VALIDATION OF NON-INVASIVE CO_2 TOLERANCE FIELD TEST

by

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ABSTRACT

INTRODUCTION: Breathwork, or the specific act of controlling breathing to elicit a desired outcome, is not only a potential way to improve one's mental well-being but also to alter adaptative responses through gas exchange at the capillary level. Increasing tolerance to carbon dioxide (CO_2) has been positively correlated with improvement in athletic performance and reduction of stress and anxiety. However, there does not exist a validated non-invasive test to accurately measure one's tolerance to CO_2. **PURPOSE:** The purpose of this study was to validate the only currently known CO_2 tolerance (CO_2T) field test against measured maximal dissolved CO_2 and maximal ventilatory carbon dioxide ($\dot{V}CO_{2max}$) in endurance athletes. **HYPOTHESIS:** It was hypothesized that the novel CO_2T field test would have a strong positive correlation to clinical CO_2 measurements, there would be no observable differences in comparative values between the CO_2T field test and clinical CO_2 measurements between sexes, and there would be a negative correlation between anaerobic capacity and CO_2T. **METHODS:** Twenty-eight (n=28, 19 males, 9 females) participants reported to the Human Performance Laboratory on two separate occasions, separated by 48 hours, for testing. Peak CO_2 levels were generated via repeated 30-second Wingate anaerobic power intervals with two minutes of rest in-between. Participants continued the Wingate tests until a plateau of peak power was achieved or volitional fatigue. CO_2 data were recorded at the end of each interval. Day one consisted of a study orientation, informed

consent, anthropometric measurements, CO_2T field test, and ventilatory CO_2 measurements during the repeated Wingate tests. Day two consisted of a second CO_2T field test, end-tidal CO_2 ($EtCO_2$), and lactate (BL) measurements during the repeated Wingate tests. **STATISTICS**: Repeated measures ANOVA and paired t-test were used to determine if differences existed between CO_2T times, $EtCO_2$, anaerobic capacity (AC), and blood BL values. Pearson correlation coefficient analysis was used to determine if there was a relationship between CO_2T times and all primary research variables. **RESULTS**: The CO_2T field test showed to have a very high degree of intra-rater reliability but did not show any validity to CO_2 measurements. Statistical analysis showed a high positive correlation between CO_2T tests one and two ($r = 0.989$, $p < 0.01$) and no significant difference so an average score was used for analysis. A moderate positive correlation to HRpeak for CO_2Tavg ($r = 0.38$, $p < 0.05$), and an indirect correlation to $EtCO_2(1)$ ($r = -0.342$, $p < 0.05$). Correlations between CO_2T tests and all other variables did not reach statistical significance. **CONCLUSION:** While reliable, the novel CO_2T field test does not seem to be a valid measurement of physiological levels of CO_2. The test was shown to have a high degree of intra-rater reliability and could be used to show trends over time but does not give an accurate portrayal of actual CO_2 production, accumulation, and tolerance.

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LIST OF ABBREVIATIONS

CO_2	Carbon Dioxide
HRpeak	Peak Heart Rate
$\dot{V}O_{2max}$	Maximum Oxygen Consumption
$EtCO_2$	End Tidal Carbon Dioxide
HR	Heart Rate
VE	Ventilation
CO_2T	Carbon Dioxide Tolerance
$\dot{V}CO_{2max}$	Maximal Carbon Dioxide Exhalation
O_2	Oxygen
VBG	Venous Blood Gas
ABG	Arterial Blood Gas
BL	Blood Lactate
AC	Anaerobic Capacity
BLrel	Relative Blood Lactate
BLabs	Absolute Blood Lactate
$EtCO_2rel$	Relative End-tidal Carbon Dioxide
$EtCO_2abs$	Absolute End-tidal Carbon Dioxide
CO_2Tavg	Average Carbon Dioxide Tolerance

CHAPTER ONE: INTRODUCTION

Production, Transportation, and Elimination of Carbon Dioxide

Cellular respiration consists of a cascade of metabolic processes in which cells use and convert glycogen, a stored form of the simple sugar glucose, into energy in the form of adenosine triphosphate (ATP). These processes may or may not require oxygen (O_2) and results in the use of ATP and generation of metabolic byproducts that must be expelled. Carbon dioxide (CO_2) is one of the major byproducts of cellular respiration. Carbon dioxide is transported in the blood by being dissolved in solution (reflected by the partial pressure of CO_2), as carbonic acid, in the form of bicarbonate, or bound to hemoglobin.[1] As energy output increases, the demand for ATP, glycogen, and oxygen increases. As more energy is used, more CO_2 is produced that must be offloaded. Responses to the increased physiological need for O_2 and increased production of CO_2 are met, in part, by increasing heart rate and ventilatory rate.[1] The increase in ventilation serves dual purposes of taking in more O_2 and offloading more CO_2.

Under normal atmospheric conditions, O_2 availability is not an issue in healthy adults. Thus, the main reason for increased ventilation is to offload the rising levels of CO_2 as elevated CO_2 levels increase perceptions of fatigue, exhaustion, and air starvation resulting in increased anxiety and decreased performance[2–4]; increasing ventilation, the metabolic cost of breathing, and the metabolic cost of physical activity[2]. Being able to tolerate higher levels of CO_2 will then, in turn, reduce the need for increased ventilation rates as exercise intensity increases resulting in improved efficiency.[5–7] This adaptation

can also be beneficial during extended breath-hold freediving as rising levels of CO_2 during the breath hold triggers an associated chemoreflex requiring cessation of the breath-hold and initiation of ventilation.[8,85]

Measurement of Carbon Dioxide

Much like training to increase lactate threshold in power and strength athletes, as well as aerobic training to increase maximal oxygen consumption ($\dot{V}O_2max$) in endurance athletes, training to increase CO_2T is gaining popularity in the freediving community.[7,9] Previous research has shown improving CO_2T to be beneficial for lowering blood acidosis, reducing oxidative stress, and improving state anxiety.[3,10–12] However, although studies have shown that CO_2T can be improved, there is no 'gold standard' test to assess an individual's actual CO_2T.

Carbon dioxide levels are measured in a variety of ways. The most common laboratory test for measuring dissolved CO_2 levels in blood (partial pressure (PCO_2)) evaluates the change in pH in venous or arterial blood, commonly known as venous blood gas (VBG) or arterial blood gas (ABG) analysis. As opposed to blood PCO_2, capnography is the measurement of exhaled gas PCO_2 and is conducted with a capnometer or standard metabolic cart.[13] Both of these testing methods have a high level of validity and reliability [14,15,16]; however, they are expensive, require a trained professional to administer the test, require specialized equipment, and are only found in laboratory or medical facilities.

Almost all 'gold standard' exercise tests traditionally occur in a controlled lab setting via expensive equipment and a trained professional. These tests include but are not limited to, testing $\dot{V}O_2max$, anaerobic capacity (AC), and body composition. To

make testing more manageable, affordable, and easier, all of these 'gold standard' tests have an equivalent validated field test possessing a high degree of reliability.[17–19] The need to validate simple field tests is paramount to training when access to a laboratory and/or equipment is not available, trained technicians are not present, or there is no surplus of money.

The only known field test used to evaluate an individual's tolerance level to CO_2 is a rudimentary never validated, single-breath exhale for time[25]. Done over two trials separated by two minutes of rest, individuals inhale to vital capacity and then immediately exhale in a slow and controlled manner for as long as possible. It is theorized that this method not only assesses pulmonary function but also one's ability to tolerate higher levels of CO_2 in that during extended exhalation, CO_2 will continue to accumulate in the body until a maximal tolerable concentration has been reached and normal ventilation must commence. This method was developed using the concepts of breath-holding creating higher levels of CO_2 in the body and the ability to control exhalation uniformly results in a high level of diaphragmatic or pulmonary control. Previous research has demonstrated this method to have a high degree of internal reliability as well a high degree of intrarater reliability and has been used to examine the role CO_2 plays in stress and anxiety and the relationship between increasing CO_2T and submaximal physical performance.[4,12] Though this test has been shown to have internal reliability[12] as well as theoretical reliability, one main limitation of these studies was that it appears that this simple field test has never been validated against well-accepted CO_2 clinical measurements.[20]

Need of the Study

As freediving has gained popularity over the years, researchers have begun to study how breath-hold divers can resist fundamental physiological urges to breathe. This resistance is, in part, due to apnea training protocols that increase tolerance to CO_2 accumulation.[21] Given that CO_2T is also a performance-limiting factor during exercise, previous studies have shown that increasing tolerance to CO_2 can improve sub-maximal performance and reduce perceptions of fatigue.[12] In addition, it can reduce fear and state anxiety, as higher levels of CO_2 have been shown to be associated with the triggering of these adverse psychological responses.[3,12] An increase in fear and anxiety, especially during exercise, can then lead to poor performance outcomes or even increased injury potential[-4,12]. The only current field test that exists for measurement of CO_2T in humans involves a timed maximal breath exhale, however there is minimal research into the validity and reliability of this test. Validation of the current CO_2T field test against standardized laboratory measurements of maximal ventilatory CO_2 could significantly impact implementation of CO_2T training protocols given the minimal required equipment and the relative ease of conducting the test.[22]

Purpose

The purpose of this study is to validate the only known current CO_2T field test against $EtCO_2$, maximal ventilatory carbon dioxide ($\dot{V}CO_{2max}$), BL production, and AC in endurance athletes.

Hypothesis

This study has three related hypotheses (H):

H1: The CO_2T Field test will have a strong positive correlation to clinical CO_2 measurements.

H2: Differences in comparative values between the CO_2T field test and clinical CO_2 measurements will not be observed between sexes.

H3: There will be a negative correlation between AC and CO_2T.

Operational Definitions

Endurance Athletes

As defined for this study, "Endurance Athletes" participate in non-team sports where key muscle groups are worked sub-maximally for prolonged periods of time. These sports include but are not limited to road cycling, mountain biking, cyclocross, gravel cycling, cycle touring, any running event at or longer than 5km, cross country skiing, swimming, and race walking.[23]

Dissolved CO_2

Dissolved CO_2 is the amount of CO_2 present in arterial and venous blood. Carbon dioxide is transported back to the lungs primarily as bicarbonate as well as buffered by water as carbonic acid, bound to hemoglobin, or directly dissolved in the blood.[24]

Maximal CO_2

Maximal CO_2 output is a measurement of the maximal volume of expired CO_2 during exercise. Typically expressed as $\dot{V}CO_2$ and measured as either absolute; milliliters per minute (ml/min) or relative; ml/kg/min. Measurements are collected from expired gases during a graded exercise test (GXT). Gases are collected in the mixing chamber of a standard metabolic cart and then analyzed against supplied room air concentrations at standard temperature and pressure.[14]

CO_2 Tolerance

CO_2T can be viewed as either simple; the length of time an individual can hold their breath, or complex, the ability to endure progressively rising levels of CO_2 without resulting in unwanted psycho-metabolic reactivity.[25,26]

Limitations/Delimitations

The current study population includes endurance athletes, excluding the general population and anaerobic athletes; previous studies have shown that increasing tolerance to CO_2 has had little to no effect on performance in anaerobic athletes, thusly excluding these and the general populations can be considered a delimitation. The study population's age range of over 18 years and under 65 years, is considered a limitation due to physiological changes that occur as adults age.

Study Significance

This study's completion will validate or invalidate the only known CO_2T field test currently being used, a timed controlled single breath exhale. Given the relative ease and non-invasive nature of the current CO_2T-test, validating the results will allow the field test to be accurately applied to further research, various clinical applications, and as an assessment tool for training CO_2T.

CHAPTER TWO: REVIEW OF LITTERATURE

Introduction

Exercise testing has long been used to measure various functional capacities and intensity tolerance levels, diagnose cardiopulmonary diseases, identify responses to disease interventions, and for exercise/performance testing and prescription. There are many widely accepted clinical laboratory exercise tests, as well as validated field tests.[17,18,27] However, when measuring or calculating physiological variables, such as $\dot{V}O_2$max, there exist potential drawbacks when comparing gold standard laboratory tests to field tests. Field tests inherently do not provide as valid and reliable values; however, they are easier to administer, cost-effective, and generally do not require special equipment or training.[17,27] As $\dot{V}O_2$max is the quintessential measurement of cardiovascular fitness, many previous studies have sought to bridge the gap between clinical tests and field tests through the comparative analysis of results. This has led to many $\dot{V}O_2$max field tests being validated against the accepted gold standard, opening doors for more testing opportunities. However, maximal oxygen uptake is not the only crucial cardiorespiratory variable that can limit performance. This review will examine the impact of CO_2T on human physiology, examine the various ways CO_2 is measured, and explain the importance and need for a standardized, non-invasive, valid, and reliable CO_2T field test.

Recent studies have shown that increasing tolerance to CO_2 can have sub-maximal performance benefits for endurance athletes[12], and studies have long shown that

increasing tolerance to CO_2 is critical for breath-hold divers.[21,22] However, outside of clinical laboratory tests, there does not appear to exist a validated method for measuring CO_2T. Using the rationale that with a constant increase in the duration of exhalation, CO_2 will continue to accumulate in the body until a maximal tolerable concentration has been reached and normal ventilation must commence. In theory, a timed single breath exhale could measure CO_2T.[2–4] Though using a timed breath-exhale is a cost-effective and straightforward method compared with standard clinical tests, it has neither been validated nor implemented enough in a controlled setting to be considered truly reliable or valid. The purpose of this study is to validate the only currently known CO_2T field test against measured exhaled $\dot{V}CO_2$max in endurance athletes.

Many factors control the breathing or ventilation rate. The primary ventilatory drive is hydrogen ions (H^+) and secondarily bicarbonate levels (HCO_3^-) and arterial CO_2 levels. An increase in cellular respiration metabolites leads to an overall decrease in blood pH (blood tends to be slightly basic at a pH of 7.35 – 7.45).[28] An acute increase in respiratory metabolites will trigger both peripheral and central chemoreceptors located in the carotid bodies and the medulla oblongata, respectively. The relationship between rising cellular respiration products and ventilatory response is highlighted in a review by Dr. James Duffin. In this review, Dr. Duffin showed a progressive linear increase in ventilation as PCO_2 rose.[28] This correlation is significant in understanding how ventilation increases in response to exercise.

