RESEARCH ARTICLE

Muscle Oxidative Capacity in the Arms and Legs of Various Types of Endurance Trained Athletes

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Abstract:

Our study used near-infrared spectroscopy (NIRS) to measure muscle oxidative capacity (mVmax) in the medial gastrocnemius, vastus lateralis, biceps brachii, and wrist flexor muscles in Cross-country (LEG-T) and Swimmer/Rowers (WHOLE-T) and controls. Young male adults: cross-country LEG-T (n=6) and swimmers/rowers (n=5), moderately fit (CONTROL, n=7) were tested. mVmax was measured as the rate of post-exercise recovery of oxygen consumption after a short bout of exercise using NIRS. Whole-body peak oxygen uptake (VO2peak) was determined during a continuous treadmill protocol. The lower limb muscles had 42% higher mVmax than upper limb muscles in all subjects, with significant differences in 10 of 12 pairwise comparisons ($p< 0.05$). The LEG-T group had higher mVmax values in both legs than CONTROL group ($p< 0.05$), while the WHOLE-T group had higher mVmax in the vastus lateralis ($p = 0.048$). There were no differences in the arm muscles of between the groups. The combined mVmax of both leg muscles in all groups correlated with VO2peak ($r2=0.597$). Muscle oxidative capacity was consistent with training status, and leg mitochondrial capacity correlated with maximal whole body oxidative capacity. These results support the use of NIRS measurements to characterize oxidative capacity in skeletal muscles of athletic populations.

Keywords: Mitochondrial Capacity, Endurance Training, Maximal Oxygen Uptake, Competitive Athletes

1. Introduction

Skeletal muscle oxidative metabolism increases 10 and 100 fold during exercise, and is an important component determining athletic performance [1, 2]. In addition, skeletal muscle oxidative capacity is also recognized as a key component of pathologies such as spinal cord injury, diabetes and peripheral arterial disease [3-5]. Historically, muscle oxidative capacity has been measured from muscle tissue samples obtained by biopsy[6], or by using ^{31}P magnetic resonance spectroscopy (^{31}P MRS) [7]. These studies have shown that endurance athletes can have 50-100% more muscle mitochondria or mitochondrial capacity compared to controls[8-11]. While valuable, wide spread use of these approaches have been limited by the cost and procedural limitations associated with these methods.

Recently muscle oxidative capacity has been evaluated using near infrared spectroscopy (NIRS) [12-14]. This approach as used the rate of recovery of muscle metabolism after a short bout of exercise to characterize muscle oxidative capacity. This approach has been shown to be consistent with both biopsy and ^{31}P MRS measurements of muscle oxidative metabolism[15, 16]. This NIRS based approach has been shown to detect training induced changes in muscle oxidative metabolism[17, 18]. The advantage of the NIRS method is that in these studies nine measurement time points were obtained in two months. Previous studies have also use the NIRS method to evaluate muscle oxidative capacity in athlete populations [19]. The advantage of the NIRS device is that it is relatively inexpensive and portable, in addition to being noninvasive.

The majority of studies of muscle oxidative capacity have evaluated one muscle group, typically the vastus lateralis muscle. A few studies have evaluated multiple muscle areas using both muscle biopsy and ^{31}P MRS [20-22]. Because many of the sports and athletic training programs involve other muscle groups, it would be useful to evaluate more muscle groups[23, 24]. The aim of this study was to evaluate four different muscles groups in the arms and legs of two groups of athletes who either train specifically with their legs or involve both arms and legs in their training. We hypothesized that athletes that performed only leg training would have higher leg muscle oxidative capacity than controls; and then athletes who performed arm and leg training would have higher arm and leg muscle mVmax compared to controls. We also hypothesized that muscle oxidative capacity measured in the legs with NIRS would correlate with whole body peak oxidative capacity (VO_{2Peak}).

2. Materials and Methods

2.1 Participants

Eighteen subjects (all male) were tested in this study. The subjects were recruited and grouped based on self-reported training histories into a control group, a cross-country runner (LEG-T) group, and an upper body endurance training (WHOLE-T) group. The control group (n = 7) reported minimal specific endurance exercises (≤ 180 min/wk). The LEG-T group (n = 6) reported regular endurance exercise that was predominately running (range = 300-600 min/wk). Lastly, the WHOLE-T group (n = 5) reported regular endurance exercise that was predominately

swimming/rowing (range = 270-525 min/wk). The study was conducted with the approval of the institutional review board at the University of Georgia (Athens, GA), and all subjects gave written informed consent before testing.

