Acute Effects of Interrupting Prolonged Sitting on Vascular Function in Type 2 Diabetes

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ABSTRACT

In healthy and overweight/obese adults, interrupting prolonged sitting with activity bouts mitigates impairment in vascular function. However, it is unknown whether these benefits extend to those with type 2 diabetes (T2D); nor, whether an optimal frequency of activity interruptions exist. We examined the acute effects on vascular function in T2D of interrupting prolonged sitting with simple resistance activities (SRA) at different frequencies. In a randomized crossover trial, 24 adults with T2D (35-70 years) completed three 7-hour conditions: 1) uninterrupted sitting (SIT); 2) sitting with 3 minute bouts of SRA every 30 min (SRA3); and, 3) sitting with 6 minute bouts of SRA every 60 min (SRA6). Femoral artery flow-mediated dilation (FMD), resting shear rate, blood flow and endothelin-1 were measured at 0h, 1h, 3.5h, 4.5h, and 6.5-7h. Mean femoral artery FMD over 7 hours was significantly higher in SRA3 (4.1 ± 0.3%) compared to SIT (3.7 ± 0.3%, $p = 0.04$), but not in SRA6. Mean resting femoral shear rate over 7 hours was increased significantly for SRA3 (45.3±4.1/s, $p<0.001$) and SRA6 (46.2±4.1/s, $p<0.001$) relative to SIT (33.1±4.1/s). Endothelin-1 concentrations were not statistically different between conditions. Interrupting sitting with activity breaks every 30 minutes, but not 60 minutes, significantly increased mean femoral artery FMD over 7 hours, relative to SIT. Our findings suggest that more-frequent and shorter breaks may be more beneficial than longer, less-frequent breaks for vascular health in those with T2D.

KEY WORDS

Arteries; blood flow, sedentary behavior

54 **NEW AND NOTEWORTHY**

55 This is the first trial to examine both the effects of interrupting prolonged sitting on vascular
56 function in T2D, but also the effects of the frequency and duration of interruptions. Brief
57 simple resistance activity bouts every 30 minutes, but not every 60 minutes, increased mean
58 femoral artery FMD over 7 hours, relative to SIT. With further supporting evidence, these
59 initial findings can have important implications for cardiovascular health in T2D.

60

61

INTRODUCTION

Those who are living with type 2 diabetes (T2D) are disproportionately affected by cardiovascular disease (CVD), with two-fold increased risk of CVD mortality compared to those without T2D (19, 46). This is largely attributable to atherosclerotic complications, including increased risk of myocardial infarction, stroke and microvascular diseases (3). The importance of the endothelium in maintaining healthy vascular function is now well accepted (7, 21). Vascular impairment is recognized as an important early event in the progression of CVD, preceding obesity and diabetes (17, 27). Measurement of vascular function, via endothelium-dependent vasodilation, is a widely used prognostic marker for the progression of CVD risk (50). In T2D, vascular reactivity is usually, but not invariably, reduced (56). Consequently, interventions that improve endothelial vasodilator function can provide antiatherogenic benefit and may be considered an integral tool of diabetic management (29, 32, 43).

Lifestyle modification, including increasing physical activity (PA), is considered a cornerstone for the prevention and management of T2D. Despite the known cardiometabolic benefits of PA(57), meeting recommended levels (at least 150 minutes of moderate to vigorous activity weekly) continues to be challenging in T2D, with numerous barriers to exercise reported (10, 60). Further, in the social and economic context of rapidly advancing technologies in workplaces, transportation and home entertainment, fewer opportunities exist for incidental activity, creating many contexts of daily life that are conducive to prolonged sitting. Sedentary behaviors, defined as seated posture with low energy expenditure $\leq$1.5 METS, are now recognized as being strongly associated with all-cause and CVD-related mortality (23, 54). In particular, the deleterious consequences of prolonged periods of time spent sitting have been highlighted, with acute experimental studies reporting that prolonged

uninterrupted sitting exacerbates postprandial cardiometabolic risk biomarkers (13), and may decrease vasodilatory function (9, 35, 53) via reduced bioavailability of vasodilators (i.e. nitric oxide), and increased production of vasoconstrictors (i.e. endothelin-1 [ET-1]) (35). Elevated ET-1 may be a marker of microvascular complications in those with T2D (6), and evidence suggests endothelin receptor blockade reduces blood pressure and protects against renal events in patients with T2D (20, 34). Given that those with T2D report high basal ET-1 levels, are at increased risk of microvascular and macrovascular complications (19, 46), and that they report high levels of time spent sedentary and low levels of participation in PA (55, 60), further research is needed to find practical strategies that may contribute to reducing CVD risk.

Recent experimental evidence shows that reducing and interrupting prolonged sitting time with brief bouts of light intensity activity can negate the adverse effects of prolonged sitting on lower limb vascular function in healthy and overweight/obese adults (9, 39, 51). However, it is unknown whether interrupting sitting time can positively influence vascular function in those with T2D. Further, while several studies (5, 8, 33, 47) have reported the effects of interrupting sitting using different break frequencies and intensities, none have directly compared two different activity protocols with equivalent activity duration in those with T2D. This type of evidence is required to inform larger trials and to produce more specific public health guidelines around the optimal timing and duration of interruptions in sitting.

We examined the effects of interrupting prolonged sitting for (i) 3 min every 30 min; and (ii) 6 min every 60 min with simple resistance activities (SRA), on vascular function in those with T2D. As an exploratory outcome, we also examined the effect of interrupting prolonged sitting on plasma ET-1 levels, as a marker of vasoconstriction, in the same population. We

hypothesized that regular interruptions involving SRA would acutely improve vascular function relative to uninterrupted sitting, and that breaking up sitting either every 30 or 60 minutes (with equivalent total activity duration) would be efficacious in improving vascular function compared to prolonged sitting *per se*. Further, we anticipated that regularly interrupting sitting every 30 or 60 minutes would decrease plasma ET-1 compared to prolonged sitting.

