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Effects of a Bacteriological Filter on VO₂max Measured by a Computerized Metabolic System

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To the Graduate Council:

I am submitting herewith a thesis written by Christopher William Bach entitled "Effects of a Bacteriological Filter on VO₂max Measured by a Computerized Metabolic System." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Kinesiology.

David R. Bassett Jr., Major Professor

We have read this thesis and recommend its acceptance:

Dr. Dixie L. Thompson, Dr. Dawn P. Coe

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

Effects of a Bacteriological Filter on $VO_{2\max}$ Measured by a
Computerized Metabolic System

A Thesis Presented for the
Master of Science
Degree

The University of Tennessee, Knoxville

Christopher William Bach

August 2012

DEDICATION

This work is dedicated to my parents who have provided me with unwavering support over the past 24 years. Without their guidance, I would not be half the man that I am today. I love you both and I am forever grateful for your support.

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First off, I would like to acknowledge my major professor, Dr. David Bassett Jr. who has guided me through this process with incredible support. While my schedule over the past couple of years has certainly not made me the easiest student to work with, Dr. Bassett has shown remarkable patience and a true desire to help out this inexperienced graduate student. His knowledge in the field is remarkable and I can say it has truly been a pleasure to work with him on this project. He is one of only a few people in this world that I have met who can match my enthusiasm about a variety of topics. I have truly enjoyed developing a friendship with him, and I can only hope it continues to grow over the coming years.

I would also like to thank my other committee members for their support and guidance through this process. Dr. Dixie Lee Thompson has had a major impact on my life, as it was her class that inspired me to enter into the exercise physiology field. My other committee member, Dr. Dawn Coe, has also been a great help to me throughout this process. I am grateful to have learned under such amazing teachers during my time at Tennessee.

Of course this project would not have been possible without the help of my fellow graduate students, especially Jenny Flynn. She has been unbelievable to me over the past year, as she helped me any time I had troubles or questions. Jenny has a talent for dealing with people, and I am proud to call her a friend.

ABSTRACT

Purpose: To determine the effects of a purple bacteriological filter (PF) on the measured maximal oxygen uptake (VO_{2max}) during a maximal treadmill test as well as its effect on the expiratory flow measurements during a pulmonary function test. **Methods:** Male ($n=7$) and female ($n=7$) runners (18-35 years old) completed two continuous graded exercise tests (GXTs) followed by pulmonary function tests (PFT); one with a purple filter (PF) and one with no filter (NF). The GXT consisted of running at a constant speed on a treadmill while gradually increasing the grade until volitional exhaustion was reached. Following each of the GXTs two pulmonary function tests were performed under the same filter conditions used in the GXT. Testing was conducted over two consecutive days, and the order of the testing was randomized. **Results:** Mean values for maximal ventilation (V_{Emax}), VO_{2max} , and test duration were larger for the NF condition, however peak expiratory flow (PEF) was the only variable that proved to be statistically significant ($p<0.05$). Under the NF condition, V_{Emax} was 4.33 ± 8.93 L/min higher and VO_{2max} was 1.1 ± 2.7 ml/kg/min higher, compared to the PF condition. There was a significant interaction between order of testing and V_{Emax} ($p<0.001$), and between order of testing and VO_{2max} ($p<0.015$). Mean values of FVC, FEV₁, and FEV₁/FVC% were 0.04 ± 0.11 L, 0.06 ± 0.25 L, and 0.54 ± 4.57 % higher in the NF conditions, but were insignificant. PEF values increased significantly by 0.41 ± 0.63 L/sec, from PF to NF conditions ($P<0.031$). **Conclusion:** The PF does not appear to have a large effect on VO_{2max} , FVC, FEV₁, and FEV₁/FVC% values. However, PEF was significantly affected and V_{Emax} trended towards significance with a near-significant p-value of 0.051. When

including order in the analysis, there does appear to be an order effect as participants increased VO_{2max} and V_{Emax} values from day 1 to day 2 regardless of condition order. Overall, VO_{2max} and V_{Emax} were not greatly affected by the presence of the purple bacteriological filter inserted between the expired gas hose and pneumotachometer.

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CHAPTER I

INTRODUCTION AND GENERAL INFORMATION

Maximal oxygen uptake (VO_{2max}) is defined as the highest rate at which oxygen can be taken in and consumed by the body, during strenuous, dynamic exercise ^[1]. VO_{2max} has long been considered the gold standard for assessing cardio-respiratory fitness (i.e. aerobic power) in humans and has been linked to numerous health and performance outcomes. In addition, VO_{2max} is viewed as an important determinant of endurance athletic performance. Medical professionals use VO_{2max} for diagnostic purposes, and health and fitness professionals often use it as the basis for prescribing exercise.

