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PULMONARY DIFFUSING CAPACITY IS UNALTERED IN ELITE SWIMMERS AFTER RESTRICTED BREATHING TRAINING

By

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B.S., University of Louisville, 2013

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ABSTRACT

PULMONARY DIFFUSING CAPACITY IS UNALTERED IN ELITE SWIMMERS AFTER RESTRICTED BREATHING TRAINING

Benjamin T. Ogle

May 10, 2015

Controlled frequency breath (CFB) holding is a swim training modality that involves holding one's breath for ~12 strokes before taking another breath. We looked to examine the effects of CFB training on pulmonary diffusing capacity for nitric oxide (DLNO) and carbon monoxide (DLCO). Elite swimmers (n = 25) were divided into either the CFB or a group that breathed regularly, every ~3rd stroke. The training intervention included 16 sessions of 12 x 50-m repetitions with either breathing pattern. Approximately 60% of the males and ~20% of the females were above the upper limits of normal for diffusing capacity at baseline. However, neither DLNO nor DLCO was altered after ~4 weeks of training. The CFB and control group exhibited no differences for any of the chosen parameters following intervention. In conclusion, DLNO and DLCO is unaffected by a four week period of CFB training.

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INTRODUCTION

The benefits of increased physical activity have been studied and researched to great extent. Muscle oxidative capacity, muscle buffering capacity, resting muscle glycogen levels, lipid oxidation, and aerobic capacity are just some of the parameters that can be improved with exercise training (Burgomaster et al., 2008; Gibala et al., 2006). When evaluating the increased physiological demands of exercise, it is the job of the pulmonary system to supply the body with sufficient oxygen to meet increased metabolic demands. Like any other physiological system, an increase in pulmonary efficiency is to be expected with training. In spite of this assumption, there has been no consequential evidence showing a relationship between improved aerobic capacity and changes in lung structure (Flaherty et al., 2014). In the absence of structural lung adaptations to exercise, where does the increase in performance come from? One logical explanation is an increase in the lungs' ability to transfer oxygen and carbon dioxide across the alveolar-capillary membrane. This increase in diffusing capacity would allow for greater gas exchange over a reduced period of time resulting in more efficient respiration.

A study recently examined the effects of controlled frequency breathing (CFB) on respiratory muscle fatigue, diffusing capacity and running economy in novice swimmers (Lavin, Guenette, Smoliga, & Zavorsky, 2015) . They found that after four weeks of CFB training, novice swimmers were able to improve their maximum static expiratory pressure which, along with maximum inspiratory pressure, can be used as a marker for improved respiratory strength. The CFB group also showed significant decreases in a 150 yard time trial as a test of performance post training. However, the results for diffusing capacity showed no statistically significant difference after the intervention (Lavin et al., 2015). Other studies demonstrate that diffusing capacity remains unaltered in adults after a training period at sea level or in a hypoxic environment (Dempsey et al., 1977; Reuschlein, Reddan, Burpee, Gee, & Rankin, 1968). Conversely, other longitudinal studies do show a small improvement in pulmonary diffusing capacity after a training program (Flaherty, Smoliga, & Zavorsky, 2014; Hanson, 1969; Kaufmann & Swenson, 1981). Thus, there is controversy on whether diffusing capacity can be altered in an adult population with strenuous exercise training.

The data collected from this study may provide evidence that CFB protocols, which stimulate increased effort and a build-up of carbon dioxide (CO_2) levels in the blood (Woorons, Gamelin, Lamberto, Pichon, & Richalet, 2014), termed hypercapnia, may be a viable mechanism for improving pulmonary diffusing capacity in elite level athletes. It was hypothesized that CFB would increase the training stimulus, due to the greater exertion during exercise, which would lead to an increase in aerobic capacity,

ultimately resulting in an increase in DLNO. The results of this study may alter the methodology of collegiate training programs and it may produce scientific evidence that diffusing capacity is in fact subject to improvement following physical activity protocols that utilize CFB.

Purpose of the Study

The purpose of the current study was to investigate the effect of a four week controlled frequency breathing program on lung function, specifically, pulmonary diffusing capacity for nitric oxide (DLNO) in a group of National Collegiate Athletic Association (NCAA) Division I swimmers. We chose DLNO as the primary dependent variable as this is a relatively novel estimate of alveolar-capillary membrane function. Since resistance of NO transfer lies within the red cell and in the thickness of the alveolar-capillary membrane (C. Borland, Bottrill, Jones, Sparkes, & Vuylsteke, 2014), any improvement in DLNO may represent increased alveolar growth or increased permeability of the alveolar–capillary membrane (Flaherty et al., 2014). Furthermore, DLNO has not been measured in an elite adult swimming population, so establishing what is normal in NCAA swimmers adds to the scientific literature.

Research Question & Hypotheses

1. Does a controlled frequency breath holding training program improve DLNO in elite adult swimmers?

   Null Hypothesis: Controlled frequency breath holding will not alter DLNO.

Alternative Hypothesis: A CFB intervention will improve DLNO. More specifically, it is reasonable to expect that for every 1 ml/kg/min increase in aerobic capacity, DLNO will increase by ~3.7 ml/min/mmHg (Zavorsky et al., 2010). Thus, any improvement in aerobic capacity should improve DLNO.

Definition of Terms

Alveolar Membrane Diffusing Capacity for CO (DmCO): A measure of carbon monoxide (CO) transfer from alveolar blood to pulmonary tissue measured in ml of CO diffused through the alveolar-membrane per minute per mmHg of partial pressure (ml/min/mmHg). It can also be indexed to body surface area and is expressed as ml/min/mm Hg/m^2.

Alveolar Membrane Diffusing Capacity for NO (DmNO): A measure of nitric oxide (CO) transfer from alveolar blood to pulmonary tissue measured in ml of CO diffused through the alveolar-membrane per minute per mmHg of partial pressure (ml/min/mmHg). It is always greater than DLNO. It can also be indexed to body surface area and is expressed as ml/min/mm Hg/m^2.

Pulmonary Diffusing Capacity for Nitric Oxide (DLNO): A measure of alveolar-capillary membrane diffusion measured in ml of nitric oxide (NO) diffused into the blood per minute per mmHg of partial pressure (ml/min/mmHg). It can also be indexed to body surface area and is expressed as ml/min/mm Hg/m^2.

Pulmonary Diffusing Capacity for Carbon Monoxide (DLCO): A measure of total CO transfer from inspired gas to pulmonary capillary blood measured in ml of CO diffused into the blood per minute per mmHg of partial pressure (ml/min/mmHg). It can also be indexed to body surface area and is expressed as ml/min/mm Hg/m^2.

θCO: blood transfer conductance for carbon monoxide. It is the standard rate at which 1 ml of whole blood will take up CO in ml standard pressure and temperature dry (STPD) per minute per ml of mercury of partial pressure. The formula used to determine $1/\theta CO = 1.31+0.0041 \cdot P_AO_2 \cdot 14.6 \div [Hb]$ (Forster, 1987) where P_AO_2 is the partial pressure of oxygen in the alveoli (assumed to be 100 mmHg), and Hb is the hemoglobin concentration of the subject. For women, [Hb] was assumed to by 13.4 g/dl, for men it was assumed to be 14.6 g/dl (Macintyre et al., 2005).

