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Title Page

Title: Reliability of oscillometric central blood pressures responses to lower limb resistance exercise

Running title: Central blood pressure responses to exercise

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Abstract

Background and aims: Although it is well known that resistance training (RT) is beneficial for patients suffering a variety of cardiovascular diseases, it remains underutilized as a rehabilitation tool as there is no reliable way to monitor the additional stress placed on the central organs. The current study aimed to determine between-day reliability of central haemodynamic indices determined using oscillometric pulse wave analysis (PWA) during progressive sub-maximal RT. **Methods:** Nineteen healthy young males were tested on 3 different mornings in a fasted state. Central hemodynamic variables including augmentation index (AIx), AIx normalized to a heart rate of 75 beats per minute (AIx@75), central systolic blood pressure (cSBP), forwards (Pf) and backwards (Pb) wave reflection, were determined at rest, as well as during leg extension RT at 10, 15 and 20% of maximal volitional contraction (MVC), and following 1 min and 5 min passive recovery. **Results:** During RT at 10, 15 and 20% MVC, the intraclass correlation coefficient (ICC) values for AIx@75 (0.76-0.9), cSBP (0.74-0.78), Pf (0.75-0.82) and Pb (0.75-0.83) exceeded the criteria (0.75) for excellent reliability. During 5 min recovery the ICC values for AIx@75 (0.87-0.87), cSBP (0.69-0.7), Pf (0.63-0.67) and Pb (0.63-0.66) indicated good to excellent reliability. **Conclusions:** Clinically meaningful changes in central hemodynamic indices can be obtained during resistance training using oscillometric PWA devices. This technology holds potential for advancing resistance training prescription guidelines for patients with overt cardiovascular diseases.

Key words: Resistance training, Pulse wave analysis, Augmentation index

Introduction

The inclusion of low intensity resistance training (RT) into both primary and secondary cardiovascular disease-prevention programmes is associated with positive cardiovascular effects ^{1, 2}. However, the American Heart Association Scientific Advisory Statement suggests that the safety of RT in moderate-high risk cardiac patients remains largely unknown, requires further study ³, and when performed, needs close monitoring and good clinical judgement ¹. This paucity of evidence has potentially led to its limited inclusion, or exclusion, from recommended rehabilitation guidelines in many countries ⁴. RT may be more widely incorporated into cardiovascular rehabilitation guidelines with the identification of a suitable method for monitoring the acute stress that RT places on the cardiovascular system. Such a method should be practical, accurate and precise (between-day reliability). Indeed, the method should have sufficient precision to enable clinical exercise physiologists and clinicians to track clinically meaningful changes.

Although it is currently recommended that heart rate and brachial blood pressure responses are recorded during RT as part of cardiac rehabilitation ^{1, 4}, these peripheral hemodynamic responses do not accurately reflect left ventricular load or overall myocardial stress ^{1, 5, 6}. Alternatively, central blood pressures and arterial wave reflection are of high clinical importance ⁵, and can be determined relatively quickly and non-invasively with acceptable accuracy ⁷ and precision ⁸ using oscillometric pulse wave analysis (PWA) ^{9, 10}. However, while a recent study demonstrated that oscillometric PWA can be used during low intensity aerobic (cycling) exercise with acceptable precision ¹¹, no study has examined the reliability of oscillometric PWA during RT. Therefore, the purpose of this study was to determine the between-day reliability of central haemodynamic indices determined using oscillometric PWA during progressive sub-maximal RT in a healthy population.

Materials and Method

This observation study was carried out in accordance with STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines.¹²

Participants

To ascertain the upper limit of reliability, a relatively homogenous healthy cohort of 19 active males (age: 23.3 ± 4.2 yrs; BMI: 26.2 ± 3.1 kg/m²) were recruited. All participants were non-smokers, asymptomatic of any illness, physically active, and were not suffering from any metabolic diseases, nor were they taking any medication known to have vascular actions. All participants provided written informed consent prior to participating in the study. Institutional ethical approval, which conformed to the Declaration of Helsinki and the standards of the journal was obtained prior to data collection and recruitment.

