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In Vivo Oxygen Consumption and Hemoglobin Levels in Human Thyroarytenoid Muscle

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Abstract

Objectives/Hypothesis—Visible light spectroscopy (VLS) is the technology behind the Food and Drug Administration–approved TSTAT device that is used to monitor tissue oxygen (StO₂) and relative total hemoglobin (rHb) levels by measuring reflected visible light. The purpose of this novel, pilot study was to determine if VLS is a reliable and valid method of measuring StO₂ and rHb levels in the human thyroarytenoid/lateral cricoarytenoid (TA-LCA) muscle complex, thus providing information about vocal fold muscle physiology.

Study Design—Pre-test/post-test with multiple baselines and two conditions.

Methods—VLS measurements were taken at baseline, during exercise, and following recovery on six subjects using both noncontact channel-port endoscope (endo-probe) and laryngeal electromyography (LEMG) needle-guided techniques.

Results—The average baseline StO₂ was 69% (standard deviation [SD] = 3.6%) for the LEMG-guided probe and was 71.5% (SD = 2.8%) for the endo-probe. During phonation, the StO₂ for the LEMG-guided probe dropped to 59% (SD = 7%; $P = .04$). Mean rHb measured by the LEMG probe rose from a baseline of 144 μ M (SD = 165 μ M) to 214 μ M (SD = 166 μ M, $P = .34$) during phonation and back to 149 μ M (SD = 139 μ M, $P = .85$) after recovery. Mean rHb as measured using the endo-probe at baseline and after recovery was 104 μ M (SD = 30 μ M, $P = .76$).

Conclusions—VLS can be used to measure changes in StO₂ and rHb levels pre- and postexercise in the human TA-LCA muscle complex.

Keywords

Visible light spectroscopy; oxygen consumption; hemoglobin; thyroarytenoid muscle; vocal cord muscle physiology

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INTRODUCTION

Vocal fatigue, increased muscle tension, and decreased muscle activation are terms used clinically to identify the cause or symptoms of some voice disorders such as muscle tension dysphonia, vocal fold paresis, paralysis, and atrophy. Currently there is no measurable method to accurately determine if a symptom such as vocal fatigue matches actual muscle function. Most of the instruments used are perceptual and rely on an observer's subjective opinion about the status of the larynx during voice production. Furthermore, discussions regarding disorders related to muscle tension dysphonia are often teleological at best due to the inability to measure intrinsic laryngeal muscle physiology in vivo. Laryngeal electromyography (LEMG) can provide some information about if and how the laryngeal muscles are activating. The quantification of the activation of the muscles using LEMG, however, depends greatly on electrode placement.¹ LEMG is also an invasive technique, can be uncomfortable for patients, and is most accurate when the laryngologist understands LEMG and the electromyographer has knowledge about the functioning of the larynx.²

Visible light spectroscopy (VLS) is the concept behind the Food and Drug Administration–approved TSTAT device (T-Stat 303; Spectros Corp., Portola Valley, CA) that is currently intended for use as an adjunct monitor of the localized relative total hemoglobin (rtHb) and oxygen saturation (StO₂) of blood in the microvascular tissue spaces in infants, children, or adults at risk for reduced-flow and no-flow ischemic states.^{3,4} VLS has been shown to detect StO₂ changes following tissue perfusion in the brain and colon,^{5,6} as well as diagnose ischemia in the gastrointestinal tract⁷ and, most recently, in cardiac muscle.⁸ VLS uses a fiber-optic probe to deliver white light through the instrument channel of an endoscope (endo-probe) or the lumen of an electromyography needle to the target muscle. The light that is scattered back from the tissue is captured by an integrated sensor fiber and measured using a spectrometer. Differences in the absorbance spectra of oxygenated and deoxygenated hemoglobin allow the device to determine the StO₂ of the blood in the tissue and the total concentration of hemoglobin in the light path through the tissue (rtHb).