θNO: blood transfer conductance for nitric oxide. It is the standard rate at which 1 ml of whole blood will take up NO in ml standard pressure and temperature dry (STPD) per minute per ml of mercury of partial pressure. It is assumed to be 4.5 ml/min/mmHg/ml (C. Borland et al., 2014; Carlsen & Comroe, 1958).

DLNO to DLCO ratio: It provides an alternative way of investigating the blood-gas barrier and alveolar-capillary exchange (Hughes & van der Lee, 2013). It is representative of the DmCO to Vc ratio (Hughes & van der Lee, 2013). That is, this ratio is reduced in extrapulmonary restriction and chronic heart failure, and increased in interstitial and pulmonary vascular disease and in heavy smokers (Hughes & van der Lee, 2013).

Pulmonary Capillary Blood Volume (Vc): The volume of blood available for gas exchange in the pulmonary capillaries (ml).

Forced Vital Capacity (FVC): The maximum volume of air in liters (L) that can be expired during a maximal expiration attempt over 6 seconds.

Forced Expiratory Volume in One Second (FEV_1): The volume of air expired during the first second of a FVC test measured in liters (L).

Forced Expiratory Volume/Forced Vital Capacity (FEV_1/FVC): Ratio of FEV_1 to FVC in one second expressed as a percentage.

Vital Capacity (VC): The change in volume between a maximum inspiration and maximum expiration expressed in liters at body temperature and pressure saturated (BTPS).

Tidal Volume (TV): The amount of air inspired and expired during a normal breath measured in ml.

Residual Volume (RV): The volume of air that remains in the lungs following a maximal expiration measured in liters (L).

Total Lung Capacity: The sum of VC and RV measured in liters (L).

Controlled Frequency Breath Holding (CFB) Training: A method of training where athletes are required to adhere to a strict number of breaths per unit of activity. In this

case, it is holding one's breath at TLC for 8-12 strokes before being allowed to take another breath again.

Delimitations

Delimitations can include the choice to not include a novice swimming control group. Lavin *et al.* (2015) already studied tri-athletes as a novice swimming group and therefore the results of that study exist as a control group for our purposes. Literature reviewed for the purpose of intervention prescription will not include studies wherein hypoxia was used. We will encourage the athletes to hold their breath at a high pulmonary volume (TLC) to induce hypercapnia rather hypoxia (Woorons et al., 2014).

Assumptions

We assume that all participants will accurately report to all testing sessions both in the lab and at the natatorium where research will occur. Additionally we assume that the subjects will be present for, at minimum, 12 of the 16 training sessions and accurately report to the investigators their number or breaths taken and rate of perceived exertion. Anonymity through the study will be insured to encourage honestly from all participants. It is also assumed that each athlete will give maximal efforts on all pulmonary function and volume tests.

LITERATURE REVIEW

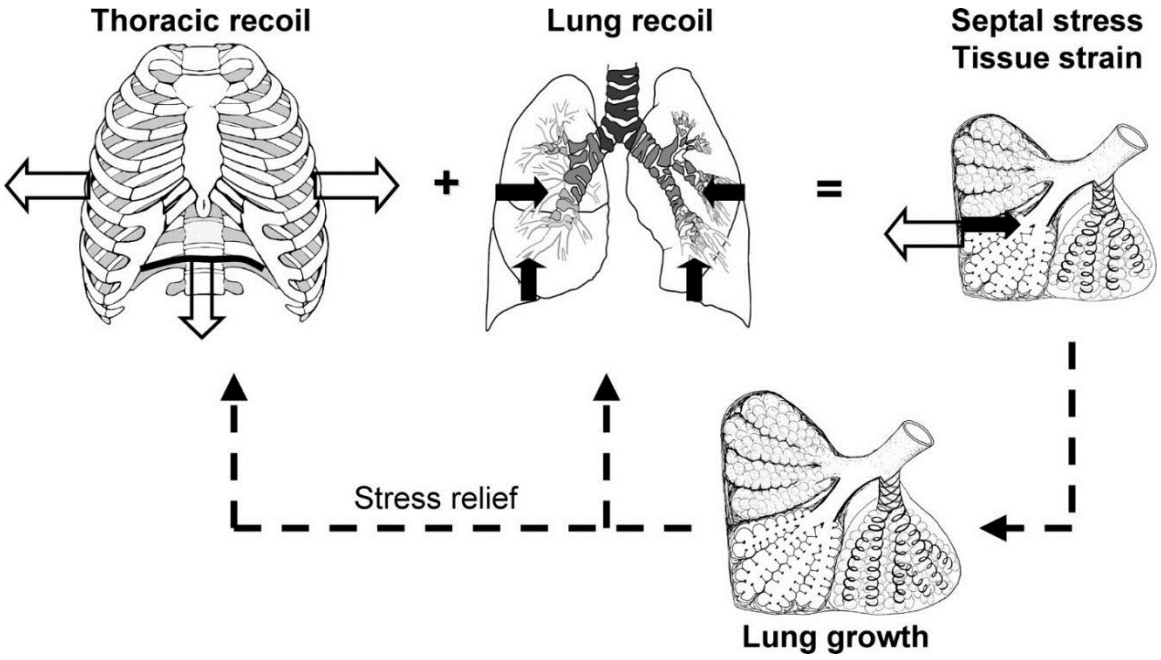
Lung Development

At its base level, the pulmonary system has two primary functions: Carbon dioxide removal and restoration of blood oxygen levels (Horsfield, 1980). These two functions are achieved via the net-like configuration of capillaries surrounding the pulmonary system. Inspired oxygen is transported to the alveolar walls where it is then diffused into the pulmonary capillaries and eventually bound to hemoglobin. Carbon dioxide follows the same process but in reverse with removal from the body occurring during expiration (Horsfield, 1980). Further transport of oxygen in the body is achieved via the integration of the lungs, blood, muscle, and heart (Wagner, 2005). "The principal O_2 transport functions undertaken by these four components are: ventilation and alveolar-capillary diffusion (in the lung), Hb binding, blood flow (in the circulation), and capillary-mitochondrial diffusion (in muscle)" (Wagner, 2005).

Development of the lungs occurs primarily during childhood and adolescence. A driving factor for this development is the expansion of the thoracic cage. As the thoracic cavity expands, mechanical stress is placed on the lungs resulting in tissue

stress. In an attempt to alleviate this stress, pulmonary adaptations occur via cellular growth mechanisms. The resulting increase in pulmonary tissue (lung size) reduces the stress incurred by the expanding thorax. **Figure 1** shows a potential mechanism for stimulation of lung growth via tissue stress. This process continues until cessation of thoracic growth occurs with the closing of the epiphyseal plates.

