Title

Multiple short bouts of exercise are better than a single continuous bout for cardiometabolic health: a randomised crossover trial

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Running Title: Accumulating exercise for cardiometabolic health.

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Abstract

Purpose: To compare the cardiometabolic responses to five consecutive days of daily postprandial exercise accumulated in three 10-minute bouts or a single 30-minute bout to a no-exercise control.

Methods. Ten insufficiently active adults completed three trials in a randomised order. Each trial comprised five consecutive days of 30 minutes of exercise either accumulated in three separate 10-minute bouts (ACC) after main meals; a single 30-minute bout after dinner (CONT); or a no-exercise control (NOEX). Glucose regulation was assessed from an oral glucose tolerance test. Applanation tonometry was used to assess pulse wave velocity (PWV) approximately 12 hours following completion of the final trial.

Results. Area under the two-hour glucose curve was similar for CONT (Mean; 95%CI: 917 $mmol \cdot L^{-1} \cdot 2h^{-1}$; 815 to 1019) and ACC (931 $mmol \cdot L^{-1} \cdot 2h^{-1}$; 794 to 1068, p = 0.671). Area under the two-hour insulin curve was greater following NOEX (70328 $pmol \cdot L^{-1} \cdot 2h^{-1}$; 30962 to 109693) than ACC (51313 $pmol \cdot L^{-1} \cdot 2h^{-1}$: 21822 to 80806, p = 0.007). Pulse wave velocity was lower for ACC (5.96 $m \cdot s^{-1}$: 5.38 to 6.53) compared to CONT (6.93 $m \cdot s^{-1}$: 5.92 to 7.94, p = 0.31) but not significantly lower for ACC compared to NOEX (6.52 $m \cdot s^{-1}$: 5.70 to 7.34, p = 0.151)

Conclusion. Moderate-intensity walking completed as three separate 10-minute bouts after breakfast, lunch, and dinner compared to a single 30-minute bout after dinner were more effective at reducing markers of cardiometabolic health risk in insufficiently active, apparently healthy adults. Both exercise prescriptions were equally as effective at reducing postprandial glucose concentrations compared to a no-exercise control. Therefore, accumulating exercise in multiple short bouts after each main meal might be more advantageous for overall cardiometabolic health than a single bout after dinner only.

Keywords. Glycaemic regulation, physical activity, accumulated exercise, vascular compliance, postprandial, insulin.

Abbreviations.

ACC	Accumulated exercise
ANOVA	Analysis of variance
AUC	Area under the curve
CAVI	Cardio-ankle vascular index
CONT	Continuous exercise
ECG	Electrocardiograph
$HbA1_c$	Glycated haemoglobin
Hg	Mercury
HRR	Heart rate reserve
HRV	Heart rate variability
MD	Mean Difference
$mmol \cdot L^{-1}$	Millimol per litre
NCD	Noncommunicable disease
NOEX	No exercise
$pmol \cdot L^{-1}$	Picamol per litre
PWV	Pulse wave velocity

T2DM	Type 2 Diabetes Mellitus
$\dot{V}O_{2peak}$	Peak volume of oxygen consumed

Author contributions

PS, MK and BG conceived and designed the study. CW analysed the food diaries and prepared the individual food plans. MK developed the software to undertake the HRV analyses. PS, DW and BG conducted the experiments, PS, MK, NT and BG analysed the data and PS wrote the manuscript. All authors edited and approved the final manuscript.

Introduction

Insufficient physical activity is a well-established risk factor for the development of noncommunicable diseases (NCD) including cardiovascular disease and type 2 diabetes (World Health Organization 2018). Furthermore, known factors that increase the risk of NCDs such as obesity and hypertension are more likely to occur in people with low levels of physical activity participation (World Health Organization 2018). International physical activity guidelines have long advocated that individuals undertake regular exercise for health and fitness, yet worldwide up to 40% of adults fail to achieve recommended amounts of physical activity (World Health Organization 2018). Guidelines around the world, including the US and Australia, suggest that adults can accumulate the recommended amount of physical activity for health in multiple short bouts throughout the day (Piercy et al. 2018; Australian Government Department of Health 2014). Accumulating exercise might be an effective strategy for overcoming the most frequently stated reason for being insufficiently active, lack of time (Netz et al. 2008). Therefore, the notion that exercise can be accumulated in multiple short bouts for health and fitness is intuitively appealing.

