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Skeletal Muscle Oxidative Metabolism during Exercise Measured with Near Infrared Spectroscopy

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Abstract: This study characterized the level of oxidative metabolism in skeletal muscle during whole-body activity as a percentage of the muscle's maximum oxidative rate (mVO_2max) using near-infrared spectroscopy (NIRS). Ten healthy participants completed a progressive work test and whole-body walking and lunge exercises, while oxygen saturation was collected from the vastus lateralis muscle using near-infrared spectroscopy (NIRS). Muscle oxygen consumption (mVO_2) was determined using arterial occlusions following each exercise. mVO_2max was extrapolated from the mVO_2 values determined from the progressive exercise test. mVO_2max was 11.3 ± 3.3%/s on day one and 12.0 ± 2.9%/s on day two ($p = 0.07$). mVO_2max had similar variation (ICC = 0.95, CV = 6.4%) to NIRS measures of oxidative metabolism. There was a progressive increase in mVO_2 with walking at 3.2 Km/h, 4.8 km/h, 6.4 Km/h, and with lunges (15.8 ± 6.6%, 20.5 ± 7.2%, 26.0 ± 6.6%, and 57.4 ± 15.4% of mVO_2max, respectively). Lunges showed a high reliability (ICC = 0.81, CV = 10.2%). Muscle oxidative metabolism in response to whole-body exercise can be reproducibly measured with arterial occlusions and NIRS. This method may be used to further research on mitochondrial activation within a single muscle during whole-body exercise.

Keywords: near-infrared spectroscopy; metabolic rate; walking; maximal oxidative capacity

Citation: McCully, K.K.; Stoddard, S.N.; Reynolds, M.A.; Ryan, T.E. Skeletal Muscle Oxidative Metabolism during Exercise Measured with Near Infrared Spectroscopy. *NDT* **2024**, *2*, 417–429. https://doi.org/10.3390/ndt2040025

Academic Editor: Fabio Tosti

Received: 6 August 2024
Revised: 4 October 2024
Accepted: 10 October 2024
Published: 11 October 2024

1. Introduction

Whole-body aerobic capacity, commonly termed VO_2max, is a frequently used measure to evaluate health and athletic performance [1,2]. Skeletal muscle oxidative metabolism is a key factor that contributes to whole-body VO_2max. Increasingly, the health of skeletal muscle, and in particular skeletal muscle mitochondrial function, has also been shown to be important in health and disease [3,4]. This is true for resting muscle metabolism [5], but also for muscle metabolism during exercise [6].

Traditionally mitochondrial function has been measured using biochemical approaches in vitro with muscle biopsies [7–9]. Because of the invasive nature of the muscle biopsy procedure and artificial conditions employed during in vitro biochemical assessments, several noninvasive methods have been developed to assess skeletal muscle oxygen consumption in vivo. Magnetic resonance spectroscopy (^{31}P MRS) can characterize muscle oxidative metabolism and changes in muscle pH [10–12]. However, ^{31}P MRS is limited to exercise that can be performed in the constraints of the magnet bore. The advancement of near-infrared spectroscopy (NIRS) provides an additional noninvasive means of monitoring skeletal muscle oxygenation [13,14]. Skeletal muscle oxygen consumption (mVO_2) can be measured by NIRS at rest and during exercise using an acute arterial occlusion [15–22]. However, most studies have been performed using an exercise ergometer in a seated or supine position. Measurements of muscle oxygen levels can be made during free-standing or untethered exercise [23]. What is needed is to incorporate measurements of muscle metabolism into free-standing exercise.

The purpose of this study was to characterize muscle oxygen consumption (mVO_2) in the vastus lateralis muscle during free-moving exercise: consisting of walking on a treadmill at different speeds and free-standing bodyweight lunges. Values of mVO_2 during walking and lunging exercise were normalized to maximal mVO_2 estimated from mVO_2 measurements made during a progressive knee extension work test. We repeated the measurements on a separate day to determine day-to-day variability. We hypothesized that mVO_2 would increase proportionally with walking speed and that bodyweight lunging would require great levels of mVO_2 than walking.

2. Materials and Methods

- Participants

Young, healthy adults between 18 and 33 years of age were recruited to participate in this study. This study was approved by the Institutional Review Board at the University of Georgia (Athens, GA, USA). All participants provided written informed consent prior to testing. All testing was conducted in accordance with the Declaration of Helinski (2008).

- Study Design

We used a repeated-measures one group experimental design that consisted of two testing sessions on non-consecutive days within a period of seven days (Figure 1). Each testing session consisted of a progressive work knee extension protocol, an increasing intensity treadmill walking protocol, and bodyweight lunges.

