STUDY PROTOCOL:

Study Design: A single-centre, randomised and counter-balanced, two-arm cross-over study.

Population: 32 adults with T1D aged between 18-70 years. Age is restricted to a minimum of 18 years due to the well documented hormonal effects on glycaemic control in adolescents and young adults.

Inclusion criteria: A diagnosis of T1D for a minimum of 5 years; Currently treated on a stable insulin regimen for a minimum of 6 months consisting of continuous subcutaneous insulin infusion (CSII) therapy or multiple daily injections (MDI) of a combination of rapid-acting and long-acting insulin; Familiar and currently using the carbohydrate-counting method for determining mealtime insulin dose; Not currently pregnant; No overt diabetes complications including end stage renal failure requiring dialysis; Free from hypoglycaemia unawareness assessed through a combination of the Clarke[64] and Gold[65] methods[66]; No recent (<6-months) history of diabetic ketoacidosis (DKA); Free from medical conditions relating to a haematological disorder, gut mobility or digestion; No history of anorexia, bulimia, or any other disordered eating; No history of deep vein thrombosis; No history of heart attack or stroke within 6 months prior to recruitment; No history of malignancy; No existing medical or psychiatric conditions likely to interfere with the study; No severe functional limitations that contraindicate prolonged episodes of sitting (i.e. back pain) or short frequent bouts of light-intensity walking (i.e. mobility issues); No dietary allergies or intolerances likely to interfere with the study; Able to understand written English and provide written informed consent; Do not currently meet the recommendations for moderate-to-vigorous physical activity (150 min/wk) in adults.

Setting and recruitment strategy: Potential participants will be identified via recruitment using University/charity recruitment materials/websites. Once potential patients have been identified, eligibility checked, and informed consent obtained, participants will be randomised by computer programme to determine the sequence of two cross-over arms. Our team is research active and has significant expertise and experience in recruiting to clinical studies (including those involving CGM) in both T1D and T2D (GLADIS, REPLACE, LIBERATES). Over the 12-month recruitment period of the proposed study, the team will see >1400 individuals with T1D, of which 25-35% are likely to be eligible. Based on our track record, we estimate a participation rate of 20% of those eligible is readily achievable based upon the studies we have previously conducted. Using a conservative estimate, we anticipate recruiting 32 participants (to target) within 6 months, which is, on average 1 participant per week. It should be clear that we have been developing this proposal, and the data to support it, for a long time prior to this call, and collectively have the experience and track-record to ensure successful completion. All reasonable travel expenses will be reimbursed to study participants, and individuals will receive a £100 gift voucher as compensation for their time.

Study Protocol:

Preliminary visit: Patients will attend a preliminary visit to our research facility to assess eligibility and obtain written informed consent. At this visit, participants will be fitted with a blinded real-time CGM and an accelerometer and provided with a food diary. During this visit, patients will complete a questionnaire to assess their medical history and physical activity. Both CGM and accelerometer devices will be worn by participants for the duration of their study involvement; both devices complete with data capture will be returned by participants to the research team either in person or via post (using a prepaid envelope).

CGM measurement: A small CGM device (CGM; Freestyle Libre Pro; Abbott Diabetes Care) will be inserted during the preliminary visit to the laboratory. It is a small discreet, water resistant sensor, with a thin filament (<0.4m thick) that is inserted (painlessly) 5mm beneath the skin surface on the upper arm. The sensor will be inserted into the subcutaneous tissue on the anterior of the upper arm. The insertion site will be taken as equidistant between the most medial portion of the upper arm and marked with indelible ink so that initial placement can be replicated on subsequent insertions in the unlikely event that a sensor should fail. The sensor is factory calibrated making it far more convenient and removing the risk for sensor inaccuracies from calibration user error (capillary meter inaccuracy, not washing hands etc) seen with other CGM devices. Glucose data generated by the CGM is masked (i.e. participants cannot see their glucose data and thus cannot change their treatment regimen based on this information). A single reader device is used to activate and retrieve the data. The accuracy and safety of this device has been established in T1D across the full range of glucose levels likely to be observed in this study, including those in the normal range[67]; the latest generation of the CGM sensor has a MARD of <9% as of 2018. Moreover, as an un-blinded version of this device is now made available to people with T1D with treatment indications through the NHS, the use of this technology is deemed acceptable to people with T1D. Study participants who are using Freestyle Libre to monitor their glucose patterns can continue to use the device as usual but will be asked to wear an additional blinded sensor.

Accelerometer: Participants will be issued and fitted with an accelerometer (GeneActiv, worn on the arm) which will provide a comprehensive assessment of sitting/standing patterns (i.e. posture allocated) *and* physical activity levels. The data collected from this device will be used to estimate daily energy expenditure (using a priori cut points) and calculate physical activity levels including time spent in different postures and activities during discrete parts of the day both before and after each laboratory visit. This will allow for quantification, standardisation and replication of physical activity patterns during free-living episodes.

