Research Square

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Establishing Normative Data for SmO2 Equilibrium in Relation to Leg Elevation and Activity Levels: A Near-Infrared Spectroscopy (NIRS) Analysis

Andrew Moya
AdventHealth Orlando
Pooya Tehrany
pmtehrany@yahoo.com

AdventHealth Orlando
Neel Sandella
AdventHealth Orlando
Ramakanth Yakkanti
AdventHealth Orlando
Amir Kachooei
AdventHealth Orlando

Research Article

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Abstract

Purpose: Muscle oxygen saturation (SmO_2), measured using near-infrared spectroscopy (NIRS), is a non-invasive marker of local tissue perfusion. While explored in sports medicine, normative data for SmO_2 equilibrium time in healthy lower limbs are lacking. We aim to explore this crucial data for interpreting postoperative and pathologic changes

Methods: In this cross-sectional study, 48 healthy adults (18–65 years) underwent SmO_2 measurement with the Moxy Muscle Oxygen Monitor® (Hutchinson, MN, USA) on the medial gastrocnemius. Six functional conditions were tested: supine, 20° leg elevation, dangling, standing, walking (3 mph), and running (5 mph). Equilibrium was defined as a stable SmO_2 for 3 minutes. Times were compared using repeated measures ANOVA and post-hoc analyses.

Results: Equilibrium times differed significantly across conditions ($p < 0.001$). Supine and standing showed longer stabilization, while running had the shortest mean equilibrium time (44 seconds ± 13). Differences were both statistically and clinically significant. All data were formatted to two significant digits for clinical interpretability.

Conclusions: This study establishes normative equilibrium times for SmO_2 under various leg positions and activities. These values provide a benchmark for clinical comparisons in foot and ankle patients. NIRS shows potential for monitoring postoperative recovery and vascular health.

Level of Evidence: Level II, Cross-Sectional Study

Introduction

Skeletal muscle, comprising about 40% of body volume, is critical for mobility, stability, and overall function. It receives a significant share of cardiac output, underscoring its role in oxygen use and metabolic balance [1, 2]. SmO_2 indicates muscle perfusion and oxidative capacity, especially in lower limbs where vascular health is essential for recovery and performance [3–6]. Near-infrared spectroscopy (NIRS) has emerged as a non-invasive, real-time monitoring tool to assess SmO_2 dynamics, providing insight into muscle perfusion, ischemic conditions, and post-operative recovery pathways in lower extremity surgery [7–13]. However, the equilibration time required for SmO_2 to reach a stable state under different leg elevations and activity levels remains poorly characterized, limiting its clinical applicability in orthopedics. This knowledge gap presents a significant challenge for clinicians seeking to optimize patient recovery and understand muscle physiological responses during rehabilitation.

Understanding SmO_2 equilibrium across various lower limb positions and movements is crucial for effective post-surgical rehabilitation and vascular monitoring. In foot and ankle surgery, vascular integrity is vital for healing in procedures such as Achilles tendon repair, ankle fracture fixation, and ligament reconstructions, where compromised blood flow can delay recovery or lead to nonunion [14–16]. Rehabilitation strategies, including limb positioning and ambulation protocols, rely on muscle

oxygenation stability, highlighting the need for reference SmO_2 equilibrium values [17, 18]. Without standardized data, clinicians lack objective criteria to optimize perfusion, exercise progression, and vascular assessments in orthopedic patients [19].

Despite advances in muscle perfusion assessment, current methodologies present significant limitations. Phosphorus magnetic resonance spectroscopy (P-MRS)^{31} and similar methods reveal mitochondrial oxidative capacity, but they are invasive, costly, and not practical for routine orthopedic use [19–23]. While NIRS-based SmO_2 assessments are validated against ^{31}P-MRS, a critical gap exists in understanding equilibrium times for SmO_2 across various lower limb positions and movements [24, 25]. This gap limits evidence-based decisions in postoperative care, return-to-sport assessments, and vascular monitoring in orthopedics [26].