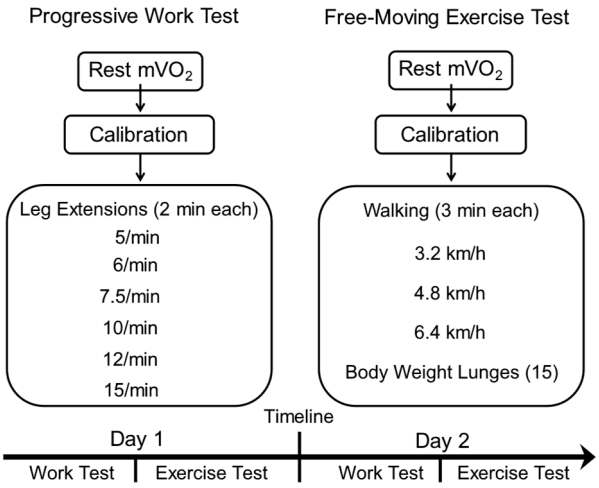

Figure 1. Experimental timeline. The order of test administration was randomized between subjects and between testing days.

- Muscle assessments

Skeletal muscle oxygen consumption (mVO_2) was assessed using the change in oxygenated hemoglobin/myoglobin (O_2Hb) signal during brief arterial occlusions [24]. Arterial occlusions were performed using a blood pressure cuff (Hokanson SC12D, Bellevue, WA, USA) inflated using a rapid inflation system (Hokanson E20 Cuff Inflator, Bellevue, WA, USA) powered by a 10-gallon commercially available air compressor (California Air Tools 210DLV, San Diego, CA, USA). Oxygen signals were obtained using a continuous-wave NIRS device (PortaMon, Artinis Medical Systems, Zetten, The Netherlands) with 3 LED transmitters that each emit two wavelengths (760 nm and 850 nm) of light at three set distances (30, 35, and 40 mm) from the receiver. All data analyses were performed at 40 mm separation distances. The depth of penetration of the light was assumed to be approximately half of the distance between the transmitter and receiver [25]. All NIRS data were collected at a sampling rate of 10 Hz. NIRS data were scaled (expressed as a percentage) to the maximal physiological range obtained during the ischemic calibration.

NIRS signals were corrected for blood volume changes as previously described [24]. mVO_2 was calculated as the rate of change in the NIRS oxygenated (O_2Hb) signal during arterial occlusions using a simple linear regression [21].

B-mode ultrasound imaging (LOGIQ e: GE HealthCare, Wauwatosa, WI, USA) was used to measure the adipose tissue thickness (ATT) under the NIRS probe. ATT thickness has been shown to influence NIRS signals in previous studies [18,26,27].

Muscle electromyography (EMG) signals were collected using a Biopac Systems MP100A-CE, with the ECG100C unit). Electrodes were placed across the vastus lateralis muscle and on a bony landmark. The raw signal was collected at 200 Hz; the magnitude of the signal (squared and square root) was determined.

- Knee extension exercise protocol

The knee extension protocol consisted of performing single-leg knee extensions in the self-reported dominant leg at increasing frequencies of contraction. Resistance level was set at ~30% of the one repetition maximum. A leg extension fitness machine (Magnum Fitness Systems, Badger Fitness, South Milwaukee, WI, USA) was used to perform knee extensions (Figure 2A). The blood pressure cuff was placed as high as anatomically possible on the thigh on the self-reported dominant leg, with the NIRS device secured to the vastus lateralis muscle of the same leg using Velcro straps. The placement of the NIRS device was chosen to allow the maximum amount of distance between the device and the blood pressure cuff to attenuate any motion artifact in the NIRS signal that would be caused by inflation of the blood pressure cuff. Placement of NIRS device was recorded using measures relative to boney landmarks of the patella and hip bones to reproduce device placement as close as possible for the second day of testing. The following contraction frequencies were used: 5, 6, 7.5, 10, 12, and 15 leg extensions per minute. Single-leg knee extension exercises at each of the above contraction frequencies were performed for two minutes to ensure participants reached steady state mVO_2. At the end of each two-minute bout of knee extension exercise, mVO_2 was measured as the rate of change in the NIRS signal during a 10-s arterial occlusion. After the last set of knee extensions, an ischemic calibration was performed to scale NIRS data to a maximum physiological range as previously described [21]. Briefly, 5–8 leg extensions were performed immediately followed by a 5–6 min arterial occlusion and 3–5 min of reactive hyperemia after the release of the occlusion. All arterial occlusions were performed with the leg in a relaxed, fully extended position.

- Free-moving exercise protocols

Free-moving exercise protocols consisted of both treadmill walking and bodyweight lunge exercises. A blood pressure cuff was placed as high as anatomically possible on the thigh of the self-reported dominant leg and attached to elastic suspenders to ensure the blood pressure cuff remained in place during the exercises (Figure 2B). The NIRS device was placed on the vastus lateralis of the same leg, allowing for the maximum amount of distance between the NIRS device and blood pressure cuff. Bony landmarks were used to record placement of the device to replicate placement location on the second day of testing.