Medical history: To include: age, gender, duration of diabetes, insulin therapy (average total daily dose and basal insulin dose), associated medical conditions and family history of T2D and cardiovascular disease. Presence of diabetes complications will be carefully evaluated where possible, including assessment of latest retinal screenings, renal dysfunction (proteinuria or reduced eGFR) and neuropathy (clinical examination), history or symptoms suggestive or coronary artery, and peripheral or cerebrovascular disease.

Physical activity assessment: Patients will complete the IPAQ (International Physical Activity Questionnaire), and an Exercise Benefits/Barriers Scale Questionnaire which will assess current levels of physical activity participation, as well as general and diabetes-specific enablers and barriers to physical activity.

Anthropometrics: Anthropometric data will include: weight, height, waist circumference and waist-hip-ratio. Body composition will be determined using the bioelectrical impedance method [Seca mBCA 525] as detailed above. Blood pressure will be taken alongside ankle brachial index measurements. Estimated glucose disposal rate (eGDR; a measure of insulin resistance) will be calculated for all patients.

Experimental visits

Pre-laboratory phase: Patients will be required to use their dietary recording sheets to replicate their dietary patterns in the 48-hours prior to each experimental laboratory visit. For standardisation of glycaemic control, as well as biochemical parameters, participants will be provided with a standardised meal to be consumed on the evening before each experimental laboratory visit based upon their weight (~635kcal; vegetarian lasagne; Tesco). Since an acute exercise session may enhance insulin action for up to 48-hours, participants will be asked to reframe from moderate-to-vigorous physical activity/exercise during the 48-hours preceding each laboratory visit, in order to avoid the potential impact of this on glycaemia and hypoglycaemia risk. Additionally, during the 24-hour prior to each laboratory visit, participants will be asked to reframe from caffeine and alcohol which are known to influence glucose levels.

To avoid any confounding influence of hyper/hypoglycaemia or illness, a researcher will call the morning of each testing day – if blood glucose is outside the range of 4-12 mmol/L or if the participant is unwell with blood ketones >0.6 mmol/L, the experimental visit will be postponed. The dosage and timing of insulin administrations as well as any supplements/vitamins being taken will be standardised for the duration of each trial period. To control for the potential confounding effect of insulin regimen (CSII vs MDI) and medication, insulin regimen and medication will be included as a covariate in the statistical model. Participants using CSII will be advised to avoid making changes to their insulin pump delivery settings and insulin regimen between study visits. Participants treated with MDI will be advised not to change their insulin regimen between study visits. Due to the likelihood of changes in glycaemic control and/or insulin requirements over longer periods of time, pre-menopausal women will complete each condition with a 1-week washout, as for all other participants. Menstrual phase will be recorded for each condition and controlled for as a covariate in statistical models.

Main experimental visits: In a randomised crossover design, patients will attend two separate morning-time (~08:00am) laboratory-based visits (lasting ~7-hours in duration) within our research facility, each interspersed by one week. Each experimental condition will be conducted in a controlled laboratory setting to maximise internal validity and minimise the inherent variability of less-controlled 'real-world' settings. Except for toilet breaks, participants will be seated in a comfortable lounge-chair and instructed to minimise excessive movement.

On each occasion and upon arrival to the laboratory following an overnight fast, participants will assume a seated and rested position while a 20-guage cannula (Vasofix, B. Braun, Melsungen AG, Melsungen, Germany) is inserted into the antecubital vein of their non-dominant arm; resting, fasted venous blood samples will be collected prior to experimental testing. Following the initial blood sample, patients will be given a standardised mixed-macronutrient breakfast-based meal which will be identical on each occasion. At 3.5-hours post-breakfast, participants will be provided with a standardised mixed-macronutrient lunch meal. Participants will remain in the laboratory under observation for a further 3.5-hours before being discharged home.

Trial sequencing will be randomly assigned with the use of a computerised random-number generator such that participants will perform two experimental conditions (Arm 1 = **Sit**; Arm 2 = **Sit-Less**); a schematic of the experimental design is provided in Figure 2.

Arm 1 –Sit (control): Following baseline measurements participants will sit in a lounge chair for 7-hours, with breakfast provided upon beginning the experimental trial (0-hours), and lunch at 3.5-hours (Figure 2).

