Breaking Up Sitting Time after Stroke (BUST-Stroke). A within-person randomised controlled trial

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Contributorship statement

Authors English, Dunstan and Bernhardt developed the research question and obtained funding and provided strategic direction and oversight to the trial. Authors English, Janssen, Crowfoot, Callister, Dunn, Patterson and Walker developed the trial protocol, oversaw and directly contributed to data collection. Authors Walker and Ong advised on, and oversaw the analyses of blood biomarkers. Author Spratt provided clinical interpretation of the data. Authors Oldmeadow and Palazzi developed the statistical analysis plan and acted as consultants overseeing all data

analyses. Authors English and Crowfoot ran all data analyses. Author English lead the writing of the manuscript with all named authors contributing and providing final approval.

Data sharing statement

Extra data is available by emailing Associate Professor Coralie English (Coralie.English@newcastle.edu.au).

Ethics

The trial is registered (ANZTR 12615001189516), and was approved by the Hunter New England Local Health District (#15/10/21/4.05) and University of Newcastle Human Research Ethics Committees (#H-2015-0437). All participants provided written informed consent.

Conflicts of interest and sources of funding

The trial was supported by a Stroke Foundation of Australia Seeding Grant (2015) and John Hunter Hospital Charitable Trust Grant (2016). Associate Professor English was supported by National Heart Foundation Future Leaders Fellowship (2017-2020). Prof Dunstan was supported by an NHMRC Senior Research Fellowship (NHMRC #1078360). Prof Bernhardt was supported by a National Health and Medical Research Council Established Research Fellowship (#1058635). The Florey Institute of Neuroscience and Mental Health and Baker Institute acknowledge infrastructure support from the Victorian State Government. Authors have no conflicts of interest to declare. The results of this trial are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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Abstract

Objectives

People with stroke sit for long periods each day, which may compromise blood glucose control and increase risk of recurrent stroke. Studies in other populations have found regular activity breaks have a significant acute (within-day) positive effect on glucose metabolism. We examined the effects of breaking up uninterrupted sitting with short, regular activity breaks in people with stroke on post-prandial plasma glucose and insulin.

Methods

Randomised within-participant crossover trial. We included people between 3 months and 10 years post-stroke, ambulant with minimal assistance and not taking diabetic medication other than metformin. The 3 experimental conditions (completed in random order) were: uninterrupted sitting (8 hours), sitting + half-hourly 3-minute light-intensity exercise while standing, or sitting + half-hourly 3-minute walking breaks. Meals were standardised and bloods were collected half- to one-hourly via an intravenous cannula.

Results

19 participants (9 female, mean [SD] age 68.2 [10.2]) completed the trial. The majority (n=12, 63%) had mild stroke symptoms (National Institutes of Stroke Scale score 0-13). There was no significant effect of experimental condition on glucose (mean [SD] positive incremental area [+iAUC] mmol·L·h-1 under the curve during sitting 42.3 [29.5], standing 47.4 [23.1], walking 44.6 [26.5], p=0.563) or insulin (mean +iAUC pmol·L·h-1 sitting 14,161 [7,560], standing 14,043 [8,312], walking 14,008 [8,269], p=0.987).

Conclusion

Short regular activity breaks doing simple activities did not have a significant effect on glucose metabolism in this sample of people with stroke. Further studies are needed to identify strategies that improve inactivity-related glucose metabolism after stroke.

Article summary – strengths and limitations

- The trial was fully powered randomised, within-participant cross-over trial, conducted in accordance with the CONSORT statement.
- Confounding variables, including food and water intake and physical activity both prior to and during the experimental conditions were tightly controlled and monitored.
- Reasons for the null result in this population is unclear and may include changes in muscle physiology and glucose regulation after stroke.
- Higher energy expenditure during standing and walking, and the high intra-assay coefficient of variation for glucose may have influenced the results for some participants.