Treadmill exercise consisted of walking at 3.2, 4.8, and 6.4 km/h for three minutes each to achieve close to steady level exercise. At the end of each three-minute bout of walking exercise, the treadmill was stopped to allow the participant to safely step to the side of the treadmill belt and transfer all body weight into the non-dominant leg. Once the participant was in a stable position (usually 2–3 s), a 10-s arterial occlusion was performed to measure mVO_2.

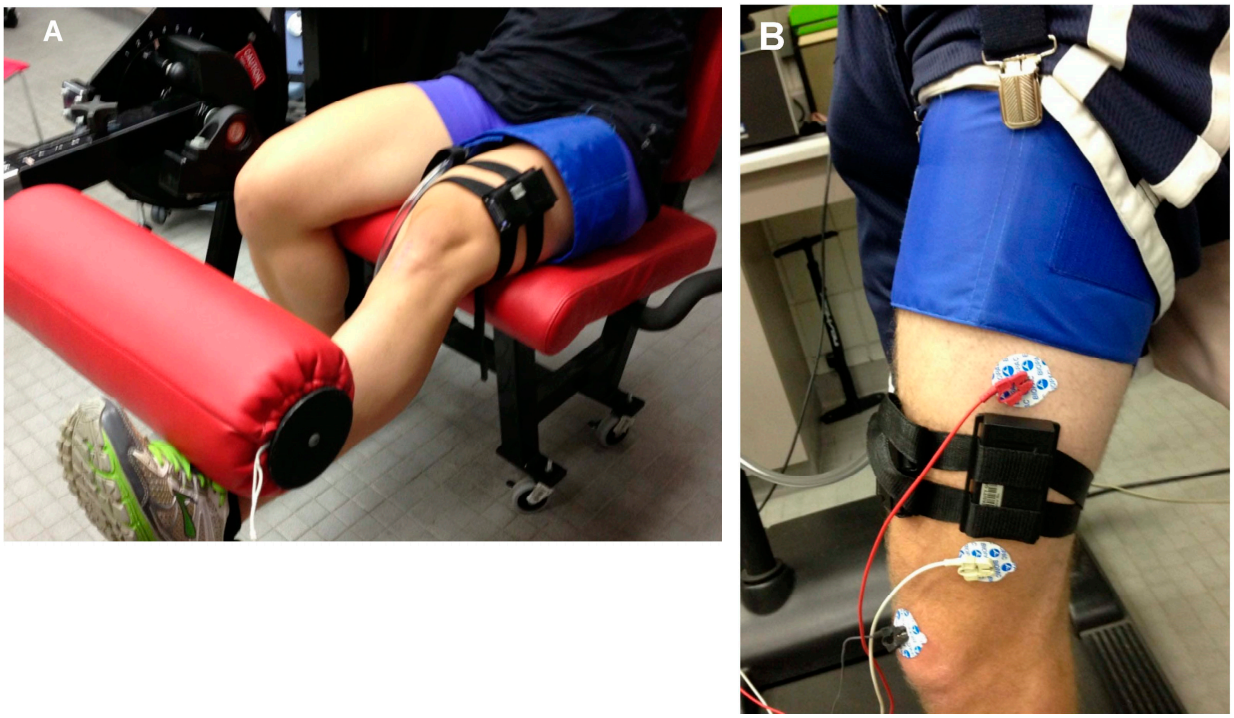

Figure 2. (**A**) The experimental setup for the progressive exercise test. The NIRS device was placed on the vastus lateralis using straps. The blood pressure cuff was placed proximally to the NIRS device. The leg is shown lifting the weight. For NIRS measurements, the leg was placed on top of the padding in the horizontal position to allow the muscle to relax during the measurements. (**B**) Placement of the NIRS device, EMG electrodes, and ischemic cuff for the treadmill walking and lunging measurements.

Participants performed 15 bodyweight lunge exercises with the self-reported dominant leg forward to measure the level of oxidative metabolism after a short bout of exercise. Participants were instructed to perform 15 lunges, defined as a 90-degree bend in both the forward and behind leg with the back knee touching the floor, at a self-selected pace (between 0.7 and 0.9 lunges per second). Immediately following the lunges, the participant was instructed to stand with their body weight supported by their non-dominant leg and a 10 s arterial occlusion was performed to measure mVO_2. After both treadmill and lunge protocols were completed, an ischemic calibration was performed as previously described [21]. Briefly, the participant was instructed to perform ~5 lunges and immediately stand with their body weight supported by the non-dominant leg. A 5–6 min arterial occlusion was performed followed by 3–5 min of reactive hyperemia after the release of the occlusion to obtain the ischemic calibration.

- NIRS data analysis

The metabolic rates of the increasing frequencies of leg extensions were plotted, producing an exponential curve (see Figure 3). The point at which the curve plateaued and no longer increased represented the mVO_2max. Eadie Hoftsee plots were used to extrapolate mVO_2max [28]. The metabolic rates obtained from the whole-body activities were then characterized as a percentage of mVO_2max, with resting metabolism representing 0% and the calculated mVO_2max representing 100%.