Figure 1: Proposed mechanism for lung growth via tissue strain



Mechanical interaction between the thorax and lung plays a major role in lung growth. During somatic maturation, recoil generated by enlarging thorax (open arrows) creates a negative intrathoracic pressure that opposes lung elastic recoil (solid black arrows). The resulting tissue stress and strain sustain cellular activities of lung growth; growth in turn relieves stress and strain in a feedback loop that continues until somatic maturity, when the bony epiphyses close. Thereafter, mechanical signals diminish, cellular growth ceases, and thoracopulmonary dimensions become fixed. (Hsia, 2004)

There is a promising body of evidence that points to increased expression of lung growth genes when pulmonary tissue is subjected to hyperinflation by means of a

lung resection or positive pressure ventilation (Wagner, 2005). This response is stronger during childhood than the response exhibited during adulthood (Landesberg, Ramalingam, Lee, Rosengart, & Crystal, 2001). However, it should be noted that in lung resection experimentation, growth could not be substantiated until 50% or more of the lung had been removed (Wagner, 2005). The observable growth occurs primarily at the alveolar level rather than the conducting or blood vessel level (Hsia et al., 2003).

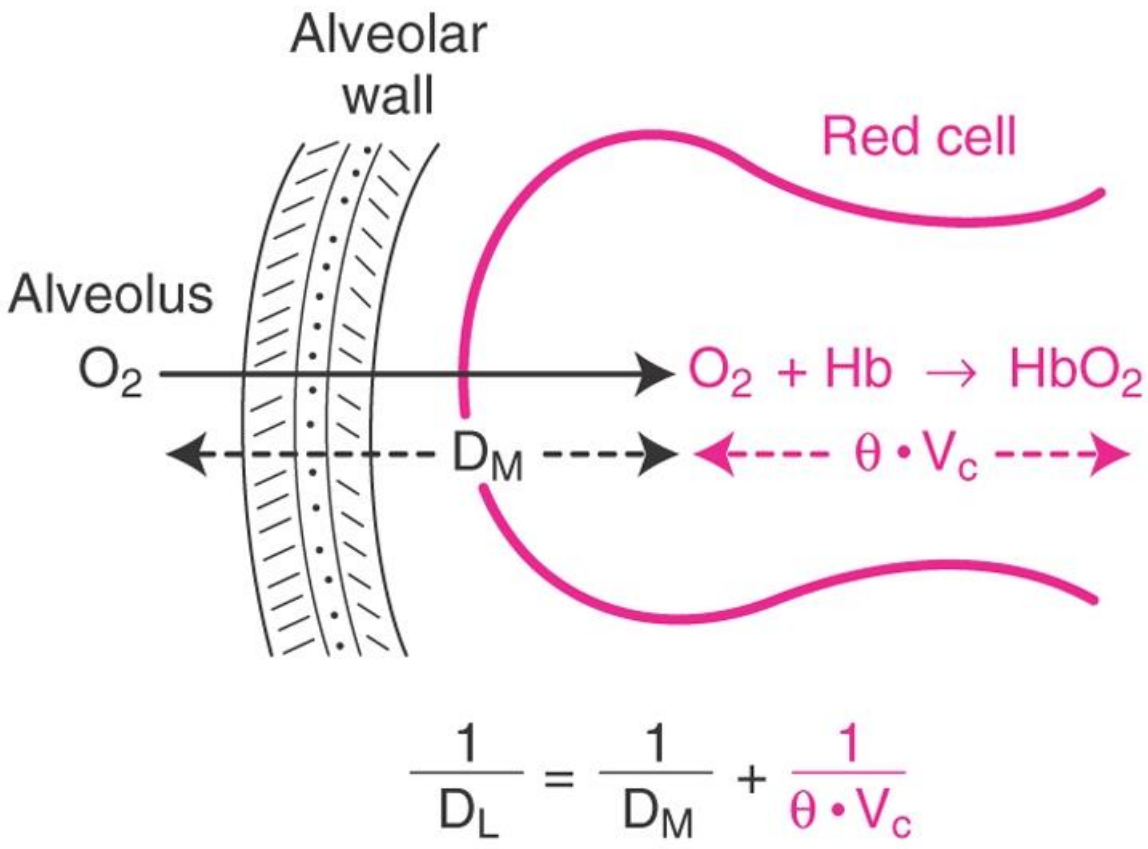
Pulmonary Diffusing Capacity

"The rate at which oxygen is taken up by erythrocytes in pulmonary capillaries is termed lung diffusing capacity, and is affected by several geometric and functional factors" (Roy & Secomb, 2014). At rest, the lung has a higher diffusion potential than is necessary to perform low intensity activity. However, for intense exercise, hypoxic, or diseased states diffusing capacity could be a limiting factor (Roy & Secomb, 2014). The rate of diffusion through tissues (pulmonary in this respect) can be defined by Fick's law which states that the rate of diffusion through a given tissue is proportional to the surface are and the difference in partial pressure between the two sides of the membrane for a given gas. Additionally, the rate of diffusion for a gas is inversely proportional to the thickness of the membrane the gas must pass through. "Pulmonary diffusing capacity [specifically] measures the transfer of a diffusion-limited gas (e.g. O_2, CO) across the alveolar capillary membrane and to the capillary blood" (Flaherty et al., 2014). There are inherent difficulties associated with measuring the diffusing

capacity for oxygen because it is subject to rapid changes in partial pressures as it crosses the capillary membrane. Due to this anomaly, carbon monoxide is used more frequently to determine an approximation for the movement of oxygen across the alveolar-capillary membrane (Flaherty et al., 2014).

Figure 2 is a representative of the diffusing capacity model and equation for the alveolar-capillary membrane. For the equation in **Figure 2,** the D_L value (diffusing capacity for the lung) is most commonly evaluated when measuring for diffusing capacity. However, in recent studies, data has shown that the D_M (alveolar-membrane component of the equation) correlates with DLNO. NO has been identified as a good indicator of D_M diffusion across the alveolar-capillary membrane (C. Borland et al., 2014) because it reacts rapidly with the hemoglobin in the pulmonary capillary. In fact, the affinity of NO for hemoglobin is about 1,500 times that of CO, chiefly due to the slow breakdown of iron nitrosyl hemoglobin (NOHb) (Gibson & Roughton, 1957).

Figure 2: Pulmonary Diffusing Capacity Model



The diffusing capacity of the lung (D_L) is made up of two components: that due to the diffusion process itself, and that attributable to the time taken for O_2 (or CO) to react with hemoglobin. From West JB: Respiratory Physiology: The Essentials. 9th Edition Baltimore: Williams & Wilkins, 2009, p. 32.

As previously stated, diffusing capacity is related to aerobic capacity in such a way that for each 1 mL/kg/min increase in VO_2 there is a corresponding increase in DLNO of 3.7 mL/min/mmHg (Zavorsky et al., 2010). Despite the increase in DLNO observed with increasing VO_2 values, there is a lack of evidence showing that any changes in

lung structure occur as a result of increased physical fitness (Wagner, 2005). Currently, swimming is the only training modality that has been shown to significantly alter DLCO (Zinman & Gaultier, 1987). However, it is theorized that many of the diffusing capacity adaptations associated with swimming are established around the onset of puberty (Zinman & Gaultier, 1987).