Key words

Sedentary behavior

Physical Activity

Rehabilitation

Secondary prevention

Introduction

High sitting time is associated with an increased risk of cardiovascular disease and metabolic disorders [1]. A recent large (n=1, 005, 791) meta-analysis [2] found that people in the lowest quartile of activity (average 5 min/day MVPA and sitting > 8 hours/day) had 59% higher mortality rate compared to the most active group. People with stroke living in the community fit this profile [3-6]. Estimates of daily time spent sitting/lying in people with stroke range from 81% across a 24-hour period [3 6] to 75% of waking hours [4 7], with most of it in prolonged, unbroken bouts [3 4]. Reports of accelerometer measures of time spent in MVPA range from 5 [4] to 10 [5] min/day and daily step counts in this population are less than half that of age-matched peers [8 9]. It is important to note that these estimates of sitting time are based on people who are able to walk, at least short distances, and that degree of difficulty walking is only weakly associated with sitting time in this population [10]. Breaking up sitting time is a promising new target for intervention, particularly for people with minimal walking disability after stroke, and is clinically important, given their elevated risk of cardiovascular disease and recurrent stroke [11].

Several studies have examined the acute (within-day) effects of breaking up sitting time with short bursts of light intensity activity (such as walking at comfortable pace and active standing exercises) in populations including overweight and obese, Type 2 diabetes and healthy adults. In these studies, regular activity breaks led to reductions in post-prandial glucose and insulin excursions, compared to uninterrupted sitting [12-15]. However, no studies have explored this approach in people with stroke. Reducing post-prandial glucose levels is important as large swings in glucose leads to oxidative stress and endothelial dysfunction [16] and is a risk factor for cardiovascular disease [17 18].

We investigated the acute (within-day) effects of breaking up prolonged, uninterrupted sitting with regular short activity breaks (active standing or walking) on metabolic and cardiovascular markers in people with stroke. We hypothesised that compared with uninterrupted sitting;
(1) regular activity breaks will reduce post-prandial glucose and insulin levels, and
(2) the experimental protocol will be safe and feasible

Methods

Trial population and settings

The full trial protocol is published [19] and registered (ANZTR 12615001189516). Briefly, people with self-reported stroke between 2 months and 10 years previously, aged >18 years, who were able to walk with minimal assistance (Functional Ambulation Classification (FAC) ≥ 2) were invited to participate. Exclusion criteria included self-reported sitting < 4 hour/day or >150 min/week MVPA, body mass index (BMI) >45 kg/m2 and taking diabetic medication other than metformin. Recruitment occurred between January and November 2016. The trial was approved by the Hunter New England Local Health District (#15/10/21/4.05) and University of Newcastle Human Research Ethics Committees (#H-2015-0437) and all participants provided written informed consent. All data were collected in the Clinical Trials Unit, Hunter Medical Research Institute.

Trial Design

Randomised, within-participant cross-over design in accordance with the CONSORT statement. Figure 1 presents the trial protocol. The three conditions were (a) uninterrupted sitting for eight

158 hours (SIT), (b) sitting + light-intensity exercise while standing (STAND-EX) and (c) sitting +
159 walking breaks (WALK). A person independent of the trial prepared a computer-generated
160 randomisation sequence for condition order in sequentially numbered, sealed, opaque envelopes
161 which were opened at the end of the familiarisation visit.
162
163 Participant demographics and other pre-specified data
164 Demographic and baseline data included age, sex, BMI, risk of diabetes (The Australian Type 2
165 Diabetes Risk Assessment tool (AUSDRISK), co-morbidities, medications, stroke severity
166 (National Institutes of Health Stroke Scale [NIHSS]), time since stroke, stroke type (Oxfordshire
167 Stroke Classification (OSC)), walking ability (comfortable speed over 5 meters, Functional
168 Ambulation Classification and cognition (Montreal Cognitive Assessment (MoCA).
169
170 Standardisation of dietary intake and physical activity
171 Meals were standardised for the day prior to and during each testing day and matched to
172 individual energy requirements. Physical activity was measured for a minimum of 3 days prior to
173 and during each experimental day using the activPAL3 (PAL Technologies Ltd) and Actigraph
174 GT3x+ (Actigraph Penascola FL). Participants were instructed to abstain from caffeine and
175 alcohol and MVPA for 48 hours prior to each experimental day.
176
177 Experimental day protocol
178 Two blood samples, 30 minutes apart, were collected at the beginning of the day via an
179 intravenous catheter (steady state baseline) and continued throughout the day prior to scheduled
180 activity breaks (Figure 1). Experimental day meals (breakfast and lunch) contained

approximately one third of the participant's energy requirements each, and the full day's meals combined had macronutrient profile of 17% protein, 23% fat and 57% carbohydrate. Breakfast consisted of a pre-packaged single serve breakfast cereal and milk, one slice of white bread toast with butter and jam or honey, 200 ml apple juice and decaffeinated tea or coffee. Lunch consisted of a pre-packaged frozen meal, 170g individual tub of canned fruit and 30g cheese and cracker snack pack.

