

Depth Penetration of Near Infrared Spectroscopy in the Obese

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Abstract

Near-Infrared Spectroscopy (NIRS) measures to a depth of 2 to 3 cm below the skin, raising concern over the utility of NIRS in the obese patient. The purpose of this prospective study is to investigate the effect of overlying adipose tissue thickness (ATT) on NIRS oxygenation measurements of skeletal muscle. ATT was measured by ultrasound. NIRS sensors were placed over the anterior and superficial posterior compartments of one leg during exercise and the change in regional oxygen saturation was calculated for each compartment. There was a decreasing trend in change of rSO₂ from baseline with increasing ATT. Extremely obese patients (BMI >40) had significantly smaller changes in rSO₂ from baseline as compared to otherwise similar patients in both the compartments (p<0.01). As ATT increased, the change of the NIRS values from baseline decreased. There was not a specific BMI or ATT determined to be incapable of being monitored.

Keywords: Near infrared spectroscopy; Adipose tissue thickness; Compartment syndrome

Introduction

Clinical diagnosis of Acute Compartment Syndrome (ACS) is customarily made based on clinical symptoms and occurs when increased pressure within a muscle compartment causes muscle ischemia and ultimately death if left untreated [1,2]. If the diagnosis of ACS is uncertain after clinical evaluation, the Intramuscular Pressure (IMP) within the compartment can be measured to identify the area of high pressure [2]. However, the procedure is invasive and can lead to inaccurate values if not performed correctly [3]. Near-infrared spectroscopy (NIRS) allows for continuous, non-invasive measurement of tissue oxygen saturation [3,4]. NIRS uses light transmission and absorption to measure the percentage of hemoglobin saturated with oxygen in the tissue roughly 2 to 3 cm below the skin [3,5]. This technology has the capacity to provide data on oxygen perfusion in an affected compartment. Skeletal muscles also deoxygenate during exercise and NIRS can be used to monitor these metabolic changes as well [6]. The ability of NIRS to measure only to a depth of 2 to 3 cm below the skin has raised concern over its utility in the obese patient [5,7]. Adipose tissue metabolism is lower than muscle metabolism, leading to an inaccurate estimation of muscle oxygen consumption. The subcutaneous adipose tissue layer can fluctuate among individuals and may confound NIRS measurements made in muscles underlying the adipose layer [7]. Prior research has made mention that ATT could impact NIRS values [8,9]. A study was designed to investigate how varying depths of overlying adipose tissue affect the ability of NIRS to measure muscle oxygenation. By measuring the adipose depth in both the anterior and superficial posterior compartments of the leg and then measuring the decrease in tissue oxygenation caused by muscle contraction during exercise in each compartment, the ability of NIRS to measure muscle oxygenation and not adipose tissue was determined. The hypothesis that NIRS values of the activated compartment would decrease significantly from baseline if the adipose depth is less than 2 cm was examined.

Materials and Methods

The study population consisted of 120 uninjured volunteers between 18 and 60 years of age, who provided written informed consent in accordance with institutional review board approval. Exclusion

criteria included subjects with a diagnosis of peripheral vascular disease or pulmonary disease, subjects with type I or type II diabetes mellitus, tattoos over the area the NIRS sensors placement, or a prior diagnosis of compartment syndrome. Subjects were categorized according to the National Institutes of Health Classification of Overweight and Obesity by body mass index (BMI). There were 24 subjects in each of the five classifications (<25, 25-29.99, 30-34.99, 35-39.99 and greater than 40 kg/m²). Potential subjects were randomly screened for eligibility based on their age, height, and weight to ensure equal numbers of subjects within each BMI classification, and gender to ensure equal numbers of males and females [10-12].

