Reliability of near-infrared spectroscopy for measuring *biceps brachii* oxygenation during sustained and repeated isometric contractions

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Abstract. We examine the test–retest reliability of *biceps brachii* tissue oxygenation index (TOI) parameters measured by near-infrared spectroscopy during a 10-s sustained and a 30-repeated (1-s contraction, 1-s relaxation) isometric contraction task at 30% of maximal voluntary contraction (30% MVC) and maximal (100% MVC) intensities. Eight healthy men (23 to 33 yr) were tested on three sessions separated by 3 h and 24 h, and the within-subject reliability of torque and each TOI parameter were determined by Bland-Altman$\pm$2 SD limits of agreement plots and coefficient of variation (CV). No significant ($P>0.05$) differences between the three sessions were found for mean values of torque and TOI parameters during the sustained and repeated tasks at both contraction intensities. All TOI parameters were within$\pm$2 SD limits of agreement. The CVs for torque integral were similar between the sustained and repeated task at both intensities (4 to 7%); however, the CVs for TOI parameters during the sustained and repeated task were lower for 100% MVC (7 to 11%) than for 30% MVC (22 to 36%). It is concluded that the reliability of the *biceps brachii* NIRS parameters during both sustained and repeated isometric contraction tasks is acceptable. [DOI: 10.1117/1.3309746]

Keywords: tissue oxygenation index; oxidative metabolism; coefficient of variation; Bland-Altman plots.

Paper 09391RR received Sep. 1, 2009; revised manuscript received Nov. 24, 2009; accepted for publication Dec. 8, 2009; published online Feb. 18, 2010.

1 Introduction

Near-infrared spectroscopy (NIRS) has been used in exercise physiology and clinical medicine for noninvasive investigation of muscle oxygenation and hemodynamics during exercise.[1,2] NIRS directly measures the oxygen (O_2)–dependent absorption of hemoglobin (Hb) in the microcirculation blood vessels (i.e., arterioles, capillaries, and venules) and myoglobin (Mb) in the muscle cytoplasm.[3] The muscle utilization of the oxidative metabolic system is represented by NIRS as concentration changes in oxy-Hb (O_2Hb), deoxy-Hb (HHb), and total Hb volume ($tHb=O_2Hb+HHb$), and as O_2Hb saturation ($SmO_2=O_2Hb/tHb\times 100$). SmO_2 reflects the dynamic balance of O_2 supply by the muscle microcirculation and O_2 consumption/demand by the muscle.[2,4] tHb is related to the changes in muscle microcirculation blood volume and is considered a qualitative indicator of changes in local muscle blood flow/O_2 supply.[1]

During exercise, skeletal muscle increases the metabolic demand for O_2, resulting in an increase in O_2 supply and O_2 consumption.[1,2] In order to characterize muscle oxidative metabolism using NIRS, sustained and/or repeated isometric contractions have been used.[5–9] It has been reported that during sustained isometric contractions at 50% maximal voluntary contraction (MVC), blood flow/O_2 supply to the *biceps brachii* is completely impeded due to increased intramuscular pressure compressing blood vessels.[10] The rate of decrease in SmO_2 and minimum SmO_2 amplitude represent muscle O_2 demand, which is considered as a measure of energy consumption for muscle force production.[7,11] When repeated isometric contractions are performed, in which short periods of muscle contraction alternate with periods of relaxation, reoxygenation is permitted during the relaxation phases. Consequently, amplitude and kinetic changes in SmO_2 represent the dynamics of O_2 supply and O_2 demand.[9] Therefore, during sustained isometric contractions above 50% MVC, the changes in SmO_2 primarily provide information about muscle O_2 demand, while during repeated isometric contractions, the changes in SmO_2 provide additional information about the

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1083-3668/2010/15(1)/017008/8/$25.00 © 2010 SPIE

regulation of O_2 supply to meet the muscle metabolic demand for O_2.[9]

Felici et al.[7] have recently reported that changes in *biceps brachii* SmO_2, represented as tissue oxygenation index (TOI) parameters, and tHb during 30-s sustained and 15-repeated (1-s contraction, 1-s relaxation) isometric contractions of the elbow flexors were related to the exercise intensity (20, 40, 60, and 80% MVC). Although TOI could be used to quantify oxygenation, tHb is only a qualitative indicator of hemodynamics.[2] However, currently no data are available regarding the reliability of *biceps brachii* TOI parameters during exercise. To use the TOI parameters as indicators of the effects of an intervention such as exercise or medical therapy on muscle metabolism/microcirculation, the test–retest reliability of the TOI parameters, especially within-subject reliability, needs to be established.

Therefore, the purpose of this study was to investigate the test–retest reliability of *biceps brachii* TOI parameters during sustained and repeated isometric contractions of the elbow flexors on three separate occasions. Based on the previous study by Felici et al.,[7] the present study used two different intensities (30% MVC and 100% MVC) that were expected to result in contrasting responses of *biceps brachii* TOI parameters during the sustained and repeated tasks.