Pulmonary Function Testing

Spirometry

Forced vital capacity (FVC) and the forced expiratory volume over one second (FEV_1) are the two main components of spirometry. Both of these parameters are classified as direct measurement of lung volumes. In clinical settings, spirometry is used to identify signs of obstructive and restrictive airway diseases. For diagnostic purposes, the equation FEV_1/FVC allows for healthcare professionals to identify the differences between restrictive and obstructive airway disorders based on the relationship that exists between peak expiratory flow rate and mean forced expiratory flow during a FVC test (Miller et al., 2005).

Static Lung Volumes

The inspiratory and expiratory lung volumes obtained via spirometry are beneficial in identifying and classifying the severity of varying lung diseases (Wanger et al., 2005). Measuring absolute lung volumes such as residual volume (RV), functional residual capacity (FRC), and total lung capacity (TLC) is more difficult than

spirometry so the clinical applications of these measurements are limited (Wanger et al., 2005).

Diffusion Capacity for CO and NO

The diffusion rates of CO and NO (DLCO and DLNO) are being evaluated using the five second NO/CO method, where the subject simultaneously inhales ~40 to 60 ppm NO and 0.3% CO. The following evidence supports this method modified one-step method: DLCO has traditionally been defined by the Roughton and Forster equation (see **Figure 2**) so that DmCO is representative of alveolar-membrane diffusing capacity for CO, θ is the blood transfer conductance for CO, and Vc is volume of blood in the pulmonary capillaries (Roughton & Forster, 1957). Normally, membrane resistance (1/DmCO) and red blood cell resistance [1/(θCO·Vc)] play an equal role in the total resistance to diffusion across the lung (Hsia, Ramanathan, & Estrera, 1992).

The Roughton and Forster two-step method of measurement is considered to be antiquated because it is both uncomfortable (especially during exercise) and time consuming to complete. This is due in part to the fact that the testing procedures require DLCO be measured at two different points of oxygen partial pressure and the breath-hold is required to be about 10 seconds. In an attempt to find a more efficient method of testing, recent studies have found that DLNO and DLCO measurements allow for the interpolation of DmCO and Vc in a single 5-s breath-hold maneuver. This in turn reduces the amount of trials and time required of a subject during testing (C. D.

Borland & Higenbottam, 1989). This new method has been identified as the modified one-step Roughton and Forster Method. DLNO is relatively hemoglobin independent clinically (van der Lee, Zanen, Biesma, & van den Bosch, 2005) and, therefore, it closely reflects DmNO (alveolar–capillary membrane diffusing capacity for NO). As the diffusivity of NO is about twice that ofCO, then DLNO ≈ DmNO ≈ 2 × DmCO [see editorial by G. S. Zavorsky for a summary of the simultaneous measurement DLNO and DLCO; (Zavorsky, 2010)].

Being able to approximate DmCO and Vc from a one-step DLCO and DLNO measurement has many advantages when compared to the original two-step method. The first being that a single-step test records the DLNO and DLCO values at the same cardiac output. In contrast, the traditional two-step method measures DLCO at different oxygen tensions which can alter cardiac output. This is an issue because the results of the two trials are evaluated assuming one cardiac output value when in fact, there could be a discrepancy between the trials and DmCO and Vc could be misinterpreted (Phansalkar, Hanson, Shakir, Johnson, & Hsia, 2004). Second, the distribution of the CO gas throughout the lung may be different between two different inspirations, thus altering the DLCO between two tests misinterpreting DmCO and Vc. Third, the build-up of CO in the blood is greater with the original Roughton and Forster method as one needs to perform at least two tests to obtain DmCO and Vc, and the breath-hold time is longer compared to the modified technique (Zavorsky, 2013). A build-up of CO in the blood reduces oxygen carrying capacity especially when performing multiple measurements in a single session. Fourth, inspiring a small amount

of NO does not affect cardiac output, gas exchange, or DLCO (Sheel, Edwards, Hunte, & McKenzie, 2001; Tamhane, Johnson, & Hsia, 2001). As such, this modified one-step method is advantageous compared to the traditional Roughton and Forster technique.

Pulmonary Function in Swimming

When evaluating the stress placed on the respiratory system as a result of physical activity, swimming has often been studied due to the unique development of the lungs. In the early 90's multiple studies were conducted evaluating pulmonary function of swimmers. When compared against age and height matched runners and control groups, swimmers exhibit larger static lung volumes by ~15-20% (Cordain, Tucker, Moon, & Stager, 1990). Increased pulmonary diffusing capacity in swimmers has also been recorded at rest and at exercise (Cordain & Stager, 1988). Swimmers have further demonstrated higher PEF, FVC, FEV_1 against land based athletes and sedentary control groups (Doherty & Dimitriou, 1997). There is no known reason for these adaptations, but it is hypothesized that the unique tissue stress and hypoxic demands of the sport may play a role.

It has been suggested that five different factors of swimming that contribute to higher pulmonary function values; two of which are worth noting for the present study. The first being that submersion in the water may present a slight load on the inspiratory muscles due to transthoracic pressure across the lungs. (Cordain & Stager, 1988). This pressure taxes the respiratory muscles in a way that they are strengthened via

swimming. The end result would be greater force generation during inspiration and expiration. This in turn would elevate tissue stress and potentially promote lung growth (see **Figure 1**). Second, breathing in swimming is a rapid, forced maneuver due to limited opportunities to breathe within the context of arm strokes (Cordain & Stager, 1988). Minute ventilation is reduced at high swimming intensities, with respect to land based sports, favoring hypercapnia and enhanced oxygen extraction (Dempsey et al., 1977). With this and the benefits to the lungs with prone exercise, "larger than normal capillary to alveolar partial pressures of carbon dioxide and oxygen gradients may routinely be incurred" (Cordain & Stager, 1988). The restrictive breathing patterns inherent to swimming logically mandate that diffusing capacity be increased. Reducing the available supply of oxygen in pulmonary tissues should lead to an increased diffusion of gas across alveolar membranes.

Research Summary

Table 1 gives an overview of studies that have evaluated the effects of physical activity on pulmonary diffusing capacity. The most significant gains associated with DLCO were in studies that incorporated swimming in one form or another. Andrew and colleagues were able to show a 53% increase in DLCO for adolescents after three years of training when compared to controls. This falls in line with Zinman & Gaultier stating that the majority of adaptations to swimming occur during puberty. Furthermore, Flaherty *et al.* (2014) and Hanson *et al.* (1969) were able to improve DLCO via training modalities other than swimming. (**Table 1**).

