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#### ABSTRACT

Near-infrared spectroscopy (NIRS) can potentially be used to assess the cardiovascular autonomic system by monitoring orthostatic challenge-induced shifts in lower limb blood volume. However, in order to be of clinical utility the test must be valid, reliable, and relatively simple to conduct. **Purpose:** To induce lower limb blood volume shifts using a 10min 70° head-up tilt, and: (1) in the soleus, determine the validity of an inexpensive continuous wave (cw)-NIRS device by comparing to a criterion frequency-domain (fd-) NIRS device, (2) determine the between-day reliability of soleus assessments obtained from cw-NIRS and fd-NIRS; and, (3) compare the between-day reliability for fd-NIRS assessments obtained at the soleus (standard) and gastrocnemius (simpler alternative). Methods: Fifteen non-smoking healthy adults were tested on 3 different mornings, under standardized conditions, separated by a maximum of 7 days. Total haemoglobin concentration (tHb) was continuously monitored bi-laterally in the medial soleus using cw-NIRS and fd-NIRS. For site comparison, tHb was measured in the medial gastrocnemius using fd-NIRS. **Results:** (1) The area under the curve (AUC) for cw-NIRS and fd-NIRS assessments at the soleus were not significantly different (p = .619). (2) The criterion (0.75) intra-class correlation coefficient (ICC) was exceeded for both cw-NIRS and fd-NIRS. (3) The criterion ICC was exceeded for both soleus and gastrocnemius assessments. Conclusion: Continuous-wave NIRS can be used to monitor orthostatic stress-induced shifts in lower leg blood volume with acceptable validity and reliability. This orthostatic test may present a relatively simple and inexpensive approach for assessing the cardiovascular autonomic nervous system.

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The validity and reliability of continuous-wave near -infrared spectroscopy for the assessment of leg blood volume during an orthostatic challenge.

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Running head: Validity and reliability of continuous-wave NIRS

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#### **INTRODUCTION**

Autonomic dysfunction has been linked to a number of cardiovascular disturbances, including hypertension and stroke (16, 19), and can be detected by evaluating responses to orthostatic stress (10). During an orthostatic challenge blood pooling in the small vessels of the legs contributes to a reduction in central venous return and a transient fall in stroke volume and blood pressure (10, 24). In the healthy adult, these events stimulate the baroreflex, which increases sympathetic activity leading to an increase in heart rate and vasoconstriction of the resistance and capacitance vessels in the lower limbs (4). These mechanisms act to restrict the degree of blood pooling and permit the restoration of cardiac output and blood pressure (24, 28). In contrast, a blunted baroreflex response or a failure to elevate vascular resistance leads to excessive blood pooling in the legs and indicates an impaired autonomic nervous system (25, 26). This blood pooling phenomena can be assessed using near-infrared spectroscopy (NIRS); however, in order to be of value in a clinical setting, these assessments must be accurate (valid), precise (reliable), and relatively simple to conduct.

The re-distribution of blood during orthostatic stress can be assessed by using NIRS to determine changes in total haemoglobin concentration (tHb) (18, 29, 32). This technique relies on the relative transparency of tissue to infrared light and oxygen-dependent absorption characteristics of haemoglobin to determine concentrations of oxy-haemoglobin (O<sub>2</sub>Hb) and deoxy-haemoglobin (HHb), the sum of which is tHb (14, 22). While frequency-domain NIRS (fd-NIRS) is capable of assessing absolute values of tHb (5, 8), these devices are expensive and complex to use. Alternatively, continuous-wave NIRS (cw-NIRS) is particularly suited for use in a range of

settings due to its simplicity and affordability. However, unlike fd-NIRS, cw-NIRS assumes constant and homogenous tissue optical properties in order to calculate absolute concentrations (6, 14, 20, 27). Currently, it is unclear whether these assumptions are valid and if not, whether this may impact the validity and reliability of assessments (15).

If cw-NIRS assessments of leg blood volume (tHb) shifts during an orthostatic challenge are shown to be valid and reliable, this would represent a potentially viable clinical tool. A final consideration, with regards to simplicity of use, is the measurement site. Previously, orthostatic stress-induced blood volume shifts have been assessed in the soleus (29) because this muscle consists predominantly of type I muscle fibres and is highly vascularized (11). While assessments of the soleus demonstrate good between-day reliability (29), it can be difficult to locate, particularly in physically inactive individuals. Also, most of the soleus muscle mass lies beneath the gastrocnemius, which may be beyond the depth penetration of NIR light. The more prominent gastrocnemius muscle may be a suitable alternative.

