# Use of Diffuse Optical Spectroscopy to Monitor Muscle and Brain Oxygenation Dynamics During Isometric and Isokinetic Exercise

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## ABSTRACT

The use of near-infrared time-resolved spectroscopy (TRS-20, Hamamatsu Corporation) in two resistance type exercise applications in human subjects is described. First, using isometric flexion of the biceps, we compared the magnitude and relevance of tissue hemoglobin concentration and oxygen saturation (stO2) changes when assuming constant scattering versus continuous measurement of reduced scattering coefficients at three wavelengths. It was found that the assumption of constant scattering resulted in significant errors in hemoglobin concentration assessment during sustained isometric contractions. Secondly, we tested the effect of blood flow restriction (BFR) on oxygenation in a muscle (vastus medialis oblique, VMO) and in the prefrontal cortex (PFC) of the brain. The BFR training technique resulted in considerably more fatigability in subjects, and correlated with reduced muscle stO2 between sets of exertion. Additionally, exercise with BFR resulted in greater PFC deoxygenation than a condition with equivalent work performance but no BFR. These experiments demonstrate novel applications for diffuse optical spectroscopy in strength testing and targeted muscle rehabilitation.

**Keywords:** resistance training, exercise, blood flow restriction, fatigue, spectroscopy, scattering

## 1. INTRODUCTION

### 1.1 Background

Near Infrared Spectroscopic (NIRS) techniques have been widely used in exercise physiology and muscle function in research for many years.[1, 2] NIRS techniques are well suited to exercise applications because both oxygen extraction and oxygen delivery can be non-invasively assessed through the measured tissue changes in deoxy-hemoglobin and oxy-hemoglobin concentrations, respectively. Well-designed optical probes have shown good performance even during subject motion.[3] Various NIRS instruments have provided insight into important exercise-related mechanisms such as blood flow regulation and oxygen delivery/extraction in response to variety of different exercise protocols. While the majority of NIRS studies have been conducted on skeletal muscle tissues, there are also active efforts to characterize the brain responses to exercise and related stimuli.[4] The clinical and physiological applications of NIRS are numerous, and there is significant potential in cardio-pulmonary exercise testing (CPET)[5]. The assessment of early hemodynamic changes in muscle tissue that may be predictive of future cardiovascular disease is especially desirable.[6]

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Despite these documented strengths of NIRS techniques, barriers to their applicability and adoption in the clinic persist. Important limitations include (but are not limited to): inability to resolve myoglobin from hemoglobin,[7, 8] difficulty separating the contributions of adipose and skin from the muscle of interest,[9] tissue spatial heterogeneity,[10] and resolving the individual-specific changes in optical path length due to tissue scattering properties changes that occur during exercise.[11] For this reason, there is active work in the refinement and development of NIRS-related technologies.

The overall purpose of this reported work was to use a relatively new commercial prototype NIRS instrument that features the use of time resolved spectroscopy (TRS-20, Hamamatsu Corporation) to quantify tissue oxygenation changes during two different exercise protocols that target specific muscles; one involved isometric contractions, and the other involved isokinetic contractions with or without blood flow restriction. The TRS-20 allows for quantitative determination of oxy- and deoxyhemoglobin concentrations using three wavelengths (759, 796, and 833 nm) in two independent channels. Importantly, the TRS-20 uses time-resolved photon counting to measure tissue path length, and subsequently resolve absorption from and scattering, at all three wavelengths[12]. The device has produced published results in the fields of exercise physiology[10], oncology[13], and cerebral oximetry[14]. Using this prototype, we were interested in quantitatively measuring simultaneous hemodynamic changes in the exercising muscle and prefrontal cortex, as well as quantifying the effects changing tissue scattering properties on the measured hemodynamics (which may affect physiological interpretation of the results).

### 1.2 Exercise Protocols

The two exercise protocols featured in this study were: Isometric Contraction (Biceps Brachii) and Blood Flow Restriction (BFR) during exercise (Vastus Medialis Oblique). In both exercise protocols, the muscle exercised as well as the prefrontal cortex (PFC) were simultaneously measured with the TRS-20 using the second independent measurement channel. To our knowledge, the application of NIRS to BRF in exercise has not been reported.