Experimental Procedure

Participants visited the laboratory on four separate occasions. Each session was at least 24 hours apart and all sessions were completed within 10 days from the initial visit. Visit one was used to: 1) determine maximal volitional contraction (MVC) on a double leg-press resistance machine (Pulse Fitness; Congleaton, UK), and 2) familiarise participants with the exercise protocol at 10, 15 and 20% of MVC. For visits 2, 3 and 4 participants attended between the hours of 0730 and 1000 following an overnight fast, consuming only water and having refrained from caffeine for 12 hours and alcohol for 24 hours prior. For each session, baseline measures were collected in an upright-seated position following a minimum of 20 min of quiet rest. This was followed by progressive intensity double leg-press resistance exercise (10, 15 and 20% MVC), with each stage lasting for 5 min. Exercise intensities of 10, 15 and 20% MVC were chosen as clinical exercise physiologists and clinicians are advised to start RT at low intensities during cardiac rehabilitation ¹, in part because this reduces the chance of performing a potentially dangerous valsalva manoeuvre ¹³. For this initial study, the order of exercise

intensity was progressive, and not randomized, to avoid a carry-over effect. At each exercise intensity, brachial blood pressure was assessed on the left arm after 3 min, with the participant continuing to perform leg extensions throughout the cuff inflation and deflation. Following the brachial blood pressure assessment a sub-diastolic recording was measured, during which the participant remained completely still with their legs, arms and head in a fixed, but relaxed position for ~10 s. After both the brachial and sub-diastolic pressures were assessed, which lasted between 60-90s in total, the participant continued to exercise at the same intensity until the 5 min stage was completed. Once all three exercise intensities were completed (15 min in total), participants were asked to rest in a seated upright position on the leg-press machine while a PWA assessment was conducted at post 1 and 5 min.

Determination of one repetition maximum

Each participant's one repetition maximum was predicted from a submaximal double leg-press performance using the Brzycki equation ¹⁴. In brief, the protocol consisted of a pulse raiser and exercise specific warm-up (6-10 repetitions at approximately 50% MVC) followed by 2 min of rest. Succeeding this, starting at a self-selected resistance, participants attempted to lift the heaviest weight possible whilst ensuring failure occurred between 7 and 10 repetitions ^{14, 15}. The following equation was used to determine 1RM.

$$\text{One repetition maximum} = \frac{100 \times \text{LOAD}}{(102.78 - 2.78 \times \text{REPS})}$$

LOAD = amount of resistance on the machine in kg
REPS = number of repetitions performed

Exercise protocol

Once seated on the leg-press machine, participants were asked to listen to a metronome and make one complete contraction cycle (knee flexion to 90 degrees and a near complete extension) at 0.33 Hz (one contraction every 3 s), ensuring a smooth movement during both extension and flexion occurred.

Pulse wave analysis

Following standard manufacturer guidelines¹⁶, oscillometric pressure waveforms were recorded on the upper left arm using the SphygmoCor XCEL device (AtCor Medical, Sydney, Australia). Each measurement cycle consisted of a brachial blood pressure recording lasting approximately 60 s, followed by a 10 s sub-diastolic recording. A corresponding aortic waveform was generated using a validated transfer function¹⁷, from which central: systolic blood pressure (cSBP), diastolic blood pressure (cDBP), pulse pressure (cPP), augmentation pressure (cAP), augmentation index (Alx), augmentation index normalized to a heart rate of 75 bpm (Alx@75), forward aortic pressure (Pf), backward aortic pressure (Pb), reflection magnitude percentage (RM%), and sub-endocardial viability ratio (SEVR) were derived. Heart rate and double product (DbPr), an index of myocardial oxygen consumption, were also determined.

Sample size

Sample size calculations were based on the primary outcome cSBP, and presuming a typical error of 6.4 mmHg derived from a previous PWA reliability study using healthy subjects¹⁸. Using magnitude-based inference¹⁹ to estimate the sample size required to detect the smallest beneficial (or detrimental) in a cross-over study, with the maximum chances of a type 1 and 2 error set at 5 and 20% (i.e., very unlikely-unlikely), approximately 15 participants are required to detect a 5 mmHg change (based on the smallest change reported in previous blood pressure studies²⁰). To account for the novel paradigm we oversampled (20 participants).

