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Title

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Permalink

<https://escholarship.org/uc/item/1085860j>

Journal

Medicine & Science in Sports & Exercise, 47(1)

ISSN

0195-9131

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Publication Date

2015

DOI

10.1249/mss.0000000000000393

Peer reviewed

Published in final edited form as:

Med Sci Sports Exerc. 2015 January ; 47(1): 185–193. doi:10.1249/MSS.0000000000000393.

Effect of Blood Flow Restriction on Tissue Oxygenation during Knee Extension

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Abstract

Purpose—Time-Resolved Near Infrared Spectroscopy (TR-NIRS) was used to quantify tissue oxy- and deoxy-hemoglobin concentrations ([HbO₂], [HbR]), and O₂ saturation (stO₂) in the oblique fibers of the vastus medialis muscle (VMO) and brain prefrontal cortex (PFC) during knee extension with and without blood flow restriction (BFR).

Methods—Six young healthy males performed three sets of knee extensions on a dynamometer (50% 1 RM), separated by 90 sec rest periods, in three conditions: 1) until fatigue without BFR (Fatigue); 2) until fatigue with BFR (100 mm Hg cuff constriction around thigh, BFR); 3) same number of repetitions from condition 2, without BFR (Matched). Each condition was performed on a separate visit.

Results—BFR was associated with higher VMO [HbR] (rest 1: 57.8 μM BFR vs. 35.0 μM Matched, $p < 0.0001$) and a significantly lower stO₂ during recovery periods between sets (7.5 – 11.2 % lower than non-BFR conditions for rest 1 and 2, $p < 0.0001$). Using a piecewise linear spline method, a spike in [HbR] was observed before the onset of HbR clearance during recovery, causing HbR clearance to begin at a higher concentration (BFR: 81 μM vs. Matched: 62 μM, $p = 0.029$). [HbO₂] kinetics during recovery were also affected by BFR, with longer duration (BFR: 51 s, Matched: 31 s, $p = 0.047$) but lower rate of increase (BFR: 58 μM/min, Matched: 89 μM/min, $p = 0.004$) during recovery. In the PFC, BFR was associated with increased [HbR], diminished increase in [HbO₂], and higher subjective exertion.

Conclusions—These findings yield insight into possible physiological mechanisms of BFR, and suggest a role of TR-NIRS in monitoring and optimization of BFR exercise on an individual basis.

Keywords

resistance training; exercise; blood flow restriction; fatigue; diffuse optics; tissue oxygenation

INTRODUCTION

In recent years, considerable attention has been drawn to the potential utility of blood flow restriction (BFR) in enhancing the skeletal muscle response to resistance exercise. Exercise with BFR (sometimes called Kaatsu training(32)) has been observed to cause greater increases in muscle size compared to non-BFR resistance exercise of comparable intensity/duration(22, 39). ACSM guidelines recommend sets of exercise at 70–85% of 1-repetition maximum (1RM) to achieve hypertrophy in untrained individuals (2), whereas it has been shown that intensities as low as 20% 1RM can induce hypertrophy with concomitant BFR (37). These and other published findings suggest a use of BFR in treatment of muscle atrophy (6, 19, 40).

The molecular mechanisms for BFR enhancement of training are being actively investigated. Among the proteins with postulated signaling roles are mammalian target of rapamycin (m-TOR), heat shock proteins, and nitric oxide synthase(23). However, it is not known precisely how BFR exerts an effect on these signaling pathways. We hypothesized that measurements of muscle hemodynamics and oxygenation during BFR and similar non-BFR exercise would provide insight into this process.

Near-Infrared Spectroscopy (NIRS) is a non-invasive technique used to measure tissue concentrations of oxy- and deoxyhemoglobin ($[HbO_2]$, $[HbR]$), which together provide information about tissue-specific O_2 availability and utilization, as well as blood flow. NIRS has numerous applications in sports science (13). Considerable work has been done to characterize muscle NIRS responses in a variety of resistance exercise conditions (9, 29). NIRS has also been used to assess cerebral function during resistance exercise (24). Based on the range of currently available commercial instrumentation, most studies have utilized continuous wave NIRS (CW-NIRS) techniques (29), which report relative changes in tissue hemoglobin concentration and oxygen saturation. In contrast to CW-NIRS, time-resolved NIRS (TR-NIRS) is able to quantitatively separate light absorption from scattering and measure absolute hemoglobin concentrations in tissue. This is accomplished by measuring and fitting the temporal point spread function of emitted light signals to a model of light transport in tissue (26). These features make TR-NIRS particularly attractive for the current study because it allows compensation for differences in path length and scattering (14) that might occur with large changes in blood volume, and would therefore result in more accurate measurements.

To gain insight into physiological mechanisms for the effect of BFR, we conducted a study of isokinetic knee extension exercise with and without partial BFR (occlusion pressure of 100 mm Hg) using a two-channel commercial TR-NIRS instrument (TRS-20, Hamamatsu KK, Japan) (41). For the muscle studies, we measured the oblique fibers of the vastus medialis muscle (VMO). These muscle fibers insert medially on the patella and are thought to be important in preventing injuries such as subluxation and lateral displacement of the patella, and in the assessment of patello-femoral pain syndrome (28, 31). There have been efforts to study the VMO using EMG (33), but no published studies to our knowledge using NIRS to assess VMO oxygenation in knee extension. There is ongoing interest in whether therapies can specifically target the VMO for hypertrophy (34).