The Biodex Dynamometer (Biodex Corp., Shirley, NY) was used in all reported studies. It is a device that allows for controlled isometric and isokinetic maneuvers at many joints. The Biodex Dynamometer is commonly used in physical therapy and strength testing applications. For the first set of studies, we chose to study muscle oxygenation in isometric contractions. This is because isometric conditions allow for the isolation of muscle contraction from effects of movement around the joint. Additionally, isometric contractions can be prolonged, in this case to a duration of 30 s.

BFR in exercise involves the use of a pressure cuff to occlude vascular flow proximal to a muscle of interest. This technique, known also as KAATSU training, has been shown to enhance the beneficial adaptations of exercise training at lower work intensities than would be otherwise required.[15] American College of Sports Medicine guidelines recommend sets of exercise at 70-85% of 1-repetition max (1 RM max) to achieve hypertrophy in novices or intermediates,[16] whereas it has been shown that even intensities as low as 20% of 1 RM max can induce hypertrophy with concomitant BFR.[17] Given these benefits, exercise with BFR holds numerous clinical and fitness advantages. Most basically, BFR allows for training with lower loads to minimize stress on vulnerable joints or bones, while still leading to hypertrophy and strength gains.[18] BFR, even without an exercise stimulus, have been proven effective to reduce the amount of disuse atrophy after surgical operation, such as ACL reconstruction.[19] The elderly population may benefit from the use of low-intensity occlusion training, as many older adults are unable to withstand the loads associated with high intensity resistance training. Occlusion training may help to slow down or even reverse the loss of muscle mass with increasing age, as strength gains have been seen in individuals over the age of 50 with resistance as low as 20% of 1RM.[20] While the precise mechanisms by which BFR serves to enhance training adaptation have not been fully understood, it is thought that the impaired egress of exercise metabolites from the muscle, and the effect of these metabolites on signaling pathways such as hypoxia inducible factor, mTOR, and Nitric Oxide Synthase, play a significant role.[15]

To evaluate the effectiveness of BFR in exercise, we recorded changes in oxygenation in the vastus medialis oblique (VMO) muscle using the TRS-20 during occlusion of the thigh. The VMO is part of the quadriceps group, consisting of distal fibers of the vastus medialis, and inserting on the quadriceps tendon and medial patella. Weakness of this muscle is thought to contribute to knee instability and patella-femoral pain syndrome (PFPS).[21] Exercises that preferentially activate this muscle would be desirable in physical therapy and knee rehabilitation, and potentially in treatment of PFPS.

The VMO has been implicated in proper patellar tracking, in its role in opposition to the Vastus Lateralis.[22] We hypothesized that NIRS measurements that distinguish absorption from scattering, such as the TRS-20, could determine whether adding BFR to knee extension exercise would increase deoxygenation and metabolic stress on the VMO.

# 2. METHODS

## 2.1 Exercise 1: Isometric Contraction Exercise in the Bicep Brachii

Studies were conducted under an institutional-approved human-subjects protocol and carried out in the Human Performance Laboratory (HPL) at the University of California- Irvine (Irvine, CA). Subjects were healthy males (n = 5 for both sets of studies) between the ages of 18-30 and were recruited by word of mouth or by response to email recruitment messages. All subjects signed informed consent forms, and were allowed time for familiarization with study procedures before participating. For isometric testing, the Biodex dynamometer was set up to assess torque production during flexion of the right elbow, with no movement of the joint. Each subject was placed in a chair and immobilized except for the right arm. Pads were placed to support the upper arm, and each subject gripped a handle connected to the torque-measuring arm of the dynamometer. TRS-20 measurements were done on the belly of the biceps brachii muscle, and on the left prefrontal cortex, just inferior to the hairline on the lateral forehead. The source-detector separation used was 4 cm for both channels, and measurements were integrated over 3 seconds. Both probes were secured with medical tape, and in the case of the forehead probe, an elastic band around the head.