2.2 Experimental Design

Testing occurred over one or two visits to the laboratory. Subjects were instructed not to consume tobacco on the day of the test or consume alcohol or perform moderate or heavy physical activity at least 24 h before testing. Adipose tissue thickness (ATT) of all four muscle locations were measured using ultrasound (LOGIQe; GE HealthCare, Waukesha, WI).

2.3 NIRS measurements of oxidative capacity.

Testing was performed based on previous studies [14, 19]. The four muscle groups tested were the medial gastrocnemius, the vastus lateralis, the biceps brachii, and the medial wrist flexors (flexor carpi radialis, palmaris longus and flexor carpi ulnaris). All tested muscles were located on the participant's right side. The NIRS assessment were conducted on the gastrocnemius muscle as previously described as shown in Figure 1A. NIRS assessment for the vastus lateralis muscle were performed as previously described [25] as shown in Figure 1B. For the biceps brachii muscle, the optode was placed perpendicular to the muscle fibers on the biceps brachii muscle, 6-12 cm proximal to the elbow flexion crease (Fig. 1C). NIRS assessment for the wrist flexors muscle were made as previously described [17] as shown in Figure 1D. NIRS signals were obtained using a continuous-wave NIRS device (Oxymon MK III; Artinis Medical Systems, Zetten, The Netherlands), which consists of two channels (two equivalent pulsed light sources and two avalanche photodiode detectors, shielding from ambient light), uses intensity-modulated light at a frequency of 1 MHz and laser diodes at three wavelengths (850, and 760 nm) corresponding to the absorption wavelengths of oxyhemoglobin (O_2Hb) and deoxyhemoglobin, with an autosensing power supply (approximately 40W at 110–240V). The probe was set for two source-detector separation distances after the measurement of adipose tissue thickness (ATT). The deep source-detector pair separation distance was always 1 cm greater than the shallow separation distance. NIRS data were collected at 10 Hz. A straight segmental blood pressure cuff (SC12D-medial gastrocnemius, SC12L-vastus lateralis, SC5-biceps brachii and wrist flexors; Hokanson, Bellevue, WA) was placed proximal to the NIRS optodes.

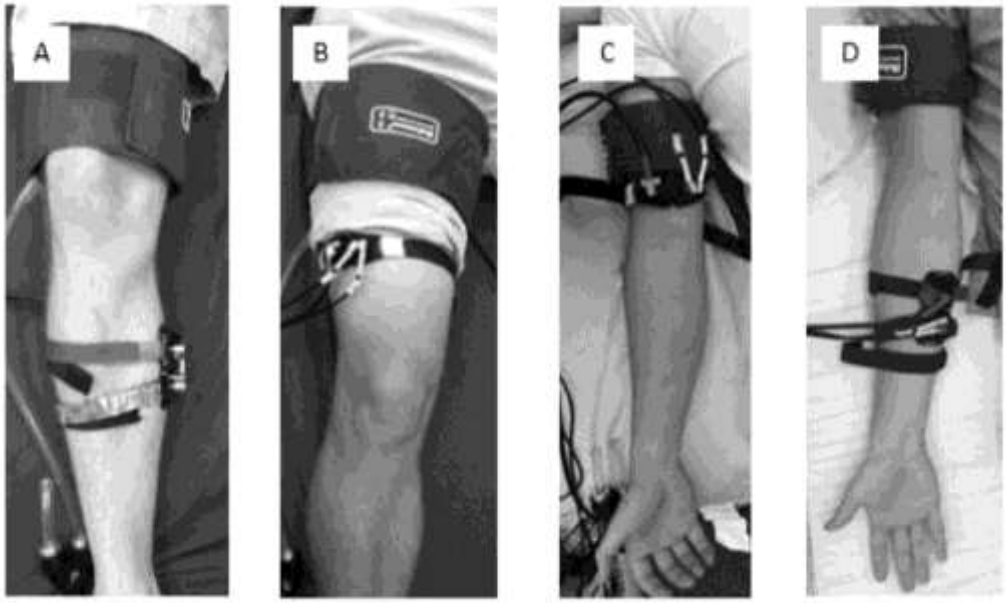

Figure 1. Illustrations of the optode and cuf placements for the four testing sites: A. medial gastrocnemius muscle, B. vastus lateralis muscle, C. biceps brachii muscle, D. flexor carpi radialis muscles. In all cases the NIRS probes were placed in the center of the muscle, and the blood pressure cuff was placed proximal to the testing site.

