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Abstract

Purpose. Whole-body vibration therapy (WBV) has been reported to potentially act as an exercise mimetic by improving muscle function and exercise capacity in a variety of healthy and clinical populations. Considering the important role that microvascular blood flow plays in muscle metabolism and exercise capacity, we investigated the muscle microvascular responses of acute WBV to knee extension exercise (KEX) in healthy individuals. **Methods.** Eleven healthy adults (age: 33 ± 2 years; body mass index: 23.6 ± 1.1 kg/m^2) underwent 3 min of WBV, or 3 min of KEX at 25% of 1-repetition maximum, in a randomised order separated by a minimum of one week. Femoral arterial blood flow was measured via Doppler ultrasound and thigh muscle microvascular blood flow was measured via contrast enhanced ultrasound at baseline and throughout the 3-min post-intervention recovery period. **Results.** Both WBV and KEX significantly increased peak microvascular blood flow (WBV: 5.6-fold; KEX: 21-fold; both $P < 0.05$) during the 3-min recovery period. Despite a similar increase in femoral arterial blood flow (~4-fold; both $P<0.05$ versus baseline) and whole-body oxygen consumption measured by indirect calorimetry (WBV: 48%; KEX: 60%, both $P<0.05$ versus baseline) in both conditions, microvascular blood flow was stimulated to a greater extent after KEX. **Conclusion.** A single 3-min session of WBV in healthy individuals is sufficient to significantly enhance muscle microvascular blood flow. Despite KEX providing a more potent stimulus, WBV may be an effective method for improving microvascular blood flow in populations reported to exhibit microvascular dysfunction such as patients with type 2 diabetes.

Key Words: ultrasound, microcirculation, hyperemia, femoral artery blood flow, oxygen consumption, muscle activation

Introduction

Exercise has many health benefits including reducing the risk of chronic diseases (1). Whole-body vibration (WBV) has recently received attention as an alternative or a supplement to traditional exercise due to its proposed exercise mimetic actions. The ability of WBV to act as an exercise mimetic is in part due to oscillations from the vibration causing a reactionary activation of skeletal muscles, activation of sensory and motor neurons (2, 3) increased cardiovascular demand (4-6) and oxygen consumption (5, 6). This has led to the use of WBV for both acute (7) and long-term therapy (8-10) in healthy and clinical populations. While it has long been established that muscle performance during exercise is highly dependent on adequate blood flow to the working muscles (11), the muscle hemodynamic responses of WBV have been poorly characterised.

Muscle contraction (or exercise) stimulates both total limb and microvascular blood flow in skeletal muscle to facilitate the delivery of nutrients, hormones and gases (12, 13). Although exercise increases both macro- and micro-vascular blood flow, it is the effect on the microvasculature that is particularly important for nutrient exchange. This is due to the microvasculature (or capillaries) being in intimate contact with the myocyte allowing for amplified delivery of nutrients during metabolic demand such as contraction (14, 15). Muscle contraction potently increases microvascular blood flow which occurs even with low levels of muscle contraction (16-19). For example, forearm contraction at 25% of maximal voluntary strength every 20 seconds has been shown to maximally recruit the microvasculature (~3-fold above baseline) in healthy humans (18, 19). Other exercises such as calf raises (plantar-flexion exercise) (20, 21), elicit a marked increase in skeletal muscle microvascular blood volume and

flow. Importantly, many of these studies have shown that increases in total limb blood flow through conduit arteries do not always reflect increases in microvascular blood flow (18, 22-24). While studies have shown that an acute bout of WBV increases total limb blood flow (4, 25, 26), it is not known whether WBV also has exercise mimetic actions to increase microvascular blood flow which is important for nutrient exchange.

It is important to determine whether WBV stimulates microvascular blood flow because of the microvascular-based benefits of traditional exercise. For example, muscle microvascular blood flow in response to insulin (or a meal) is blunted in type 2 diabetes which results in impaired muscle glucose uptake and poor glucose regulation (27). Exercise training improves postprandial-related microvascular responsiveness and this directly improves glycemic regulation in people with type 2 diabetes (28). Given the importance of exercise on microvascular-related health outcomes, it is important to characterise the effect of WBV on microvascular blood flow to determine whether WBV can be used as an alternative or a supplement to traditional exercise, particularly in people who are unable or have limited capacity to exercise. However, prior to this it is first necessary to characterise the muscle hemodynamic responses of WBV in healthy individuals. The aim of this study was to characterise the muscle microvascular responses of an acute bout of WBV compared to knee extension exercise (KEX) when matched for the same femoral artery blood flow response in apparently healthy individuals.

Methods

Participants and screening. Twelve healthy adults were recruited though community advertisement of which 11 (5 males and 6 females) completed the study with 1 withdrawal. Females were recruited and tested at any stage during their menstrual cycle and estradiol levels were not measured. Participant characteristics are presented in Table 1. Exclusion criteria for participation included current or previous history of cardiometabolic disease, smoking, and musculoskeletal or other conditions that prevent daily activity. Verbal and written explanations about the study were provided prior to obtaining written informed consent. Participants were asked to refrain from moderate to vigorous physical activity (48 hours) and alcohol and caffeine ingestion (24 hours) prior to all testing sessions. The study was approved by the Deakin University Human Research Ethics Committee. To confirm participants did not have any cardiometabolic risk factors, participants were screened via a medical history, resting blood pressure, and a fasting blood sample which was analyzed at an accredited commercial pathology lab (Australian Clinical Labs, Victoria, Australia) using standard protocols for glucose, insulin, total cholesterol, triglycerides, and glycated hemoglobin (HbA1c). Eligible participants were then invited to attend a familiarization session prior to completing three experimental sessions, separated by a minimum of 72 hours.