2 Methods

2.1 *Subjects*

Eight healthy men participated in this study, and their mean ($\pm$SD) age, height, and body mass were 27.1 ± 3.1 yr, 176.8 ± 5.0 cm, and 78.3 ± 7.0 kg, respectively. All subjects had no known health problems (e.g., metabolic or neuromuscular disorders) or any upper extremity muscle or joint injuries. Subjects were asked not to perform any strenuous exercise for at least 48 h prior to and during the experimental period, not to take any medication or nutritional supplements during the experimental period, and not to consume any caffeine at least 1 h prior to any testing session. The study conformed to the recommendations of the local Human Research Ethics Committee in accordance with the Declaration of Helsinki. A written informed consent form was signed by each subject.

2.2 *Study Design*

All subjects performed a sustained and a repeated isometric contraction task at two intensities (30% MVC and 100% MVC) with the same arm over three sessions; two sessions on the same day separated by 3 h (sessions I, II), and one session 24 h later (session III). The use of the dominant or nondominant arm was counterbalanced among subjects. The 30% MVC tasks were performed prior to the 100% MVC tasks, and the sustained tasks prior to the repeated tasks, in order to minimize the influence of the previous task on the next task, since it was expected that metabolic demand was greater for the 100% MVC tasks than for the 30% tasks, and for the repeated task than for the sustained task at 100% MVC.

2.3 *Protocol*

Each subject was seated on a preacher arm curl bench, which placed the upper arm at 45-deg shoulder flexion and aligned the elbow joint with the axis of rotation of an isokinetic dynamometer (Cybex6000, Lumex, Inc., Ronkonkoma, New York, with HUMAC-2004 software, Computer Sports Medicine, Inc., Sloughton, MA). The lever arm of the dynamometer was secured to the subject's wrist in a supinated position with an elbow joint angle of 90 deg.

All subjects performed two 3-s MVCs with a 45-s rest between contractions, and the peak torque of the two contractions was used to set the 30% MVC torque values for the sustained and repeated tasks. A line representing 30% MVC target torque level was displayed on the computer screen along with the actual torque output, which ensured real-time feedback on torque levels to the experimenter and subject. After 90 s of recovery, subjects performed a 10-s sustained isometric contraction task at 30% MVC followed by a 10-s sustained isometric contraction at 100% MVC separated by a 90-s rest. After a 180-s rest, subjects performed a 30-repeated (1-s contraction, 1-s relaxation) isometric contractions task (60-s duration) at 30% MVC. Following a 150-s rest, subjects performed a 30-repeated (1-s contraction, 1-s relaxation) isometric contraction task at 100% MVC. Torque was indicated visually on the computer screen, and the tone from the computer was used to indicate the timing of the contraction and relaxation. Torque signals were collected onto a data acquisition system (PowerLab16/30 with Chart 5.5.5 software, AD Instruments, Bella Vista, Australia) at a sampling rate of 200 Hz.

2.4 *Near-Infrared Spectroscopy*

This study used a NIRO-200 oximeter (Hamamatsu Photonics K.K., Hamamatsu, Japan), which measures concentration changes in O_2Hb, HHb, and tHb expressed in $\mu M \cdot cm$, and SmO_2 as tissue oxygenation index (TOI) expressed in %.)[12] The optical probe, consisting of one emitter and one detector 4 cm apart supported by a rubber shell, was firmly attached to the skin at the medial side over the midbelly of the *biceps brachii* muscle, parallel to the major axis of the arm by a double-sided adhesive tape, which ensured no sliding of the probe on the skin. The rubber shell was covered by a soft black cloth, and all wires were taped down to minimize movement during exercise. The position of the NIRS probe was marked on the skin with a semipermanent ink marker to obtain consistent measures over the subsequent two testing sessions.

A B-mode ultrasound apparatus fitted with a 7.5-MHz linear probe (SSD-1000, Aloka Co. Ltd., Tokyo, Japan) was used to measure the subcutaneous fat layer thickness of the area over which the NIRS probe unit was placed. The adipose tissue thickness (2.7 ± 0.6 mm) did not change over sessions, and considering that the penetration depth of the NIRS signal is approximately half of the emitter–detector distance (4 cm), the changes in TOI and tHb reflected mainly the muscle metabolic and hemodynamic changes, respectively, of the superficial muscle volume of the *biceps brachii*.[7]

Prior to each testing session, an initialization procedure on the NIRO-200 was carried out, which set each laser power automatically to establish the optimum measurement conditions. The zero set procedure was adopted to return tHb to the zero value. This procedure does not affect the TOI values, since TOI is measured as absolute values instead of a change

