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**TITLE**

Venous occlusion plethysmography vs. Doppler ultrasound in the assessment of leg blood flow kinetics during different intensities of calf exercise

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**Abstract:**

*Purpose* It has recently been shown that venous occlusion plethysmography (VOP) can successfully assess the rate of increase in leg blood flow (LBF) (LBF kinetics) responses during calf exercise, but there is lack of data supporting its validity. *Methods* Using Doppler ultrasound (DU) as a criterion standard technique, we tested the hypothesis that VOP would provide similar estimates of LBF kinetics responses as DU during calf plantar-flexion exercise at a range of different intensities. Ten healthy men performed repeated intermittent calf plantar-flexion contractions (3 s duty cycles, 1 s contraction / 2 s relaxation) at 30, 50 and 70% maximum voluntary contraction (MVC) on different days. *Results* Resting LBF values were significantly ( $P < 0.05$ ) larger for DU than VOP but the overall mean LBF responses during exercise were not different ( $P > 0.05$ ) between DU and VOP (30% MVC:  $330 \pm 78$  vs.  $313 \pm 92$  ml/min; 50% MVC:  $515 \pm 145$  vs.  $483 \pm 164$  ml/min; 70% MVC:  $733 \pm 218$  vs.  $616 \pm 229$  ml/min). LBF kinetics analyses revealed that the end-amplitude at the highest intensity (70% MVC) was significantly higher when measured by DU compared with VOP, but all other kinetics parameters were not different between VOP and DU. *Conclusions* Given that these slight differences in amplitude observed during exercise can be explained by differences in vascular regions which the two techniques assess, our results suggest that VOP can accurately assess LBF kinetics responses during calf plantar-flexion exercise at intensities between 30 and 70% MVC.

**Keywords:**

Blood flow, exercise, venous occlusion plethysmography, Doppler ultrasound, validity

**Abbreviations:**

A: Amplitude.

ANOVA: Analysis of variance

CV: Coefficient of variation.

DBP: Diastolic blood pressure.

DU: Doppler ultrasound.

ECG: Electrocardiogram.

ICC: Intraclass correlation coefficient.

LBF: Leg blood flow.

MAP: Mean arterial blood pressure.

MVC: Maximal voluntary contraction.

PBV: Popliteal blood velocity.

PDiam: Popliteal arterial diameter.

SBP: Systolic blood pressure.

SD: Standard deviation

TD: Time delay.

VOP: Venous occlusion plethysmography.

## **Introduction**

The rate at which leg muscle blood flow (LBF) is increased at the onset of exercise (LBF kinetics) has been shown to be closely related to the rate of increase in muscle/pulmonary oxygen uptake at exercise onset (i.e.  $\dot{V}O_2$  kinetics) (MacDonald et al. 1998; duManoir et al. 2010). Given that blunted or slowed  $\dot{V}O_2$  kinetics responses are related to earlier onset of fatigue and reduced exercise tolerance, which is related to morbidity and mortality in all ages (Paterson et al. 2007), it is paramount to accurately quantify and assess the initial increase in the LBF response during exercise. This is particularly relevant for patients with peripheral vascular disease whereby information about the leg hyperaemic response during exercise is of clinical importance in the diagnosis and monitoring of those patients (Green 2002).

Various techniques have been employed to quantify this hyperaemic response to exercise (Radegran 1999) including magnetic resonance imaging (Anderson et al. 2009) and position emission tomography (Heinonen et al. 2007). The most common techniques, often considered as criterion standards, are thermodilution and Doppler ultrasound (DU) (Andersen and Saltin 1985; Gonzalez-Alonso et al. 2006; Koskolou et al. 1997; Radegran and Saltin 1998; Saunders et al. 2005). While these two techniques provide similar estimates of leg blood flow during exercise (Radegran 1997), thermodilution is invasive and has poor temporal resolution for the measurement of the hyperaemic response (Saunders et al. 2005). DU permits continuous measurements on a beat-by-beat basis due to its higher temporal resolution allowing blood flow measurements during exercise; however, it is restricted to single conduit arteries, and thus, it is not suited to assess arterial inflow into limbs that present arterial collateralization due to vascular disease.

Venous occlusion plethysmography (VOP) is a common non-invasive, blood flow measurement technique that measures whole limb blood flow by assessing the changes in limb circumference and is therefore, a particularly useful method for patients with diseases affecting vascularisation of the lower limb. VOP is a well established technique in the study of vascular control and adaptation that is generally used under resting or immediately after exercise conditions given that the method requires that the limb is immobilized during the period of assessment (Joyner et al. 2001). Under these conditions conflicting evidence exists regarding the accuracy of this technique and the correlation between VOP and other blood flow measurement techniques, including DU, radioisotope washout or dye-dilution methods (Adiseshiah et al. 1984; Bystrom et al. 1998; Saltin 2007; Bernink et al. 1982; Hiatt et al. 1989; Jorfeldt and Rutberg 1990; Lubbers et al. 1979; Pallares et al. 1994; Tschakovsky et al. 1995; Radegran 1999).

However, Green et al. (2011) recently reported similar estimates of LBF responses during calf exercise between VOP and DU during relaxation periods between intermittent contractions performed across a wide range of intensities (Green et al. 2011). In addition, confirming early findings by Barcroft and Millen (Barcroft and Millen 1939), Green et al. (2011) showed that LBF responses during contractions at different intensities were not greater than LBF values at rest demonstrating that the hyperaemic response is limited during the contraction and it manifests mainly during relaxation (Green et al. 2011).

We have recently shown that VOP can successfully be used to assess LBF kinetics responses during calf exercise in healthy participants (Egaña and Green 2005; Reeder and Green 2012) as well as in participants with type 2 diabetes (MacAnaney et al. 2011; Kiely et al. 2014); but there is lack of data supporting the validity of VOP in measurements of limb blood flow kinetics. Accordingly, the aim of the present study was to investigate the accuracy of VOP as a measurement of leg blood flow kinetics during calf plantar-flexion exercise at different exercise intensities using simultaneous DU measurements as a criterion standard technique. We hypothesised that VOP would provide similar estimates of LBF kinetics responses to exercise as DU.