This study also included TR-NIRS measurements of cerebral oxygenation. Blood flow and oxygenation in critical cerebral areas, such as the pre-frontal cortex (PFC), have been hypothesized as determinants in the ability to sustain longer exercise challenges. This may be especially relevant during conditions of BFR when the subjective perception of fatigue may be amplified (17). We hypothesized that if BFR exercise results in greater subjective exertion, it may also alter the PFC oxygenation response to exercise when compared with control, non-BFR exercise.

METHODS

This study was 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). Seven healthy male subjects were enrolled and each provided written informed consent.

Familiarization period

Each subject was familiarized with all elements of the experimental protocol before the first testing condition. During the first visit, body mass and resting blood pressure were measured and each subject was screened for possible contraindications to exercise. The Biodex dynamometer (System 3, Biodex Corp., Shirley, NY) was used for all exercise sessions and measurements. Isokinetic extension of the dominant knee joint was conducted at 30° per second with a range of motion (ROM) of 90°. Maximal torque production for knee extension (1 repetition maximum, 1RM) was obtained from the highest torque produced during five repetitions. Subjects were familiarized with real-time visual feedback of peak torque production until they could reliably produce torque as close to 50% of 1RM as possible, which was used for all subsequent testing.

Testing visits

Testing sessions were conducted during morning hours in a fasted state. The three conditions for this exercise study are as follows: 1) exercise to volitional fatigue without blood-flow restriction (Fatigue); 2) exercise to volitional fatigue with BFR (BFR); 3) exercise without BFR with the same number of repetitions as in the BFR condition (Matched). The subjects were randomly chosen to perform the three conditions in one of two orders: 1) BFR, Matched, Fatigue or 2) Fatigue, BFR, Matched, with each session separated by at least 48 hours. Each exercise test was preceded by a brief warm-up. Rest periods between sets were 90 seconds and the minimum number of repetitions per set was 10. For the BFR and Fatigue conditions, sets were terminated when two consecutive repetitions produced torque >10% lower than 50% 1RM. For the BFR condition, an 85 cm cuff was wrapped around the proximal thigh and connected to a rapid inflator (E20 Rapid Cuff Inflator, Hokanson Inc.) set to 100 mm Hg. Cuff inflation occurred 30 seconds before the start of the first set and deflation occurred immediately after cessation of the third set. The number of repetitions achieved during the BFR condition was used to determine the number of repetitions in the Matched condition. The protocol for the Fatigue condition was identical to that for BFR, but with no cuff inflation. At the end of each exercise set, subjects were asked to provide a rating of perceived exertion (RPE), expressed as a number between 6 and

20 (3). One subject was excluded from data analysis, due to failure to adequately follow experimental instructions regarding consistent torque production.

TR-NIRS Measurements

Prior to exercise testing, TRS-20 silicone fiber holders were placed over the VMO (parallel to muscle fibers) and PFC (superolateral forehead), and secured directly to the skin with double-sided clear tape. The VMO probe was further secured with overlying tape and the PFC probe with an elastic headband to prevent motion. Source-detector separation was 30mm for the VMO and 40mm for the PFC. Continuous measurements in both tissues were made in parallel every three seconds. TR-NIRS, as implemented in the TRS-20 and described in a recent publication (41), uses a single-photon counting method to record individual reflected pulses from tissue. From these, the intensity of reflectance, optical path length and absorbance change can all be derived without model assumptions. A least-squared method is used to fit a curve derived from the diffusion equation to the obtained reflectance, allowing determination of reduced scattering coefficient (μ_s') and absorption coefficient (μ_a). From absorption coefficients, path length calculations and known molar extinction coefficients at the three wavelengths, micromolar concentrations of oxyhemoglobin ($[\text{HbO}_2]$), deoxyhemoglobin ($[\text{HbR}]$), total hemoglobin ($[\text{THb}]$), and oxygen saturation (stO_2) are calculated using the Beer-Lambert law (41). stO_2 is defined as the ratio of $[\text{HbO}_2]/[\text{THb}]$ and expressed as a percentage.