Following an extended phonatory task, it is hypothesized that the thyroarytenoid/lateral cricoarytenoid (TA-LCA) muscle complex would display a decrease in StO₂ during phonation (exercise) and a recovery back to baseline after cessation of the task. VLS may be a minimally invasive method to quantitatively measure in vivo vocal fold muscle physiology in individuals with and without voice disorders. The purpose of this pilot study was to determine if VLS, using the TSTAT device, is a reliable and valid method of measuring in vivo StO₂ and hemoglobin levels in the human TA-LCA muscle complex to provide information about muscle function pre-, during, and postexercise (phonation).

MATERIALS AND METHODS

Institutional review board approval was obtained at both the University of Pittsburgh Medical Center and Misericordia University. The experimental portion of the study consisted of two tests. The endo-probe readings were obtained first. Flexible laryngoscopy was performed using a 4.9-mm channel port flexible laryngoscope (Olympus ENF-VT2; Olympus Corp., Tokyo, Japan). A 1.5-mm VLS sensor (CTH-060-END 2.3m; Spectros Corp., Portola Valley, CA) was placed through the working channel of the flexible laryngoscope to obtain StO₂ and rtHb data in a noncontact fashion (Fig. 1). To determine the reliability and validity of the endo-probe readings, a 21-gauge hollow LEMG needle (Aiglette model DIN-75 75 mm × 22 gauge) (Technomed Europe, The Netherlands) was inserted into the TA-LCA muscle complex under LEMG guidance. A different fiber-optic VLS probe, which was fabricated specifically for this experiment, was guided through the

LEMG needle to obtain the StO₂ and rHb measurements from directly within the muscle. The LEMG needle-guided probe was used to assess the reliability and validity of the endo-probe data. Results were displayed and recorded on the TSTAT device.

Six subjects were enrolled in the study. Subjects were included if they were female, had no prior or current vocal complaints, and scored 0–1 on the Voice Handicap Index-10 indicating that they have no perception of voice handicap.⁹ The subjects' nares were topically anesthetized with cotton pledgets soaked in a mixture of 2% lidocaine and oxymetazoline. A topical, nebulized treatment of 3 mL of 4% lidocaine was administered. Flexible fiber-optic laryngoscopy with stroboscopy was performed before the VLS probe was inserted to confirm that the participants had normal vocal fold mobility and no abnormalities of the membranous vocal folds. An additional 1 to 3 mL of plain 4% lidocaine was dripped onto the endolarynx to achieve complete topical laryngeal anesthesia. The VLS fiber-optic probe was then guided through the channel-port of the endoscope.

The endo-probe was positioned as close and as consistently as possible to approximately 1 mm above the vocal fold being tested and was aimed at the TA-LCA muscle complex using the visible light emanating from the probe (Fig. 2). The probe was positioned perpendicular to the superior surface of the posterior half of the membranous vocal fold, away from the free edge. A baseline measurement of StO₂ and rHb levels was obtained. Participants were then asked to sustain “ah” as loudly as possible for 60 seconds. If participants needed to breathe, they were instructed to take a quick breath and resume the phonatory task. A sound pressure level meter was used to ensure the participants stayed between 90 and 100 dB during the phonatory task. Measurements were not obtainable during the phonatory task owing to movement of the larynx and an inability to position the probe consistently. Upon completion of the task, the probe was again positioned as described, and postexercise measurements were obtained. The probe did not contact the mucosa of the vocal fold. Data were recorded until StO₂ and rHb levels returned to baseline. After a 3-minute period of voice rest, the task was repeated. Phonatory task and readings were recorded on the same muscle two to four times depending on the accuracy of the readings per subject.