Elevated levels of CO_2 in the bloodstream, otherwise known as hypercapnia, are typically caused by poor or inadequate ventilation. Hypercapnia can be self-induced, as seen in competitive breath-hold divers and during exercise or result from pulmonary

conditions such as interstitial lung disease or chronic obstructive pulmonary disease. Being hypercapnic is undesirable as many physiological and psychological problems can occur. In severe cases, referred to as acute hypercapnic respiratory failure, it can be a medical emergency brought on by conditions associated with diminished neuromuscular transmission, defects of the ribcage, or as a sudden result of chronic hypercapnia.[29] Chronic hypercapnia is typically not seen in healthy individuals as some form of impairment within the pulmonary system that limits alveolar ventilation and reduces CO_2 offloading, in turn causing blood concentrations to rise, needs to exist.[30] Chronic hypercapnia results in a consistent lack of energy, wheezing, dyspnea, and frequent respiratory infections. This condition also makes it very difficult to exercise, as even minor increased energy expenditure will exacerbate the condition.[31]

The general population and athletes alike can also experience adverse symptoms of acute hypercapnia such as dyspnea (breathlessness), anxiety, and diminished performance in sports and activities[4,12,21,26,31]. An increase in CO_2 concentration in the blood will lead to respiratory acidosis. Normally, the body will regulate through metabolic compensation, wherein a sudden increase of H^+ secretion from the kidneys occurs. Higher concentrations of H^+ ions increase ventilatory drive with the end goal of off-loading excess CO_2 to rebalance blood pH. A sudden shift, or increase, in ventilatory drive, results in increased breathing depth and frequency. In athletes, a decrease in energy expenditure is necessary to decrease the amount of CO_2 that is being produced from cellular respiration as respiratory rate and depth will plateau.[28,32] These shifts in ventilatory drive, cardiac output, and CO_2 directly correlate with an individual's ventilatory threshold. Ventilatory threshold is the point at which ventilation

exponentially increases in relation to O_2 consumption. This threshold reflects BL levels (lactate threshold) and anaerobiosis. The amount of time an individual can withstand this shift in ventilatory drive can be seen as their ability to tolerate ever-rising levels of CO_2 in their system, and thus as a measure of CO_2T.[4,12,25]

CO_2 Chemosensitivity

Chemoresponsiveness is influenced by age, sex, genetics, and apnea conditions. A recent review by Chowdhuri and Badr (2017) showed the importance of cerebrovascular responsiveness in ventilation. Decreases in cerebral blood flow allow an accumulation of CO_2, which then stimulates the medulla and increases ventilation rates. This decrease in cerebral blood flow can be attributed to specific gene mutations, leading researchers to believe that one cause of sleep apnea and sudden infant death syndrome can be traced back to these genetic mutations. Apnea conditions, mainly central sleep apnea, increases blood CO_2 and is more commonly found in men with low testosterone. As humans age, they experience a decrease in cerebrovascular responsiveness and higher occurrences of altered sleep states, resulting in drastic oscillations in sleep that can lead to a greater chemosensitivity and trigger an increase in ventilation at only slightly elevated CO_2 levels.[33]

This is further illustrated in a study that examined hypercapnic ventilatory responsiveness. Fukuoka et al. (2003) sought to understand the effect that peripheral and central chemoreceptors have on ventilation during graded and steady-state exercise. Eleven healthy adults each performed two exercise tests under normoxic (21% O_2) and hypoxic conditions (12% O_2). To establish a ventilatory threshold for each subject, participants performed an incremental exercise test until reaching a heart rate of 160 or

more beats per minute or until they reached 80-90% exhaustion on the Borg fatigue scale.[34] The provocative tests then consisted of constant exercise at 40%, 60%, and 80% of the ventilatory threshold – conducted under both the normoxic and hypoxic conditions. Fukuoka et al discovered that hypercapnic ventilatory responsiveness and hypoxic ventilatory response increased up to 70% of ventilatory dynamics resulting from exercise, regardless of the increased work rate and under both normoxic and hypoxic conditions. This study showed a drastic rise in blood CO_2 levels from steady-state and graded cardiovascular exercise occurs regardless of O_2 availability that resulted in dynamic increases in ventilatory drive. Practically speaking, O_2 uptake is solely affected by O_2 availability and is minimally affected by CO_2 production; meaning regardless of O_2 availability, CO_2 production will continue to trigger chemoreceptors and increase ventilatory drive as work rate increases. Similar results were found in later studies on chemoresponsiveness and CO_2 sensitivity in athletes under exercise-induced hypoxemia.[8,35–38]

Carbon dioxide sensitivity or an individual's tolerance to CO_2 has been challenging to assess as there exists no easily administered or standard field test to measure the amount of CO_2 building up inside the body at any given moment. The effects of CO_2T have been researched for decades.[32] Nevertheless, scientists recently started looking beyond the chemoreceptor response and investigating CO_2 sensitivity as a potentially trainable performance variable. As previously stated CO_2T can be viewed simply as the length of time an individual can hold their breath, or as complexly as "the ability to endure progressively rising levels of CO_2 without resulting in unwanted psycho-metabolic reactivity".[12]

Psychological Effects of CO_2

Correlations between rising circulatory CO_2 levels and adverse psychological effects have been observed. Stoeckel et al. (2017) conducted a study examining the role CO_2 plays in developing and controlling dyspnea.[3] Dyspnea can result from a variety of causes including exercise, stress, anxiety, pulmonary diseases, and even the anticipation of dyspnea. Sixty-six healthy volunteers underwent 20 blocks of inspiratory resistive load breathing while undergoing Functional Magnetic Resistance Imaging. Resistive breathing loads were set to trigger severe or mild dyspnea and were administered in an alternating fashion. Each instance was either visually or non-visually cued to assess the difference between the anticipation of dyspnea and unanticipated dyspnea respectively, as the visual cue would signal the participant that they were about to experience resistive breathing. The researchers found that with the anticipation of dyspnea, there was a marked increase in activity in the anterior cingulate cortex.

The anterior cingulate cortex's primary psychological functions are decision making, emotion, impulse control, and attention allocation while its main physiological functions are blood pressure and heart rate regulation, particularly during a flight or fight response. Subjective ratings reported from the State-Trait Anxiety Inventory for intensity of dyspnea were significantly higher during severe as compared to mild dyspnea (mean ± SD 0.75 ± 0.63 and 0.64 ± 0.72, respectively, $t(65) = 22.80$ and $t(65) = 16.45$, $p < 0.001$). The Breathless Catastrophizing Scale showed a strong positive correlation with the anticipatory activation of the anterior cingulate cortex ($p = 0.003$) and remained constant when controlling for age ($r = 0.05$, $p < 0.001$). This increase in activity was also

found to be present in participants who directly experienced dyspnea without any anticipatory visual cues.[10]

The results of this study point directly to a potential mechanism for assisting populations that suffer from chronic stress and anxiety. Since the cognitive anticipation of being out of breath triggers dyspnea and the rise of serum CO_2 levels, this anticipation can create a positive feedback loop and further dyspnea – resulting in higher levels of stress and anxiety due to the feeling of being out of breath, and so on. Thus, it has been proposed that possessing a higher tolerance to CO_2 and having normal pulmonary function could interrupt this positive feedback loop, allowing individuals to avoid or shorten the act of experiencing dyspnea and regain normal ventilation.[4] Interrupting the catastrophizing dyspnea loop with a breathing intervention has potential benefits for populations other than athletes such as individuals who experience a generalized anxiety disorder, acute anxiety attacks, or even those who simply experience more than average stress during any particular moment.[4,26,39,40]

Manipulation of Tolerance to CO_2

Aside from controlling breathing, and improving breathing mechanisms, apnea or breath-holding protocols have been shown to increase tolerance to CO_2. Freedivers have long used these protocols to increase their tolerance to CO_2 and increase their ability to use O_2.[10,41] Combining these breath-hold protocols with glossopharyngeal insufflation (GI) – a breathing technique initially developed for patients experiencing insufficiency due to poliomyelitis to increase their time being off a ventilator). The glossopharyngeal insufflation technique works by increasing air that is pumped into the lungs by contractions of the upper pharyngeal muscles[3] which is accomplished by taking rapid

forceful breaths, "piston breathing", to fill the lungs with a higher volume of air than can be accomplished by normal ventilation. Using glossopharyngeal insufflation contributes to competitive breath-hold divers' ability to stay underwater for a remarkable amount of time, upwards of 11 minutes. Walterspacher et al. (2011) asked 12 elite competitive breath-hold divers to complete a battery of lung function tests to establish baseline values for glossopharyngeal insufflation's effects on the pulmonary functions. Baseline values included vital capacity before, during, and after glossopharyngeal insufflation; esophageal pressure, static lung compliance (being a measurement of the lungs' ability to expand and stretch), minute ventilation, breathing frequency, and CO_2/O_2 blood gas levels. Measurements for static lung compliance were taken at rest, immediately after glossopharyngeal insufflation, and every minute for five minutes after GI for a total of three trials. Lastly, the investigators looked at the effect CO_2 has on ventilatory drive (breathing rate). Participants were fitted with an oral mouthpiece, and nasal breathing was occluded. Mixtures of 6% CO_2 and 9% CO_2 were administered using the steady-state method.[42]

Walterspacher et al. (2011) collected respiratory drive measurements after participants reached steady-state in minute ventilation and breathing frequency. Of the 12 original participants, four had been previously tested under the same conditions three years prior and were assessed under the current conditions to observe glossopharyngeal insufflation and apnea breathing's long-term effects on the pulmonary system. There was no significant difference between the original and three-year follow-up participants for static lung compliance at rest, static lung compliance at glossopharyngeal insufflation, static lung compliance at 1 min post glossopharyngeal insufflation, and static lung

compliance at 3 min post glossopharyngeal insufflation. There were statistically significant increases in breathing frequency between 6% CO_2 and 9% CO_2 compared with un-occluded free-breathing of normal room air (both $p < 0.05$). There were also significant increases in minute ventilation between 6% CO_2 and free-breathing, 9% CO_2 and free-breathing, and between 6% CO_2 and 9% CO_2 (all $p < 0.001$). These results indicate that long-term glossopharyngeal insufflation in breath-hold diving does not affect pulmonary function and, more importantly, as it applies to examining CO_2 there was a distinctly blunted response to elevated CO_2 levels in breath-hold divers supporting the investigators' hypothesis that CO_2T can be trained and is not simply an inherited trait.[42]

Measurement of CO_2

As the previous articles have shown, there are many reasons to measure or monitor a patient or athlete's CO_2 production. However, measurement techniques are limited, and each has its benefits and drawbacks. Arterial blood gas measurement became the gold standard in measuring levels of blood CO_2 in 1924.[43] Currently, samples are gathered using vented plastic syringes where all ambient air is removed from the sample. Analysis is rapidly performed using an arterial blood gas analyzer with electrodes that measure PO_2, PCO_2 and pH. Other values such as bicarbonate and total CO_2 are calculated from the measured values. Ventilation being the flow of air in and out of the alveoli, and perfusion being the flow of blood to the alveoli. Clinically the ventilation/perfusion ratio influences the degree to which an individual is experiencing hypercapnia or an excess of CO_2. However, this method is very invasive, painful, costly,

only gives a moment-to-moment analysis, and requires highly skilled staff to administer and process the sample.[15,31]

Because ABG collection relies on a highly trained respiratory therapist or clinical laboratory technologist to administer the test, as well as being invasive, researchers have looked at viable alternatives to the standard ABG test to measure CO_2 levels. From a clinical standpoint, blood sampling will consistently give the most accurate and reliable results if standard testing procedures are followed. A common alternative is VBG, and previous studies have shown that VBG measurements, when combined with pulse oximetry, have a high positive correlation to ABG measurements.[45,46] In a study involving 129 participants, Zeserson et. al. collected ABG and VBG samples and compared PO_2, PCO_2, pH, bicarbonate, and pulse oximetry between the two blood draws. Arterial blood was taken from an existing arterial line or the femoral artery, venous blood was taken from either a venous catheter or a peripheral venipuncture. Researchers found that there was a strong positive correlation between arterial and venous pH levels ($r = 0.94$) and PCO_2 ($r = 0.93$). The mean difference between pH levels between venous and arterial blood gasses was 0.03 (95% confidence interval, 0.03-0.04). The mean difference between arterial and venous PCO_2 was 4.8 mmHg (95% confidence interval, 3.7-6.0 mmHg).[45] These results show that VBG can be used in place of ABG for CO_2 analysis and would be preferable outside of the control of a hospital setting.[44,47,48]

Arterialized capillary blood (ACB) sampling is another standardized method used to analyze blood gas levels. Traditionally ACB is used in infants or elderly individuals who are not suited for an ABG puncture. [49] Capillary blood collection is a fairly common practice in and out of clinical settings. Like measuring blood sugar or BL, blood is

obtained by a skin puncture 1-2.5mm deep using a lancet or automated incision device. The process is considered to cause minimal pain and discomfort. It is also the least invasive of the blood collection methods. Blood is "arterialized" when the skin is warmed just prior to puncture and collection either the fingertip or the earlobe can be used.[50]

Many studies have shown that ACB not only has a high correlation to ABG measurements but is also suitable to be used outside of traditional clinical settings in more austere environments.[51] In a study of 20 healthy adults Lumholdt et al. sought to validate the collection and analysis of arterialized venous blood as a valid collection method for blood gas analysis when compared to ABG. Blood samples were processed using three different methods; the first was held for five minutes and then analyzed, the second was mixed for five minutes and then analyzed seven minutes after collection, and the third was held and then processed 15 minutes after collection. No statically significant differences were found between processing methods. Researchers analyzed blood samples for PCO_2, pH levels, and PO_2 and found that though ACB was not a suitable alternative for PO_2, both pH and PCO_2 comparisons were statistically significant ($p < 0.05$, $r = 0.941$ and $r = 0.924$ respectively) and concluded that ACB is a valid alternative to ABG for pH and PCO_2 measurements.[52]

Capnography, or capnometry, is the collection and analysis of exhaled gases for CO_2 content. After being fitted with a mouthpiece and having the nasopharynx occluded, exhaled gasses are warmed and dehumidified – exhaled gasses contain 100% humidity and CO_2 must be dried for accurate results. This method is non-invasive and painless.[43] There is also no need for a highly skilled technician to administer the test. Another

advantage of capnometry is the ability to accurately measure $EtCO_2$. Capnometry; however, suffers from the need for expensive equipment and handheld versions are less accurate due to the volume of gasses inhaled vs. exhaled.[44]

The last of the common CO_2 measuring modalities is done via a transcutaneous sensor. These wearables can either be attached to the arm, chest, or ear. These devices use a nondispersive infrared sensor, a type of simple spectroscope sensor traditionally used to measure gases. The basic principle of a transcutaneous CO_2 measurement device works by putting a heated electrode securely and directly onto the skin. This then causes the natural microcirculation to arterialize. Using the Henderson-Hasselbalch equation, the production of carbonic acid provides a pH reading which can then be interpreted as arterial CO_2 concentration.[53] These devices are non-invasive, convenient, and accurately measure capillary CO_2 blood gas. [14,15,16,44] Ekkernkamp et al. (2015) investigated the correlation between the most common invasive and non-invasive arterial and capillary PCO_2 measurement methods. 83 patients and 17 healthy adults volunteered for both invasive and non-invasive PCO_2 measuring methods. The invasive method included a standard blood draw for ABG and an earlobe prick to access capillary blood gas. Non-invasive methods included a transcutaneous sensor placed on the right side of the chest. Mean differences between ABG and transcutaneous CO_2 were 11.9 ± 15.0 mmHg. Mean difference between right earlobe capillary PCO_2 and arterial values was 5.6 ± 7.2 mm Hg, though acceptable agreements between ABG and capillary PCO_2 could not be found. Researchers concluded that the non-invasive transcutaneous measurement of PCO_2 is a viable alternative to more painful and invasive methods based on a further comparative analysis, qualitative observations, and the acknowledged limitations of the study.[54]

Though these previous results were positive, more research was needed to assess if this would be a viable method for CO_2 monitoring during exercise. In a preliminary pilot study, Grangeat et al. (2019) tested a new and innovative wearable device for continuous monitoring of PCO_2 called the CAPNO. Researchers used a standard cardiopulmonary exercise test to evaluate the feasibility and accuracy of a forearm mounted device for transcutaneous CO_2 measurement; as previous methods have been limited to devices mounted on the chest and ear lobe, both of which are difficult to implement across a wide range of people and have adherence issues during exercise. Investigators found a positive correlation ($r = 0.74$) between the CAPNO and transcutaneous CO_2 measurement at the ear, as well as $EtCO_2$ measured via standard metabolic cart ($p = 0.81$). $EtCO_2$ is a typical marker for dead-space ventilation which is used to evaluate individuals for possible pulmonary emboli. Overall $EtCO_2$ can provide very useful information regarding CO_2 production and offload. The major limitation of this study is the general nature of case studies. With only one healthy participant involved, more research needs to be done to evaluate this device fully. However, given Granger et al. (2019) found a positive correlation to transcutaneous CO_2 measured at the forearm and earlobe, it is plausible to say that along with the results of the previous study, transcutaneous CO_2 measured at the forearm with the CAPNO device would also have a positive correlation to standard ABG measurements. Another interesting finding is the strong positive correlation between transcutaneous CO_2 and $EtCO_2$.[55]