Therefore, the aims of the study were to induce lower limb blood volume shifts using a 10min 70° head-up tilt, and: (1) determine the validity of cw-NIRS soleus assessments by comparing to the criterion fd-NIRS, (2) determine the between-day reliability of soleus assessments obtained from cw-NIRS and fd-NIRS; and, (3) compare the between-day reliability for fd-NIRS assessments obtained at the soleus and gastrocnemius.

#### **METHODS**

#### **Study Population**

Fifteen healthy males (age: 25.5 yrs, SD 4.6; height 181.2 cm, SD 5.1; body mass 79.5 kg, SD 12) volunteered and provided written informed consent to participate in the study. All participants were non-smokers and were not taking any vascular acting medications. Before commencing recruitment or testing, all experimental procedures were approved by the host institution which met the standards of the Journal and the Declaration of Helsinki of the World Medical Association.

#### **Experimental Design and Protocol**

Participants were tested in a climate controlled laboratory between the hours of 7am and 10am on three separate days, separated by a maximum of 7 days (mean 4.2 d, SD 1.7). Prior to the start of data collection, participants were familiarised with all experimental procedures. The laboratory was quiet and dimly lit, with temperature being maintained between 21 to 23 °C. All participants were overnight fasted, consuming water only, and refrained from caffeine, alcohol and physical activity for 12 hours prior to experimentation. Following NIRS placement, each participant rested in a supine position for at least 15 min prior to the start of testing. Resting measures were then collected over a 5 min period. Following rest, the participant was rapidly (~10 s) tilted to a 70-degree upright position for 10 min using a tilt table fitted with a foot board, before being returned to a supine position for 5 min.

#### **Near-infrared Spectroscopy**

Near-infrared spectroscopy was used to continuously track changes in leg blood volume induced by the head-up tilt. Changes in the absolute tHb were taken to represent alterations in leg blood volume. To compare cw-NIRS and fd-NIRS, tHb was measured at the soleus of the dominant leg using cw-NIRS ( $CS_{tHb}$ ) and the non-dominant leg using fd-NIRS ( $FS_{tHb}$ ) (Figure 1). To compare the reliability of measures obtained at the soleus (standard assessment site) and gastrocnemius (simpler alternative), tHb was measured at the gastrocnemius on the non-dominant leg using fd-NIRS ( $FG_{tHb}$ ).



**Figure 1. Near-infrared spectroscopy (NIRS) probe locations (medial view).** cw-NIRS, continuous-wave NIRS; fd-NIRS, frequency-domain NIRS.

Continuous-wave NIRS devices emit infrared light at a constant intensity and measure the attenuation of light through the tissue (22). The cw-NIRS device used in this study was an Artinis Portalite (Artinis Medical Systems, BV, Zetten, The Netherlands). The Portalite comprised of a single wireless optode consisting of three light-emitting diodes, positioned 30mm, 35mm and

40mm from a single receiver, which transmitted infrared light at two-wavelengths (760nm and 850nm). The Portalite optode was fixed to the skin with bi-adhesive tape and covered with an opaque cloth to prevent signal contamination by ambient light. In order to determine absolute haemoglobin concentrations, the Portalite employs spatially-resolved spectroscopy (SRS). In SRS the spatial profile of the intensity of backscattered light is measured as a function of the distance from the light transmitter, with the shape of this function being related to the absorption coefficient, from which absolute haemoglobin concentrations can be calculated (20, 27). As cw-NIRS cannot measure the scattering of light in tissue, a reasonable and constant light scattering coefficient ( $\mu_s$ ) must be assumed (14, 17, 22). It is also assumed that the tissue being probed is homogenous, i.e. it possesses identical optical properties throughout its volume.