Statistical analysis

Statistical analyses were performed using Statistical Package for Social Sciences Version 22 (SPSS, INC., Chicago, USA). All data are presented as mean (X) and standard deviation (SD), unless otherwise stated. For each hemodynamic variable a one-way repeated measures ANOVA was used to determine whether a significant change occurred between baseline, exercise and recovery. Effect sizes are reported using partial eta-squared η^2_p , where 0.01, 0.06, and 0.14 represent a small, medium, and large effect, respectively.

Between-day reliability of central haemodynamic parameters was determined by calculating the intra-class correlations coefficient (ICC), standard error of measurement (SEM), and smallest detectable change (SDC). The ICC was calculated using the formula: $SD_b^2 / (SD_b^2 + SD_w^2)$, where SD_b^2 and SD_w^2 are the between and within-subject variance. In accordance with Fleiss²¹, an ICC value above 0.75 is considered to indicate excellent reliability, 0.4 – 0.74 fair to good reliability, and <0.4 poor reliability. The SDC is defined as the critical difference in a parameter that must be exceeded between two sequential results in order for a statistically significant change to occur in an individual²¹. Absolute SDC was calculated using the formula: $1.96 \times SEM \times \sqrt{2}$, where 1.96 corresponds to 95% confidence interval, and SEM was calculated using the equation: $SD_b \times \sqrt{(1-ICC)}$ ²².

Results

Twenty individuals were recruited but the data for 19 participants are reported. All 20 participants completed each exercise protocol, but for 1 participant the XCEL PWA device did not calculate AIx or AIx75 during one visit. The participant who was removed did not discernibly differ from the remainder of the group in terms of demographics or hemodynamic responses.

Leg press responses

Table 1 provides a summary of mean (SD) values for central hemodynamic variables following 20-min seated rest and exercise at 10, 15 and 20% MVC as well as following 1 min and 5 min recovery. With the exception of Alx , there was a large (η^2_p 0.273 – 0.839) and significant ($p < 0.05$) change from baseline to post 5 min recovery.

Table 1. Mean (SD) hemodynamic responses determined during rest, exercise and recovery

		Baseline	10% MVC	15% MVC	20% MVC	Recovery 1 min	Recovery 5 min	P value	Partial η^2
HR (BPM)	X	67	75	84	90	79	74	< 0.0001	0.746
	SD	(12)	(14)	(16)	(16)	(14)	(14)		
MAP (mmHg)	X	86	98	101	106	95	93	< 0.0001	0.715
	SD	(5)	(9)	(8)	(7)	(5)	(6)		
SBP (mm Hg)	X	120	140	147	154	127	124	< 0.0001	0.839
	SD	(6)	(7)	(11)	(13)	(7)	(6)		
cSBP (mm Hg)	X	103	119	121	127	112	109	< 0.0001	0.715
	SD	(4)	(8)	(10)	(9)	(7)	(6)		
DBP (mm Hg)	X	76	84	86	92	83	83	< 0.0001	0.641
	SD	(4)	(7)	(7)	(7)	(5)	(5)		
cPP (mm Hg)	X	28	34	35	36	28	27	< 0.0001	0.569
	SD	(3)	(6)	(6)	(7)	(5)	(3)		
Alx (%)	X	-3	-4	-4	-3	-3	-4	0.986	0.120
	SD	(8)	(7)	(12)	(12)	(13)	(11)		

Alx @ 75bpm (%)	X	-7	-4	-1	3	0	-6	< 0.0001	0.273
	SD	(8)	(7)	(10)	(11)	(12)	(11)		
SEVR (%)	X	177	131	118	110	154	167	< 0.0001	0.838
	SD	(34)	(32)	(28)	(23)	(36)	(38)		
DbPr	X	6945	8902	10107	11460	8907	8153	< 0.0001	0.810
	SD	(1338)	(1835)	(2038)	(2217)	(1756)	(1827)		
Pf (mmHg)	X	25	31	31	33	25	24	< 0.0001	0.578
	SD	(3)	(4)	(5)	(7)	(4)	(4)		
Pb (mmHg)	X	11	13	13	13	11	10	< 0.0001	0.311
	SD	(1)	(2)	(3)	(3)	(2)	(2)		
RM (%)	X	44	41	41	39	45	43	< 0.0001	0.308
	SD	(4)	(4)	(6)	(6)	(4)	(3)		

HR = heart rate; MAP = Mean arterial pressure; cSBP = central systolic blood pressure; DBP = diastolic blood pressure; SBP = systolic blood pressure;

cPP = pulse pressure; Alx = Augmentation index; Alx @75 Augmentation index normalised to a heart rate of 75bpm;