The three experimental conditions were:

A) Uninterrupted sitting (SIT) – sitting for eight hours uninterrupted in a comfortable lounge chair.

B) Standing breaks (STAND-EX) – sitting for eight hours with 3-minute light-intensity exercise while standing (marching on spot, small amplitude squats, calf-raises) every 30 minutes.

C) Walking breaks (WALK) – sitting for eight hours with 3-minute walking breaks (self-selected pace) every 30 minutes.

Adherence to protocol measures

Time on task, heart rate and self-perceived exertion (Borg rating of perceived exertion), were measured immediately after activity breaks. Fatigue was assessed using a visual analogue scale at the beginning and end of the day. Toilet breaks outside of scheduled activity breaks were recorded. To minimise variation in plasma volume, water intake was recorded and participants encouraged to maintain standardised intake across conditions.

Blood sampling and analysis

Blood samples were coded, refrigerated immediately, centrifuged between >1 and < 2 hours post-collection, aliquoted and stored at -80 degrees. Plasma glucose and insulin were determined using commercially available Glucose Hexokinase assay (TR15421, ThermoScientific) and Human Insulin ELISA kit (KAQ1251, Invitrogen) respectively, according to manufacturers' instructions and by technicians blinded to condition. All samples were assessed in duplicate along with standards and controls. Intra-assay coefficients of variation were < 35% for glucose and <10% for insulin.

Trial outcomes

Our co-primary outcomes were differences in post-prandial glucose and insulin responses (within-participant, between condition differences in positive incremental area under the curve [+iAUC] [20]). Safety and feasibility outcomes included adverse events, number of people screened for eligibility, reasons for exclusion, number of experimental conditions completed, beginning and end of day fatigue, and degree of difficulty completing each experimental condition.

Statistical analysis

The trial was powered to detect differences in post-prandial glucose and insulin incremental area under the curve expressed as a Cohen's d of 0.8. Based on previous estimates of population variability (SD 1% glucose and 30% insulin) [21], 19 sets of observations (ie participants) provides power of 0.8 to detect a difference of 0.8% in glucose and 24% in insulin iAUC (two tailed testing, α=0.05).

A statistical analysis plan was prepared prior to analysis. Glucose and insulin trajectories were summarised for each participant as the +iAUC, using the trapezoidal rule. Analyses of the primary outcome (glucose) and secondary outcome (insulin) were blinded. Between condition differences were analysed using linear mixed models including fixed effects for condition, period, and order, and random intercept to account for repeated measures. Where significant differences between conditions were found, we examined comparisons of estimated fixed effects between pairs of conditions. The influence of pre-specified potential effect modifiers (measured at baseline, including walking speed, habitual sitting time, AUSDRISK score, BMI, sex, stroke severity, metformin as current medication and diagnosed diabetes) were explored individually. A statistical significance threshold of 5% was set for all analyses and data from all individuals randomised were analysed (intention to treat). All data were entered into an excel spreadsheet by one person and checked against original documentation by another person. Glucose and insulin data were checked a third time. All analyses were undertaken in SPSS version 23.

Results

Twenty-two participants were randomised, 19 completed at least two conditions and 18 completed all three conditions (see Figure 2 for trial flow). Participant characteristics are shown in Table 1. Participants were on average (SD) 68.2 (10.2) years old and 47 (37) months since stroke. At baseline, average sitting time (waking hours) was 577 (132) min/day and average time spent in MVPA was 11.6 (18.4) min/day (see Table 2). Physical activity levels between experimental condition days were not significantly different to baseline (Table 2).

Adherence to trial protocol

Time spent sitting, standing and stepping, Borg scores for perceived exertion and average heart rate during each experimental condition are presented in Table 3. There were no between condition differences in the number of toilet breaks taken (mean [SD] 3 [2] for all conditions, range 0 to 6 for STAND-EX and 0 to 8 for SIT and WALK) or water consumption between conditions (mean [SD] intake per condition in mL: SIT 903 [566], STAND-EX 847 [518], WALK 948 [477], p=0.457).