The criterion NIRS device used in this study was an ISS Oxiplex TS (Oxiplex TS, ISS Inc., Champaign, USA). The Oxiplex TS is a frequency-domain NIRS (fd-NIRS) device which modulates emitted light intensity and measures the phase shift (time of flight) as well as the attenuation in the intensity of light (5). Accordingly,  $\mu_s$  can be measured when determining absolute tHb. The OxiplexTS comprised two independent optodes, which consisted of eight light-emitting diodes, positioned 20, 25, 30 and 35mm from a single receiver, which transmitted at two wavelengths (690 and 830 nm). The instrument was calibrated before each measurement by positioning the optical probes on calibration and check blocks of known scattering ( $\mu_s$ ) and absorption coefficient ( $\mu_a$ ) properties. The Oxiplex TS probes were fixed to the skin with biadhesive tape, stabilised using Velcro straps inherent to the system and covered with an opaque cloth to prevent signal contamination by ambient light.

For soleus assessments, optodes were placed at the medial aspect of the soleus with the transmitting and receiving optodes positioned in the vertical plane along the muscle. For the gastrocnemius assessment, the optode was placed in the vertical plane over the central belly of the gastrocnemius at the position of maximum circumference. A practising physiotherapist who was a member of Chartered Society of Physiotherapy assisted with the placement of the NIRS optodes. Leg dominance was determined by asking participants which leg they would prefer to use when kicking a ball. The location of the optodes on the skin was marked to ensure identical placement on the second and third testing days. To determine the potential influence of subcutaneous adipose tissue on NIRS signal (30), skinfold thickness was measured by a qualified ISAK practitioner using a skinfold calliper (Harpenden, London, UK). Adipose thickness was determined by dividing the observed values by 2. Mean skinfold thickness of the dominant soleus, non-dominant soleus and gastrocnemius sites was 5.3 mm, SD 1.6; 5.1 mm, SD 1.5; and 5.2 mm SD 1.9, respectively. Given the low skinfold values observed, the effects of excessive adipose tissue on the NIRS signal was considered (8).

The primary measures used to compare cw-NIRS and fd-NIRS, and to determine the reliability of assessment sites were: absolute tHb area under the curve during tilt (AUC,  $\mu$ mol) and relative change in tHb from baseline to minute ten of the head-up tilt ( $\Delta$ Tilt<sub>10</sub>,  $\mu$ mol).

#### **Haemodynamic Measurements**

Haemodynamic responses to head-up tilt were measured continuously using servo-controlled, finger cuff infrared photo-plethysmography (Portapres Model-2, FMS, Amsterdam, The Netherlands). The finger cuff was placed at the middle phalanx of the middle finger on the left hand. Haemodynamic responses were monitored in real-time and recorded using a blood pressure waveform analysis package (Beatscope 1.1, FMS). Beat-to-beat values for mean arterial pressure (MAP) were computed as the integral over one beat, and heart rate (HR) was the inverse of the pulse interval. Stroke volume (SV) is estimated form the aortic waveform using the Modelflow method (31) inherent to the analysis package. Cardiac Output (CO) is calculated as SV x HR and total peripheral resistance (TPR) is calculated as MAP / CO. As haemodynamic responses to head-up tilt were not a primary outcome, measurements were only performed during the first tilt trial in order to confirm participants exhibited a typical response to head-up tilt.

#### **Sample Size**

Truijen *et al.* previously reported change in tHb values at eight minutes post tilt using cw-NIRS measurements at the soleus (29). The change in tHb and SD were approximately 25  $\mu$ mol and 5  $\mu$ mol, respectively (values estimated from graph). Based on these values, to detect a one SD  $\Delta$ Tilt<sub>10</sub>, using 5% alpha and 80% beta error rates, 9 patients was deemed necessary. This assumed an equitable SD for both NIRS devices; to account for this unknown know source of variation, the sample size was inflated to 15.

#### **Statistical Analysis**

All analysis was performed using Statistical Package for Social Sciences (SPSS, IBM, Version 21). All statistical tests were two-sided, with type I error rate fixed at 0.05.

**Haemodynamic Measures.** One way repeated measures ANOVA's or non-parametric Friedman tests were used to examine haemodynamic changes during head-up tilt, following normality examination using Shapiro-Wilk tests. Bonferroni or Bonferroni adjusted Wilcoxon-signed rank *post hoc* tests were used to determine the level of significance when a significant main effect was found.