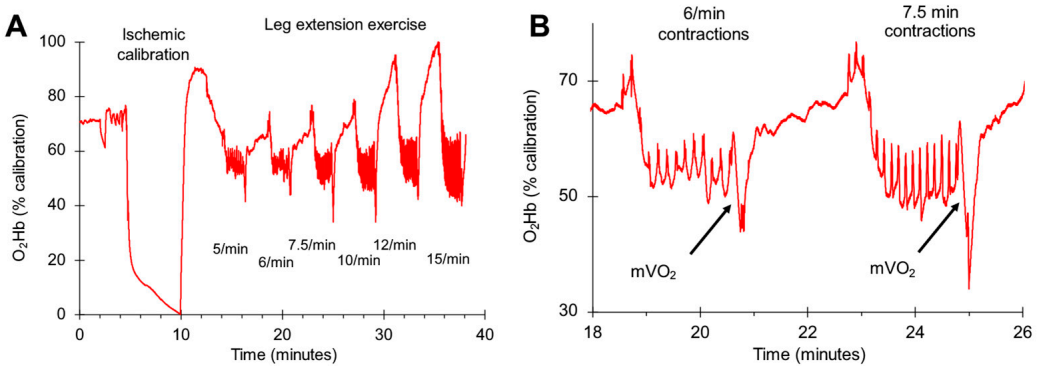

Figure 3. Representative oxygen levels (O_2Hb) for one subject during the progressive exercise test. An ischemic calibration was performed to normalize the oxygen levels to a range of 0–100%. (**A**) shows the complete protocol with six different exercise intensities. (**B**) shows an expanded view of two mVO_2 slope measurements for two exercise intensities. The arrows indicate where the mVO_2 measurements were made.

- Statistical analyses

All data are presented as means and standard deviations. Differences between days were assessed using paired T tests. We assumed the significant differences with p values less than 0.05. Day-to-day reproducibility was characterized by calculating the coefficient of variation (CV) and the intraclass correlation coefficient (ICC). The CV was calculated as the standard deviation of between-day tests divided by the mean of between-day tests, expressed as a percentage of the mean value. The ICC was calculated using a two-way mixed model analysis of variance. Pearson correlations were performed when examining the relationship between two variables. Equivalency plots were made for the mVO_2max measurements [29].

3. Results

Ten young, healthy adults (seven male, three female) were recruited to participate in this study (22.8 ± 2.4 years of age, BMI of 24.5 ± 2.5). All participants self-reported to be recreationally active, defined as participating in physical exercise two days or more per week. Average adipose tissue thicknesses (ATT), measured by ultrasound over the vastus lateralis muscle was 0.66 mm (range = 0.35 to 0.86 mm). ATT measurements of the same location were highly correlated for day one and day two (r^2 = 0.94). No significant relationships were seen between ATT and mVO_2 measurements (p = 0.88).

A representative example of relative O_2Hb levels during the progressive knee extension test is shown in Figure 3. A representative example of relative O_2Hb levels during the whole-body activities are shown in Figure 4. Relative O_2Hb levels for each of the measurements are shown in Figure 5. Relative O_2Hb levels were not significantly different between day one and day two for any of the measurements ($p > 0.05$). The time from the last muscle contraction or step to the start of the NIRS slope measurement ranged from 2.2 to 3 s and was not different between day one and day two (Table 1).

Increasing the contraction frequency during the progressive work test resulted in increases in mVO_2 (Figure 6). The extrapolated mVO_2max was 11.3 ± 3.3%/s on day one and 12.0 ± 2.9%/s on day two (p = 0.07). For each work level, the coefficients of variation ranged from 5.1 to 10.1% of the mean value, and the ICC values varied from 0.25 to 0.79. mVO_2max had a coefficient of variation of 6.4% and an ICC value of 0.95. The correlation of day one and day two for mVO_2max and the equivalency plot are shown in Figure 7.

