites characteristics, be considered to assess the study results?

Answer/Response:

Study Procedures-Biomedical Research

1. What will be done in this protocol?

INSTRUCTIONS:
This should include everything that will be done as part of this protocol. Do not repeat information that is included in other sections such as Background or Hypothesis sections.
This section should include an indication of which research interventions if any offer a prospect for direct benefit and which interventions (invasive measurements, collection of blood, tissue, data, surveys, etc.) are being done solely to answer a research question and generate generalizable knowledge. If the interventions done solely for research purposes are associated with greater than minimal risk they need to be justified. Describe and justify any control and experimental arm and include method, dose, and duration of drug administration. Reference any claim of clinical equipoise if applicable.

If you are obtaining specimens or data, provide information regarding the type of specimen/data, amount of specimen needed and how the specimen/data will be obtained and what analysis will be done with the specimen/data.

<u>Special note for studies with waiver of consent/waiver of documentation of consent:</u>
Include a statement regarding how subjects will be recruited. For other studies this information is captured in Recruitment does not need to be duplicated in this section.

Study visits may be performed in-person, phone, or via a secure WebEx.

Participants will be screened and inclusion/exclusion will be reviewed. A urine pregnancy will be performed. The subject will also be asked for their medical history, preferably with a physical exam, will be requested but not required. Height, weight, and vitals may be self-reported. Medical history may be obtained from data collected during prior clinical studies conducted from the past 24 months at CDT. Subjects will also have an HbA1c test completed upon enrollment. If the screening is performed remotely, the pregnancy test will be mailed to the participant. The

pregnancy test must be negative to proceed in the trial. While optional, participants will be asked to provide 3-months of pre-study personal CGM data.

Eligible participants will start a 3-month home data collection. During data collection, subjects will be asked to use a study-provided Dexcom G6 continuous glucose monitor, personal insulin pump, and a study-provided activity tracker. Participants who are not on oral contraceptive will be asked to use an ovulation kit to confirm ovulation. All participants will be asked to record the carbohydrates consumed each day (i.e. meals, snacks, carbohydrate treatments). Unbolused carbohydrates will be recorded in the Dexcom app or receiver, not in the participant's insulin pump. Participants will also be instructed to record Day 1 of their menstrual cycle each month into the Clue App. Free cycling participants will be asked to enter the monthly ovulation test results in the app as well.

Some study participants have prior use of diabetes data aggregators (e.g., Tidepool, Nightscout) and do want to switch to another data aggregator. The study team did not request that they use Tidepool or Nightscout; however, so not to interrupt their standard of care, the study may access study data stored at Tidepool and Nightscout in order to save it on a UVA server for analysis. This may require participants sharing personal login information to the study team to access data or study participants may share this data with the team.

If the subject owns a personal laptop device, the participant will be asked to bring it to the visit for the study team to download specific software to be used during data collection if an in person screening is performed; otherwise, the study team will instruct the subject how to download the software from online website or the study team may provide the subject with a memory drive (USB) storing the appropriate resources to be used at home will be provided. Participants will be instructed on how to upload the equipment (i.e. insulin pump, CGM, Activity Tracker, Clue App, etc.). Participants will be asked to provide uploaded data periodically during the data collection period (approximately 1-2 times per month) using a local diabetes device management software (e.g., Dexcom Studio). Subjects will have the option of using their personal smartphone or receive a study smartphone to use in order to collect the data from the devices.

Participants will be asked to wear the study activity monitor during the data collection phase. Participants will also wear the activity monitor to collect information on activity, exercise, heart rate, and sleep. The commercially available app associated with the activity monitor will be placed on the smartphone to facilitate weekly downloading of data.

The study team will contact the participant via phone/email/text at least one time month to assess:

- Adverse events and device issues
- Review download of CGM and activity monitor to assess occurrence of glucose values <60 mg/dL and >300 mg/dL
- Verify Clue App monthly download – the study team logging into the subject's study account to verify its use and download data

Study Schedule

	Screening	Monthly Monitoring	Final
Study Days	1*	2-90	~90
Visit	1	2,3,4	5
Informed consent	x		
Review study eligibility	x		
Medical history (optional)	x		
Pregnancy test	x		x
Physical exam (optional), height, weight, and vital signs (self-reported)	x		
HbA1c	x		x
CGM use	x		
Activity tracker use		x	
Ovulation test (if applicable)		x	
Record menses in app		x	
Data uploads provided to study team		x	x
Monthly check-ins with staff		x	
Return study equipment			x

* Visit 1 may be split over 2 days

Participants will return all study equipment and remaining supplies at the end of the study. A hemoglobin A1c value will be collected and assessed on a point of care machine, a local laboratory, or derived from blood glucose data.