## **Materials and Methods**

### *Participants*

Ten young men (mean  $\pm$  SD; age:  $28 \pm 4$  yr; height:  $180 \pm 9$  cm; body mass:  $80 \pm 11$  kg, body mass index:  $25.1 \pm 2.2$  kg·m<sup>-2</sup>; leg volume:  $3.38 \pm 0.56$  L) volunteered to take part in this study. All participants were non-smokers with no apparent cardiovascular disease (assessed by medical questionnaire and physical examination as well as ankle-brachial index measurement) and regularly undertook moderate to vigorous exercise at least 2-3 times per week. To avoid diurnal fluctuations in fatigue and overall exercise performance, all experimental sessions were held at the same time of day and participants were required to refrain from heavy exercise and caffeine or alcohol consumption for 24 h before each visit to the laboratory. Each participant gave written informed consent to participate in this study, which was conducted according to the Declaration of Helsinki and approved by the Faculty of Health Science Research Ethics Committee, Trinity College Dublin.

### *Experimental design*

All participants visited the cardiovascular performance laboratory at Trinity College Dublin on two occasions separated by at least 48 hours. On *visit one*, participants were initially familiarised with the calf plantar-flexion

exercise protocol and performed 3 maximal voluntary contractions (MVC) in the prone position separated by one minute from which the highest effort was taken as MVC. Participants rested for ~20 min and then performed five intermittent plantar-flexion exercise bouts of 6 min. The first two bouts were performed at 30% MVC, the 3<sup>rd</sup> and 4<sup>th</sup> bouts at 50% MVC and the last bout at 70% MVC. Each exercise bout was separated by a resting period of 10 min. On *visit two*, participants repeated the same five exercise bouts following the same order and resting periods as per visit one. Testing was conducted in a quiet, darkened room where room temperature was kept constant at ~22°C.

#### *Calf exercise*

Intermittent (3 s duty cycle: 1 s contraction / 2 s relaxation) isometric plantar-flexion exercise was performed with the right and dominant leg while using a custom-built calf ergometer as previously described (Egaña and Green 2007, 2005). The set-up of the ergometer was slightly modified so that participants in the present study were in the prone position (instead of the supine position used in our previous studies). The prone position was used to better identify the popliteal artery for the DU measurements. All measurements were recorded in the horizontal position to remove the effects of hydrostatic pressure and to minimise cardiopulmonary baroreceptor-mediated changes in muscle sympathetic nerve activity (Ray et al. 1993). Briefly, the participant was secured to the ergometer with a harness that minimised the backward displacement of the body during each contraction while hand gripping was not allowed. The participant's foot was attached to an immobile footplate, which is connected to its own strain gauge and against which force can be applied when the participant plantar-flexes the foot. The resultant force was amplified and sampled at 40 Hz before being processed by a PowerLab analog-to-digital converter (ML 795, AD Instruments) and displayed on a screen visible to the participant (Chart v6.0, AD Instruments). Participants used this visual display to control their effort and to conform to specific instructions given by the investigator.

#### *Leg blood flow*

LBF was assessed at rest (only during the 60 s period preceding the first exercise bout at 30% MVC) and during exercise (i.e. during the relaxation periods between intermittent contractions) using VOP and DU simultaneously (see immediately below). LBF measurements were performed by the same operator on both visits.

*VOP and leg blood flow:* Briefly, a mercury-in-silastic strain gauge (Hokanson EC-6, USA) was placed around the widest part of the subject's right calf and connected to the plethysmograph (Hokanson EC-6, USA). A cuff

(Hokanson, USA) placed around the upper right thigh of the subject was inflated to a constant pressure of ~55 mmHg for the duration of the exercise. The pressure of ~55 mmHg was chosen to occlude venous return without interfering with arterial BF (Egaña and Green 2005; Hunt et al. 2016). LBF during exercise was assessed by measuring the change in leg volume detected by the strain gauge over the 2 s relaxation period between contractions (Egaña and Green 2005). To record resting LBF values, the cuff was inflated to ~55 mmHg at 10s intervals for 1 min while the participant rested. The plethysmographic estimates of LBF, expressed relative to the resting limb volume ( $\text{ml} \cdot 100\text{ml}^{-1} \cdot \text{min}^{-1}$ ) were converted to millilitres per minute (ml/min) using an estimate of each participant's leg volume obtained from the water displacement method of the leg (Kaulesar Sukul et al. 1993).

*DU and leg blood flow:* Doppler ultrasound imaging was performed using a Zonare Z.One Ultra apparatus operated in pulsed wave mode (3.5 MHz) with a flat L8-3 transducer, operating at a frequency of 7 MHz. Ultrasound images were triggered on the R-wave pulse of an electrocardiogram (ECG) signal captured using a video capture card (Matrox Orion HD, Matrox, Quebec, Canada) and recorded with a vascular imaging software (Vascular Imager HD: 6.3.3, IO, USA). The gate width was set to 11 mm for all participants and all blood flow measurements were insonated at a  $60^\circ$  angle to standardise measurements (Radegran 1999). To determine transducer placement, the popliteal artery was located using 2D ultrasound with an L3 transducer operating in B-mode. The transducer was manipulated to establish correct alignment along its longitudinal axis and to ensure full isolation of the artery lumen without contamination from the venous signal. Transducer position was recorded by directly marking the skin to assist repositioning of the probe during the test and on subsequent tests. During the test, the investigator manipulated the transducer to preserve the best Doppler signal directed by auditory and visual feedback. Popliteal artery mean blood velocity (PBV) and luminal diameter (PDiam, i.e. end-diastolic diameter) were recorded at each cardiac cycle and analyzed for each duty cycle (i.e. averaged over the 2 s relaxation period) with a custom-designed edge detection and wall tracking software (Medical Imaging Applications, Vascular Research Tools 5, IO, USA). The popliteal blood velocity (PBV) ( $\text{cm} \cdot \text{s}^{-1}$ ) and the PDiam (radius in cm) were used to calculate LBF (ml/min) using DU, as  $\text{LBF} = \text{PBV} \pi r^2 60$ , where  $r$  is the vessel radius. Resting LBF values were recorded at 10 s intervals for 1 min and then averaged.

