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Protocol

Breaking up sedentary time to improve glucose control in a population at risk for developing type 2 diabetes (BURST2D study): a randomized controlled trial

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ABSTRACT

Background: To compare the acute and chronic effects of frequent, short physical activity (PA) bouts spread throughout the day to a time-matched intervention consisting in a single continuous daily bout of PA on glucose control and potential underlying mechanisms in adults at risk of developing type 2 diabetes (T2D).
Methods: BURST2D is a single-center, parallel-group, randomized controlled trial, in which sedentary adults with overweight/obesity and pre-diabetes (18-45 y, BMI: 25-40 kg/m^2, fasting glucose: 100-125 mg/dL or 2h glucose: 140-199 mg/dL or HbA1c: 5.7-6.4%) will be randomly assigned to one of two 3-month PA interventions: BREAK, nine bouts of 5-min brisk walking performed every hour for nine consecutive hours (45-min/d total), 5 days/wk; ONE, one continuous 45-min bout of brisk walking, 5 days/wk. Primary outcomes will be daily glycemic mean and variability, fasting glucose and HbA1c, postprandial plasma glucose and insulin, glucose kinetics, and content of skeletal muscle proteins related to insulin signaling and glucose uptake. Secondary outcomes will be whole-body insulin sensitivity, 24-h total substrate oxidation, postprandial triglycerides, daily PA and sedentary behavior (SB) patterns, knowledge and attitude towards PA and SB, barriers and facilitators to intervention compliance, self-perceived appetite, mood, and sleep. Outcomes will be assessed at baseline and after one month and/or three months of intervention.
Conclusions: This study will establish the acute and chronic effects of breaking up SB, independent of increases in PA, on glucose control and underlying mechanisms in adults with pre-diabetes. Results will advance the science of T2D prevention.
Trial registration: This study is registered with the ClinicalTrials.gov, registry number NTC05041491.

Keywords: Sedentary behavior, Pre-diabetes, Obesity, Physical activity, Active breaks, Glucose kinetics, Stable isotope tracers, Skeletal muscle, Insulin sensitivity, Substrates use

INTRODUCTION

Approximately 88 million American adults have pre-diabetes.[1] Almost 1 in 5 will progress to type 2 diabetes (T2D) per year.[1] At this rate, it is estimated that 642 million people will have T2D by 2040.[2] There is a need to consider what strategies might be safe, acceptable, cost-effective, and clinically useful population-wide to improve glucose metabolism and reduce the risk for T2D. Meta-analyses suggest there is a 112% relative risk of T2D in those with high levels of sedentary behaviors (SB; any waking behavior characterized by a low energy expenditure (≤1.5 MET) while sitting, reclining or lying down), independent of time spent in physical activity (PA).[3,4] Importantly, the elevated relative risk of T2D in those with high SB is independent of time spent in moderate-to-vigorous PA (MVPA; >3 METs). This highlights the importance of reducing SB in addition to promoting participation in MVPA.Reducing total sedentary time with frequent, short active bouts seems to lower metabolic risk even in adults who regularly exercise.[5-7] For anyone with T2D, inclusion of taking more frequent breaks to SB is likely to bestow even greater glycemic benefits.[8,9] Significant improvements in postprandial glycemia and insulinemia have been observed following frequent, short (≤5 min) light-or moderate-intensity walking breaks to sitting, with MVPA being the most potent health-enhancing behavior.[5-14] These acute experimental studies showed that regular brief interruptions to SB can improve glucose control in healthy adults but to a greater extent in those with impaired glucose regulation.[15] However, it is still unclear whether benefits related to glucose control are similar or more pronounced following frequent breaks compared to a calorie-matched continuous PA bout. Recent data suggest that frequent 5-min bouts of PA spread throughout the day may be better for glycemic control than performing a single 45-min continuous bouts of PA, even when interventions are matched for total active time and total energy expenditure (TEE).[16,17] We found in sedentary adults with overweight/obesity that 4 days of frequent hourly 5-min brisk walking interruptions in prolonged sitting (45 min/d, BREAK) leads to greater reliance upon carbohydrates as fuel both after a meal and over 24h as compared to a sedentary control condition. In contrast, a single 45-min isoenergetic continuous bout of brisk walking (ONE) increased 24h fat oxidation despite a similar increase in TEE, as measured by whole room calorimetry.[16]