2.4 NIRS Protocol

The range of NIRS based oxygen levels was measured by a short bout of exercise to increase metabolic rate followed by 3-5 minutes arterial occlusion to completely deoxygenate the tissue under the optode (assumed to be 0% oxygenation), and the peak hyperemic response upon release of the cuff was used to indicate 100% oxygenation. The arterial occlusion was performed prior to measurements of muscle oxidative capacity to make sure the exercise used to stimulate metabolic rate for those measurements did not desaturate the muscle beyond 50% of the range. For measurements of mVmax, stimulation of metabolic activity within the muscles was activated by 10-15s of rapid muscle contractions against a low load performed by the tested muscle/muscle group. The gastrocnemius was exercised by repeated plantar flexion against a stationary foot pedal while the subject was in supine position. The vastus lateralis was exercised by full leg extension from 90° flexion while simultaneously hip-flexed at 45-60°. The biceps brachii was exercised by arm flexion to 90° and complete extension (partial arm curl). Similarly, the wrist flexors were exercised by wrist flexion to 70-90° and complete extension (wrist curl). Immediately after each bout of exercise stimulation, a series of 15-20 brief (5–10s) arterial occlusions using a blood pressure cuff were applied to measure the rate of recovery of mVO_2 back to resting levels. The repeated arterial occlusions were performed as follows: cuffs 1–5 (5s on 5s off), cuffs 6–10 (7s on 7s off), and cuffs 11 – ~20 (10s on - 10s off) (Figure 2). The total time to test all four muscles using the described NIRS protocol ranged from 2.5-3 hours.

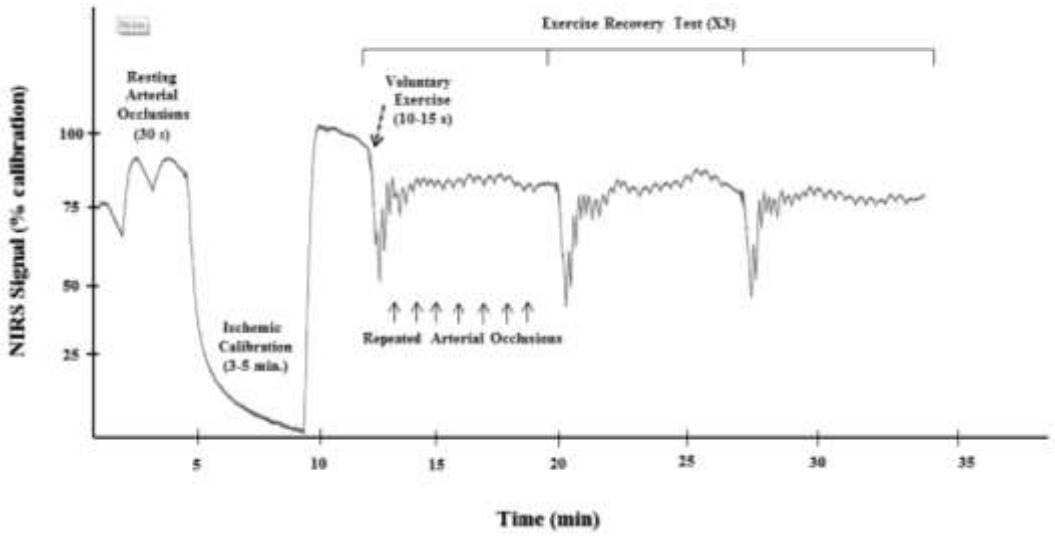

Figure 2. Figure shows a representative NIRS protocol, including resting measurements, ischemic calibration and three oxidative capacity recovery tests. Note the testing protocol lasted 35 minutes, and included one five minute ischemic period and approximately 50-60 short duration ischemic periods.