Each subject performed a set of short (5 and 10 second long) isometric contractions as a warm-up to compensate for differences in baseline status. The primary assessment was done during a 30-s maximal isometric flexion of the elbow. Tissue oxyhemoglobin concentration (ctHbO2), deoxyhemoglobin concentration (ctHHb), Total Hemoglobin concentration (ctTHb), and Hemoglobin Saturation (stO2) were recorded continuously on both channels. Additionally, absorption ($\mu_a$) and scattering ($\mu_s'$) coefficients were also computed and analyzed. Peak torque production was also recorded and analyzed using the dynamometer software.

## 2.2 Exercise 2: Blood Flow Restriction During Exercise in the Vastus Medialis Oblique

These second set of studies were also conducted under an institutional-approved human-subjects protocol and carried out in the Human Performance Laboratory (HPL) at the University of California- Irvine (Irvine, CA). For these studies, a total of 5 healthy male subjects were recruited, and each provided written informed consent. This study required three separate visits to the HPL with a preliminary consent/familiarization session. Subject blood pressure was assessed, and each subject was screened for possible contraindications to exercise. For these studies, the Biodex dynamometer was set up to conduct isokinetic extension of the knee joint at a speed of 30 degrees per second, with 90 degrees of joint motion. Flexion speed was 90 degrees per second, and subjects were instructed not to exert themselves during flexion. During the preliminary session, each subject performed five repetitions of this exercise at maximal exertion to calculate the peak torque for this motion (1 RM). On subsequent visits, each subject was instructed to and achieve as close to 50% of the 1 RM as possible. Torque production was monitored in real-time using the Biodex software and queues from the operator. Subjects were also instructed to abstain from resistance exercise while participating and from exercise generally on the day before each study visit. Testing occurred in the morning with subjects fasting and without having consumed caffeine-containing products.

**Conditions**: The three subsequent visits involved three sets of knee extension in one of the three following conditions: (1) three sets to fatigue with occlusion, (2) three sets without occlusion matched to the occlusion condition, and (3) three sets to fatigue without occlusion. For each of these visits, 90 seconds of recovery time between sets was provided. A minimal threshold of 10% below the 50% 1 RM was set to determine the termination of the set. Termination occurred when two consecutive repetitions were below the minimum threshold. Subjects had visual feedback regarding the 50% 1 RM and minimum thresholds and were verbally encouraged by the operator to maintain torque production at 50% 1RM. Following a rest period after condition (2), the effect of 10 minutes of occlusion by itself was assessed.

**Occlusion**: The occlusion cuff was wrapped around the subject's proximal thigh, and connected to a rapid inflator (E20 Rapid Cuff Inflator, Hokanson Inc.). The pressure was maintained at 100 mm Hg during the exercise protocol. The cuff was inflated 30 seconds before the beginning of the first set, and released immediately after the end of the third set.

**Optical Measurements**: The dual-channel Hamamatsu TRS-20 was used for measurements of tissue hemodynamics. One probe was placed over the VMO muscle on the dominant leg, which is located on the medial thigh, superior to the patella. The second probe was placed on the superior-lateral forehead on the side opposite the dominant leg. The source-detector separation for the muscle probe was 3 cm, while for the brain, 4 cm was used.

### 2.3 Data Analysis

Tissue optical properties were calculated from the time-resolved photon counting statistics using the software provided by Hamamatsu. Calculation of the optical properties was performed using the same data but two different modes: Fit All Data (FAD) and Fit and Change (FAC). In the FAD method, the time-resolved data was used to recover absorption and scattering independently at each measured timepoint. In the FAC method, a baseline period (6 measurements, 18 seconds) was used to measure the scattering; after this point the scattering was assumed to be constant and all changes in intensity were assumed due to absorption. Plots of chromophore concentrations, absorption, and scattering coefficients were produced using Microsoft Excel, and so were calculations of differences and averages. Statistical analysis was performed in SPSS (Version 11, IBM) and some plots were constructed in JMP (version 10, SAS).