The greater use of carbohydrates during BREAK intervention may explain the reduced postprandial glycemia observed following active breaks.[5,10-12] The mechanistic underpinnings of the beneficial effects of breaking up SB are likely to be multifactorial, and involve peripheral organs that play a key role in the regulation of intermediary metabolism. Skeletal muscle is the largest glucose consuming organ in the body, accounting for more than 80% of the insulin-stimulated glucose disposal and is quantitatively the predominant tissue during exercise.[18] In adults with overweight/obesity favorable changes in skeletal muscle gene and protein expression were observed acute (5-h) and short-term (3-4 days) exposure to breaking up SB with short bouts of PA that likely contribute to improved cellular glucose uptake, insulin signaling cascade and oxidative capacity.[19,20] These changes include some which align with, and others which are distinct from, the known effects of continuous acute PA.[19-21]

The 2016 position statement on PA and diabetes from the American Diabetes Association, the 2018 U.S. PA guidelines and the American college of sports medicine PA recommendations for T2D include recommendations to reduce and interrupt prolonged sitting.[22-24] Most studies on the effects of reducing and interrupting prolonged SB on glycemic control have mostly been acute (<1-4 d) and conducted in controlled laboratory settings or derived from population-level evidence. Current gaps in knowledge include: establishing whether the acute metabolic benefits of interrupting SB are sustained or mitigated over time; establishing whether frequently interrupting prolonged sedentary periods is more beneficial than or like performing a time-matched single continuous bout of PA for glucose homeostasis; and better understanding the underlying mechanisms.

Objectives and hypothesis

Our objective is to compare the acute and chronic effects of frequent PA breaks vs a single continuous bout of PA on glucose control and potential mechanisms in adults at risk for T2D. Our aim was to compare the acute and 1-month effect of breaking up SB (BREAK) vs. a time-matched continuous PA bout (ONE) on; daily glycemia and insulin sensitivity, glucose fluxes, and skeletal muscle insulin-dependent and independent oxidative and non-oxidative glucose disposal pathways. Our hypothesis is that both acute and one-month exposure to BREAK will lower postprandial glycemia and insulinemia in response to standard meals, lower daily glycemic variability and improve insulin sensitivity to a greater extent than ONE.

These changes will be associated with greater increases in both endogenous and exogenous carbohydrate uptake and oxidation following BREAK than ONE. Finally, both BREAK and ONE will enhance the amount and phosphorylation of proteins involved in insulin-dependent glucose uptake and non-oxidative glucose pathways in skeletal muscle, but insulin-independent pathway and glucose transport signaling proteins and phosphorylation will be greater after BREAK than ONE. We will also pursue two exploratory aims. We will compare the 3-month effects of BREAK vs ONE on daily patterns of SB and PA, glucose control, insulin sensitivity, lipid profile, body composition, waist circumference, appetite, vigor, fatigue, mood, and sleep. We will further assess the changes in knowledge and attitude towards SB and PA, the barriers, challenges, and facilitators following one month and three months of BREAK or ONE.

METHODS

Trial design

BURST2D is a single-center, parallel-group, randomized controlled trial. Eligible participants will be randomly assigned into one of the two arms: BREAK or ONE. Primary and secondary outcome measures will take place at baseline and after one month of intervention. Exploratory outcome measures will take place after one month and three months of interventions. Overall study design is illustrated in (Figure 1).

Study setting

The study will be conducted at the University of Colorado Denver Anschutz medical campus (CU-AMC) and all study visits (except Visit A) will occur at the University of Colorado hospital clinical and translational research center (CTRC). The current protocol is approved by the Colorado multiple institutional review board (COMIRB) of the CU-AMC (identifier 20-1900). This study is registered at ClinicalTrials.gov (identifier NTC05041491).