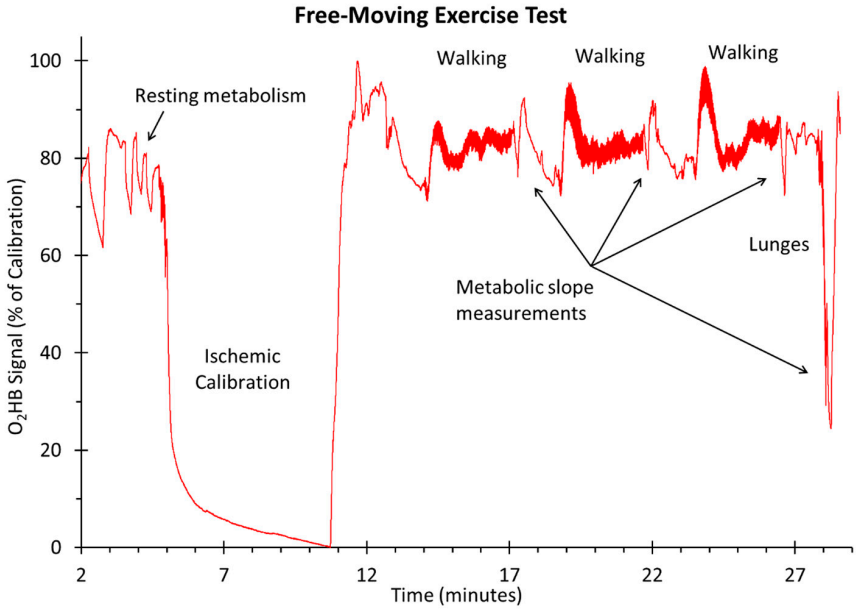

Figure 4. Representative oxygen levels (O_2Hb) for one subject during the walking and lunging exercise.

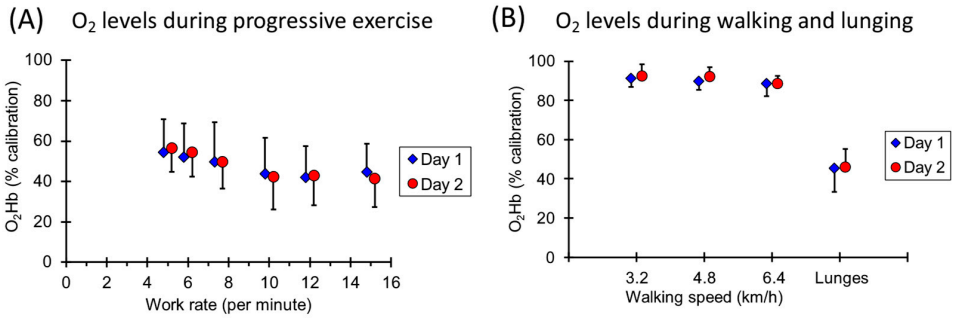

Figure 5. (**A**) Oxygen levels at the start of the slope measurements for mVO_2 during the progressive walking test. (**B**) Oxygen levels at the start of the slope measurements for mVO_2 during the walking and lunging exercise. Values are means and standard deviations and have been offset on the x axis for clarity.

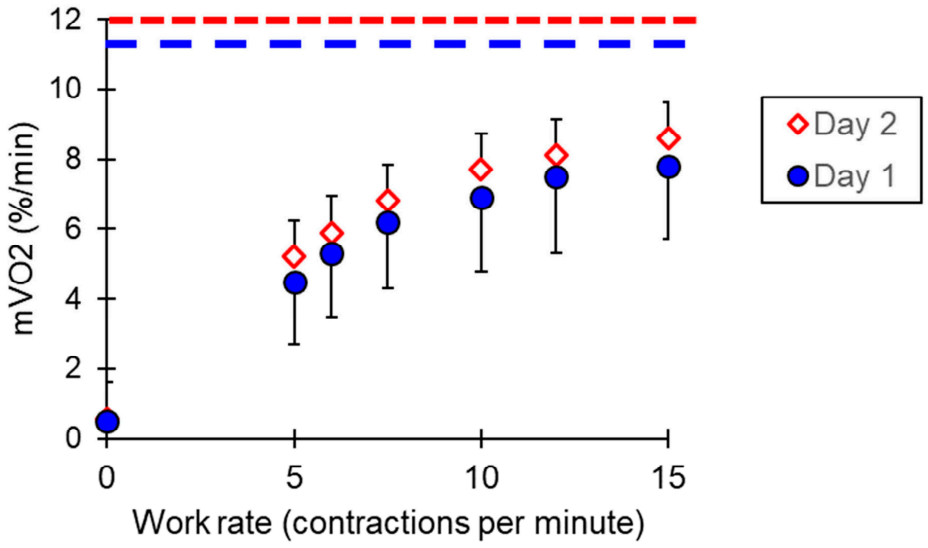

Figure 6. mVO_2 values for the progressive exercise test. The dotted lines represent the calculated mVO_2max values for each test day. Values are means and standard deviations.

Table 1. Times from the last muscle contraction to the start of the slope measurements.

Progressive work test: time from the last knee extension				
Exercise	**Day 1**		**Day 2**	
Rate	Time	SD	Mean	SD
#/minute	seconds	seconds	seconds	seconds
5	2.8	1.2	3.0	0.7
6	2.8	0.9	2.9	0.9
7.5	2.8	0.8	2.9	1.1
10	2.8	0.4	3.2	0.8
12	2.7	0.8	3.2	0.9
15	2.6	0.6	2.9	0.6
Exercise test: time from the last leg movement				
Walking	**Day 1**		**Day 2**	
speed	Time	SD	Mean	SD
MPH	seconds	seconds	seconds	seconds
2	2.6	1.1	2.9	1.3
3	2.2	0.7	2.1	0.8
4	1.9	0.5	2.4	0.9
lunges	2.5	0.7	2.3	0.6