Previous research comparing the effects of accumulating aerobic exercise in multiple short bouts throughout the day to a single bout once daily differ in their approach with respect to the number, intensity and duration of the bouts. In a 24-week study involving 134 healthy, sedentary postmenopausal women the mean blood glucose declined more in the group undertaking two 15-minute bouts of walking than a group undertaking a single 30-minute bout without any stastically significant changes in fasting plasma insulin or the 2-hour insulin following a glucose tolerance test (Asikainen et al. 2003). Similarly, in a study involving women with type 2 diabetes mellitus (T2DM) Baynard et al. (2005) reported that a single day of 30 minutes of aerobic exercise undertaken as either a single bout or three 10-minute bouts did not differentially alter glucose control following an oral glucose tolerance test (OGTT). However, when studying nine people (seven male, two female) with insulin

resistance, six one-minute work bouts of treadmill walking at 90% HR_{max} with one minute recovery were reported to be as effective as a single 30-minute moderate-intensity bout at lowering glucose (Francois et al. 2014). Both Pahra et al. (2017) and Reynolds et al. (2016) concluded that accumulating exercise was more beneficial for measures of glucose regulation in participants with T2DM when the total amount (45 minutes and 30 minutes respectively) was completed in three separate bouts as opposed to one continuous bout, however it was unclear whether the intensity of each condition was matched. While exercise is widely accepted as a means to improve cardiometabolic health including glucose control and regulation, it has not yet been determined if accumulating exercise in multiple short bouts throughout the day is more or less benefical than a single bout undertaken once daily.

There is also yet to be consensus for when exercise should be completed in relation to a meal. Exercise in a fasted state has been suggested to reduce the lipaemic response to a meal while postprandial exercise reduces the glycaemic response to a meal (Haxhi et al. 2012). In the limited investigations of accumulated and single-bout exercise, both postprandial (Dipietro et al. 2013) and preprandial (Francois et al. 2014) exercise has been conducted with similar outcomes reported. However, the glycaemic response is likely to be transient with pre-breakfast exercise having no influence on postprandial glucose concentrations following lunch and minimal influence on postprandial glucose response to dinner (Francois et al. 2014). This supports earlier research suggesting that 45 minutes of moderate-intensity exercise initiated 45 minutes after consuming breakfast was effective at reducing glucose and insulin concentrations following breakfast but the effect did not persist following the lunch meal in adults with type 2 diabetes (Larsen et al. 1997). There is evidence to suggest that walking after an evening meal lowers blood glucose concentrations more than walking before the evening meal in adults with type 2 diabetes (Colberg et al. 2009). Therefore, to maximise glycaemic responses and to minimise differences associated with transient responses to exercise completed at different times of day, investigations of

accumulated and single-bout aerobic exercise might be better conducted with the single-bout completed after the evening meal on the same day.

Poor glycaemic control and regulation adversely affect heart rate variability (HRV) and pulse wave velocity (PWV: Benichou et al. 2018; Jarczok et al. 2013; Pober et al. 2004; Salomaa et al. 1995; Ashor et al. 2014). Pulse wave velocity, a surrogate measure of arterial stiffness (Van Bortel et al. 2012) was reported to be improved following aerobic exercise, with further improvements obtained at higher intensity (Ashor et al. 2014). Furthermore, Salomaa et al. (1995) reported that elevated insulin and glucose concentrations were negatively associated with arterial stiffness in a cross-sectional study involving over 4700 men and women aged 45 to 64 years. Similarly, Zheng et al. (2015) reported 30 minutes of aerobic moderate-intensity exercise completed as a single bout and accumulated through two 15-minute bouts, with either 20 minutes or 60 minutes between bouts, reduced arterial stiffness amongst healthy young men. Exercise might be an effective intervention to reduce the negative effects of glycaemic dysfunction on HRV and PWV. Pober et al. (2004) reported that 60 minutes of continuous aerobic cycling improved cardiac autonomic function in healthy male volunteers.

Although the benefits of exercise on cardiometabolic health have been confirmed, there has been no agreement on how exercise should be prescribed (Shambrook et al. 2018; Oja et al. 2018) or whether exercise should be accumulated in multiple bouts throughout the day or completed as a single-continuous bout of equal duration. Therefore the primary aim of this study was to compare the glycaemic and cardiovascular responses to five days of 30 minutes of daily aerobic exercise accumulated in three 10-minute bouts after breakfast, lunch, and dinner, with a single 30-minute bout after dinner, and a no-exercise control in apparently healthy adults who did not meet current activity guidelines (Australian

171 Government Department of Health 2014; Piercy et al. 2018). A secondary aim was to
172 investigate whether participants preferred exercise completed in multiple short bouts or as a
173 single bout.

174

175 Method

176 Individuals were recruited to participate in this three-arm randomised controlled, cross-over
177 trial. Adults aged 18 to 65 years were recruited through social media, posters on community
178 noticeboards, and word-of-mouth. Participants were eligible to take part in the study if they
179 were not meeting current physical activity recommendations (Piercy et al. 2018), were free
180 from any diagnosed cardiometabolic disorder, not taking any medication for cardiac or
181 glycaemic disorders, and had no contraindications to walking. Prospective participants were
182 sent information about the study along with a seven-day food diary to be completed before
183 pre-screening and baseline assessment. Participants provided written informed consent
184 before any measures were collected. The study was approved by the La Trobe University
185 Human Ethics Committee (HEC 15-034) and conducted in accordance with the Declaration
186 of Helsinki.

187

188 A random number generator (www.randomization.com) was used to generate a block-
189 randomised trial order; the outcomes of which were placed in sealed opaque envelopes.
190 The trial order was revealed to the participant and researchers on successful completion of
191 pre-screening and baseline assessment. It was not possible to blind the participants or
192 experimenters to the trial.

