

Supplementary Procedures

The MOVE study (*Mitigating the effects of sitting on vascular dysfunction in type 2 diabetes*) was prospectively registered with the Australian New Zealand Clinical Trials Registry (No. ACTRN12617000392369). Details on all outcomes collected as part of the study are listed here, and below.

Details of all outcome measures collected

In addition to the outcomes reported as the central aim of this manuscript, the MOVE Study also collected data on the clinically relevant outcomes of;

- Endothelial function of the femoral artery, as measured with flow-mediated dilation using high resolution doppler ultrasound. Five measures were collected during each 7 h experimental condition (0, 1, 3.5, 4.5 and 7 h) (Taylor et al 2020; Manuscript currently under review)
- Blood pressure was measured using an OMRON HEM-907 automatic digital blood pressure machine. Eight measures were collected during the 7 h experimental condition (0, 0.5, 1.5, 2.5, 3.5, 4.5, 5.5, and 6.5 h). The average of three measurements collected over 8 min was recorded. (Taylor et al 2020; Manuscript currently under review)
- 22 h glycaemic control as measured by Flash Continuous Glucose Monitoring Systems (Abbott Freestyle Libre) from the beginning of each experimental condition until the next day wake. Homer et al. 2020; Manuscript currently under review.
- Additional plasma from blood samples collected for the measurement of the metabolic outcomes (glucose, insulin and triglycerides) was stored at -80°C for the later exploratory analysis of vasoconstrictive metabolites (Endothelin-1) (Taylor et al

2020; Manuscript currently under review), Catecholamines and inflammatory biomarkers (RNase Protection Assay, Inter-Cellular Adhesion Molecules (ICAMs), Vascular Cell Adhesion Molecules (VCAMs)) and lipidomics. Data yet to be analysed.

Amendments to original eligibility criteria

At its original conception, the study was designed to recruit a sample population which closely replicated that of previous work conducted by the laboratory (1). However, due to extremely slow rates of recruitment and higher than expected screening failure rates (2 successful screenings in the first 6 months of active recruitment), a decision was made by the investigators to have the eligibility criteria widened to be more inclusive of a general T2D population (and subsequently enhance recruitment). The original eligibility criteria with the respective amendments in parentheses (*italicised and underlined*) is presented below. Human research ethics amendment approval was granted prior to the implementation of these changes.

Eligibility criteria

- Aged 35-65 years (*amended to 35-70 years*)
- BMI 25-35 kg m² (*amended to 25-40 kg m²*)
- Diagnosed with T2D for at least 3 months
- Diet- or metformin-controlled (*amended to taking at least one, but a maximum of three anti-hyperglycaemic medications*)
- English speaking

Exclusion criteria

- HbA1c <7% or >10% (amended to <6.5% or >10%)
- Taking insulin
- Pregnancy
- Current smoker
- Self-reported sitting <5 h per day
- Regularly engaging in moderate physical activity for ≥ 150 min per week, or vigorous physical activity for ≥ 75 min per week; for >3 months
- Any known physical activity contraindications or major illness/physical problems (acute or chronic) that may limit participation in physical activity
- Lost or gained more than 5kg in the previous 3 months.

Device-based assessment of sedentary behaviour and physical activity

For the entire duration of this study (i.e. from the end of the familiarisation visit, until the day following the last experimental condition) participants were fitted with two activity monitors for the assessment of sedentary behaviour, physical activity and sleep across the habitual period as well as during the restricted lead-in periods, and the experimental condition.

The ActivPAL4 monitor was worn in a waterproof dressing, fixed to the anterior midpoint of the right thigh. Participants were instructed to wear this monitor at all times, with the exception of situations where the participant was submerged in water for long periods of time (e.g. swimming or bathing) to minimise risk of the monitor being lost. Participants were given take home materials so they could change the dressing should they feel the need to. A space in the participant handbook was provided for participants to note down any periods of time >15 min that the device was not worn. ActivPAL data was processed in PAL software