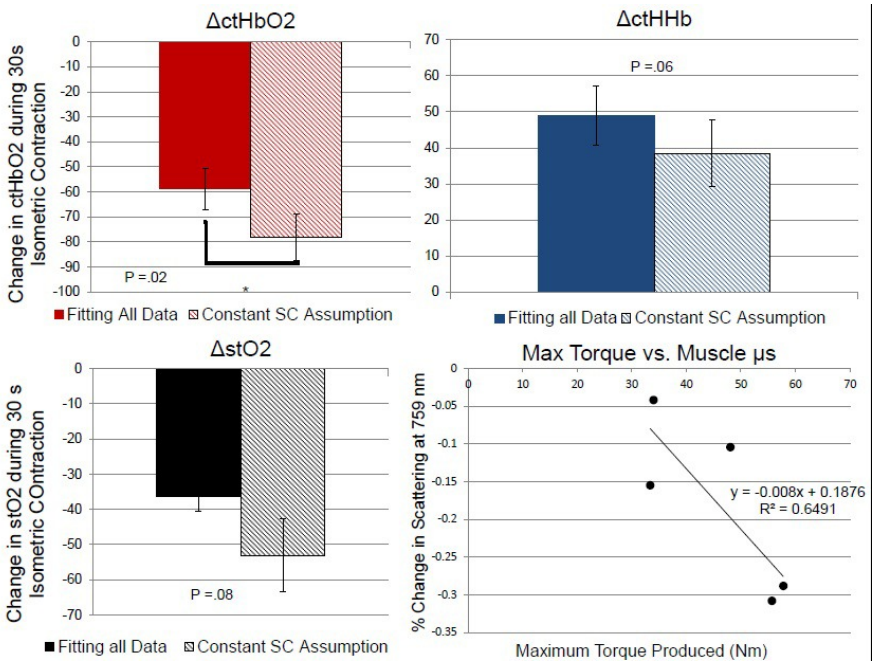


Figure 1. Top Left Panel: In 30 s isometric contraction, measured muscle ctHbO2 decreases and ctHHb increases. If scattering changes are not accounted for (FAC), the decrease in ctHbO2 is overestimated (p = .02), and the increase in ctHHb is underestimated (p = .06), and therefore, the decline in stO2 is overestimated (p = .08, bottom left panel). Bottom Right Panel: For five subjects, the fractional magnitude of the decrease in scattering coefficient at 759 nm showed correlation with peak torque output (R-squared value = .649).

## 3. RESULTS

### 3.1 Exercise 1: Isometric Contraction Exercise in the Bicep Brachii

Figure 1 (left) provides in the different values for ctHbO2 and in the muscle using the two NIRS data processing methods (FAD and FAC). 30-s isometric contraction decreased muscle ctHbO2, and increased muscle ctHHb due to simultaneous O2 extraction and compression of the muscle, inhibiting arterial flow. This resulted in a large drop in stO2 for both analysis methods. We observed that the 30-s contraction also caused significant decreases in scattering coefficients at all three wavelengths (18 ± 5%, 21 ± 4 %, and 22 ± 4% at 759, 796, and 833 nm respectively). If these

changes were not taken into account by assuming fixed scattering coefficients (FAC), it resulted in an overestimation of the stO2 decrease by an average of 16% (Figure 1, bottom left panel, p = .08). This resulted from a significant overestimation of the ctHbO2 decline (p = .02 paired 2-tailed t-test), and an underestimation in ctHHb increase (p = .06).

Figure 1 (right) demonstrates that changes in tissue scattering were proportional to the maximum torque generated by the exercise. Using the biodex software, the maximum torque production during isometric elbow flexion was recorded for each subject. In each case, a decrease in the measured reduced scattering coefficient ($\mu_s$') at 759 nm was also observed. It was found that there is a generally linear correlation between maximal torque and the percentage reduction in $\mu_s$'.