Upon completion of data collection, data will be analyzed to identify patterns in glucose metabolism across the menstrual cycle, and contrast free-cycling females to females using oral contraceptives.

If a subject is considered a screen failure, they will be replaced. Subjects who withdraws from the study will be replaced per the Principal Investigator's discretion. Should any study subject receive

a positive test result for COVID-19, they should tell the study team but will continue to use the study equipment as it is commercially available equipment.

2. **If this protocol involves study treatment, explain how a subject will be transitioned from study treatment when they have completed their participation in the study.**

> **Example:** If the subject will be taking an investigational drug, will they need to be put back on an approved drug when they have completed the study? If yes, explain how this will be accomplished and who will cover the cost. If the subject has a device implanted will it be removed? Again- who will cover the cost of the removal?
> **Instructions:** Answer NA if this study does not involve a study treatment.

The study equipment provided to participants during the trial are commercially available items and will be used per labeling instructions. Participants will return to their usual care at study end.

Subject Compliance with Study Procedures

1. **Explain how the study team will monitor the subject for compliance with the study procedures.**
   (e.g. study team will administer study drug/ study interventions, study drug inventory of dispensed and returned drug, diary etc.)
   Answer/Response: Study team will regularly check the Dexcom Clarity account to verify usage of the study CGM. Participants will be asked to call the study team as needed during the trial, specifically to report medical issues, medical visits, etc. The study team will also have monthly check-in with the participant.

2. **Describe criteria for when a subject is considered to be non-compliant with study procedures.**
   (e.g. subject returns more than 20% of the study drug, subject misses 20% of study visits)
   Answer/Response:
   - subject does not use the glucose sensor for more than 25% of the study
   - subject does not download devices per study schedule

Bibliography

INSTRUCTIONS: Provide a current bibliography supporting the hypothesis, background and methodology including references to papers and abstracts that have resulted from previous work by the investigator and references to the work of others.

[1] American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 2014;37(S1):S81-90.

[2] Klonoff DC, Prahalad P. Performance of Cleared Blood Glucose Meters. J Diabetes Sci Technol. 2015;9(4):895-910.

[3] Klonoff DC, Ahn D, Drincic A. Continuous Glucose Monitoring: A Review of the Technology and Clinical Use. Diabetes Res Clin Pract. 2017;133:178-192.

[4] Breton MD, Patek SD, Lv D, Schertz E, Robic J, Pinnata J, Kollar L, Barnett C, Wakeman C, Oliveri M, Fabris C, Chernavvsky D, Kovatchev BP, Anderson SM. Continuous Glucose Monitoring and Insulin Informed Advisory System with Automated Titration and Dosing of Insulin Reduces Glucose Variability in Type 1 Diabetes Mellitus. Diabetes Technol Ther. 2018;20(8):531-40.

[5] Nimri R, Ochs AR, Pinsker JE, Phillip M, Dassau E. Decision Support Systems and Closed Loop. Diabetes Technol Ther. 2019;21(S1):S42-56.

[6] Schiavon M, Dalla Man C, Kudva YC, Basu A, Cobelli C. Quantitative Estimation of Insulin Sensitivity in Type 1 Diabetic Subjects Wearing a Sensor-Augmented Insulin Pump. Diabetes Care. 2014;37(5):1216-23.

[7] screeLi L, Li X, Zhou W, Messina JL. Acute Psychological Stress Results in the Rapid Development of Insulin Resistance. J Endocrinol. 2013;217(2):175-84.

[8] Dalla Man C, Breton MD, Cobelli C. Physical Activity into the Meal Glucose-Insulin Model of Type 1 Diabetes: In Silico Studies. J Diabetes Sci Technol. 2009;3(1):56-67.

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[11] Moberg E, Kollind M, Lins PE, Adamson U. Day-To-Day Variation of Insulin Sensitivity in Patients with Type 1 Diabetes: Role of Gender and Menstrual Cycle. Diabet Med. 1995;12(3):224-8.

[12] Trout KK, Rickels MR, Schutta MH, Petrova M, Freeman EW, Tkacs NC, Teff KL. Menstrual Cycle Effects on Insulin Sensitivity in Women with Type 1 Diabetes: A Pilot Study. Diabetes Technol Ther. 2007;9(2):176-82.

[13] Brown SA, Jiang B, McElwee-Malloy M, Wakeman C, Breton MD. Fluctuations of Hyperglycemia and Insulin Sensitivity Are Linked to Menstrual Cycle Phases in Women With T1D. J Diabetes Sci Technol. 2015;9(6):1192-9.