Study Objectives

This study quantifies SmO_2 equilibrium time in different lower limb positions and activities, providing normative data for lower extremity surgical care. Using NIRS-based real-time monitoring, we assessed SmO_2 stability in six conditions: supine, 20-degree elevation, dangling, standing, walking (3 mph), and running (5 mph). Reference equilibrium times will guide post-surgical limb positioning, rehabilitation, and vascular monitoring. These findings will help orthopedic surgeons and rehabilitation professionals optimize lower limb recovery and perfusion management after surgery [27].

Materials and Methods

This cross-sectional, non-randomized study used Near-Infrared Spectroscopy (NIRS) to measure the time to reach stable muscle oxygenation (SmO_2) equilibrium across different leg heights and exercise intensities. From October 2, 2023 until February 2, 2025, students studied. Determining normative SmO_2 equilibrium data in healthy individuals provides reference values for lower extremity surgical rehabilitation programs. The institutional ethics committee approved the study in accordance with the World Medical Association's Declaration of Helsinki (IRB# 2102822). Participant informed consent was obtained before enrollment. The primary outcome measure was the time to equilibrium (in seconds) for SmO_2 levels to stabilize in all positions for three consecutive minutes. SmO_2 equilibrium was compared under six conditions: sitting, 20-degree elevation, dangling, standing, walking (3 mph), and running (5 mph) [14, 19, 27].

Study Participants and Selection Criteria

Participants were recruited using ads, email invitations, and healthcare facility brochures. Participants had to be 18–65 years old and not have had lower limb surgery, peripheral vascular disease, or other circulation-affecting disorder. Candidates completed a rigorous medical history questionnaire and a clinical assessment to ensure eligibility. Participants who reported bilateral or unilateral lower limb injuries requiring surgery, cast, or boot immobilization were excluded. A sample size calculation using

G*Power software determined that 48 participants were needed to detect statistically significant differences in SmO_2 across six conditions, with 24 males and 24 females. The average age of participants was 34 ± 7.8 years (range 18-65 years). To identify potential confounders, age, sex, BMI, baseline physical activity, and smoking status were recorded. A heterogeneous participant pool allows application to postoperative and rehabilitative settings [1, 7].

Measurement of Muscle Oxygen Saturation (SmO_2)

To detect muscle oxygen saturation (SmO_2), the Moxy Muscle Oxygen Monitor® (Hutchinson, Minnesota, USA), a portable NIRS device, was used in sports science, rehabilitation, and clinical research [28]. To ensure accuracy, the device was calibrated before each session per manufacturer instructions [29]. The monitor was secured to the medial head of the gastrocnemius muscle on both legs with hypoallergenic adhesive tape to minimize ambient light interference [Figure 1]. This muscle was chosen for its superficial location, excellent vascularization, and direct lower extremity function [30]. Before recording, participants performed an isometric contraction to assure device position and perfusion to the monitored area. The 1 Hz data were wirelessly sent to a laptop for storage and processing using the VO2 Master application (Vernon, British Columbia, Canada). An example of the Moxy Muscle Monitor device's output onto the VO2 Master application can be appreciated in Figure 2. NIRS reliability was confirmed using test-retest repeatability measures performed in a subset of participants [31].

Study Protocol and Data Collection

The study protocol included six standardized conditions: [Figure 3 a-f]

- Supine – participant lying flat
- 20° elevation – both legs elevated 20° from supine
- Dangling – sitting with both legs hanging freely from a bed
- Standing – standing in an upright position
- Walking – walking on a treadmill at 3 mph
- Running – running on a treadmill at 5 mph [32]

Data collection began immediately after position or activity change and lasted for three minutes until SmO_2 equilibrium was achieved for each condition. After reaching equilibrium, SmO_2 was measured every second for three minutes to assess stability. To ensure data reliability, measurements were taken in a temperature-controlled laboratory (22°C ± 1°C) at the same time of day (morning) to minimize variability. For muscle perfusion consistency, participants were told to avoid caffeine and intense activity 12 hours before testing.