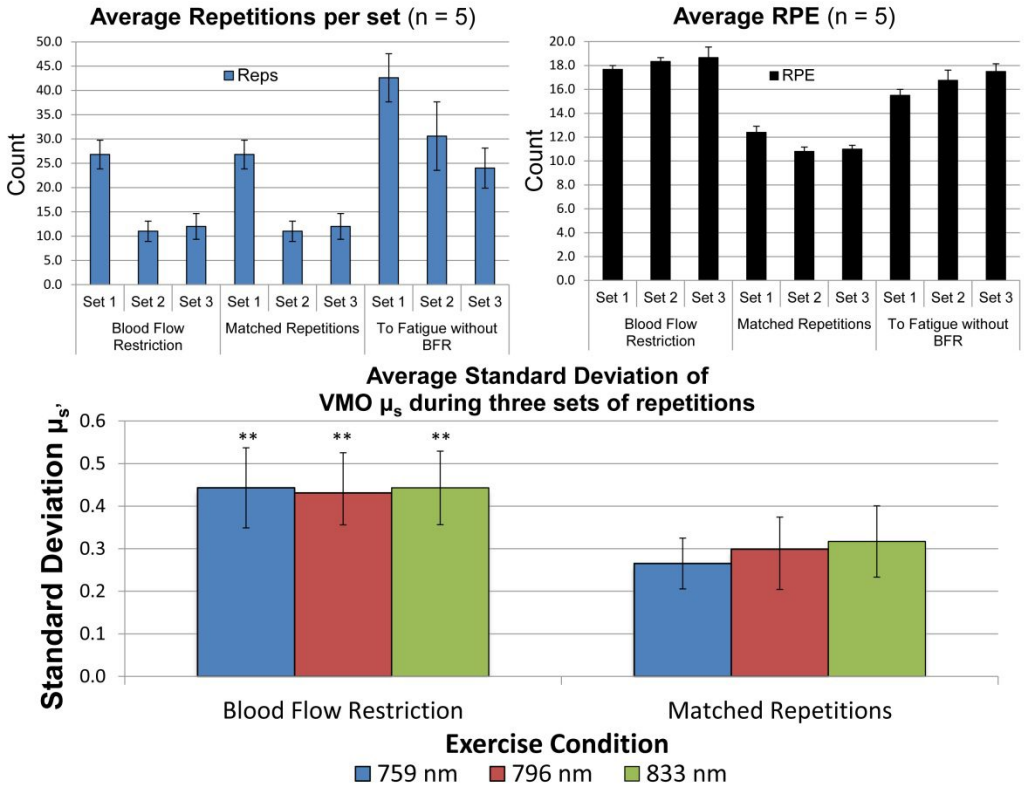


Figure 2. Top Panels: Average numbers of repetitions (blue) and response to Rating of Perceived Exertion questionnaire (black) are shown for all three sets, in each of three experimental conditions. Occlusion and matched conditions had identical numbers of repetitions by design. Average RPE was highest in the occlusion condition for all three sets, but drastically higher than in the matched condition. Bottom Panel: The average sample standard deviation of reduced scattering coefficients was measured during the three sets and intervening recovery periods. Only the BFR and matched conditions are compared here due to the identical number of repetitions, and therefore very similar sample numbers.

### 3.2 Exercise #2: Blood Flow Restriction During Exercise in the Vastus Medialis Oblique

Oxygenation measures in the Vastus Medialis Oblique (VMO) muscle and in the contralateral prefrontal cortex (PFC) were studied under three separate conditions:

(1) The occlusion condition entailed three sets of knee extension to fatigue with proximal cuff occlusion of 100 mm Hg.

(2) The matched condition consisted of the same number of repetitions achieved with occlusion, but with no blood flow restriction.

(3) The fatigue condition also had no flow restriction, but consisted of three sets of knee extension to fatigue.

For all three conditions, the targeted exertion was 50% of maximal torque, which was measured for each individual previously.

Figure 2 demonstrates the average number of repetitions achieved in each of the conditions, as well as the average responses to the Rating of Perceived Exertion scale (RPE).[23] The RPE was used as a subjective index of fatigue after each exercise set. The RPE was used to verify that the difficulty of the exercise was enhanced by BFR. On average, despite a much lower number of repetitions, all three sets done under BFR elicited higher responses on the RPE, indicating a greater degree of fatigue when compared to the other conditions, but especially the matched condition as was expected. TRS-20 measurements indicated that the average standard deviation of measured $\mu_s$' values was significantly higher in the BFR condition ($p < .01$ for all three wavelengths), compared with matched repetitions. Only these two conditions are compared because a similar number of measurements were collected in each over the course of the three sets. This indicates greater fluctuation in optical scattering over the course of three sets of exercise, which was likely due to the increased blood volume present in the leg as a result of the occlusion.