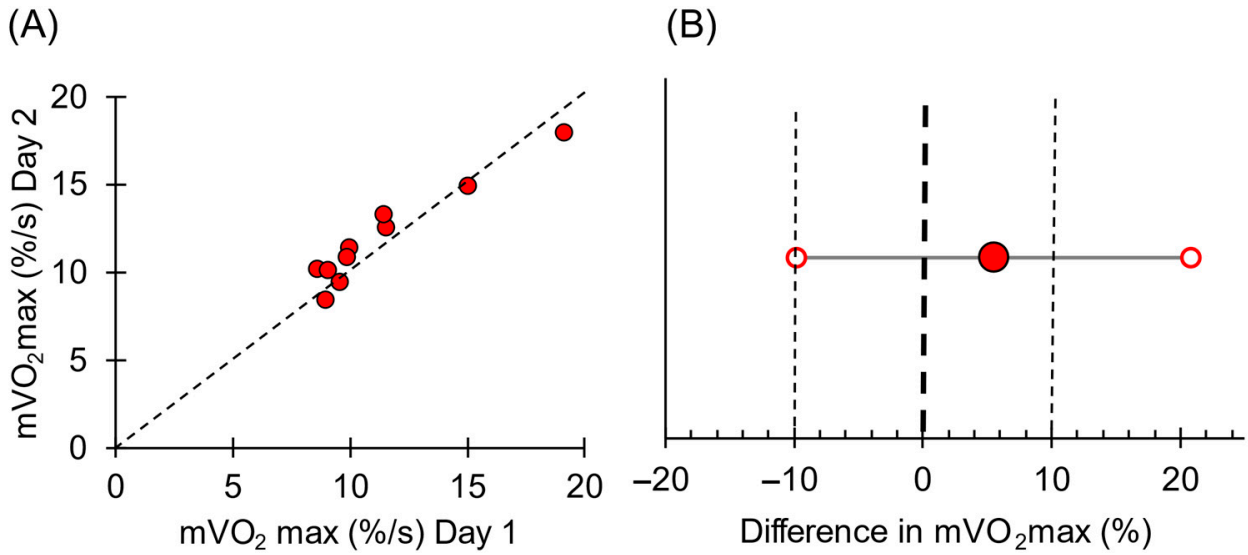

Figure 7. (**A**) Individual mVO_2 values for day 1 and day 2. The dotted line is the line of identity for reference. (**B**) Equivalency plot of mVO2max values for the two testing days. Solid circle is the mean difference; open circles are the 95% confidence interval. Bold dotted line represents zero difference between days. The light dotted lines represent a 10% difference between days.

The average percentage of mVO_2max produced by walking at 3.2 km/h, 4.8 km/h, and 6.4 km/h, and for lunges is shown in Figure 8A. Average EMG signals are shown in Figure 8B. A total of 16.6 ± 0.8 lunges were completed in 23.4 ± 3.1 s on day 1 and 16.3 ± 1.2 lunges in 21.0 ± 3.1 s on day 2. Lunges showed a high reliability for percentage of mVO_2max (ICC = 0.81, CV = 10.2%).

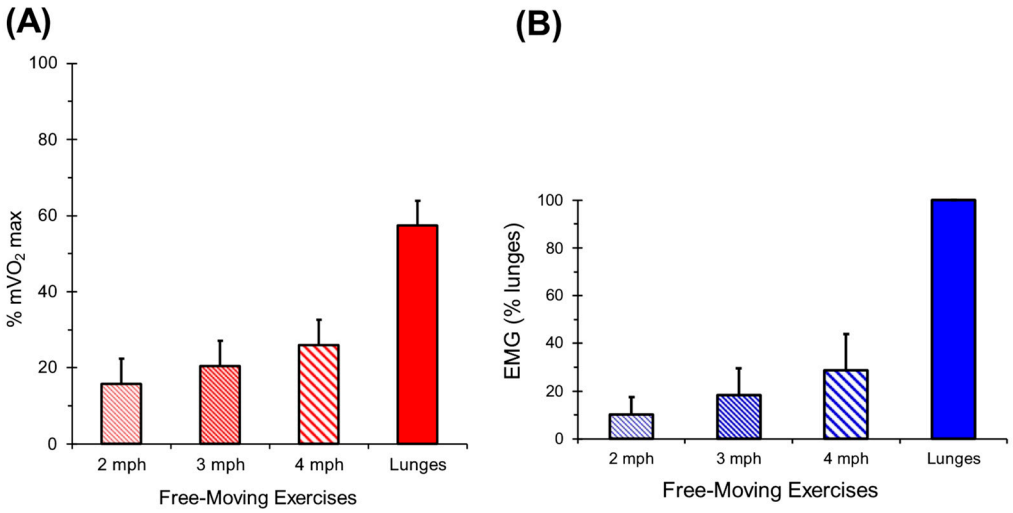

Figure 8. (**A**) Values for walking and lunging exercise for mVO_2. Values are means and standard deviations. (**B**) Valu es for walking and lunging exercise for rectified EMG. Values are means and standard deviations.