Following completion of the VLS measures of the vocal fold via the endo-probe, three subjects underwent direct measurement of the StO₂ of the TA-LCA muscle complex through the LEMG-guided probe. Subjects were placed in a semirecumbent position, and the skin and soft tissue overlying the cricothyroid membrane was anesthetized with a subcutaneous injection of 1 mL of 1% lidocaine with 1:100,000 epinephrine. The LEMG needle was inserted through the cricothyroid space and into the TA-LCA muscle complex being tested. Optimal needle localization within the TA-LCA complex was confirmed by electromyography using a phonatory task. The TA-LCA complex that was analyzed using the endo-probe was then analyzed with the LEMG-guided probe. Exact knowledge of what part of the TA-LCA complex was being measured with either probe was impossible, but an attempt was made to analyze within or above the bulk of the TA-LCA complex with each respective probe. We also made an effort to consistently place the probes within or above the TA-LCA complex from subject to subject. After confirmation of needle placement, a 0.38-mm-diameter fiber-optic probe was placed through the lumen of the electromyography needle. A baseline measure of StO₂ and rHb levels was obtained. Participants were asked to perform the same phonatory task as was used for the endo-probe measurements. Vocal intensity remained between 90 and 100 dB SPL. StO₂ and rHb measures were able to be recorded during phonation with the LEMG probe. StO₂ and rHb were recorded after phonation until measurements returned to baseline. After a 3-minute period of voice rest, the task was repeated.

Endo-probe and LEMG-guided tissue saturation and rHb measured after phonation were compared to baseline values using a two-tailed paired *t* test, with threshold for significance of .05. Similarly, phonation and postphonation measures of muscle saturation and rHb were compared to baseline levels using a two-tailed paired *t* test with the same threshold for significance.

RESULTS

Each of the six subjects performed two to four phonation tasks with a minimum of 3 minutes recovery period between tasks. In all, 15 baseline and 15 postphonation measurements were endoscopically made of the vocal folds. Examples of the StO₂ and rHb measurements made are shown in Fig. 3A and Fig. 3B. The average baseline saturation was 71.5% (SD = 2.8%), and the average postphonation saturation was 73.1% (SD = 3.6%, *P* = .10). The baseline rHb was 106 μM (SD = 32 μM), and the postphonation rHb measured was 104 μM (SD = 30 μM, *P* = .76). (Fig. 4A and 4B).

Direct, continuous measurement using the LEMG needle-guided probe of the StO₂ and rHb on the TA-LCA muscle complex was performed in three subjects before, during, and after the phonatory task. In both the endoprobe and LEMG guided-needle probe, an overshoot of StO₂ past baseline was noted after cessation of the phonatory task. Fig. 5A and Fig. 5B demonstrate this overshoot in one of the subjects during the LEMG guided-probe data. Range of recovery time was 10 to 60 seconds. At time series 19:07 to 19:09, StO₂ was approximately 70%, and rHb was 330 μM. At the onset of phonatory task, the StO₂ decreased to 60% while the rHb increased steadily to more than 400 μM. After cessation of the phonatory task, StO₂ increased beyond baseline levels, then returned to baseline, and rHb decreased close to baseline within 2 minutes of recovery (Fig. 5A and 5B).

The average baseline StO₂ for all three subjects with the LEMG needle-guided probe in the TA-LCA muscle was 69% (SD = 3.6%). During exercise, StO₂ dropped to a value of 59% (SD = 7.2%, *P* = .04). Postphonation StO₂ returned to a baseline level of 72% (SD = 8.2%, *P* = .44). The average baseline rHb in the TA-LCA was 144 μM (SD = 165 μM). During phonation, the rHb level trended higher at 214 μM (SD = 166 μM, *P* = .34) but did not reach statistical significance owing to the limited number of subjects. After phonation, the measured rHb was 149 μM (SD = 139 μM, *P* = .85) (Fig. 6A and 6B).

Pre- and postphonation StO₂ were similar as measured by the endo-probe and the needle probe placed within the TA-LCA muscle. Comparisons of the mean values are shown in Figure 6A and Figure 6B.