2.5 Calculation of muscle oxygen consumption.

mV_{max} was calculated as the slope of change in O_2Hb and deoxyhemoglobin during the arterial occlusion using simple linear regression. This measurement was made at rest and repeated 15-20 times after exercise. The post-exercise repeated measurements of mVO_2 were fit to a monoexponential curve according to the following formula:

$$y = End - Delta \times e^{-k*T} \quad [1]$$

For this equation, y represents mVO_2 during the arterial occlusion, End is the mVO_2 from the first cuff inflation after the cessation of exercise, Delta is the change in mV_{max} from rest to end exercise cuff inflation, and k is the fitted rate constant. k presented in units of 1/min was used as our indicator of mV_{max}.

2.6 Whole-body peak oxygen uptake.

Whole-body peak oxygen uptake (VO_{2peak}) was used to characterize the metabolic differences between the three participant groups [26]. VO_{2peak} was determined by indirect calorimetry during a continuous ramp protocol to exhaustion via treadmill (model Trackmaster, TMX 3030C. Eastlake, OH). The ramp protocol consisted of a 2 minute warm-up stage (3.5 mph, 10% incline), immediately followed by a transition to stage 1 (7.5 mph, 0% incline). Following stage 1, the speed (7.5 mph) and duration (2 min.) were consistent throughout the protocol; however the incline would increase in increments of 2.5% between stages until exhaustion. Oxygen consumption and carbon dioxide production were measured continuously by open-circuit spirometry and analyzed using a ParvoMedics metabolic measurement system (model TrueMax 2400; ParvoMedics, Sandy, UT) that was calibrated before each experimental LEG-T. Heart Rate

(HR) was monitored by a Polar HR monitor (Polar Beat, Port Washington, NY). Rate of Perceived Exertion (RPE) were assessed during the last 30 s of every 2 minute stage during the ramp protocol using Gunnar Borg's 6–20 RPE scale [27]. For the test to be considered an acceptable measurement of physiological VO_{2peak}, two of the following criteria had to be met: 1) Respiratory Exchange Rate (RER) > 1.05, 2) maximal HR within 10 beats of age predicted maximum, and 3) RPE >18.

2.7 Data Analysis

Data are presented as means ± SD. Statistical analyses were performed using the Statistical Package for the Social Sciences (version 22 IBM ®, Armonk, NY). An ANOVA was performed using a 3 group by 4 muscle design. Data was analyzed to test whether significant differences existed in mV_{max}: 1) within the three groups for the four different of muscles, and, 2) between the three groups for the four different muscles. Pairwise comparisons were made using least significant differences. Significance was assumed with a $p < 0.05$. With a population variance of 12-14% [28], sample sizes of approximately six per group should allow adequate power (β >0.80) for differences greater than 25%. Expected differences in mVmax values between arms and legs of highly fit subjects has been shown to be approximately 70% [29].

3. Results

Eighteen of the 19 subjects that were enrolled in the study were able to complete the entire study protocol. A single participant was lost to follow up after completing one of two scheduled sessions. No adverse events took place during the study. The physical characteristics and VO_{2peak} values of participants are shown in Table 1.

Table 1.

Participant Characteristics						Adipose Tissue Thickness (mm)			
Group	Age (yrs)	Height (cm)	Weight (kg)	BMI ($kg \cdot m^{-2}$)	VO_{2peak} ($mL \cdot kg^{-1} \cdot min^{-1}$)	MG	VL	BB	WF
Control (*n*=7)	22.8 ± 2.1	181.6 ± 8.3	79.4 ± 8.2	24.0 ± 1.9	46.8 ± 6.6	4.6 ± 1.2	4.7 ± 1.2	3.2 ± 1.1	2.9 ± 0.7
LEG-T (*n*=6)	19.3 ± 0.8	175.9 ± 7.7	57.3 ± 6.1	18.5 ± 1.9	68.1 ± 5.1	4.1 ± 0.5	3.8 ± 0.5	2.9 ± 0.4	2.6 ± 0.3
WHOLE-T (*n*=5)	26.0 ± 6.3	178.98 ± 11.0	79.0 ± 11.0	24.6 ± 2.7	53.2 ± 7.9	4.6 ± 0.7	4.2 ± 0.7	3.4 ± 0.3	3.0 ± 0.5

Data are presented as means ± SD. Medial Gastrocnemius (MG), Vastus Lateralis (VL), Biceps Brachii (BB), Wrist flexors (WF). LEG-T is the lower body endurance trained, WHOLE-T is upper and lower body endurance trained. Adipose tissue thickness was measured over the testing site with ultrasound.