Overview of study design. Participants completed a familiarization session and three experimental sessions (Fig. 1). The first experimental session was designed to determine and match femoral arterial (total limb) blood flow responses between WBV and knee extension. This was achieved by measuring femoral arterial blood flow of the right leg after a range of vibration frequencies and comparing and matching this response to that elicited by the KEX protocol.

Once completed, participants were then randomized (and subsequently crossed-over) to undergo two experimental sessions, separated by a minimum of one week, to measure and compare the muscle microvascular blood flow responses to both vibration exposure and knee extension when matched for total leg blood flow.

Familiarization session: Whole-body vibration familiarization and determination of 25% 1-RM knee extension. Participants arrived in the Deakin University exercise laboratory and their height and weight were measured via a standard weight scale and stadiometer. A pneumatic bi-lateral knee extension machine (AIR250; Keiser Corporation, Fresno, CA, USA) was than adjusted for each individual participant and seat position and leg length settings were documented. Participants were provided instructions on correct knee extension technique and allowed to familiarize themselves with the range of motion of the unloaded machine. The right leg was used for all participants and experimental sessions. After a 5-min warm-up on a cycle ergometer at 50-75 Watts, participants returned to the knee extension machine and an initial resistance was selected that was estimated to correspond to their 4-10 repetition maximum. If participants successfully completed more than 10 repetitions, the resistance was incrementally increased between sets, with 5-10 min rest periods, until participant's achieved their 4-10 repetition maximum. Twenty-five percent of 1-RM was calculated using the Brzycki equation (Weight ÷ (1.0278 - (0.0278 × Number of repetitions))*0.25) (29). After adequate rest, participants were then familiarized with the whole-body vibration machine by asking them to stand on a synchronous, side-alternating vibration platform (Galileo Sport, Novotec Medical GmbH, Pforzheim, Germany). Participants were instructed to stand as natural as possible with their feet shoulder width apart with a slight bend in their knees to absorb the vibration and

minimize discomfort at higher frequencies. Participants wore sport shoes, stood flat-footed on both legs in an upright position. They were instructed to look forward and not down at the vibration platform and to rest their hands on the support bar but not to grip it. The standing position was not made too prescriptive to ensure that participants would mimic how they would use these platforms at home, in a gym or a clinic. For this reason foot position was not regimented between participants and thus it is not possible to report on specific amplitude of vibration or acceleration parameters. Based on estimated foot positions, peak-to-peak displacement would be in the range of 6-8 mm which at 12.5 Hz gives an acceleration range of 1.9 g - 2.5 g (where $g=9.81 m \cdot s^{-2}$). The vibration frequency was progressively increased from 5 Hz to 15 Hz, over a 5 min period, to ensure participants were comfortable with the range of frequencies used in the study.

Experimental session 1: The effects of acute whole-body vibration and knee extension on superficial femoral artery blood flow. Participants arrived in the laboratory and rested for 10 min prior to measurement of resting heart rate, blood pressure, and baseline diameter and blood flow velocity of the superficial femoral artery of the right leg via a high frequency L12-5 linear array transducer interfaced to an ultrasound machine (Philips iU22 Ultrasound Machine; Philips Medical Systems, Bothell, WA). Participants then underwent 3-min periods of standing on the vibration platform at a frequency set to 5 Hz, 7.5 Hz, 10 Hz, 12.5 Hz and 15 Hz, in a randomized order and interspersed by a minimum of 5 min recovery between frequencies. Following the last frequency, and after a minimum of 5 min rest, participants then underwent a 3-min 25% 1-RM knee extension protocol consisting of three initial contractions over 3 seconds, followed by a single contraction every 15 seconds (15 total contractions). Heart rate, blood pressure and

superficial femoral artery blood flow (artery diameter and blood flow velocity) were measured before and 15 seconds after each individual vibration frequency and after the KEX protocol. The vibration frequency of 12.5 Hz produced a similar peripheral hemodynamic response (femoral arterial blood flow) to that of the KEX protocol (see Results and Fig. 2). As such, the frequency of 12.5 Hz was selected to be the prescribed frequency for the 3-min WBV intervention in the subsequent experimental session.

Experimental sessions 2 and 3: The effects of acute whole-body vibration and knee extension on skeletal muscle microvascular blood flow and whole-body energy expenditure. On two separate days, participants arrived in the laboratory after an overnight fast and rested on a hospital bed while an intravenous cannula was inserted into an antecubital vein. While rested, a perflutren lipid microsphere injectable suspension contrast agent (Definity, Lantheus Medical Imaging, North Billerica, Massachusetts) was infused intravenously to measure resting thigh (vastus lateralis) skeletal muscle microvascular blood flow of the right leg. Participants then underwent either the 3-min WBV or the 3-min KEX in a randomized order and then crossed-over for the subsequent visit. Microvascular blood flow was assessed before, immediately after the 3-min intervention and subsequently at 0.5, 1, 2 and 3 min of recovery after cessation of the WBV or KEX protocols (Fig. 1B). During the KEX we were able to acquire contrast enhanced ultrasound (CEU) measurements at 2 and 2.5 min during the KEX when the leg remained rested between contractions. This gave insight as to whether microvascular blood flow had reached steady-state by the end of the 3 min. Due to the continuous leg movements during WBV it is not possible to collect CEU measurements simultaneously while WBV is occurring. Whole-body

oxygen consumption (VO_2) and carbon dioxide production were measured during the protocol via an indirect calorimetry system (Cortex MetaMax 3B, Cortex Biophysik, Leipzig, Germany).