SEVR = subendocardial viability ratio; DbPr = Double product; Pf = Pressure forwards; Pb = Pressure backwards;

RM% = Reflection magnitude; *P* = level of statistical significance for a one way repeated measures ANOVA; X = mean average;

SD = Standard deviation

Reliability

Reliability data for heart rate and central blood pressures are presented in Table 2. All resting baseline measures meet the ICC criterion value (0.75) for excellent reliability (ICC 0.75 – 0.89). All variables assessed during each exercise intensity (10, 15 and 20% MVC) also had excellent reliability (0.75 – 0.93), with the exception of cPP which was considered good (ICC 0.72) at 20% MVC. During recovery, heart rate, SEVR, DbPr and SBP all met the criterion for excellent (ICC 0.86 – 0.92), and MAP, cSBP, DBP and cPP met the criteria for good reliability (ICC 0.67 – 0.70).

Table 2. Reliability data for heart rate and blood pressure responses to rest, exercise and recovery

	Baseline			10% MVIC			15% MVIC			20% MVIC			Recovery 1 min			Recovery 5 min		
	ICC	SEM	SDC	ICC	SEM	SDC	ICC	SEM	SDC	ICC	SEM	SDC	ICC	SEM	SDC	ICC	SEM	SDC
HR (beat min⁻¹)	0.88	4.05	11.24	0.91	4.32	11.97	0.93	4.29	11.89	0.90	5.07	14.04	0.87	5.03	13.95	0.91	4.11	11.39
MAP (mmHg)	0.77	2.14	5.94	0.79	3.94	10.93	0.75	3.94	10.93	0.75	3.66	10.15	0.69	2.74	7.58	0.62	3.57	9.90
SBP (mm Hg)	0.82	2.4	6.7	0.78	3.49	9.67	0.87	3.79	10.51	0.79	6.01	16.67	0.81	3.09	8.58	0.77	3.14	8.69
cSBP (mm Hg)	0.75	2.18	6.03	0.77	3.95	10.95	0.74	4.96	13.74	0.78	4.46	12.38	0.70	3.59	9.96	0.69	3.30	9.14
DBP (mm Hg)	0.79	2.04	5.67	0.76	3.57	9.89	0.75	3.49	9.66	0.75	3.45	9.57	0.66	2.74	7.58	0.75	2.33	6.45
cPP (mm Hg)	0.75	1.37	3.81	0.75	3.02	8.38	0.76	3.17	8.78	0.72	3.91	10.84	0.67	2.62	7.28	0.57	2.21	6.12
SEVR (%)	0.89	11.13	30.85	0.87	11.29	31.30	0.88	9.57	26.53	0.82	9.76	27.05	0.91	11.18	30.98	0.92	10.65	29.52
DbPr (%)	0.89	451	1252	0.90	588	1631	0.89	672	1864	0.87	797	2209	0.86	652	1808	0.89	597	1657

HR = heart rate; MAP = Mean arterial pressure; cSBP = central systolic blood pressure; DBP diastolic blood pressure; SBP = systolic blood pressure; cPP = pulse pressure; SEVR = subendocardial viability ratio; DbPr = Double product; ICC = Intra-class correlation coefficient; SEM = Standard error of measurement; SDC = smallest detectable change.

Reliability data for wave reflection response are presented in Table 3. All resting baseline measures met the ICC criterion for excellent reliability (0.75 – 0.89), with the exception of Pf for which the reliability was good (ICC 0.73). All central haemodynamic parameters demonstrated excellent ICC values during each of the exercise intensities (10, 15 and 20% MVC). During recovery, Alx and Alx @75 demonstrated excellent reliability (ICC 0.86-0.9), while Pf, Pb and RM% were considered good (ICC 0.53 – 0.67).