3.1 NIRS measurements.

The mV_{max} values are presented for the three groups and four muscles groups in

Figure 3. When comparing the leg muscles between groups, there was a main effect of group for the VL (F = 6.56, P = 0.009) and MG (F= 5.96, P = 0.012). Post Hoc comparisons showed significant differences in mVmax between the LEG-T group and the control group for both the VL (P = 0.013) and the MG muscles (P = 0.008). No other Post Hoc comparisons were significant (P > 0.05). When comparing the arm muscles between groups, there were no main effects of group for the BB (F = 0.23, P = 0.796) or the WF (F= 1.90, P = 0.184) muscles.

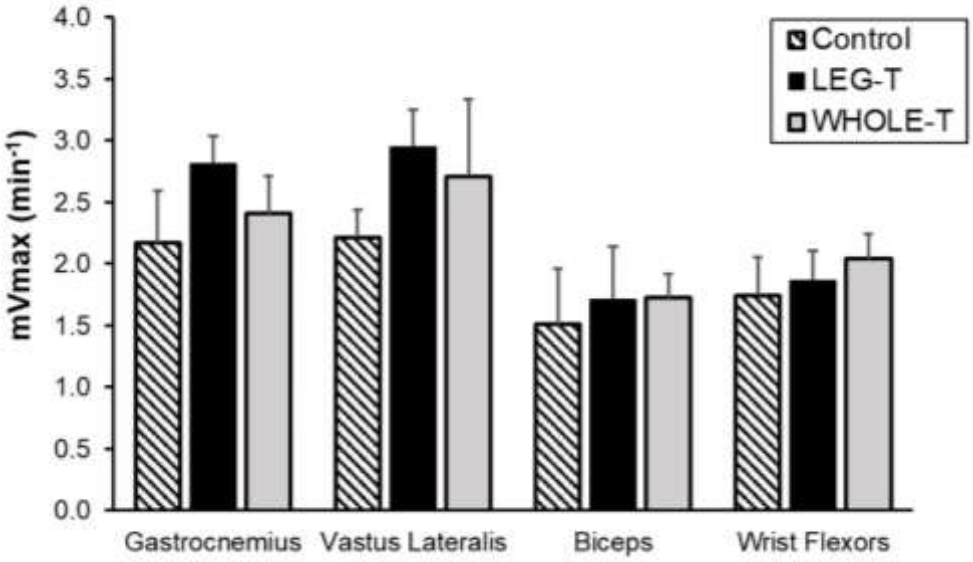

Figure 3. Average muscle oxidative capacity (mVmax, min^{-1}) of MG, VL, BB and WF muscle for LEG-T, WHOLE-T and control groups. Data are means and standard deviations. Stars indicate significantly different from arm muscles ($p < 0.05$).

3.2 VO_2peak measurements.

VO_2peak values were significantly higher in the LEG-T group compared to both the WHOLE-T and Control groups ($p < 0.001$ and $p = 0.002$, respectively), however there was not a significant difference between the WHOLE-T and Control groups ($p = 0.054$) (Table 1). There was a significant correlation between VO_{2peak} and the combined mV_{max} for the VL and MG muscles ($r^2 = 0.60$) (Figure 4).