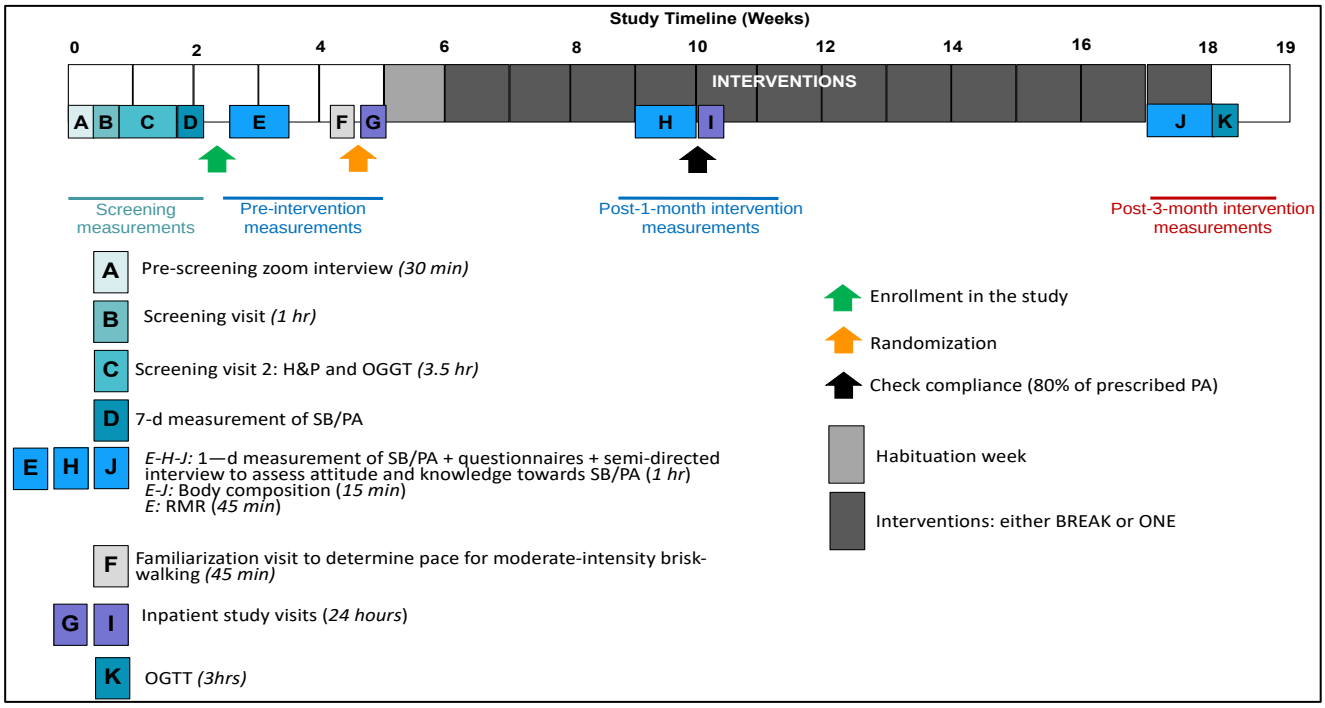

Figure 1: Study timeline.

Eligibility criteria

The trial inclusion and exclusion criteria are described in (Table 1).

Recruitment

The recruitment strategies will include direct mailing, e-mail newsletters, newspapers advertisement, study pamphlets, social media websites, university e-newsletters, and participation in health fairs and walk-in clinics. If needed, other recruitment strategies may be explored.

Consent

A detailed description of the screening visits is provided in (Figure 1). Participants will complete a pre-screening questionnaire. If they meet study eligibility criteria, the informed consent and risk-benefits of the study will be discussed via a private Zoom meeting (Visit A). A fasting blood sample will be drawn for measurement of a comprehensive metabolic panel, lipid profiles, complete blood count, and fasting glucose (Visit B). Eligible participants will be invited to attend a second screening visit that will consist in a history and physical examination (H&P) and a 75g standard oral glucose tolerance test (OGTT). Plasma HbA1c will be measured in fasting state and blood glucose at 0, 30, 60, 90 and 120min post glucose load.[25] Participants will be asked to wear a pedometer on their waist and maintain their habitual daily steps for 5 days including at least one weekend day. They will also be asked to perform the ONE intervention on one day and the BREAK intervention on another day to make sure they understand what the interventions are about. Their habitual SB/PA will be assessed by filling out the short version of the International Physical Activity Questionnaire. Eligible participants will be invited to participate in the study.

Interventions

Participants will be randomly assigned using a randomization table (1:1 ratio) for each sex to one of the two following 3-month interventions: BREAK: Participants will be asked to break up their prolonged sitting in daily living with 5-min of brisk walking performed every hour for nine consecutive hours, 5 d/wk. ONE: Participants will be asked to perform 45 consecutive minutes of brisk walking, 5 d/wk. The timing and days the intervention will be performed will be of their choosing. Pace will be determined at the familiarization visit (Visit F).