Data Analysis

Repetitions, RPE, and total work output were recorded and tabulated for each set. All TR-NIRS output variables ($[\text{HbR}]$, $[\text{HbO}_2]$, $[\text{THb}]$, and stO_2) were averaged for the baseline period, all sets, and all rest periods in both tissues. Average values were calculated for each set and rest period and tabulated using Prism (Version 6, Graphpad Software, San Diego, CA.). The same program was also used for ANOVA and subsequent Bonferroni-corrected multiple comparisons. Mean optical path length (PL) and μ_s' at 796 nm in VMO were also analyzed using coefficients of variation (CV) calculated over the course of the whole exercise challenge in the BFR and Matched conditions. CV values were compared between conditions using paired, two-tailed t-test. For analysis of oxygenation kinetics, $[\text{HbO}_2]$ and $[\text{HbR}]$ for the first set and recovery period of exercise by each subject were imported to MATLAB (Version R2013A, Mathworks). The beginning of the rapid phase of deoxygenation for both $[\text{HbR}]$ and $[\text{HbO}_2]$ was identified and used as the starting point for subsequent fitting. The Shape Language Modeling tool (SLM, D'Errico, John. (2009) Shape Language Modeling. <http://www.mathworks.com/matlabcentral/fileexchange/24443>. Retrieved 2/19/2013) was used to apply a sequential piecewise linear model to each $[\text{HbO}_2]$ and $[\text{HbR}]$ curve. We chose to apply this method in the first set and recovery because it was the longest set in repetitions, and it would allow for determination of the effect of BFR independent of any prolonged effects of occlusion. The output of each of these fits is an R-squared value, and a series of "breakpoints" (BP) time t , which represent the optimized times (and corresponding concentrations) of transition between the phases. Using this approach, $[\text{HbR}]$ and $[\text{HbO}_2]$ were modeled as following two-phase kinetics during both exercise and recovery: an initial fast phase, followed by a longer, and slower second phase. The values for fit parameters (transition concentrations and rapid-phase slopes) were

averaged for all subjects across two conditions (BFR and Matched, the conditions in which overall timing was similar due to similar number of repetitions) and compared using paired, two-tailed t-tests.

RESULTS

Exercise Parameters

The Fatigue condition was associated with a higher number of repetitions (Fig. 1A, $p < 0.001$), and therefore higher total work (Fig. 1B), as compared to the other two conditions. There was not a significant difference in work output between BFR and Matched conditions (Fig. 1B). BFR was associated with a significantly higher RPE (Fig. 1C) than the Matched condition for all sets (16.8 v. 13.6 for set 1, 18.4 v. 12.8 for set 2, and 18.6 v. 13.0 for set 3, $p < 0.0001$), but only higher than the Fatigue condition in set 2 (18.4 v. 16.8, $p = 0.0128$).

VMO Oxygenation

In figure 2, average values for each stage of exercise across six subjects are shown. For all four variables, there were significant F-values for interaction between exercise condition and stage of exercise. BFR exercise was associated with a significantly higher average [THb] than other conditions at each stage from the first rest period until the end of the third set when occlusion was released ($p < 0.0001$ in all cases, Fig. 2A). During rest periods 1 and 2, this was driven by a significantly higher [HbR] in exercise with BFR over other conditions (Fig. 2C, BFR v. Fatigue: rest 1 and rest 2 ($p < 0.0001$ for both), BFR v. Matched: rest 1 and rest 2 ($p < 0.0001$)). During sets 2 and 3, the increased [THb] was driven by a higher [HbO₂] (Fig. 2D, set 2 and set 3: BFR vs. Matched and Fatigue, $p < 0.01$ for all). The average [HbO₂] was also significantly higher in the Fatigue condition than the BFR condition in the third rest period ($p = 0.0008$), after the release of the occlusion. BFR was associated with a nearly 10% lower average stO₂ during rest periods 1 and 2 than the other conditions (Fig. 2B, $p < 0.0001$ in both cases). Reduced scattering coefficient (μ_s') and PL at 796 nm were also analyzed in the BFR and Matched conditions. It was found that the CV for PL was significantly higher for BFR than Matched condition over the whole duration of exercise (4.9% v. 3.0%, $p = 0.002$). There was also a trend toward a difference in CV of μ_s' at 796 nm, but it did not reach statistical significance (6.0% for BFR v. 4.0% Matched, $p = 0.09$). Finally, in comparison with the Matched condition, the percentage change in PL from baseline to final recovery was different (-2.7% BFR v. -0.6% Matched, $p = 0.03$).

Two-Phase Linear Fitting Results

Figure 3 shows representative results from a single subject for two-phase piecewise linear fitting of [HbO₂] and [HbR] during set 1 and rest 1. Panels A and B show data for [HbO₂] in Matched and BFR conditions, respectively, whereas panels C and D show [HbR] fits in the same conditions. For all variables, the slope and duration of the rapid first phase (phase 1) and the concentration at the transition between phases during both exercise and recovery were analyzed, as shown in Table 1. In subject 6, the rapid phases in [HbO₂] seemed to occur faster than the measurement time of the TRS (3 s) and therefore parameters for this phase were not included in the analysis. Significantly different fit parameters were identified in analysis of [HbR] during rest 1. Specifically, the concentrations at which both rapid and

slow phases of [HbR] clearance began were higher in the presence of BFR ($p = 0.029$ and $p = 0.011$ respectively). This reflected the fact that in BFR, the rapid phase of [HbR] clearance during recovery was preceded by a “spike” of [HbR] contributing to the higher average concentration (Fig. 2C). To accommodate this, an extra breakpoint was included in the fitting procedure for the BFR condition. This [HbR] “spike” was detected in all six subjects analyzed in the BFR condition and to some extent in the Matched condition for only 2 subjects. For [HbO₂] during recovery, the duration of the first phase was significantly longer in BFR vs. Matched conditions (51 vs. 31 s, $p = 0.047$). However, the slope of [HbO₂] increase was also significantly lower (56 vs. 89 $\mu\text{M/s}$, $p = 0.004$). Finally, in the BFR condition (but not Matched) there was a correlation between the magnitude of increase in [THb] from first set exercise to recovery, and the slope of rapid-phase clearance of [HbR] during recovery ($r = -0.88$, $p = 0.02$).