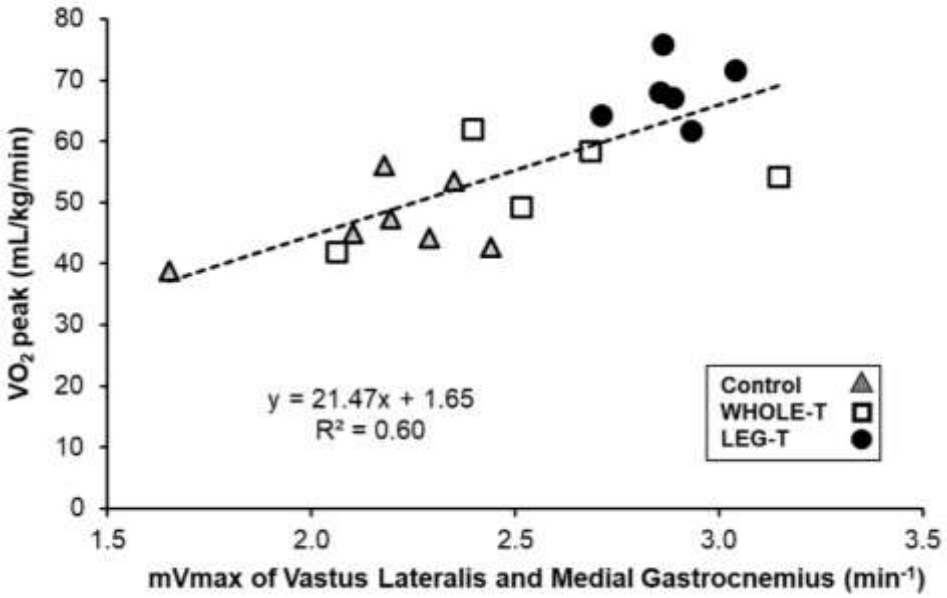

Figure 4. Correlation between whole body (VO_{2peak}) and muscle (mVmax) of the combined leg muscle groups (VL and MG).

4. Discussion

The principal finding of this study was the demonstration of NIRS based measurements of muscle oxidative capacity (mV_{max}) in four different muscle groups of a single person. We found that lower body muscles had higher oxidative capacities than upper body muscles in all subjects regardless of specificity of training. These results are consistent with previous studies that reported faster NIRS recovery rates in for the calf and thigh muscles [19, 25, 28, 30], compared to the wrist flexor muscles [17, 29]. The difference in our study was that the measurements of muscle oxidative capacity were usually made on the same participants, where the previous studies are comparisons of different study groups across different experiments. The results are also consistent with a study comparing the arms and legs of highly trained cross-country skiers using biochemical analysis of muscle biopsies [23]. The 53% difference between the arms and legs of the skiers was consistent with the ~62% difference seen in the current study. Our results are also consistent with the ~70% difference in mVax values in the legs of women with mixed training activities compared to inactive controls [29].

We did not find significant differences between the two examined lower body muscles (MG vs VL). The lack of difference between the lower body muscles was in agreement with Green et al. [31] who performed histochemical and biochemical analysis on both the vastus lateralis and gastrocnemius and showed strong similarities between fiber proportion and size, as well as citrate synthase activities of the muscles. However, Larsen et al.[32] found differences in the lower limb muscles where comparing the VL to the Tibialis anterior. This result is consistent with Gregory et al.[33] who reported slightly lower quantitative SDH levels from biopsies in the TA compared to both the VL and lateral gastrocnemius. Gregory et al. [33] reported

similar quantitative SDH values for the VL and lateral gastrocnemius, consist with the current study.

We observed ~40% higher muscle oxidative capacities in the lower body muscles of LEG-T athletes compared to the less well trained control group. Previous studies using ^{31}P MRS [10, 11] as well as NIRS [19] have shown approximately 70-100% higher oxidative capacity in endurance trained populations compared to inactive populations in the wrist flexor muscles [10], gastrocnemius [11], and the vastus lateralis [19]. Similar two-fold differences between trained and untrained subjects were reported in studies involving enzyme activities such as citrate synthase [2], succinate dehydrogenase [9], and oxogluterate dehydrogenase [8] derived from biochemical assays of muscle biopsy tissue. The relative lower difference between the trained and untrained group in our study in oxidative capacity that seen in previous studies could be explained by the relatively higher training status of our untrained group. Our control population VO_{2peak} values (46.8 ml/kg/min) was higher than what is usually reported for untrained populations, for example 33.7 ml/kg/min in the study by Brizendine et al. [19]. Our two training groups have VO_{2peak} values less than those of the previous study with correspondingly lower mV_{max} values. Overall, the differences between our trained and control groups were consistent with the literature.