Table 1: The effects of physical activity on DLCO and DLNO

Studies	Sample size and type of subjects	Intervention	Result	Percent Improvement
(Andrew et al., 1972)	12 boys and 12 girls ages 8-18 years with competitive swim experience	Initial testing with follow up after one year of competitive swim training. Subjects retested over the course of 3 year	Absolute exercise DLCO was significantly higher compared to control subjects. DLCO of 17.1 mL/min/mmHG	+53% higher exercise DLCO compared to controls
(Hanson, 1969)	10 male long distance runners and 5 male non-exercising control subjects	9 weeks physical training	Experimental group DLCO increased 3.7 mL/min/mmHg 9 weeks post-training (n=10) when measured at 3 mph (7% grade) Control group DLCO (n=5) also increased by 4.2 mL/min/mmHg	+9% DLCO experimental group +10% DLCO control group
(Reuschlein et al., 1968)	8 male university crew athletes and 8 male non-exercising university control subjetcs	5 months vigorous physical training	Resting DLCO decreased in both training and control groups 5 months after the baseline test (by 6 and 2 mL/min/mmHg in training and control groups).	0% DLCO
(Flaherty et al., 2014)	28 sedentary females randomly assigned to control and intervention	6 weeks of high intensity interval training. 3 sessions per week	Aerobic capacity and DLNO values increased for the HIT group but not control	+8% aerobic capacity +4% DLNO

Obviously, there are serious gaps in the literature when considering the effect of CFB intervention on DLCO. To date, the Lavin study is the only one of its kind that incorporated an intervention which modifies breathing patterns in swimming. Unfortunately, these results found no improvement in DLCO values for the experimental group (Lavin et al., 2015). As such, it is the purpose of this study to evaluate the effects of CFB training in elite college-level swimmers in hopes of

quantifying a relationship between the interventions and diffusing capacity. The lack of consequential evidence regarding this topic means that any findings will of great importance to researchers interested in potential mechanisms associated with improvements in pulmonary diffusing capacity.

METHODS

This study was conducted at the University of Louisville. Because of this, our subject selection was be comprised of readily available athletes on the University's swimming and diving team. Members of this team were considered as elite level athletes since they competed on a team that was 11th at the NCAA Division I Championships for the men and 15th for the women in 2014. These rankings placed each program within the top 10% for Division I eligible programs.

To be eligible for this study, a subject had to have competed for the University at some point during the 2013-2014 swim season. No time standards were set as requirements for entry into the study, i.e. USA Swimming national standards.

Settings

All lung function testing took place in Room 17A in Crawford Gym (Dr. Zavorsky's lab) while the swimming training was conducted at the University of Louisville's Ralph Wright Natatorium. During the swimming portion, pool water temperature was closely monitored to be kept between 78-80° F per competitive swimming guidelines set by USA Swimming, the national governing body for

swimming (Nelson & Nelson, 2010). Air temperature was also maintained to match pool temperature, 78-80° F.

Testing

Each subject was required to perform lung function testing on two different days: at baseline, and after the four week intervention. During baseline testing, age (y) and anthropometric data such as height (m), weight (kg), body mass index (BMI), body surface area (m^2), and percent body fat was recorded. The body fat percentage was measured via hydrostatic (underwater) weighing. Residual volume was approximated in the Siri and Brozek equations for hydrostatic weighing; body composition was recorded as the average of the two equations (Brozek, Grande, Anderson, & Keys, 1963; Siri, 1993).

This study was approved by the Institutional Review Board of the University of Louisville (#14.0103). Every subject signed an informed consent document detailing the responsibilities and risks associated with participating in the study. After an investigator explained the form and questions were answered, signatures and entry into the study were finalized.

Pulmonary function testing was conducted using a HypAir pulmonary function system (Medisoft, Dinant, Belgium) seated in a standard office chair. Spirometry was measured according to ATS/ERS standardization of spirometry guidelines (Miller et al., 2005). Forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1),

forced expiratory flow rate over the middle half of expiration (FEF_{25-75}), and peak expiratory flow rate (PEF) were measured as part of the spirometry battery. The subjects' values were compared against reference equations (Hankinson, Odencrantz, & Fedan, 1999). Pulmonary diffusing capacity for nitric oxide (DLNO) and carbon monoxide (DLCO) were also measured according to the methods described elsewhere (Zavorsky, Cao, & Murias, 2008), and subjects' values were also compared against reference equations (Zavorsky et al., 2008). Pulmonary capillary blood volume (Vc) was determined based on the following: Alveolar PO_2 (P_AO_2) = 100 mmHg (Zavorsky et al., 2008), the blood transfer conductance for NO (θNO) = 4.5 mL/min/mmHg/mL (C. Borland et al., 2014; Carlsen & Comroe, 1958), the blood transfer conductance for CO (θCO) = 0.584 mL/min/mmHg/mL when male hemoglobin concentration = 14.6 g/dL, and 0.537 mL/min/mmHg/mL when female hemoglobin concentration = 13.4 g/dL). This was estimated on the blood transfer conductance equation by Forster, 1987 (Forster, 1987): $1/\theta CO = (1.3 + 0.0041 \cdot P_AO_2) \cdot (14.6 \div \text{subject's Hb})$. Furthermore, the alveolar-membrane diffusing capacity for carbon monoxide (DmCO) was calculated as the alveolar membrane diffusing capacity for nitric oxide (DmNO) divided by 1.97. Thus, DLNO < DmNO (C. Borland et al., 2014; C. D. Borland et al., 2010; Zavorsky, 2010). The ratio of DLNO to DLCO was assumed to be an adequate surrogate for the DmCO to Vc ratio (Hughes & van der Lee, 2013).

Baseline and post-testing of the swimmers aerobic capacity was performed after the completion of all pulmonary function tests. This data collection was simultaneously performed in conjunction with another study examining running economy of swimmers. The Human Performance Laboratory in Crawford Gym was where aerobic capacity was

assessed. First, three, 5-minute submaximal running stages were performed. Running at all speeds was conducted on 0% incline. Submaximal stage one was conducted at 6 mph and 5.5 mph for male and female subjects, respectively. Submaximal stage two was conducted at 7mph and 6.5mph for male and female subjects, respectively. The third submaximal stage was conducted at 8mph male subjects, 7.5mph for female subjects. Between the first two submaximal stages, and between the second and third submaximal stage, a passive rest period of five to seven minutes was permitted, with all subjects beginning the next stage in no fewer than five, and no more than 6.5 minutes. Participants did not perform any active recovery or physical activity during these inter-stage recovery periods (Sims, 2014).

At the end of the third submaximal stage, subjects did not participate in a passive recovery period, but rather proceeded on a graded exercise protocol up to maximum volitional fatigue. After the five minutes at the third submaximal stage, the graded exercise progressed every two minutes with a 1.0 mph increase until maximal fatigue was achieved. Aerobic capacity was defined as the highest averaged minute for oxygen consumption. All tests were conducted on the Woodway ELG treadmill (Woodway USA, Waukesha, WI). Metabolic testing was conducted using the PARVO Medics TrueOne 2400 metabolic cart (PARVO Medics, Sandy, UT).