Effect of conditions on post-prandial glucose and insulin

There was no significant effect of experimental condition on glucose (mean +iAUC mmol·L·h-1 SIT 42.3 [29.5], STAND-EX 47.4 [23.1], WALK 44.6 [26.5], p=0.563). See Table 3 and Figure 3. None of the effect modifiers were significant when added to the linear mixed models. Results for insulin mirrored that of glucose (mean +iAUC pmol·L·h-1 SIT 14,161 [7,560], STAND-EX 14,043 [8,312], WALK 14,008 [8,269], p=0.987).

Safety and feasibility

Self-reported fatigue at the end of the experimental day was highest for the standing condition (mean [SD] 4.3 [2.8] cm) compared to SIT (3.2 [2.6] cm) and WALK conditions (3.3 [2.6] cm), although differences were not statistically significant (p=0.143). There were six minor adverse events, none of which led to deviations to the trial protocol. These included: bruising/pain at the cannulation site n=3, non-injurious fall during WALK condition n=1, minor skin tear n=1, delayed onset muscle soreness after STAND-EX condition n=1.

Discussion

We found that compared to eight hours of uninterrupted sitting, breaking up sitting time with 3-minute bouts of either light-intensity exercise while standing or walking every half-hour did not significantly alter post-prandial glucose and insulin excursions in people with stroke, regardless of whether participants were at higher risk of or had diagnosed Type 2 diabetes. Other potential effect modifiers, including habitual sitting time behaviours, walking speed, BMI and stroke severity did not alter results.

Our findings on post-prandial glucose and insulin responses were unexpected. Our trial was powered to detect a moderate effect size for between condition differences, used a similar sample size to previous trials, and the small mean differences and large p values suggest statistical power was not an issue. Two systematic reviews and more recent primary studies have found consistent evidence in people without stroke that interrupting prolonged sitting with frequent activity breaks attenuates post-prandial glucose and insulin rises in healthy, overweight/obese and type 2 diabetic or insulin resistant populations [12 22-24]. We chose to use real food as opposed to a test drink, to strengthen the ecological validity of our findings and found glucose excursions in the order of 2-3 mmol·L following a standardised breakfast meal from fasting which is similar to previous studies that used either fluid replacement meals [21 23], real food meals [22] or a combination [24] in overweight/obese [21 24], healthy adult [22] or Type 2 diabetic [14] populations. The mean baseline fasting glucose level across all conditions in our trial was 6.2 (SD 1.1) mmol·L, which is somewhat higher than that reported in previous studies of healthy (mean 4.7 mmol·L [22]) or overweight/obese groups (5.0 mmol·L [21]), but lower than that reported in similar studies in

Type 2 diabetes populations (8.0 mmol·L [14]), and is therefore also not able to explain the disparity in our results compared to previous studies.

Changes in muscle physiology after stroke may be a potential reason for our surprising results. Stimulation of the GLUT-4 transporter protein in contracting skeletal muscle is the most likely mechanism by which breaking up sitting time attenuates post-prandial glucose spikes [25].. In people with stroke (>6 months post-stroke), skeletal muscle mass is significantly reduced in paretic compared to non-paretic limbs [26], with loss of muscle mass over time within individuals varying depending on how quickly walking ability is recovered [26]. There is also an increase in inflammatory cytokine tumor necrosis factor-α and a switch to greater proportion of fast twitch (type II) muscle fibres in the paretic limbs of people with stroke [27]. These alterations in skeletal muscle morphology and physiology after stroke may mean that activation of larger muscle groups for longer or at a higher intensity is required in people with stroke compared to other population groups to achieve the same benefits in post-prandial glucose responses.