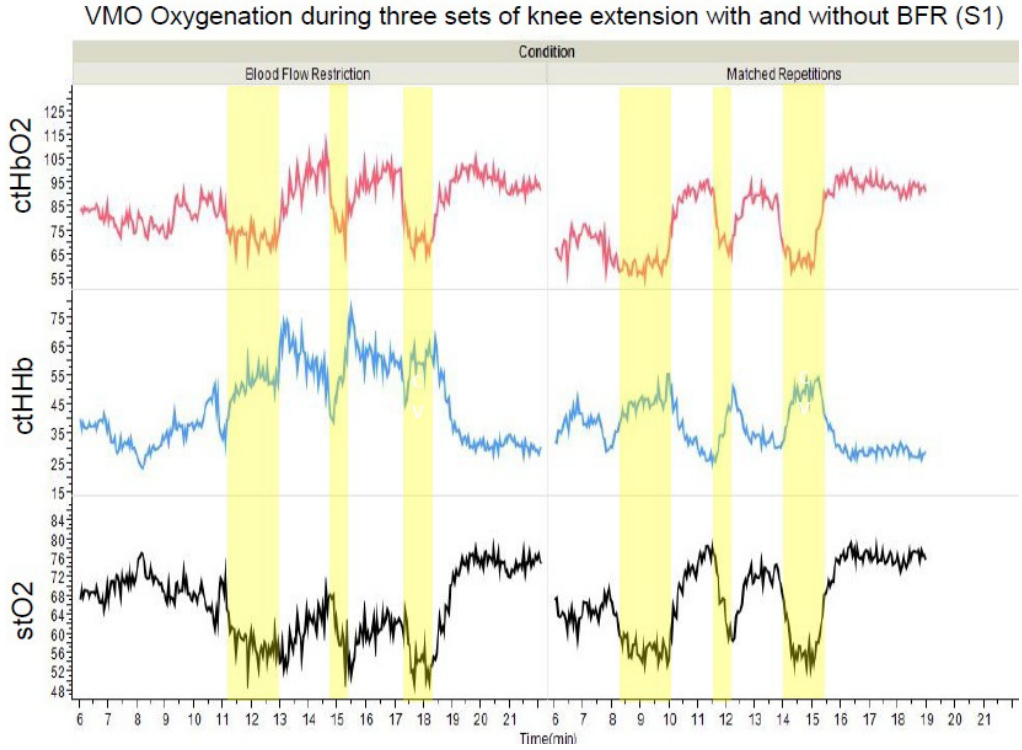


Figure 3: Representative data tracings for VMO oxygenation during three sets under two experimental conditions for one representative subject. Each set of exercises is shown in the yellow shaded regions, with 90-s recovery periods in between sets is associated with a rise in ctHHb, a decrease in ctHbO2, and a decrease in stO2. Conversely, recovery periods demonstrate the opposite trends. The two conditions were collected on separate days on the same muscle.

Figure 3 shows a representative set of tracings for VMO oxygenation using the TRS-20 in each of the three experimental conditions. In the occlusion condition, it appears that recovery increase in ctHbO2 is impeded slightly, if at all, whereas the clearance of ctHHb is much less than in the matched condition. As a result, there is a much smaller hyperemic increase in VMO stO2 during recovery after the first two sets. The absolute level of stO2 during each set of exercise does not appear to be significantly different between the two conditions.

Figure 4 shows average stO2 values during each set of exercises and during the 90-s period following each set. The primary finding is that the VMO demonstrates substantially decreased stO2 during recovery from the first two sets, primarily due to sustained levels of deoxyhemoglobin. This is despite the fact that there is, on average, no difference in the average stO2 during the exercise set. stO2 increases the most during recovery from fatiguing sets, and less so from

matched sets. Occlusion causes a significant suppression of the increase in stO2 after sets 1 and 2 (Repeated Measures ANOVA, overall significance with $p < .01$, paired t-test shows $p < .01$ for both sets compared to matched conditions), which is normalized when the occlusion is released after the third set.