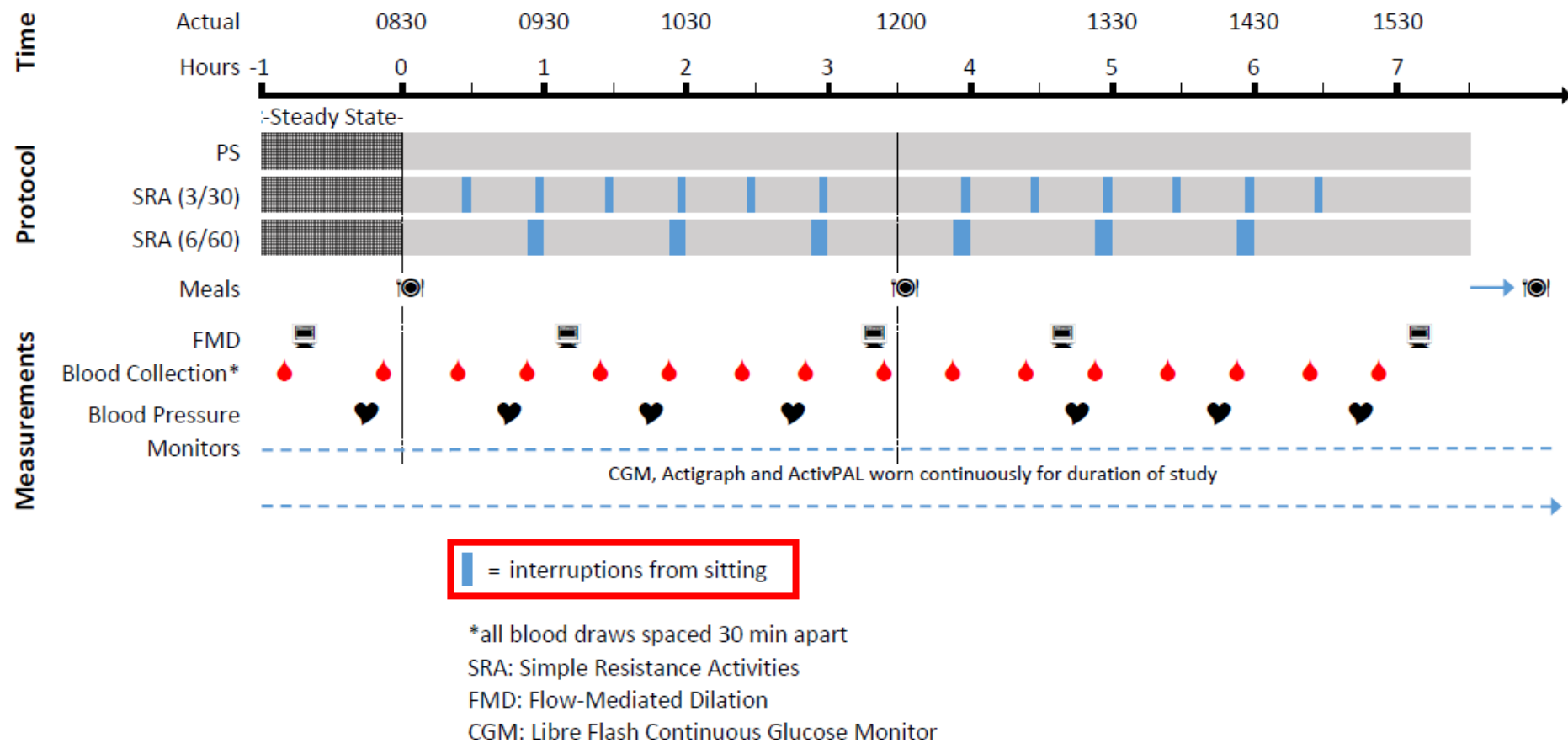
(version 8) and daily summaries were generated from which time spent sitting, standing, stepping, and stepping at a cadence of greater than 100 steps per minute for longer than 1 minute (indicative of moderate-intensity activity). The ActivPAL monitor has been previously validated to accurately classify time spent in physical activities of different intensities (2). However, the authors acknowledge that this validation study was performed in healthy adults and as such these results may not extend to use for the sample of adults with type 2 diabetes reported on here.

Wrist-worn ActiGraph MVPA was calculated using the R package GGIR (3) and time spent in MVPA was identified using Hildebrand Euclidean Norm Minus One (ENMO) cutpoints of 100.6 for moderate and 428.8mg for vigorous physical activity (4) it is well-accepted that wrist-worn accelerometry, despite having higher compliance, has limitations for the assessment of physical activity (5-8). Specifically, when compared to hip-worn accelerometry, wrist-worn (and the cut points used to characterize the data (4)) tend to overestimate the amount of physical activity performed (5-8). As such, the level of confidence with which we report pre-experimental activity data (Table 1) and results of the sensitivity analyses (Supplementary Table S6) is low.