**Validity.** Three-way repeated measures analysis of variance (ANOVA) was used to make comparisons of the primary measures between assessment sites, where FS<sub>tHb</sub> was specified as the reference category (CS<sub>tHb</sub> *vs.* FS<sub>tHb</sub> and FS<sub>tHb</sub> *vs.* FG<sub>tHb</sub>). The normality distribution of the data was checked using the Shapiro-Wilk test. Bonferroni post-hoc tests were employed to determine the level of significance when a significant *F*-ratio was found. Homogeneity of variance was evaluated using Mauchly's test of sphericity and when violated the Greenhouse-Geisser adjustment was used. Partial eta squared ( $\eta^2_p$ ) was used as a measure of effect size, where 0.0099, 0.0588 and 0.1379 represent a small, medium and large effect, respectively (3). In addition, agreement between the two NIRS methodologies (CS<sub>tHb</sub> *vs.* FS<sub>tHb</sub>) was assessed by calculating the standard error of estimate (SEE), then dividing the SSE by the SD of the criterion (fd-NIRS) to provide a standardized indicator of error, whereby <0.20 is considered a trivial difference, 0.2-0.6 small, 0.6-1.2 moderate, 1.2-2.0 large, and >2.0 very large difference (13). The relative standard error (RSE) was also calculated by expressing SSE relative to the mean of the criterion.

**Reliability.** To determine the between-day reliability of cw-NIRS and fd-NIRS soleus assessments and compare fd-NIRS soleus and gastrocnemius assessments, reproducibility of primary measures were assessed by calculating the coefficient of variation (CV), intra-class correlation coefficient (ICC) and standard error of measurement (SEM). The CV was calculated as  $SD_w/\mu$ . The ICC was calculated according to 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 general, ICC values above 0.75 are considered to indicate excellent reproducibility (7). The SEM was calculated according to the formula:  $SD^* \sqrt{(1-ICC)}$  (9).

#### RESULTS

## **Haemodynamic Measures**

Haemodynamic responses to head-up tilt are presented in Table 1. Significant time effects were observed for HR (P < .0001), MAP (P = .006), SV (P < .0001) and TPR (P = .002). Heart rate (P = .001) and MAP (P = .005) increased from baseline to tilt<sub>10</sub>, whilst SV was reduced (P < .0001). Despite a significant main effect, *post hoc* analysis revealed a non-significant trend for increased TPR during the tilt (P = .09).

#### Table 1. Haemodynamic variables during the head-up tilt.

	-	Baseline	Tilt <sub>10</sub>	Recovery <sub>5</sub>
HR (BPM)	Х	52	67 *	49 <sup>†</sup>
	SD	8	11	8
MAP (mmHg)	Х	67	77 *	72 *
	SD	14	16	16
SV (ml)	Х	102	78 *	111 <sup>†</sup>
	SD	19	17	22
CO (I min⁻¹)	Х	5.2	5.2	5.4
	SD	1.4	1.0	0.9
TPR (mm Hg ml-1 s)	Х	0.85	1.00	0.85 <sup>†</sup>
	SD	0.31	0.41	0.30

**Abbreviations:** HR, heart rate; MAP, mean arterial pressure; SV, stroke volume; CO, cardiac output; TPR, total peripheral resistance; Tilt<sub>10</sub>, variables at minute ten of tilt; Recovery<sub>5</sub>, variables at minute five of recovery. Values are means  $\pm$  SD. \* *P* <0.05 *vs*. Baseline.  $\dagger P$  <0.05 *vs*. Tilt<sub>10</sub>.

#### Validity

Mean absolute tHb response during the head-up tilt for CS<sub>tHb</sub>, FS<sub>tHb</sub> and FG<sub>tHb</sub> assessments is presented in Figure 2. Significant main effects (group) were observed between assessment sites for AUC (P = .012) and  $\Delta$ Tilt<sub>10</sub> (P = .0001) during the head-up tilt (Table 2). For cw-NIRS and fd-NIRS comparisons, post-hoc analysis revealed that there was no significant difference in AUC (P= .619,  $\eta^2_p = .006$ ) or  $\Delta$ Tilt<sub>10</sub> (P = .198,  $\eta^2_p = .075$ ) between CS<sub>tHb</sub> and FS<sub>tHb</sub>. For soleus and gastrocnemius fd-NIRS comparisons, post hoc analysis revealed that AUC (P = .003,  $\eta^2_p = .182$ ) and  $\Delta$ Tilt<sub>10</sub> (P = .00004,  $\eta^2_p = .322$ ) for FG<sub>tHb</sub> were both significantly smaller than FS<sub>tHb</sub>, demonstrating large effects for each primary measure. The standardized SSE (Table 3) indicated that the difference between the two devices (cw-NIRS vs. fd-NIRS), for measurements collected on the soleus, was small for Tilt<sub>10</sub>, Rec<sub>5</sub> and AUC, and moderate for  $\Delta$ Tilt<sub>10</sub>.