193

194 Sample size estimates were based on published data for glucose AUC from a study using
195 similar exercise trials (Baynard et al. 2005). *A priori* power analysis using GPower 3.1

196 (Heinreich-Heine University, Dusseldorf, Germany) calculated that a sample size of 10 was
197 sufficient to determine a medium effect (partial eta^2 0.06 – 0.13) for a three-group repeated
198 measures trial with a power of 0.80 and alpha level of 0.05.

199

200 Pre-screening and baseline assessment were conducted during two separate laboratory
201 visits. On the first visit, participants attended following an overnight fast and having
202 abstained from any exercise for 48 hours. An Adult Pre-exercise Screening Tool (Exercise
203 and Sport Science Australia et al. 2011) was used for health screening and risk
204 stratification. Leisure-time physical activity was assessed using the long-form International
205 Physical Activity Questionnaire (IPAQ Group 2010). Blood samples were collected from an
206 antecubital vein to determine blood lipids, glycated haemoglobin (HbA1c), fasting plasma
207 glucose, and fasting serum insulin. Baseline measurements of stature (Portable
208 Stadiometer; Holtain, Wales), body mass (Model 770 1321004; Seca GmbH & Co.,
209 Germany), and waist and hip girths were recorded. Participants unfamiliar with using a
210 motorised treadmill were familiarised with this equipment.

211

212 On the second visit, participants were fitted with a 12-lead electrocardiograph (ECG;
213 Cardiovit CS-200 Touch; Schiller AG, Baar, Switzerland) to monitor heart rate and rhythm
214 during a graded exercise test. Participants undertook the Balke-Ware graded exercise text
215 (Pescatello et al. 2013) to volitional exhaustion to determine $\dot{V}O_{2peak}$. Respiratory gases
216 were collected and analysed throughout the graded exercise text with an automated
217 metabolic cart (ParvoMedics TrueOne 2400; Sandy, UT). Participants' rating of perceived
218 exertion (Borg's 6 - 20 scale; Borg 1982), heart rate, and manually obtained blood pressure
219 were recorded at three-minute intervals throughout the test. The highest 30-second
220 continuous recorded values obtained during the graded exercise text were used to
221 determine $\dot{V}O_{2peak}$. Following completion of the pre-screening and baseline assessment, an

222 accredited practising dietitian provided individual meal plans from the participants' seven-
223 day food diaries. Meal plans were individually designed to simulate participants' usual diet
224 and matched to their average energy intake and macronutrient composition. All food was
225 produced and sourced from a local kitchen. The same individualised meal plan was
226 provided to each participant for each trial week. Participants were fitted with an
227 accelerometer (Actigraph GTX3; Actigraph LLC, Florida, USA), worn on a wrist band on the
228 participants' non-dominant arm to record physical activity during each trial week.
229 Participants were instructed to remove the accelerometer only to avoid immersion in water.

230

231 Participants completed each of the three trials for five consecutive days, 30 minutes after
232 meals. The exercise trials required participants to undertake 30 minutes of moderate-
233 intensity walking at 55 - 70% of heart rate reserve (HRR: Karvonen et al. (1957)) equivalent
234 to a brisk walk at 4.0 - 6.5 $km \cdot h^{-1}$ (Piercy et al. 2018), as a single bout, 30 minutes after
235 consuming the evening meal (CONT), or three separate 10-minute bouts, 30 minutes after
236 breakfast, lunch, and dinner (ACC). Heart rate (FT60; Polar OY, Finland) was recorded
237 every minute throughout each trial and adjustments made as necessary to the treadmill
238 speed or incline to ensure the required intensity was maintained. For the no-exercise control
239 (NOEX), participants were required to remain seated for a total of 60 minutes following the
240 dinner meal. A wash-out period of at least 14 days was imposed between trials to minimise
241 potential carry-over effects between trials as previously implemented (Bally et al. 2016;
242 Figure 1).

243

244 For all trials, participants attended the laboratory each Monday to Friday morning in a fasted
245 state to consume their prescribed breakfast and then remain seated for 30 minutes.
246 Participants completing NOEX or CONT trials were given lunch and allowed to leave with
247 instructions to eat lunch at approximately midday, then remain seated for 30 minutes.

Participants completing the ACC trial completed the first 10-minute bout of moderate-intensity walking on the treadmill before leaving the laboratory. Participants completing ACC returned for their lunch, consumed their meal, then sat for 30 minutes before undertaking 10 minutes of moderate-intensity walking. All participants returned to the laboratory to consume their evening meal and sit for 30 minutes before completing the relevant condition. On the final morning of each trial week before eating breakfast, participants were fitted with a five-lead ambulatory ECG (FD12 plus; Schiller AG, Switzerland) to record cardiac electrical activity at a sampling rate of 1000 Hz for 24 hours. After the final exercise bout for the week, participants also completed the Physical Activity Enjoyment Scale (Kendzierski and DeCarlo 1991) to evaluate any preference towards either exercise mode. Participants were requested not to change their physical activity or dietary habits throughout the duration of their involvement in this study, which was confirmed verbally at the start of each trial week.