PFC Oxygenation

Figure 4 shows results for PFC oxygenation parameters. Panels A and B show representative BFR and Matched raw data for [HbR] in two subjects, with the whole three sets of exercise shown by dashed lines. Generally, exercise was associated with small but consistent increases in both PFC [HbR] and [HbO₂], as shown by maximal changes from baseline ([HbR] and [HbO₂]) in figure panels 4C and 4D. The BFR condition was associated with a significantly lower [HbO₂] than the Matched and Fatigue conditions during all sets (Fig. 4C), as well as the second rest period ($p < 0.01$ for all). During the first rest period, the [HbO₂] was lower in BFR than in Matched ($p = 0.0008$). BFR was associated with a significantly higher [HbR] (Fig. 4D) than Matched during the first rest period and all subsequent stages (and $p = 0.0014$ for set 2, $p < 0.0001$ for others). [HbR] was higher in BFR than Fatigue during rests 2 and 3 as well ($p < 0.0001$ and $p = 0.02$ respectively). Panels E-G show average absolute stO₂ at each experimental stage, with conditions analyzed separately by one-way ANOVA, and post-hoc Dunnett’s test against baseline values. For Fatigue (Fig. 4E), there was a significant increase in stO₂ during set 1 ($p = 0.0176$). For the Matched condition (Fig. 4F), stO₂ at every stage was higher than at baseline ($p < .01$ for all). There were no PFC stO₂ changes with BFR (Fig. 4G).

DISCUSSION

VMO Oxygenation

We have assessed tissue hemoglobin content and oxygenation using TR-NIRS with and without BFR in order to understand the effects of occlusion on muscle oxygenation during knee extension. The typical patterns observed during NIRS measurements of exercising muscles are shown in the Matched condition in Figure 3. Exercise is associated with increased [HbR] and reduced [HbO₂], with both reversing during recovery. Recovery is associated with a hyperemic supra-exercise [HbO₂] and stO₂, due to both vasodilation and increased demand to pay back O₂ deficit. [HbR] and [HbO₂] together reflect the balance between O₂ utilization and muscle blood flow (15). To our knowledge, there are few published studies of muscle oxygenation during BFR exercise, and none in the VMO muscle. In a 2011 study, Kacin and Strazar, using a CW-NIRS system, compared oxygenation dynamics of the vastus lateralis (VL) before and after a BFR training program

(18). They reported no acute differences in VL oxygenation with full arterial occlusion (230 mm Hg), but training resulted in larger increases in exercise-associated [THb] and [HbO₂] during non-BFR exercise. They did not report kinetics of oxygenation during recovery (18). We observed that knee extension with sub-systolic BFR (100 mm Hg) results in exposure of the VMO to substantially decreased stO₂ values (range: 7.5 to 11.2% lower) during recovery from each set. This reduction in stO₂ occurs in the context of greater [THb] indicating perhaps an increased capacity for post-exercise O₂ consumption. Part of this phenomenon may be driven by increased downstream muscle O₂ demand in the period immediately following exercise. Analysis of exercise and recovery kinetics of [HbO₂] and [HbR] showed that while BFR slowed VMO [HbO₂] hyperemia during recovery (56 μM/min with BFR vs. 89 μM/min in Matched, $p = 0.004$), it did not inhibit the average increase in [HbO₂]. BFR exercise was, however, associated with a spike in [HbR] before the onset of rapid clearance during recovery, contributing to the higher average [HbR] (49% higher for BFR during rest 1). To come to these conclusions, we employed an empirical piecewise regression approach to identify transitions between VMO oxygenation phases. Typically, muscle [HbR] kinetics are modeled with phasic mono-exponential regression(4, 11, 20). Recent work has shown, in the context of cycling ramp exercise, that piecewise linear fitting may be a valid approach (35). Qualitatively, the piecewise approach provides an advantage of greater descriptive utility and potentially useful comparisons between parameters such as phase duration and slope. While VMO [HbR] and [HbO₂] kinetics in knee extension have not been extensively characterized, our results would seem to indicate that the piecewise approach is valid. However, our study was limited by the acquisition time of the TR-NIRS instrument (~3s/measurement) and therefore a loss of sensitivity to transient changes.