Preliminary testing revealed that LBF flow responses using both techniques were not different between the two days of testing, and were repeatable as demonstrated by moderate variability, good agreement and consistency. The coefficient of variation for LBF at rest and between min 4 and 6 of the constant load bouts (i.e. steady-state



condition) was between 25 and 28% for DU and between 30 and 35% for VOP, with intraclass correlation coefficients (ICC) between 0.70-0.86 for DU and 0.60-0.91 for VOP.

### *Data Analysis*

LBF responses estimated by DU and VOP for the exercise bouts at each intensity from both exercise days were averaged to yield a temporal profile of LBF for each subject. It was evident that in addition to the two growth phases commonly observed in the literature during low to moderate intensity hyperaemia (Radegran and Saltin 1998; Saunders et al. 2005), a fast decay phase followed the first growth phase in most responses, as recently described (Kiely et al. 2014; Reeder and Green 2012). In addition, a second slow decay (after the second growth phase) was also evident in many participants (30% MVC,  $n=6$ ; 50% MVC,  $n=7$ ; 70% MVC,  $n=3$ ). Therefore, to better represent the physiological responses and to enable a more accurate estimate of the parameters defining the first three phases, a quadraphasic model was fitted in participants in whom a second decay was apparent, but the second decay was not compared across measurement techniques. The following two empirical models represented by their corresponding functions (see below) were fitted to the individual time series of LBF:

Triphasic (two growth and one decay phases) model:

$$y(t) = A_0 + A_1(1 - e^{-(t-TD1)/\tau1})F1 - A_2(1 - e^{-(t-TD2)/\tau2})F2 + A_3(1 - e^{-(t-TD3)/\tau3})F3$$

Quadraphasic (two growth and two decay phases) model:

$$y(t) = A_0 + A_1(1 - e^{-(t-TD1)/\tau1})F1 - A_2(1 - e^{-(t-TD2)/\tau2})F2 + A_3(1 - e^{-(t-TD3)/\tau3})F3 - A_4(1 - e^{-(t-TD4)/\tau4})F4$$

where  $F1 = 0$  for  $t < TD1$  and  $F1 = 1$  for  $t \geq TD1$

$F2 = 0$  for  $t < TD2$  and  $F2 = 1$  for  $t \geq TD2$

$F3 = 0$  for  $t < TD3$  and  $F3 = 1$  for  $t \geq TD3$

$F4 = 0$  for  $t < TD4$  and  $F4 = 1$  for  $t \geq TD4$

$y(t)$  is LBF at time  $t$ ,  $A_0$  is the baseline amplitude,  $A_1$ ,  $A_2$ ,  $A_3$ , and  $A_4$  are the amplitudes,  $TD_1$ ,  $TD_2$ ,  $TD_3$ , and  $TD_4$  are the time delays and  $\tau_1$ ,  $\tau_2$ ,  $\tau_3$ , and  $\tau_4$  are the time constants of the first (rapid growth), second (rapid decay), third (slow growth), and fourth (slow decay) phases respectively. The parameters  $F1 - F4$  are conditional expressions that limit the fitting of a particular phase to the period at and beyond the time delay associated with that phase. The two models were fitted to LBF data using a weighted least-squares nonlinear regression procedure and the

Marquardt-Levenberg algorithm (TableCurve 2D, Jandel Scientific, USA). For all models, data that exceeded the 95% prediction intervals during an initial fit of a model were excluded. The steady-state amplitude, referred to as End A, for the two models was calculated as follows:

Triphasic model:

$$\text{End A} = A_0 + A_1(1 - e^{-(360\text{-TD1}/\tau_1)}) - A_2(1 - e^{-(360\text{-TD2}/\tau_2)}) + A_3(1 - e^{-(360\text{-TD3}/\tau_3)})$$

Quadruphasic model:

$$\text{End A} = A_0 + A_1(1 - e^{-(360\text{-TD1}/\tau_1)}) - A_2(1 - e^{-(360\text{-TD2}/\tau_2)}) + A_3(1 - e^{-(360\text{-TD3}/\tau_3)}) - A_4(1 - e^{-(360\text{-TD4}/\tau_4)})$$

### *Statistical Analyses*

Estimated parameters of the LBF kinetics responses as well as mean LBF responses between VOP and DU at each exercise intensity (30, 50 and 70% MVC) were compared using a two-way repeated measures ANOVA and Tukey's post-hoc test. Similarly, PDiam values at rest, end A<sub>1</sub> and end exercise (using DU) at each exercise intensity were compared using a two-way repeated measures ANOVA and Tukey's post-hoc test. Mean PDiam values at each of the three intensities were compared using the non-parametric Friedman test and Student-Newman Keuls test. To test the consistency of LBF measurements between the two days of testing coefficient of variation (CV = standard deviation/mean\*100) and ICC were performed whereas to test the level of agreement between the two days of testing as well as between the two measurement techniques Bland-Altman plots were constructed. All statistical analyses were performed using SigmaPlot (v12; Systat Software, CA, USA) with the level of significance set to  $P < 0.05$ . All values are expressed as mean  $\pm$  SD (parametric data) or median and interquartile ranges (non-parametric data).