Approximately 12 hours after the final exercise bout of each trial week, participants attended the laboratory following an overnight fast for measurement of arterial stiffness as indicated by PWV, and to complete an OGTT. On arrival, participants assumed a supine position and rested for a minimum of five minutes in a darkened environment. Pulse wave velocity was determined using applanation tonometry according to the manufacturer's instructions (Sphygmocor® XCEL PWA & PWV; AtCor Medical Holdings Ltd, Australia). A teflon cannula was inserted into an antecubital vein to allow regular blood sampling. An intial blood draw was taken for the analysis of fasting glucose and insulin. Participants had five minutes to consume an OGTT solution containing 75 g of glucose in 300 ml of water (Gluco Scan; Daniels Health, Australia). Additional blood samples were taken at 30, 60, 90, and 120 minutes following consumption of the solution for the analysis of plasma glucose and serum insulin. Participants remained seated throughout the OGTT. All blood was collected in BD vaccutainers™ (BD, UK) which were taken to a local pathology laboratory as soon as

possible after collection. The accelerometer, canula, and five-lead ambulatory ECG were removed after completion of the OGTT.

Two-hour glucose and insulin area under the curve (AUC) were calculated using the trapezoidal rule. Insulin sensitivity from the OGTT was determined using the Stumvoll insulin sensitivity index (SISI) (Stumvoll et al. 2000). Data from the SphygmoCor® applanation tonometry were downloaded to assess PWV, a surrogate indicator of arterial stiffness (Vlachopoulos et al. 2010), using Sphygmocor® XCEL software (AtCor Medical Holdings Ltd, Australia). Non-sinus cardiac cycles were excluded from the ECG data that were downloaded and analysed using Medilog Darwin V2 software (Schiller AG, Switzerland). The R-R intervals for all sinus beats (N-N intervals) along with the corresponding time co-ordinates (determined from the time at the beginning of each N-N interval) were imported into a customised software package designed using LabVIEW software (LabVIEW 2016; National Instruments, UK). As previously reported (Kingsley et al. 2005), a 4 Hz sampling frequency was used to re-sample the RR interval data to provide uniformaly sampled data, before being de-trended and windowed using a Hanning window followed by Fast Fourier Transformation of 1 minute segments to provide an estimate of the power spectral density in HF bandwidth (0.15 - 0.40 Hz) and LF bandwith (0.04 – 0.15 Hz). The recommendations of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (Camm et al. 1996) were followed to calculate frequency domain (LF, HF LF/HF power) and time domain (NNmean, NNSD, RMSSD NN50, pNN50) parameters.

Statistical analyses were carried out using IBM SPSS for Macintosh (V25 software; IBM Corp, Armonk, US). One-way repeated measures ANOVAs were performed to compare trial effects. Where appropriate, Bonferroni corrections were applied to account for multiple

300 comparisons. A paired samples t-test was conducted to evaluate participants' preference for
301 the exercise trials. Tests were deemed statistically significant at alpha ≤ 0.05. Unless
302 otherwise stated, all data are reported as mean ± standard deviation or mean difference
303 (95% confidence interval). Small 0.01–0.05, medium 0.06-0.13, and large > 0.14 magnitude
304 of effects were reported using partial eta^2 (Cohen 1988).

305

306 Results

307 Ten adults (m = 8, f = 2 postmenopausal) completed all aspects of the study; their baseline
308 characteristics are at Table 1. Participants were free of any diagnosed cardiometabolic
309 disorder. From the IPAQ, only two participants reported they undertook moderate-intensity
310 leisure-time exercise (for 80 minutes and 100 minutes per week respectively), and zero
311 participants reported completing vigorous intensity exercise. Mean speed and treadmill
312 incline for the ACC trial was 5.4 ± 0.8 $km \cdot h^{-1}$ and 7.7 ± 2.9% respectively, and for the CONT
313 trial 5.1 ± 0.6 $km \cdot h^{-1}$ and 6.4 ± 1.9% respectively. Participants were provided with the same
314 individualised meal plans for each trial week comprising 230 ± 54 $g \cdot day^{-1}$ carbohydrate, 114
315 ± 18 $g \cdot day^{-1}$ protein, and 88 ± 19 $g \cdot day^{-1}$ fat for a mean daily energy content of 9072 ± 1205
316 $kJ \cdot day^{-1}$. Overall physical activity (step count) was not different between trials (NOEX 58039
317 ± 14585, CONT 61732 ± 15462, ACC 64644 ± 13253, p = 0.268, partial eta^2 = 0.338).
318 Participants did not express a preference for either CONT (82 ± 19) or ACC (83 ± 18; $t_{(9)}$ =
319 2.26, p = 0.885) exercise trials.