There may be other impacts of stroke on glucose regulation. Hyperglycaemia in the first few days after stroke is common [28] and glucose dysregulation persists in the longer term [29]. While we did not collect direct data relating to glucose tolerance in this trial, AUSDRISK scores (mean 16.4 (5.5), range 8 to 29) indicate our cohort were at high risk for diabetes. Chronic stress and stress hormones (cortisol, adrenaline, nor adrenaline) also play a role in glucose regulation, although evidence is conflicting [30 31]. The experimental protocol itself may have induced a stress response in some participants, which may have influenced results. Stroke in the insular cortex has been associated with glucose dysregulation in some [32] but not all [33] studies. We did not

316 collect imaging data for our participants, and therefore were not able to explore the potential
317 influence of stroke location on glucose and insulin responses to activity breaks.
318
319 The activity break paradigms used in previous studies range in mode (walking, cycling, standing
320 exercises), intensity (light to moderate), duration (1.5 to 6 min) and frequency (every 20 to 60
321 minutes) [12 13 15 21 34]. In exploratory secondary analyses of experimental trials, Larsen [35] found
322 greater improvements in postprandial glucose and insulin responses with activity breaks of
323 higher estimated energy expenditure (light and moderate intensity walking) compared to
324 standing breaks. This suggests a dose-response relationship between activity break intensity and
325 glucose response. Further investigation of the effect of higher doses (both greater intensity and
326 longer duration of activity bouts) of activity breaks for people with stroke is warranted.
327
328 Regular activity breaks may have other beneficial health effects. Regular aerobic exercise and/or
329 resistance training reduces blood pressure [36 37]. Hypertension is the leading risk factor for stroke
330 [38], and others have shown beneficial effects of regular activity breaks on blood pressure control
331 compared to uninterrupted sitting. Blood pressure was a key secondary outcome in this trial, and
332 the results will be reported in a separate paper.
333
334 Strengths and limitations
335 A key strength of this trial was the tight control of potential confounding variables between
336 experimental conditions. Food and water intake for the 48 hours prior to and during experimental
337 conditions were tightly controlled. We objectively measured physical activity levels for a
338 minimum of three days prior to each condition and found little variation over the course of the

trial. Our analyses were robust, with a pre-established statistical analysis plan, blinded analysis of glucose and insulin data and consideration of pre-specified potential effect modifiers.

Energy expenditure during standing and walking is higher for people with stroke with residual gait deficits [39 40] and this could have influenced results for some of our participants. There was a large range in time since stroke which may have influenced our results, although the degree of stroke severity and residual disability within our sample was relatively homogenous. Finally, the high intra-assay co-efficient of variation for glucose may have influenced results.

Conclusion

In people with stroke frequent, short activity breaks (3 minutes of light-intensity activity every 30 minutes) did not reduce postprandial glucose and insulin levels compared to eight hours of uninterrupted sitting. Further work is required to examine both the acute (within-day) and longer-term effects of different break paradigms on glucose control.