Subjects were screened for eligibility based on age, height, and weight. Once enrolled, gender, race, and BMI were recorded. An ultrasound was conducted using a BodyMetrix Professional – BX2000 (IntelaMetrix, Livermore, CA) ultrasound device on the anterior and superficial posterior compartments to measure the adipose tissue depth overlying the muscle groups [5,13-17]. NIRS values were obtained using an Equinox 7600 Oximeter (Nonin Medical, Inc, Plymouth, MN). The sensor used in this study has two sensor depths, which by design allow the superficial depth to be subtracted from the deeper values in order to isolate oxygenation values in the deeper tissue. Values are displayed as the percentage of hemoglobin saturated with oxygen (rSO₂). Consequently, a higher reading indicates a higher tissue oxygenation level. The device was calibrated during manufacturing and did not require recalibration before each use. The NIRS sensors were placed over the middle one-third of the tibia for the anterior and superficial posterior compartments of the leg (directly posterior). The anterior compartment was located by palpating the anterior tibial ridge and placing the sensor lateral approximately 2 cm [5,13-17]. The superficial posterior compartment measurement was located by placing

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the sensor directly posterior. Based on adipose tissue distribution, the largest deposit of subcutaneous adipose tissue is located over the superficial posterior compartment. The least amount of subcutaneous tissue is located over the anterior compartment. Therefore, the anterior compartment would be used as an internal control for the posterior compartment. Additionally, each of these compartments is easily isolated through specific and simple exercises. The deep posterior and lateral compartments were not monitored in this study.

The sensors were applied to the leg and rSO₂ was monitored for approximately thirty to sixty seconds to obtain a stable reading to serve as a baseline measurement for each compartment. In order to isolate the compartments, subjects were asked to perform specific exercises intended to activate only the desired muscle group. The exercises were performed with the subject sitting with the legs extended on an exam table. A 6-foot length special heavy resistance exercise band (Thera-Band, Akron, OH) was folded once and used to provide resistance to enhance muscle activation. The participant performed each exercise for 30 to 60 seconds followed by a period of about 60 seconds of rest to allow the rSO₂ values to return to baseline. To activate the anterior compartment, the exercise band was placed around the dorsal aspect of the toes and pulled about two feet away from the feet of the subject. Subjects were instructed to quickly pump the foot in a dorsiflexed position (pull their toes towards their nose) to isolate the anterior compartment. The exercise was repeated until the subject fatigued or the time of exercising reached one minute. The superficial posterior compartment was activated by placing the exercise band around the plantar aspect of the toes and then pulling the exercise band to the knee of the patient. Subjects were instructed to quickly pump the foot in a plantar flexed motion (similar to a calf raise against resistance) against the resistance to isolate the superficial posterior compartment muscle group. The exercise was again repeated until the subject fatigued or the time of exercising reached one minute. For each exercise the lowest NIRS measurement reached during activation and the duration of exercise were recorded.

Statistical analysis

The change in rSO₂ was calculated separately for each compartment based on the difference between NIRS values pre and post exercise. Significance of this pre- and post-test difference in rSO₂ was tested using ANOVA. BMI was calculated using the following equation (weight (lbs.) / 2.20462262) / (Height (in) / 39.3700787)². The change in rSO₂ was plotted across increasing BMI and fat-depth. Subjects were divided into BMI groups of <25, 25-30, 30-35, 35-40 and >40. Fat-depth categories were <5 mm, 5-10 mm, 10-15 mm and >15 mm. ANOVA testing with Bonferroni correction for multiple comparisons was used to test whether the mean change in rSO₂ differed significantly by BMI group or fat-depth category. Pearson correlation was calculated for comparing rSO₂ and ATT. All statistical calculations were performed using STATA statistical software version 12.

Results

One hundred twenty adult volunteers were recruited to participate with ages ranging from 18 to 60 (mean: 39.4) years (Table 1). There were 60 male and 60 female patients. The majority of patients were Caucasian (approximately 86%). No significant trends were found based on age or race. Males were found to show a greater change in rSO₂ after exercise for both the anterior and poster compartments (p=0.000). Subjects were asked to perform the exercises until fatigue. On average patients completed 49.4 (range: 14-60) seconds of exercise for superficial posterior activation and 36.3 (range: 13-67) seconds for anterior activation.

Characteristics	Mean (SD)	
Age	39.38 (12.99)	
Gender	Male (N=60)	Female (N=60)
BMI	32.81 (6.92)	32.38 (8.94)
Anterior Fat Depth	4.12 (1.54)	7.05 (3.03)
Posterior Fat Depth	5.65 (2.63)	10.00 (5)

Table 1: Subject characteristics.