Figure

2.

#### Absolute total

**haemoglobin during and following the head-up tilt.**  $CS_{tHb}$ , continuous-wave NIRS total haemoglobin concentration at the soleus;  $FS_{tHb}$ , frequency-domain NIRS total haemoglobin concentration at the soleus;  $FG_{tHb}$ , frequency-domain NIRS total haemoglobin concentration at the gastrocnemius.

	-		Change			
		Baseline	Tilt <sub>10</sub>	Recovery <sub>5</sub>	AUC	$\Delta Tilt_{10}$
CS <sub>tHb</sub> (µmol)	Х	61	82	65	783	21
	SD	14	20	15	196	9
	CV	13	15	13	13	24
	ICC	0.74	0.72	0.75	0.78	0.75
	SEM	7	10	7	93	4
FS <sub>tHb</sub> (µmol)	X	60	85	62	806	25
	SD	22	33	23	299	13
	CV	7	8	7	8	19
	ICC	0.97	0.96	0.97	0.96	0.88
	SEM	4	6	4	62	4
FG <sub>tHb</sub> (µmol)	Х	56	72	59	678 *	16 *
	SD	22	28	23	258	7
	CV	8	5	6	5	17
	ICC	0.96	0.98	0.98	0.98	0.89
	SEM	4	4	3	35	2

## Table 2. Total haemoglobin and reliability values for near-infrared spectroscopy assessments during the head-up tilt.

**Abbreviations:**  $CS_{tHb}$ , continuous-wave NIRS total haemoglobin concentration at the soleus;  $FS_{tHb}$ , frequencydomain NIRS total haemoglobin concentration at the soleus;  $FG_{tHb}$ , frequency-domain NIRS total haemoglobin concentration at the gastrocnemius;  $Tilt_{10}$ , total haemoglobin at minute ten of tilt; Recovery<sub>5</sub>, total haemoglobin at minute five of recovery; AUC, area under the curve during the tilt;  $\Delta Tilt_{10}$ , change in total haemoglobin from baseline to minute ten of tilt; CV, coefficient of variation; ICC, intra-class correlation coefficient; SEM, standard error of measurement. \* *P* <0.05 *vs*. FS<sub>tHb</sub>.

# Table 3. Comparison of total haemoglobin values obtained from the soleus during head-up tilt with continuous wave and frequency domain near-infrared spectroscopy.

	Baseline	Tilt <sub>10</sub>	Recovery <sub>5</sub>	AUC	$\Delta Tilt_{10}$
Mean Diff. (CS <sub>thb</sub> vs. FS <sub>thb</sub> )	1.6	-3.2	2.2	-23.0	-4.7
SEE (Absol.)	12.0	18.0	12.0	177.0	8.6
SSE (Stand.)	0.5	0.6	0.5	0.6	0.7
RSE %	20.0	22.0	20.0	22.0	34.0

**Abbreviations:**  $CS_{thb}$ , continuous-wave NIRS total haemoglobin concentration at the soleus;  $FS_{thb}$ , frequency-domain NIRS total haemoglobin concentration at the soleus; Tilt<sub>10</sub>, total haemoglobin at minute ten of tilt; Recovery<sub>5</sub>, total haemoglobin at minute five of recovery; AUC, area under the curve during the tilt;  $\Delta Tilt_{10}$ , change in total haemoglobin from baseline to minute ten of tilt; SEE (absol.), standard error of estimate in absolute terms; SEE (stand.), standard error of estimate relative to the standard deviation of the criterion (frequency domain); RSE, relative standard error.

## Reliability

For the primary measures (AUC,  $\Delta$ Tilt<sub>10</sub>), ICC values for CS<sub>tHb</sub> and FS<sub>tHb</sub> were above the 0.75 criterion (Table 1), indicating excellent between-day reliability (Table 2). Similarly, FG<sub>tHb</sub> derived ICC's for the primary measures were above the 0.75 criterion.