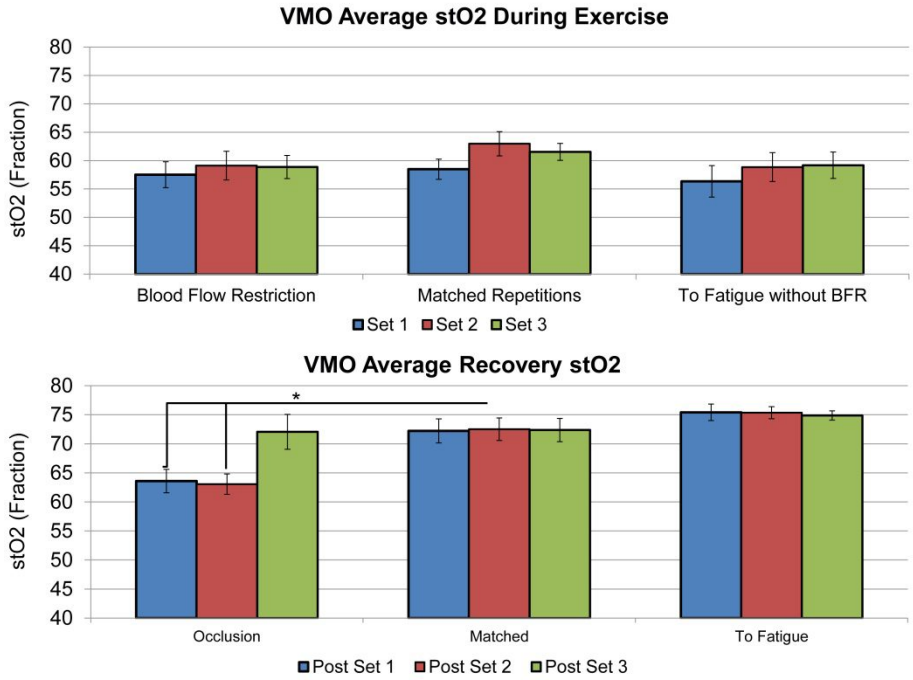


Figure 4: Top Panel: Average stO2 for all subjects across three conditions, during each set of exercise. No significant differences were found. Bottom panel: Average SO2 during 90 s of recovery after each set, under all three conditions.

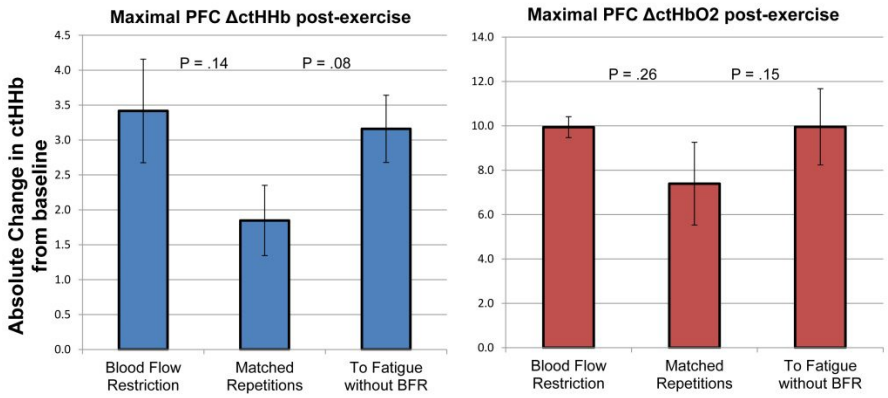


Figure 5: Maximum absolute increases in ctHHb and ctHbO2 in the prefrontal cortex during recovery from exercise. Both ctHHb and ctHbO2 increase during recovery under each condition, but the magnitude of these changes was higher in BFR and fatigue conditions relative to the matched condition. The baseline value referenced is the average concentration of each chromophore during the rest period before exercise.