Primary and Secondary Outcome Measures

The primary outcome measure was the time to SmO_2 equilibrium, which is the time needed for muscle oxygenation levels to stabilize for three minutes. Secondary outcomes included SmO_2 levels across

conditions, demographic characteristics (age, sex, BMI, baseline activity level), and correlations between SmO_2 trends and physical activity intensity. The study assessed SmO_2 trends during postoperative recovery, as impaired vascular integrity can delay wound healing and raise complication risks.

Statistical Analysis

Statistical analyses were conducted using IBM Statistical Packages for Social Sciences Statistics (SPSS)(Version 30.0.0). Paired t-test or Wilcoxon signed-rank test was used to compare SmO_2 equilibrium times under various settings. One-way ANOVA with post-hoc Bonferroni correction was used to analyze equilibrium timings across all six conditions. A statistical significance level of $\alpha = 0.05$ and power (β) of 0.80 were used to identify significant differences. Multiple techniques for imputation were used to manage missing data for statistical robustness.

We used a Kaplan-Meier survival analysis to compare time to SmO_2 stabilization across settings, adding to the validity of equilibrium dynamics. Effect size calculations (Cohen's d) assessed the extent of SmO_2 equilibrium time differences between conditions. Sensitivity analyses assessed data against potential confounding factors as BMI, age, smoking status, and baseline cardiovascular fitness. SmO_2 measurements were compared using Bland-Altman plots to ensure data reliability and measurement consistency across situations. A multivariate regression model was used to identify predictors of SmO_2 stabilization time, such as age, sex, and baseline activity level, to improve post-surgical rehabilitation programs for vascular recovery.

Results

The study analyzed data from 48 healthy adults (mean age = 30. ± 9.15 years, range: 18-61), with a 30:18 male-to-female ratio. The race distribution was 60% Caucasian, 25% Asian, 8.3% Black, 6.3% Other, and 63% non-Hispanic [Table 1]. Participants' demographics ensured a representative sample, reducing SmO_2 equilibrium measurement confounding variables.

Table 1: Participant Demographics

Category	Mean ± SD / %	n
Age (years)	30 ± 9.2	48
Sex		
Male	63%	30
Female	38%	18
Race		
Caucasian	60%	29
Asian	25%	12
Black	8.3%	4
Other	6.3%	3
Ethnicity		
Non-Hispanic	63%	30
Hispanic	38%	18

Time to Equilibrium Across Different Conditions

The mean time to equilibrium (SmO_2) varied significantly between leg heights and exercise intensities. Supine positioning had a mean equilibrium duration of 100.00 ± 33.41 seconds, whereas elevating to 20 degrees increased it to 123 ± 36.35 seconds. With legs dangling, the time remained elevated at 121.25 ± 51.85 seconds. Dynamic activities like walking (65.31 ± 26.81 seconds) and running (44.37 ± 13.39 seconds) shortened the equilibrium period, whereas standing reduced it to 99.69 ± 30.34 seconds [Table 2]. The results indicate a decrease in SmO_2 stabilization time as metabolic demand and muscle activation increase.

Table 2: Time to Equilibrium Across Different Conditions

Condition	Mean Time to Equil (secs)	Std. Deviation
Supine	100	33
20° Elevation	123	36
Dangling	121	52
Standing	100	30
Walking	65	27
Jogging	44	13

Paired Comparisons and Statistical Significance

Paired sample comparisons confirmed differences in SmO_2 equilibrium times between conditions [Table 3]. The transition from supine to 20-degree elevation significantly increased equilibrium time by 23± 45.60 seconds (p = 0.001). However, differences between 20-degree elevation and dangling were minimal (1.77 ± 55.93 seconds, p = 0.827). Standing significantly reduced equilibrium time compared to dangling (21.56 ± 58.29 seconds, p = 0.014). Notably, the reduction from standing to walking was highly significant, with equilibrium time decreasing by 34.37 ± 45.71 seconds (p < 0.001), indicating the strong influence of dynamic movement on oxygenation stability.