Table 1: Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Male and female	Pregnancy or breast-feeding for women based on serum pregnancy test for females.
Overweight and obese BMI of 25-40 kg/m^2 and weight stable (i.e., a weight change of <3% of body weight over the previous 6 months).	Being considered unsafe to participate as determined by the study physician.
Age, 18-64 years old.	Ever having a history of systemic, psychiatric, neurological disease, or drug and alcohol abuse.
Fasting glucose of 100-125 mg/dl or fasting HbA1c of 5.7-6.4%, or 2h OGTT blood glucose of 140-199mg/dl based on the American diabetes association criteria for pre-diabetes.	History of cardiovascular disease, diabetes, uncontrolled hypertension, untreated thyroid, renal, hepatic diseases, dyslipidemia, or any other medical condition affecting weight or lipid metabolism.
More than 6 hours/day of sitting time and less than 150 min/week of MVPA, as self-reported by the volunteers using the IPAQ, or less than 6500 of steps per day as measured by a pedometer over 5 days, including at least 1 weekend day.	Being positive for human immunodeficiency virus or hepatitis B or C.
Passing medical and physical screening, and analysis of blood and urine pregnancy screening test.	Taking medications affecting weight, triglycerides, energy intake/energy expenditure, or sleep in the last 3 months.
Low-moderate caffeine use (<3 cups/day).	Having abnormal blood chemistry and/or hematology as deemed significant by the study physician.
Agree to refrain from alcohol for 72 h before and during the inpatient CTRC visits.	Being a smoker or having been a smoker in the 3 months prior to their screening visit.
Agree to maintain their habitual PA and not start any new structured exercise and or PA.	Having donated over 400 mL of blood within 3 months (90 days) of screening for the study.
Agree to eat control diets for 3 days before and during the CTRC visits.	Working night shifts or traveling across more than 2 time zones within 1 month of and throughout the study.
Agree to refrain from taking any over the counter (including nonsteroidal anti-inflammatory drugs) or prescribed medication (apart from oral contraceptives) for 3 days prior to the inpatient CTRC visits.	Not completing the trial days of BREAK and ONE during the screening period to assess the willingness and ability of the participant to perform each of the interventions.
Agree to wear a Fitbit activity monitor and upload data on the website daily for the whole duration of the study.	Not being fully vaccinated against COVID-19 based on CDC definition.
Agree to follow the PA interventions and to be randomly assigned to one of the two arms of the study.	
Agree to complete all the study procedures.	

BMI- Body Mass Index; BREAK- breaking up prolonged sitting in daily living with 5-min of brisk walking performed every hour for nine consecutive hours, 5 d/wk; COVID- Coronavirus disease; CDC- Center for Disease Control and Prevention; CTRC- Clinical and Translational Research Centers; PA- Physical Activity; OGTT- Oral Glucose Tolerance Test; ONE- performance of 45 consecutive minutes of brisk walking, 5 d/wk.

Familiarization visit

Participants will perform a sub-maximal exercise test on a treadmill to determine a walking pace corresponding to moderate intensity. The walking test will start at a pace of 2.0 mph and the pace will increase by 0.3 mph every minute. At each level, participants will report their rate of perceived exertion (RPE) on a Borg scale from 6 (very light) to 20 (maximal exertion). The aim will be to identify the speed associated with a RPE of 13 (somewhat hard). The walking test will stop when the participant rates the speed of the treadmill with RPE of 15 (hard). Volunteers will be asked to perform a moderate-intensity bout during the inpatient study visits

while in the whole-room calorimeter. For safety reasons and following hospital policies, a stepping equivalent (beats per min-bpm) to the treadmill will be performed inside the chamber.

A stepping test will therefore be performed during Visit F to identify the stepping pace equivalent to the walking speed. The stepping test will be conducted following the same protocol of the walking test, excepted by starting at a pace of 55-70 bpm and the pace will increase by 5 bpm every minute. In both tests heart rate and breath-to-breath gas exchange will be measured by using a heart rate monitor placed on the chest (polar, polar electro, US) and indirect calorimetry (Parvo Medics True One 2400, Salt Lake City), respectively. Rate of oxygen consumption (kcal/min) and heart rate will be used to match walking speed and stepping pace.

Strategies to improve adherence to interventions

Participants will perform one week of habituation, to incorporate to their routine and troubleshoot any eventual problems that may raise from starting the new intervention. To verify compliance with PA interventions, each subject will be equipped with a FitBit Inspire 2 (San Francisco, CA, US) and a personal online account. The study team will follow-up with participants on a weekly basis with phone or Zoom call, check Fitbit online accounts and send regular motivational text messages to ensure adherence to the intervention protocol throughout the study. Monetary compensation incentives according to the number of visits and compliance to intervention will be used to ensure adherence to their new lifestyle and study procedures. At the end of the study, we will provide to each subject an individual subject report containing results from study procedures.