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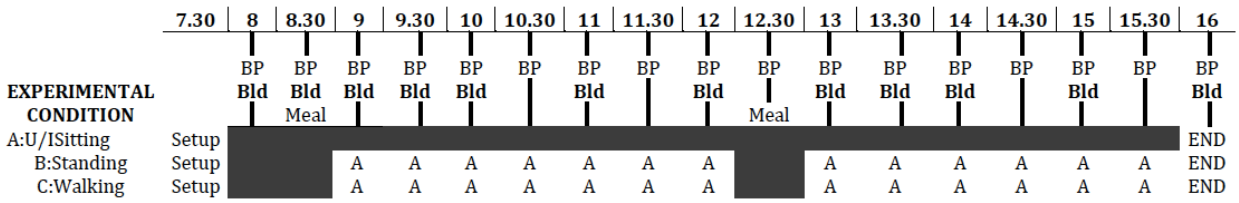
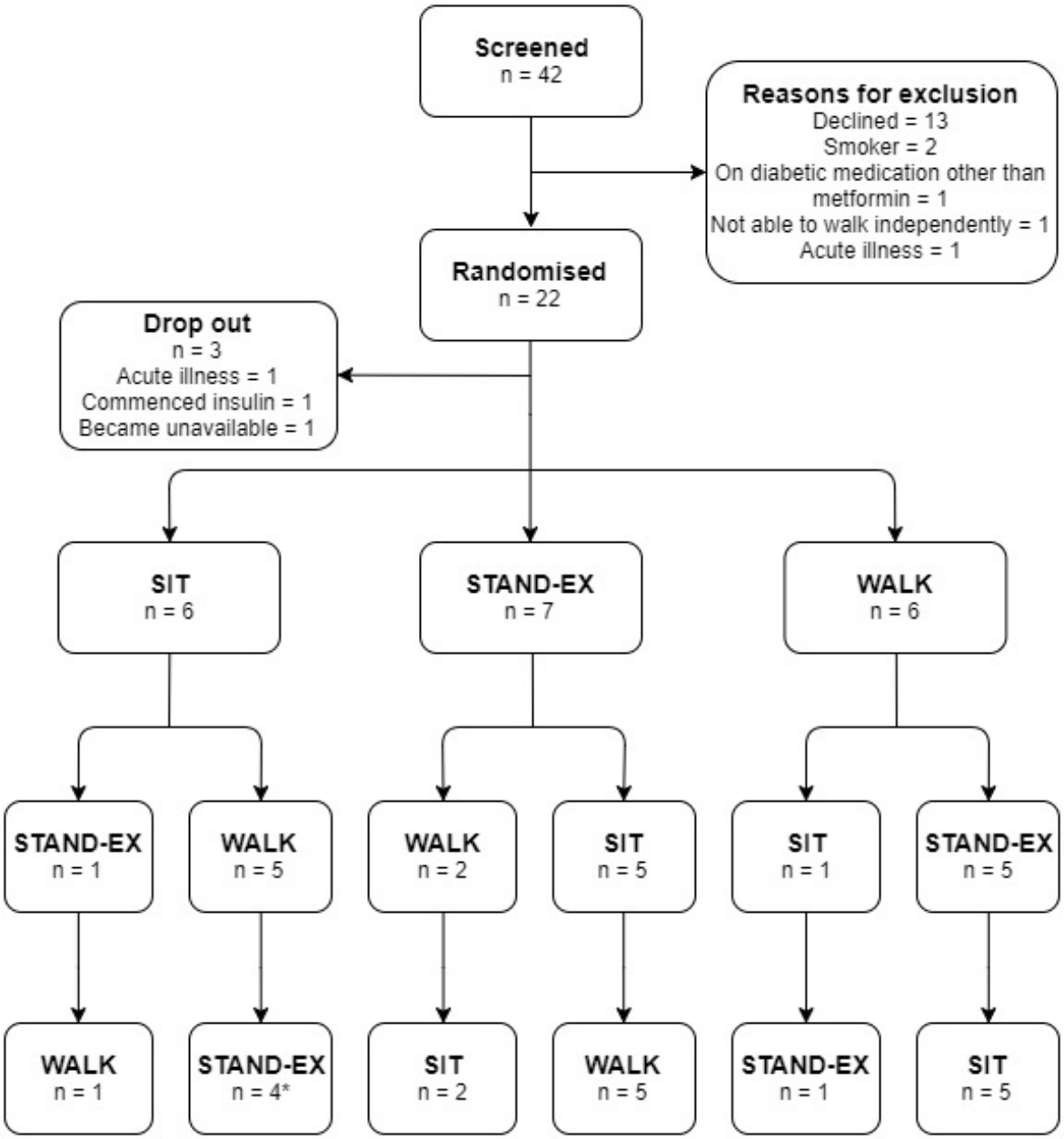
List of tables and figures



BP= blood pressure, Bld=blood draw, U/ISitting= uninterrupted sitting, A=activity

Figure 1 Trial protocol



511

512 Figure 2 Trial CONSORT diagram showing condition order

513 *one participant could not complete STAND-EX condition due to repeated cannulation failure

514

515 Table 1. Participant characteristics

Characteristic	Mean (SD) or n (%)	Range
Age (years)	68.2 (10.2)	45 - 84
Sex M:F	10:9	
Months since stroke	47.2 (36.8)	2 - 118
Body Mass Index (kg·m2)	29.9 (5.1)	23.8 – 46.1
Waist circumference (cm)	102.1 (15.4)	81.1 – 147.0
Anticoagulants (% yes)	15 (79)	
Antihypertensives (% yes)	11 (58)	
Cholesterol (% yes)	14 (74)	
Antidepressants (% yes)	6 (32)	
Metformin (% yes)	2 (10.5)	
AUSDRISK[1] score	16.4 (5.5)	8.0 – 29.0
AUSDRISK[1] categories		
Intermediate risk	3 (16)	
High Risk	16 (84)	
Living situation	Alone: 4 (21)	
	With spouse/other: 15 (79)	
Side of hemiparesis	Left: 5 (26)	
	Right: 11 (58)	
	No hemiparesis: 3 (16)	
NIHSS[2]	3.6 (3.4)	0 – 13
(measured at trial enrolment)	Median 3.0 IQR 4.0	