METHODS

Participants

Twenty-four men and women (BMI, 25–40 kg/m^2) aged 35-70 years with T2D (1-3 hypoglycemic medications, ≥ 3 months' duration [based on the American Diabetes Association diagnostic criteria](1)) were recruited from local community advertisements, social media and the Baker Heart and Diabetes Institute (ACTRN12617000392369). To be eligible, participants were required to be inactive (currently sitting for ≥ 5h/day and not meeting PA guidelines of ≥150 min/week of moderate-intensity exercise or high-intensity exercise ≥ 75 min/week for >3 months). Exclusion criteria included HbA1c<6.5% or >10%; current use, or use within the last three months, of insulin medication/s; current smoker; pregnancy; or major acute or chronic illness that may limit their ability to perform SRA. Based on previously published work a sample size of 24 individuals (allowing for 15% attrition) would provide >90% power, assuming two-tailed alpha=0.05 (G*Power v3.1.2) and a standard deviation of 1% between individuals, to detect a change in FMD of 1% between the intervention (interrupting sitting with activity breaks) and control (prolonged sitting) conditions (59). For longitudinal observational studies across heterogeneous populations, a

1% difference in FMD is associated with a clinically meaningful ~7-13% difference in cardiovascular events (17, 22).

Study Overview and Randomization

This three-arm randomized crossover trial took place at the Baker Heart and Diabetes Institute between July 2017 and April 2019. The study was approved by the Alfred Human Research Ethics Committee (50-17). Volunteers were initially screened via a telephone questionnaire to determine their eligibility, during which they were asked to verbally confirm medical diagnosis of T2D for ≥ 3 months, physical activity time of < 150 minutes a week and sitting time of >5 hours a day. Eligible participants provided written informed consent and attended the laboratory on four separate occasions: medical screening/familiarization, and three trial condition visits in a randomized order: 1) prolonged, uninterrupted sitting (SIT); 2) 3-min simple resistance activities every 30 minutes (SRA3); and 3) 6-min simple resistance activities every 60 minutes (SRA6).

The medical screening occurred 3-6 days prior to the first visit and included; HbA1c and anthropometric measurements, resting blood pressure, resting 12-lead electrocardiogram, and a physical examination performed by the study physician (NDC). Participants also provided information about medical history and current medications, and were familiarized with the SRA. Additionally they were familiarized with the study procedure including weighed food diaries and activity records, activity monitors, cuff occlusion and requirements for the restrictive lead-in phase and fasting prior to each trial condition.

158 Experimental condition was randomly assigned by an independent third-party using computer
159 generated random numbers and sealed in envelopes (balanced block randomization).
160 Participants were blinded to the condition order until the start of the second visit.
161
162 **Study Protocol**
163 *Experimental conditions*
164 Figure 1 shows the overall experimental protocol. A 6-day washout period between
165 conditions was used to address any potential carry over effects. Each participant completed
166 three 8-hour experimental conditions, including an initial one-hour steady state period. To
167 mimic a free-living setting habitual, unstandardized upper and lower body movements were
168 permitted (e.g. reading a book, use of phone, readjusting seated position if uncomfortable).
169 To minimize walking distance, participants were transported in a wheelchair for bathroom
170 breaks. Participants completed the SRA in time with the video demonstration which can be
171 found at this link: https://www.youtube.com/watch?v=Ieb3wqDD_7Y&t=1s. The video was
172 run twice for the SRA6 condition.
173
174 The choice for the activities was informed by previously published studies (9, 12). Exercises
175 were selected on the basis of engaging large muscles in the lower body to promote increased
176 leg blood flow and reduce vascular impairment (35). Additionally, these exercises were
177 selected on the basis that that they can be performed in a static position with no equipment
178 and therefore can be practical choice for most adults._Regarding the break frequency,
179 interrupting sitting every 30 minutes has previously been shown to improve lower limb
180 vascular function (9). However, the 60 minute break was used to overcome issues relating to
181 the feasibility/practicalities of high frequency interruptions. To ensure that the activity was

matched for duration, the 60 minute break was doubled so that both conditions undertook identical total amount of activity.

Participants were asked to refrain from moderate- to vigorous- PA for 48 hours, and caffeine and alcohol for 24 hours prior to each experimental condition and completed questions at the start of each experimental visit to ensure compliance. Participants resumed their habitual PA and dietary patterns during the washout period between experimental conditions. To objectively monitor daily activity levels throughout the study, participants wore an activPAL^3 triaxial PA monitor (PAL Technologies Ltd., Glasgow, Scotland).

INSERT FIGURE 1 ABOUT HERE

To minimize any potential diet-induced variability, participants were provided with standardized meals from the night before each trial visit to the end of the trial visit day. Consistent with previous investigations in our lab (9, 12), all meals provided 33.3% of estimated energy requirements (Schofield equation (41), 1.5 activity factor) with a target macronutrient profile of 12-15% energy from protein, 30-33% energy from fat, and 53-55% energy from carbohydrate. Participants were instructed to eat their standardized evening meal between 19:00 and 21:00 hours.

On each experimental day participants arrived at the laboratory at 0730 in a fasted state (>10h). After participants voided and were weighed, they were asked to remain seated in an upright chair, and minimize movement, for the duration of the visit. Each experimental visit started with a 1-h “steady-state” period. During this time baseline blood samples, including

glucose and insulin, were collected, blood pressure (BP) was measured and femoral artery flow-mediated dilation (FMD) recorded. Participants received a standardized breakfast and lunch at 0h, and 3.5h respectively and were given up to 20 min to consume. Breakfast options included bran-based cereals, ham-and-cheese croissant, fruit salad and juice. Lunch options included a salad and meat bread roll, sweet biscuits and a juice. A note was made regarding each individuals' meal choice and replicated for subsequent visits. Participants were advised to take medications as normal. Blood samples were collected at 0h (fasted), 1h, 3.5h, 4.5h and 7h for the analyses of ET-1. Due to the distance of the bathrooms from the clinic rooms, a wheelchair was used to take participants to the toilet. One participant's data was excluded from this analysis as the blood draws were unable to be completed on the study days.