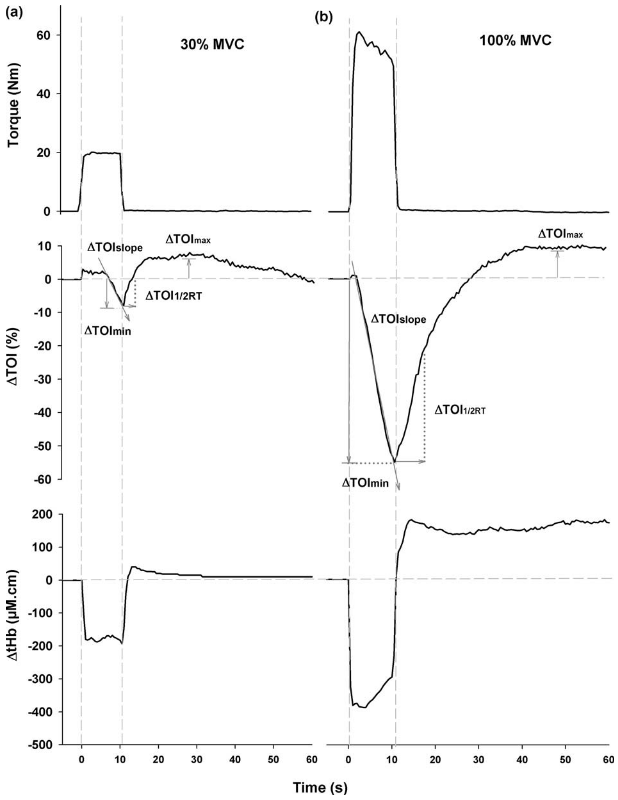

Fig. 1 Typical changes in elbow flexor torque and *biceps brachii* tissue oxygenation index (ΔTOI) and total hemoglobin volume (ΔtHb) during 10-s sustained isometric contraction at (a) 30% MVC and (b) 100% MVC. The vertical lines represent the onset and the end of the contraction phase. ΔTOI_{slope}: ΔTOI desaturation slope; ΔTOI_{min}: minimum ΔTOI amplitude; ΔTOI_{max}: maximum ΔTOI amplitude; and $\Delta TOI_{1/2RT}$: ΔTOI half resaturation time.

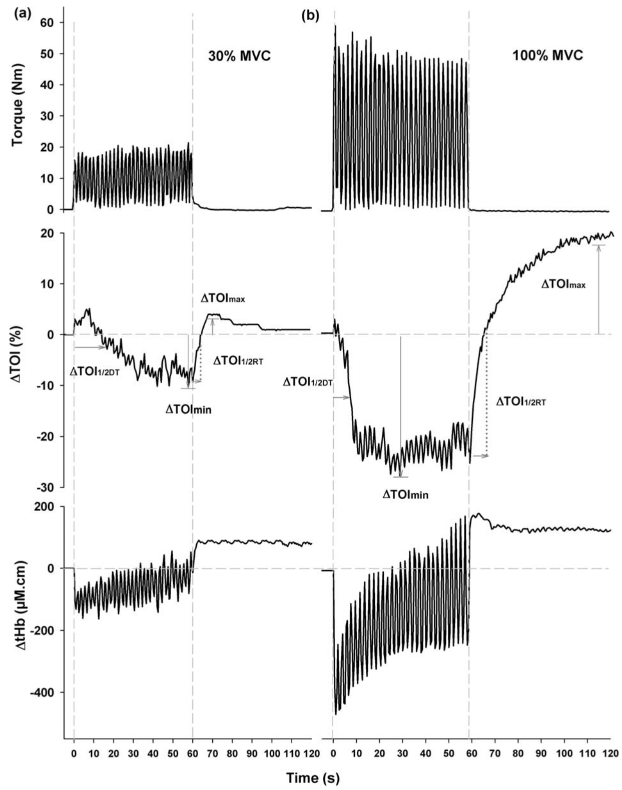

Fig. 2 Typical changes in elbow flexor torque and *biceps brachii* tissue oxygenation index (ΔTOI) and total hemoglobin volume (ΔtHb) during 30 repeated isometric contractions at (a) 30% MVC and (b) 100% MVC. The vertical lines represent the onset and the end of the 30 contractions. $\Delta TOI_{1/2DT}$: ΔTOI half desaturation time; ΔTOI_{min}: minimum ΔTOI amplitude during the 30 contractions; and $\Delta TOI_{1/2RT}$: ΔTOI half resaturation time.

with respect to the arbitrary initial zero value. NIRS signals were sampled at 6 Hz by the NIRO-200 and collected simultaneously with torque data onto the PowerLab system and stored on the computer for later analysis using the analysis software.