DISCUSSION

The aim of the present study was to determine if VLS can be used to reliably measure muscle function in the larynx through measures of StO₂ and rHb levels in the intrinsic laryngeal muscles. Average StO₂ values were very similar between the flexible laryngoscopy-guided probe (endo-probe) and LEMG needle-guided probes at baseline and after phonation. This result indicates that both probes were able to accurately measure resting saturation and hemoglobin levels before and after exercise. This validates the endo-probe location and depth of measuring from the TA-LCA muscle complex. The LEMG-guided probe showed a drop in StO₂ values and an increase in rHb values during phonation, with a return to baseline after cessation of the phonatory task. The results are consistent with exercise physiology literature stating StO₂ levels drop during exercise because the muscle is working, and after exercise StO₂ levels should return to baseline.¹⁰ With both probes it was often noted that StO₂ levels showed an overshoot past baseline before returning to baseline

values after 10 seconds to 1 minute of recovery. Unfortunately it was impossible to accurately obtain fiber-optic-probe data during phonation.

Most of the research on limb skeletal muscle physiology has been conducted using near-infrared spectroscopy (NIRS).^{11–13} NIRS and VLS are two technologies that exist to measure StO₂ and hemoglobin levels within human muscle. In theory, VLS is similar to NIRS with the significant exception that the absorption of visible light in tissue (mostly hemoglobin) is 100 times greater than NIRS, and so NIRS is more deeply penetrating. The penetration depth of VLS is approximately 2 mm, but the NIRS devices are around 2.5 cm. In practice, there are a number of other substantial differences. One of these differences is that NIRS devices currently available are designed to maximize the path length and therefore separate the light source and detector by 3 to 4 cm, making the probe itself very large. This deeply penetrating large sensor is good for skeletal muscle but would not work well on small laryngeal muscles.³

One-to-one correlations of StO₂ and rtHb baseline levels, however, cannot be made between limb skeletal muscle and intrinsic laryngeal muscle. This is because how much these levels change with exercise and how long it will take for them to recover depends so much on the visualizing instrument, the type of exercise performed, and the muscle itself. Because VLS and NIRS measure similar properties, though, it is reasonable to conclude that results from the present study on intrinsic laryngeal muscle using VLS showed consistency with the trends found in limb skeletal muscle visualized through NIRS.

The histologic properties of the intrinsic laryngeal muscles, especially the TA-LCA muscle complex, are also different from limb skeletal muscles,^{14,15} making it difficult to make direct comparisons to known exercise physiology literature. The results of our study, however, indicated that as the muscle contracted, StO₂ decreased as the muscle worked to extract oxygen from the blood.¹⁰ Hemoglobin levels increased as oxygen was extracted from oxyhemoglobin to meet the increasing demands of oxygen from the muscle, and the muscle showed an overshoot of blood oxygenation postexercise. These findings are consistent with exercise physiology literature.¹⁰

A limitation of our study was that accurate recovery times to baseline after the phonatory task were not obtained with both the endo-probe and the needle-guided probe. The VLS device recorded recovery times ranging from 10 to 60 seconds, although observed recovery times by the investigators was closer to 10 seconds. Adjustments have been made to the device to more accurately record recovery times for ongoing studies. An average recovery time in healthy muscle will be an important parameter to have to compare to patients with voice disorders.

Voice disorders are a common occurrence in the general population, with as many as 3% to 9% of people experiencing some type of voice dysfunction in their lifetime.¹⁶ People who participate in professions that rely on extensive voice use (e.g., teachers, actors, singers) experience voice disorders more often than the general population and report symptoms such as vocal fatigue and muscle tension.^{17,18} The literature in voice disorders has focused primarily on tension in the extrinsic and intrinsic laryngeal muscles related to disorders such as muscle tension dysphonia, adductor and abductor spasmodic dysphonia, functional dysphonia, psychogenic voice disorders, and vocal fold paresis and paralysis. Excessive muscle tension has been proposed to cause significant phonotrauma.¹⁹ There are many possible reasons why excessive muscle tension is presumed to occur in these disorders; however, the actual muscle physiology of the proposed “laryngeal muscle tension” is not known or is difficult to identify.²⁰