Real-time Contrast Enhanced Ultrasound. Skeletal muscle microvascular blood flow was determined by CEU as previously described (23, 28). 1.5 ml of Definity contrast agent was diluted in 19 ml of saline and infused intravenously at a constant rate of 1.5–2.2 mL/min (infusion rate based on body weight) using a rotating syringe pump (Vue Ject, BR-inf 100; Bracco, Geneva, Switzerland). After 4 min of infusion to allow for whole-body contrast agent equilibrium, baseline microvascular blood flow was measured via CEU using an L9-3 linear transducer positioned (cross-section) over the right vastus lateralis muscle. A series of 3 x 45-second CEU real-time video captures were recorded prior to commencement of the protocol. Single 30-second CEU videos were captured immediately after the protocol, and at 0.5, 1, 2, and 3 min post-protocol. Settings for mechanical index (0.11 for continuous and 1.30 for flash), gain (75%), depth, and focus, were identical between trials.

Digital images were analyzed offline using Qlab (QLAB, Philips Medical Systems, Andover, MA, USA). Raw acoustic intensity was background subtracted (0.5 second frame for baseline and 0.25 second frame for post-protocol) to eliminate signal from larger vessels and tissue artefacts (e.g., tissue and fascia). The acoustic intensity measured from a suitable region of interest was then plotted over time and fitted using the equation $y=A\ (1-e^{-\beta(t-tb)})$, where y is acoustic intensity at time t, tb is the background time, A is plateau acoustic intensity (microvascular blood volume; MBV), and β is the rate constant (a measure of microvascular refilling rate). Microvascular blood flow (MBF) was determined by $A \times \beta$ (30).

Statistical Methods. Data was analyzed using GraphPad Prism version 8.0.0 (GraphPad Prism, La Jolla, California, USA). For time course MBF responses, comparisons of multiple means within a condition were examined using a repeated measures analysis of variance (ANOVA) with a Holm-Sidak post hoc to control for multiple comparisons (all time points versus baseline). Comparisons between interventions of baseline and peak responses for MBF measures, femoral artery blood flow, VO_2, heart rate and blood pressure were performed by a two-factor (Protocol x Timepoint) repeated measures ANOVA followed by a Holm-Sidak post hoc test where appropriate. A priori sample size calculation determined that 12 participants would be needed to detect a 2-fold increase in MBF following WBV (α= 0.05 and power = 90%). One participant withdrew due to a vasovagal episode and therefore only data from the 11 participants who completed the study were used in the final analysis. Data are reported as mean ± standard error (SEM) and statistical analysis conducted at the 95% level of significance ($P \leq 0.05$).

Results

Participant Characteristics

Six females and five males completed the study (age: 33 ± 2 years; body mass index: 23.6 ± 1.1 kg/m^2). All participants were apparently healthy with normal fasting blood glucose (< 5.5 mmol/L), HbA_{1c} (< 6.5 %) and insulin (< 25 mU/L) levels. Participants were not on any medication known to affect glucose or lipid levels.

Superficial femoral artery blood flow

The first testing visit was used to determine which vibration frequency would elicit a similar femoral artery blood flow response to the KEX protocol. Compared to standing with no vibration, femoral artery blood flow was significantly higher after all vibration intensities (Fig. 2A). The KEX protocol elicited a 4-fold increase in femoral artery blood flow (Fig. 2B). Femoral artery blood flow at 12.5 Hz was the lowest frequency that stimulated a comparable 4-fold increase compared to 25% 1-RM knee extension exercise (Fig. 2B). There was no further increase in femoral artery blood flow at the higher frequency of 15 Hz (Fig. 2A). The effect of WBV and contraction on femoral artery blood flow was acute and flow returned to baseline values after 5 min (Fig. 2A). In summary, the vibration frequency of 12.5 Hz was used for comparison to the KEX in the main trial.

Skeletal muscle microvascular blood flow responses

Baseline muscle microvascular responses (MBV, β and MBF) were similar between KEX and WBV (MBV: 7.2 ± 1.4 vs 8.2 ± 1.9 AI; β: 0.09 ± 0.01 vs 0.08 ± 0.01 1/sec; MBF: 0.68 ± 0.23 vs 0.65 ± 0.18 AI/sec, $P>0.05$, Fig. 3) indicating that MBF was not affected by standing on the non-vibrating platform.

Compared to baseline, KEX elicited a significant increase in MBV (4-fold, $P<0.05$) and β (6-fold, $P<0.05$; Fig. 3A and 3B respectively). Consequently, MBF (which is the product of MBV and β) was markedly elevated above baseline (~23-fold, $P<0.05$, Fig. 3C). All microvascular responses to exercise were similar in magnitude during the last minute of real-time contraction (2 min and 2.5 min) and immediately following cessation of KEX (3 min time point) (Fig. 3A-C).

There was a time dependent decay in microvascular responses (MBV, β and MBF) which reached baseline levels 3 min after cessation of KEX (Fig. 3A-C).

Three minutes of WBV did not change MBV when data from all participants were collated together for each time point following WBV (Fig. 3D). However, each participant had a different microvascular time course following WBV and all participants displayed an increase in MBV at some time point. Five participants had an immediate increase in MBV in response to WBV when compared to baseline which declined over time (see Figure, Supplemental Digital Content 1, microvascular blood volume response after 3 min WBV, http://links.lww.com/MSS/C80). The other six participants had the opposite time course with no increase in MBV immediately after WBV when compared to baseline but increasing and peaking to a level above baseline over time (see Figure, Supplemental Digital Content 1, microvascular blood volume response after 3 min WBV, http://links.lww.com/MSS/C80). Due to the different microvascular time course in response to WBV, the data were also analysed by assessing the peak response of each participant. Peak muscle MBV in response to WBV was elevated by 2.5-fold above baseline ($P<0.05$; Fig. 3G) compared to the 4.0-fold increase with KEX ($P<0.05$). Although WBV produced an exercise mimetic action on MBV, the response to leg exercise was significantly higher that WBV ($P<0.05$, Fig. 3).

Three minutes of WBV increased microvascular blood flow velocity (β) 3.4-fold compared to baseline (Fig. 3E) and quickly returned to baseline values within 30 seconds. Peak β in response to WBV was 3.6-fold higher than baseline ($P<0.05$; Fig. 3H) whereas KEX elicited a 6.2-fold

increase from baseline (P<0.05; Fig. 3H). Although WBV produced an exercise mimetic action on □, the response to KEX was significantly higher than WBV (P<0.05, Fig. 3).