Table 3: Paired Sample Comparisons of Time to Equilibrium

Pair	Mean Difference (secs)	Std. Deviation	p-value
Supine vs. 20° Elevation	23	46	0.001
20° Elevation vs. Dangling	1.8	56	0.83
Dangling vs. Standing	22	58	0.014
Standing vs. Walking	34	46	0.0

Correlation Analysis

SmO_2 equilibrium times between conditions were weakly correlated [Table 4]. The correlation between supine and 20-degree elevation was r = 0.147 (p = 0.318), while 20-degree elevation and dangling showed a moderate correlation (r = 0.234, p = 0.110). Standing and walking were inversely correlated (r = -0.277, p = 0.057), suggesting that greater muscle activation facilitates faster oxygenation stabilization. These findings indicate that equilibrium times are condition-dependent, and individual factors such as baseline circulation and activity level likely influence SmO_2 stabilization.

Table 4: Correlation Analysis of SmO_2 Equilibrium Times

Pair	Correlation (r)	p-value
Supine vs. 20° Elevation	0.15	0.32
20° Elevation vs. Dangling	0.23	0.11
Dangling vs. Standing	0.067	0.65
Standing vs. Walking	-0.28	0.057

Unexpected Findings

While we hypothesized that higher limb elevation would proportionally increase equilibrium times, there was no significant difference between 20-degree elevation and dangling (p = 0.827). This suggests that venous return in a dependent limb position does not substantially delay SmO_2 stabilization compared to

mild elevation. Additionally, the dramatic reduction in equilibrium time observed in walking and running ($p < 0.001$) highlights the role of active muscle contraction in enhancing oxygenation stability.

These findings provide insight into SmO_2 regulation in lower limb positions and mobility, potentially enhancing surgical rehabilitation. Future studies should investigate equilibrium trends in clinical populations, particularly in patients recovering from lower extremity pathologies, where perfusion status is a key determinant of healing outcomes.

Discussion

This study quantified the time to achieve stable muscle oxygen saturation using near-infrared spectroscopy across various leg positions and activity levels. Results indicate that dynamic activities, such as walking and running, expedite stabilization compared to static postures like leg elevation and dangling. These findings suggest that increased metabolic demand and muscle pump activity enhance microvascular perfusion and oxygen utilization, supporting the use of near-infrared spectroscopy monitoring in orthopedic rehabilitation settings.

Our findings corroborate prior research indicating that dynamic muscle contractions enhance muscle oxygen saturation through improved venous return and vasodilation [9, 33–35]. This study advances existing knowledge by quantifying the time to achieve muscle oxygen saturation equilibrium across six functional positions. While previous studies assessed trends during static and active muscle use, few have examined equilibrium time as a physiological endpoint or compared both elevation and activity conditions [24, 36]. Measuring stabilization time in healthy individuals provides novel insights into perfusion regulation. Equilibrium time may serve as a reproducible biomarker in postoperative rehabilitation following lower extremity procedures, where tissue oxygenation is critical for recovery [37–39].

Unexpectedly, equilibrium times did not differ significantly between 20-degree leg elevation and dangling. Contrary to expectations that dependent positioning would impede venous return and delay oxygen equilibration, this was not observed. This may reflect compensatory autonomic mechanisms preserving perfusion in passive limb positions, as suggested by prior studies on peripheral tissue oxygenation under varying pressure and flow states [40, 41]. The weak correlation of equilibrium times across positions suggests that microvascular dynamics are posture-dependent, influenced by systemic and local regulatory factors. These findings underscore the necessity for condition-specific analysis when applying muscle oxygen saturation metrics in clinical care.