Measures

Arterial function

All vascular function assessments were performed in a quiet, dim-lit, temperature-controlled (22°C –25°C) room. Participants rested in the seated position for ~15 minutes prior to assessment, and were instructed to place both feet flat on the floor. The superficial femoral artery was assessed in the right leg using a 10-Mhz multi frequency linear array probe in conjunction with a high-resolution duplex ultrasound (Terason t3200, Teratech, Burlington, MA) machine at an isonation angle of 60°. A rapid inflatable cuff (SC-12-D, D.E. Hokanson Inc., Bellevue, WA) was placed around the thigh (distal femur). Once an optimal image of the artery was obtained, a 1-min recording of continuous resting vessel diameter and blood velocity was measured (live duplex mode). The cuff was then inflated (~220mmHg) for 5 minutes. Following 5 min of inflation, the cuff was released to induce reactive hyperemia, and duplex ultrasound recording continued for a further 3 min to observe the post-deflation

diameter and peak velocity response. All FMD measures occurred before the SRA to avoid any transient effects of the SRA that may have influenced the measurement. Placement of the probe was marked and recorded on the first scan at the first visit and replicated for corresponding vascular measurements.

Data from four participants was excluded from this analysis as complete valid datasets were not available due to poor image quality from patient movement or imaging artefact. Datasets were considered invalid if more than 3 out of the 5 tests could not be assessed. Analysis of femoral artery diameter and blood velocity was performed using offline, automated edge detection and wall tracking software, by one scanner (59). Analysis of ultrasound recordings were performed using (LabVIEW 6.02, National Instruments). This software has previously been demonstrated to overcome methodological issues, reproducing diameter measurements that significantly reduce observer error with an intra-observer CV of 6.7% (59). FMD was calculated as the percentage rise in peak diameter from the preceding baseline diameter. Blood flow was defined as the product of cross-sectional area and velocity. Shear rate (s^{-1}), derived from blood velocity and diameter, was used as an estimate of shear stress on the artery wall. The shear stimulus was calculated as the shear rate area under the curve (AUC) from time of cuff release to peak dilation, using the sum of trapezoids method (2). Our sonographer has a between-visit reproducibility of 4.5%.

Resting BP

Seated, resting brachial BP was measured at hourly intervals from -30min with an additional measurement at 3h. Measurements were taken in triplicate, at 1-min intervals using an automated oscillometric BP monitor (HEM-907, Omron, Kyoto, Japan) and an appropriately sized cuff, as per recommended guidelines (8). All measurements were repeated on the same

arm for both conditions. An average of the three measurements at each hour was used in analysis. Blood pressure measurements were taken immediately after the SRA (Figure 1).

Biochemical analysis

Whole blood samples were drawn into EDTA tubes and centrifuged within 5 min of collection, and the plasma fraction was separated and stored at -80°C. ET-1 samples were analyzed using sandwich immunoassay technique with DET-100 kits from R&D systems (Minneapolis, MN) according to the manufacturer's instructions. The final product of the ELISA was quantified using a Benchmark Plus Microplate spectrophotometer and standard curve (Bio-Rad Laboratories, Hercules, CA) at 450 nm (16).

Statistical analysis

All analyses were performed using R statistical programming language (Version 3.6.1, 2019, USA) (48). The total AUC across the 7-h protocol on each day was calculated for ET-1 using the trapezoidal method, where area is taken from a plasma concentration of zero. Generalized linear mixed models were used to examine FMD and hemodynamic measurements for 1) the average for each condition across all scans, excluding baseline, in the 7h period, and 2) between- and within-condition effects (i.e. condition x time interaction). Further analysis was undertaken to examine meal-specific responses on mean FMD. Generalized linear mixed models were used to examine mean femoral artery FMD for the pre- (1h + 3.5h) and post- (4.5h and 7h) lunch time periods. The generalized linear mixed models had the following fixed effects; age, sex, BMI, values and 0h and conditioner order. We modelled participants as random effects. Additional fixed effects for resting diameter and shear stimulus were used on FMD models (50). To account for any residual effects of activity preceding the experimental conditions, primary outcomes were additionally adjusted for number of steps in

the restrictive period. A condition-by-time interaction with post comparisons was used to compare individual time point's between- and within-conditions relative to 0h. Post hoc comparisons between time points were adjusted for multiple comparisons using a Šidák corrections. Associations between variables were assessed using Spearman's rank correlation coefficients at each time point. Descriptive data are presented as mean ± standard deviation (SD), and output from mixed model analysis are presented as marginal means ± standard error. $P \leq 0.05$ was considered statistically significant.

RESULTS

Participant characteristics

Of the 25 participants included, 24 randomized participants completed all three study arms (Figure 2). Participant characteristics are presented in Table 1. All female participants within the study were post-menopausal. Pre-experimental period data on time spent sitting, standing and stepping (inferred using activPAL data from a stepping cadence of >100 steps per minute for >1 min) and diet are presented in Table S2 of the supplementary material. Prior to the SRA3 condition, the total number of steps and total stepping time was significantly higher in the restricted period, but not the habitual period. No other significant differences were observed in activity level, sitting time and dietary indices between pre-experimental periods.

There were 14 participants on antihypertensive medication. All participants maintained their baseline antihypertensive treatment and other medications (Table 1) on the experimental day and throughout the course of the trial.