Immediately following WBV skeletal muscle MBF was 5.1-fold higher than baseline (P<0.05, Fig. 3F). MBF decreased quickly in the 30 sec following WBV, and while it appeared to remain above baseline for the 3 min into recovery, none of the other time points were statistically different from baseline. However, as above, participants had a varied response in the time course of MBF response following WBV. When the peak MBF□for each participant was calculated, irrespective of the time, WBV elicited a 5.6-fold increase in MBF above baseline (P<0.05; Fig. 3I). However the response to KEX (23-fold increase) was significantly higher than WBV (P<0.05, Fig. 3). Representative ultrasound images of the vastus lateralis and representative data with corresponding curve fit results for baseline and post-intervention are shown in Figure 4.

Oxygen consumption, heart rate, and blood pressure during WBV and knee extension

Whole-body VO_2, heart rate and blood pressure were similar at baseline between WBV and KEX. VO_2 increased to a similar degree in the first minute for both KEX and WBV, and remained significantly elevated throughout the 3-min intervention (P<0.05; Fig. 5). Heart rate did not increase from baseline during WBV (72 ± 3.1 vs 69 ± 2.6 bpm, P>0.05), but was significantly elevated following KEX compared to baseline (73 ± 2.6 vs 81 ± 3.1 bpm, P<0.05). Systolic blood pressure did not change significantly from baseline following either condition (WBV: 119 ± 3.2 vs 120 ± 4.4 mmHg; KEX: 119 ± 3.2 vs 122 ± 3.1 mmHg; P>0.05). Diastolic blood pressure however was elevated slightly from baseline following both conditions (WBV: 75 ± 2.6 vs 80 ± 2.2 mmHg; KEX: 75 ± 2.6 vs 81 ± 3.0 mmHg; both P<0.05).

Discussion

Microvascular perfusion represents the capacity for exchange of nutrients, hormones and gases as it is the interface between the blood in the capillaries and the tissue. The major findings of this study are that WBV significantly increases muscle microvascular blood flow but to a much smaller magnitude than exercise, despite WBV and exercise eliciting similar increases in femoral artery blood flow and whole body oxygen consumption. Although 3 min of WBV produced these hemodynamic and metabolic exercise mimetic actions, knee extension contraction produced stronger effects on the microcirculation with greater increases in MBV, β and MBF. WBV may be a therapeutic option for improving microvascular perfusion in at-risk populations who find it difficult to do traditional exercise.

While the main objective of this study was to determine if WBV could increase skeletal muscle microvascular perfusion, it was first necessary to determine a vibration frequency that elicited an increase in leg blood flow, as measured in the superficial femoral artery. We found that leg blood flow increased above baseline (standing quietly on the vibration platform) even at the low frequency of 5Hz, but a higher blood flow response was achieved at 12.5 Hz with no further detectable increase at 15 Hz (Fig. 2A). Our observations of an approximate 3-fold increase in femoral artery blood flow are not too dissimilar with other studies showing a doubling of popliteal artery blood flow velocity following 26 Hz vibration exposure (4) and ~3-5-fold increases in femoral artery mean blood velocity at frequencies of 5-30Hz (26). Lythgo et al. (2009) also performed a frequency dose-response, however, these were done during 1-min intervals in an isometric squat position and thus difficult to directly compare to this study. While they found a main effect of vibration to increase mean blood velocity, they did not detect

significant differences between the frequencies (26). An increase in skin blood flow in response to WBV has been observed previously in people with type 2 diabetes (31) and during supine whole-body vibration (32), however, skin blood flow does not mirror the effects of limb blood flow (28, 32). Since 12.5 Hz was the lowest frequency to stimulate a comparable increase in femoral artery blood flow to knee extension contraction, this frequency was chosen to investigate the effects of WBV on skeletal muscle microvascular perfusion.

This is the first study to our knowledge to directly measure microvascular perfusion following WBV and present the novel finding that three min of WBV at 12.5 Hz increases vastus lateralis muscle microvascular responses. Furthermore, individual time-course responses after WBV are highly variable as evident by the large variation in the timing of the increase in MBV post-WBV. These different time courses may be reflective of the body position while standing on the platform and the degree of loading on the quadriceps while standing during and after the vibration. A limitation of the study is that acceleration parameters during WBV for each participant were not recorded. Relating acceleration to macrovascular and microvascular responses will be important to follow-up in future studies. An investigation of group characteristics found no group differences in body mass index, body mass or physical activity status between immediate and delayed responders (see Table, Supplemental Digital Content 2, Participant characteristics for groups identified as immediate or delayed responders, http://links.lww.com/MSS/C81). However, there was a trend for more males to be in the immediate response group (4M, 1F) and more females in the delayed response group (1M, 5F; see Figure, Supplemental Digital Content 1, microvascular blood volume response after 3 min WBV, http://links.lww.com/MSS/C80). We are unaware of any sex-specific explanation for a

differential time course in microvascular response but do note that the baseline MBV in the females appear to be higher. Despite an apparent difference in timing of response, the total peak response was similar between the groups, so in this regard the significance in sex differences may be minimal. While this study is not statistically powered for sex specific comparisons, this observation is worth noting and worthy of future investigation. A limitation of the study is that females were not tested during the same phase of the menstrual cycle and estradiol levels were not measured which are important to follow-up.