$EtCO_2$ measurements collected during $\dot{V}O_2$ max tests are shown to be very valid and reliable during graded exercise tests. This is another area where a theoretical correlation could be made between expired CO_2 and levels of dissolved CO_2. Unlike

other inert gases, CO_2 very quickly and thoroughly diffuses across cell membranes. In a recent study by Fijimoto et al. (2019) researchers sought to investigate correlations between $EtCO_2$, arterial, venous, and transcutaneous CO_2. Thirty participants underwent a total of 60 sample trials in which arterial CO_2, venous CO_2, and transcutaneous CO_2 were collected. The resulting values were then compared with continuous monitoring of $EtCO_2$ via capnometer. Investigators found a strong positive correlation between $EtCO_2$ and ABG measurements ($p < 0.001$) with a minimal bias of 6.48 mmHg, whereas a weak positive correlation between ABG and transcutaneous CO_2 was found ($p = 0.037$). Investigators concluded that ABG agreed with $EtCO_2$ as well as or better than VBG and transcutaneous CO_2 measurements, suggesting that $EtCO_2$ can be used, with minimal loss of accuracy, in place of ABG measurements.[24]

The main limitation of using expired gases to evaluate CO_2T would be the amount of CO_2 leftover in the blood and the anatomical dead space. End-expiratory lung volume and anatomical dead space in the lungs are determined by the inhalation and exhalation of nitrogen gas. Because nitrogen is an inert gas and therefore, does not possess a charge, it is not quickly diffused into blood from the lungs. The resulting level of expired gas can be taken from the total inhaled gas to determine the amount of end-expiratory lung volume and anatomical dead space present in the lungs. CO_2 is not used to determine end-expiratory lung volume because of the ease with which it diffuses out of blood, into the lungs, and then offloaded through alveolar ventilation. Because CO_2 is easily transported and transferred there is a potentially strong positive correlation between expired levels of CO_2 or the amount of CO_2 an individual can offload and the amount of circulating CO_2 an individual can sustain during maximal or sub-maximal effort.[56] Tang

et al. (2012) showed that standard modeling for end-expiratory lung volume and anatomical dead space relies on the amount of exhaled CO_2. Researchers found that this N_2 depletion model can be used to measure end-expiratory lung volume, also resulting in insignificant amounts of CO_2 being left behind. This supports the theoretical validity of a single timed-breath exhale for the evaluation of CO_2T.[55–57]

CO_2 and Physical Activity

As previously explored, dyspnea is a common result of physical exertion; and is primarily influenced by the rising levels of H^+ and secondarily from a rise in CO_2. From a clinical standpoint, it is vital to use the above-mentioned modalities to evaluate levels of CO_2 as a controlled clinical laboratory setting will undoubtedly provide the highest level of validity and reliability. A standard graded exercise test, or as previously mentioned, a cardiopulmonary exercise test, can be used to increase levels of CO_2 production. Datta et al. (2015) reviewed the most common cardiopulmonary exercise test protocols and CO_2 testing modalities to evaluate exercise-induced dyspnea from a clinical research perspective. Investigators evaluated results from both field and laboratory tests, including a six-minute walk, incremental, and endurance walking tests as field options and a cardiopulmonary exercise test in a laboratory setting. They concluded that though the field tests were easy to administer, relate to normal activities of daily living, and may be able to predict morbidity or mortality, they do not give much by way of actual physiological measurements, whereas the cardiopulmonary exercise test provides a wealth of physiological data.[58] These conclusions highlight the need for reliable physiological measurements when assessing and treating individuals who suffer from

exercise-induced dyspnea while also giving rise to difficulties due to the need for expensive equipment and specialized training.

Validation of Field Tests

Because of the usefulness of field tests to evaluate cardiopulmonary physiological measurements for athletic testing or clinical application[27], it is important to not only evaluate the validity and reliability of specific field tests with that of gold standard laboratory tests but also determine the ease with which these tests can be implemented across various populations. Field tests are an important part of exercise testing as they not only are easily implemented but also are extremely cost-effective and generally do not require a high degree of training. A review of 43 studies covering a total of six commonly used field cardiopulmonary exercise tests included a 20-meter shuttle run (SR-20m), 550 m run, a 1-mile run, and timed runs for distance durations of 6, 9, and 12 minutes. Further examination into each field test gave even more correlative data behind various algorithms used to compute test results. Researchers concluded that the SR-20m to be the most valid of the six cardiopulmonary exercise tests tested when using the equation supplied by Barnett et al. (1993). Of the three equations supplied by Barnett et al. (1993), $\dot{V}O_2$peak = 24.2-5.0 (gender: M = 0; F = 1) – 0.8(age) + 3.4(MS) where MS is maximal speed calculated by the total number of laps completed during the course of the test, showed to be the most statically significant results ($p< 0.01$).[59] They also observed that the 1-mile run could be a suitable alternative to the SR-20m but lacks repeatability due to the increased testing distance. As subjects were limited to youth athletes, other equations might need to be used with the same test to evaluate cardiopulmonary performance in adults or individuals with compromised respiratory systems.

CO_2 accumulation plays a significant role in limiting cardiovascular and cardiopulmonary exercise and testing[12,32,60]. An increased blood CO_2 level results in increased ventilatory rate and cardiac output. As previously referenced; elevated CO_2 levels, ventilatory rate, and cardiac output cannot be sustained for long periods of time once the ventilatory threshold is surpassed. This limits maximal exercise and effort must be decreased to continue physical exertion. Though limited, previous studies have shown that the ability to have a positive hypercapnic response during activity, i.e. the ability to withstand higher levels of CO_2 without experiencing adverse psychophysiological responses, is linked to genetics,[61] not unlike how $\dot{V}O_2$max is also largely genetic, and trainable.[62] With these factors in mind, the concept of how CO_2T can potentially affect performance outcomes in endurance athletes is applicable and warrants further investigation into the extent or limits to which this mechanism is trainable.

Studying, training, and improving CO_2T is a relativity new area of research. There have only been a handful of studies that have specifically tried to assess an individual's CO_2T and quantify that measurement. As most CO_2 measurement methods either require a high level of invasiveness or the use of specialized training and equipment, the limitations are significant when assessing CO_2T outside of a clinical environment. Few studies have used the non-validated CO_2T-test of a timed breath exhale. In a recent study, Murphey, J. & Lafrenz, A. (2020) sought to evaluate the effects that increasing tolerance to CO_2 had on sub-maximal performance in trained cyclists.[12] Twenty-four well-trained cyclists were tested for anthropomorphic, maximal cardiorespiratory, and ventilatory values, and sub-maximal power output before and after a six-week breathing intervention. Twelve participants served as control and were given

simple meditative breathing exercises to complete throughout the six-week intervention. An experimental group consisting of twelve participants was given specific apnea breathing protocols designed to increase tolerance to CO_2.

Participants in both groups were administered the previously mentioned CO_2T field test to assess CO_2T, the State-Trait Anxiety Index to measure anxiety levels, and a Functional Threshold Power (FTP) test in an orally restricted breathing environment to measure sub-maximal performance under elevated CO_2 levels. Because participants could only breathe through the nasopharynx, less CO_2 was offloaded during the test. Their power output was thus limited by their ability to withstand constantly rising levels of CO_2. The researchers found that breathing protocols increased CO_2T and positively affected sub-maximal threshold power while negatively correlating to state anxiety. There was a strong positive correlation between pre and post CO_2T ($r = 0.9$) across all participants in both the experimental and control groups, regardless of breathing intervention. This high level of internal reliability shows that a timed breath exhale is a potentially viable field test.[12]

As previously stated, there is a strong rationale to implement CO_2T training in non-athletic populations. Spirmont P. et al. (2019) and Bentley TGK et al. (2020) both used the same un-validated timed breath exhale as an assessment of CO_2T in their investigations of CO_2 and State-Trait Anxiety.[2,4] Spirmont et al. (2019) showed a negative correlation between performance on the CO_2T and state anxiety, while Bentley et al. showed positive results in implementing slow breathing as an anxiety reduction method in high school-aged children.[2,4]

Conclusion

The information presented in the preceding articles demonstrates the importance of evaluating CO_2T levels in populations including high-level competitive athletes, individuals suffering from COPD, breath-hold divers, and amateur athletes looking to add to their training protocols. Though there is still a great need for more research in this area, enough evidence has been shown to justify the need for a valid and reliable field test to accurately assess ones' tolerance to CO_2. Future studies could look at how more specific populations are affected by breathwork interventions, examining which breathing protocols are useful for training and different competition modalities and any potential benefits from increasing tolerance to CO_2 under anaerobic conditions.

These findings could also be particularly useful outside of athletics and clinical application. Many studies have shown how meditation can lower heart rate and blood pressure alongside alleviating stress and anxiety. Having the ability to assess CO_2T and then create training protocols to lower CO_2 sensitivity could improve the overall quality of life, especially for those who suffer from chronic stress and anxiety. Future studies in this area could then examine the psychological effects CO_2T has on individuals in their everyday lives, the psychological aspect of competition, and finally, individuals that work in high-stress, high-risk situations such as police, military, and firefighters.

CHAPTER THREE METHODS

Participants

Based on a statistical power analysis (IMB SPSS 2020, College Armonk, NY) twenty-eight, (n=28, 19 males, 9 females) healthy female and male endurance athletes between the ages of 18-65 were recruited for this study. Seventeen of which participated in triathlons (swimming, cycling, and running), eight participated in cycling only, and three were distance runners only. All participants were currently cardiovascular training 2-4 times per week and strength training 1-3 times per week and were free of any musculoskeletal, metabolic, and cardiovascular injuries or conditions. Subjects' training or racing status in their chosen endurance sport was self-reported on the initial visit. All potential participants were deemed low risk via the American College of Sports Medicine (ACSM) screening protocol[5,6]. The ACSM pre-exercise screening protocol is widely accepted across various populations as a valid and reliable survey highlighting potential health risks from strenuous exercise. [63–65] All participants read and signed an informed consent approved by Boise State University Institutional Review Board (approval #186-MED21-007 9/20/2021).

Measurements

Anthropometrics and Medical History

Height (cm) and mass (kg) was measured to the 0.1 cm and 0.01 Kg, respectively (Charder Height Measurement, Charder Electronic CO, Tanita 300, Tanita Corp., Arlington Heights, IL). Body fat (BF%) and lean body mass (LBM) were calculated via

BodPod (Life Measurement Incorporated, Concord, CA) using the SIRI formula.[77] Before each subject testing session, BodPod calibration was performed per the manufacturer's recommendation.[66] Using air displacement, the BodPod has been validated against the gold standard in body composition, hydrostatic weighing. The BodPod is considered a reliable and less expensive alternative with minimal variability (± 3.0 %). [66]

Anaerobic Graded Exercise Test:

The 30-second Wingate test was conducted on an electronically braked cycle ergometer (Velotron bicycle ergometer, Quarq, Chicago, IL) to assess maximum anaerobic power and increase CO_2 production. Resistance loads were calculated and applied via standard Wingate Ergometer Test specific to each subject (Velotron Wingate Ergometer Test, Velotron Wingate software). Resistance loads were set at 7.5% of the study participants' body mass in kg for females and 8% for males (kg BM). These testing figures have been shown to produce valid and reliable results as stated by the coaching and sport science division of United States Cycling. [67,68]

Once all anthropometric data were collected for each subject, the Velotron seat and handlebars were adjusted to each participant's comfort. Participants completed five minutes of warm-up interspersed with five 4-6 second sprints at their discretion. Each subject rested for two minutes after the warm-up before the start of the testing period. The standard Wingate protocol (30-WT) was used for each testing interval.[68] Upon completing the 30-WT, participants passive rested for two minutes and then completed another 30-WT, followed by another rest period. The 30-WT tests continued until participants reached a plateau of $\dot{V}CO_2$max, volitional fatigue, or at their own discretion.[67,69]

Blood Lactate

BL accumulation was measured at the end of each 30-WT. Samples were obtained via a finger prick using a sterile lancet and a single drop of blood placed onto a lactate meter (Lactate +, Nova Biomedical, USA) strip and inserted into the meter. Its ease of use and relatively cheap cost to operate the Lactate Plus© meter provides immediate and accurate results.[70]

Maximal Heart Rate

A Polar® heart rate monitor chest strap (Polar Electro, Finland) was used to collect peak heart rate. The chest strap was adjusted by the subject themselves for comfort before testing.

CO_2 Measurement

End-Tidal CO_2 ($EtCO_2$)

End-tidal CO_2 was measured via Capnograph (RespSence, Nonin, Plymouth, MN). Participants were fitted with a disposable nasal collection cannula (CO_2 Sampling Cannula, Salter Labs, Lake Forest IL) and all anthropomorphic data were entered into the machine. Sampling began immediately upon testing and continued until the testing intervals were over.

Maximal Expired CO_2 ($\dot{V}CO_2$ max)

Participants completed the Wingate protocol as previously described on a cycle ergometer (Velotron bicycle ergometer, Quarq-SRAM, Chicago, IL) while their exhaled gases were measured. For the duration of the test, participants were fitted with a Hans Rudolph 7900 series mouth-only mask to capture and measure their exhaled gases and a padded nose clip to ensure that all exhaled gases went through the mask. Expired gasses were analyzed via a Parvo Medics TrueOne 2400 metabolic cart (Parvo Medics, Salt Lake City, UT); leading industry gold standard for clinical testing of cardiovascular

measurements. The TrueOne has been shown to provide consistent and valid measurement of all metabolic processes through the use of indirect calorimetry [73,74]. Peak $\dot{V}CO_2$ levels were noted at the end of each interval.

CO_2T Field Test

Participants performed two trials, each separated by a 5-minute rest. They took three normal breaths and on the fourth breath, participants inhaled through the nose to total lung capacity and exhaled slowly and continually through the nose for as long as possible. Using a stopwatch (Accusplit, PRO SURVIVOR 601x, MA, USA), the investigator observed and timed the participants' exhale duration. Participants were told that the trial ends if they either: 1) pause, stop, or otherwise interrupt the continuous exhale, or 2) inhale. Participants signaled to the researcher with a simple agreed-upon hand gesture (a "thumbs up") when they began their initial slow exhalation and the moment the continuous exhale ended.[25] Participants were given the opportunity to practice the procedure prior to actual testing to eliminate results being affected by the learned testing effect. This method has shown to have a high degree of internal reliability, but no studies have been conducted on this test's validity. [4,12,26]

Procedure

Participants reported to the Human Performance Laboratory (HPL) at Boise State University on two separate occasions, each day separated by at least 48 hours of rest.

Day 1: Orientation/Informed Consent/anthropometric measurements/CO_2T Field Test/ Ventilatory CO_2

This visit consisted of study orientation and informed consent, anthropometric measurements, and baseline maximal anaerobic threshold testing. Anthropometric data can be found in Table 2. The CO_2T field test was used to estimate CO_2T. Participants

then performed 30-WT intervals while all expired gases were collected and analyzed for peak $\dot{V}CO_2$ levels. The test was terminated when a plateau of $\dot{V}CO_2$ max and BL levels were reached, volitional fatigue, or at the subject's discretion. Peak heart rate was also used as a comparative value and to establish anaerobic threshold. Anaerobic capacity was defined at a plateau in $\dot{V}CO_{2max}$ followed by a 20% decrease in maximal power output between intervals. The number of 30-WT intervals was noted for the second day of testing.