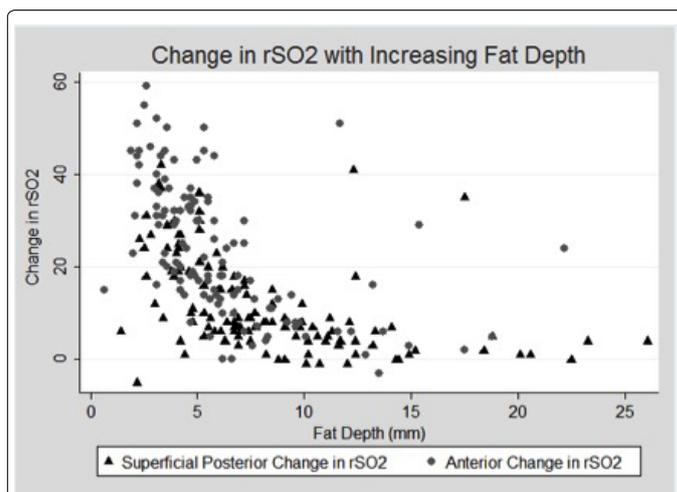


Figure 1: Change in rSO₂ with increasing adipose tissue thickness. As the depth of fat increases the average change in muscle oxygenation after exercise decreases (r=-0.5105; p=0.000). Outliers are generally males, with BMI greater than 34 kg/m².

Anterior Compartment rSO ₂			
F-Stat			0.000
FD Group	Comparison FD Group	Mean difference in change in rSO ₂	P-value
<5 mm	5-10 mm	-13.885*	0.000
	>10 mm	-20.477*	0.000
5-10 mm	>10 mm	-6.592	0.252

* The mean difference is significant at the 0.05 Level

Table 2: Fat-depth category rSO₂ comparison – anterior compartment.

Superficial Posterior Compartment rSO ₂			
F-Stat			0.000
FD Group	Comparison FD Group	Mean difference in change in rSO ₂	P-value
<5 mm	5-10 mm	-9.480*	0.000
	>10 mm	-14.819*	0.000
5-10 mm	>10 mm	-5.339*	0.026

* The mean difference is significant at the 0.05 Level

Table 3: Fat-depth Category rSO₂ Comparison – Superficial Posterior Compartment.

The change in rSO₂ as fat-depth increases can be seen in Figure 1. Both anterior (r=-0.5312) and posterior (r=-0.5105) changes in NIRS values after exercise show a negative correlation with moderate magnitude. Tables 1-4 show the results of the ANOVA analysis. No statistically significant difference was found between fat-depth and NIRS values after 1 cm of subcutaneous fat. However, a statistically significant drop in the average change in rSO₂ was seen when the subject's subcutaneous-depth was greater than 5 mm in the anterior compartment (Table 2). In the superficial compartment, a significant drop in NIRS values was seen when fat-depth increased from <5 mm to

5-10 mm (-9.480; p=0.000) and again when greater than 1 cm (-5.339; p<0.05) as seen in Table 3. Change in NIRS values after exercise in subjects with BMI greater than 40 kg/m² differed significantly than those with BMI less than 25 kg/m² in the superficial posterior compartment (-9.25, p<0.05; Table 4) and was also significantly less in those less than 30 kg/m² in the anterior compartment (<25: -15.583, p<0.01; 25-30: -13.083, p<0.05; Table 5).

Discussion

Near infrared spectroscopy has previously been validated to measure tissue oxygenation in humans [10,11] and further described by several studies as a method of correlating muscle oxygenation to compartment pressures in acute compartment syndrome of the leg [5,12-16]. It has also been shown that NIRS has the ability to isolate among specific muscle groups with proper sensor placement over individual compartments [17]. The displayed NIRS values are unique for each compartment; contracted compartments have decreasing NIRS values while compartments at rest show little or no change [17]. However, previous studies do not address the concern about the efficacy of the device's penetration depth. The ability of NIRS to be used in obese patients is still unknown [9].