Figure 5 shows average maximum increases from baseline in ctHbO2 and ctHHb in the prefrontal cortex during recovery from three training conditions. ctHbO2 and ctHHb increased during recovery for each training condition, which led yo minimal changes in stO2 (data not shown). However, the matched condition produced smaller concentration changes during recovery on average, but not to the level of statistical significance. We hypothesize that larger increases in ctHHb correlates with the greater tendency of producing fatigue.

## 4. DISCUSSION

In these studies we have demonstrated two distinct applications for NIRS using the TRS-20 in the study of exercise physiology. First, we demonstrated that optical scattering changes can obscure hemodynamic measurements during isometric contraction. The assumption of constant scattering during isometric contraction results in substantially different changes in hemoglobin concentrations and therefore introduces potentially large errors in stO2. In a 2007 study, Ferreira *et al*. investigated the same question in a different context. They found using a multi-distance frequency domain NIRS system that the assumption of constant scattering results in overestimation of muscle hemoglobin concentrations in cycling exercise. Furthermore, they reported that scattering changes were significant at maximal intensity.[24] We demonstrated that exercise conducted with proximal vascular occlusion results in a larger range of scattering changes muscle. This is possibly due to the presence of greater fluctuations in blood volume in the tissue.

Additionally, we report that the scattering coefficient itself may have some value as a corollary to force production. In the isometric elbow flexion experiment, we found a correlation between the magnitude of isometric torque and reduction in $\mu_s$' at 759 nm. The sample size involved (n = 5) is too small to draw a definitive conclusion, but it might suggest a use for time-resolved/frequency domain NIRS technologies to be used in studies of muscle strength. The changes in muscle fibers during contraction may account for these measured tissue scattering changes.

Second, we have applied TRS-20 to the study of muscle oxygenation in the context of exercise with blood flow restriction. These studies involved a complex experimental paradigm in which subjects were required to visit the laboratory on three separate occasions, and measurements were repeated on the same tissue areas. The absolute concentrations of HHb and HbO2 reported by the TRS-20 allowed for robust comparisons between the tissues measured under different experimental conditions. It is thought that the enhancement of training adaptation in the muscle subjected to BFR is due to the backup of metabolites in the venous blood.[15] We demonstrated that knee extension done with BFR can result in exposure of the VMO to substantially decreased stO2 values during recovery from each set. Our measurements suggest that the presence of BFR does not significantly inhibit the increase in ctHbO2 to the VMO following each set of exercise. The primary effect appears to be the insufficient clearing of ctHHb. It has been suggested by other researchers that the increase in arterial blood flow following exercise with BFR is not responsible for the enhanced training effects [25]. Our findings would seem to confirm this finding as there were no significant differences in stO2 in the muscle after the third set between the three conditions. If the mechanism of BFR enhancement of training is indeed through the impaired egress of exercise metabolites, this would have implications for potential therapeutic applications of BFR. If BFR interventions are used to specifically target VMO hypertrophy, the pressure applied should be individually determined such that the maximal deoxygenation can be achieved in the muscle.

In these measurements, we have also shown small but consistent increases in ctHHb in the prefrontal cortical areas of subjects performing these exercises. In the more fatiguing conditions (BFR, to fatigue without BFR), this increase in [Hb] is significantly higher, lending some credence to the notion that PFC ctHHb is related to activity-dependent fatigue [4, 26].

## 5. CONCLUSION

In these studies, we have used a 2-channel time-resolved spectroscopy device to measure the influence of scattering coefficients in determination of muscle oxygenation changes during isometric contraction. The evidence from these studies suggests that in this context, changes in optical path length due to scattering may have a significant effect on measured oxyhemoglobin concentrations. Secondly, we have used the same technique to demonstrate the effects of blood flow restriction on oxygenation changes in a leg muscle and in the prefrontal cortex over multiple experimental sessions and conditions, which is to our knowledge a novel and potentially useful application in the field of rehabilitation medicine. These measurements allow for novel insights into the mechanisms of training enhancement by BFR, and in the understanding of exercise-induced changes in prefrontal cortex hemodynamics.

## 6. ACKNOWLEDGMENTS

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