Day 2 – 48 hrs of rest from day 1: CO_2T Field Test/$EtCO_2$ /BL Measurements

Participants completed the CO_2T field test for internal and intra-rater reliability upon arrival. After a 5 min rest, participants then completed 30-WT intervals under the same testing procedures as the previous day of testing. End-tidal CO_2 was collected via $EtCO_2$ testing procedure during the 30-WT intervals, and measurements were recorded at the end of each interval. Testing was completed when the same number of 30-WT intervals from the previous day of testing had been reached or at the subject's discretion.

Data Analysis

Means and standard deviations were calculated for essential anthropometric characteristics. Physical characteristics, anaerobic capacity, $EtCO_2$, and CO_2T changes across the clinical CO_2 tests and CO_2T field tests were compared for statistical significance. Analysis of variance was used to examine differences within groups (sex); if statistical significance was found, a Tukey post-hoc analysis was used to locate where the differences were. Differences, within and between tests, were performed using ANCOVA with baseline variables as covariates to adjust for any differences at pre-test CO_2T between groups. Statistical tests were computed using SPSS 27 (IMB SPSS 2020, Armonk, NY), and a p-value of < 0.05 was considered statistically significant.

CHAPTER FOUR: VALIDATION OF NON-INVASIVE CO2 TOLERANCE FIELD TEST

Introduction

Exercise testing has long been used to measure various functional capacities and intensity tolerance levels, diagnose cardiopulmonary diseases, identify responses to disease interventions, and for exercise effectiveness and prescription. There are many widely accepted clinical laboratory exercise tests, as well as validated field tests. However, when measuring or calculating physiological variables, such as maximal oxygen uptake $\dot{V}O_2max$, there exist potential drawbacks when comparing gold standard laboratory tests to field tests. Field tests inherently do not provide as valid and reliable values; however, they are much easier to administer, cost-effective, and do not generally require special equipment or training.

Recent studies have shown that increasing tolerance to CO_2 can have sub-maximal performance benefits in endurance athletes[12], and studies have long shown that increasing tolerance to CO_2 is critical for breath-hold divers.[21,22] Much like training can affect the level of lactate build up one can tolerate as seen in lactate threshold training in power and strength athletes, and training to increase $\dot{V}O_2max$ for endurance athletes, training to increase CO_2T has gained popularity in recent years in the freediving community.[7,9] Previous research has also shown improving CO_2T to be beneficial for lowering blood acidosis, reducing oxidative stress, and improving state anxiety.[3,10–12] However, although

studies have shown that CO_2T can be improved, there is no 'gold standard' test to assess an individual's actual CO_2T.

Outside of clinical laboratory tests, there does not appear to exist a validated method for measuring CO_2T. The previously mentioned studies have used a novel timed single-breath exhale to evaluate CO_2T among their subjects. Using the rationale that the longer exhalation is limited, CO_2 will continue to accumulate in the body until a maximal tolerable concentration has been reached and normal ventilation must commence. Though using a timed breath-exhale is a cost-effective and straightforward method compared with standard clinical tests, it has neither been validated nor implemented in a controlled setting to be considered truly reliable or valid. The purpose of this study is to validate the only known current CO_2T field test against $EtCO_2$, $\dot{V}CO_{2max}$, BL production, and AC in endurance athletes.

Methods

Participants

Thirty-two (n = 32) healthy individuals with no previous experience in breath-hold training were recruited. Four participants did not complete the whole study; thus, the data of 28 participants (n=28, 19 males, 9 females, Table 2.) were used for analysis. All procedures were approved by the Institutional Review Board (approval #186-MED21-007 9/20/2021) and participants provided consent and completed the PAR-Q and ACSM health questionnaire.[5,7]

Anaerobic Threshold Test

The 30-second Wingate test on an electronically braked cycle ergometer (Velotron bicycle ergometer, Quarq, Chicago, IL) was used to assess maximum anaerobic power[8]. Resistance loads were calculated at:

Females: Test load (kg) = body mass (kg) * 0.075

OR

Males: Test load (kg) = body mass (kg) * 0.083

Table 1 Anaerobic Threshold Test[68]

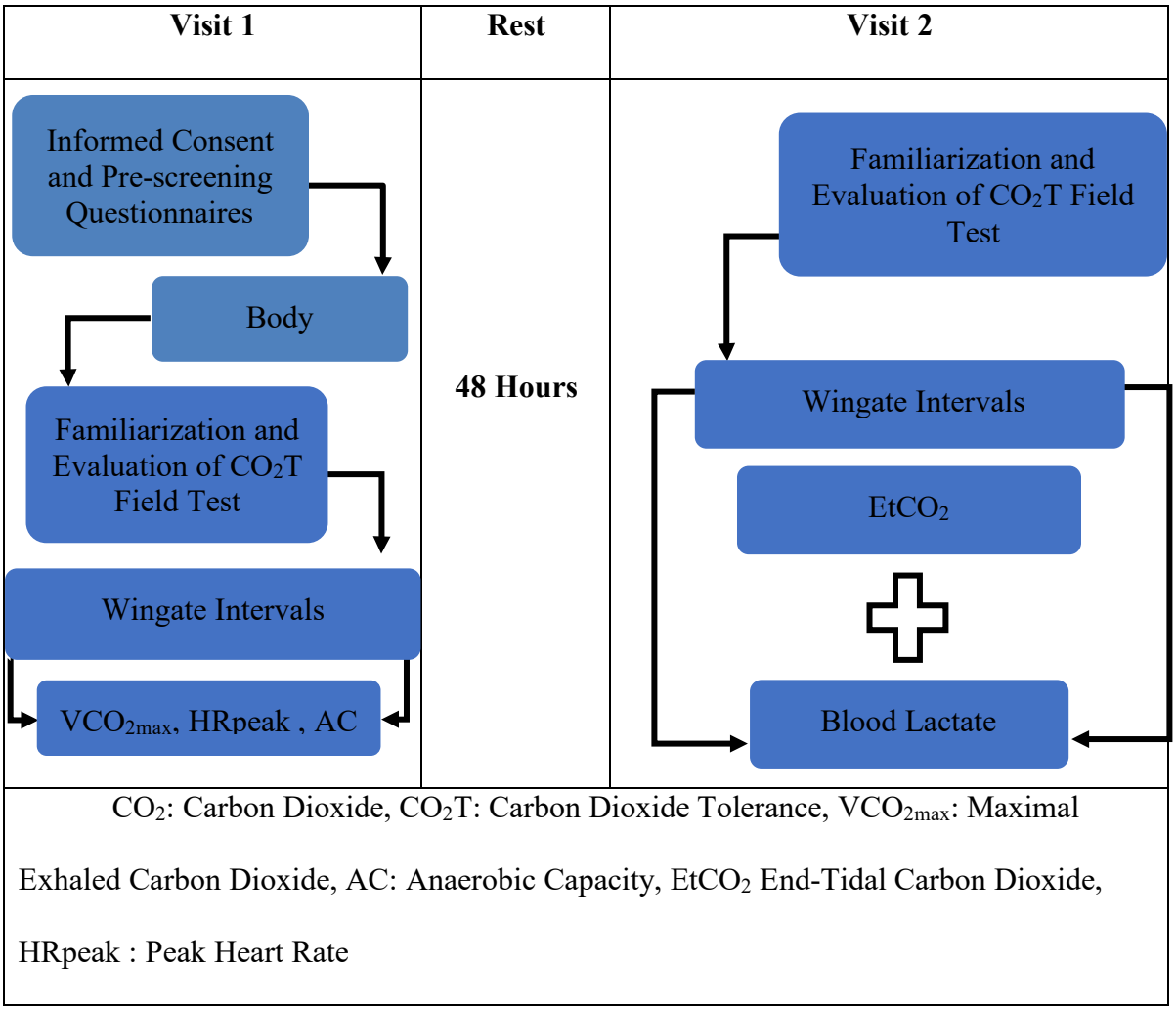
Procedure	**Resistance (% kgBM)**	**Data Collected**
20 sec resistance ramp-up @ 2-5% kgBM	8.3 - males	BL
5 sec cadence ramp-up @ 0% kgBM	7.0- females	HRpeak
30 second sprint @ Rx'ed resistance		$\dot{V}CO_2$ max
2 min passive recovery @ 2% kgBM		$EtCO_2$

Procedures

Data were collected on two separate occasions, each day separated by at least 48 hours of rest. Height (cm) and mass (kg) were measured to the 0.1 cm and 0.01 Kg, respectively (Charder Height Measurement, Charder Electronic CO, Tanita 300, Tanita Corp., Arlington Heights, IL). Body fat (BF%) and lean body mass (LBM) were calculated via BodPod (Life Measurement Incorporated, Concord, CA) using the SIRI[77] formula. Participants were also familiarized with the CO_2T field test on both days of testing. See Figure 1. for the study outline.

Day 1 Testing

The first day of testing consisted of study orientation and informed consent, anthropometric measurements, and baseline maximal anaerobic threshold testing. Participants performed repeated 30 sec WT intervals while expired gases were collected and analyzed. Peak $\dot{V}CO_2$, peak heart rate (HRpeak), and power output were monitored and used as a to establish anaerobic threshold (AT). AT is the point at which a curvilinear increase in minute ventilation is observed (Owles Point)[9], critical power is reached, and HRpeak or HR_{max} was observed.[10,11] Participants completed Wingate intervals until HRpeak and plateau of peak power was achieved, volitional fatigue occurred, or at the discretion of the participant. Each participant passively rested two minutes between testing intervals. The number of repeated 30 sec WT intervals was noted for the second day of testing. AC was noted at the end of each 30 sec WT interval and the highest value was recorded and used to help determine when anaerobic threshold was met.



CO_2: Carbon Dioxide, CO_2T: Carbon Dioxide Tolerance, VCO_{2max}: Maximal Exhaled Carbon Dioxide, AC: Anaerobic Capacity, $EtCO_2$ End-Tidal Carbon Dioxide, HRpeak : Peak Heart Rate

Figure 1 Study Outline of Visits and Variables Measured

Day 2 Testing

On day 2, participants completed a second CO_2T field test for internal and intra-rater reliability. After a 5 min rest, participants then repeated the 30 sec WT intervals using the same testing protocol. $EtCO_2$ values were collected via capnometer and at the end of each interval, BL was measured via blood obtained from a finger prick. Testing was terminated when the same number of 30-sec WT intervals from the previous day was reached or at the subject's discretion.

Data Analysis

Mean and standard deviations were calculated for anthropometric characteristics. Physical characteristics, anaerobic capacity, $EtCO_2$, and CO_2T changes across the clinical CO_2 tests and CO_2T field tests were compared for statistical significance (SPSS 27 (IMB SPSS 2020, Armonk, NY). Analysis of variance was used to examine differences within groups (sex); a Tukey post-hoc analysis was used to identify where the ANOVA differences were. Differences, within and between tests were determined using ANCOVA with baseline variables as covariates to adjust for any differences at pre-test CO_2T between groups. Data are presented in means ± SD with an alpha level ≤ 0.05.

Results

All subject and group demographics can be found in Table 2. Mean ± SD for all primary and secondary research variables can be found in Table 3. All subjects who completed both days of testing met end-range criteria for established maximal CO_2 production.

Table 2 Study Participant Characteristic

	Male Mean±SD	Female Mean±SD
Height (cm)	180.85 ± 6.25	165.98 ± 2.17
Mass (Kg)	77.47 ± 7.69	61.43 ± 6.28
Age (years)	37.05 ± 9.60	37.89 ± 10.28
BF (Kg)	16.33 ± 7.79	22.00 ± 6.25
LBM (Kg)	79.57 ± 18.53	78.02 ± 6.22

Table 3 Mean ± SD for all Research Variables Between Sex

Group (sex) Statistics			
Sex		Mean±SD	Std. DV
AC (W/kg)	Male	7.96 ± 1.25	0.29
	Female	7.26 ± 1.19	0.40
EtCO2rel (mmHg)	Male	0.31 ± 0.14	0.03
	Female	0.24 ± 0.09	0.03
EtCO2abs (mmHg)	Male	8.47 ± 5.15	1.18
	Female	6.44 ± 3.13	1.04
$\dot{V}CO_2$max (ml/kg/min)	Male	51.59 ± 6.75	1.55
	Female	47.68 ± 7.58	2.53
BLrel (mmol)	Male	0.55 ± 0.30	0.07
	Female	0.57 ± 0.33	0.11
BLabs (mmol)	Male	5.18 ± 1.61	0.37
	Female	5.22 ± 2.27	0.76
HRpeak (BPM)	Male	179.58 ± 8.01	1.84
	Female	176.22 ± 10.63	3.54
CO_2Tavg (Sec)	Male	43.81 ± 27.49	6.31
	Female	40.00 ± 12.06	4.02

CO_2 Tolerance

Participants completed two CO_2T trials and an average was taken each day. Day 1 and Day 2 CO_2T times were not found to be statically different ($p = 0.181$) and showed a high positive correlation to each other. ($r = 0.989$, $p < 0.001$). Therefore, an average of the scores was used for comparisons to primary and secondary research variables. There was a direct correlation to HRpeak ($r = 0.380$, $p < 0.05$) and an inverse relationship to $EtCO_2(1)$ ($r = -0.393$, $p < 0.05$). Differences in means between sexes was shown to be significant ($t(26) = 0.395$, $p < 0.05$, figure 2). No significant correlations were found between average CO_2T and all primary research variables (BL_{abs}, BL_{rel}, $EtCO_{2abs}$, and $EtCO_{2rel}$, and $\dot{V}CO_2$max).

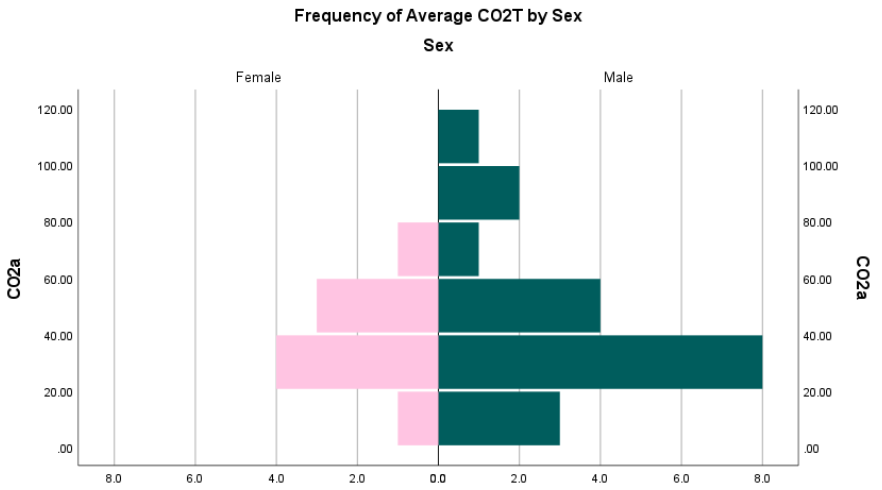


Figure 2 CO_2T Distribution by Sex

Primary Research Variables

End-Tidal CO_2 values for all participants decreased from intervals 1-4 whereas BL values increased (see Figs. 2 and 3). A one-way ANOVA showed that $EtCO_2$ and BL values between intervals were significantly different ($p < 0.001$) within groups (sex), but not between groups ($p = 0.729$ and $p = 0.703$ respectively). Because of the wide variety in individual absolute values, relative values were calculated for $EtCO_2$ and BL and used for comparisons. All four $EtCO_2$ values were also cross correlated to CO_2Tavg times. $EtCO_2(1)$ showed a moderate negative correlation to CO_2T ($r = -0.393$, $p < 0.05$). No other significant correlations or differences were observed between CO_2T and any other primary or secondary research variable including AC ($r = 0.005$, $p < 0.491$). Table 4 shows all correlations between CO_2T and all primary research variables.