The initial understanding of oxygen consumption by the muscles occurred in 1923 when A.V. Hill investigated the relationship between the study of isolated frog muscle and its application to human muscle. Through his work with isolated frog muscle in conditions of both the absence of oxygen and in the presence of oxygen, Hill was the first to establish that there are three different quantities with regards to oxygen and muscle: oxygen intake, oxygen consumption, and oxygen requirement. Hill's work with muscles in various oxygen-leveled environments and his study of lactic acid also allowed him to become the first to distinguish between aerobic and anaerobic mechanisms. These two findings were critical in the basic understanding of VO_2 ^[2]. Just one year later, Hill published further work on the topic and investigated the relationship between oxygen intake and the severity of physical exertion. Using himself as a subject, he was able to see

oxygen consumption, ventilation, and CO₂ output increased as work load increased until a maximal level was reached ^[3].

Early studies examining gas exchange variables utilized the Douglas bag technique ^[4]. ^[5]. This method involves the collection of expired air in a large bag during pre-selected time periods of time. Upon completion of gas collection, the gas content in the bag is analyzed to determine its precise gas composition. The expiratory volume (V_E) can be used to compute the inspiratory volume (V_I). The gas analyzers determine the fraction of expired air that is oxygen (FEO₂) and the fraction of expired air that is carbon dioxide (FECO₂). Measurement of V_E, FEO₂ and FECO₂ values are critical for the determination of an accurate VO₂.

While the Douglas bag technique is still considered the gold standard for gas analysis today, it is extremely time consuming. As a result, gas analysis techniques and systems have developed into computerized metabolic systems which are widely used today. These computer systems are often times extremely expensive in comparison to the Douglas bag method, but they allow for extremely precise measurements to be made in a less time-consuming manner than the Douglas bag technique ^[6]. This transition to widespread computerized measurements makes the study of these methods/systems even more important to ensure the measurements of VO₂ are still valid and reliable.

Automated systems measure ventilation using two different types of flow meters: turbines and pneumotachometers. Turbine flowmeters are sometimes inaccurate as they have shown to elicit higher than acceptable error for VO₂ and VCO₂. In fact a study by Yeh et al. showed that there is up to a 20% error at rest, but that error decreases as workload increases ^[7]. Some of the issues with the turbine flowmeter are that it exhibits a

“Lag-before-start” effect and a “spin-after-stop effect”. The lag-before-start effect essentially means that there is a small time delay at the beginning of flow through the turbine, which makes the turbine lag behind the measurements of a pneumo-tachometer at the end of inspiration and expiration. The “spin-after-stop” effect means that even after the expired breath is completed, the turbine is continuing to record an exhalation as it spins to a stop. This effect causes an increased CO₂ output, which in turn causes inaccurate VCO₂ measurements ^[7].

A pneumotachometer does not exhibit these same characteristics due to the fact that it uses pressure differences to measure flow rates instead of a turbine. The Hans Rudolph pneumotachometer uses three fine, mesh screens that provide minimal resistance to flow. In a pneumotachometer, the flow rate is determined by measuring the pressure differences on either side of the central mesh screen which allows for more accurate, instantaneous measurements in comparison to the turbine flowmeter.

For decades, it was believed that pneumotachometers provide a linear pressure-flow relationship, but in actuality the relationship is not linear. There was a major advancement in understanding of the pressure-flow relationship in 1982 when Yeh et al. challenged the linearity characteristics of pneumotachometers. Yeh et al. showed that “linear or quadratic fitting of the pressure-flow characteristics do not provide accurate correction” ^[8]. Instead of using the typical pressure-flow curve to determine the characteristics of the pneumotach, they utilized a conductance-pressure curve based on a weighted-averaging technique. The weighted-averaging technique is based on conductance values established by repeated strokes with a calibrated syringe. The reason that this advancement was so critical is because it allowed the instantaneous flow rate to

be measured by multiplying the conductance value (based on hundreds of strokes by a calibrated syringe) by the instantaneous pressure difference across the fine mesh filter. This advanced method developed by Yeh et al. provided a mean absolute percent error (MAPE) of less than $\pm 0.5\%$ [8]. Rather than assuming a constant level of resistance at different flow rates as was previously done, Yeh's method calibrates the resistance over a range of flows and pressures, providing more accurate measurements.

The ParvoMedics TrueOne 2400 (formerly known as the ParvoMedics TrueMax2400) is one of the most widely used computerized metabolic measurement systems in exercise physiology laboratories. One of the standard accessories of this system is a purple, disposable bacteriological filter (Creative BioTech Clear Advantage Pulmonary Function Filter, Kansas City, MO). With the ParvoMedics system, ventilation is usually measured on the expired side of the equipment. The filter is placed between the expired air hose and the Hans-Rudolf pneumotachometer that adjoins the mixing chamber. The purpose of the filter is to serve as a bacterial shield, and perhaps to reduce measurement issues that may arise from the accumulation of condensation on the pneumotachometer.