Furthermore, 200-yard freestyle swim tests were performed in conjunction with another study (Sims, 2014). These swimmers completed a 200m freestyle swim time trial at maximal volitional effort at baseline and post-training in order to investigate correlation between running economy and swimming performance as well as to investigate performance improvements (Sims, 2014).

Training Intervention

Each of the 16 sessions lasted approximately thirty-five minutes; each subject was responsible for completing a standardized 1000-m warm up of easy, mixed swimming. The training intervention consisted of 12 reps of a 50-m swim on a one minute interval for the first week. Weeks two and three decreased the interval by five seconds to :55 per rep. An additional five second decrease during the final week of training set the intervals at :50 per rep (see **Table 2**).

Table 2: Training intervention

Training Progression	Group Instructions
Week 1: 12x50m Front Crawl @ 1:00 per 50m Weeks 2, 3: 12x50m Front Crawl @ :55 per 50m Week 4: 12x50m Front Crawl @ :50 per 50m	Experimental: Limit breathing to 2-3 breaths per 50m Ideally, 24-30 breaths per workout Control: Breath every 2-3 strokes per 50m Therefore, 105-120 breaths per workout

Only breaths taken while swimming were countable breaths during data collection. The controlled frequency breathing group was encouraged to limit their breathing to two breaths per lap resulting in about 24 breaths per workout. The control group was instructed to breathe on a stroke-matched basis, breathing every 2-3 strokes accumulating 10-12 breaths per lap. At the end of each workout, the subjects self-reported their number of breaths taken during the working along with a rating of

perceived exertion (RPE) based upon the 6-20 Borg scale (Borg, 1982). Training sessions were supervised by at least one member of the University of Louisville swimming coaching staff.

Research Design

The research design implemented for this study was a pre-post test design with control group. This was a quasi-experimental design in which a convenient sample of elite college swimmers was used. To examine changes in diffusing capacity, a 2 x 2 repeated measures analysis of variance was used. The independent variable was the training program [Experimental Group = CFB training group; Control group = stroke matched (SM) group] and the number of measurements per variable (two measurements per variable: baseline, and post-testing). The Lee notation was represented as: $S_{12}\cdot(G_2)\cdot T_2$ in which subjects were nested within group (2 groups, CFB, SM) and crossed with time (familiarization, baseline, post-testing).

Statistical Analyses

Sample size calculation was estimated from the mean overall changes for aerobic capacity with interval training of 8% (Burgomaster et al., 2008) . For every 1 mL/kg/min improvement in aerobic capacity, DLNO is increased by ~4 ml/kg/min (Zavorsky et al., 2010). Thus, with an improvement of 8% in aerobic capacity, DLNO should be increased by 11 ml/min/mmHg. Using online statistical software (G*Power Version 3.1.7, Universität Kiel, Germany), the following was calculated for the within-between interaction for repeated measures ANOVA: statistical power was set at 80%, type I error rate at 5% ($\alpha = 0.05$), correlation among repeated measures = 0.70, and

effect size $f = 0.25$. A total of 22 subjects was estimated. Twenty six subjects were recruited into the study to allow for an approximate 10% attrition rate.

The data was analyzed with the SPSS statistical software package (SPSS Version 21.0, IBM SPSS Statistics Inc., Chicago, IL). Statistical significance was be declared when $p < 0.05$ unless otherwise noted.

Data Management and Storage

All data for pulmonary function testing was recorded digitally within the password protected hard drive associated with the Hyp'Air pulmonary function system. All data pertaining to the study was kept within a locked room in a locked filing cabinet with access granted only to the investigators managing the study.

RESULTS

Twenty-five subjects were recruited for participation during this study, eleven women and fourteen men. Subjects were randomly placed into either control (n=12) or experimental (n=13). During the course of the study, seven subjects were lost due to attrition. Therefore, eighteen subjects were retained through the end of the study. Nine of these were experimental group (five men and five women) and nine in control (five men and four women). All subjects completed pre and post intervention data collection. The subjects' baseline anthropometric data at baseline is described below. All data was normally distributed except for age. There were no differences between groups for any of the parameters listed in Table 3.

Table 3: Subjects at baseline

Variables	Control, SM (n = 12)	Experimental, CFB (n = 13)	*p* -value	Combined Mean (n = 25)
Age (yrs)	19 (1) [18 to 22]	20 (1) [19 to 22]	0.13	20 (1) [18 to 22]
Weight (kg)	78.3 (10.3) [63.0 to 93.9]	76.8 (10.5) [56.8 to 89.8]	0.71	77.6 (10.2) [56.8 to 93.9]
Height (cm)	176 (8) [162 to 189]	178 (11) [156 to 191]	0.64	177 (9) [156 to 191]
BMI (kg/m^2)	23.4 (1.4) [21.4 to 25.9]	22.8 (1.8) [20.2 to 26.5]	0.33	23.1 (1.6) [20.2 to 26.5]
Body fat percentage	17 (6) [9 to 26]	15 (3) [9.8 to 22.3]	0.51	16 (5) [9 to 26]
Wing span (cm)	183 (11) [165 to 199]	184 (13) [158 to 199]	0.88	183 (12) [158 to 199]
Wing span divided by height (%)	104 (2) [98 to 106]	103 (2) [100 to 108]	0.53	104 (2) [98 to 108]

Mean (SD), [Range]

Pulmonary Function

Baseline and follow-up testing both occurred within one week of the intervention. There were 35 (5) days between baseline and follow-up testing. Spirometry measures were recorded in addition to diffusion capacity parameters for both testing sessions. At baseline, spirometry was evaluated by sex rather than group to determine significance of predicted values compared to recorded values. In all parameters, with the exception of PEF (L/min) for males, recorded values were statistically significant ($p < 0.05$) in difference from age predicted values (Table 4).

Table 4: Baseline spirometry

Variables	Female n=11	Female %Pred.	Male n=14	Male %Pred.	Combined n=25	Combined %Pred.
FEV_1 (L)	4.3 (0.6) [3.8-5.6]	121% (10%)* [104%-140%]	5.4 (0.4) [4.7-6.0]	110% (7%)* [97%-123%]	4.9 (0.7) [3.8-6.0]	115% (10%)* [97%-140%]
FVC (L)	5.4 (0.6) [4.7-6.5]	131% (14%)* [112%-163%]	7.18 (0.48) [6.55-8.18]	121% (8%)* [112%-139%]	6.4 (1.1) [4.7-8.2]	125% (12%)* [112%-163%]
FEV_1/FVC	0.80 (0.06) [0.69-0.87]	93% (7%)* [80%-101%]	0.76 (0.05) [0.69-0.86]	90% (5%)* [82%-102%]	0.78 (0.06) [.069-0.87]	91% (6%)* [80%-102%]
PEF (L/min)	7.9 (1.2) [5.7-9.0]	110% (16%)* [77%-131%]	10.1 (0.9) [8.2-11.4]	96% (10%) [73%-113%]	9.1 (1.5) [5.7-11.4]	102% (15%) [73%-131%]
FEF 25-75	4.7 (0.9) [3.7-6.6]	121% (20%)* [94%-159%]	5.6 (0.7) [4.5-7.1]	109% (14%)* [90%-139%]	5.2 (0.9) [3.7-7.1]	114% (18%)* [90%-159%]

Mean (SD), [Range] *statistically significant within each sex

In contrast to spirometry, baseline diffusing capacity was evaluated using group to group comparison. There were no differences between the groups at baseline for any of the chosen parameters (Table 5). However, it is important to note that for DLCO 10 subjects (9 males, or 64% of males) were above the upper limits of normal for predicted values. In addition, 11 subjects (8 males, or 57% of the males) were above the upper limits of normal for recorded DLNO values. The Zavorsky et al. reference equations were used to determine predicted values which were then compared to recorded values (Zavorsky et al., 2008). Furthermore the observed values were compared against additional reference equations from Europe to insure validity (Aguilaniu, Maitre, Glenet, Gegout-Petit, & Guenard, 2008). There were no significant differences in percent predicted values for DLCO and DLNO between the two reference equations.