## DISCUSSION

The main findings of this study are that; (1) when compared to the criterion fd-NIRS, cw-NIRS measures of leg blood volume (tHb) during an orthostatic challenge demonstrate good validity, (2) cw-NIRS and fd-NIRS measurements at the soleus demonstrate excellent reliability, and (3) the gastrocnemius is a suitable alternative measurement site, demonstrating reliability that is excellent and highly comparable to that of the soleus.

To the author's knowledge, this study is the first to document the validity and reliability of cw-NIRS in determining absolute leg blood volume during an orthostatic challenge. A novel finding is the indication that, despite the assumptions made by cw-NIRS when determining absolute haemoglobin concentrations using SRS (20, 27), cw-NIRS and fd-NIRS derived measures of leg blood volume (tHb) were not different during head-up tilt. Typically, cw-NIRS has been used to determine relative change in haemoglobin concentrations during orthostatic stress (18, 29, 32). This is due to the inherent limitation of cw-NIRS; its inability to measure the scattering of NIR-light ( $\mu$ s) as it travels through tissue. However, if a reasonable evidence based (17) and constant  $\mu$ s is assumed when using SRS, the determination of absolute haemoglobin values is possible using cw-NIRS (14, 22). In the present study, we have demonstrated that such assumptions are valid during head-up tilt and absolute tHb can be accurately determined using cw-NIRS.

The ICC values we observed for cw-NIRS primary measures (0.75-0.78) were lower than those of fd-NIRS (0.85 - 0.96) at the soleus during the head-up tilt, but exceeded the criterion for excellent reproducibility (0.75) (7). Further, the ICC's are consistent with those previously reported where the relative change in tHb has been measured using cw-NIRS at the soleus (29). Collectively, the findings of the present study suggest that cw-NIRS may be a valid and reliable clinical tool for monitoring leg blood volume changes during an orthostatic challenge.

With regards to the simplicity of using NIRS to determine leg blood volume changes, this study has identified that the gastrocnemius is a suitable alternative measurement site to the soleus. The absolute tHb AUC and  $\Delta Tilt_{10}$  at the gastrocnemius were both significantly smaller than those at the soleus, which is likely attributable to the extent of capillarisation and capillary arrangement that exists between the muscles (1, 11). However, the ICC and CV values of the gastrocnemius were comparable to that of the soleus (0.89 – 0.98). Accordingly, the superficial positon and larger surface area of the gastrocnemius are likely to make it easier to determine appropriate NIRS probe placement than at the soleus, particularly in clinical populations. The degree of blood pooling in the lower limb vasculature during orthostatic stress is influenced by an interaction of both autonomic (sympathetic activity) and regional (vasoconstriction) mechanisms (24). Accordingly, excessive blood pooling in the legs may be caused by a number of factors including a blunted barorreflex or impaired arterial vasoconstriction (2, 12, 25, 26), leading to enhanced venous filling. Although unable to provide a specific diagnosis, assessing blood pooling during an orthostatic challenge could be a useful test of cardiovascular control and provide objective confirmation of the presence of impaired autonomic function. Further, the use of cw-NIRS to determine absolute tHb, rather than change from an arbitrary baseline, are likely to be of greater use in shaping thresholds for diagnosing the presence of autonomic dysfunction.

#### LIMITATIONS

The homogenous cohort of young, healthy males was recruited in order to determine the validity and upper limits of reliability for the assessment of leg blood volume using cw-NIRS. Further studies are therefore required in order to generalise any findings of the present study in clinical populations of varying gender, age and health states. Additionally, bilateral assessments were conducted in order to directly compare NIRS devices; some error variance may be attributable to asymmetrical differences. However, asymmetrical differences are likely to have been minimal considering the current population were young, homogenous, and did not participate in regular unilateral exercise (21, 23).

#### CONCLUSION

Findings of this study suggest that, at least in healthy young males, cw-NIRS measurements of leg blood volume (tHb) during an orthostatic challenge are valid (compared to the criterion) and demonstrate a reliability that exceeds the criterion for acceptable between-day reliability. Furthermore, the gastrocnemius presents a practical alternative measurement site to the soleus given that it can be identified more easily. Accordingly, cw-NIRS leg blood volume measurements during orthostatic stress could present a relatively simple and low cost opportunity for the clinician or clinical research scientist to identify the presence of autonomic nervous system dysfunction.

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None to declare.

#### **CONFLICT OF INTEREST**

The authors confirm that there is no conflict of interest associated with this article.

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