There were no significant differences between $\dot{V}CO_2$max values between sexes ($p < 0.181$). Peak exhaled CO_2 showed a moderate positive correlation to LBM ($r = 0.513$, $p < 0.01$), VE ($r = 0.429$, $p < 0.05$), $\dot{V}CO_2$ ($r = 0.403$, $p < 0.01$), and height ($r = 0.368$, $p < 0.05$); and an inverse relationship to BF ($r = -0.538$, $p < 0.01$). There was no correlation to CO_2T; however, the level of significance was not met ($r = 0.005$, $p = 0.491$) There were no significant differences between $\dot{V}CO_2$max values between sex ($p < 0.181$).

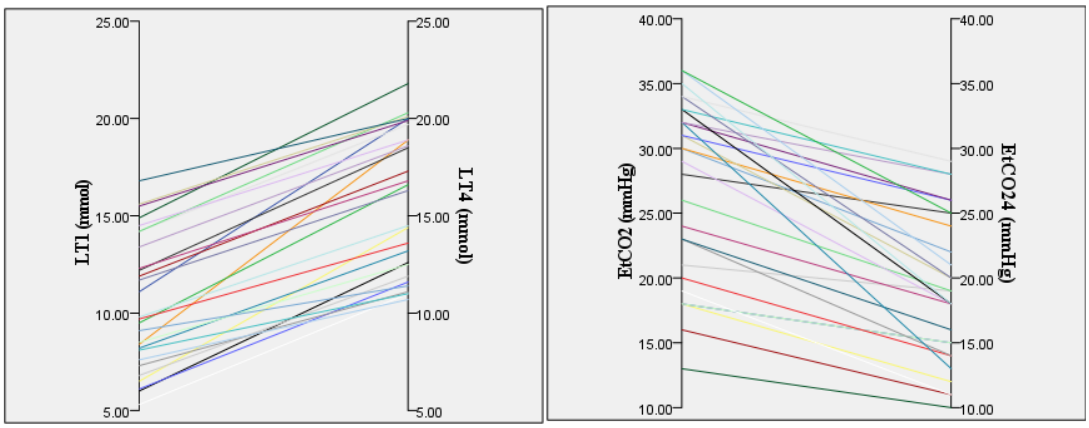


Figure 3 and Figure 4 Changes in BL and $EtCO_2$ across intervals

Table 4 Correlations between CO_2Tavg and all Research Variables

Average CO2T Correlations

		CO2avg	BLavg	Blrel	PeakHR	VCO2max	EtCO2avg	EtCO2rel	AC
CO2Tavg	Pearson Correlation	1	0.116	0.089	.380*	0.115	0.012	0.193	0.005
	Sig. (1-tailed)		0.278	0.327	0.023	0.280	0.475	0.163	0.491
	N	28	28	28	28	28	28	28	28

*. Correlation is significant at the 0.05 level (1-tailed).

**. Correlation is significant at the 0.01 level (1-tailed).

Discussion

The purpose of this study was to validate the only currently known CO_2T field test against measured CO_2, maximal ventilatory CO_2, and anaerobic capacity in endurance athletes. We hypothesized that the CO_2T field test would have a strong positive correlation to clinical CO_2 measurements, there would be no observed differences between groups among any measured variable, and anaerobic capacity would have a negative correlation to CO_2T. Based on the results here, we reject all three of the hypotheses.

This study did not find any meaningful relationship to the duration of a single-breath exhale and clinical CO_2 measurements. There was a strong positive correlation between the two CO_2T tests and this is consistent with previous studies which used the timed single-breath exhale test to measure individuals' CO_2T.[2–4] These results show that the single-breath exhale has a high degree of intrarater reliability but does not speak to the validity of the measurements. Previous studies have validated the CO_2T test as a measure of state and trait anxiety however these results only correlated exhale times to survey scores from the State-Trait Anxiety Index and did not use any clinical CO_2 measurements giving little insight into what the novel CO_2T test is actually measuring.

One possibility as to why none of the CO_2T values showed any correlations to clinical CO_2 measurements could be within the test itself. Though each individual participant was coached on how to complete the test and was given as many practice times as needed, the test relies heavily on mental restriction of exhalation as opposed to mechanical restriction. Investigators observed an increase in time over the practice sessions for all participants. Once exhalation times started to plateau the coaching was stopped and participants completed one more test and values were recorded. Comparisons of $EtCO_2$ measurements and CO_2T times showed no statically significant results. All participants showed a decrease in $EtCO_2$ across the testing intervals, which is to be expected as for output to continue, ventilation would have to increase and $EtCO_2$ would subsequently decrease. If the CO_2T test was a valid measure of CO_2, then regardless of the overall decrease in $EtCO_2$, participants with overall higher $EtCO_2$ values should have been able to exhale for a longer duration of time which was not measured or observed and can been seen in Fig 5. It is more likely that the CO_2T test is measuring

one's ability to simply control exhalation and resist re-breathing from a psychological standpoint rather than actual levels of CO_2.

Previous studies have shown that strenuous aerobic training can lead to an increase in pulmonary function measures such as vital capacity, maximal voluntary ventilation, and total lung capacity, regardless of age.[75] As age was not shown to be a factor in CO_2T test outcomes or $EtCO_2$ values and there was no control for the level at which the participants were at in their respective sport, it is plausible that the participants who were highly trained had the ability to ventilate more efficiently during maximal efforts. Though the testing modality was not aerobic by nature many mid to high level endurance athletes purposely train strenuous anaerobic intervals as anaerobic training can lead to increases in aerobic capacity.[76] Thus, when tasked with repeated anaerobic threshold intervals the participants who were highly trained were able to control the single-breath exhale for an extended period of time and were more efficient at offloading CO_2.

Some limitations should be considered in the present study. As previously stated not controlling for participant training or ability can be seen as a limitation and could have affected the outcome of the results. Our results for CO_2T could not be compared to gold standard CO_2 measurements for actual ABG or VBG. Even though $EtCO_2$ can be used as a comparative value[79], actual blood gas measurements may have shown a more accurate correlation. Under normal circumstances, $EtCO_2$ measurements are slightly lower than partial pressure arterial CO_2 and will decrease with exercise, which was observed across all participants. Though these observations align with previous research, there were no significant correlations between any $EtCO_2$ values and CO_2T test times.

Regardless of the decrease in $EtCO_2$ times over the testing intervals and because $EtCO_2$ values accurately correlate to arterial levels of CO_2; if the CO_2T test was accurately predicting CO_2 levels, then participants with higher CO_2T times should also have higher overall $EtCO_2$ values showing their ability to function under higher levels of CO_2; the opposite of this was observed. This could be due to the collection method, as $EtCO_2$ was measured via nasal cannula, and as previously participants with higher CO_2T times may be better at controlling pulmonary function and are simply better at ventilating during maximal efforts, resulting in lower $EtCO_2$ values. As previous research correlating $EtCO_2$ and arterial CO_2 pressure has primarily been done on subjects with cardio-pulmonary dysfunction, it is possible that $EtCO_2$ values were not accurate due to increased ventilation from higher output in normal functioning individuals during exercise.

The results from the present study can only be generalized to endurance athletes between the ages of 18 and 65. This athletic population can be considered a delimitation and future research should include non-athletic populations including those with and without a cardio-pulmonary afflictions. There has been substantial previous research on the effects CO_2 has on individuals with COPD, however these studies are highly clinical by nature.[80-82] As all the participants in the present study were able to effectively off-load CO_2 during exercise and may have altered their CO_2T, the possibility exists that the novel CO_2T test may have a higher correlation to clinical CO_2 values in the untrained or those who already possess a high degree of CO_2 sensitivity. Each of these populations have been shown to have diminished pulmonary functions when compared to athletes; correlations between the CO_2T test and clinical measurements among these populations

would also support the conclusion that the novel CO_2T is a better measure of pulmonary function in athletes as opposed to actual CO_2 levels.[30,83,84]

Other future research should consider comparing the CO_2T field test results to maximal breath holds and maximal breath holds to these same clinical measures of CO_2 production. As previously explained, CO_2 will continue to build regardless of O_2 availability, and ventilatory response must increase if output is maintained or increased.[85] Because of this, there is a possibility of a maximal breath-hold having a higher degree of correlation to actual CO_2 levels.[78] Because the CO_2T test seems to function more as a measure of pulmonary control rather than actually measuring circulating levels of CO_2 future studies should focus on comparing outcomes from the CO_2T test to pulmonary function tests to determine if the field test provides a better correlation. As highlighted from previous literature the "crossover point" or ventilatory threshold might be a better indicator of CO_2T; future studies attempting to correlate the CO_2T field test to clinical cardiopulmonary measurements should include ventilatory threshold as a primary research variable. Lastly, future studies attempting to implement or validate the novel CO_2T test should test participants during or after exercise. Because exhalation at rest in healthy individuals offloads all unnecessary CO_2 it is plausible that CO_2T values after anaerobic intervals, or during aerobic exercise could be more representative of CO_2 levels when breathing is compromised from increased physical exertion.

In summary, though the application of a timed single-breath exhale has a high degree of reliability, there is no evidence to support the notion that this novel field test accurately represents levels of produced CO_2. Furthermore, as ventilation during exercise in healthy adults provides ample CO_2 offloading, there does not seem to be a

need to evaluate CO_2 for the purposes of aerobic training under normal respiration conditions. As previous findings have shown it is possible to train CO_2 tolerance through the practice of apnea breathing protocols within the freediving community, more research needs to be done to support the timed single breath field test as a valid measure of tolerance to CO_2 and/or to find a more valid field test.

REFERENCES

1. Zhang X, Wargocki P, Lian Z. Physiological responses during exposure to carbon dioxide and bioeffluents at levels typically occurring indoors. *Indoor Air*. 20[1]7;27(1):65-77. doi:10.1111/ina.12286
2. Bently T, Wilson R, MacKenzie B, Russell S. Anxiety, CO2 tolerance, and breath practice in high school students: feasibility and observations from a 6-week slow breathing program. 2020. https://static1.squarespace.com/static/5bf8b5c6297114fff40f5fc4/t/5edace57d16497 5b782d560e/1591397982242/Bentley_ICIMH_2020.pdf
3. Stoeckel MC, Esser RW, Gamer M, Büchel C, von Leupoldt A. Dyspnea catastrophizing and neural activations during the anticipation and perception of dyspnea. *Psychophysiology*. 2018;55(4):e13004. doi:10.1111/psyp.13004
4. Spirmont P. The Use of a Timed Exhale CO2 Tolerance Test as an Index of State/Trait Anxiety in Healthy Adults. Published online 2019.
5. Sebert P, Barthelemy L, Mialon P. CO_2 Chemoreflex Drive of Ventilation in Man: Effects of Hyperoxia and Sex Differences. *Respiration*. 1990;57(4):264-267. doi:10.1159/000195853
6. Kurra S, Lathtinen, Kimmo, Nissinen, Ari. *Freediving*. 1st ed. Deep Ideas Oy; 2015.
7. Lemaître F, Joulia F, Chollet D. Apnea: A new training method in sport? *Med Hypotheses*. 2010;74(3):413-415. doi:10.1016/j.mehy.2009.09.051
8. Santin JM. How important is the CO2 chemoreflex for the control of breathing? Environmental and evolutionary considerations. *Comp Biochem Physiol A Mol Integr Physiol*. 2018;215:6-19. doi:10.1016/j.cbpa.2017.09.015

9. Skow RJ, Day TA, Fuller JE, Bruce CD, Steinback CD. The ins and outs of breath holding: simple demonstrations of complex respiratory physiology. *Adv Physiol Educ*. 2015;39(3):223-231. doi:10.1152/advan.00030.2015

10. Joulia F, Steinberg JG, Faucher M, et al. Breath-hold training of humans reduces oxidative stress and blood acidosis after static and dynamic apnea. *Respir Physiol Neurobiol*. 2003;137(1):19-27. doi:10.1016/S1569-9048(03)00110-1

11. Martarelli D, Cocchioni M, Scuri S, Pompei P. Diaphragmatic Breathing Reduces Exercise-Induced Oxidative Stress. *Evid Based Complement Alternat Med*. 2011;2011:1-10. doi:10.1093/ecam/nep169

12. Murphey J, Lafrenz A. Effects of Nasal Only and Apnea Breathing on Performance in Trained Cyclists. Published online 2020.

13. McArdle WD, Katch FI, Katch VL. *Exercise Physiology: Nutrition, Energy, and Human Performance*. Eighth edition. Wolters Kluwer Health/Lippincott Williams & Wilkins; 2015.

14. Conway A, Tipton E, Liu WH, et al. Accuracy and precision of transcutaneous carbon dioxide monitoring: a systematic review and meta-analysis. *Thorax*. 2019;74(2):157-163. doi:10.1136/thoraxjnl-2017-211466

15. Gerdung CA, Adeleye A, Kirk VG. Noninvasive monitoring of CO2 during polysomnography: a review of the recent literature. *Curr Opin Pulm Med*. 2016;22(6):527-534. doi:10.1097/MCP.0000000000000320

16. Gerlach G, Guth U, Oelßner W. CO2 Sensing in Medicine. In: *Carbon Dioxide Sensing: Fundamentals, Principles, and Applications*. 1st ed. Wiley; 2019:391-413. doi:10.1002/9783527688302

17. D. Mitchell R, Crandall C. Validation of the 15 Minute Balke Field Test for Competitive, Adult 5K Runners: From Treadmill VO_{2max} Testing to Enhancing Performance. *Am J Sports Sci Med*. 2017;5(3):44-47. doi:10.12691/ajssm-5-3-1

18. Hazir T, Kose MG, Kin-Isler A. The validity of Running Anaerobic Sprint Test to assess anaerobic power in young soccer players. *Isokinet Exerc Sci*. 2018;26(3):201-209. doi:10.3233/IES-182117

19. Steinberg A, Manlhiot C, Li P, et al. Development and Validation of Bioelectrical Impedance Analysis Equations in Adolescents with Severe Obesity. *J Nutr*. 2019;149(7):1288-1293. doi:10.1093/jn/nxz063

20. Trembach N, Zabolotskikh I. The Influence of Age on Interaction between Breath-Holding Test and Single-Breath Carbon Dioxide Test. *BioMed Res Int*. 2017; 1-5. doi:10.1155/2017/1010289

21. Stanford N. Apnea Training and Physical Characteristics: Enhancement of the Dive Response, Apneic Time, and Recovery. Published online May 2019. doi:10.18122/td/1544/boisestate

22. Lourenço SM de S. The psychology of freediving: psychological strategies used by elite freedivers. Published online 2018.