Table 5: Baseline diffusing capacity

Variables	**Control** (n=12)	**% Pred.**	**Exp.** (n=13)	**% Pred.**	**Combined** (n=25)
DLCO[1]	41.8 (9.4) [27.0-54.4]	119 (14)* [92-140]	42.9 (8.6) [28.6-54.2]	121 (12)* [100-139]	42.3 (8.9) [27-54.2]
DLCO/VA	5.1 (0.6) [4.1-6.0]		5.0 (0.3) [4.3-5.5]		5.09 (0.47) [4.10-6.01]
DLCO/BSA	21.3 (3.4) [15.8-25.8]		22.0 (2.9) [16.5-26.7]		21.60 (3.13) [15.8-26.7]
DLNO[2]	207 (40) [152-262]	115 (11)* [90-127]	211 (42) [133-273]	116 (12)* [91-136]	209 (40) [133-273]
DLNO/VA	25.4 (2.2) [20.6-28.7]		24.9 (2.2) [22.1-29.3]		25.19 (2.15) [20.6-29.3]
DLNO/BSA	105.4 (13.3) [86.9-123.6]		108.2 (14.4) [76.9-130.6]		106.8 (13.6) [76.9-130.6]
DLNO/DLCO	5.0 (0.4) [4.3-5.8]		4.9 (0.2) [4.5-5.4]		5.0 (0.3) [4.27-5.53]
Vc	99 (19) [58-122]		95 (16) [71-113]		94 (17) [58-122]
DmCO	211 (49) [133-306]		213 (52) [115-297]		212 (49) [115-306]
DmCO/Vc	2.3 (0.5) [1.5-3.3]		2.2 (0.3) [1.6-2.6]		2.30 (0.40) [1.5-3.3]
DmNO	416 (96) [263-603]		421 (102) [227-585]		419 (97) [227-603]

Mean (SD), [Range]

1. One female (9%) and nine males (64%) were above the ULN for predicted values.
2. Three females (27%) and eight males (57%) were above the ULN for predicted values.

Baseline testing and follow-up both occurred within one week of the intervention beginning and ending, respectively. The average amount of days between baseline testing and follow-up was 38 (8). Each subject completed at least the minimum of twelve training sessions with a group average at 14 (2) sessions. The number of

breaths taken during the intervention period was not normally distributed so a Mann-Whitney U test was run to assess statistical differences. RPE was normally distributed. There was an overall difference between groups for both RPE and the number of breaths taken in total per workout (Table 6). There were no differences ($p > 0.05$) for in spirometry values following the intervention.

Table 6: Intervention data

		Weekly Interval Progression					
Group		1:00	:55	:55	:50	Average	*p*-value
Experimental	Breaths	24 (2)	24 (2)	25 (1)	27 (6)	25 (3)	<0.001
	RPE	14 (1)	15 (1)	15 (1)	17 (1)	15 (1)	
Control	Breaths	113 (13)	111 (9)	111 (6)	114 (9)	112 (9)	<0.001
	RPE	10 (1)	11 (1)	10 (1)	12 (2)	11 (1)	

Mean (SD)

After data collection was complete, a correlation matrix was performed to determine if there was any relationship between diffusion capacity parameters and 200-yard freestyle swim time performance. Out of seven predictors (sex where 0 is female and 1 is male, DLCO, DLNO, FVC, height, MIP, MEP), we chose the highest three correlations to swim times (FVC, r = -0.86; sex, r = -0.84; DLCO, r = -0.78). A stepwise multiple linear regression was run. It was found that only FVC was the best predictor of swim time. The equation is as follows:

Swim time (sec) = 150.6 – 5.66*(FVC)

[n=25, Standard error of the estimate = 3.6 seconds, Adjusted R^2=0.73, F (1,23) = 65.5, $p < 0.001$].

For every 100 mL improvement in FVC, swim times improve (decrease) by ~0.6 s

Table 7 details the effects of the intervention on all diffusion capacity parameters. The data showed there to be no significant difference between groups as a result of control frequency breath holding. It is also important to note that for certain parameters (DmCO, DmCO to Vc ratio, DLNO to DLCO ratio) the control group experienced larger positive change than the intervention subjects (**Table 7**).

Table 7: Pulmonary diffusing capacity and its components pre and post intervention

Variables	Control Pre	Control Post	Change	Exp Pre	Exp Post	Change	*p*-value
DLCO	42.7 (9.3)	41.5 (9.5)	-1.2 (3.7) [-4.0, 1.6]	43.4 (8.9)	44.9 (12.0)	1.5 (4.4) [-1.9, 4.9]	0.18
DLNO	214 (41)	216 (46)	2 (15) [-9, 15]	213 (44)	222 (62)	9 (25) [-10, 28]	0.53
DLCO/VA	5.2 (0.5)	5.1 (0.5)	-0.1 (0.4) [-0.4, 0.2]	5.2 (0.3)	5.4 (0.5)	0.2 (0.4) [-0.1, 0.5]	0.12
DLCO/BSA	21.6 (3.5)	20.9 (3.6)	-0.6 (1.8) [-2.0, 0.8]	22.1 (3.3)	22.8 (4.8)	0.7 (2.2) [-1.0, -2.4]	0.18
DLNO/VA	26.2(1.4)	26.9 (2.6)	0.7 (1.8) [-0.7, 2.1]	25.5 (2.2)	26.5 (2.2)	1.0 (1.9) [-0.4, 2.4]	0.71
DLNO/BSA	108 (14)	109 (17)	1 (8) [-5, 7]	109 (16)	113 (25)	4 (13) [-6, 14]	0.58
VA	8.1 (1.3)	8.0 (1.4)	-0.1 (0.5) [-0.5, 0.3]	8.4 (1.7)	8.3 (2.0)	-0.1 (0.4) [-0.4, 0.2]	0.86
DmCO	223 (51)	240 (58)	17 (28) [-4, 39]	215 (56)	228 (81)	13 (37) [-16, 42]	0.78
DmCO/Vc	2.4 (0.5)	2.7 (0.2)	0.3 (0.5) [-1.0, 0.7]	2.2 (0.4)	2.3 (0.4)	0.0 (0.4) [-0.3, 0.3]	0.26
DLNO to DLCO ratio	5.1 (0.4)	5.2 (0.2)	0.2 (0.3) [-0.1, 0.4]	4.9 (0.2)	4.9 (0.2)	0.0 (0.3) [-0.2, 0.2]	0.22