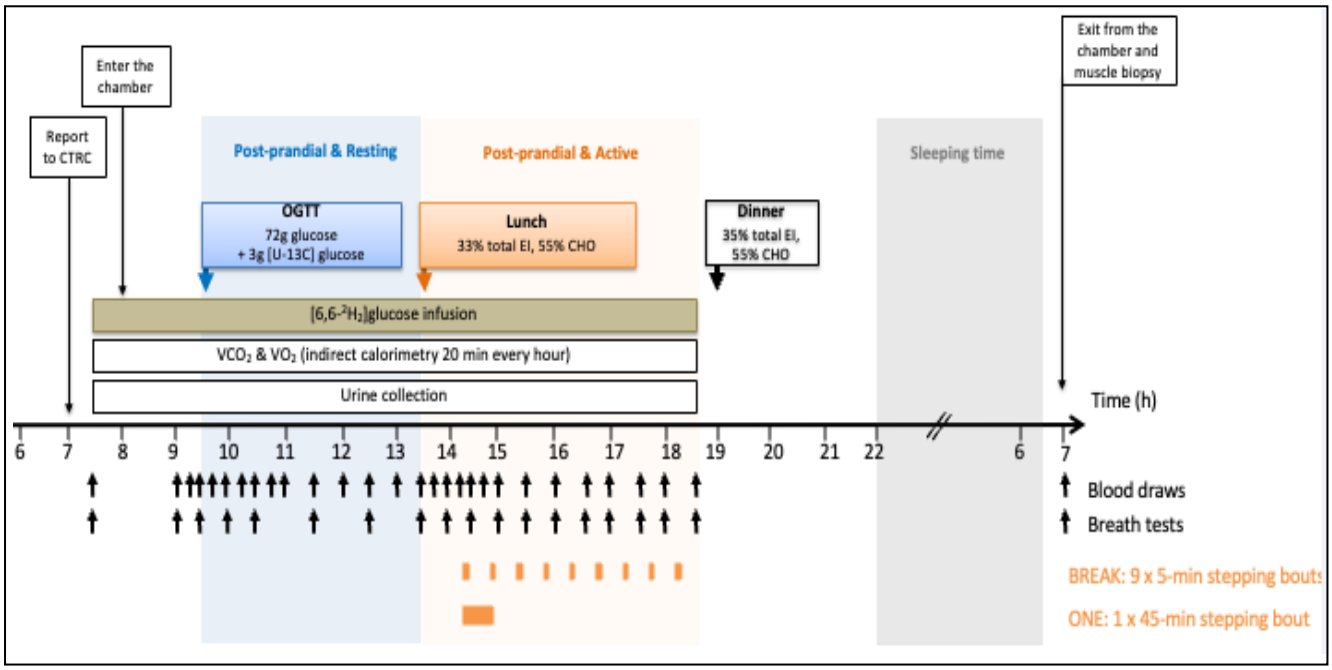

Figure 2: Overview of the whole-room calorimeter inpatient visits (visits G and I) protocol.

Compliance

A participant will be considered compliant if more than 80% of the instructed PA is achieved based on the FitBit data. This will correspond to ≥7 PA bouts/d, ≥36 min/d of structured PA, ≥4 d/wk in the BREAK arm and to ≥36 min/day of structured PA, ≥4 d/wk in the ONE arm.

Outcomes

Daily pattern of time spent sedentary and physically active: before and after 1- and 3-month of intervention, participants will be fitted with two accelerometers for 10 days to assess daily patterns of SB/PA (activPAL; PALTechnologies: Glasgow, Scotland; ActiGraph GT3X+, LLC, Fort Walton Beach, FL). They will be asked to fill out a dairy log to record sleeping and waking times and any period during which they removed the devices for >15 min. SB/PA data will be analysed as previously published.[26] Daily glycemia: Over the same 10 days, participants will be equipped with a continuous glucose monitor (CGM, FreeStyle LibrePro, Abbott) to assess mean glucose levels and daily glucose oscillations before and after one and three months of intervention. Body weight, body composition and waist circumference: before, and after 1- and 3-month of intervention, body weight will be measured in undergarments using a standard calibrated scale. At baseline and after three months of intervention, total, lean, and fat mass will be measured by DXA (Hologic Delphi W, Bedford, MA, software version 11.2) as previously described.[27] Waist circumference will be measured at the smallest circumference between the lowest margin of the ribs and the upper margin of the iliac crest. 24h substrates use,

glucose kinetics and whole-body insulin sensitivity: run-in period prior to the inpatient visits; to reduce inter-individual variability and the impact of unprescribed PA and diet, a 3-day run-in period will precede the inpatient visits.

At baseline, participants will be instructed to maintain their sedentary lifestyle before the inpatient visit. In post intervention, they will be asked to perform their prescribed PA until the day prior to the inpatient visit. During this period, participants will be provided with a personalized calorie-matched standardized diet. To estimate the daily energy content of the diet, we will calculate the average kcal from: free-living energy requirements as 24h energy intake (EI): resting metabolic rate (RMR) x an activity factor and estimated RMR: ((23.9 x FFM in kg) + 372) x an activity factor. RMR will be measured on Visit E in fasting state by indirect calorimetry (ParvoMedics TrueOne 2400, Salt Lake City), as done previously.[16,28,29] The macronutrient composition of the diet will be: 55% CHO, 30% fat, and 15% protein (percent daily EI: 30% breakfast, 35% lunch, 35% dinner and the remaining energy of the total calories will be a snack). Participants will be instructed to consume only the meals prepared and provided by the study, and nothing else but water. They will be asked to bring any leftover.