Arm 2 - Sit-Less: As described above for Arm 1, with the exception of:
After 1-hour post-breakfast the participants will complete a 3-minute bout of light-intensity walking, then return to the seated position. This procedure will be completed on a further 5 occasions at 30-minute intervals up to (and including) 3.5-hours post-breakfast. At 3.5-hours, the lunch meal will be consumed. At 1-hour post-lunch, (4.5-hours post-breakfast), and every 30-minutes thereafter up to and including 6.5-hours post-breakfast, a 3-minute bout of light-intensity walking will be performed (equalling 11x3 minutes bouts of walking; i.e. 36 minutes of light-intensity activity across the 7-hour day) (Figure 2). During the two 1-hour periods immediately following breakfast and lunch, participants will remain in a seated rested position to limit the risk of hypoglycaemia during peak insulin absorption time before commencing the 3-minute walking break on 60-minutes.

Laboratory meals: Both meals will seek to replicate a typical westernised diet with an energy density of ~855kcal and a macronutrient profile of ~42% energy from carbohydrate, ~16% energy from protein, and ~42% energy from fat. The carbohydrate content of each meal will equate 1g.carbohydrate.kg.BM^{-1}. Each meal will contain ~40g of fat as this is typical of westernised dietary patterns and we have previously shown that meals with a similarly high fat content invoke an adverse response in postprandial glycaemic, lipaemic, and inflammatory parameters[68,69]. The breakfast meal will comprise of cereal with milk and a slice of toast with jam and the lunch meal will comprise of a sandwich. Patients will be asked to consume each meal within a 20-minute window. Meals will be preceded by an insulin bolus, calculated from each participant's usual insulin-to-carbohydrate ratio (with no correction dose), administered 10-minutes before eating, with those treated on MDI standardising insulin injection site. The timing and amount of water consumed with each meal, and ad libitum throughout the duration of the first visit will be recorded so that this can be replicated on subsequent visits.

Blood sampling: Periodic blood samples will be drawn at 60-minute intervals, for a total of 7-hours (commencing immediately prior to the consumption of the breakfast meal). At each time point, 10mL of venous whole blood will be taken and dispensed into Vacutainers before centrifugation at 3000 rev/min for 15-minutes at 4ºC. Plasma will be separated and stored at -80 degrees centigrade for retrospective analysis of insulin and triglycerides. At each time point, a panel of vascular inflammatory and thrombotic parameters, including plasma levels of C-reactive protein (CRP), complement C3, fibrinogen, plasminogen activator inhibitors (PAI)-1, interleukin 1b, will be assessed using standard ELISA techniques. CRP has been shown to predict future vascular events (ref), while C3 appears to have a role in both vascular inflammation and thrombosis potential, particularly in individuals with diabetes. Fibrinogen and PAI-1 levels can determine thrombosis risk in diabetes, and consequently predisposition to atherothrombotic disease. Moreover, recent work by the group has shown that lysis time of clots made from plasma samples is an independent predictor of cardiovascular mortality following coronary ischemia[72], and therefore we will study plasma clot formation and lysis using a validated turbidimetric assay[73-75]. Work from the group has shown that fibrin clot lysis is impaired in people with T1D[73,76], representing one potential mechanism for the increased risk of vascular complications in this population. We have also shown that lowering glucose levels improves the hypofibrinolytic environment in diabetes, provided hypoglycaemia is avoided. An additional 5mL of venous blood will be taken at rest on one arm of the study for C-peptide and routine blood tests (including HbA_{1c}, U&Es, LFTs, full lipid profile, urinary albumin creatinine ratio (unless these have been done within three months of enrolment). A total of 8 blood samples will be taken across the course of each study visit equating to a total of 85ml, which is approximately 1/5 of a full blood donation. The total volume of blood to be taken is safe, and, is similar to other studies that we have performed previously in people with T1D. Further, we have worked hard with our PPI representatives to minimise the participant burden of all study procedures; the volume and frequency of blood sampling has been deemed acceptable by our PPI representatives.

Post-laboratory observation period:
Following the last blood sample at 7-hours post-breakfast, participants will be discharged from the laboratory. Glycaemia will continue to be monitored under free-living conditions for a total of 48-hours post-intervention using CGM. As we are interested in the 48-hour glucose responses following our intervention (including the dawn phenomenon), participants will be given two standardised meals to consume as their evening meal and breakfast, and be required to replicate their dietary intake across trials

for the duration of the 48-hour post-intervention period, in order to control for the potentially confounding effects of diet on glycaemic control. This will provide the unique opportunity to investigate the legacy effects of interrupted sitting on glycaemic parameters, including the dawn phenomenon.