320

321 Fasting glucose and fasting insulin were not different between the trials (Table 2). There
322 were large intervention effects for glucose AUC ($F_{(2,8)}$ = 4.21, p = 0.010, partial eta^2 = 0.40;
323 Figure 2A) and insulin AUC ($F_{(2,8)}$ = 5.29, p = 0.057, partial eta^2 = 0.27; Figure 2B). Glucose
324 AUC following CONT (917 ± 142 $mmol \cdot L^{-1} \cdot 2h^{-1}$) was not different from ACC (931 ± 191
325 $mmol \cdot L^{-1} \cdot 2h^{-1}$; p = 0.671). However, glucose AUC following NOEX (1037 ± 151 $mmol \cdot L^{-1} \cdot 2h^{-}$

^{1}) was higher than CONT (p = 0.025) and higher than ACC (p = 0.016). Insulin AUC was lower following ACC (51313 ± 13037 pmol·L^{-1}·2h^{-1}) than NOEX (70328 ± 17401 pmol·L^{-1}·2h; p = 0.007). However, insulin AUC following CONT (66150 ± 19185 pmol·L^{-1}·2h^{-1}) was not different to that for NOEX (70328 ± 17401 pmol·L^{-1}·2h-1; p = 0.627). There was a large trial effect for insulin sensitivity that did not reach statistical significance (SISI: $F_{(2,8)}$ = 2.61, p = 0.114; partial eta^2 = 0.225, Table 2).

A large intervention effect was observed for PWV ($F_{(2,8)}$ = 2.91, p = 0.047; partial eta^2 = 0.29). Pairwise comparisons indicated a difference between CONT (6.9 ± 1.4 m·s^{-1}) and ACC (6.0 ± 0.8 m·s^{-1}; p = 0.031), but not between NOEX (6.5 ± 1.2 m·s^{-1}) and CONT (p = 0.265) or NOEX and ACC (p = 0.151).

The exercise completed in these trials (CONT and ACC) did not result in a systematic change in HRV over the 24-hour measurement period when compared to no-exercise. The 24-hour HRV data for the time and frequency domains were not systematically different between the three trials (Table 3).

Discussion

The key finding from this study was that five days of moderate-intensity walking completed as three separate 10-minute bouts after breakfast, lunch, and dinner compared to a single 30-minute bout after dinner were more effective at reducing insulin requirements and pulse wave velocity in insufficiently active, apparently healthy adults. Both exercise prescriptions were equally as effective at reducing postprandial glucose concentrations compared to a no-exercise control. Furthermore, ACC (but not CONT) resulted in a lower insulin requirement than NOEX, even with similar reductions in glucose. It was also determined

351 that PWV was lower for ACC compared to CONT. This is an important finding as studies
352 involving the effects of exercise on arterial stiffness have generally focused on longer
353 duration continuous exercise bouts of 30 minutes or more. Taken together, these novel data
354 suggest that accumulating exercise in multiple bouts throughout the day might be more
355 beneficial for cardiometabolic health than a single bout of similar total duration after the
356 evening meal only. However, whether these results would occur if exercise was completed
357 prior to meal consumption was not assessed.

358

359 In the current study, only the ACC trial was associated with a reduced insulin requirement to
360 facilitate a reduction in glucose. Elevated blood glucose has been demonstrated to increase
361 arterial stiffness (Salomaa et al. 1995). As both the ACC and CONT trials experienced a
362 reduction in glucose AUC, it might be inferred that the reduced insulin requirement following
363 accumulated exercise in this study drove the reduction in arterial stiffness. This hypothesis
364 is consistent with other research investigating the effects of increased insulin concentration
365 on arterial stiffness. In a cross-sectional study of people with T2DM or prediabetes,
366 Salomaa et al. (1995) reported that increases in fasting insulin were positively associated
367 with increased arterial stiffness. This might be due to the residual effects of the insulin
368 released following meal consumption during the separate exercise bouts having a
369 cumulative effect greater on PWV than that from the single 30-minute bout.

370

371 Previous research has shown that the risk of cardiocascular events is reduced by 12-14%
372 for every 1 $m \cdot s^{-1}$ reduction in PWV (Vlachopoulos et al. 2010). In the current study, PWV
373 was 0.9 $m \cdot s^{-1}$ lower when exercise was accumulated throughout the day compared to a
374 single continuous bout, suggesting the result is likely clinically significant. In a systematic
375 review and meta-analysis, Ashor et al. (2014) reported that aerobic exercise improved PWV
376 noting that greater imporvements were observed at higher exercise intensities and in trials

of 10 weeks or more without noting whether the exercise was continuous or accumulated. Two 15-minute bouts of cycling separated by 20 minutes of rest acutely improved PWV compared to a resting control group but the absence of another group undertaking a single 30-minute bout of exercise render it difficult to make any comparision (Kobayashi et al. 2018). Although there has been minimal investigation of the effect of accumulating exercise on arterial stiffness, Okamoto et al. (2018) demonstrated that 30 minutes of treadmill walking, comprising five sets of 3 minutes at 30% VO_{2max} and five sets of 3 minutes at 70% VO_{2max}, resulted in a more persistent reduction in PWV in healthy adults when compared to a single bout of continuous walking for 30 minutes at 50% VO_{2max}. The results from Okamoto et al. (2018) suggest that interval exercise might be better than continuous exercise for reducing PWV. Further research should investigate the effects of habitual accumulation of exercise compared to continuous exercise in order to allow a better determination of which method might be more efficacious in the long-term.