23. Pate RR, Branch JD. Training for endurance sport. *Med Sci Sports Exerc*. 1992;24(9 Suppl):S340-343.

24. Fujimoto S, Suzuki M, Sakamoto K, et al. Comparison of End-Tidal, Arterial, Venous, and Transcutaneous P_{CO2} . *Respir Care*. 2019;64(10):1208-1214. doi:10.4187/respcare.06094

25. CO2 Tolerance Breath Test. SH//FT. Accessed November 24, 2020. https://shiftadapt.com/breath-test-calculator/

26. Bently T, Wilson R, MacKenzie B, Russell S. Anxiety, CO2 tolerance, and breath practice in high school students: feasibility and observations from a 6-week slow breathing program. In: ; 2020. https://static1.squarespace.com/static/5bf8b5c6297114fff40f5fc4/t/5edace57d164975b782d560e/1591397982242/Bentley_ICIMH_2020.pdf

27. Batista MB, Romanzini CLP, Castro-Piñero J, Ronque ERV. VALIDITY OF FIELD TESTS TO ESTIMATE CARDIORESPIRATORY FITNESS IN CHILDREN AND ADOLESCENTS: A SYSTEMATIC REVIEW. *Rev Paul Pediatr Orgao Of Soc Pediatr Sao Paulo*. 2017;35(2):222-233. doi:10.1590/1984-0462/;2017;35;2;00002

28. Duffin J. The chemoreflex control of breathing and its measurement. *Can J Anaesth*. 1990;37(8):933-942. doi:10.1007/BF03006641

29. Roussos C, Koutsoukou A. Respiratory failure. *Eur Respir J*. 2003;22(Supplement 47):3s-14s. doi:10.1183/09031936.03.00038503

30. Burgraff NJ, Neumueller SE, Buchholz K, et al. Ventilatory and integrated physiological responses to chronic hypercapnia in goats: Ventilatory and physiological responses to chronic hypercapnia. *J Physiol*. 2018;596(22):5343-5363. doi:10.1113/JP276666

31. Spruit MA, Burtin C, De Boever P, et al. COPD and exercise: does it make a difference? *Breathe*. 2016;12(2):e38-e49. doi:10.1183/20734735.003916

32. Clark TJH, Godfrey S. The effect of CO_2 on ventilation and breath-holding during exercise and while breathing through an added resistance. *J Physiol*. 1969;201(3):551-566. doi:10.1113/jphysiol.1969.sp008772

33. Chowdhuri S, Badr MS. Control of Ventilation in Health and Disease. *Chest*. 2017;151(4):917-929. doi:10.1016/j.chest.2016.12.002

34. Borg G. *Borg's Perceived Exertion and Pain Scales*. Human Kinetics; 1998.

35. Fukuoka Y, Endo M, Oishi Y, Ikegami H. Chemoreflex Drive and the Dynamics of Ventilation and Gas Exchange during Exercise at Hypoxia. *Am J Respir Crit Care Med*. 2003;168(9):1115-1122. doi:10.1164/rccm.2202027

36. Granger EA, Cooper TK, Hopkins SR, McKenzie DC, Dominelli P. Peripheral chemoresponsiveness during exercise in male athletes with exercise-induced arterial hypoxaemia. *Exp Physiol*. 2020;105(11):1960-1970. doi:10.1113/EP088639

37. Harms CA, Stager JM. Low chemoresponsiveness and inadequate hyperventilation contribute to exercise-induced hypoxemia. *J Appl Physiol*. 1995;79(2):575-580. doi:10.1152/jappl.1995.79.2.575

38. Weil JV, Byrne-Quinn E, Sodal IE, Kline JS, McCullough RE, Filley GF. Augmentation of chemosensitivity during mild exercise in normal man. *J Appl Physiol*. 1972;33(6):813-819. doi:10.1152/jappl.1972.33.6.813

39. Guz A. Brain, breathing and breathlessness. *Respir Physiol*. 1997;109(3):197-204. doi:10.1016/S0034-5687(97)00050-9

40. Pal GK, Velkumary S, Madanmohan null. Effect of short-term practice of breathing exercises on autonomic functions in normal human volunteers. *Indian J Med Res*. 2004;120(2):115-121.

41. Lemaitre F, Fahlman A, Gardette B, Kohshi K. Decompression sickness in breath-hold divers: A review. *J Sports Sci*. 2009;27(14):1519-1534. doi:10.1080/02640410903121351

42. Walterspacher S, Scholz T, Tetzlaff K, Sorichter S. Breath-Hold Diving: Respiratory Function on the Longer Term. *Med Sci Sports Exerc*. 2011;43(7):1214-1219. doi:10.1249/MSS.0b013e31820a4e0c

43. Gerlach G. *Carbon Dioxide Sensing.* Wiley Vch; 2015.

44. Huttmann SE, Windisch W, Storre JH. Techniques for the Measurement and Monitoring of Carbon Dioxide in the Blood. *Ann Am Thorac Soc*. 2014;11(4):645-652. doi:10.1513/AnnalsATS.201311-387FR

45. Zeserson E, Goodgame B, Hess JD, et al. Correlation of Venous Blood Gas and Pulse Oximetry With Arterial Blood Gas in the Undifferentiated Critically Ill Patient. *J Intensive Care Med*. 2018;33(3):176-181. doi:10.1177/0885066616652597

46. Byrne AL, Bennett M, Chatterji R, Symons R, Pace NL, Thomas PS. Peripheral venous and arterial blood gas analysis in adults: are they comparable? A systematic review and meta-analysis: Systematic review of PVBG and ABG analysis. *Respirology*. 2014;19(2):168-175. doi:10.1111/resp.12225

47. Raffin TA. Diagnostic Decision: Indications for Arterial Blood Gas Analysis. *Ann Intern Med*. 1986;105(3):390. doi:10.7326/0003-4819-105-3-390

48. Potter L. How to take an Arterial Blood Gas (ABG) - OSCE Guide. Geeky Medics. Accessed November 24, 2020. https://geekymedics.com/arterial-blood-gas-sampling/

49. Hughes J. Blood gas estimations from arterialized capillary blood versus arterial puncture: are they different? *Eur Respir J*. 1996;9(2):184.

50. Vaquer S, Masip J, Gili G, et al. Earlobe arterialized capillary blood gas analysis in the intensive care unit: a pilot study. *Ann Intensive Care*. 2014;4(1):11. doi:10.1186/2110-5820-4-11

51. Lewis CT, Malein WL, Chesner I, Clarke S. High altitude arterialised capillary earlobe blood gas measurement using the Abbott i-STAT. *J R Army Med Corps*. 2018;164(5):335-337. doi:10.1136/jramc-2017-000902

52. Lumholdt M, Damgaard KA, Christensen EF, Leutscher PDC. Mathematical arterialisation of peripheral venous blood gas for obtainment of arterial blood gas values: a methodological validation study in the clinical setting. *J Clin Monit Comput*. 2019;33(4):733-740. doi:10.1007/s10877-018-0197-1

53. Wadhwa V, Vargo JJ. Sedation and Monitoring in Endoscopy. In: *Clinical Gastrointestinal Endoscopy*. Elsevier; 2019:81-90.e2. doi:10.1016/B978-0-323-41509-5.00007-4

54. Ekkernkamp E, Welte L, Schmoor C, et al. Spot Check Analysis of Gas Exchange: Invasive versus Noninvasive Methods. *Respiration*. 2015;89(4):294-303. doi:10.1159/000371769

55. Grangeat P, Gharbi S, Accensi M, Grateau H. First Evaluation of a Transcutaneous Carbon Dioxide Monitoring Wristband Device during a Cardiopulmonary Exercise Test *. In: *2019 41st Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC)*. IEEE; 2019:3352-3355. doi:10.1109/EMBC.2019.8857020

56. Tang R, Huang Y, Chen Q, et al. The Effect of Alveolar Dead Space on the Measurement of End-Expiratory Lung Volume by Modified Nitrogen Wash-Out/Wash-In in Lavage-Induced Lung Injury. *Respir Care*. 2012;57(12):2074. doi:10.4187/respcare.01800

57. Stege G, van den Elshout FJJ, Heijdra YF, van de Ven MJT, Dekhuijzen PNR, Vos PJE. Accuracy of Transcutaneous Carbon Dioxide Tension Measurements during Cardiopulmonary Exercise Testing. *Respiration*. 2009;78(2):147-153. doi:10.1159/000187631

58. Datta D, Normandin E, ZuWallack R. Cardiopulmonary exercise testing in the assessment of exertional dyspnea. *Ann Thorac Med*. 2015;10(2):77. doi:10.4103/1817-1737.151438

59. Melo X, Santa-Clara H, Almeida JP, et al. Comparing several equations that predict peak VO2 using the 20-m multistage-shuttle run-test in 8–10-year-old children. *Eur J Appl Physiol*. 2011;111(5):839-849. doi:10.1007/s00421-010-1708-z

60. Gurton WH, Gough LA, Sparks SA, Faghy MA, Reed KE. Sodium Bicarbonate Ingestion Improves Time-to-Exhaustion Cycling Performance and Alters Estimated Energy System Contribution: A Dose-Response Investigation. *Front Nutr*. 2020;7:154. doi:10.3389/fnut.2020.00154

61. Battaglia M, Pesenti-Gritti P, Spatola CAM, Ogliari A, Tambs K. A twin study of the common vulnerability between heightened sensitivity to hypercapnia and panic disorder. *Am J Med Genet B Neuropsychiatr Genet*. 2008;147B(5):586-593. doi:10.1002/ajmg.b.30647

62. Lundby C, Montero D, Joyner M. Biology of VO_2 max: looking under the physiology lamp. *Acta Physiol*. 2017;220(2):218-228. doi:10.1111/apha.12827

63. *Acsm's Guidelines for Exercise Testing and Prescription.* Lippincott Williams & Wilkins; 2017.

64. Price OJ, Tsakirides C, Gray M, Stavropoulos-Kalinoglou A. ACSM Preparticipation Health Screening Guidelines: A UK University Cohort Perspective. *Med Sci Sports Exerc*. 2019;51(5):1047-1054. doi:10.1249/MSS.0000000000001868

65. Whitfield GP, Riebe D, Magal M, Liguori G. Applying the ACSM Preparticipation Screening Algorithm to U.S. Adults: National Health and Nutrition Examination Survey 2001–2004. *Med Sci Sports Exerc*. 2017;49(10):2056-2063. doi:10.1249/MSS.0000000000001331

66. Bailey BW, LeCheminant G, Hope T, Bell M, Tucker LA. A comparison of the agreement, internal consistency, and 2-day test stability of the InBody 720, GE iDXA, and BOD POD ® gold standard for assessing body composition. *Meas Phys Educ Exerc Sci*. 2018;22(3):231-238. doi:10.1080/1091367X.2017.1422129

67. Hernández-Belmonte A, Buendía-Romero Á, Martínez-Cava A, Courel-Ibáñez J, Mora-Rodríguez R, Pallarés JG. Wingate Test, When Time and Overdue Fatigue Matter: Validity and Sensitivity of Two Time-Shortened Versions. *Appl Sci*. 2020;10(22):8002. doi:10.3390/app10228002

68. Wingate Anaerobic Test. https://educationalathletics.weebly.com/uploads/2/9/9/0/29907311/wingate_testing.pdf

69. Bertuzzi R, Kiss MAPDM, Damasceno M, Oliveira RSF, Lima-Silva AE. Association between anaerobic components of the maximal accumulated oxygen deficit and 30-second Wingate test. *Braz J Med Biol Res*. 2015;48(3):261-266. doi:10.1590/1414-431x20144043

70. Hart S, Drevets K, Alford M, Salacinski A, Hunt BE. A method-comparison study regarding the validity and reliability of the Lactate Plus analyzer. *BMJ Open*. 2013;3(2): e001899. doi:10.1136/bmjopen-2012-001899

71. Baig MA, Shahzad H, Hussain E, Mian A. Validating a point of care lactate meter in adult patients with sepsis presenting to the emergency department of a tertiary care hospital of a low- to middle-income country. *World J Emerg Med*. 2017;8(3):184-189. doi:10.5847/wjem.j.1920-8642.2017.03.004

72. School of Sport & Exercise, Massey University, Palmerston North, New Zealand, Miller MC, Macdermid PW, School of Sport & Exercise, Massey University, Palmerston North, New Zealand. Predictive Validity of Critical Power, The Onset of Blood Lactate and Anaerobic Capacity for Cross-Country Mountain Bike Race Performance. *Sports Exerc Med - Open J*. 2015;1(4):105-110. doi:10.17140/SEMOJ-1-116

73. Crouter SE, Antczak A, Hudak JR, DellaValle DM, Haas JD. Accuracy and reliability of the ParvoMedics TrueOne 2400 and MedGraphics VO2000 metabolic systems. *Eur J Appl Physiol*. 2006;98(2):139-151. doi:10.1007/s00421-006-0255-0

74. Woods AL, Garvican-Lewis LA, Rice AJ, Thompson KG. The Ventilation-Corrected ParvoMedics TrueOne 2400 Provides a Valid and Reliable Assessment of Resting Metabolic Rate (RMR) in Athletes Compared With the Douglas Bag Method. *Int J Sport Nutr Exerc Metab*. 2016;26(5):454-463. doi:10.1123/ijsnem.2015-0315

75. Hagberg JM, Yerg JE, Seals DR. Pulmonary function in young and older athletes and untrained men. Journal of Applied Physiology. 1988;65(1):101-105. doi:10.1152/jappl.1988.65.1.101

76. Sözen H, Akyildiz C. The Effects of Aerobic and Anaerobic Training on Aerobic and Anaerobic Capacity. Uluslararası Anadolu Spor Bilimleri Dergisi. 2018;3(3):331-337.

77. Siri WE. Body composition from fluid spaces and density: analysis of methods. 1961. *Nutrition*. 1993;9(5):480-491; discussion 480, 492.

78. Lindholm P, Lundgren CEG. Alveolar gas composition before and after maximal breath-holds in competitive divers. *Undersea Hyperb Med J Undersea Hyperb Med Soc Inc*. 2006;33(6):463-467.

79. Fujimoto S, Suzuki M, Sakamoto K, et al. Comparison of End-Tidal, Arterial, Venous, and Transcutaneous P CO2 . *Respir Care*. 2019;64(10):1208-1214. doi:10.4187/respcare.06094

80. Campoi H, Campoi E, Lopes R, Regueiro E. Effects of physical activity on aerobic capacity, pulmonary function and respiratory muscle strength of football athletes and sedentary individuals. Is there a correlation between these variables? *J Phys Educ Sport*. 2019;2019(04).

81. Attarzadeh Hosseini SR, Department of Exercise Physiology, Faculty of Sport Sciences, Ferdowsi University of Mashhad, Mashhad, Iran., Hejazi K, Department of Exercise Physiology, Faculty of Sport Sciences, Ferdowsi University of Mashhad, Mashhad, Iran. The Relationship Between Peak Oxygen Consumption and Pulmonary Function Indices: Measured by Spirometry and Allometry Equation in Male Students. *Phys Treat - Specif Phys Ther*. 2016;6(3):137-142. doi:10.18869/nrip.ptj.6.3.137

82. Goldberg S, Ollila HM, Lin L, et al. Analysis of Hypoxic and Hypercapnic Ventilatory Response in Healthy Volunteers. Penzel T, ed. *PLOS ONE*. 2017;12(1):e0168930. doi:10.1371/journal.pone.0168930

83. Wang J, Guo S, Zeng M, Yu P, Mo W. Observation of the curative effect of device-guided rehabilitation on respiratory function in stable patients with chronic obstructive pulmonary disease. *Medicine (Baltimore)*. 2019;98(8):e14034. doi:10.1097/MD.0000000000014034

84. Spruit MA, Burtin C, De Boever P, et al. COPD and exercise: does it make a difference? *Breathe*. 2016;12(2):e38-e49. doi:10.1183/20734735.003916

85. Lindholm P, Lundgren CE. The physiology and pathophysiology of human breath-hold diving. *J Appl Physiol*. 2009;106(1):284-292. doi:10.1152/japplphysiol.90991.2008

APPENDIX A

Additional Results

Physical Characteristics

Participant age was inversely correlated to HRpeak ($r = -0.431$, $p < 0.05$), LBM ($r = -0.426$, $p < 0.05$); and was directly correlated to BF ($r = 0.363$, $p < 0.05$). Lean body mass showed a positive correlation to $\dot{V}CO_2$max ($r = 0.513$, $p < 0.01$) and height ($r = 0.444$, $p < 0.01$). Subject demographics can be found in Table 2. Group statistics for all primary and secondary research variables can be found in Table 3.