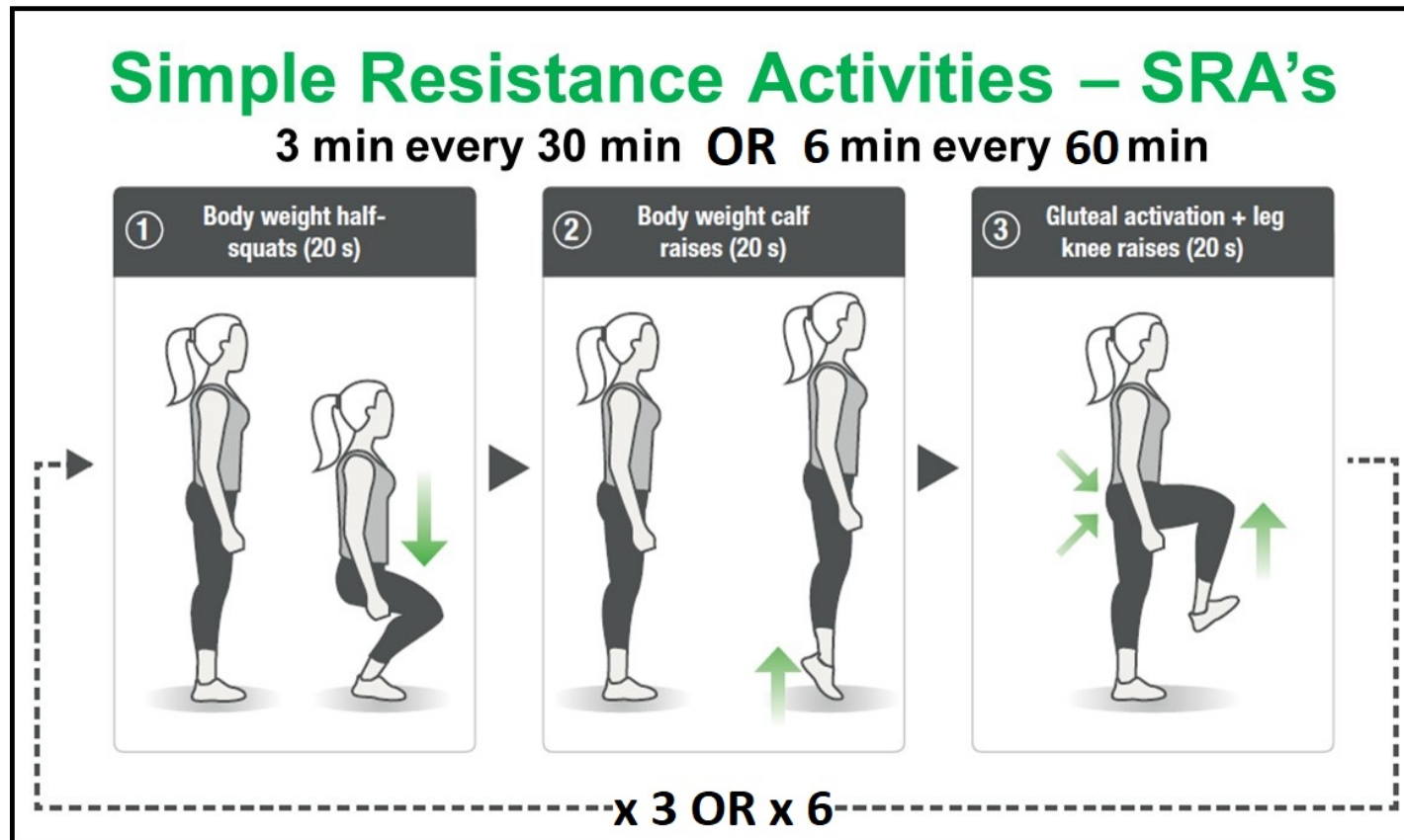
Experimental protocol

Each participant completed three 8 h experimental conditions comprised of a 1 h seated steady state period, followed by a 7 h protocol. During the experimental conditions, whilst seated, participants had access to magazines, TV, DVDs and WIFI, had access to their mobile phones, and were permitted to work or use streaming services from a personal device. Activities performed whilst seated during the experimental protocol were consistent between conditions. Importantly, participants were strictly supervised during all experimental conditions to ensure adherence to these protocols.

An expanded figure containing all of the outcome measurements collected is given in Figure S1. This figure details the timing of the SRAs, meals, blood draws, FMD and blood pressure measurements. Additionally, a visual representation of the SRA exercises is provided in Figure S2. Briefly, participants completed 20 second rotations of bodyweight half-squats, calf raises, and single-leg knee lifts (i.e. stationary march) separately by standing gluteal contractions.



Supplementary Figure S1: Expanded experimental protocol for the MOVE study, inclusive of all outcome measurements and their respective collection time points. Specifically, outcomes not reported on here; Flow Mediated Dilation (vascular function), Blood Pressure, Flash Continuous Glucose Monitor and ActiGraph GTX3+ accelerometer data.



Supplementary Figure S2: The Simple Resistance Activity (SRA) protocol used in this study. Note; during the SRA3 condition these exercises were performed for 3 rotations, totalling 3 minutes, every 30 minutes; during SRA6 they were performed for 6 rotations, totalling 6 minutes, every 60 minutes. The total activity accumulated across the 7 h experimental condition was 36 minutes for both protocols. Figure adapted from original by Maria Brännholm Syrjälä, Umeå University, Sweden. A demonstration of the exercises can be viewed here:

https://www.youtube.com/watch?v=Ieb3wqDD_7Y&t=1s

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