2.5 *Data Analysis*

Typical torque, TOI, and tHb changes during the sustained task at 30% MVC and 100% MVC are shown in Fig. 1. The contraction onset was identified as the torque value 4% MVC above the baseline, and the end of contraction was identified as the time when the torque value became lower than 4% MVC. The 4% MVC ($\sim$3 Nm) was the minimum threshold of torque level necessary to determine the contraction onset and offset criteria by the Chart data analysis software (AD Instruments, Bella Vista, Australia). The torque integral was determined as area under the torque traces during the contraction phase. The baseline of TOI and tHb was determined as the mean value over 30 s before the onset of contraction. Since the baseline was stable, the TOI and tHb baselines were expressed as zero, and the TOI and tHb parameters are presented as the magnitude of change from the respective baseline in the figures. As shown in Fig. 1, ΔTOI desaturation slope (ΔTOI_{slope}) was determined as the negative slope of the least-squares regression line of ΔTOI during the contraction phase. A higher ΔTOI_{slope} would represent a greater muscle O_2 demand, and consequently a greater energy consumption.[7,11,13] Minimum ΔTOI amplitude (ΔTOI_{min}) was the difference between the minimum TOI value reached during the contraction phase and TOI baseline. Lower ΔTOI_{min} values represent greater O_2 demand relative to O_2 supply.[7,14] Maximum ΔTOI amplitude (ΔTOI_{max}) was the difference between the maximum TOI value reached during the relaxation phase and TOI baseline. Higher ΔTOI_{max} values indicate increased O_2 supply relative to O_2 demand.[14] ΔTOI half recovery time ($\Delta TOI_{1/2RT}$) was determined as the time to reach 50% of the difference between ΔTOI at the end of the contraction phase and ΔTOI_{max} in the recovery period. $\Delta TOI_{1/2RT}$ has previously been used as an index to evaluate muscle oxidative capacity.[2,15–17]

Figure 2 shows typical torque, TOI, and tHb changes during the repeated task at 30% MVC and 100% MVC. The mean torque integral was determined as the sum of the area under the torque traces during each of the 30 contractions. In addition to the TOI parameters described earlier for the sustained contractions, the following parameters were included for the repeated contractions. TOI_{min} was the difference between the minimum TOI value reached during the 30 contractions and TOI baseline. A given ΔTOI_{min} value represents the dynamic balance of O_2 supply to match O_2 consumption.[9]