Inpatient visits

The protocol of the inpatient study visits (Visits G and I) is summarized in Figure 2. Participants will be asked to report to the CTRC at 0630h. They will be asked to void. An IV will be placed on one arm for glucose tracer infusion and another one on the hand of the other arm for serial blood draws. A baseline blood sample will be taken for metabolic substrates, hormones, and isotopic background. A primed (13.5 μmol/kg) infusion (0.35 μmol/kg/min) of (6,6-$^{2}H_2$) glucose (99%, Cambridge Isotope Laboratories-CIL, MA) will begin at 0700-0730h and continue until 1835h. Participants will enter a whole-room calorimeter at 0800h for measurement of 24h energy expenditure and nutrient oxidation.[31]

At 0930h, an OGTT will be performed by giving participants 72g of glucose + 3 g of [U-^{13}C]-glucose (99%, Cambridge Isotope Laboratories-CIL, MA). Blood glucose and insulin will be measured. Matsuda index, HOMA-IR and HOMA-B will be calculated as surrogates of insulin sensitivity, insulin resistance, and beta-cell function.[25,30,32] Participants will be asked to remain sedentary except to void; this will be the resting period of the test. At 1335h, they will receive a standard lunch (55% CHO, 30% fat and 15% protein, 35% of estimated energy needs). Then, participants in the BREAK group will complete 9 bouts of 5-min moderate intensity stepping every 30 minutes from 1410h to 1815h and participants in the ONE group will perform a 45-min moderate intensity stepping from 1410h to 1455h. For everyone, the stepping pace will correspond to the pace identified during the familiarization visit and will be imposed by a metronome. Finally, participants will be offered a standardized dinner at 1900h and a snack at 2100 h. Bedtime will be at 2200 h. Participants will be awakened at 0630 h and exit the room at 0700h. Vastus lateralis skeletal muscle biopsy will be collected from participants by the Bergstrom technique in fasting state upon exiting the chamber and immediately frozen in liquid N_2 and stored for protein analyses.[33] During the inpatient visit, serial blood sampling (Figure 2) will be performed for measurements of metabolites, hormones, and glucose kinetics. Participants will collect their own breath at 15-60 min intervals sampling for $^{13}CO_2$ by blowing through a straw into two 15ml Vacutainer. Finally, urine will be collected from 0730h to 0730h the following day. Of note, only compliant participants will be asked to complete visit I.

Using collected data and samples, the following analysis will be done:

Total substrate oxidation

Energy expenditure and substrate oxidation will be calculated from O_2 consumption, RER, and urinary nitrogen excretion as previously published.[31] Metabolites and hormones: Blood samples will be used for the analysis of insulin, FFA, and triglycerides, all data will be analyzed by the CTRC core lab using standard methods. Fasting and postprandial glycemia and insulinemia: Areas under the curve (AUC) post OGTT (from 0930h to 1330h), post lunch (from 1335h to 1835h) and for the whole study visit period (from 0800h to day+1 0700h) will be calculated.

Exogenous glucose oxidation

Breath CO_2 will be sampled directly from the Vacutainer with a syringe, and $^{13}CO_2$ /$^{12}CO_2$ measured with isotope-ratio mass spectrometer (IRMS, Delta V, Thermo Electron, Bremen, Germany). The results will be calculated as previously published.[34] Isotopic glucose measurement: the absolute concentration of both unlabeled and labelled glucose will be measured in plasma samples by gas chromatography/mass spectrometry (GCMS, model 6890 series II and mass spectrometry model 5973A; Hewlett-Packard) through a dual acquisition program in single ion monitoring m/z ratios of 331 (unlabeled glucose) with either 333 for (6,6-$^{2}H_2$) glucose or 337 for (U-^{13}C) glucose, and calculated by reference to internal standards added to the plasma.

The concentration of (6,6-$^{2}H_2$) glucose and (U-^{13}C) glucose will be calculated by multiplying the respective molar percent enrichment by the concentration of total glucose, as done previously.[35] Glucose from the meals will be obtained by acid hydrolysis and be quantified by GC/MS after derivatization in glucose pentaacetate. This glucose derivative will then be analyzed by GC/C/IRMS for ^{13}C enrichment.

Calculations

Steele's equation for non-steady-state will be used to compute RaT and RaE, as well as the rates of disappearance (RdT and RdE) from the percentage of (6,6-$^{2}H_{2}$) glucose[36] and of ^{13}C-glucose in plasma glucose.[37] Endogenous glucose production will be computed as RaT-RaE. Nonoxidative glucose disposal will be calculated by subtracting total carbohydrate oxidation from (RdT+RdE). Plasma glucose utilization will be assumed to be equivalent to RdT as has been confirmed previously.[38] Muscle glycogen utilization during the active period will be calculated as total carbohydrate utilization during exercise minus plasma glucose utilization during PA.