NIHSS[2] Categories		
No Stroke Symptoms (score 0)	1 (5)	
Mild (score 1-4)	12 (63)	
Moderate (score 5-14)	6 (32)	
Walk at admission to hospital[3] (% yes)	6 (32)	
Oxfordshire Stroke Classification (measured at trial enrolment)	TACI: 0 (0) PACI: 7 (37) LACI: 4 (21) POCI: 2 (11) Haemorrhage: 6 (32)	
Fatigue Assessment Scale score	4.6 (0.8)	2 - 5
Fatigue Assessment Scale categories		
Fatigue not identified	13 (68)	
Fatigue identified	6 (32)	
Montreal Cognitive Assessment score	21.7 (4.1), 13 – 28 Median 22 IQR 6	
Montreal Cognitive Assessment categories		
Cognitive impairment identified	16 (84)	
No impairment identified	3 (16)	
Functional Ambulation Classification		

Score 2	1 (5)
Score 3	1 (5)
Score 4	2 (11)
Score 5	15 (79)
Walking aid used (%yes)	9 (47)
Walking speed (m·s)	0.94 (0.48)
	Median 1.0 IQR 0.83
Timed Up and Go	20.4 (18.9)
	Median 10.9 IQR 15.0

[1]AUSDRISK = Australian Type 2 Diabetes Risk Assessment tool

[2]National Institutes of Health Stroke Scale

[3]Determined by asking participants "Could you walk immediately after your stroke?"

519

520 Table 2 Physical activity at baseline and between experimental conditions

	Physical activity baseline and condition 1 (n=15) Mean (SD) (range)	Physical activity between condition 1 and condition 2 (n=16)	Physical activity between condition 2 and condition 3 (n=16)	p-value[1]
activPAL derived variables				
Waking wear time (min·day)	854 (64) (709 - 961)	858 (63) (724 – 954)	836 (84) (696 – 985)	0.672
Wake sitting time (min·day)	577 (132) (316 – 862)	578 (133) (399 – 856)	568 (120) (403 – 850)	0.497
Percentage wake sitting time (%)	68.1 (17.1) (35 – 97)	67.8 (16.4) (43 – 97)	68.6 (15.3) (47 – 93)	0.428
Wake sitting time in bouts ≥ 30 min (min·day)	379 (172) (86 – 693)	395 (169) (122 – 742)	365 (146) (169 – 649)	0.498
Wake standing time (min·day)	208 (129) (11 to 493)	217 (128) (122 – 742)	204 (112) (47 – 378)	0.316

Wake stepping time	70 (39)	63 (36)	64 (39)	0.656
(min·day)	(1 – 168)	(1 – 133)	(2 – 157)	
Step counts (n)	2455 (1641)	1800 (1507)	2361 (616)	0.491
	(26 - 6831)	(19 – 5473)	(39 – 6584)	
Actigraph derived variables				
Monitored days	6.0 (1.0)	6.0 (0.4)	5.7 (0.7)	0.442
	(4 – 8)	(5 – 7)	(4 – 6)	
MVPA[2] (min·day)	11.6 (18.4)	7.50 (15.5)	8.4 (13.6)	0.538
	(0 – 70)	(0 – 55.2)	(0 – 49)	

[1]Linear mixed models (fixed effects for condition, period, and order, and random intercept to account for repeated measures)

[2]Moderate to vigorous physical activity as defined by ≥1952 counts per minute [41]