Clinical CO_2 Measurements

Maximal Exhaled CO_2

Maximal exhaled CO_2 values were recorded five seconds post 30-WT to allow for delay in exhalation and instrument capture. There were no significant differences between $\dot{V}CO_2$ max values between participants or groups ($p < 0.01$, $p < 0.05$ respectively). There was a small positive correlation to CO_2T but did not reach statistical significance ($r = 0.115$, $p = 0.280$).

End-Tidal CO_2

A paired t-test reviled $EtCO_2$ values between tests were significantly different (-1.10 ± 2.54, $t(27) = -1.37$, $p < 0.001$). Absolute and relative change values were calculated across all four $EtCO_2$ tests and Mean±SD are reported in Table 2. Neither relative nor absolute $EtCO_2$ showed a significant correlation to CO_2T. All four $EtCO_2$ values were cross correlated to CO_2T times and $EtCO_2(1)$ showed a moderate negative correlation to CO_2T ($r = -0.393$, $p < 0.05$). There were no observed differences between groups for any $EtCO_2$ measurement. All participants experienced a decrease in $EtCO_2$ across intervals 1-4 as seen in Fig 3. Figure 4 represents the relationship between CO_2T times and $EtCO_2$ beginning and ending values.

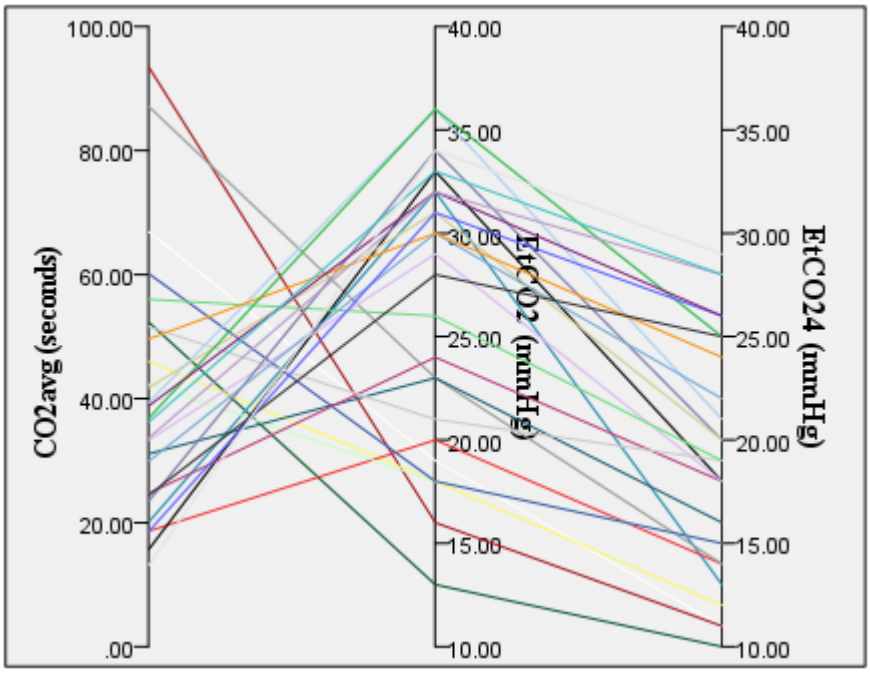


Figure 5 CO_2T and $EtCO_2$ Comparison

Secondary Variables

Peak Heart Rate

Peak heart rate was recorded at the end of each testing session. No significant differences were found between testing days ($p = 0.435$). The average between testing days was used for comparisons. A moderately positive correlation was found between HRpeak and CO_2T ($r = 0.380$, $p < 0.01$) and $\dot{V}CO_{2max}$ ($r = 0.335$, $p < 0.05$) and a moderate negative correlation between age ($r = -0.431$, $p < 0.05$) and BF ($r = -0.341$, $p < 0.05$) was also observed. There were no statically significant differences between groups for HRpeak. Mean ± SD are reported for participants and groups in Table 3.

Anaerobic Capacity

Aerobic Capacity was noted after each interval and the highest value was recorded. Anaerobic capacity showed no correlation to any of the primary or secondary testing variables at or beyond significance level. A test of between group effects showed no significant differences ($t(26) = 1.41$, $p = 0.171$).

Lactic Acid

A paired t-test reviled BL values between tests were significantly different (-4.89 ± 2.54, $t(27) = -10.16$, $p < 0.001$). Absolute and relative change values were calculated across all four BL tests and Mean±SD are reported in Table 3. No correlations between BL and CO_2T, or any other research variable reached statistical significance. Absolute and relative BL measurements showed no significant differences between groups. Lactic acid increased across all participants from intervals 1-4 as seen in Fig 2.

APPENDIX B

Additional Discussion

As previously stated $EtCO_2$ fell across all intervals among all participants and BL rose in a similar fashion, which can be seen in Figures 2 and 3, which is to be expected as participants tried to maintain anaerobic output throughout the duration of the graded exercise test. Likewise, AC was used to determine when the participants had reached a plateau and AC values consistently decreased over time. It was hypothesized that AC would have a negative correlation to CO_2T times, meaning the longer the duration of exhalation the lower AC would be. The rationale behind this lies in differences between pure endurance athletes and though that have a greater ability to produce anaerobic power. Previous studies have shown there to be no correlation between anaerobic efforts and CO_2T[14,15] where as others have shown a positive relationship among endurance athletes[2].

Though these studies used CO_2T as a measurement value moreover the focus was on nasal vs. oral breathing. Since the participants were breathing primarily through their nose during the $EtCO_2$ collection phase, the distribution of participants who had higher AC should have had lower overall CO_2T scores, lower $EtCO_2$, and higher BL, which was either observed or measured. Again, one possible reason for this could be the previously described nature of the CO_2T test, or simply that the CO_2T test does not measure an individual's level of CO_2 but their ability to control breathing rates, especially when nasal breathing.

APPENDIX C

IRB Approval

Date: September 20, 2021

To: Shawn Simonson

cc:Joshua Murphey

From: Biomedical Institutional Review Board

(MED-IRB) c/o Office of Research

Compliance (ORC)

Subject: MED-IRB Notification of Approval - Original – 186-MED21-007

Validation of Non-Invasive CO2 Tolerance Field Test

The Boise State University IRB has approved your protocol submission. Your protocol is in compliance with this institution's Federal Wide Assurance (#0000097) and the DHHS Regulations for the Protection of Human Subjects (45 CFR 46).

Protocol Number:	**186-MED21-007**	Received:	7/26/2021	Review:	Expedited
		Approved:	9/20/2021	Category:	2, 4
Expires:	**9/19/2022**				

Your approved protocol is effective until 9/19/2022. To remain open, your protocol must be renewed on an annual basis and cannot be renewed beyond 9/19/2024. For the activities to continue beyond 9/19/2024, a new protocol application must be submitted.

ORC will notify you of the protocol's upcoming expiration roughly 30 days prior to 9/19/2022. You, as the PI, have the primary responsibility to ensure any forms are submitted in a timely manner for the approved activities to continue. If the protocol is not renewed before 9/19/2022, the protocol will be closed. If you wish to continue the activities after the protocol is closed, you must submit a new protocol application for MED-IRB review and approval.

You must notify the MED-IRB of any changes to your approved protocol and the committee must review and approve these changes prior to their commencement. You should also notify the committee if your activities are complete or discontinued.

Current forms are available on the ORC website at http://goo.gl/D2FYTV

Please direct any questions or concerns to ORC at 426-5401 or humansubjects@boisestate.edu. Thank you and good luck with your research.

1910 University Drive Boise, Idaho 83725-1139
Phone (208) 426-5401 orc@boisestate.edu

APPENDIX D

Informed Consent

INFORMED CONSENT FORM
Study Title: **Validation of a Non-Invasive CO_2 Tolerance Field Test**
Principal Investigator: **Josh Murphey**
Co-Principal Investigator: **Dr. Shawn Simonson**
Sponsor: **Boise State University**

You are invited to participate in a research study. This consent form will provide you the information you will need to understand why this study is being done and why you are being invited to participate. It will also describe what will be expected of you as a participant, as well as any known risks, inconveniences or discomforts that you may have while participating. We encourage you to ask questions at any time. If you decide to participate, you will be asked to sign this form and it will be a record of your agreement to participate. You will be given a copy of this form to keep.

PURPOSE AND BACKGROUND

The purpose of this study is to validate the only known current CO_2 tolerance field test against measured maximal dissolved CO_2 and maximal ventilatory carbon dioxide in endurance athletes. We expect approximately 22 volunteers. No one will be paid to be in the study. We will begin enrollment on 08/01/2021 and end enrollment on 12/01/2021.

PROCEDURES

If you agree to be in this study, you will participate in the following:

We will set up a time for you to meet one of the Boise State University Human Performance Lab investigators on two separate occasions, each day separated by at least 48 hours of rest. Day one will consist of anthropomorphic measurements, a timed breath exhale, venous blood gas measurement, and measurement of maximal ventilatory CO_2 during a maximal anaerobic threshold testing. Day two will consist of blood lactate measurement and measurement of end-tidal CO_2 during maximal anaerobic threshold testing. Day three will consist of a threshold CO_2 Tolerance test. Data collection will be led by the Primary Investigator, Joshua Murphey, Co-Investigator, Professor Dr. Shawn Simonson at Boise State University will also assist in data collection.

RISKS

Maximal effort exercise testing can cause feelings of light-headedness, dizziness, nausea, exhaustion, and possible muscle soreness. A proper warm-up and cool-down will help diminish the risk of these side effects. Anaerobic threshold testing used in this study is a common practice in the lab and the field of exercise science in general.

Risks of timed exhale and breath-hold Tests: The breath-hold tests and timed exhale tests can cause temporary light-headedness and minor discomfort. Due to the nature of the testing, participants will likely experience feelings of breathlessness and the associated discomforts during the tests. In very rare cases, prolonged breath-holding may lead to faintness or momentary loss of consciousness. However, participants will be explicitly instructed to cease the breath-hold if they encounter faintness. In addition, participants may terminate the test at any time. Also, due to the nature of the test (to measure participants' physical and mental capacities for breath-hold-associated sensations), participants will cease testing at the limits of their capacities and not beyond. The researcher will be monitoring the duration of the test to ensure the safety of the participant

Occasionally one or more of the following potential side effects of taking blood samples may occur: pain, bruising, slight bleeding, light-headedness, fainting, and (rarely) an infection. A trained technician will be drawing the blood. The treatment or procedure may involve risks that are currently unforeseeable. There are no known risks or discomforts from the saliva collection technique.

There are no other risks aside from collecting your information.

BENEFITS

You will not derive any direct benefit, and with the information you provide, the protentional to society would be the validation of a CO_2 Tolerance Field test which could have further beneficial research and training applications.

EXTENT OF CONFIDENTIALITY

Reasonable efforts will be made to keep the personal information in our research records private and confidential. Any identifiable information obtained in connection with this study will remain confidential and will be disclosed only with your permission or as required by law. The members of the research team and the Boise State University Office of Research Compliance (ORC) may access the data. The ORC monitors research studies to protect the rights and welfare of research participants.

Your name will not be used in any written reports or publications which result from this research.

Data will be kept for at least 3 years (per federal regulations) after the study is complete and then destroyed.

PAYMENT/COMPENSATION

You will not be paid or compensated for your participation in this research study.

PARTICIPATION IS VOLUNTARY

Your decision to participate in this research study is entirely voluntary. Your participation is greatly appreciated, but we acknowledge that the tasks being performed may have the potential to cause physical or emotional duress. You are free at any point to choose not to engage with or stop the study. You may choose not to participate in any portion of the preliminary testing, study intervention, or post-testing. This study is not required, and there is no penalty for not participating. If at any time you experience a negative emotion or physical effect from any portion of the preliminary testing, study intervention, or post-testing, we will stop any test being performed, or you may choose to withdraw yourself\

QUESTIONS

If you have any questions or concerns about your participation in this study, you may contact the Principal Investigator, Dr. Missy Elliott: 208-426-0000 or _ or Dr. Shawn Simonson:

This study has been reviewed and approved by the Boise State University IRB (IRB). If you have questions about your rights as a research participant, you may contact the IRB, which is concerned with the protection of volunteers in research projects. You may reach the board through the Office of Research Compliance by calling (208) 426-5401 or emailing humansubjects@boisestate.edu.

DOCUMENTATION OF CONSENT

I have read this form and the descriptions of this research study. I have been informed of the risks and benefits involved and all of my questions have been answered to my satisfaction. Furthermore, I have been assured that any future questions I may have will also be answered by a member of the research team. I understand I can withdraw at any time. I voluntarily agree to take part in this research study.

Printed Name of Study Participant	**Signature** of Study Participant	Date

Signature of Person Obtaining Consent	Date

APPENDIX E

Participant Screening Questionnaires

ACSM HEALTH STATUS & HEALTH HISTORY QUESTIONNAIRE

This form includes several questions regarding your physical health – please answer every question as accurately as possible. Please ask us if you have any questions. Your responses will be treated in a confidential manner.

PERSONAL INFORMATION

Last Name: _____ First Name: _____

Gender: F M

Mobile: _____ Email:

Date of Birth ____/ ____ / Height _____ Weight _____

YES NO (ACSM HEALTH SCREEN)

☐ ☐ Do you have any personal history of heart disease (coronary or atherosclerotic disease)?

☐ ☐ Any personal history of diabetes or other metabolic disease (thyroid, renal, liver)?

☐ ☐ Any personal history of pulmonary disease, asthma, interstitial lung disease or cystic fibrosis?

☐ ☐ Have you experienced pain or discomfort in your chest apparently due to blood flow deficiency?

☐ ☐ Any unaccustomed shortness of breath (perhaps during light exercise)?

☐ ☐ Have you had any problems with dizziness or fainting?

☐ ☐ Do you have difficulty breathing while standing or sudden breathing problems at night?

☐ ☐ Have you experienced a rapid throbbing or fluttering of the heart?

☐ ☐ Do you suffer from ankle edema (swelling of the ankles)?

☐ ☐ Have you experienced severe pain in leg muscles during walking?

☐ ☐ Do you have a known heart murmur?

☐ ☐ Has your serum cholesterol been measured at greater than 200 mg/dl?

☐ ☐ Are you a cigarette smoker?

☐ ☐ Has your HDL (the "good" cholesterol) been measured at greater than 60 mg/dl?

☐ ☐ Would you characterize your lifestyle as "sedentary"?

☐ ☐ Have you had a high fasting blood glucose level on 2 or more occasions (>=110mg/dl)?

☐ ☐ Are you 20% or more overweight or have you been told your “BMI” was greater than 30?

☐ Have you been assessed as hypertensive on at least 2 occasions (systolic > 140mmHg or diastolic > 90mmHg)?

☐ Do you have any family history of cardiac or pulmonary disease prior to age 55?

MEDICAL HISTORY

☐ Are you currently being treated for high blood pressure?

If you know your average blood pressure, please enter: _____/_____

Please check all conditions or diagnoses that apply:

☐Abnormal EKG?

☐Abnormal Chest X-Ray?

☐ Rheumatic Fever?

☐ Low Blood Pressure?

☐ Asthma?

☐ Bronchitis?

☐ Emphysema?

☐ Other Lung Problems?

☐ Recently Broken Bones?

☐ Limited Range of Motion?

☐ Arthritis?

☐ Bursitis?

☐ Swollen or Painful Joints?

☐ Foot Problems?

☐ Knee Problems?

☐ Back Problems?

☐ Shoulder Problems?

☐ Stroke?