INSERT FIGURE 2 ABOUT HERE

306

307 **FMD and hemodynamics**

308 The hemodynamic and absolute (i.e., unadjusted) FMD data are presented in Supplementary
309 material, Table S1. Table 2 displays adjusted data with statistical comparisons. Femoral
310 artery FMD averaged across the 7 hours was significantly different between SIT: 3.7 ± 0.3%
311 and SRA3: 4.1 ± 0.3%, $p = 0.04$; however, there were no significant differences between SIT
312 and SRA6, or SRA3 and SRA6 (Figure 3B). When the day was split into pre and post lunch
313 periods, there was a statistically significant difference in the average of the 2 pre-lunch
314 measures between SRA6: 3.7 ± 0.3% and SRA3: 4.4 ± 0.3%, $p < 0.001$; and SRA6 and SIT:
315 4.3 ± 0.3%, $p = 0.005$. The average of post-lunch measures revealed a statistically significant
316 difference between SIT: 3.1 ± 0.5% and SRA6: 3.7 ± 0.5%, $p = 0.02$ suggesting that the
317 impact of SIT was greater as the day progressed (Figure 3A). Additional adjustments for
318 resting diameter and shear stimulus did not change the interpretation of the results. There
319 were no statistically significant differences for between- or within- condition (i.e. condition x
320 time interaction) effects at any of the time points for FMD (Table 2). However, within the
321 SRA3 condition there was a trend for femoral artery FMD to increase following a meal at the
322 3.5 h and 7 h time points (increase of 1.5% from 0h – 3.5h, and an increase of 1.3% from
323 4.5h - 7h). A similar trend was not observed in the SRA6 or SIT condition, with FMD levels
324 remaining relatively constant across the day (Table 2).

325

326 INSERT FIGURE 3ABOUT HERE

327

328 Mean resting femoral shear rate, averaged across the 7h, was significantly lower in the SIT
329 condition (33.1 ± 4.1/s) relative to SRA3 (45.3 ± 4.1/s, $P < 0.001$), and SRA6 (46.2 ± 4.1/s, P
330 < 0.001). Mean resting femoral blood flow averaged across 7h, was significantly lower in the

SIT condition (71.1 ± 9.2 ml/min) relative to SRA3 (96.4 ± 9.2 ml/min, P < 0.001), and SRA6 (92.5 ± 9.1 ml/min, P < 0.001). No differences in resting systolic or diastolic BP averaged across 7h between the SIT and SRA3 (121 ± 3 vs 121 ± 3 mmHg, $p = 0.95$; 68 ± 1 vs 69 ± 1 mmHg, $p = 0.81$, respectively) or SIT and SRA6 (121 ± 3 vs 120 ± 3mmHg, $p = 0.93$; 68 ± 1 vs 67 ± 1 mmHg, $p = 0.57$, respectively) conditions were observed. Additionally, there were no significant differences in resting mean HR over 7h (SIT: 74 ± 2 bpm; SRA3: 75 ± 2 bpm; SRA6: 75 ± 2 bpm; $p > 0.26$ for all).

Blood biomarkers

Plasma ET-1 total AUC's were 4% lower in the SIT (10.1 ± 0.9 pg·hr·ml^{-1}) condition relative to SRA3 (10.5 ± 0.9 pg·hr·ml^{-1}), and 1% lower relative to SRA6 (10.2 ± 0.9 pg·hr·ml^{-1}); however, neither were statistically different (Figure 4B). ET-1 concentrations were not significantly different between conditions (P > 0.17 for all). ET-1 at 7 hours was significantly lower relative to respective baselines for all conditions (p < 0.007). Change relative to baseline was not statistically different between conditions ($p > 0.93$). A weak positive relationship was observed across the day between resting femoral blood flow and ET-1 (r_s=0.062, $p = 0.02$), and resting femoral shear rate and ET-1 (r_s= 0.163, $p < 0.001$).

INSERT FIGURE 4 ABOUT HERE

DISCUSSION

To our knowledge, this is the first study to examine the effect of interrupting prolonged sitting with different SRA break frequencies on vascular function in those with T2D. When averaged across 7h, femoral artery function (measured via FMD) significantly increased in the SRA3 condition, but not the SRA6 condition, relative to SIT. Whilst no between- or

within-condition differences in femoral artery function were observed at specific time points (Table 2), this average difference reflected increases in FMD in the SRA3 condition that occurred ~3 hours after meal ingestion. Blood flow and resting shear rate were also significantly higher across the day in the SRA3 and SRA6 conditions, relative to SIT. These findings provide insights into the effects of interrupting prolonged sitting on lower-limb vasculature in adults with T2D.

While FMD tended to increase across the day (baseline to 7h) in the SRA3 condition, only small to negligible changes were observed for SRA6 and SIT. Others have reported similar findings in healthy desk workers and young, healthy males, observing an increase in FMD when prolonged sitting was broken up with PA, despite not reaching statistical significance relative to baseline (5, 51). Nevertheless, a 1.5% increase across the day (baseline to 7h) was observed in the SRA3 condition (Table 2). Whilst this was not statistically significant, a FMD increase of 1% is considered clinically meaningful given that it decreases the risk of cardiovascular events by 13% (22). Certainly, previous studies have reported breaking up prolonged sitting prevents superficial femoral artery endothelial dysfunction (9, 36, 51).

The increase in superficial femoral artery FMD across the day in the SRA3 condition, relative to SRA6 condition, suggests the frequency of SRA may be more important than the duration of SRA. This contradicts previous work which has found that infrequent walking breaks maintained superficial femoral artery endothelial function at 30-, 90- and 150 min (51) and 120- and 240 min (5). However, it should be noted that these two studies were examining endothelial function in healthy males and females whereas participants in this study had T2D with overweight and/or obesity. Given that there is a progressive impairment in vascular function throughout the pathogenesis of T2D (27), it is possible that more frequent interruptions to sitting are needed to preserve leg blood flow (35), and therefore mitigate

sitting induced vascular impairments, in this population. Further research that investigates interrupting prolonged sitting with activity breaks across the spectrum of dysmetabolism (healthy individuals to those with T2D and complications) is needed to confirm this hypothesis.