Strengths of this study include standardized measurement protocols, utilization of a validated near-infrared spectroscopy device (Moxy Monitor), and data collection within a temperature-controlled environment. Prior research has confirmed the reliability and precision of wearable near-infrared spectroscopy in assessing muscle oxygenation under both controlled and stress conditions [42–44]. The study's inclusion of six functional conditions pertinent to foot and ankle rehabilitation, along with a demographically balanced cohort, enhances its applicability to orthopedic populations. Analytical rigor

was maintained through appropriate use of paired testing, ANOVA, and correlation modeling. Real-time, second-by-second muscle oxygen saturation monitoring provided high temporal resolution in identifying stabilization points, a feature not commonly reported in previous near-infrared spectroscopy studies [45, 46].

Active muscle contractions during walking and running expedite muscle oxygen saturation stabilization, likely due to enhanced arterial inflow and venous return via the muscle pump mechanism. Conversely, passive positions like elevation and dangling challenge microcirculatory oxygen delivery. These findings suggest a direct relationship between muscular workload and time to oxygen equilibrium, informing post-surgical mobilization protocols. The consistent observation of shorter equilibrium times during movement supports the benefits of early ambulation in enhanced recovery protocols. Notably, prior literature has primarily focused on response amplitude or peak recovery values, whereas this study emphasizes equilibrium time as a critical parameter [47, 48], our study shifts focus to the timing of these changes, offering a complementary but distinct perspective.

This study has limitations. The exclusive inclusion of healthy participants may restrict applicability to orthopedic patients with vascular compromise, diabetes, or other comorbidities. While the gastrocnemius was chosen for its functional relevance and accessibility, oxygenation patterns may differ in other lower limb muscles. Furthermore, equilibrium time was measured acutely, not capturing longitudinal recovery changes, thus limiting its predictive value over time. Nonetheless, these findings provide a foundational baseline for future research into perfusion dynamics in pathological conditions.

Equilibrium time serves as a functional perfusion marker, potentially guiding postoperative protocols in lower extremity surgery. Delayed stabilization in passive positions may indicate risk for wound healing complications or venous stasis, whereas accelerated equilibrium during movement supports early mobilization strategies. This aligns with the emphasis on real-time, non-invasive, patient-centered monitoring in orthopedic recovery planning [47, 49]. Identifying patients with abnormal equilibrium times may enable clinicians to customize rehabilitation intensity and optimize recovery timelines. Furthermore, equilibrium time could serve as a stratification tool for preoperative risk assessment or postoperative monitoring, especially in cases with compromised vascularity, such as diabetic foot reconstruction or trauma with delayed healing.

In conclusion, this study defines normative equilibrium times for SmO_2 across various lower limb positions and activity intensities in healthy individuals. Our results support NIRS as a practical tool for perfusion monitoring and establish equilibrium time as a relevant endpoint for microvascular adaptation. Integrating these metrics into postoperative care may enhance recovery and personalize rehabilitation. These insights expand the clinical utility of wearable NIRS by introducing equilibrium time as a novel marker of tissue perfusion in orthopedic practice.

Declarations

Ethics Statement:

This study was reviewed and approved by the Institutional Review Board of AdventHealth (IRB# 2102822). All participants provided written informed consent prior to enrollment.

Funding Statement:

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Conflict of Interest:

The authors declare no conflicts of interest related to this work.

Author Contribution

A.R.M. and P.T. contributed to the conception and design of the study. A.R.M., P.T., and N.S. contributed to data collection, data analysis, and interpretation of the results. A.R.M. and P.T drafted the manuscript. N.S prepared Figures 1-3. R.Y. and A.R.K. provided study supervision and critically revised the manuscript for important intellectual content. All authors reviewed and approved the final manuscript.

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Figures

Figure 1

The Moxy Muscle Oxygen Monitor was placed over the medial gastrocnemius and secured with hypoallergenic skin tape to ensure adequate skin contact and reduced light interference.