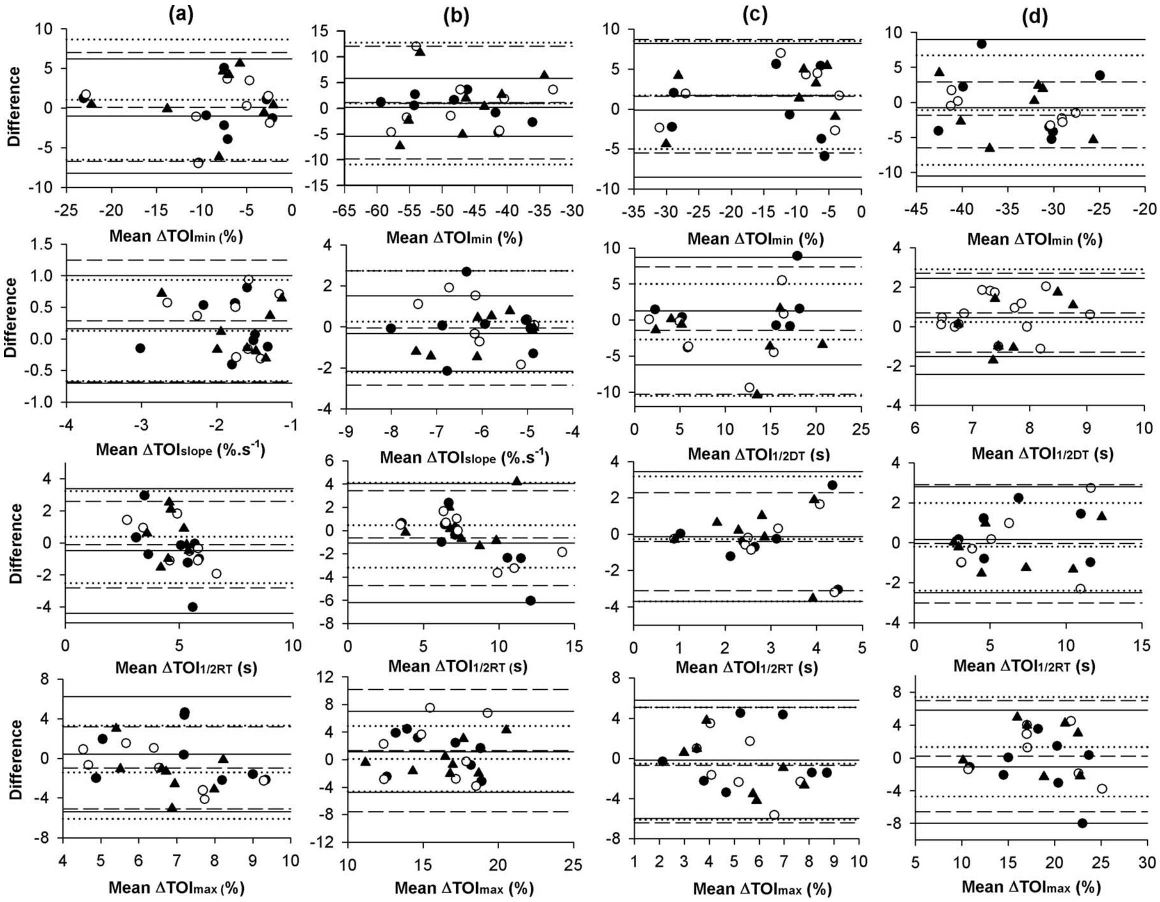

Fig. 3 Bland-Altman plots of TOI parameters for the sustained task at (a) 30% MVC and (b) 100% MVC, and repeated task at (c) 30% MVC and (d) 100% MVC. Every plot represents the difference vs mean value measured in three separate testing sessions (I, II, III). ●: Session I vs session II (the straight lines represent mean difference [central line] and mean ± 2 SD [upper and lower lines]). ○: Session I vs session III (the dashed lines). ▲: Session II vs session III (the dotted lines). ΔTOI_{min}: minimum ΔTOI amplitude; ΔTOI_{slope}: ΔTOI desaturation slope; $\Delta TOI_{1/2DT}$: ΔTOI half desaturation time; ΔTOI_{max}: maximum ΔTOI amplitude; and $\Delta TOI_{1/2RT}$: ΔTOI half resaturation time. For further details, see Sec. 2.

ΔTOI half desaturation time ($\Delta TOI_{1/2DT}$) was the time difference between contraction onset until TOI reached 50% of the difference value between TOI baseline and minimum TOI value reached during the first 15 contractions. At the rest-exercise transition (i.e., initial 30 s), a longer duration $\Delta TOI_{1/2DT}$ for a similar minimum ΔTOI amplitude would indicate a slower desaturation rate, indicating that O_2 demand was better matched by O_2 supply.[2]

2.6 *Statistical Analyses*

A Shapiro-Wilk test was used to confirm that all dependent variables were normally distributed ($P < 0.01$). To test the within-day and between-day reproducibility of the mean values of torque and TOI parameters, a one-way ANOVA with repeated measurements over the three sessions (I, II, and III) was performed. As an indication of within-subject variation in the form of absolute measurement error, Bland and Altman ± 2 SD limits of agreement plots were calculated[18] on all torque and TOI parameter data to demonstrate the distribution of measurement error between the three sessions (I, II, and III). Furthermore, a coefficient of variation (CV) was calculated for each subject across the three sessions, and the mean value of the subjects was used as an indicator of the test–retest reliability.[19] A paired *t*-test was also used to compare between the sustained and repeated conditions for the torque integral and common TOI parameters (TOI_{min}, $\Delta TOI_{1/2RT}$, and ΔTOI_{max}) on the combined values over the three sessions (i.e., 8 subjects × 3 sessions = 24 measurements). Significance was set at $P < 0.05$. Data are presented as mean ± SD.

3 Results

All variables passed the Shapiro-Wilk normality test. No significant difference in MVC was found among sessions I (71.6 ± 9.4 Nm), II (69.2 ± 9.2 Nm), and III (69.0 ± 10.3 Nm). The CV of the MVC over the three sessions was 3.1 ± 1.9%.

All torque and TOI parameters were within the ± 2 SD limits of agreement. Figure 3 shows the Bland-Altman plots of five TOI parameters over the three sessions during the sustained and repeated isometric contraction tasks at 30% MVC and 100% MVC.

The mean torque integral and TOI values measured over the three sessions of the sustained isometric contraction tasks and their CVs are presented in Table 1. The torque integral during the 30% MVC and 100% MVC did not change signifi-

Table 1 Torque integral and biceps brachii tissue oxygenation index (ΔTOI) parameters during 10-s sustained isometric contraction over three testing sessions (I, II, III) at 30% MVC and 100% MVC intensity, and their coefficient of variation (CV). Mean±SD (n=8).

	Session			
	I	II	III	CV (%)
30% MVC				
Torque integral (Nm·s)	228.9±38.4	219.0±33.2	217.6±40.2	7.2±5.6
TOI baseline (%)	57.5±5.9	57.9±4.3	58.3±4.9	3.0±1.5
ΔTOI_{slope} ($\% \cdot s^{-1}$)	−1.9±0.6	−1.9±0.9	−1.6±0.5	21.9±13.1
ΔTOI_{min} (%)	−8.3±6.8	−8.8±7.0	−8.2±7.0	36.0±17.5
$\Delta TOI_{1/2RT}$ (s)	5.0±1.9	4.5±1.0	4.9±0.9	20.4±13.7
ΔTOI_{max} (%)	7.0±2.5	7.5±1.9	6.1±1.2	24.6±10.9
100% MVC				
Torque integral (Nm·s)	651.1±92.2	625.2±100.6	616.5±100.0	4.6±1.7
TOI baseline (%)	58.4±4.8	59.1±4.4	58.0±5.4	4.4±2.1
ΔTOI_{slope} ($\% \cdot s^{-1}$)	−6.1±1.3	−5.8±0.8	−6.1±1.3	11.1±7.6
ΔTOI_{min} (%)	−47.8±10.1	−47.6±7.1	−46.7±9.2	6.9±4.1
$\Delta TOI_{1/2RT}$ (s)	8.6±4.1	7.5±2.1	7.9±2.7	13.8±7.3
ΔTOI_{max} (%)	15.9±4.2	16.5±2.5	16.7±3.5	13.4±5.7