## **Results**

### *Mean estimates of leg blood flow using DU and VOP*

The mean LBF responses obtained during the relaxation phases in each duty cycle (every 3 s) employing both VOP and DU are presented in Fig. 1. Mean resting LBF values were significantly ( $P < 0.05$ ) larger for DU ( $172 \pm 47$  ml/min) compared with VOP ( $73 \pm 35$  ml/min), but the overall mean LBF responses averaged over the 6 min bouts at the 3 exercise intensities were not different between the two techniques (30% MVC, DU:  $330 \pm 78$  ml/min, VOP:  $313 \pm 92$  ml/min; 50% MVC: DU:  $515 \pm 145$  ml/min, VOP:  $483 \pm 164$  ml/min; 70% MVC: DU:  $733 \pm 218$  ml/min,

VOP:  $616 \pm 229$  ml/min).

#### *Mean estimates of popliteal artery diameter and blood velocity using DU*

The mean PDiam and PBV during the relaxation phases in each duty cycle (every 3 s) employing DU are presented in Fig. 3. The overall PDiam averaged over the 6 min bouts at the 3 intensities were significantly ( $P < 0.05$ ) larger at 70% (5.52 [5.47 – 5.58]) and 50% (5.40 [5.38 – 5.42]) compared with 30% MVC (5.30 [5.21 – 5.35]) and were also significantly larger ( $P < 0.05$ ) at 70% compared with 50% MVC intensities. PDiam at the end of exercise were significantly ( $P < 0.05$ ) larger than resting PDiam, and PDiam at the end of A<sub>1</sub> tended to be larger than resting PDiam ( $P = 0.09$ ) within each of the three intensities (main effect time,  $P < 0.05$ ), but were not different among intensities (30% MVC, rest:  $5.26 \pm 0.54$  mm, A<sub>1</sub>:  $5.28 \pm 0.41$  mm, end exercise:  $5.37 \pm 0.51$  mm; 50% MVC: rest:  $5.23 \pm 0.48$  mm, A<sub>1</sub>:  $5.37 \pm 0.45$  mm, end exercise:  $5.37 \pm 0.50$  mm; 70% MVC: rest:  $5.23 \pm 0.48$  mm, A<sub>1</sub>:  $5.50 \pm 0.66$  mm, end exercise:  $5.54 \pm 0.64$  mm).

#### *Leg blood flow kinetics responses using DU and VOP*

LBF kinetics responses obtained using VOP and DU during plantar-flexion exercise at 30%, 50% and 70% MVC are presented in Table 1 and individual plots from a representative individual are presented in Fig. 2 In addition, Bland Altman plots for A<sub>1</sub> and End A parameters for all three intensities between the two measurement techniques are shown in Fig 4. DU estimates of resting blood flow were significantly larger than those measured with VOP ( $P < 0.05$ ). The end amplitude (End A) at 70% MVC was also significantly larger ( $P < 0.05$ ) for DU than VOP. No other parameters of the LBF kinetics responses were different between DU and VOP.

## **Discussion**

To our knowledge this is the first time that leg blood flow kinetics during exercise at a range of different intensities were compared between VOP and DU. In agreement with our hypothesis, the current study provides evidence that VOP is an acceptable measurement technique for the assessment of the dynamic response characteristics of leg blood flow during calf plantar-flexion exercise. Nevertheless, resting LBF values and end-exercise responses at the highest intensity were larger when using DU.

#### *LBF kinetics during exercise*

Immediately at the onset of exercise, the rate of metabolic processes increases significantly. By identifying the

discrete phases of the dynamic response of blood flow to exercise and by quantifying the three parameters of the adaptive response; namely, the time delay from onset of stimulus to onset of response, the time constant or rate of adaptation of the response, and the gain or amplitude of the response, we can better understand its control. Thus, in order to explore the accuracy of VOP as a technique to assess the hyperaemic response of the lower leg, we compared the parameters of these discrete phases with those obtained using DU.

Up until the work of Reeder and Green (Reeder and Green 2012), it was assumed that the dynamic response of blood flow during rest-exercise transitions only consisted of growth phases (Shoemaker et al. 1996a; Radegran and Saltin 1998; Hughson and Tschakovsky 1999; Saunders et al. 2005). However, Reeder and Green (Reeder and Green 2012) showed in healthy participants that this hyperaemic response to exercise is more dynamic than previously thought and consists, in addition to growth phases, of an early rapid decay phase and, depending upon the intensity, a later and slower decay phase; patterns that were also recently observed in individuals with type 2 diabetes (Kiely et al. 2014). The tri- and quadruphasic responses, including growth and decay phases, reported in the current study and obtained using both DU and VOP techniques, are therefore in agreement with this notion. In the current study, as in the study of Reeder and Green (Reeder and Green 2012) short duty cycles (1 s contraction / 2 s relaxation) allowed for better empirical modelling to determine these phases. It is possible that the longer duty cycles used in previous research (Shoemaker et al. 1996a; Radegran and Saltin 1998; Hughson and Tschakovsky 1999; Saunders et al. 2005) may help explain why growth-only LBF responses were observed. As previously reported by Reeder and Green (Reeder and Green 2012), in the present study evidence for the biphasic decay in blood flow was more apparent at 30 and 50% MVC intensities than at the 70% MVC intensity, which may be due to a higher increase in MAP over the latter part of exercise as was the case in the present study (data not shown). This is most likely as a result of an increase in vascular resistance in inactive regions due to an increase in sympathetic nervous activity. Further possibilities may relate to activation of antagonist muscles or the increased variability of blood flow responses at higher intensities.

In the present study, despite some relatively small differences in amplitudes of LBF responses (see immediately below), importantly, the time constant and time delay of all phases of the hyperaemic response at the 3 different intensities were similar between DU and VOP techniques. This suggests that VOP and DU provide similar trends on estimates of the dynamic response of blood flow during plantar-flexion exercise at intensities between 30 and 70% MVC.