4. Discussion

This study examined the ability of NIRS to reliably measure muscle oxygen consumption in the vastus lateralis muscle during progressive walking and lunging exercises. Oxygen consumption was measured using the arterial occlusion method [15,30,31]. The uniqueness of our study was making the measurements during free-moving exercise rather than with special leg ergometers [17,32–35]. We also normalized our oxygen consumption measurements to estimated maximal oxygen consumption values. Much like the way various whole-body exercises can be presented as a percentage of whole-body maximal oxidative capacity (VO_2max), we presented our muscle exercise results (walking and lunging) as a percentage of muscle maximal oxidative capacity (mVO_2max). This study found the vastus lateralis had relatively low mVO_2 values with walking (16–26% of mVO_2max). Low mVO_2 values during walking were consistent with relatively low EMG levels, indicating low muscle activation. The low EMG activation agreed with previous studies [36,37]. Lunges resulted in greater muscle activation, which produced higher mVO_2 values. Our value of 57% of maximal metabolic rate during lunging is consistent with surface EMG data from Kooistra et al., who observed up to 80% muscle activation of the vastus lateralis during knee extensor exercise [38]. The agreement in relative activation levels between our mVO_2 values, EMG values, and comparisons with the literature suggest the NIRS approach can successfully measure muscle metabolism during exercise (immediately post exercise).

This study calculated the maximal muscle oxidative metabolism (mVO_2max) using a progress exercise protocol to extrapolate a maximal rate of oxygen consumption. The mVO_2max values calculated in this study were greater than those reported by Erickson et al. and consistent with testing a younger, more physically active population [32]. This approach was similar to previous studies which used ^{31}P MRS to measure the ratio of Pi/PCr or the calculated concentrations of ADP during progressive exercise [39,40]. NIRS measurements of muscle metabolism have been shown to provide similar metabolic relationships to ^{31}P-MRS measurements [34,41]. The limitation of the ^{31}P-MRS approach is that Pi/PCr ratio is influenced by muscle pH, which is known to decrease at higher work levels. NIRS measurements of muscle metabolism do not appear to have the same sensitivity to changes in muscle pH [42]. A major limitation of the progressive exercise approach to determine mVO_2max is that it involves extrapolation from the work levels to determine the maximal values. Estimates of mVO_2max using the recovery from exercise approach use interpolation to determine a constant rate [17,32–35]. In addition, the calculation of mVO_2max from a progressive work test assumes the work measured on the ergometer is being performed by the muscle being tested. This assumes there were no significant

changes in the work of synergistic muscles during the progressively increasing exercise intensities. An advantage of using the progressive work test approach to measure relative work levels (%mVO_2max) is that the mVO_2 and mVO_2max values are in the same units, making direct comparisons possible.

This study found that the reproducibility of both the VO_2max values and the values during exercise were excellent. We found the CV for our mVO_2 measurements during exercise to be around ~10%, consistent with the CV reported for exercise using muscle ergometers [21,43]. Our CV for maximal mVO_2 determined from recovery tests was consistent with these previous studies [21,44].

This is consistent with previous studies of muscle metabolism and of mitochondrial capacity using NIRS [15,45]. Excellent reproducibility supports the utility of measuring mVO_2 during free-standing exercise. A limitation to the measurements of mVO_2 during lunges was that the duration of the lunging exercise was short (17–28 s). It is possible that oxidative metabolism was not activated long enough during the lunges to ready steady levels. Thus, the mVO_2 measurements during lunges could be an underestimate of the true steady-state oxidative demand for this exercise.

A key aspect of measuring muscle metabolism with the ischemic cuff method was the time between the cessation of exercise and the measurement of mVO_2. In this study, the time delay from last muscle contraction to the metabolic measurement was 2.5–3 s. During this time delay, muscle metabolism is expected to be 'recovering' towards resting metabolism. Figure 9 shows theoretical curves illustrating this effect for people with higher and lower values of mVO_2max. In both higher and lower examples, there would be a change in the metabolic values between 2 and 3% per second immediately post-exercise. With the time delays seen in this study, the actual muscle mVO_2 values could be expected to be ~5% higher than the values that were measured. This study did not find significant differences in the delay times between testing days, which contributed to the excellent reproducibility values seen. However, future studies should record the actual time delays and account for potential differences in time delays when making post-exercise measurements of mVO_2.