It was expected that the change in rSO₂ for the obese groups would be smaller than the changes in the normal and overweight groups based upon physiologic differences. More lean muscle enables better stimulation of the muscle compartment and further lowers rSO₂ from

baseline. The ability to elicit a stronger contraction increases energy costs and lowers oxygen saturation values [18]. Also, intense exercise training increases mitochondrial gene expression [19], leading to a metabolic advantage in leaner and more trained individuals which would be expected to be found in lower BMI subjects. This finding possibly influences the data to show lower rSO₂ levels in the subjects with less adipose tissue or lower BMI. Additionally, subjects in the Class II and Class III obesity groups likely do not have the effects of increased mitochondrial expression attributed to training, contributing to the expected decrease in observed change of NIRS values from baseline in these groups. Therefore, obese subject would not be expected to have as great of a drop in NIRS values due to their inability to perform exercises efficiently nor extract the supplied oxygen effectively. This trend was observed in this study. The more obese the subjects, the less the drop in NIRS values was observed. The reduced effect of exercise in the obese could be explained in part by the inability of NIRS to measure changes in muscle in the obese. While this factor may play a partial role in the observed results, a complete failure of NIRS to monitor muscle oxygenation would result in no change in rSO₂ values. If measurements came from solely subcutaneous fat, not change (decrease in rSO₂) would be seen in these subjects as subcutaneous fat does not play a role in exercise. The fact that in all subjects, a decrease in NIRS values was recorded with exercise indicates the NIRS device was monitoring muscle below the subcutaneous fat at least in part.

The group with the lowest BMI has distinct physiologic characteristics hindering their use as the reference standard in the study. Tanner et al. found that leaner muscle has more type I (slow-twitch) fibers and tends to be oxidative and vascularized [20] while Gavin et al. found that obese muscle has a lower capillary density than lean skeletal muscle [21]. Capillary density and muscle fiber recruitment can contribute to deoxygenation levels and affect NIRS readings [18,22]. Subjects with a BMI of 25-30 have less lean muscle (along with fewer of the corresponding physiological changes) and therefore have less of a drastic impact on the NIRS readings, enabling their use as a reference standard to best represent the general population. Despite an attempt to recruit extremely obese subjects, 95% of the study population had a fat depth of less than 2 cm in either compartment. This finding suggests that having a depth of adipose tissue greater than 2 cm is very rare. The deposition of fat in the lower leg region is quite low in humans; therefore, demonstrating the lower leg is an ideal location to monitor muscle perfusion. The NIRS device displayed rSO₂ changes at increasing fat depths beyond 2 cm indicating the ability of NIRS to measure muscle rSO₂ changes despite the extreme amounts of subcutaneous fat that was specifically sought and selected for in this study. This type of subject is not typically found in the general population.

Figure 1 shows a moderate correlation between ATT and the change in rSO₂ from baseline following exercise (r= -0.5105). As expected, there was a trend showing that as ATT increased, the change in rSO₂ from baseline decreased. Increasing ATT was expected to correlate with less muscle training. These results are statistically significant and can be seen in Tables 1 and 2. A mean decrease in change of approximately 9.5 percentage points in rSO₂ can be seen when subjects have <5 mm of adipose tissue versus 5-10 mm, and decreases an average 5.3 points above 10 mm in the superficial posterior compartment. There was also a decrease in change of rSO₂ from baseline with increasing BMI. This difference became statistically significant between the overweight group (BMI between 25 and 30) and the extremely obese group (BMI >40) in the anterior compartment (p<0.05), as well as between the normal BMI and extremely obese groups in both compartments (p<0.05). The study also found that the baseline NIRS reading is not a predictor

Anterior Compartment rSO ₂			
F-Stat			0.0007
BMI Group	Comparison BMI Group	Mean difference in change in rSO ₂	P-value
<25 kg/m ²	25-30 kg/m ²	2.500	1.000
	30-35 kg/m ²	-5.667	1.000
	35-40 kg/m ²	-10.667	0.072
	>40 kg/m ²	-15.583*	0.001
25-30 kg/m ²	30-35 kg/m ²	-3.167	1.000
	35-40 kg/m ²	-8.167	0.384
	>40 kg/m ²	-13.083*	0.011
30-35 kg/m ²	35-40 kg/m ²	-5.000	1.000
	>40 kg/m ²	-9.917	0.123
35-40 kg/m ²	>40 kg/m ²	-4.917	1.000

* The mean difference is significant at the 0.05 Level

Table 4: BMI category rSO₂ comparison – anterior compartment.