Note: See Sec. 2 and Fig. 1 for details of the TOI parameters.

cantly across the three testing sessions, and the CV was less than 7%. The ANOVA did not show significant changes in the TOI parameters (TOI baseline, ΔTOI_{slope}, ΔTOI_{min}, $\Delta TOI_{1/2RT}$, and ΔTOI_{max}) over the three sessions for the sustained task at both 30% MVC and 100% MVC. The CV for TOI baseline was low for both 30% MVC and 100% MVC. The CV values for ΔTOI_{slope}, ΔTOI_{min}, $\Delta TOI_{1/2RT}$, and ΔTOI_{max} in 100% MVC (6.9 to 13.8%) were smaller than those in 30% MVC (20.4 to 36.0%).

Table 2 shows the mean torque integral and TOI values over the three sessions for the repeated isometric contraction tasks and their CV. The torque integral during the 30% MVC and 100% MVC task did not change significantly over the three sessions, and the CV was less than 5%. No significant differences were found for the TOI parameters (TOI baseline, $\Delta TOI_{1/2DT}$, ΔTOI_{min}, $\Delta TOI_{1/2RT}$, and ΔTOI_{max}) across the three sessions at both 30% MVC and 100% MVC. The CV values of TOI baseline were low for 30% MVC (2.4%) and 100% MVC (3.2%); however, the CV values of $\Delta TOI_{1/2DT}$, ΔTOI_{min}, $\Delta TOI_{1/2RT}$, ΔTOI_{max} were greater than 25% for 30% MVC (Table 2).

Table 3 shows the comparison between the sustained and repeated tasks at 30% MVC and 100% MVC. When we compared the common TOI parameters between the sustained and repeated tasks at 30% MVC, ΔTOI_{min}, $\Delta TOI_{1/2RT}$, and ΔTOI_{max} were significantly ($P<0.05$) smaller for the sustained than for the repeated task. When comparing the sustained and repeated tasks at 100% MVC, changes in ΔTOI_{min} were significantly ($P<0.05$) smaller for the repeated than for the sustained task. During the recovery period, ΔTOI_{max} was significantly greater for the repeated than for the sustained task, but $\Delta TOI_{1/2RT}$ was not significantly different between conditions.

4 Discussion

This is the first investigation to report the test–retest reliability of *biceps brachii* oxygenation (TOI) during sustained and repeated isometric contraction tasks at submaximal (30% MVC) and maximal (100% MVC) intensity. The results showed no significant differences in mean values of torque and TOI parameters during the sustained and repeated tasks at both intensities over the three testing sessions, suggesting that the within-day and between-day reproducibility was good. The absolute measure of within-subject variability as demonstrated by the Bland-Altman ±2 SD limits of agreement plots indicated that torque and TOI parameters during sustained and repeated tasks at 30% MVC and 100% MVC intensity were within acceptable limits (Fig. 3). However, in order to be able to detect any change caused by an intervention and/or treatment, any potential increases or decreases in TOI parameters in subsequent testing sessions would have to be greater than

Table 2 Torque integral and *biceps brachii* tissue oxygenation index (ΔTOI) parameters during 30 repeated isometric contractions over three testing sessions (I, II, III) at 30% MVC and 100% MVC intensity, and their coefficient of variation (CV). Mean±SD (n=8).

	Session			
	I	II	III	CV (%)
30% MVC				
Torque integral (Nm·s)	696.7±112.9	694.8±113.1	691.9±103.3	4.1±3.0
TOI baseline (%)	58.9±5.0	60.7±6.2	60.1±6.6	2.4±0.8
$\Delta TOI_{1/2DT}$ (s)	10.6±6.3	12.5±8.3	9.2±6.4	25.8±16.4
ΔTOI_{min} (%)	−13.1±10.7	−13.2±10.1	−11.5±11.2	31.5±20.1
$\Delta TOI_{1/2RT}$ (s)	3.2±1.9	2.9±1.5	2.6±1.2	31.4±17.9
ΔTOI_{max} (%)	5.4±2.6	5.2±3.0	4.7±1.3	37.7±14.1
100% MVC				
Torque integral (Nm·s)	1545.0±275.7	1468.8±215.6	1492.8±198.9	5.3±2.5
TOI baseline (%)	59.3±4.8	60.8±5.2	59.5±6.9	3.2±2.3
$\Delta TOI_{1/2DT}$ (s)	7.1±0.9	7.6±0.7	7.8±1.1	10.1±4.3
ΔTOI_{min} (%)	−35.2±8.2	−35.9±8.5	−37.7±10.0	8.1±2.9
$\Delta TOI_{1/2RT}$ (s)	7.2±4.3	7.4±4.2	7.2±4.4	11.9±6.1
ΔTOI_{max} (%)	18.8±5.1	17.7±4.4	19.0±4.4	11.3±4.2