### *Amplitudes of LBF at rest and during exercise*

Prior to this study, Green et al. (2011) compared VOP and DU estimates of leg blood flow during relaxation periods between intermittent calf contractions at a wide range of intensities (50 to 400 N) and observed a strong association (Pearson  $r = 0.91$ ) between all paired responses reporting similar LBF values between techniques (248 and 278 ml/min for VOP and DU) (Green et al. 2011). In that study ordinary least products (OLP) regression revealed a slope of 0.942 indicating a proportional bias of ~6% between measurements techniques, VOP showing lower estimates of the hyperaemic response. In addition, OLP regression revealed a y-intercept of -13 ml/min indicating a fixed bias between measurements and that VOP estimates were consistently lower by ~13 ml/min, a difference in LBF that was most likely established at rest (resting DU: 89 ml/min vs. resting VOP: 65 ml/min  $P < 0.05$ ). Green and colleagues suggested that these fixed and proportional biases could be attributed to the fact that DU measures blood flow into vascular regions in the leg and foot which are not assessed by VOP (for more details see (Green et al. 2011)). In particular, the proportional bias between measurement techniques was attributed to the dilation of these additional vascular regions during exercise (Boushel et al. 2000) leading to a larger estimate of the hyperaemic response using DU than VOP (Green et al. 2011).

In agreement with Green et al. (2011), in the present study resting LBF values were significantly lower using VOP compared with DU (72 vs. 172 ml/min); however, the magnitude of the difference was greater in the present study than in the study by Green and co-workers (Green et al. 2011). Resting LBF values using VOP reported in the present study are similar to those observed by previous studies in healthy male participants (Kiely et al. 2014; Green et al. 2011). However, previous observations of resting leg (i.e. popliteal) blood flow values using DU in healthy males are much more variable, as values of 45-50 ml/min (Villar and Hughson 2013), 89 ml/min (Green et al. 2011), 240 ml/min (Hunt et al. 2013) or up to 272 ml/min (Hunt et al. 2016) have been reported in the recent literature. This would suggest that in addition to the differences in the vascular regions assessed by both techniques, errors associated with both techniques (perhaps more so to DU, see *limitations* below) have influenced the values at rest.

Regarding LBF responses during exercise, in the present study hyperaemic responses at intensities equivalent to 30% and 50% MVC were ~6% larger for DU compared with VOP. These findings are consistent with a similar proportional bias (~6%) shown by Green et al. (2011) during calf plantar-flexion exercise at a range of intensities

up to ~50% MVC (Green et al. 2011). At the intensity of 70% MVC in the present study the magnitude of the difference between techniques was larger (~16% larger for DU than VOP) with end-amplitudes at this higher intensity being significantly lower for VOP compared with DU (Table 1, Fig 4 D-F). It is possible that further dilation of the above-mentioned additional vascular regions which are measured by DU but not by VOP and that have been suggested to contribute to the 6% bias up to the intensities of 50% MVC might contribute to this larger proportional bias of 16% at the higher intensities of 70% MVC. The significantly larger overall popliteal artery diameters observed at 70% MVC compared with the 2 lower intensities in the present study supports that further dilation of additional active vascular regions at this highest intensity is likely, although the increase in diameter might also be due to increased blood pressure in the popliteal artery. An alternative reason for this larger proportional bias between techniques at 70% MVC is the build up of venous volume, and therefore, venous pressure, which can result in a reduction of arterial inflow (Tschakovsky et al. 1995) and/or a rise to leakage with the mechanical effect of muscle contraction (Lorentsen et al. 1970; Pallares et al. 1994; Egaña and Green 2005), possibly through the deep femoral vein if the cuff pressure becomes too low to collapse deep veins. Both conditions would further underestimate VOP measurements more than DU measurements. However, in the present study we did not observe evidence of venous outflow (i.e. presence of a plateau or reduction in the pulsatile change in leg volume between contractions) in the VOP recordings at any of the intensities. Even if the amplitudes of the first growth phase ( $A_1$ ) were not significantly different between techniques they tended to be larger for VOP (Table 1, Fig 4 A-C). The reasons for this difference between techniques is not known, but one of the possible reasons, particularly at the lowest workload, relates to an early redistribution of the flow from leg/foot regions to calf muscles which would be detected by VOP but not by DU. The fact that End A estimates detected by VOP were not larger than estimates detected by DU is consistent with this and suggests that this redistributed flow is similar to the additional flow measured at rest by DU.

### *Limitations*

VOP cannot assess blood flow during contraction and this represents a limitation. Another limitation relating to VOP is the fact that the use of the venous occlusion cuff could affect LBF responses during exercise (Radegran 1999; Tschakovsky et al. 1995). Tschakovsky et al. (1995) observed a significant decrease in the blood flow response (measured using DU) from the second to the fourth cardiac cycle when a venous occlusion pressure of 50 mmHg was used compared with no cuff pressure following a sustained contraction of the forearm; however, the initial cardiac cycle was unaffected by the cuff pressure (Tschakovsky et al. 1995). Given that in the present study

each relaxation period included 1 or, at the most, 2 cardiac cycles, it is likely that the use of cuff inflation did not restrict estimates of LBF (or restrictions were very small). Furthermore, Green et al. (2011) compared intermittent plantar-flexion contractions at intensities between 200 to 600 N with and without cuff inflation (to 55 mmHg) using 1s contraction – 2 s relaxation duty cycles, and demonstrated that cuff inflation did not decrease LBF responses (Green et al. 2011).

Using DU, systematic errors in the measurement of arterial diameter, insonation angle and sample volume can cause large errors in measurement of blood flow during exercise, although the extent of these errors and the subsequent impact on the LBF measurements has not been quantified. During low-intensity (8-13% MVC) intermittent exercise muscle contraction has been shown to induce variability in the angle of insonation of ~5% (Shoemaker et al. 1996b), where an error of 3° when the angle of insonation was 55° would overestimate femoral and axillary blood velocities by 8% (Amundsen et al. 2002). This variability in the angle of insonation was likely to be larger in the current study given that higher intensities were investigated and the popliteal artery was employed.