The findings from the current study confirm that exercise is beneficial for improving arterial stiffness. However, this study extends the findings of Zheng et al. (2015), as the results suggest that following multiple days of exercise, changes in PWV can be observed for at least 12 hours after the final exercise bout. Zheng et al. (2015) investigated the acute effects of single sessions of 30 minute continuous exercise bouts or two 15-minute bouts separated by 20 and 60 minutes respectively on arterial stiffness using the cardio-ankle vascular index (CAVI), measured immediately and 60 minutes after the trials. All exercise trials improved CAVI when measured immediately after the exercise had finished. However only the accumulated exercise with the bouts separated by 20 minutes showed a reduction in arterial stiffness when measured 60 minutes after the exercise had finished (Zheng et al. 2015). Therefore, it is possible that repeated bouts of exercise have an additive, accumulated effect on arterial function/compliance that is further enhanced if the exercise is accumulated in short bouts throughout the day on multiple consecutive days.

The current study and that by Zheng et al. (2015) both detected changes in PWV but used different measurement techniques that might make it difficult to directly compare the results. The CAVI is derived from a stiffness parameter (ß) determined from the time and distance of pulses from the heart valve, popliteal, brachial, and ankle pulses. Blood-pressure cuffs with sensors are placed on both ankles, right knee, and upper arms. A phonocardiogram is placed at the second intercostal space on the sternal border and electrocardiogram leads placed on the wrists. In contrast the applanation tonometry technique of the Sphygmocor® utilises a cuff on the femoral artery and a tonometer placed over the carotid artery to determine the femoral-carotid pulse transit time. When comparing different techniques used to assess arterial stiffness Huck et al. (2007) suggested that the requirement to place various sensors and cuffs might have contributed to the greater variance in readings from CAVI technique compared to applanation tonometry measured PWV. Therefore, although both this study and that of Zheng et al. (2015) demonstrated that accumulating exercise had a beneficial effect on arterial stiffness, further research using similar exercise methodologies and techniques to measure arterial stiffness are required in order to confirm or refute the results.

In the current study, there was no evidence to suggest that five days of completing 30 minutes of moderate-intensity aerobic exercise, whether undertaken as a single bout or accumulated in multiple bouts, was sufficient to systematically influence HRV throughout the 24-hour period of measurement that included exercise trials with the same overall volume and intensity in both the CONT and ACC trials but also a no-exercise control. In a previous study of healthy young males completing 60 minutes of moderate-intensity aerobic exercise while consuming standardised meals, positive HRV changes were reported when collected under controlled resting conditions (Pober et al. 2004). In the current study HRV data was collected over a 24-hour period to ensure any changes in HRV resulting from the different

exercise trials were captured that might have dissipated had the data been collected the following morning only. Interpretation of HRV during exercise is difficult as measures of sympathovagal activity at rest behave differently during exercise (Sandercock and Brodie 2006). Therefore, despite the inherent risks of introducing high levels of variance, it might be preferable to consider HRV data gathered over longer durations that embrace exercise as this reflects normal life activities. In the current study there was a high degree of variance in all measurements of HRV recorded, possibly as a result of the largely free-living conditions under which the participants took part in the study and also the apparently good health of the participants. Understanding how trials impact on free-living people is important, as research outcomes in well-controlled experiments might not be replicable beyond the well-controlled conditions of the laboratory. Furthermore, insights might be gained in how exercise can be used to reduce the risks of healthy people developing adverse cardiometabolic outcomes.

Although the results from this study suggest that postprandial exercise can reduce the risk of developing cardiometabolic health conditions, there are some limitations which should be considered. Only the effects of exercise completed 30 minutes after meals were assessed in this study. Future research should consider whether exercising sooner or later than 30 minutes after meals, and also before meals, might have different outcomes. Walking might not affect lipids or lipoproteins as neither were reported to change following 12 weeks of walking three days per week either accumulated as two 10-minute walks, or one 20-minute walk in healthy adults (Murtagh et al. 2005). Furthermore, accumulated exercise and single-bout exercise might be equally as effective at attenuating postprandial lipaemia (Murphy et al. 2009). Therefore, we did not assess the effects of walking on lipid profiles in this study but measuring it in future might be useful to add to the limited evidence base. While all participants consumed the same individually prepared diet for all trials, no attempt was made to match energy intakes or macronutrient content between participants or relative to

458 body mass. Neither was there any attempt to control how much energy participants
459 expended beyond the laboratory other than recording their steps with an accelerometer and
460 asking them to refrain from any exercise they did not normally undertake throughout their
461 involvement in the study. Analysis of blood gases were not undertaken as part of this study
462 as no changes were anticipated due to the exercise intensity (Burton et al. 2004). Blood gas
463 analysis should be considered in future studies involving higher intensity exercise or
464 participants with diabetes or other cardiometabolic disorders that might impact on the acid-
465 base balance or gas partial pressures during exercise. Power was not calculated *a priori* for
466 HRV, therefore differences might exist, but the study was possibly insufficiently powered to
467 detect them.