Expression of proteins involved in the regulation of insulin action and glucose metabolism

Before and after 1-month of intervention muscle samples will be used to measure insulin signaling proteins associated with insulin sensitivity and glucose uptake using Simple Western Jess: Serine phosphorylation of insulin receptor substrate (IRS)1 (Ser312), total protein content of IRS1, phosphorylation of protein kinase B (Akt) (Aktser473/total), and phosphorylation of glycogen synthase kinase (GSK)3ß (GSK3ß Ser9/total). We will also measure phosphorylation of ACC (ACCS79/total) as an index of changes in AMPK and of changes in contraction-mediated glucose uptake regulation membrane will be assessed by changes in phosphorylation of TBC1D4 (AS160/ total) that is regulated by both insulin-dependent and insulin-independent pathways. Content proteins involved in mitochondrial activities/density (OXPHOS, COX4) and central regulator of energy fuel (PPARg, PGC1a) will also be measured.

Attitudes and knowledge towards PA/SB, self-perceived mood, appetite, and sleep

Before, after 1- and 3-month of intervention, participants will be asked to complete questionnaires in REDCap (Research Electronic Data Capture), a secure, HIPAA-compliant web-based application designed for data collection for research studies. During the three days of run-in diet, participants will be asked to fill in visual analog scales to rate their hunger and appetite before and after each meal. Vigor, fatigue, and mood will be assessed using the profile of mood states.[26,39] Sleep duration and quality will be assessed using the Pittsburgh sleep quality index questionnaire, and daytime sleepiness will be assessed using the Epworth sleepiness questionnaire and the Karolinska sleepiness questionnaire.[40,41] Participants will be asked to complete seven questionnaires related to SB/PA. The Hu Self-Efficacy in Walking Questionnaire will assess self-confidence of the ability to walk increasing periods at a moderately fast pace.[42] The Behavioral Regulation in Walking Questionnaire will assess motivation for walking.[43] The low-level physical activity recall questionnaire will measure time spent in work activities, household/yard activities, and recreational/ leisure activities in the past week.[44] Participants will also complete a questionnaire designed by KM, MOW and AB to assess confidence/self-efficacy to reduce SB (confidence to reduce sedentary behavior questionnaire). The social environmental support survey will be used to understand social and environmental support for engaging in healthy behaviors.[45] The interpersonal support evaluation list-12 assesses perceived social support.[46] Finally, the Meaning in Life questionnaire has two subscales that assess participants' sense of presence of life meaning/purpose and search for meaning/purpose.[47] Participants will participate in a semi-structured interview to assess knowledge and attitudes towards SB/PA and potential moderators.

Qualitative data analysis

Atlas.ti (scientific software development GmbH, Berlin, Germany) will be used to facilitate qualitative analysis. All interviews will be transcribed verbatim. Content analysis will be used to analyze the data.[48] We will read the interviews transcripts individually and carefully to identify categories that appear to represent key concepts or ideas. Once all interviews have been read at least three times, we will begin to identify patterns or similarities across the interviews. We will then return to the text of the interviews and documents and use Atlas.ti to code representative sections of text. This will allow for identification of the frequency of specific codes, and identification of exemplars that will be used in reporting study findings. Each decision made throughout data analysis, along with the rationale, will be recorded. The categories, coding, and themes identified will be reviewed with the study investigators to determine if there is agreement. Finally, we will compare themes between BREAK and ONE.

Sample size

Effect sizes for aims 1 and 2 were estimated based on our preliminary study with similar design, in sedentary adults with overweight/obesity (13 ONE and 11 BREAK participants who reached >80% compliance). The effect sizes (ES) were 0.87 and 0.83 common SD of the individual change score for postprandial glycemia and 24h exogenous carbohydrate oxidation, respectively. We conservatively elected to power the study on detecting a smaller but clinically meaningful effect size of 0.80. Twenty-six participants per treatment arm is required to ensure 80% power at 5% significance for linear mixed effect model (LMM) analysis. An attrition rate of 21% (N=7) of participants per arm will be assumed; this is more than twice higher than the rate observed in our pilot study.

Thirty-three participants (50% male) per arm (total N=66) will be enrolled. Changes in the total amount of

TBC1D4 protein will be the primary outcome for aim 3. ES was estimated based on published data from a cross-over study during which adults with overweight/obesity were asked to either perform moderate-intensity active breaks for three days or remain sedentary for three days; a skeletal muscle biopsy was collected at the end of the study.[19] ES was 1.46 common SD of the individual change score for total TBC1D4. Eleven participants per treatment arm (total N=22) is required to ensure 80% power at 5% significance for LMM analysis. With 19 participants per treatment arm (total N=38), we will reach a power of 95% at 5% significance with LMM analysis.