Despite a significant increase in microvascular responses with WBV, the increase in the microcirculation with knee extension was substantially greater. Contraction at 25% of 1-RM every 15 seconds increased perfusion (MBF) by greater than 20-fold even though the task was of light-to-moderate intensity and not demanding. This level of contraction was used in the current project because it has been shown in the forearm flexors to produce near maximal microvascular recruitment, otherwise known as MBV (18, 19). These disparities in microvascular responses between WBV and knee extension exercise occurred despite a comparable increase in femoral artery blood flow in both conditions. As we have pointed out that changes in MBF do not always mirror changes in femoral artery blood flow, it is possible that higher vibration frequencies could induce greater microvascular blood flow responses than reported here (12.5 Hz), even though femoral artery blood flow does not increase. Limits on the number/volume of microbubbles than can be safely infused into humans prevented us from measuring MBF at different vibration frequencies, but this is worthy of further investigation.

It is well known that skeletal muscle blood flow increases to meet the metabolic demand of contracting muscle (33-35) although the precise molecular pathways are not fully resolved. Metabolic bi-products (eg. inorganic phosphate, carbon dioxide, nitric oxide) and reduced oxygen tension which occurs during contraction can cause vasodilation of the precapillary arterioles increasing the number of open capillaries (capillary recruitment) (13, 36, 37) and reducing vascular resistance. Whether WBV increases skeletal muscle blood flow using similar mechanisms is unknown. The reason why WBV elicited a similar but smaller microvascular response than KEX, despite comparable whole-body energy demands, is likely due to the fact KEX exercise (a dynamic movement) was isolated to a small muscle group of higher metabolic demand which was the specific site that microvascular measurements were taken whereas WBV (a more static movement) likely activated muscles of the entire lower limb and stabilizing muscles of the trunk. Although we did not measure it directly, standing with WBV likely requires much less than 25% of vastus lateralis muscle strength which was required by KEX. We, and others, have shown that bulk blood flow does not always reflect skeletal muscle microvascular blood flow (17, 18, 23, 24) for which these data further support such disparity.

To this end, it is noteworthy that both the WBV and knee extension elicited similar total leg blood flow (Fig. 2B) and oxygen consumption (Fig. 5), suggesting similar metabolic demands for both activities. Whole body VO_2 and femoral artery blood flow were increased following WBV which corroborates other published data (4, 6). In possibly the most convincing data for WBV increasing VO_2, Rittweger et al. used 3 conditions of increasing load with and without WBV and found across all 3 conditions a ~3.5mL/min/kg increase in VO_2 with the addition of WBV. In our study, we observed a slightly lower increase of ~2.1 mL/min/kg, which may be

explained by the lower vibration frequency in our study compared to theirs (12.5 Hz versus 26 Hz). It has been reported that the increases in peripheral blood flow with WBV are minimized with vibration frequencies above 25 Hz (25, 26), potentially through greater sympathetic stimulation (25). We report that WBV did not result in an increase in heart rate or blood pressure compared with standing quietly, an observation seen by others of similar study design (4).

This study is the first to show that even just a short 3-min bout of WBV can increase muscle microvascular perfusion in healthy individuals. Although knee extension exercise provides a more potent stimulus for muscle microvascular blood flow, WBV may be a suitable and effective method for stimulating muscle microvascular blood flow in populations which commonly exhibit microvascular dysfunction, such as patients with type 2 diabetes or heart failure. Future research is required to establish whether the reported cardiometabolic benefits, both acute and chronic, of WBV therapy are in part due to muscle microvascular related improvements or adaptations. For example, it would be useful to know if an acute exposure to WBV was able to improve glycemic control after a meal or if long-term repeated exposure could improve microvascular networks and/or function.

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Figures

FIGURE 1. A. Overall flow of the study design and participant testing.

B. Experimental session overview. Participants underwent 3 min of knee extension exercise, or in a randomized cross-over fashion, 3 min of WBV at 12.5 Hz. Skeletal muscle microvascular blood flow of the thigh was measured before, immediately after, and throughout the 3-min post-intervention recovery period. Indirect calorimetry was measured via a portable metabolic cart at baseline and during the intervention.

WBV, whole-body vibration; KEX, knee extension exercise; FBF, femoral artery blood flow; 1-RM, 1-repetition maximal

FIGURE 2. Femoral artery blood flow A) following 3 min WBV at different vibration frequencies (solid bars) and showing return to baseline by 5 min (grey bars); B) following 3 min of KEX compared with 12.5 Hz WBV; Values are Mean ± SEM. *P<0.05 vs baseline;$ P<0.05 vs 10 Hz.

WBV, whole body vibration; KEX, knee extension exercise

FIGURE 3. CEU results following 3 min of KEX (top panels) or WBV (middle panels). Microvascular blood volume (A, D), β (B, E) and microvascular blood flow (C, F). Note the different y-axis scale for microvascular blood flow (C, F) when comparing KEX vs WBV. Panels G, H and I are the peak values recorded after the intervention for microvascular blood volume, β and microvascular blood flow respectively for both KEX and WBV. For WBV, it was not possible to perform CEU measurements during the vibration so there are no values for 2 and

2.5 min. Values are Mean ± SEM. *P<0.05 vs Base; $^{+}$P<0.05 vs WBV; Base, baseline; nd, not determined

WBV, whole body vibration; KEX, knee extension exercise; CEU, contrast-enhanced ultrasound

FIGURE 4. Representative images of the vastus lateralis showing intensity of microbubbles and curve fits at baseline and following 3 min of WBV or KEX.

Images showing intensity of microbubbles at baseline prior to WBV (A) and knee extension exercise (D) and following 3 min of WBV (B) or knee extension (E). Right side panels showing representative curve fits at baseline and following WBV (C) and KEX (F).

FIGURE 5. VO_2 response during the 3-min KEX and WBV intervention (Mean ± SEM). There was a main effect for time (*, P<0.05 for all time points vs Baseline within each condition) and no difference in the response between conditions (WBV vs KEX, P>0.05).