We also observed differences in measured PL between experimental conditions. The CV for PL at 796 nm was significantly higher in BFR vs. Matched, indicating greater variability over the course of exercise and recovery. There were also larger oscillations in μ_s' in BFR, although the CV difference was not statistically significant ($p = 0.09$). This observation is likely due to larger blood volume oscillations in BFR exercise, with a likely contribution of other molecular species (14) which accumulate to a higher degree in the BFR state. Also, the change in PL from baseline to final recovery (after release of BFR) was larger in BFR than in Matched, perhaps indicating a persistent effect on VMO optical properties after BFR exercise. Both PL and μ_s' can affect reported [HbO₂] and [HbR] by the Beer-Lambert law. Another issue that must be considered is the effect of skin and subcutaneous adipose tissue (SAT) on measured optical properties. While all reflectance NIRS measurements include non-negligible contributions from the superficial layers, several factors mitigate this fact in this case: firstly, the overlying tissue above the VMO is relatively thin (3–6 mm, author's unpublished observations) compared to the source detector separation (30 mm). Secondly, the time-resolved approach discriminates between early and late-arriving photons, which allows for greater intrinsic sensitivity to absorption in deeper tissues. Finally, the level of [THb] recorded in this study (baseline VMO [THb] = 148.5 ± 6.8 μM) is consistent between subjects, indicating similar distribution of muscle in the sample volume. While the influence of superficial changes in blood flow and blood volume on reported kinetics cannot be discounted(8), others have shown these to be minimal in the case of brain TR-NIRS measurements (1). Furthermore, it has been shown that using spatially-resolved NIRS,

another technique capable of compensating for changes in PL, reduces sensitivity to skin blood flow in muscle [THb] measurements(25). Future studies may help quantify the effects of SAT thickness and skin blood flow on kinetics of VMO TR-NIRS signals, as has been done with other muscle groups (5).

Mechanisms of BFR effect

One hypothesis for the increased BFR-induced training adaptation and hypertrophy in muscle is the effect of metabolite accumulation in venous blood (23) and subsequent release of circulating factors (6, 38). It is thought that reduced O₂ delivery is also likely to contribute to the BFR effect, as systemic hypoxia during resistance exercise has been shown to enhance hormonal responses (21). Given the persistently low muscle stO₂ during recovery with BFR, it is possible that hypoxic signaling may be stimulated in this condition. However, while we observed a decrease in [HbO₂] recovery slope, there is no evidence to suggest an O₂ delivery limitation during BFR because there was no overall reduction in [HbO₂] during exercise or rest. In fact, [HbO₂] is elevated during sets 2 and 3 of exercise in BFR over other conditions. It is possible that BFR, by slowing the egress of deoxygenated blood facilitated O₂ utilization, contributing to the observed [HbR] spike. Further studies are required to determine whether the lower stO₂ observed during recovery leads to hypoxic signaling or if it is simply a consequence of greater O₂ availability and extraction.

Additionally, it has been demonstrated that BFR training can enhance post-occlusive hyperemic blood flow in muscle (27), possibly as a result of increased angiogenesis. The persistent increase in blood volume caused by BFR, as evidenced by elevated [THb], may act to enhance the shear stimulus on the vessel wall, thereby promoting angiogenesis (18, 30). The level of hypertrophy achieved with a given dosage of resistance exercise is also dependent on the duration of rest periods, with shorter intervals likely inducing larger gains (10). It is possible that the spike in [HbR] we observe in BFR exercise acts in a way analogous to reduction in recovery time, i.e. by slowing metabolite clearance.

Prefrontal Cortex Oxygenation

We have also shown small but consistent increases in [HbR] and [HbO₂] in the PFC of subjects with and without BFR. In BFR and Fatigue conditions, when RPE is higher, this increase in PFC [HbR] is relatively larger, suggesting that PFC [HbR] may be related to activity-dependent fatigue (12, 36). Additionally, during BFR, the increase in [HbO₂] during exercise is smaller than in the other conditions and therefore stO₂ does not increase. Our results correspond to published data indicating that knee extension is associated with increases in PFC stO₂ and blood volume ([THb]) (24). The observation that BFR abolished the PFC stO₂ increase during exercise suggests that it causes a different metabolic response for a given increase in [THb]. It has been observed (17) that lighter exercise with moderate occlusion is associated with perceptual responses akin to heavier-load, non-BFR exercise, possibly due to increased compression of peripheral nerves. In a 2003 study, it was shown that cycling exercise with occlusion could alter cerebral metabolism (7), possibly by altered sensory input from skeletal muscle. It is not clear whether small PFC stO₂ changes (< 5%) such as those we observed would impact perception of fatigue. It seems that at minimum, moderate exercise with BFR can affect O₂ utilization and blood flow in the PFC in a manner

that correlates with increased RPE. In summary, the results of these studies indicate that muscle hemodynamics and oxygenation may play a role in the enhancement of resistance exercise outcomes by BFR and that BFR can modulate exercise-induced changes in PFC hemodynamics in combination with perceived exertion during isokinetic resistance exercise.

Note: Preliminary data from this set of studies was published in the Proceedings of SPIE conference journal, 2013(16).

Acknowledgments

This research was funded by the National Institutes of Health through a TL-1 fellowship, 8UL1TR000153 (GG), and grants P01D048721 (PG), and P41EB015890 (BJT).; and the Arnold and Mabel Beckman Foundation. BJT and AEC report patents, assigned to the University of California Regents, on Diffuse Optical Spectroscopy and Imaging technologies. We gratefully acknowledge Hamamatsu Photonics, Inc. for the loan of the TRS-20 TR-NIRS instrument used in these studies. The project described was supported by the National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health, through grant UL1 TR000153. JAC was supported by the NIH training grant 2T32AR047752 to the Multidisciplinary Exercise Science Training Program at UC Irvine. The results of this study do not constitute endorsement of the techniques used by the ACSM.