There are several possible reasons why we did not observe statistically-significant differences in condition x time interactions in FMD between conditions. Previous studies have employed tighter control over restricted leg movements (51, 52) during sitting in comparison to this current study, thus it is plausible that the habitual, unstandardized lower leg motion allowed in our study contributed to the lack of statistically significance difference in time x condition interaction (5). Indeed, low level muscular contractions whilst sitting for 3h vs sitting interrupted with leg fidgeting have been shown to prevent popliteal endothelial dysfunction that would have otherwise occurred in young, healthy participants (33). Our approach emulates a "real world" circumstance, and on average we observed improvements with interruptions to sitting across the day. Additionally, all of our FMD measurements were collected whilst retaining the unbroken seated position. Whilst we acknowledge that current FMD guidelines stipulate assessments are performed in the supine position, movement between seating and supine would necessitate muscular activity that may impact FMD measures (51). It should also be noted that offline analysis was not performed by a blinded observer. However, we used automated edge detection and wall tracking software, in keeping with published guidelines (49, 50), which is designed to minimise the potential for investigator influence and bias (59) compared to manual methods of analysis. Nonetheless, our findings are in line with previous studies investigating FMD (%) in overweight adults (5), and we still observed a change in average FMD, shear stress and blood flow across the day. It is also possible that our sample size may have been marginal to detect between-condition

405 differences at individual time points. Finally, we cannot exclude the possibility of some
406 variability in responsiveness to meals between participants, although our meals were
407 standardised and the same meals were consumed for all three conditions.
408
409 The significant differences in vascular function we observed between conditions when data
410 were averaged across time periods reflects a trend (Figure 3A) in the repetitive response in
411 FMD to the meals, particularly in the SRA3 condition. In line with previous findings, this
412 suggests that the effect of the relative insulin resistance (induced by prolonged sitting after a
413 meal (8, 12, 15)) on vascular function, could be ameliorated by frequently interrupting sitting
414 with activity breaks. This trend was in line with previous studies that have observed femoral
415 artery FMD to increase when sitting was frequently interrupted after a meal or a snack (5, 8).
416 However, given that relatively few studies explore the effect of interrupting sitting on
417 vascular function in a non-fasted state over a 7 hour timeframe (5, 9, 25), and that our study
418 is the first to observe the effects in adults with T2D, future research should determine if
419 frequently interrupting sitting following a meal is beneficial for adults with T2D.
420
421 Resting femoral shear rate and resting blood flow significantly increased from baseline across
422 7h, in both the SRA3 and SRA6 conditions (Table 2). Shear stress is recognized as an
423 important physiological factor in maintaining endothelial health (35, 42) and sitting-induced
424 decreases in blood flow and shear stress may contribute to vascular dysfunction (35, 37, 42).
425 Episodic increases in blood flow and shear stress that accompany activity breaks may provide
426 an antiatherogenic stimulus over the long-term (18), particularly given that the lower limb is
427 susceptible to atherosclerosis (35). The magnitude of increase in blood flow and
428 consequently, shear stress, needed to induce a clinically meaningful improvement in vascular
429 function is unknown. However, in adults with overweight and obesity, resting blood flow and

shear rate increased from baseline nearly four- and three-fold, respectively, corresponding to a FMD increase of 3.1% across 5 hours in the SRA condition (9). Given we did not observe an increase of this magnitude for blood flow and shear rate in our participants (Table 2), it is possible, that a larger increase is needed to stimulate a significant improvement in femoral artery FMD in those with T2D. Previous research has indicated shear and function relationships decline with long-term exposure to CVD risk factors (44) where individuals with T2D are more resistant to the beneficial vascular adaptations of PA relative to healthy controls (43). This may partly explain the relatively small increase in blood flow and shear in our T2D participants, relative to other studies assessing those with overweight and obesity who are otherwise healthy (9). Future research should explore the long-term impacts of interrupting prolonged sitting on lower limb blood flow and vascular function in populations across the spectrum of diabetes risk.

We observed high basal levels of plasma vasoconstrictor ET-1 in our participants with T2D (38, 40) and the observation of no significant changes in ET-1 levels is consistent with what others have reported following PA (28). The utility of plasma ET-1 as a marker of cellular concentrations has been questioned, since ~80% of ET-1 is secreted on the abluminal side (38). Thus, it is plausible that different results may have been observed had we directly measured ET-1 in the vessel wall. Further, we observed a weak but positive correlation between ET-1 and blood flow and shear, which may partly explain why ET-1 remained relatively sustained across the day. Previous studies have demonstrated that an ET-1 blockade significantly increases blood flow in those with T2D to a greater extent relative to individuals without T2D, suggesting that those with T2D have greater ET-1 mediated basal vasoconstrictor tone (30, 31, 45). Further, studies have demonstrated that while ET-1 antagonists improve nitric oxide bioavailability in obese individuals, these effects are not

observed at the same magnitude in those with T2D (30, 31), suggesting ET-1 blockade alone may not be enough to significantly increase nitric oxide bioavailability. These studies indirectly demonstrate the importance of insulin resistance in altering ET-1 mediated vasoconstriction. Additionally, more than half of our participants were taking anti-hypertensive medications, which may play a role in interacting with mediators (ET-1) that influence endothelial homeostasis (24). Indeed, it is possible that the elevated basal ET-1 levels found in this study may partly explain the blunted blood flow and shear response to SRA in our participants relative to overweight and obese adults (9). However, given that these are complex pathways that involve multiple integrative mechanisms and very tightly controlled experimental conditions, more comprehensive data are needed to explain the vascular mechanisms relating to interrupting sitting with activity breaks in those with T2D.

No statistically significant changes in the mean systolic BP, diastolic BP or heart rate measures were observed between conditions over the trial period. These results contrast those of previous studies that reported a systolic BP-lowering effect with short-bouts of activity (14, 26). As BP of our study population was well controlled (Table 2, Supplementary material), this may have precluded any blood pressure changes due to the interruptions.

Interventions that may be used to inform larger trials and more specific public health advice surrounding optimal timing and duration of breaks, must be feasible , so that cardiovascular health benefits from interrupting sitting are translated to high-risk populations such as office workers with T2D (5). Therefore, this study directly compared two different activity protocols with equivalent total activity duration and energy expenditure over the day. As previously noted, interrupting sitting more frequently with shorter duration SRA (SRA3) was more effective in increasing superficial femoral artery FMD, relative to less frequent, longer

duration SRA (SRA6). Given the pragmatic approach to interruptions in prolonged sitting employed in this study, which does not require people to move from their desk, nor the use of equipment, a high frequency break strategy may be suitable in sedentary workplaces. Certainly, previous studies that have examined interrupting sitting every 20- or 30 minutes have reported benefits for reducing glucose and insulin concentrations (8, 13), blood pressure (14) and maintaining cerebral blood flow (4, 58). Future research should aim to examine break frequencies that mitigate sitting-induced impairments in both vascular and metabolic function (5). This may help to inform larger trials and to produce more specific public health guidelines around the optimal timing and duration of breaks in sitting necessary to improve cardiometabolic health outcomes.