Supplemental Digital Content

Supplemental Digital Content 1-Figure-MBV_2_groups.tif

Supplemental Digital Content 2-Table-participant_characteristics_2_groups.docx

Figure 1

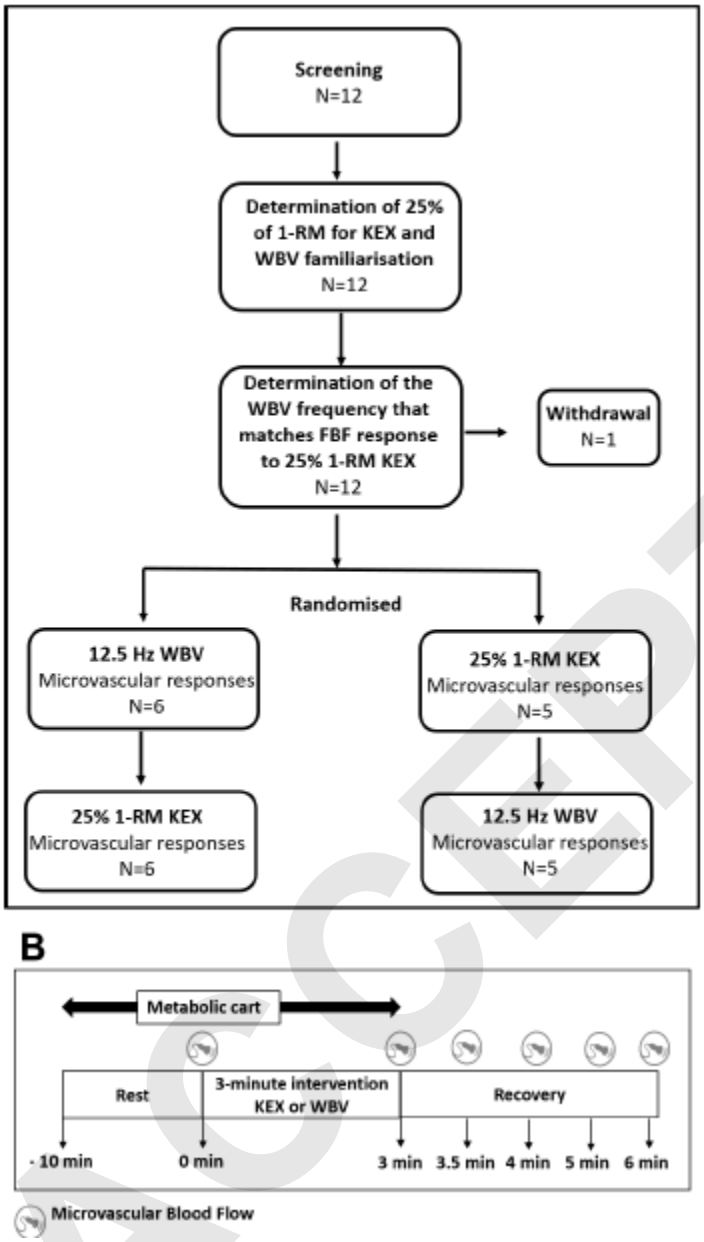


Figure 2

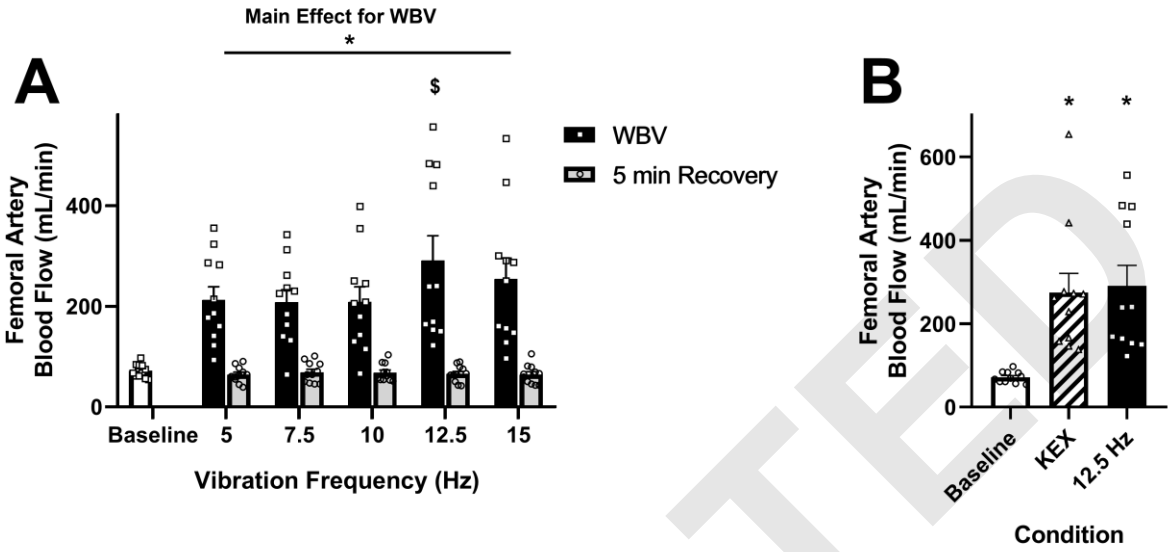


Figure 3

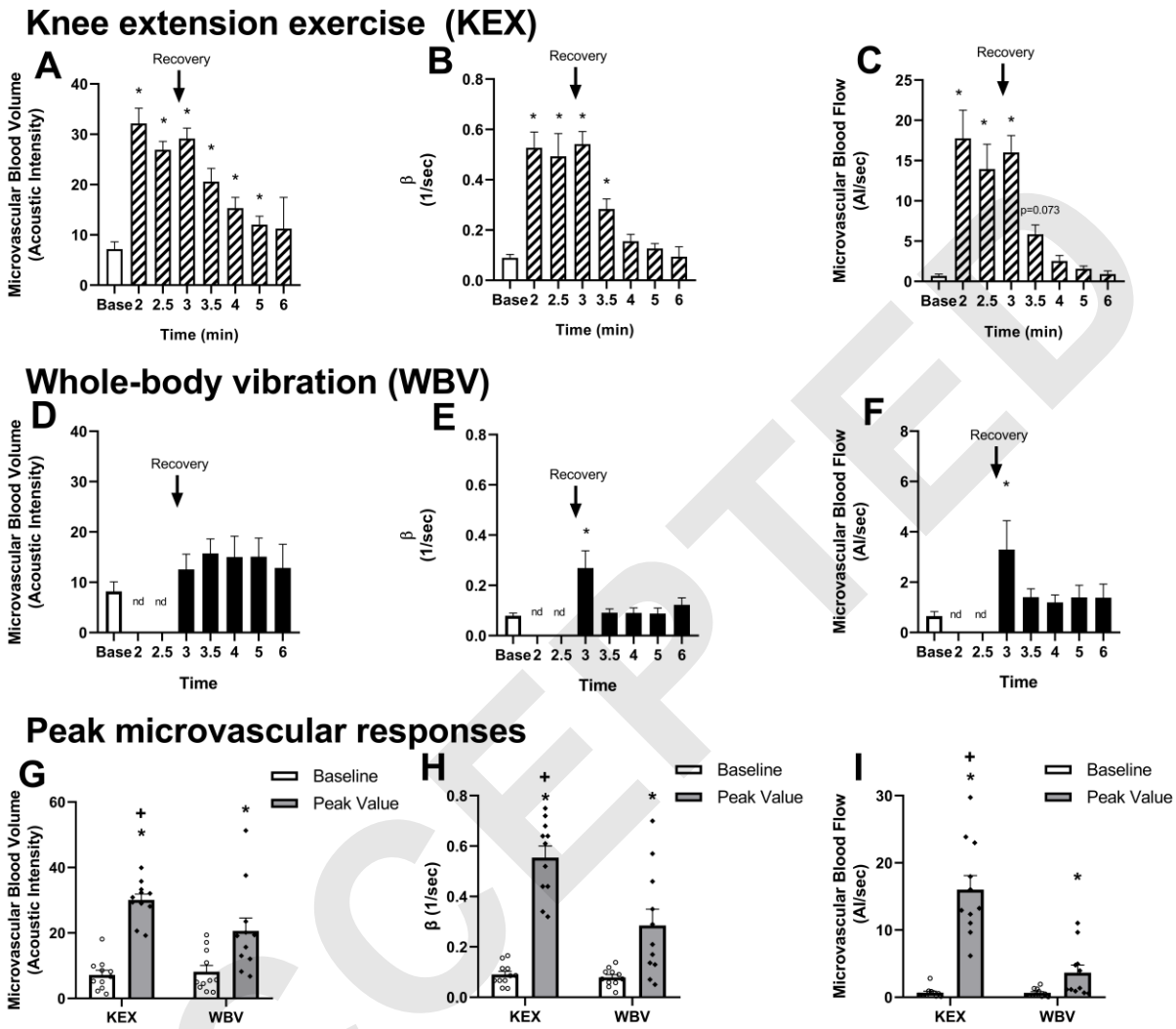