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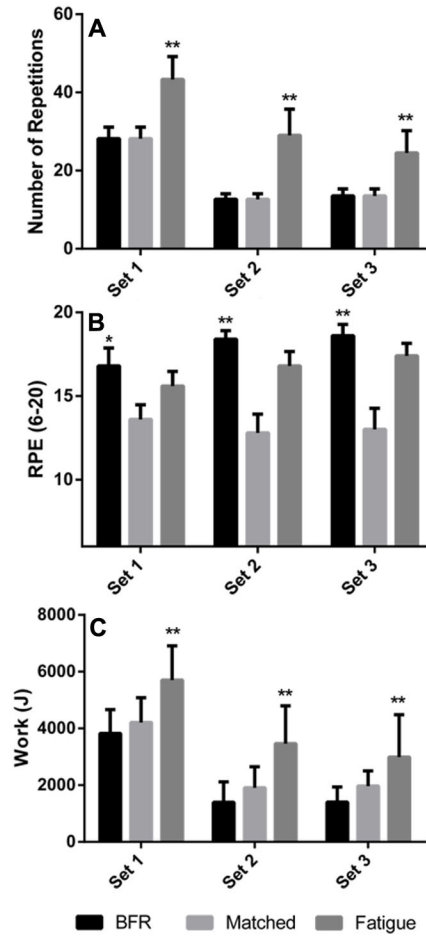


Figure 1. Average number of repetitions (A), total work output (B), and rating of perceived exertion (RPE) (C) during three sets of isokinetic knee extension in 6 healthy subjects. The 3 sets were performed by each subject in 3 conditions: to exhaustion without blood flow restriction (Fatigue); to exhaustion with blood flow restriction (BFR); and without blood flow restriction matching the workload in BFR (Matched). * $p < 0.05$, BFR vs Matched; **, $p < 0.05$, BFR vs both other conditions.

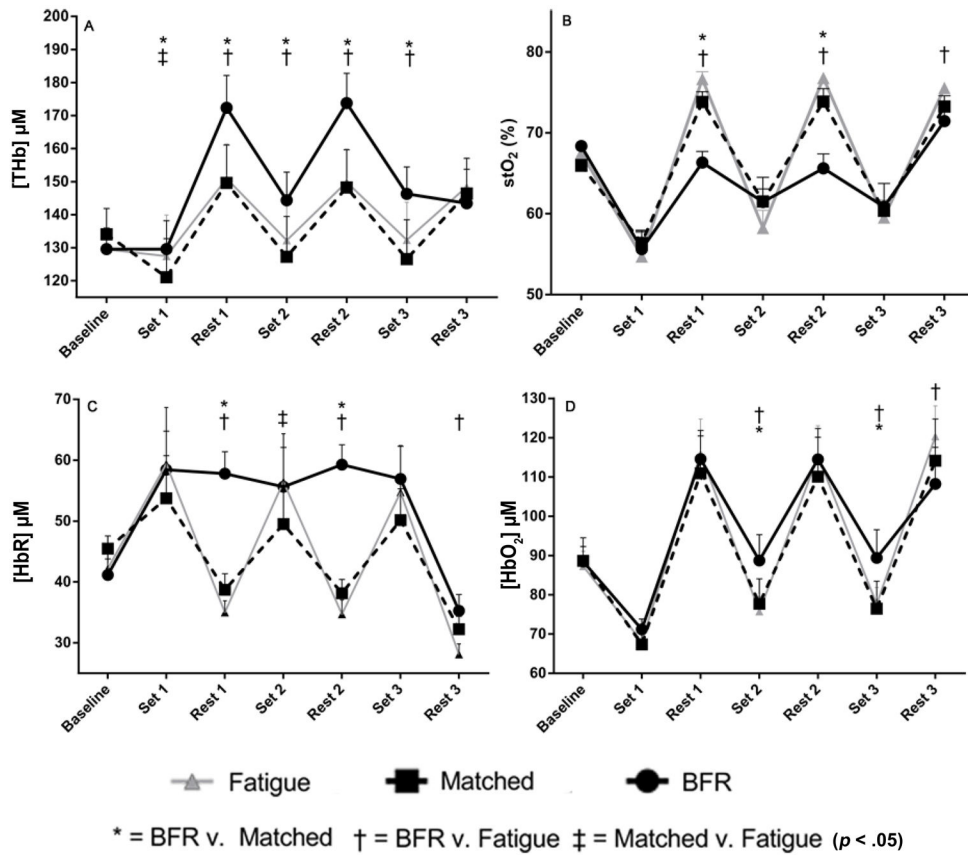


Figure 2. Average absolute [THb] (A), stO₂ (B), [HbO₂] (C), and [HbR] (D) in the VMO muscle, during three sets of isokinetic knee extension in 6 healthy subjects. The 3 sets were performed by each subject in 3 conditions: to exhaustion without blood flow restriction (Fatigue); to exhaustion with blood flow restriction (BFR); and without blood flow restriction matching the workload in BFR (Matched).