468

469 Conclusion

470 This study of insufficiently active, apparently healthy adults found that 30 minutes of
471 postprandial modetrate-intensity aerobic walking accumulated in three 10-minute bouts after
472 meals significantly improved arterial stiffness while lowering insulin requirements in
473 comparison to a single 30-minute bout after the evening meal alone. There was no
474 difference between the accumulated or continuous exercise prescriptions for 2-hour glucose
475 AUC, but both trials were different from the no-exercise control. Therefore, accumulating
476 exercise in multiple bouts throughout the day might be more beneficial than a single bout for
477 reducing the risk of developing cardiometabolic diseases such as diabetes and
478 cardiovascular disease.

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Table 1. Baseline characteristics of participants (n = 10, 8m, 2f).

Variable	Measure
Age (y)	50 ± 12.6
BMI ($kg \cdot m^{-2}$)	29.0 ± 5.4
Systolic blood pressure (mm Hg)	129 ± 13
Diastolic blood pressure (mm Hg)	86 ± 10
Waist (cm)	91.3 ± 15.8
Hip (cm)	106.1 ± 9.3
Waist/Hip ratio	0.9 ± 0.1
$\dot{V}O_{2peak}$, ($ml \cdot min^{-1} \cdot kg^{-1}$)	31.5 ± 7.1
$HbA1_c$ (%)	5.5 ± 0.2
Total Cholesterol ($mmol \cdot L^{-1}$)	6.1 ± 1.1
HDL-C ($mmol \cdot L^{-1}$)	1.3 ± 0.2
LDL-C ($mmol \cdot L^{-1}$)	3.9 ± 0.6
Triglycerides ($mmol \cdot L^{-1}$)	2.2 ± 2.4

Note: BMI = Body Mass Index; $HbA1_c$ = Glycated Haemoglobin; HDL-C = High Density Lipoprotein Cholesterol; LDL-C = Low Density Lipoprotein Cholesterol

Values are mean ± SD

609

610 Table 2. Measures of glucose control and insulin sensitivity.

Measure	NOEX	CONT	ACC	Partial eta^2	p
Fasting Glucose (mmol·L^{-1})	4.9 ± 0.3	4.8 ±0.4	5.0 ± 0.4	0.284	0.081
Fasting Insulin (pmol·L^{-1})	48.0 ± 36.1	51.0 ± 32.23	34.9 ± 17.6	0.255	0.089
Stumvoll ISI	0.07 ± 0.05	0.08 ± 0.04	0.09 ± 0.03	0.225	0.101

Note: ACC = Accumulated exercise; CONT = Continuous exercise; ISI = Insulin sensitivity index; NOEX = No exercise

Values are mean ± SD

611

Table 3. Measures of heart rate variability and vascular compliance. Heart rate variability measures obtained from 24-h ambulatory, five-lead ECG. Pulse Wave Velocity measures obtained from SphygmoCor® applanation tonomotery.

Variable	NOEX	CONT	ACC	Partial eta^2	p
NN50 (n)	4828 ± 3016	5147 ± 2998	6068 ± 4324	0.065	0.546
PNN50 (%)	4.5 ± 3.5	5.0 ± 3.3	5.9 ± 4.5	0.069	0.525
RMSSD (ms)	24.2 ± 5.7	24.1 ± 6.6	26.6 ± 8.2	0.099	0.390
NNSD (ms)	37 ± 1	38 ± 1	42 ± 1	0.150	0.232
RRmean (ms)	770 ± 9	759 ± 9	790 ± 7	0.128	0.293
VLFpower ($m \cdot s^{-1}$)	510 ± 307	526 ± 278	624 ± 301	0.128	0.293
LFpower ($m \cdot s^{-1}$)	889 ± 463	882 ± 579	1089 ± 742	0.131	0.283
HFpower ($m \cdot s^{-1}$)	200 ± 98	204 ± 106	263 ± 231	0.090	0.428
LF/HF	4.4 ± 0.9	4.7 ± 1.4	4.5 ± 1.2	0.046	0.654
PWV ($m \cdot s^{-1}$)	6.55 ± 1.2	6.9 ± 1.4	6.0 ± 0.8	0.288	0.047

Note: ACC = accumulated exercise; CONT = continuous exercise; NOEX = no exercise; n = number; NN50 = number of RR intervals differing by more than 50 milliseconds; PNN50 = percentage of RR intervals differing by more than 50 milliseconds; PWV = Pulse Wave Velocity; RMSSD = root mean squared difference of successive RR intervals; RRSD = standard deviation of RR intervals, RRmean = mean RR interval; VLF power = very low frequency power; LFpower = low frequency power; LF/HF = low frequency, high frequency ratio; PWV = pulse wave velocity.