524 Table 3 Experimental condition day data

	SIT mean (SD)	STAND mean (SD)	WALK mean (SD)	p-value[1]
Fasting plasma glucose (mmol·L)	6.1 (1.4)	6.3 (1.6)	6.1 (1.4)	0.860
Plasma glucose (mmol·L, averaged across day)	6.5 (1.4)	6.8 (1.3)	6.6 (1.5)	0.563
Plasma insulin (pmol·L, averaged across day)	387.28 (176.30)	384.21 (170.62)	374.78 (168.17)	0.814
Heart rate (beat·min, averaged across day)	64.3 (9.9)	72.5 (9.6)	73.0 (11.3)	<0.001[2]
Borg (rating, averaged across day)	0.7 (0.8)	1.6 (1.0)	1.4 (0.9)	<0.001[3]
Sitting time (mins)	457 (125) (n=16)	448 (37) (n=11)	434 (55) (n=16)	0.090[4]
Standing time (mins)	7.0 (3.9) (n=15)	32.7 (9.0) (n=11)	23.4 (37.7) (n=16)	0.066[5]
Stepping time (mins)	2.7 (1.6)	19.3 (10.8)	35.6 (15.0)	<0.001[6]

	(n=15)	(n=11)	(n=16)	
Fatigue (VAS score) end of day	3.2 (2.6)	4.3 (2.8)	3.3 (2.6)	0.143
Degree of difficulty to complete condition (VAS) score)	1.2 (1.8)	2.3 (2.6)	0.9 (2.2)	0.034[7]

[1]Linear mixed models (fixed effects for condition, period, and order, and random intercept to account for repeated measures)

[2]Significant differences were between STAND-EX and WALK (p=0.004), between SIT and STAND-EX (p<0.001) and between SIT and WALK (p<0.001) based on pairwise comparisons of estimated fixed effects

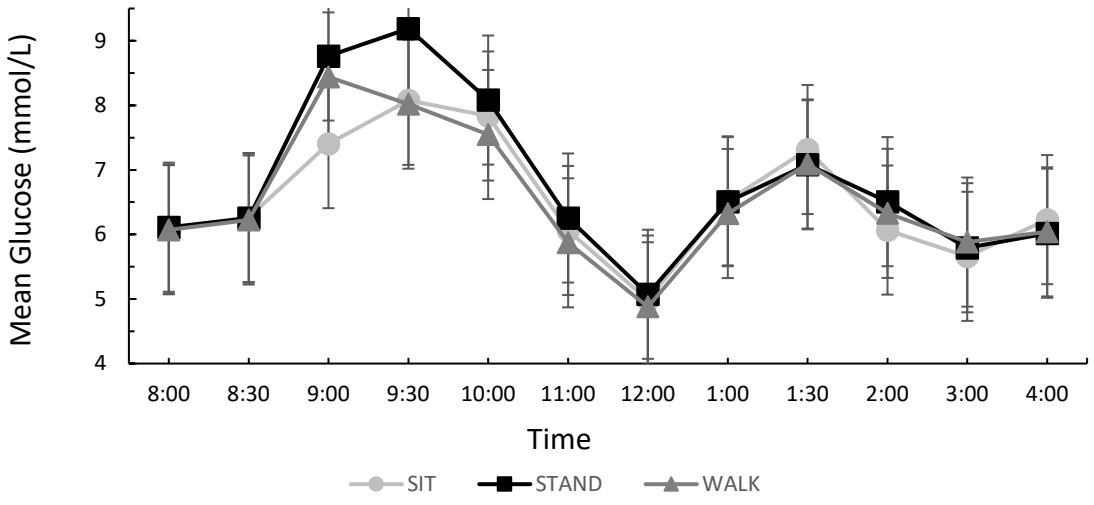
[3]Significant differences were between STAND-EX and WALK (p=0.004), between SIT and STAND-EX (p<0.001) and between SIT and WALK (p<0.001) based on pairwise comparisons of estimated fixed effects

[4]Significant differences were between SIT and STAND-EX (p=0.012) and between SIT and WALK (p=0.006) based on pairwise comparisons of estimated fixed effects

[5]Significant differences were between SIT and STAND-EX (p=0.023) based on pairwise comparisons of estimated fixed effects.

[6]Significant differences were between STAND-EX and WALK (p=0.004), between SIT and STAND-EX (p<0.001) and between SIT and WALK (p=<0.001) based on pairwise comparisons of estimated fixed effects.

[7]Significant differences were between STAND-EX and WALK (p=0.012) based on pairwise comparisons of estimated fixed effects



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537 Figure 3 Glucose mean response by condition

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