Superficial Posterior Compartment rSO ₂			
F-Stat			0.0096
BMI Group	Comparison BMI Group	Mean difference in change in rSO ₂	P-value
<25 kg/m ²	25-30 kg/m ²	2.125	1.000
	30-35 kg/m ²	-3.917	1.000
	35-40 kg/m ²	-7.958	0.078
	>40 kg/m ²	-9.25*	0.021
25-30 kg/m ²	30-35 kg/m ²	-1.792	1.000
	35-40 kg/m ²	-5.833	0.494
	>40 kg/m ²	-7.125	0.168
30-35 kg/m ²	35-40 kg/m ²	-4.042	1.000
	>40 kg/m ²	-5.333	0.720
35-40 kg/m ²	>40 kg/m ²	-1.292	1.000

* The mean difference is significant at the 0.05 level

Table 5: BMI category rSO₂ comparison– superficial posterior compartment.

of ATT and cannot be used to assess the utility of NIRS in a given patient. Baseline values varied across all thicknesses of adipose tissue in both compartments studied. There are some limitations to this study. While the study did stratify age and gender, there still could be innate physiological differences in the BMI groups that contributed to the data that were not accounted for, such as varying amounts of myoglobin and hemoglobin in skeletal muscle [19,22] or differences in vascular supply [23]. Individuals may have inherent differences in muscle fiber types, such as in aging [24], and humans have a vast blend of muscle fiber types within a given muscle group [20]. However, the study recruited a similar age distribution and mean age for each BMI category. The group factors of gender and race/ethnicity were not tested for differences and would be of interest in the future.

The inherent error of the BodyMetrix BX-2000 (IntelaMetrix Inc., Livermore, CA) used to measure the ATT is $\pm 3.5\%$. Although this is an acceptable range of error, this could influence the range of the data and cause the measured adipose thickness to be less than the actual value. There are several different NIRS devices and while this specific device (Nonin Medical, Inc) detected changes up to 2 cm and beyond, this finding may not be able to be extrapolated to other manufactures based on specific sensor configurations and settings. This study was performed in uninjured subjects which do not correlate to the acute injury setting. However, in the acute injury setting, subcutaneous fat depths have been shown to be reduced, not increased, and the swelling occurs within the compartment itself and not in the subcutaneous tissue [25]. Further research is needed to examine muscle perfusion and NIRS values in the traumatized population in a longitudinal fashion. Additionally, guidelines need to be established for normal and abnormal perfusion in the injured extremity on a continual basis in order to use NIRS as a diagnostic tool for ACS. In summary, the purpose of this study was to determine if NIRS was capable of monitoring rSO_2 , muscle oxygenation, in the general population as well as in obese subjects. First, this study found few people with an ATT of over 2 cm in either the anterior or superficial compartment, even among the Class II and Class III obesity groups indication subcutaneous fat deposits in this region of the body remain quite shallow despite extremely obese subjects being selected in this study. Second, despite specifically selecting an unnatural population of extremely obese subjects, NIRS still recorded decreased rSO_2 values with exercise indicating the ability of this specific NIRS device to monitor muscle perfusion in the most extreme patient population. These findings indicate that NIRS is capable of monitoring muscle perfusion in not only the general population, but also in the extremely obese subjects that occur quite rarely in the general population. Although the change in NIRS readings was significantly smaller in patients with >40 kg/m² BMI compared to other groups, even extremely obese subjects registered substantial changes in NIRS values during exercise. These two results indicate NIRS is not only useful in the general population where obesity is distributed in a more standard distribution, but it also recorded changes in a purposefully manipulated subset of the extremely obese population. Additionally, specifically the lower leg does not have significant depositions of subcutaneous fat depositions indicating the lower leg is an ideal location for monitoring muscle perfusion.

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