☐ Do You Suffer from Epilepsy or Seizures?

☐ Chronic Headaches or Migraines?

☐ Persistent Fatigue?

☐ Stomach Problems?

☐ Hernia?

☐ Anemia?

☐ Are You Pregnant?

☐ Has a doctor imposed any activity restrictions? If so, please describe:

_____

_____

FAMILY HISTORY

Have your mother, father, or siblings suffered from (please select all that apply):

☐ Heart attack or surgery prior to age 55.

☐ Stroke prior to age 50.

☐ Congenital heart disease or left ventricular hypertrophy.

☐ Hypertension

☐ Leukemia or cancer prior to age 60.

☐ High cholesterol

☐ Diabetes

☐ Obesity

☐ Asthma

☐ Osteoporosis

MEDICATIONS

Please Select Any Medications You Are Currently Using:

☐ Diuretics	☐ Other Cardiovascular
☐ Beta Blockers	☐ NSAIDS/Anti-inflammatories (Motrin, Advil)
☐ Vasodilators	☐ Cholesterol
☐ Alpha Blockers	☐ Diabetes/Insulin
☐ Calcium Channel Blockers	☐ Other Drugs (record below).

LIFESTYLE

☐ Are you a cigarette smoker? If so, how many per day? __

☐ Previously a cigarette smoker? If so, when did you quit? _____

How many years have you smoked or did you smoke before quitting? ___

Do you/did you smoke (Circle one): Cigarettes Cigars Pipe

Please Rate Your Daily Stress Levels (select one):

☐ Low ☐ Moderate ☐ High but I enjoy the challenge ☐ High: sometimes difficult to handle

☐ High: often difficult to handle

☐ Do you drink alcoholic

beverages?

How many units of alcohol do you consume per week: _____
(see Alcohol Units Chart)

1 pub measure of spirits (Gin, Vodka etc.)	1
1 can of beer	1.5
1 bottle of strong lager	2.5
1 can of strong lager	4
1 bottle of wine	7
1 liter bottle of wine	10
1 bottle of fortified wine (port, sherry etc.)	14
1 bottle of spirits	30

Alcohol Units Table

Type of Drink	Units
½ pint of beer	1
1 glass of wine	1

Dietary Habits. Please Select All That Apply.

☐ I seldom consume red or high-fat meats. ☐ I eat at least 5 servings of fruits/vegetables per day.

☐ I pursue a low-fat diet. ☐ I almost always eat a full, healthy breakfast.

☐ My diet includes many high-fiber foods. ☐ I rarely eat high-sugar or high-fat desserts.

OTHER HEALTH HISTORY INFORMATION

Please indicate any other medical conditions or activity restrictions that you may have, or any other information you feel is critical to understanding your readiness for exercise. It is important that this information be as accurate and complete as possible.

_____

_____

_____

HEALTH AND FITNESS GOALS

Please indicate your personal health and fitness-related goals (select all that apply):

☐ Cardiovascular Fitness ☐ Injury Rehab ☐ Muscular Strength

☐ Feel Better ☐ Look Better ☐ Reduce Stress

☐ General Fitness ☐ Lose Weight ☐ Reduce Back Pain

☐ Improve Diet ☐ Lower Cholesterol/Blood Pressure ☐ Sport-Specific Training

☐ Improve Flexibility ☐ Muscular Size ☐ Stop Smoking

Please tell us a little about your exercise patterns and goals:

What is your exercise history?

What health improvements do you need?

What are your activity preferences?

What barriers to success do you anticipate?

How will you know that you are succeeding?

What is your *motivation* level? What is your *confidence* level?

□ High □ Medium □ Low □ High □ Medium □ Low

I verify that all of the completed information is correct to the best of my knowledge. I declare that I am participating voluntarily in a performance fitness test. The maximum exertion during the test is at my discretion and I understand that I can stop the test at any time. I declare that I have no medical problems that prevent me from undertaking the fitness test and that I am not currently taking any medication that could present a danger with the performance fitness test.

_____

_____ _____ Printed Name

Signature Date

Emergency Contact: _____ Mobile: _____

2021 PAR-Q+

The Physical Activity Readiness Questionnaire for Everyone

The health benefits of regular physical activity are clear; more people should engage in physical activity every day of the week. Participating in physical activity is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

GENERAL HEALTH QUESTIONS

Please read the 7 questions below carefully and answer each one honestly: check YES or **NO**.	**YES**	**NO**
1) Has your doctor ever said that you have a heart condition ☐ **OR** high blood pressure ☐?	☐	☐
2) Do you feel pain in your chest at rest, during your daily activities of living, **OR** when you do physical activity?	☐	☐
3) Do you lose balance because of dizziness **OR** have you lost consciousness in the last 12 months? Please answer **NO** if your dizziness was associated with over-breathing (including during vigorous exercise).	☐	☐
4) Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)? **PLEASE LIST CONDITION(S) HERE:** _____	☐	☐
5) Are you currently taking prescribed medications for a chronic medical condition? **PLEASE LIST CONDITION(S) AND MEDICATIONS HERE:** _____	☐	☐
6) Do you currently have (or have had within the past 12 months) a bone, joint, or soft tissue (muscle, ligament, or tendon) problem that could be made worse by becoming more physically active? Please answer **NO** if you had a problem in the past, but it **does not limit your current ability** to be physically active. **PLEASE LIST CONDITION(S) HERE:** _____	☐	☐
7) Has your doctor ever said that you should only do medically supervised physical activity?	☐	☐

If you answered NO to all of the questions above, you are cleared for physical activity.
Please sign the PARTICIPANT DECLARATION. You do not need to complete Pages 2 and 3.

- Start becoming much more physically active – start slowly and build up gradually.
- Follow Global Physical Activity Guidelines for your age (https://www.who.int/publications/i/item/9789240015128).
- You may take part in a health and fitness appraisal.
- If you are over the age of 45 yr and NOT accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.
- If you have any further questions, contact a qualified exercise professional.

PARTICIPANT DECLARATION

If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that the community/fitness center may retain a copy of this form for its records. In these instances, it will maintain the confidentiality of the same, complying with applicable law.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

If you answered YES to one or more of the questions above, COMPLETE PAGES 2 AND 3.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ at www.eparmedx.com before becoming more physically active.
- Your health changes - answer the questions on Pages 2 and 3 of this document and/or talk to your doctor or a qualified exercise professional before continuing with any physical activity program.

01-11-2020

2021 PAR-Q+

FOLLOW-UP QUESTIONS ABOUT YOUR MEDICAL CONDITION(S)

1. Do you have Arthritis, Osteoporosis, or Back Problems?

If the above condition(s) is/are present, answer questions 1a-1c — If **NO** ☐ go to question 2

1a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
1b.	Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)?	YES ☐ NO ☐
1c.	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	YES ☐ NO ☐

2. Do you currently have Cancer of any kind?

If the above condition(s) is/are present, answer questions 2a-2b — If **NO** ☐ go to question 3

2a.	Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and/or neck?	YES ☐ NO ☐
2b.	Are you currently receiving cancer therapy (such as chemotheraphy or radiotherapy)?	YES ☐ NO ☐

3. Do you have a Heart or Cardiovascular Condition? This includes Coronary Artery Disease, Heart Failure, Diagnosed Abnormality of Heart Rhythm

If the above condition(s) is/are present, answer questions 3a-3d — If **NO** ☐ go to question 4

3a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
3b.	Do you have an irregular heart beat that requires medical management? (e.g., atrial fibrillation, premature ventricular contraction)	YES ☐ NO ☐
3c.	Do you have chronic heart failure?	YES ☐ NO ☐
3d.	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	YES ☐ NO ☐

4. Do you currently have High Blood Pressure?

If the above condition(s) is/are present, answer questions 4a-4b — If **NO** ☐ go to question 5

4a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
4b.	Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer **YES** if you do not know your resting blood pressure)	YES ☐ NO ☐

5. Do you have any Metabolic Conditions? This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes

If the above condition(s) is/are present, answer questions 5a-5e — If **NO** ☐ go to question 6

5a.	Do you often have difficulty controlling your blood sugar levels with foods, medications, or other physician-prescribed therapies?	YES ☐ NO ☐
5b.	Do you often suffer from signs and symptoms of low blood sugar (hypoglycemia) following exercise and/or during activities of daily living? Signs of hypoglycemia may include shakiness, nervousness, unusual irritability, abnormal sweating, dizziness or light-headedness, mental confusion, difficulty speaking, weakness, or sleepiness.	YES ☐ NO ☐
5c.	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, **OR** the sensation in your toes and feet?	YES ☐ NO ☐
5d.	Do you have other metabolic conditions (such as current pregnancy-related diabetes, chronic kidney disease, or liver problems)?	YES ☐ NO ☐
5e.	Are you planning to engage in what for you is unusually high (or vigorous) intensity exercise in the near future?	YES ☐ NO ☐

2021 PAR-Q+

6. Do you have any Mental Health Problems or Learning Difficulties? This includes Alzheimer's, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome

If the above condition(s) is/are present, answer questions 6a-6b If **NO** ☐ go to question 7

6a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
6b.	Do you have Down Syndrome **AND** back problems affecting nerves or muscles?	YES ☐ NO ☐

7. Do you have a Respiratory Disease? *This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure*

If the above condition(s) is/are present, answer questions 7a-7d If **NO** ☐ go to question 8

7a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
7b.	Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	YES ☐ NO ☐
7c.	If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week?	YES ☐ NO ☐
7d.	Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?	YES ☐ NO ☐

8. Do you have a Spinal Cord Injury? *This includes Tetraplegia and Paraplegia*

If the above condition(s) is/are present, answer questions 8a-8c If **NO** ☐ go to question 9

8a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
8b.	Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?	YES ☐ NO ☐
8c.	Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)?	YES ☐ NO ☐

9. Have you had a Stroke? *This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event*

If the above condition(s) is/are present, answer questions 9a-9c If **NO** ☐ go to question 10

9a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
9b.	Do you have any impairment in walking or mobility?	YES ☐ NO ☐
9c.	Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	YES ☐ NO ☐

10. Do you have any other medical condition not listed above or do you have two or more medical conditions?

If you have other medical conditions, answer questions 10a-10c If **NO** ☐ read the Page 4 recommendations

10a.	Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months **OR** have you had a diagnosed concussion within the last 12 months?	YES ☐ NO ☐
10b.	Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)?	YES ☐ NO ☐
10c.	Do you currently live with two or more medical conditions?	YES ☐ NO ☐

PLEASE LIST YOUR MEDICAL CONDITION(S) AND ANY RELATED MEDICATIONS HERE: _____

_____

GO to Page 4 for recommendations about your current medical condition(s) and sign the PARTICIPANT DECLARATION.

2021 PAR-Q+

If you answered NO to all of the FOLLOW-UP questions (pgs. 2-3) about your medical condition, you are ready to become more physically active - sign the PARTICIPANT DECLARATION below:

- It is advised that you consult a qualified exercise professional to help you develop a safe and effective physical activity plan to meet your health needs.
- You are encouraged to start slowly and build up gradually - 20 to 60 minutes of low to moderate intensity exercise, 3-5 days per week including aerobic and muscle strengthening exercises.
- As you progress, you should aim to accumulate 150 minutes or more of moderate intensity physical activity per week.
- If you are over the age of 45 yr and **NOT** accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.

If you answered **YES** to **one or more of the follow-up questions** about your medical condition:

You should seek further information before becoming more physically active or engaging in a fitness appraisal. You should complete the specially designed online screening and exercise recommendations program - the **ePARmed-X+ at www.eparmedx.com** and/or visit a qualified exercise professional to work through the ePARmed-X+ and for further information.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ **at www.eparmedx.com** before becoming more physically active.
- Your health changes - talk to your doctor or qualified exercise professional before continuing with any physical activity program.

- You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
- The authors, the PAR-Q+ Collaboration, partner organizations, and their agents assume no liability for persons who undertake physical activity and/or make use of the PAR-Q+ or ePARmed-X+. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.

PARTICIPANT DECLARATION

- All persons who have completed the PAR-Q+ please read and sign the declaration below.
- If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that the community/fitness center may retain a copy of this form for records. In these instances, it will maintain the confidentiality of the same, complying with applicable law.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

For more information, please contact
www.eparmedx.com
Email: eparmedx@gmail.com

Citation for PAR-Q+
Warburton DER, Jamnik VK, Bredin SSD, and Gledhill N on behalf of the PAR-Q+ Collaboration. The Physical Activity Readiness Questionnaire for Everyone (PAR-Q+) and Electronic Physical Activity Readiness Medical Examination (ePARmed-X+). Health & Fitness Journal of Canada 4(2):3-23, 2011.

The PAR-Q+ was created using the evidence-based AGREE process (1) by the PAR-Q+ Collaboration chaired by Dr. Darren E. R. Warburton with Dr. Norman Gledhill, Dr. Veronica Jamnik, and Dr. Donald C. McKenzie (2). Production of this document has been made possible through financial contributions from the Public Health Agency of Canada and the BC Ministry of Health Services. The views expressed herein do not necessarily represent the views of the Public Health Agency of Canada or the BC Ministry of Health Services.

Key References

1. Jamnik VK, Warburton DER, Makarski J, McKenzie DC, Shephard RJ, Stone J, and Gledhill N. Enhancing the effectiveness of clearance for physical activity participation; background and overall process. APNM 36(S1):S3-S13, 2011.
2. Warburton DER, Gledhill N, Jamnik VK, Bredin SSD, McKenzie DC, Stone J, Charlesworth S, and Shephard RJ. Evidence-based risk assessment and recommendations for physical activity clearance, Consensus Document. APNM 36(S1):S266-s298, 2011.
3. Chisholm DM, Collis ML, Kulak LL, Davenport W, and Gruber N. Physical activity readiness. British Columbia Medical Journal. 1975;17:375-378.
4. Thomas S, Reading J, and Shephard RJ. Revision of the Physical Activity Readiness Questionnaire (PAR-Q). Canadian Journal of Sport Science 1992;17:4 338-345.

01-11-2020

APPENDIX F

Debriefing Form

BOISE STATE UNIVERSITY

DEBRIEFING FORM

Study Title: Validation of Non-Invasive CO_2 Tolerance Field Test

Principal Investigator: Dr. Shawn Simonson **Co-Investigator:** Joshua Murphey

Thank you for participating in this study. This form describes the background and purpose of this investigation in more detail. **This is an ongoing project and all participants who fit the inclusion criteria are welcome. Please feel free to share your experiences during the study and have anyone interested in participating contact the principal investigator.**

The purpose of this study is to validate the only known current carbon dioxide (CO_2) tolerance field test against measured maximal dissolved CO_2 and maximal ventilatory CO_2 in endurance athletes. Validation of this field test will allow for more accurate assessment of CO_2 tolerance outside of a lab and give more individuals the ability to implement CO_2 tolerance as a part of their training. For more information about this topic, please see . Shift Adapt is solely a resource for CO_2 tolerance training and is not in any way affiliated with this project.

If you have any additional questions about the study or if you would like to receive a copy of the manuscript from this study when it becomes available, please contact Joshua Murphey at JoshMurphey@u.boisestate.edu or 216-906-2428

If you have questions about your rights as a research participant or concerns or complaints about the research, you may contact the Boise State University Institutional Review Board (IRB), which is concerned with the protection of volunteers in research projects. You may reach the board office between 8:00 AM and 5:00 PM, Monday through Friday, by calling (208) 426-5401 or by writing: Institutional Review Board, Office of Research Compliance, Boise State University, 1910 University Dr., Boise, ID 83725-1138.

We appreciate that you took the time to participate in this study.

Thanks again for your participation!

Josh Murphey
Graduate Teaching Assistant