This study was performed in a laboratory setting and utilized a well-controlled randomized crossover design, affording smaller sample sizes as it provided control for person-specific factors. Trial conditions were standardized and restrictive periods implemented prior to testing days (minimal variance in PA levels and diet – Table 2, Supplementary material). Future research should establish the effect of interrupting prolonged sitting in home-and/or work-based settings that better reflect "real-life" settings. As we did not assess changes in nitroglycerine responses, our study could not determine impacts on vascular smooth muscle function *per se*. Further, this was an acute study and we only examined responses to interrupting sitting over a 7h period. Participants in this study were also taking a diverse range of medications that may have influenced our results. Of note, 54% of participants were taking angiotensin-converting enzyme inhibitors or angiotension II receptor blockers, and 58% were under statin therapy. It is possible these medications may have modified vascular function, but we experimentally controlled for this according to established guidelines (11,

505 49, 50) by standardizing the timing and dose in our repeated measures experiment.
506 Importantly, the proof-of-concept nature of the study highlights the need for studies that
507 examine vascular response to interrupting sitting and meals in those with T2D. Longer term
508 exposures to interrupting sitting may assist in gaining a better understanding for the long-
509 term cardiovascular health adaptations in those with T2D.
510
511 In conclusion, breaking up prolonged sitting with SRA every 30 minutes significantly
512 increased mean superficial femoral artery FMD% over 7 hours relative to prolonged sitting
513 and clinically meaningful effect sizes (>1%) were evident (17). Vascular shear rate and blood
514 flow across the intervention period were also enhanced by interrupting prolonged sitting. Our
515 findings suggest that more-frequent and shorter breaks may be more beneficial than longer,
516 less frequent breaks for improvement in vascular function in those with T2D. Taken together,
517 these results provide new insights into the frequency and duration of activity breaks needed to
518 stimulate blood flow or improve vascular function during prolonged sitting. Future research
519 should aim to examine potential mechanisms and the longer-term impacts of interrupting
520 prolonged sitting in free-living settings on vascular function in adults with T2D.
521

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524

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DISCLOSURE

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

D.W.D, D.J.G, B.A.K, M.G, N.O, P.C.D, M.J.W, and R.N.L conception and design of research; F.C.T, A.R.H, M. K. T and M.J.W performed experiments; F.C.T and N.M analyzed data; F.C.T, D.W.D, P.C.D and D.J.G interpreted results of experiments; F.C.T prepared figures; F.C.T drafted manuscript; F.C.T, D.W.D, P.C.D, D.J.G, N.O, B.A.K, M.J.W, R.N.L, R.E.C, N.M, N.D.C, A.R.H, M.K.T, M.G and N.E edited and revised manuscript; F.C.T, D.W.D, P.C.D, D.J.G, N.O, B.A.K, M.J.W, R.N.L, R.E.C, N.M, N.D.C, A.R.H, M.K.T, M.G and N.E approved final version of manuscript.

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726 **TABLES**

727 Table 1. *Participant characteristics*
728
729 Table 2. *Hemodynamic and adjusted flow-mediated dilation data during 7h of uninterrupted*
730 *sitting (SIT), sitting interrupted with 3-min of simple resistance activities every 30 minutes*
731 *(SRA3), and 6-min of simple resistance activities every 60 minutes (SRA6).*
732

FIGURES

Figure 1. *Study design and protocol*. Participants were initially screened over the phone, followed by a medical screening and familiarization visit. Eligible participants then completed three experimental conditions in a random order. Grey bars represent steady-state hour where the following measures were taken: resting blood pressure and heart rate, flow-mediated dilation and fasted blood samples. Black bars in the 'SIT + SRA3' and 'SIT + SRA6' conditions represent SRAs. BP, BP, HR, heart rate, FMD, flow-mediated dilation; SIT, uninterrupted sitting condition; SRA3, sitting interrupted by simple resistance activities every 30 minutes; SRA3, sitting interrupted by simple resistance activities every 60 minutes

Figure 2. *Consort standards of reporting trials (CONSORT) diagram.*

Figure 3. *A:* time course of unadjusted femoral artery flow-mediated dilation (FMD) in the three conditions. Data are mean ± SD. *B:* unadjusted mean femoral artery FMD over 7h in uninterrupted sitting (SIT), sitting interrupted by 3-min simple resistance activities every 30 minutes (SRA3) and sitting interrupted by 6-min simple resistance activities every 60 minutes (SRA6) conditions, adjusted for values at 0h, age, body mass index, sex and treatment order. Data are marginal mean ± SEM with paired individual values. **p* = 0.04 vs SIT.

Figure 4. *A:* time course of plasma endothelin-1 in the three conditions (n=23). Data are mean ± SD. *B:* effect of uninterrupted sitting (SIT), sitting interrupted with 3-min simple resistance activities every 30 minutes (SRA3) and sitting interrupted with 6 min simple resistance activities every 60 minutes (SRA6) on plasma endothelin-1 total area under the curve (AUC) over 7h, adjusted for age, sex, body mass index and treatment order (n=23). Data are marginal means ± SEM with paired individual values.