Generalized physical tension could be a contributing factor involved in the expression of laryngeal tension or it could also be a reaction to primary laryngeal tension. Other possible contributing factors to laryngeal muscle tension could be more physiologic in nature such as unrecognized subtle glottic insufficiency from mild vocal fold atrophy, scar, paresis or a lack of strength or endurance of the intrinsic and extrinsic muscles in the phonatory system. Scant literature exists, however, on the physiology and the pathophysiology of the intrinsic laryngeal muscles in both health and disease. Developing a noninvasive in vivo method to measure laryngeal muscle physiology will open the door to assessing the role of appropriate or maladaptive muscle compensation in a variety of voice disorders that traditionally have been teleologically linked with “muscle tension.”

The perception of ‘vocal fatigue’ is also an elusive concept. From what is known about the histologic properties of the intrinsic laryngeal muscles, namely the TALCA muscle complex, they are considered to be fatigue resistant, consisting more of fast-contracting type II fibers (with more IIA than IIX) than slow-contracting type I fibers.^{14,15,21} Although results of a recent study provide evidence that the thyroarytenoid muscle shows response changes and remodeling to a more glycolytic (fatigue-resistant) capacity with constant stimulation, the most substantial limitation remains that there is no method to visualize how the muscle is responding during stimulation.²¹ Our study demonstrates that VLS is a tool that may allow the measurement of laryngeal muscle physiology in vivo in both health and disease.

Most of the voice assessment instruments used are perceptual and rely on an observer’s subjective opinion about the status of the larynx during voice production. LEMG measures muscle activation, although there are issues with 1) its ability to quantify data, 2) its invasiveness, and 3) the discomfort it affords to patients. Laryngologists and speech-language pathologists, therefore, usually make clinical diagnoses and design treatment strategies for muscle-tension voice disorders without ever knowing how the muscles are performing and why the muscles are activating in a specific manner. With the development of an instrument such as the VLS that can assess laryngeal muscle physiology directly, physicians and speech-language pathologists may be able to determine if there is muscle pathophysiology and if anything can be done to improve inefficient or overworking muscle activity during phonation.

CONCLUSION

Laryngeal evaluation of StO₂ and rHb is possible and quantifiable using VLS from the TSTAT device on the TA-LCA muscle complex. Future work is needed in a larger cohort to confirm normal StO₂ and rHb values and to investigate StO₂ and rHb in patients with a voice disorder. With the information obtained from this instrument, voice care providers could potentially identify the etiology of a functional and physiologically based voice problem, diagnose the muscles that are working inefficiently, and make clinical decisions about treatment based on observable and quantifiable data.

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Figure 1.
Channel port flexible laryngoscope with visible light spectroscopy probe.

Figure 2.
Channel port flexible laryngoscope with visible light spectroscopy probe aimed over the left true vocal fold.

Figure 3. Measurement of (A) tissue oxygen and (B) relative total hemoglobin (rtHb) of the vocal folds into the thyroarytenoid/lateral cricoarytenoid muscle complex before and after phonation for one subject with the endo-probe.

Figure 4. Mean and standard deviation of tissue oxygen (A) and relative total hemoglobin (rtHb) (B) measurements made via the endo-probe before and after phonation.

Figure 5. Time series of muscle tissue oxygen (A) and relative total hemoglobin (rtHb) (B) measurements before, during, and after phonation for one subject with the laryngeal electromyography needle-guided probe.

Figure 6. Comparison of tissue oxygen (A) and relative total hemoglobin (rtHb) (B) measurements made through the mucosal surface (endo-probe) and within the thyroarytenoid/lateral cricoarytenoid muscle (needle-guided probe).