Exp = Experimental group; DLCO, DLNO, DmCO (ml/min/mmHg); DLCO/BSA, DLNO/BSA (ml/min/mmHg/m^2); VA (ml)

DISCUSSION

The respiratory system has been shown to be a limiting factor to exercise performance in elite endurance athletes (Dempsey, Hanson, & Henderson, 1984). The goal of this study was to examine the effects of a controlled frequency breath holding training program on pulmonary diffusion capacity, specifically DLNO, in an elite population. It was hypothesized that CFB would increase the training stimulus, due to the greater exertion during exercise, which would lead to an increase in aerobic capacity, ultimately resulting in an increase in DLNO. For every 1 ml/kg/min increase in aerobic capacity, we expected DLNO would increase by approximately 3.7 ml/min/mmHg (Zavorsky et al., 2010). However, it was found that a four week intervention in collegiate swimmers left diffusing capacity parameters unchanged, because aerobic capacity was unaltered. These findings were interesting because studies have shown that an aerobic training program can improve an individual's pulmonary function with regard to diffusion capacity (**Table 1**). So, if diffusion capacity has been shown to be a malleable parameter, why was the intervention unsuccessful in altering performance? It could be the fact that more than half of the males and some females were above the upper limits of normal for both DLCO and DLNO at the start of the study (**Table 5**), thus it would be difficult to improve diffusing capacity in swimmers that are already above the 95th percentile for their age and sex.

Hence, if diffusing capacity cannot be improved in this cohort, then aerobic capacity is unlikely to improve, and if aerobic capacity is unlikely to improve, then swimming performance should be unaffected.

Pulmonary Development

As stated previously, development of the lungs occurs primarily during childhood and adolescence. A driving force for this development is the expansion of the thoracic cage. Once the epiphyseal plates have closed the maximum range of motion for the thoracic cavity is constant. This poses a potential problem for pulmonary growth because it limits possible tissue overload to a finite value. In strength training, if an individual wishes to increase the size or strength of a muscle it is feasible to continually progress the applied resistance in order to overload the muscle fibers and promote cellular growth. This process, however, is unavailable to the pulmonary system due to a constant range of motion and a predetermined volume of air. Furthermore, if you apply this model to elite aerobic athletes, the problem becomes more complex.

Pulmonary Adaptations in Swimming

When evaluating the stress placed on the respiratory system as a result of physical activity, swimming has often been studied due to the unique development of the lungs. In the early 90's multiple studies were conducted evaluating pulmonary function of swimmers. When compared against age and height matched runners and control groups, swimmers exhibit larger static lung volumes by ~15-20% (Cordain et al., 1990). The data collected for spirometry (see **Table 4**) shows that the subjects were

far above predicted values for FVC values. If you consider FVC to be constant once an individual reaches physical maturity, it is reasonable to assume that participation in swimming during adolescent development could lead to larger than normal static lung volumes which in turn can contribute to improve pulmonary function as an adult.

Performance Implications

Despite the lack of improvement in diffusion capacity following the intervention, valuable data was collected with regards to elite level swimming. The astronomically high pulmonary function values recorded in the subjects show that even within this small sample size, the pulmonary function trend for elite swimmers is that of far above average values being "normal." It was also found that swim performance can be predicted using height ($r = -0.62$), FVC ($r = -0.86$), DLCO ($r = -0.78$), DLNO ($r = -0.73$), and sex ($r = -0.84$). Nevertheless the multiple linear regression analyses demonstrated that FVC was the only significant predictor of swim times due to the fact that all the other predictors can be accounted for this one parameter. The question remains, do swimmers have high pulmonary function because of the unique characteristics of swim training during puberty and adolescence, or rather, are individuals with outstanding lung function drawn to the sport of swimming?

Study Limitations

One of the major limitations was the timeline of the study. The protocol required the subjects to participate in a four week intervention period. Considering the "elite" status of these athletes the ability to improve performance metrics in such a short time was unlikely. This study was intended to reflect the potential benefits of CFB training in

elite swimmers and therefore, results were population specific to the sport of swimming. It was expected that elite level swimmers would have high DLNO values due to physiological adaptations acquired via swim training during puberty (Flaherty et al., 2014). This could have presented a ceiling effect where the high diffusion values inherent to the athletes would limit the potential for gain, as a result of the CFB intervention. Another limitation was the small sample size. It was decided to include men and women in this intervention due to the limited amount of available subjects. Even though the study began with 25 subject attrition dropped the sample size to 18 subjects by the end of the study. Since any anticipated improvements were expected to be small in nature, having a reduced number of subjects could have contributed to less meaningful data. Furthermore, the use of RPE was a limiting factor due to its subjective nature. Utilizing heart rate monitors would have given us a more accurate representation of the difference, if any, between groups with regards to intensity. Unfortunately, due to the unique interactions that occur with the water during swimming, keeping a heart rate monitor on for the duration of a workout is not possible. An additional limitation of note was the absence of hemoglobin measurements, which affects diffusing capacity. Thus, any changes in hemoglobin values throughout the study could have precluded significant differences. However, mild changes in hemoglobin concentration (from 10 to 15 g/dL) does not affect diffusing capacity (Zavorsky, 2013).

Another limitation would be the decision to omit post hoc statistical power in the results. However, there are several shortcomings of reporting post hoc statistical power when reporting results that are not statistically significant (Hoenig & Heisey,

2001). "Because of the one-to-one relationship between *p* values and observed power, non-significant p values always correspond to low observed powers. Computing the observed power after observing the *p* value should cause nothing to change about our interpretation of the p value." (Hoenig & Heisey, 2001). Once the data is analyzed confidence intervals replace post hoc statistical power when describing results (Wilkinson, 1999).

Conclusion

Pulmonary diffusion capacity is unaltered after a controlled frequency breath holding intervention in elite Division I NCAA swimmers. It was found that in a small sample size (n=25) baseline spirometry and diffusion capacity measurements show swimmers to have high lung volumes and diffusing capacities when compared to normative values. Furthermore, pulmonary adaptations are relatively immutable in elite athletes during a four week intervention. It was found that the best overall predictor of swim performance was FVC.

Future Research

It would be beneficial to conduct a study evaluating the effect of a longer intervention period. Increasing the duration of the study would allow the controlled frequency breathing protocol more time to affect diffusing capacity. Unfortunately, in the sport of swimming training regimens are very specific and coaches have a hard time accepting changes to their programs. Because of this, it would not be feasible to expect a cohort of swimmers to participate in a season long intervention. In lieu of working with elite swimmers, a longitudinal study tracking pulmonary development in

swimmers through adolescence would help determine if the adaptations observed at the elite level are preexisting or acquired.

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