Figure 4

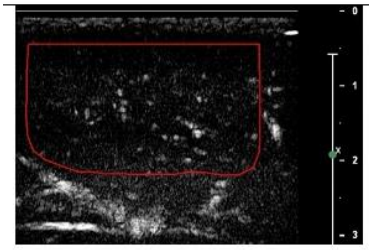
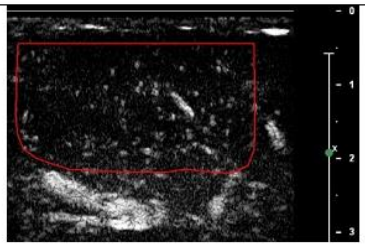
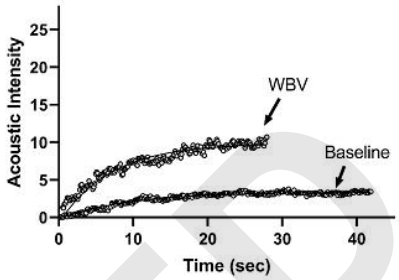
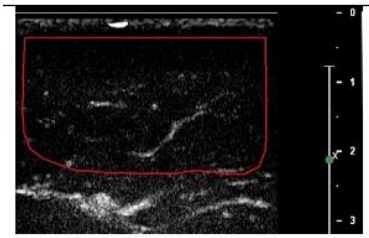
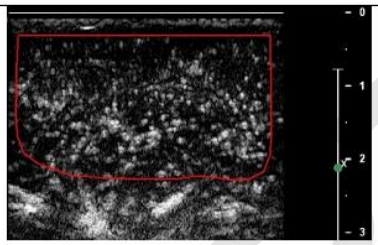
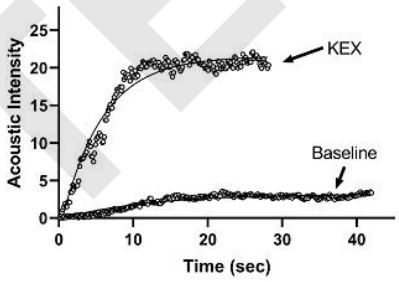
A: Image – WBV: Baseline	B: Image – WBV:3 min	C: Curve fits: WBV
		