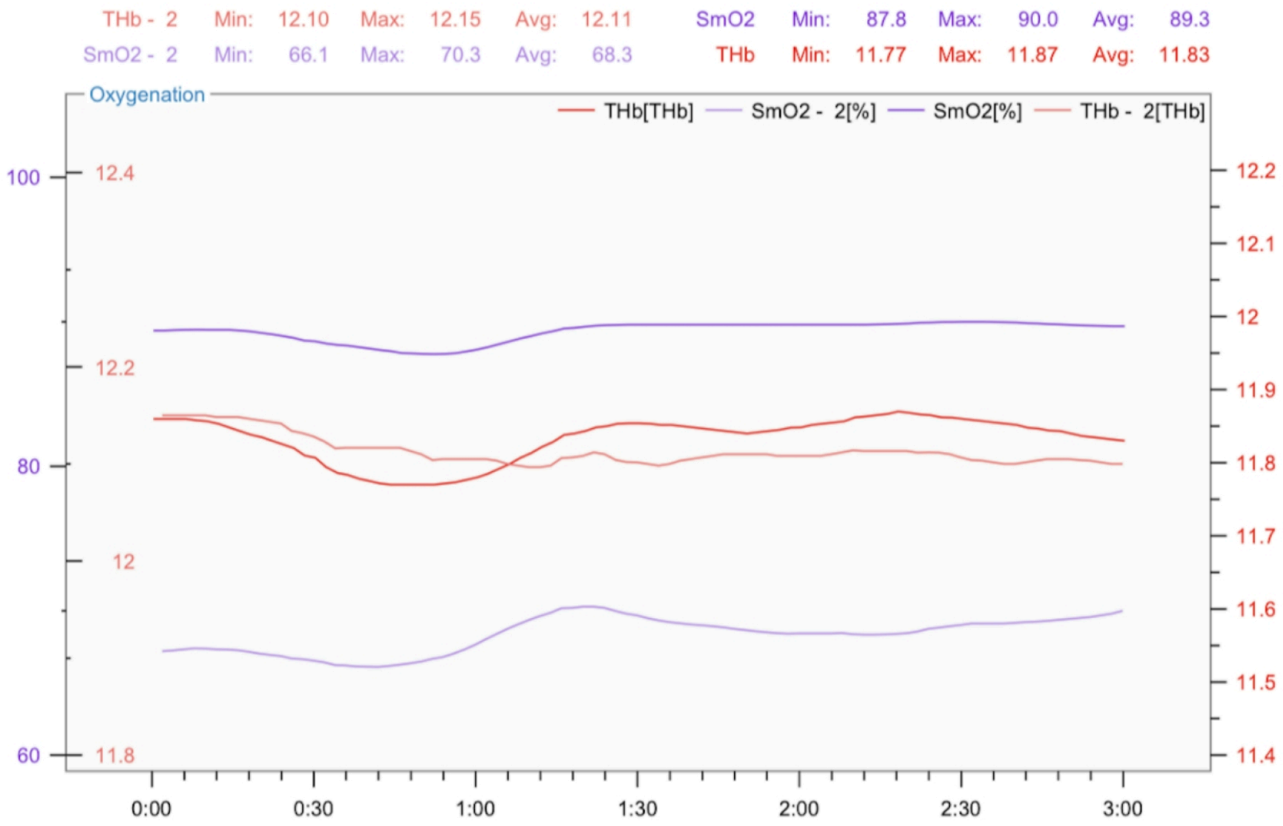

Figure 2

Graph output of the Moxy Muscle Monitor device in the VO2 Master application demonstrating SmO2 reading of three minutes with the lower extremities in 20° elevation – both legs elevated 20° while supine.

Figure 3

Standardized body positioning during six lower extremity conditions for SmO2 monitoring using near-infrared spectroscopy (NIRS). Images depict the six tested conditions in the study: (a) supine, (b) 20° leg elevation using a medical wedge, (c) dangling, (d) standing, (e) walking at 3 mph on a treadmill, and (f) running at 5 mph on a treadmill.