This study found a relatively strong correlation between mV_{max} and VO_{2peak} values. This comparison used all the subjects in our study. Given the established differences in both mVmax and VO_2peak between trained and untrained subjects [34-36], this result is to be expected. However, previous studies that have only examined highly trained subjects have not reported a relationship between muscle mitochondrial capacity values and VO_2max[2]. In addition, wide variations in muscle mitochondrial measurements have been reported in elite distance runners[37]. These results support the hypothesis that muscle mitochondrial capacity is not the main factor that determines whole body exercise capacity, other factors such as cardiac output and oxygen delivery would be more important. However, this only seems relevant for highly trained athletes. Exercise capacity (walking ability) seems linked to mitochondrial capacity in patients with low levels of physical ability[38]. At what point does the relationship between muscle mitochondrial capacity and whole body exercise capacity separate needs to be determined in future studies.

To our knowledge, our study is the first to report muscle oxidative capacity measurements in the biceps brachii using NIRS. Because the NIRS method requires producing short periods of muscle ischemia, it is difficult to place a blood pressure cuff proximal to the placement of the NIRS device on the bicep muscle. The biceps brachii muscle has been studied using muscle biopsies[39, 40], but comparisons of mitochondrial capacity to other muscles seems limited in the literature. Our results are consistent with the hypothesis that in subjects who do not actively train their arm muscles, the biceps brachii and forearm/wrist flexor muscles have similar (relatively) low oxidative capacities. We saw some evidence that the forearm/wrist flexor muscles of our arm trained group might have had higher oxidative capacities than the biceps muscles, but this result was not statistically significant

in our group. Future studies will be needed to address differences in these muscle in particular athletic groups. We did not find evidence that our arm trained group had higher muscle oxidative capacity values in the arms compared to our leg only and control groups. This was not what we predicted, and it was not consistent with previous literature reporting training and cross-sectional differences in the wrist/finger flexors muscles [10]. A possible explanation is that our arm trained group (mostly competitive swimmers) did not actually have enough arm training in the chosen muscles to produce a difference. Previous studies used highly trained rowers to evaluate training differences in the wrist flexor muscles [10]. As mentioned earlier, lower muscle mitochondrial capacity appears to remain relative to the leg muscles in athletes who train with both their arms and legs [23]. Future studies will be needed to test different groups of individuals who performed specific arm training to compare training differences between the arms and legs.

A potential limitation to the NIRS measurements are differences in superficial adipose tissue thickness (ATT) [41]. For our study, the effect of ATT was limited as the average ATT above the sites of optode placement was relatively similar between groups and relatively small (average values for muscle groups between 2.6 and 4.6 mm. It is not uncommon for ATT values over the vastus lateralis muscle in nonathletic populations to be up to 14 mm [25]. Application of this study to clinical populations with greater ATT values will need to address the issue of increased ATT. Similar to previous studies, we did not see significance differences in our oxidative capacity measurements between shallow and deep channels, or significant correlations between oxidative capacity measurements and ATT across subjects [14]. A practical limitation of the NIRS technique was that our total test protocol to evaluate four muscles groups with three tests each could be considered lengthy (2.5-3 hours). Future studies, might examine the possibility of streamlining a protocol that could accurately gauge the mitochondrial health of multiple muscle groups in a time range that would be clinically acceptable [42].

Another limitation to our study was the relatively small sample size. Given the large differences between mVmax values reported between the arms and legs of endurance trained individuals, this study was able to detect significant differences between the arm and leg muscles. The differences in mVmax between the two arm and two leg muscles within each group was small (<10%), and samples sizes to detect potential differences for those comparisons would need to be very large, which was not practical in this study.

5. Conclusions

In summary, this study demonstrated the ability to utilize NIRS to measure the muscle oxidative capacity of four different muscle groups in the same individuals. In addition, we were able to show higher muscle oxidative capacities of lower limb muscles (medial gastrocnemius, vastus lateralis) when compared to the upper limb muscles (biceps brachii, wrist flexor muscles). Finally, we observed higher oxidative capacities in the lower limb muscle of the LEG-T athletes when compared to the control group, and higher oxidative capacities in the upper limb muscles of the WHOLE-T athletes when compared to the control group, even though only the former comparison reached a level of significance. This study paves the way for a closer examination of the systemic effects

training on skeletal muscles in the arms and legs of elite athletes.

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Conflicts of Interest: Kevin McCully is the President of Infrared Rx, Inc,

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