### *Perspective*

In peripheral vascular disease metabolic control and fatigue resistance during exercise are impaired, and these effects are manifested at the onset of contracting calf muscle exercise (Barker et al. 2001; Barker et al. 2004; Bauer et al. 1999). Given that metabolic control and fatigue resistance during exercise are closely linked with the hyperaemic response in active muscles (Hughson et al. 2001), it is critically important to adequately measure the hyperaemic response in these altered muscle responses in order to elucidate the role of the hyperaemic response in these effects (Brass et al. 2004). In limbs with vascular disease complications induced by a collateralized arterial supply, arterial calcification and/or ‘jetting’ effects at the site of DU insonation make the DU technique, a criterion non-invasive technique for such assessment in healthy limbs, not well suited for these measurements in individuals with vascular disease; while VOP would not be influenced by these complications. In addition, in diseases where arteries can be affected by arteriosclerosis (e.g., coronary heart disease, diabetes), atherosclerotic changes to major arteries increase the challenge of assessing luminal diameter and blood flow with DU, whereas no adjustments are needed when using the VOP technique and thus seems to be better suited than DU to the assessment of the hyperaemic response in limbs with sclerotic arteries.

### **Conclusions**

The current study showed similar trends on estimates of dynamic responses of muscle blood flow during calf plantar-flexion exercise at intensities equivalent to 30%, 50% and 70% MVC between VOP and DU measurement techniques considering slight differences in vascular regions assessed by these techniques and the likely sources of errors in both of them. These data suggest that VOP could be used as an alternative technique to DU for the assessment of LBF kinetics in healthy limbs. Further studies are needed to elucidate if VOP is better suited than DU to assess exercise hyperaemia in limbs with vascular disease.

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### **Compliance with ethical standards**

**Conflict of interest** Authors declare that they have no conflict of interest.

### **Figure legends**

**Fig 1:** Mean ( $\pm$  SD) VOP and DU leg blood flow responses at 30% (A), 50% (B) and 70% (C) MVC intensities during calf exercise. Blood flow responses were taken during the relaxation phases of the calf exercise.

**Fig 2.** LBF responses during calf exercise using VOP (left panels) and DU (right panels) in a representative individual. Responses predicted from the fitting of either triphasic function (two growth and one decay phases) or quadruphasic function (two growth and two decay phases) are also shown with solid grey lines. A, VOP at 30% MVC; B, VOP at 50% MVC; C, VOP at 70% MVC; D, DU at 30% MVC; E, DU at 50% MVC and F, DU at 70% MVC). Blood flow responses were taken during the relaxation phases of the calf exercise.



**Fig 3.** Mean ( $\pm$  SD) DU popliteal blood velocity (PBV) and popliteal artery diameters (PDiam) at 30%, 50% and 70% MVC intensities during calf exercise. PBV and PDiam measurements were taken during the relaxation phases of the calf exercise.

**Fig 4.** Bland Altman plots comparing  $A_1$  (left panels) and End A (right panels) leg blood flow responses between VOP and DU. A,  $A_1$  at 30% MVC; B,  $A_1$  at 50% MVC; C,  $A_1$  at 70% MVC; D, End A at 30% MVC; E, End A at 50% MVC and F, End A at 70% MVC. Difference  $A_1 = A_{1DU} - A_{1VOP}$ , Average  $A_1 = (A_{1DU} - A_{1VOP})/2$ ; Difference End A = End  $A_{DU} - End A_{VOP}$ , Average End A = (End  $A_{DU} - End A_{VOP}$ )/2. Solid horizontal lines show mean values and dashed horizontal lines represent 95% confidence limits.

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Table 1: Dynamic response characteristics of the first three phases of leg blood flow obtained with VOP and DU during calf plantar-flexion at 30%, 50% and 70% MVC exercise intensities.

|                                 | 30% MVC (n = 10) |             | 50% MVC (n = 10) |            | 70% MVC (n = 10) |            |
|---------------------------------|------------------|-------------|------------------|------------|------------------|------------|
|                                 | VOP              | DU          | VOP              | DU         | VOP              | DU         |
| A <sub>0</sub> (ml/min)         | 71 ± 33*         | 168 ± 48    | 66 ± 35*         | 171 ± 49   | 73 ± 35*         | 173 ± 47   |
| A <sub>1</sub> (ml/min)         | 301 ± 132        | 210 ± 74    | 448 ± 131        | 372 ± 150  | 677 ± 177        | 656 ± 291  |
| TD <sub>1</sub> (s)             | 0.5 ± 0.3        | 0.6 ± 0.3   | 0.6 ± 0.1        | 0.7 ± 0.2  | 0.6 ± 0.2        | 0.6 ± 0.2  |
| τ <sub>1</sub> (s)              | 3.0 ± 2.6        | 2.6 ± 1.4   | 2.6 ± 1.2        | 2.7 ± 1.4  | 3.9 ± 1.6        | 3.5 ± 1.1  |
| A <sub>2</sub> (decay) (ml/min) | 116 ± 52         | 120 ± 90    | 165 ± 50         | 169 ± 95   | 265 ± 78         | 258 ± 130  |
| TD <sub>2</sub> (decay) (s)     | 11.9 ± 7.4       | 12.7 ± 4.6  | 10.7 ± 3.2       | 10.5 ± 3.5 | 12.3 ± 3.4       | 11.2 ± 2.5 |
| τ <sub>2</sub> (decay) (s)      | 7.3 ± 7.4        | 5.7 ± 2.9   | 5.7 ± 4.2        | 6.5 ± 4.9  | 5.3 ± 2.7        | 3.3 ± 2.0  |
| A <sub>3</sub> (ml/min)         | 98 ± 46          | 103 ± 82    | 185 ± 59         | 214 ± 78   | 181 ± 67         | 227 ± 91   |
| TD <sub>3</sub> (s)             | 24.7 ± 9.5       | 22.9 ± 7.3  | 22.5 ± 5.6       | 22.6 ± 6.1 | 21.4 ± 4         | 20.1 ± 4.5 |
| τ <sub>3</sub> (s)              | 20.4 ± 10.5      | 27.2 ± 19.7 | 19.0 ± 11.9      | 16.1 ± 7.3 | 9.1 ± 4.9        | 11.0 ± 3.9 |
| End A (ml/min)                  | 295 ± 92         | 307 ± 75    | 476 ± 159        | 512 ± 137  | 621 ± 217*       | 785 ± 260  |