Table 3. Reliability data for central hemodynamic variables and wave reflection responses to rest, exercise and recovery

	Baseline			10% MVIC			15% MVIC			20% MVIC			Recovery 1 min			Recovery 5 min		
	ICC	SEM	SDC	ICC	SEM	SDC	ICC	SEM	SDC	ICC	SEM	SDC	ICC	SEM	SDC	ICC	SEM	SDC
Aix (%)	0.89	2.60	7.20	0.78	3.44	9.55	0.87	4.18	11.59	0.85	4.69	12.99	0.90	4.02	11.15	0.86	4.20	11.64
Aix @75bpm (%)	0.88	2.66	7.37	0.76	3.74	10.36	0.90	3.16	8.76	0.83	4.37	12.10	0.87	4.23	11.72	0.87	4.00	11.09
Pf (mmHg)	0.73	1.31	3.64	0.82	1.70	4.71	0.80	2.44	6.77	0.75	3.34	9.27	0.67	2.08	5.76	0.63	2.35	6.52
Pb (mmHg)	0.76	0.61	1.70	0.83	0.88	2.43	0.77	1.54	4.27	0.75	1.58	4.38	0.63	1.13	3.13	0.66	0.94	2.59
RM (%)	0.75	2.22	6.14	0.77	2.15	5.95	0.76	3.10	8.60	0.77	2.86	7.93	0.63	2.66	7.37	0.53	2.30	6.38

Aix = Augmentation index; Aix @75 Augmentation index normalised to a heart rate of 75bpm; Pf = Pressure forwards; Pb = Pressure backwards; RM% = Reflection magnitude; ICC = Intra-class correlation coefficient; SEM = Standard error of measurement; SDC = smallest detectable change.

Discussion

The American Heart Association Scientific Advisory Statement suggests that the safety of resistance testing and training in moderate-high risk cardiac patients remains largely unknown and requires further study ³, and when performed it requires close monitoring and good clinical judgement ¹. Determining a safe, simple and quick way to monitor the augmented stress placed on the cardiovascular system during RT remains a barrier to clinicians, clinical exercise physiologists and clinical research scientists. The current study demonstrates that during and following RT (10, 15 and 20% MVC), oscillometric PWA can reliably estimate derived central hemodynamic parameters including AIx, AIx@75, cSBP as well as markers of arterial pulse wave reflection (Pf and Pb).

Study limitations and Strengths

In order to better contextualize the present findings, several limitations should first be addressed. First, to ensure the pressure waveforms could be captured with optimum reliability, the sub-diastolic recordings used to derive the central hemodynamic variables were taken during short (~ 10 s) pauses in leg contractions. As such, hemodynamic responses may have been underestimated. Second, to ascertain the upper limit of reliability this preliminary study used a relatively homogenous group of young males. Considering the promising findings, the next logical step is to confirm whether the current findings can be replicated in a cohort with cardiovascular disorders. Third, as the SDC of most parameters gradually rises with a concurrent increase in exercise intensity, defining set cut-off values for the upper limit of exercise may be difficult. Last, it is acknowledged that females were not included in the current study. RT was conducted over a 10 day period to ensure fatigue did not affect hemodynamic variables, over this time period the menstrual cycle may have confounded the haemodynamic responses ²³. However, the current study does indicate that PWA is suitable for monitoring central hemodynamic responses to RT in men. Subsequent investigation is required to determine whether measurement precision is equitable for women, and to determine any confounding influence of the menstrual cycle.

Comparison with previous studies

The current study found that during lower limb RT at 10, 15 and 20 % of MVC, central haemodynamic parameters including: cSBP, Alx, Alx75, Pf, Pb, DbPr and SEVR, can all be measured with excellent reliability in young healthy males. Both Holland ²⁴ and Lim ¹¹ reported excellent reliability (ICC > 0.75) during low intensity (50 watts) dynamic-aerobic exercise (cycling) for MAP, DBP, SBP, cSBP and DbPr. However, Lim ¹¹ reported only adequate (ICC 0.4 – 0.74) reliability of central augmentation at higher exercise intensities (100 and 150 W). A novel finding of the current study is that, during RT, all central hemodynamic variables can be recorded with good-excellent reliability (ICC 0.72 – 0.93).