Values are mean ± SD

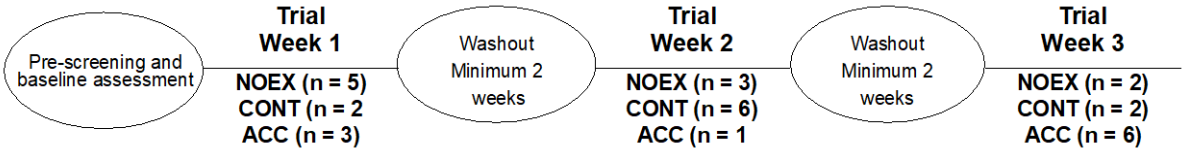

Figure 1. Study flow of participants during this three-arm randomised controlled, cross-over trial. Note: ACC = accumulated; CONT = continuous; NOEX = no exercise.

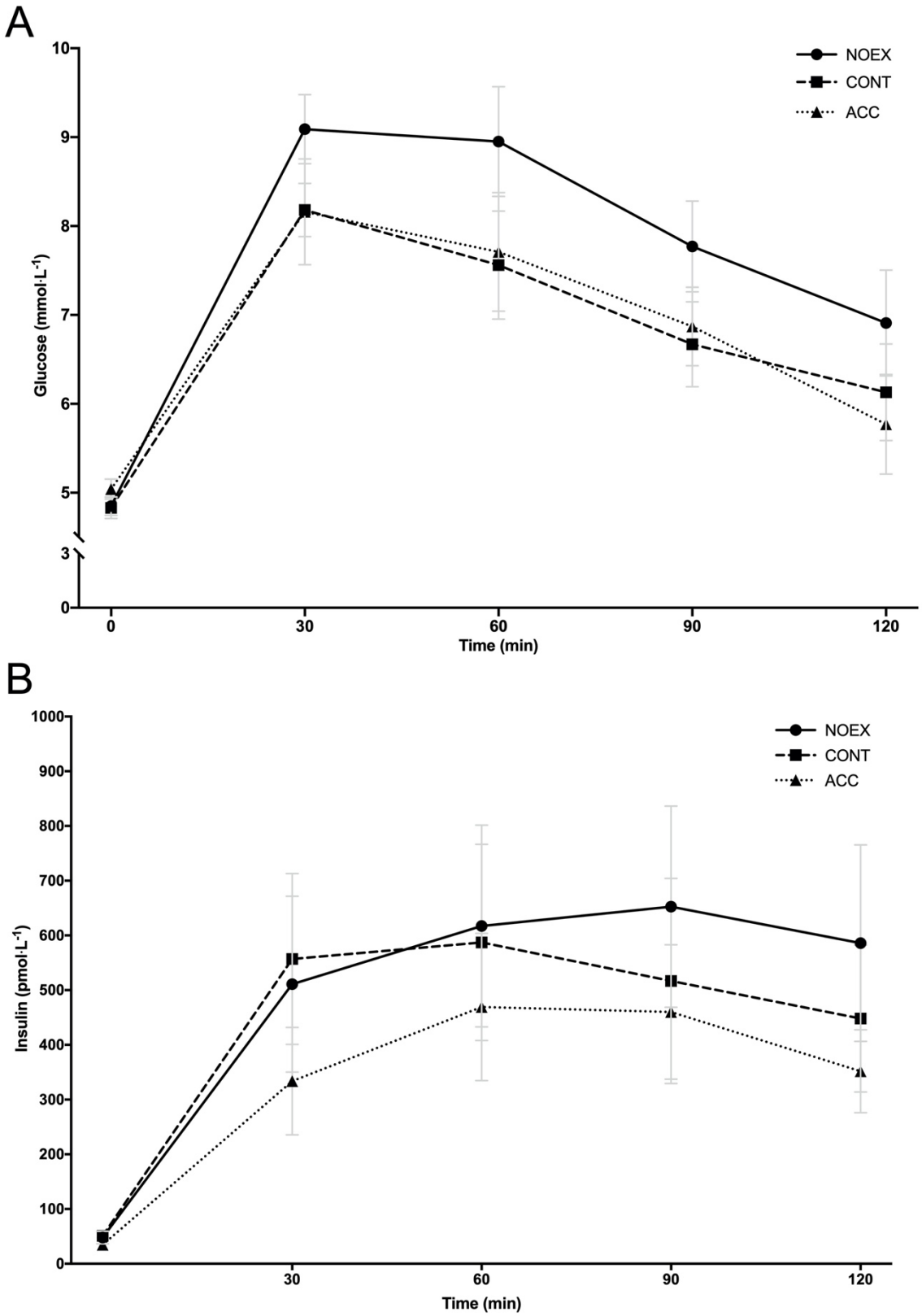

Figure 2. Two-hour oral Glucose Tolerance Test. Fasted blood draw at Time = 0. Blood drawn at 30 minutes intervals following ingestion of oral glucose tolerance test solution for

measurement of A) Glucose and B) Insulin. Note: ACC = accumulated; CONT = continuous; NOEX = no exercise.