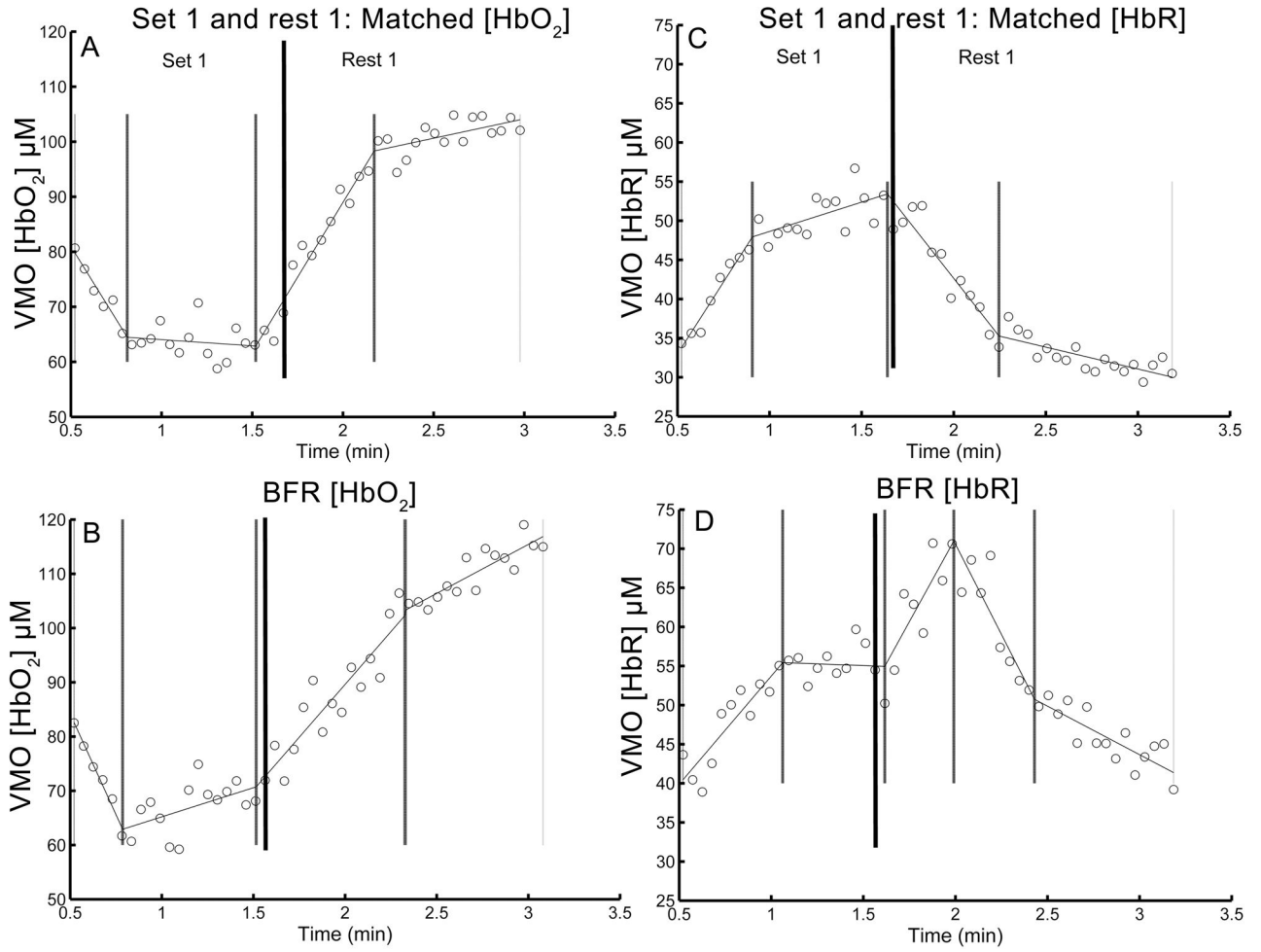


Figure 3. Representative single-subject results using piecewise linear spline fitting of the first set and rest periods. A and B show the results of [HbO₂] goodness of fit for Matched (A, $R^2 = 0.952$) and BFR conditions (C, goodness of fit: $R^2 = 0.888$). B and D show results of [HbR] for Matched (C, $R^2 = 0.976$) and BFR (D, $R^2 = 0.965$). Solid black lines represent event markers for division between exercise and recovery. Vertical narrow lines represent the position in time of optimally-fitted breakpoints between linear phases. The circles represent raw data points, and the solid lines represent the fitted linear segments.