D: Image – KEX: Baseline	**E: Image – KEX: 3 min**	**F: Curve fits: KEX**
		

Figure 5

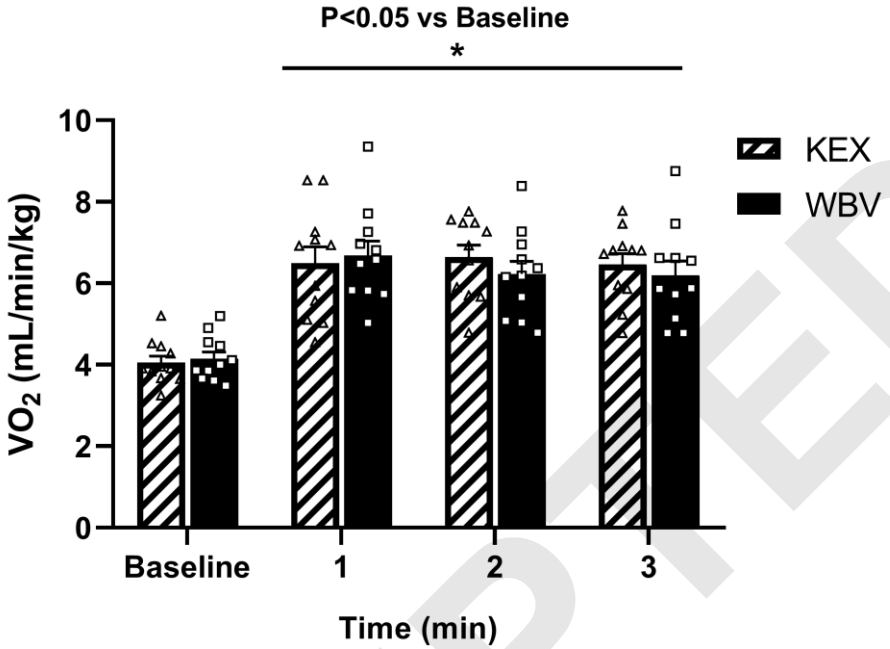
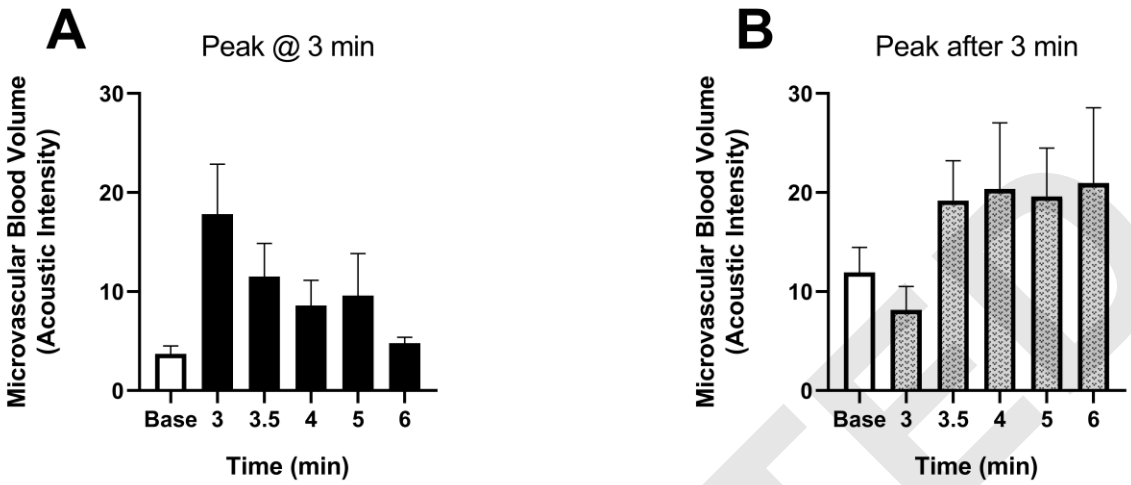


Table 1. Participant characteristics (n=11)

Anthropometrics	
Age (years)	*33 ± 2*
Body mass (kg)	*69.2 ± 4.5*
Height (cm)	*170.5 ± 2.8*
Body Mass Index (kg/m^2)	*23.6 ± 1.1*
Fasting Blood	
Glucose (mmol/L)	4.6 ± 0.1
HbA_{1c} (%)	5.0 ± 0.1
HbA_{1c} (mmol/mol)	31.4 ± 0.9
Insulin (mU/L)	5.9 ± 1.0
Total Cholesterol (mmol/L)	5.4 ± 0.3
HDL (mmol/L)	1.7 ± 0.1
LDL (mmol/L)	3.2 ± 0.3
Triglyceride (mmol/L)	1.0 ± 0.1

Abbreviations : HbA_{1c}, glycated haemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Data are mean ± SEM.

SDC 1



Supplemental Digital Content 1 Figure. Microvascular blood volume (MBV) response (Mean ± SEM) after 3 min WBV showing varied time courses in achieving peak MBV. Due to the different time course of microvascular blood volume increases post WBV, the participants were separated into two groups and their data for each time point averaged within that group to highlight the increases in MBV that are otherwise masked when all 11 participants were averaged together at each time point (Fig. 3D). Left panel (A) – the participants (n=5; 4M, 1F) who had their highest MBV values at 3 min (immediately after WBV). Right panel (B) – participants (n=6; 1M, 5F) who had their highest MBV response after 3 min (i.e. a delayed increase in MBV).

Supplementary Digital Content 2 Table.

Participant characteristics for groups defined in Supplemental Digital Content 1 (figure) as having an immediate peak in microvascular volume following WBV (n=5) and having a delayed peak in microvascular volume (n=6).

	Immediate Peak	**Delayed Peak**	
	Mean ± SEM (Range)	**Mean ± SEM (Range)**	**t-test**
Male:Female	4M:1F	1M:5F	
BMI (kg/m^2)	24.8 ± 1.20 (21.9 - 28.7)	22.6 ± 1.90 (17.9 - 30.5)	0.37
Body Mass (kg)	75 ± 6.6 (59 – 92)	64 ± 6.5 (50 – 87)	0.26
Physical Activity (min/week)	263 ± 153 (0 – 860)	311 ± 80 (105 -630)	0.78

BMI, body mass index; Physical Activity: minutes/week of moderate and vigorous intensity recored by the IPAQ Questionnaire.

Data are mean ± SEM.