Note: See Sec. 2 and Fig. 2 for details of the TOI parameters.

these limits. The CV values for TOI parameters during the sustained (ΔTOI_{min} and ΔTOI_{slope}) and repeated (ΔTOI_{min} and $\Delta TOI_{1/2DT}$) task at 100% MVC were between 7 to 11%, and the same TOI parameters at 30% MVC were higher (22 to 36%). It is stated that a measure is considered to be reliable if CV is less than 10%;[20] therefore, the TOI parameters during the sustained and repeated task at 100% MVC appear to be reliable, but caution is necessary for the TOI parameters of the 30% MVC task. It should be noted that lower intensity tasks are clinically relevant for patients, for example, with repetitive strain injury[5] or complex regional pain syndrome,[6] where maximal intensity tasks are contraindicated.

Although NIRS has been extensively used in research, little information about the reliability of the NIRS parameters has been available. To the best of our knowledge, only one previous study by Van Beekvelt et al.[9] has reported the within-subject reliability of muscle O_2 consumption derived from NIRS parameters during handgrip exercise over three testing days. They reported that the CVs of muscle O_2 consumption during 30-repeated (1-s contraction, 1-s relaxation) isomeric contractions at seven different intensities (10 to 90% MVC) ranged from 16% to 23%. Although the CV values of the TOI parameters obtained in the present study for the 30% MVC tasks were not largely different from those reported in the previous study, the CV values for the high-intensity (90% MVC in the previous study, 100% MVC in the present study) tasks were lower in the present study. Van Beekvelt et al.[9] found a relatively high CV (20.6%) for muscle O_2 consumption at the highest intensity (90% MVC), and mean values were statistically different over three testing days, indicating that the effects of fatigue and a greater contribution of other forearm flexors for force development at the 90% MVC intensity might have been the reason for the high CV. In the present study, there was no significant difference between the three exercise sessions for mean values of TOI parameters, and the CVs were less than 10%. The torque integrals during 100% MVC tasks were not significantly different over the three sessions, and the CVs were low ($\sim 5\%$). Therefore, it appears that the fatigue level and the contribution of the *biceps brachii* muscle to elbow flexor torque development during the 100% MVC tasks were consistent over the three testing sessions in the present study. Other reasons for the different CV results between the study by Van Beekvelt et al.[9] and the present study could be due to the difference in the muscle investigated (*flexor digitorum superficialis* versus *biceps brachii*) and the method for determining NIRS parameters (arterial occlusion immediately after the exercise versus during exercise).

The CVs of TOI parameters were greater during the sustained and repeated tasks at 30% MVC than 100% MVC, which cannot be explained by a measurement error. In the present study, the placement of the probe was consistent

Table 3 Comparison between sustained and repeated isometric tasks performed at 30% or 100% MVC intensity for torque integral and *biceps brachii* tissue oxygenation index (ΔTOI) parameters. Mean±SD of 24 measurements ($n=8\times3$ sessions).

	Intensity (%)	Sustained	Repeated
Torque integral (Nm·s)	30	221.8±36.1	694.5±105.0[a]
	100	630.9±94.5	1542.9±237.2[a]
ΔTOI_{min} (%)	30	−8.4±6.6	−12.6±10.2[a]
	100	−47.4±8.5	−36.3±8.6[a]
$\Delta TOI_{1/2RT}$ (s)	30	4.8±1.3	2.9±1.5[a]
	100	8.0±3.0	7.3±4.1
ΔTOI_{max} (%)	30	6.9±1.9	5.1±2.3[a]
	100	16.2±3.1	18.5±4.5[a]

[a]the repeated task is significantly ($P<0.05$) different from the sustained task.

Note: See Sec. 2 and Figs. 1 and 2 for details of the TOI parameters.

across three sessions, and adipose tissue thickness of the area where the NIRS probe was placed did not change across sessions. It should be noted that the torque integral showed some variability across the sessions (4 to 7%), and this could partially explain the variability in the TOI parameters. However, the CVs of the TOI parameters were always greater than that of the torque integral for each task (Tables 1 and 2). Thus, it seems likely that other factors also contributed to the variability in the TOI parameters, especially in the 30% MVC tasks. It could be argued that the 30% MVC tasks might have affected the 100% MVC tasks, since the order of testing was not counterbalanced; however, it seems unlikely that the reason for the greater variability of the 30% tasks was that the 30% sustained task and repeated task were performed prior to the 100% sustained task and repeated task, respectively. If the 30% sustained task had created better reliability for the 100% sustained task, we should have seen better CVs for the repeated task at 30% MVC, but this was not the case.