Data are means ± SD. A<sub>0</sub> baseline amplitude; A<sub>1</sub>, A<sub>2</sub> and A<sub>3</sub>, amplitudes, TD<sub>1</sub>, TD<sub>2</sub> and TD<sub>3</sub>, time delays and τ<sub>1</sub>, τ<sub>2</sub> and τ<sub>3</sub>, time constants of the first (rapid growth), second (rapid decay) and third (slow growth) phases respectively.

\* significantly different than DU within same exercise intensity ( $P < 0.05$ )

Figure 1

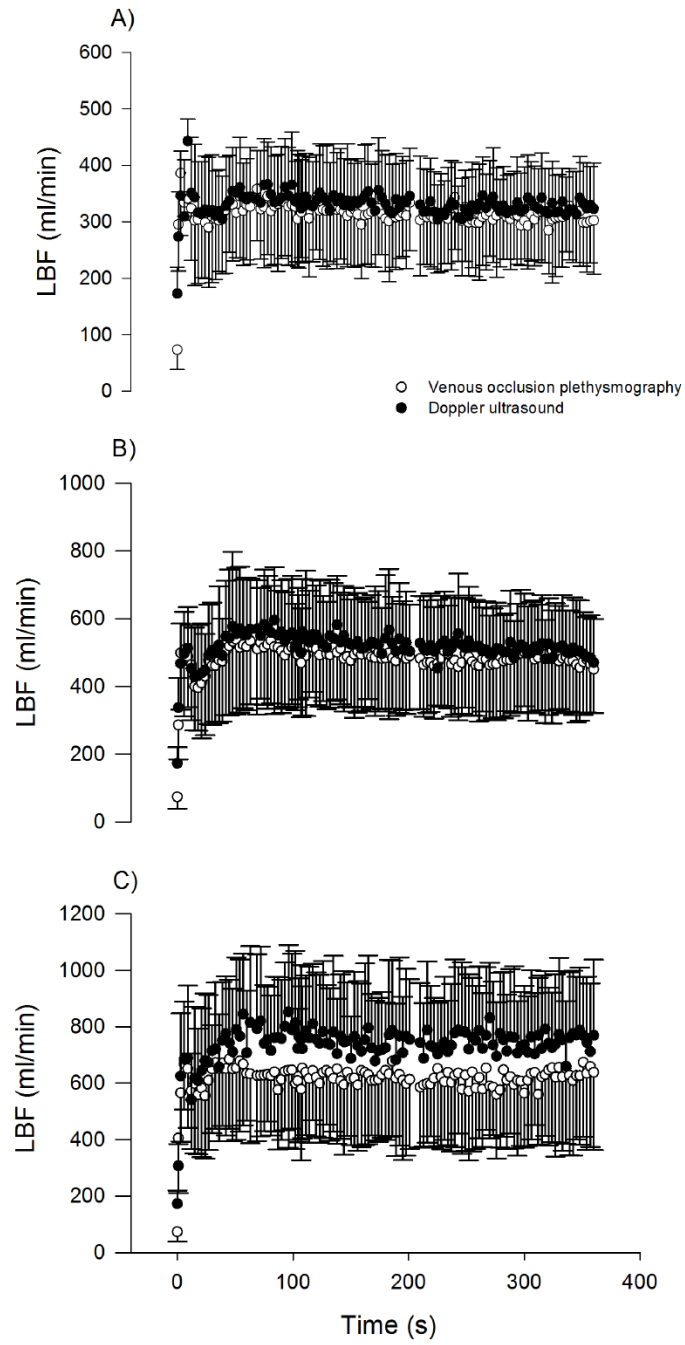


Figure 2

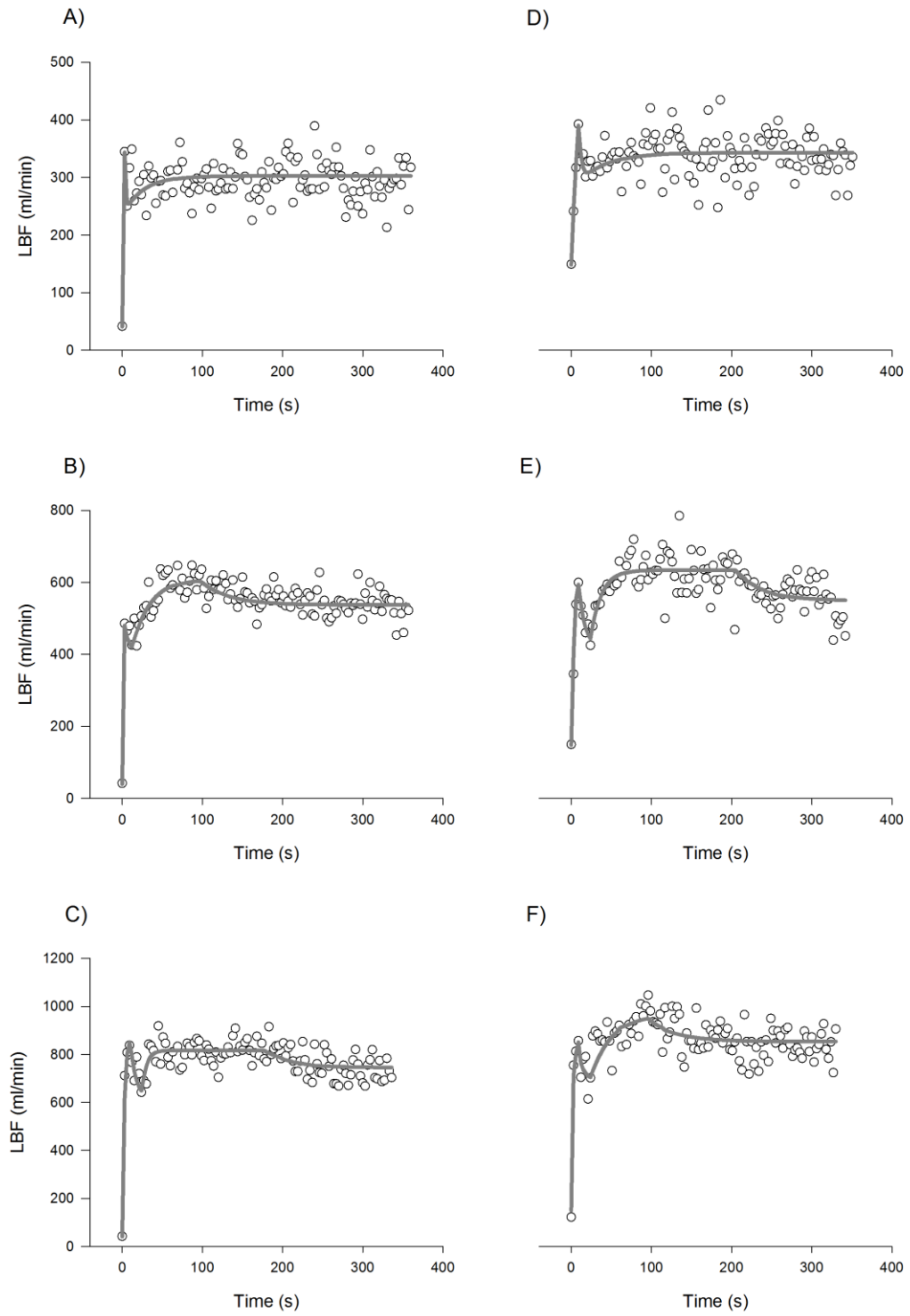


Figure 3

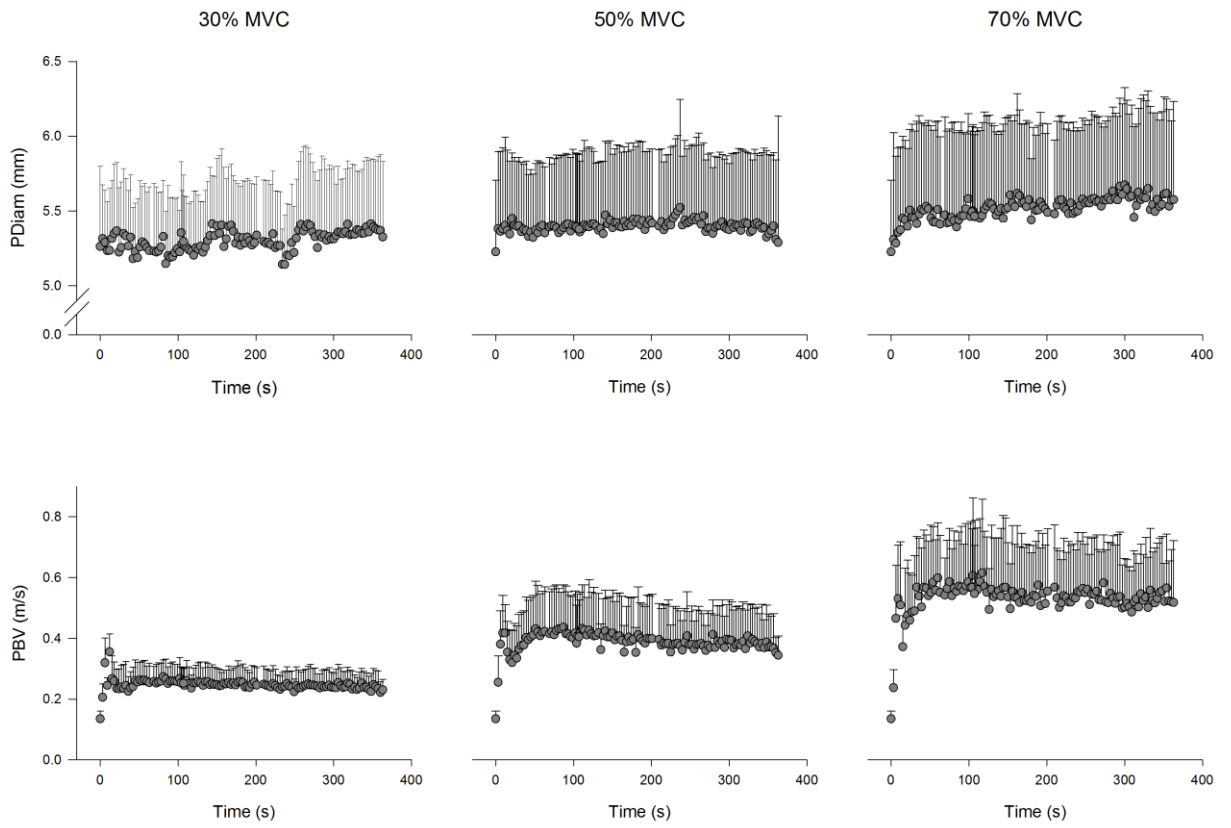




Figure 4