Statistical analysis

Participant demographics, anthropometric data, and clinical data will be summarized using descriptive statistics by study group. The primary analysis will be conducted on participants who will have successfully complied with the interventions and therefore completed the post-intervention inpatient study visit (Visit I). The between-treatment difference in the primary outcomes is the change from baseline to the end of the study (1-month and/or 3-month). It will be used to assess the effect of BREAK intervention as compared to ONE treatment. A LMM with appropriate covariance will be used to model baseline and end of treatment (EoT) outcome. This LMM consists of treatment (ONE or BREAK), time (Pre vs. Post 1-month and/or 3-month) and their interaction term as fixed effects, time as repeated factor, and participants as random factor. Between-group difference in post-intervention change in outcome will be assessed using contrast under the LMM model. If there is imbalance between two study arms with respect to potential confounding variables such as sex, age, body composition, adherence rate, time spent active and/or sedentary, baseline HAb1c, 2-h blood glucose and insulin sensitivity, or others, they will be adjusted in the LMM model as appropriate. A $p<0.05$ will be considered significant for the main effects and a $p<0.10$ will be considered significant for the interaction. No adjustment for multiple comparisons will be applied.

Data collection and management

All participants will be de-identified and data will be collected, managed, and stored using REDcap. Other digital data will be stored on a CU-AMC owned encrypted electronic driver. All the hard copies will be stored in a locked cabinet in a locked office where only study team members have access. The data collected in the study will be double-entered and/or double checked. Data records will be maintained for at least 7 years after the publication of results and most destroy after.

Oversight and monitoring

The inpatient CTRC staff will be responsible for monitoring for problems and reporting them to the PI immediately. The corresponding amendments to the protocol will be submitted to the COMIRB and other appropriate parties involved in the study. We will not implement any modifications to the study until the corresponding ethical party approves these unless the amendments prevent an immediate hazard to participants. In that case, we will proceed to protect participants and immediately report to the corresponding parties. Data and Safety Monitoring Board composed by CU-AMC physicians and researchers will oversee the clinical trial.

Dissemination plan

Results from this study will be disseminate via publications in peer-reviewed journals, as well as in abstracts and poster presentations at national and international conferences.

DISCUSSION

This study will evaluate the acute and chronic effects of implementing breaks in SB on glycemic control in participants at risk for T2D and begin to inform interventions to reduce the risk of developing T2D. The strengths of this study include the randomized controlled study design; the use of objective measures of daily SB and PA; the comprehensive clinical, physiological, and metabolic assessments using robust techniques (e.g., double-tracer approach); and the attempt of revealing new mechanisms underlying the potential differential effects of BREAK and ONE. However, the present project has some potential pitfalls that we identified, including a potential high withdrawal rate; difficulty to increase daily life PA; and recruiting participants representing the U.S. population. To circumvent these potential issues and to maximize retention and adherence; we will ask participants to complete only one arm of the study, either the BREAK or ONE; we will work with participants to ensure they understand all study procedures and aim of the study, as the more engaged the participants are the ones that are more motivated to complete the study; we will follow up with participants regularly and reschedule any missed study appointments; we have proposed a week of habituation to the interventions, frequent contacts, flexibility in the timing of performing the bouts of PA, and incentives to help participants comply with the instructed PA; we will also gather information on the barriers, challenges and facilitators to the intervention for planning future longer term interventions. Finally, we will do our best to have as many males as females and we will also target more diverse communities.

The importance of understanding the physiological and molecular mechanisms underlying the effect of breaking up SB on glucose control is significant given the current epidemic of T2D and the prevalence of cardiometabolic diseases related to the clinical problem of diabetes.[49] Physical inactivity and SB are major risk factors for the development of T2D, and obesity-related comorbidities in the general population.[4,50] It represents the fourth

cause for mortality, with an estimated three million preventable deaths annually across the world (~7%).[51] PA is a cornerstone in T2D prevention and management.

In addition to aerobic and resistance exercises, the PA guidelines now recommend that adults (with or without T2D) reduce and interrupt long periods of sitting.[22-24] An important gap is that these consensus-based guidelines lack mechanistic support. In this context, the proposed study has strong scientific, clinical, public health and economic relevance.

CONCLUSION

This study will establish the acute and chronic effects of breaking up SB, independent of increases in PA, on glucose control and underlying mechanisms in adults with pre-diabetes. Results will advance the science of T2D prevention.

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