A possible reason for the larger CV of TOI parameters for 30% MVC than 100% MVC tasks could be related to the differences in muscle O_2 demand and O_2 supply heterogeneity between the two intensities.[21,22] It has been suggested that submaximal isometric contractions are more prone to variations in blood flow/O_2 supply (i.e., reoxygenation), whereas higher intensity contractions increase intramuscular pressure, thereby cutting off the variations in O_2 supply affecting NIRS parameters.[9,23] During the sustained task, tHb was generally stable during 30% MVC [Fig. 1(a)], indicating that no reoxygenation was occurring during the task. In contrast, during the 100% MVC condition, the recovery of tHb did not affect ΔTOI_{slope} or TOI_{min}, although tHb tended to increase during the last 5 s of the contraction concomitantly to the torque decrease [Fig. 1(b)]. The relatively smaller changes in ΔTOI_{slope} and ΔTOI_{min} during 30% MVC than 100% MVC could be due to the redistribution of blood volume, allowing TOI parameters to be maintained above baseline levels for a longer duration. Therefore, it is possible that during 30% MVC, TOI parameters were influenced by both O_2 supply and O_2 demand, whereas during 100% MVC, O_2 demand was the primary factor influencing the TOI parameters and thus minimizing the variation in these parameters over testing sessions.

Another possible cause for the higher CV in TOI parameters at 30% MVC than 100% MVC might be related to the difference in the muscle activation of the region evaluated by the NIRS probe. It should be noted that NIRS detected the changes in muscle O_2 kinetics and hemodynamics for the superficial volume of the *biceps brachii*, and the contribution of other elbow flexor muscles (i.e., *brachialis*, *brachioradialis*) was not investigated in the present study. It could be that the activation of the muscle fibers under the NIRS probe was not the same across the three sessions for the 30% MVC tasks, but similar for the 100% MVC tasks (as discussed earlier). Therefore, it seems likely that the activation of the elbow flexor muscles and *biceps brachii* motor unit recruitment are not different for the maximal intensity tasks over testing sessions, but they may be more variable for the submaximal task over sessions, and this could explain the greater CVs for the 30% MVC tasks than the 100% MVC tasks. To fully understand the influence of these different muscle recruitment patterns on the variability of muscle O_2 supply and O_2 demand heterogeneity, the utilization of multichannel NIRS could be suggested in future studies.[2,21,22,24]

It was expected that the sustained and repeated tasks were complementary for each other to investigate the regulatory mechanisms of the oxidative metabolic system. It should be noted that although TOI_{min}, $\Delta TOI_{1/2RT}$, and ΔTOI_{max} parameters were common variables measured during the sustained and repeated tasks, the TOI_{min} parameter was calculated differently. The TOI parameters during the sustained task at 100% MVC were considered to provide information about muscle O_2 demand, since blood flow to the *biceps brachii* during contraction was likely to be completely prevented based on a previous study,[10] while TOI parameters during the repeated task at 100% MVC were considered to provide information about both muscle O_2 demand and O_2 supply. During the sustained and repeated task at 100% MVC, the TOI_{min} value for the repeated task was significantly smaller than for the sustained task (Table 3). Since torque integral during the repeated task was significantly greater than the sustained task, it would be expected that the absolute metabolic demand for O_2 would have been greater for the former task. It could be suggested that the smaller TOI_{min} value attained during the repeated than sustained tasks at 100% MVC were due to reoxygenation during the relaxation phases over the 30 contractions, maintaining TOI at an equilibrium [Figs. 1(b) and 2(b)]. If reoxygenation was not permitted during the repeated task, such as when intramuscular pressure prevents reoxygenation,[10,23] the TOI_{min} values would have been expected to decrease to a similar level as that attained during the sustained task.[10] During the recovery period, $\Delta TOI_{1/2RT}$ was similar and TOI_{max} was slightly but significantly greater for the repeated than sustained task at 100% MVC, suggesting that O_2 demand relative to O_2 supply in the recovery period was similar between tasks. Based on the paper by Chance et al.,[15] the similar $\Delta TOI_{1/2RT}$ for the repeated and sustained tasks at 100% MVC would indicate a similar O_2 deficit during the respective exercise, which required a similar restoration time of PCr and ATP stores to pre-exercise resting levels. Since the NIRS parameters during the contraction phase provide different metabolic and hemodynamic information than when contraction and relaxation phases are alternated, utilizing both sustained and repeated tasks in a battery of assessments is recommended to characterize the regulatory mechanisms of oxidative metabolism.

In conclusion, the results of the present study demonstrated that the test–retest reliability of TOI variables to assess *biceps brachii* oxygenation in response to sustained and repeated isometric contractions of the elbow flexors at 100% MVC intensity is acceptable, but caution is necessary for the 30% MVC intensity. However, the absolute measurement error, as represented by Bland-Altman plots, was within acceptable limits for all conditions; thus, if any potential increases or decreases in TOI parameters were caused by an intervention and/or treatment, in subsequent testing sessions, TOI parameters would have to be greater than these limits compared to the initial preintervention testing session. Furthermore, the utilization of both sustained and repeated tasks will provide complementary information on the regulation of muscle O_2 supply and O_2 demand. The ability of NIRS to identify acute changes in local muscle oxidative metabolic responses for muscle force production is beneficial for monitoring the effect of exercise and rehabilitation training programs.

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