The central hemodynamic variables that are likely to be of particular interest to clinical exercise physiologists and clinicians are cSBP, Alx75, and Pb. The SDC for cSBP during RT at 10, 15 and 20% MVC was 6, 11 and 14 mmHg respectively. Thus, 6 – 14 mmHg is the critical difference that must be exceeded in order for a significant change to be detected in an individual ²¹. Unfortunately, much of the previous research is limited to peripheral blood pressure responses in healthy patients and those with chronic diseases. However, changes in peripheral BP that occurs after exercise interventions have been shown to exceed 14 mmHg ^{25,26}. For example, previously sedentary patients suffering essential hypertension who participated in an 8-week exercise programme reduced SBP by more than 15mmHg ²⁵. While cSBP may be used to reflect overall myocardial stress, Alx75 and Pb can be used to estimate the proportion of the aortic systolic pressure that is being driven by arterial reflection from peripheral factors, including stiffness of the aorta and the degree of resistance vessel constriction ²⁷⁻²⁹. During and following exercise, it is likely that changes in Alx75 and Pb predominantly reflect changes in the tone of peripheral resistance vessels ^{11,29}. The SDCs for Alx75 and Pb indicate that a 9-12 % and 2.4-4.4 mmHg change, respectively, can be detected in a given individual. However, currently there is no available data to indicate cut points for Alx, as previous studies reported change values that have occurred during dynamic-aerobic interventions ³⁰ and not RT, and no-known study has reported on Pb. However, the SDC for Alx75 during RT in the current study, is superior to the 13-15% reported for

aerobic exercise.¹¹ In addition, the increase in exercise intensity leads to an expected increase in heart rate but Alx remains stable. In contrast, Alx75, which should be heart rate corrected, increases concurrently with exercise intensity. Recently, the appropriateness of using Alx75 has come in to question over inappropriate statistics³¹. This may in part explain the divergence between Alx and Alx75 seen in Table 1. However, questioning the validity of recognised central hemodynamic parameters is beyond the scope of this study.

Some abnormal responses in cardiac patients only occur during exercise recovery, such as abnormal ST-segment morphology³². The current study found that as well as central measures of augmentation, additional measures of myocardial oxygen supply and demand such as DbPr and SEVR which are recommended by ACSM for patient screening³³, could be determined with excellent reliability following RT. For example, SEVR has been shown to be a useful tool in determining cardiac ischemia following exercise in smokers³⁴. Accordingly, the current oscillometric device may be a useful, quick and simple to use tool in identifying post exercise risk in special populations. In addition to recovery of at risk populations, hemodynamic measures following exercise are typically used to assess physical fitness³³. Although not the primary aim of the current study, data in Table 2 and 3 suggest that measures of central augmentation can be determined post RT with excellent day-to-day reliability. As such, these parameters are of use to clinical exercise physiologists when assessing fitness over prolonged periods in at risk populations.

Clinical perspectives and future direction

Whilst it is acknowledged that further research which determines reliability in clinical populations of varying ages and health statuses is needed, the findings of the current study indicate promising utility. In high risk patients, such as (but not limited to) those with cardiovascular disease, peripheral vascular disease, stroke, or heart failure, symptoms of myocardial ischemia may ensue as a result of the elevated blood pressure and increased myocardial work caused by RT¹. As such, it is of paramount

importance to be able to measure the stress placed on the cardiovascular system. The current study suggests that central hemodynamic indices can be reliably determined during and following RT using a simple oscillometric device which could be used by clinical exercise physiologists, clinicians and research scientists to determine risk. Future research should look to determine whether: (i) these findings can be replicated in patients with overt cardiovascular diseases, and (ii) cut-off values for a safe increase in central hemodynamic load during exercise.

Conclusion

In summary, the purpose of this study was to determine the between-day reliability of central haemodynamic indices determined using oscillometric PWA during and following progressive sub-maximal RT. Findings suggest that during and following RT in a healthy population, oscillometric PWA can reliably estimate derived central hemodynamic parameters. Therefore, the use of oscillometric PWA devices may provide clinical exercise physiologists and clinicians with a practical and reliable way of obtaining important central hemodynamic information during and following RT. This technology holds potential for advancing RT prescription guidelines for patients with overt cardiovascular diseases.

Conflicts of Interest

None of the authors listed in this manuscript have any conflicts of interest

Author Contributions

Simon FRYER – Designed, collected and synthesised data, prepared manuscript

Keeron STONE – Designed, collected and synthesised data

Tabitha DICKSON – Designed, collected and synthesised data

James FAULKNER – Designed and synthesised data

Danielle LAMBRICK – Designed and synthesised data

Pablo Corres – Collected and synthesised data

Lauren JERRED – Collected and synthesised data

Lee STONER – Designed, synthesised data, aided in manuscript preparation

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