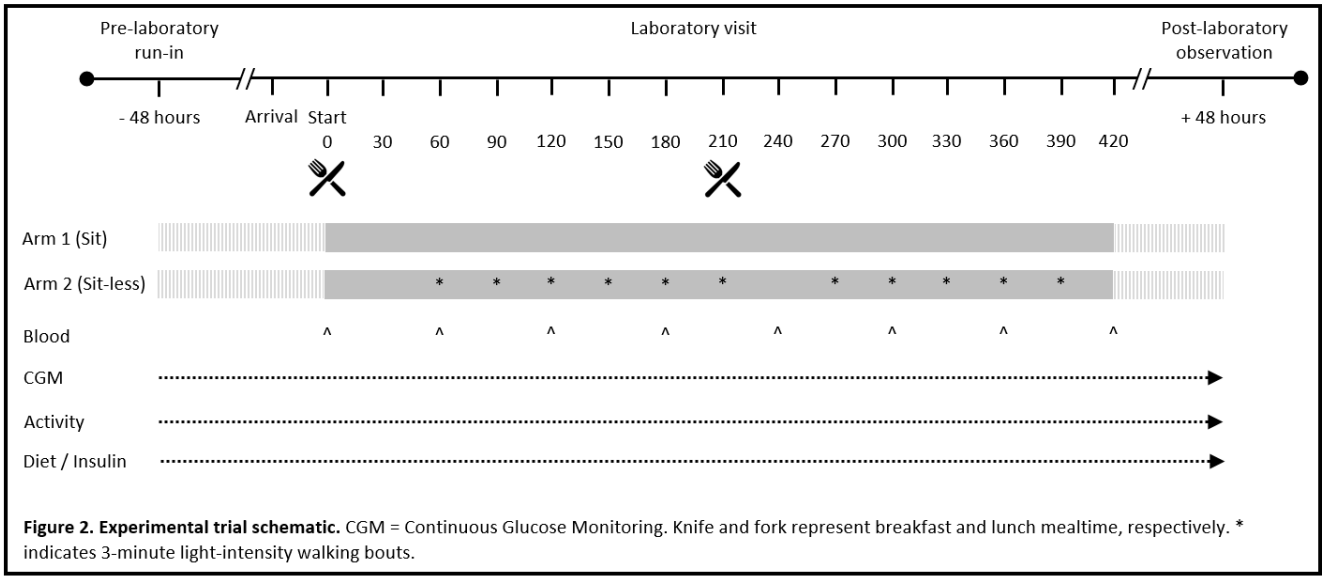

Figure 2. Experimental trial schematic. CGM = Continuous Glucose Monitoring. Knife and fork represent breakfast and lunch mealtime, respectively. * indicates 3-minute light-intensity walking bouts.

Data analysis:

Standard summary statistics of the CGM data for each condition will be calculated, including: mean CGM glucose, percentage of time spent within euglycaemia, hyperglycaemia, and hypoglycaemia; area under the curve (a measurement of participants' exposure to euglycaemia, hyperglycaemia, and hypoglycaemia levels over time) for all glucose measurements that exceed a threshold of 10 mmol/L or 6.9 mmol.L, and fall below thresholds of 3.9 mmol/L or 2.8 mmol/L; number of hyper- and hypoglycaemic excursions; glycaemic variability[75]. Functional data analysis will be performed as previously detailed to generate temporal profiles for each measurement period[61].

Online nutrition analysis software (MyFood 24, Dietary Assessment Ltd), and accelerometer software (activPAL^{3TM}, Activeinsights) will be used to determine the composition and nutritional content of pre-laboratory 24-hour dietary intakes and accelerometer data, respectively; as dietary intake and physical activity levels will be replicated across experimental visits and are not an experimental outcome, no formal statistical analysis will be undertaken.

Sample size:

Our sample size calculation is based on our preliminary data and primary outcome variable of mean 48-hour interstitial glucose assessed using FDA. Owing to the randomised crossover design of our study, participants will act as their own controls, which enhances both the internal validity and reliability of the data collected and permits a smaller sample size. To detect a significant difference in mean interstitial glucose of 1.6mmol/L with a SD of 1.5 (effect size 0.64) at P <0.05 with 95% power we will require a sample size 32. This is a clinically relevant effect size in people with diabetes; improving CGM glucose by this small, but clinically relevant margin in T1D has been shown to result a reduction of HbA1c by 1.0mmol/mol over the long-term[76]. We have shown greater improvements in interstitial glucose in people with T2D in response to interrupted sitting interventions[14,60,62]. This number of individuals is enough to detect a 26% difference in clot lysis time with a power of 90% given SD of the variable studied at 22%. This difference in lysis time reflects 18% lower risk of cardiovascular mortality in individuals with acute coronary syndrome, as we demonstrated in our recent study.

Vascular inflammatory, thrombotic and platelet responses: As these are exploratory outcomes with no previous data available on how activity breaks affect platelet biology in T1D it is difficult to provide a formal power calculation. Previously we have infused lipids into healthy subjects and then examined platelet function. In this study we found significant differences in platelet function pre- and post-lipid infusion using 20 healthy participants; as the biochemical abnormality is assumed to be higher in T1D individuals we are confident that our sample size of 32 will be adequate to detect a meaningful difference in our vascular inflammatory, thrombotic and platelet measures.