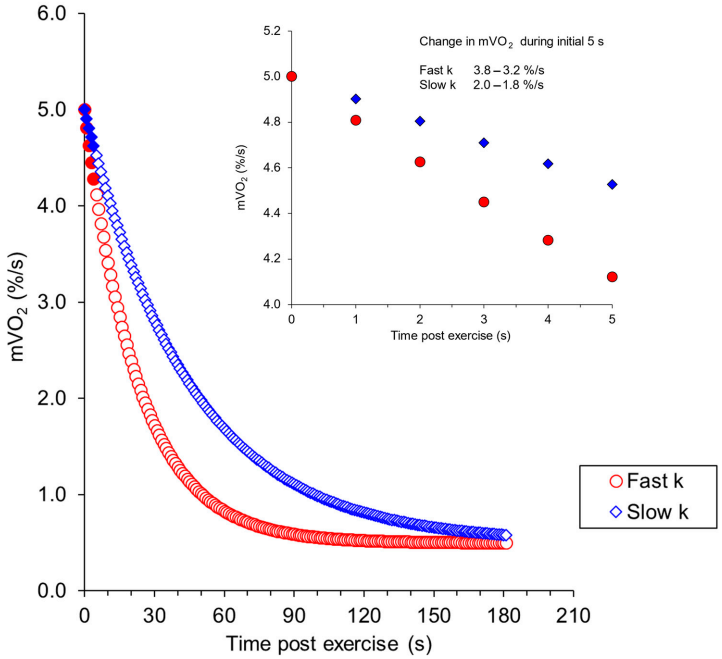

Figure 9. Calculated values of the recovery of mVO_2 starting from a metabolic rate of 5% of the ischemic range per second. The blue symbols are for a muscle with average mitochondrial capacity (rate constant of 1.33/min) and the red symbols for a muscle with higher mitochondrial capacity (2.61%/s). These values are based on previous studies [46]. The inlay shows an expanded view of the initial part of the recovery curve.

A key aspect of the ischemic slope method of measuring muscle metabolism is the presence of adequate oxygen in the muscle. Previous studies have shown that muscle metabolism is impaired when oxygen levels are low [47]. During the progressive exercise test in this study, oxygen levels did fall towards 50% or lower based on the ischemic calibration, similar to previous studies [48]. These levels could approach oxygen levels that were low enough to impair muscle metabolism. In particular, oxygen levels during the lunging exercise were potentially low enough to impair muscle metabolism. Pilotto et al. [47] reported that oxygen levels of approximately 30% of the ischemic range reduced the recovery rate constant to approximately 52% of the value with higher oxygen levels. Thus, muscle metabolism during lunges, while elevated, may have been limited by oxygen delivery. It is not clear how this can be corrected or whether changing the way lunges are performed to increase oxygen levels could increase mVO_2 during this type of exercise. Low oxygen levels have been reported during high intensity exercise using NIRS [47].

Our study provides a relatively inexpensive and translatable way to characterize individual muscle metabolic responses to free-moving exercise. The results show that this technique may be suitable for evaluating the effect of varying intensities of exercise on muscle oxidative function, such as those seen in HIIT exercise. Previous studies have measured oxygen saturation levels during free-moving exercise, such as speed skating, walking, and swimming [23,49–51]. Hesford et al. [50] show that muscle oxygen levels varied between legs consistent with muscle activation. However, they were not able to present their results relative to maximal muscle activation. The use of an extrapolated mVO_2max could allow for a normalized presentation of muscle oxidative capacity that allows comparisons across laboratories and study populations.

A limitation of the NIRS device used in this study is that only a small portion of the muscle of interest can be measured at a time. Thus, when interpreting our results, we assumed that the mVO_2 of the superficial muscle fibers measured by the NIRS device is representative of the entire vastus lateralis muscle. Previous studies have reported differences in muscle activation and muscle metabolism within a muscle during exercise [26,52,53]. Future studies will need to evaluate the impact of small variations in the placement of the NIRS probe. However, the good reproducibility of the measurements in this study as well as in other studies [15,45] suggests that small variations in location are a minimal source of variability.

5. Conclusions

In conclusion, this study measured relative muscle metabolism during whole-body exercises using the arterial occlusion approach with the NIRS method to measure oxygen levels. By using a progressive knee extension work test to estimate the mVO_2max, muscle metabolism during whole-body exercise was presented as a percentage of maximal muscle metabolism. The results agreed with expected muscle activation levels and showed good reproducibility. Future studies need to account for oxygen levels during the exercises, as well as the time delay between the end of exercise and the start of the NIRS measurements. Overall, the results herein support the use of the arterial occlusion NIRS method as a viable method to measure changes in muscle metabolism during exercise.

Author Contributions: K.K.M. designed the study, provided oversight for data collection, performed data analysis, and wrote the document. S.N.S. recruited research subjects, performed the data collection, performed data analysis, and wrote the document. M.A.R. assisted with data collection, assisted with data analysis, edited the document. T.E.R. provided oversight for data collection, assisted with data analysis, and edited the document. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of NAME OF INSTITUTE (Modification 00001723 and 7/24/2015) for studies involving humans.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Dataset available on request from the authors.

Conflicts of Interest: K.M. has a financial interest in InfraredRx, Inc.

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