SUPPLEMENTAL DATA

https://doi.org/10.6084/m9.figshare.12431696

https://figshare.com/s/13f3805314af80e22474

Table 1. *Participant characteristics*

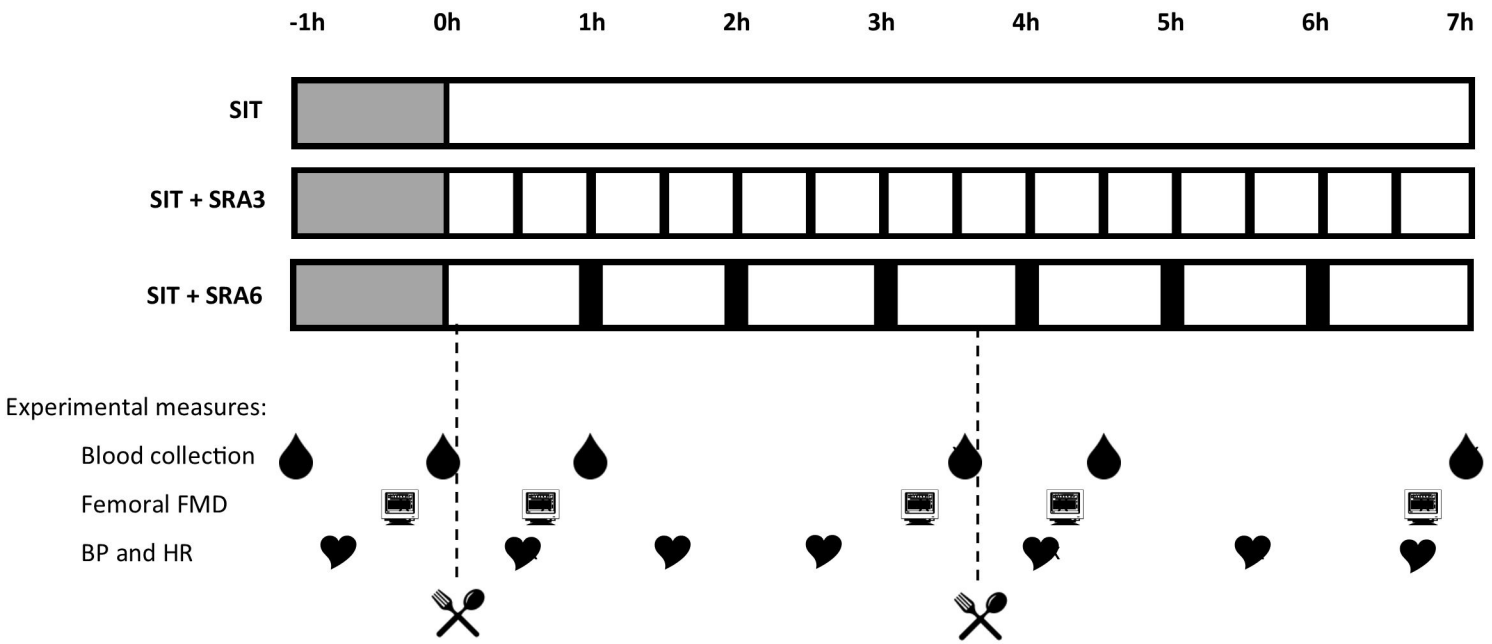
N	**24**
Sex (male/female)	13/11
Age (yr)	61.5 ± 7.8
BMI (kg/m^2)	32.6 ± 3.5
Weight (kg)	94.0 ± 13.4
Waist circumference (cm)	111.0 ± 8.8
Waist to hip ratio	1.0 ± 0.1
SBP (mmHg)	129 ± 10
DBP (mmHg)	74 ± 10
T2D duration (yr)	10.1 ± 7.0
Metabolic parameters	
Glycated hemoglobin (%)	7.6 ± 0.8
Glycated hemoglobin (mmol/mol)	59 ± 9
Fasting glucose (mmol/L)	8.1 ± 1.5
Fasting insulin (mmol/L)	76.2 ± 43.5
Fasting triglycerides (mmol/L)	1.7 ± 0.6
HOMA2-IR	1.9 ± 1.0
Medication	
Metformin, n (%)	22 (92)
DPP4, n (%)	8 (33)
Sulfonylureas, n (%)	6 (25)
SGLT2$^+$, n (%)	8 (33)
GLP agonists, n (%)	4 (17)
ACE inhibitor or ARB, n (%)	13 (54)
Calcium channel blocker, n (%)	3 (13)
Beta blocker, n (%)	3 (13)
Diuretic and other, n (%)	2 (8)
Statin, n (%)	14 (58)
Antidepressants, n (%)	6 (25)

Data are mean ± SD. ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blockers; BMI, body mass index; DBP, diastolic blood pressure; DPP4, dipeptidyl peptidase 4 inhibitors; GLP, glucagon-like peptide-1 receptor agonists; HOMA-IR; Homeostatic Model Assessment of Insulin Resistance, SBP, systolic blood pressure; SGLT2$^+$, sodium-glucose co-transpoter-2 inhibitors; T2D, Type 2 diabetes.

Table 2. *Hemodynamic and adjusted flow-mediated dilation data during 7h of uninterrupted sitting (SIT), sitting interrupted with 3-min of simple resistance activities every 30 minutes (SRA3), and 6-min of simple resistance activities every 60 minutes (SRA6).*