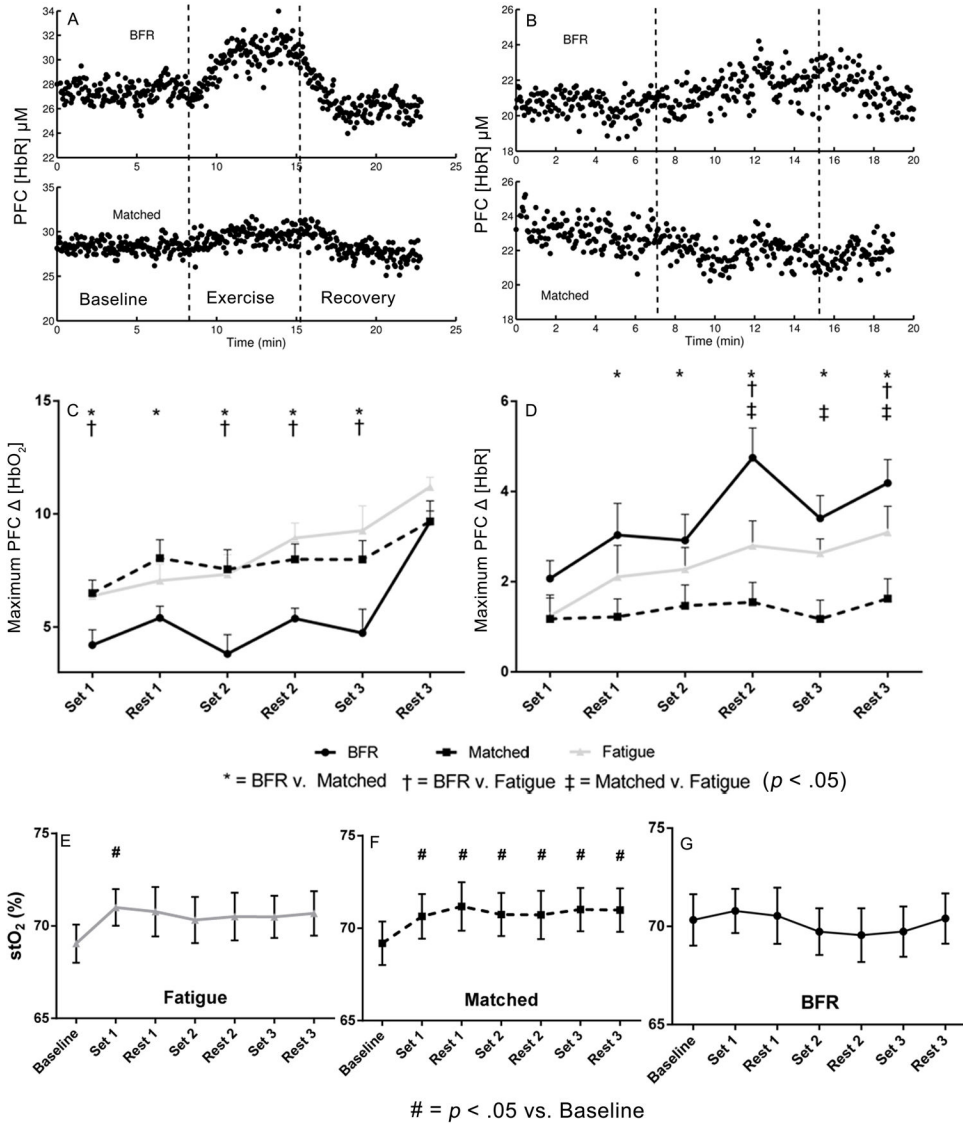


Figure 4. Sample tracings in two subjects for [HbR] in BFR (above) and Matched (below) conditions (A, B). For each exercise stage, the maximal difference from baseline was calculated for [HbO₂] and [HbR] and group averages are shown in panels C and D. Panels E-G show average stO₂ values at each stage of exercise analyzed separately in the three experimental conditions.

Summary of mean \pm SEM of fitting parameters for all subjects for the first set and rest periods. Paired, 2-tailed t-tests were used to compare mean values between 2 conditions, BFR and Matched. P-values for significant differences are underlined.

Table 1

Condition	Initial conc. (μ M)		First phase duration (s)		Transition conc. (μ M)		First phase slope (μ M/s)	
	BFR	Matched	BFR	Matched	BFR	Matched	BFR	Matched
[HbR] set 1 (average $R^2 = .895$)								
Average \pm SEM	40 \pm 4	34 \pm 4	25 \pm 5	30 \pm 7	60 \pm 7	56 \pm 7	69 \pm 29	63 \pm 20
p-Value	0.059		0.330		0.187		0.599	
[HbR] rest 1 (average $R^2 = .940$)								
Average \pm SEM	81 \pm 8	62 \pm 6	25 \pm 5	27 \pm 7	58 \pm 5	37 \pm 3	-62 \pm 13	-57 \pm 9
p-Value	<u>0.029</u>		0.781		<u>0.011</u>		0.717	
[HbO₂] set 1 (average $R^2 = .959$)								
Average \pm SEM	93 \pm 4	88 \pm 7	15 \pm 2	17 \pm 2	65 \pm 4	63 \pm 5	-133 \pm 48	-94 \pm 27
p-Value	0.371		0.296 #		0.638		0.279 #	
[HbO₂] rest 1 (average $R^2 = .977$)								
Average \pm SEM	71 \pm 2	67 \pm 4	51 \pm 7	31 \pm 2	114 \pm 9	111 \pm 9	56 \pm 10	89 \pm 14
p-Value	0.307		<u>0.047</u> #		0.441		<u>0.004</u> #	

one subject was excluded from this comparison because the fitted first phase was shorter than the TRS-20 measurement interval