	0h	1h	3.5h	4.5h	7h
SIT FMD, %	3.3 (2.1, 4.5)	5.0 (3.8, 6.2)	3.4 (2.2, 4.6)	3.4 (2.2, 4.6)	3.1 (1.8, 4.3)
SIT resting diameter, mm	6.5 (6.3, 6.8)	6.5 (6.2, 6.7)	6.8 (6.6, 7.0)	6.6 (6.4, 6.9)	6.7 (6.5, 7.0)
SIT resting blood flow, ml/min	50.2 (25.2, 75.3)	46.5 (20.9, 72.1)	58.6 (33.6, 83.7)	93.2 (67.6, 118.8)	94.6 (69.0, 120.2)
SIT resting shear rate,/s	27.3 (18.3, 36.2)	26.1 (16.9, 35.2)	30.8 (21.9, 39.8)	38.4 (29.3, 47.5)	43.6 (34.5, 52.7)
SRA3 FMD, %	3.2 (2.0, 4.4)	4.0 (2.8, 5.2)	4.7 (3.4, 5.9)	3.4 (2.2, 4.6)	4.7 (3.5, 5.9)
SRA3 resting diameter, mm	6.6 (6.4, 6.9)	6.3 (6.3, 6.7)	6.5 (6.3, 6.7)	6.6 (6.3, 6.8)	6.7 (6.5, 6.9)
SRA3 resting blood flow, ml/min	61.1 (36.0, 86.1)	63.1 (38.1, 88.2)	89.2 (63.6, 114.8)	89.9 (64.8, 114.9)	137.7 (112.1, 163.2) †
SRA3 resting shear rate,/s	29.5 (20.6, 38.4)	28.6 (19.7, 37.6)	47.6 (38.5, 56.6)	50.8 (41.9, 59.7) †	55.3 (46.2, 64.3) †
SRA6 FMD, %	3.4 (2.3, 4.6)	4.0 (2.9, 5.2)	3.3 (2.2, 4.5)	3.6 (2.5, 4.8)	3.7 (2.6, 4.9)
SRA6 resting diameter, mm	6.6 (6.4, 6.8)	6.7 (6.5, 6.9)	6.7 (6.5, 6.9)	6.6 (6.4, 6.8)	6.7 (6.5, 7.0)
SRA6 resting blood flow, ml/min	57.5 (33.0, 82.0)	43.8 (19.3, 68.4)	97.7 (73.2, 122.2)	102.1 (77.6, 126.7)	123.1 (98.6, 147.6) †
SRA6 resting shear rate,/s	30.3 (21.5, 39.0)	21.0 (12.2, 29.7)	48.9 (40.2, 57.7) †*	53.2 (44.5, 62.0) †	59.9 (51.1, 68.6) †

Data are marginal mean ± 95% CI's for each condition. All models adjusted for age, sex, body mass index, treatment order and multiple comparisons. Time points 1h, 3.5h, 4.5h, and 7h additionally adjusted for value at 0 h. FMD, flow-mediated dilation; SIT, uninterrupted sitting; SRA3, sitting interrupted with simple resistance activities every 30 mins, SRA6, sitting interrupted with simple resistance activities every 60 mins, *$p < 0.05$ relative to SIT condition, †$p < 0.05$ within condition vs. 0 h. N=20.



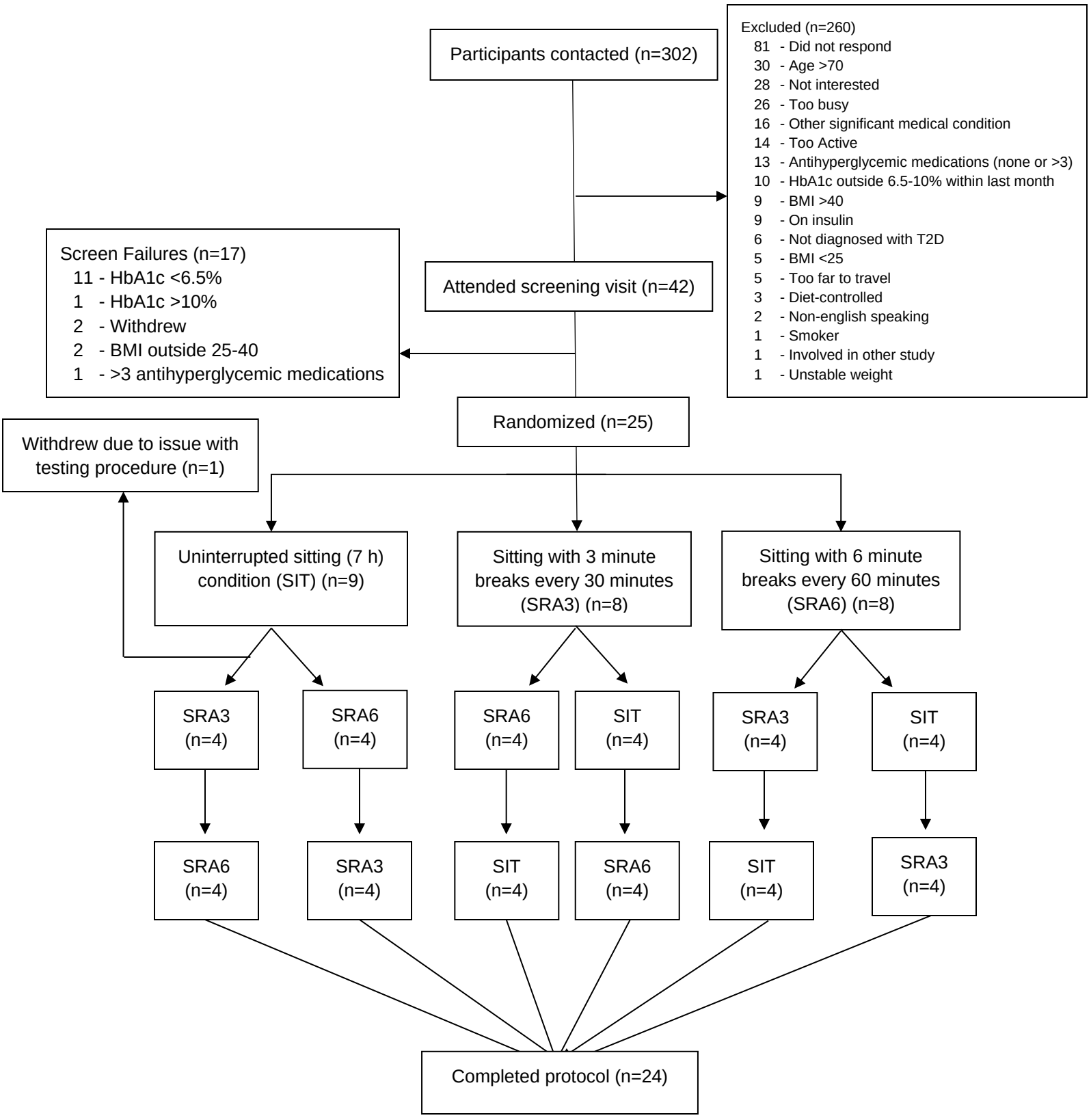


Figure 1: Participant flow diagram