The purple filter is designed for use with pulmonary function testing, including measurement of functional residual capacity (FRC) and forced expiratory volume (FEV_1). In addition to pulmonary function testing, biological filters are used with some metabolic measurement systems, such as the ParvoMedics TrueMax 2400. However, the effects of the purple filter and its impact on ventilatory parameters at high flow rates during maximal exercise testing have not previously been examined. The knowledge gained in this study could be useful to fitness professionals, athletes, and researchers,

since the use of the purple filter might impact key physiological variables, including VO_{2max} .

The primary purpose of this study was to test the effects of the purple filter on gas exchange variables during a maximal treadmill test. Specifically, we plan to assess the differences in physiological variables (VO_{2max} and V_{Emax}) with and without the purple filter. It has been commonly accepted that the filter does not alter the measured VO_{2max} values, but some subjects have reported perceived difficulties in exhaling through the purple filter at the end of a GXT.

A secondary purpose of this study was to test the effects of the purple filter on expiratory flow measurements during pulmonary function testing. Specifically, we plan to assess the differences in FVC, FEV_1 , peak expiratory flow (PEF) rates, and $FEV_1/FVC\%$.

Overall, it is hypothesized that the purple filter decreases VO_{2max} , V_{Emax} , FEV_1 , and PEF rates due to an increase in breathing resistance during exhalation.

CHAPTER II

LITERATURE REVIEW

MEASUREMENT OF ENERGY EXPENDITURE

Energy expenditure (EE) is the sum of the basal metabolic rate, dietary thermogenesis, and activity thermogenesis. The measurement of EE is important in the field of exercise physiology because it allows for the assessment of an individual's metabolic needs, the determination of that individual's fuel utilization, and the relative thermic effect of different foods and drinks. The measurement of an individual's daily energy expenditure can be useful to professionals in various fields, as it can provide the information around which a lifestyle program, whether it is nutrition, exercise or some related field, can be designed.

The measurement of energy expenditure dates back centuries to the time of eighteenth-century physicist, Antoine L. Lavoisier. Lavoisier was one of the first to study metabolism and respiration during exercise, and as a result contributed greatly to the current understanding of energy expenditure. Through the simple observation that animals breathe and give off heat, Lavoisier developed a direct calorimeter to measure the heat produced by the animal. The calorimeter was a simple design that consisted of a chamber surrounded by an inner jacket of ice and insulated by ice water. Through simple calculations based on the heat required to melt a given quantity of ice and the volume of water produced, Lavoisier was able to ascertain the heat produced by the animal^[9]. This

calorimeter, designed in 1780, is a prime example of a direct calorimeter because it measures the rate of heat produced by the subject over a given amount of time.

Only four years later, in 1784, Lavoisier developed an indirect calorimeter. Indirect calorimeters measure the oxygen consumption and/or carbon dioxide production of a subject over a given amount of time, and as a result allow for the estimation of heat production and energy expenditure. In 1784, Lavoisier developed an extremely innovative closed-circuit respirometer that consisted simply of a glass jar, a bed of mercury and NaOH. The measurement of the respirometer volume pre-, during, and post-animal habitation allowed for the measurement of O₂ consumption and CO₂ production [9].

As previously mentioned, there are multiple ways of measuring EE. The two main methods of measuring EE are indirect calorimetry and direct calorimetry. The difference between the two calorimetry methods lies in what is being measured. In indirect calorimetry (e.g. Lavoisier's respirometer), oxygen consumption and carbon dioxide production are measured and used to compute energy expenditure. Direct calorimetry (e.g. Lavoisier's ice chamber) on the other hand, directly measures the rate of heat production from the subject inside the calorimeter.

With the indirect calorimetry method, there are both open-circuit and closed-circuit systems. In an open-circuit indirect calorimeter system, the subject inspires atmospheric air and expires into an impermeable bag or mixing chamber from which the expired air is drawn. The expired air is then measured to determine O₂ consumption and CO₂ production. Closed-circuit indirect calorimetry systems are rarely used today. It

consists of a sealed respiratory gas circuit in which gaseous concentrations are measured over time ^[10].

Direct calorimetry systems are not as common as indirect systems due mainly to the fact that they are very expensive and offer only small advantages for most researchers. Isothermal, heat sink, and convection systems are the three primary types of direct calorimetry which measure heat lost from the body. As indicated, an isothermal system maintains a constant internal temperature essentially through the use of an insulating layer and a circulating fluid ^[11]. Heat sink systems, also referred to as adiabatic systems, measure the heat lost by extracting it with a liquid-cooled heat exchanger ^[12].

The third type of direct calorimetry, the convection system, once again utilizes an insulated chamber. These systems however, measure the heat loss from the subject by measuring the temperature of the precisely-ventilated air, the specific heat capacity of the air, and the temperature change in the ventilated air leaving the chamber ^[13-15]. While indirect calorimetry methods are more common today, each of these methods and systems has proven to be an adequate and precise way of measuring energy expenditure.

DOUGLAS BAG METHOD

The Douglas bag method was developed by C.G. Douglas for the measurement of O₂ consumption and CO₂ elimination while he was preparing for an expedition to Pike's Peak in Colorado ^[4]. Despite being developed over a century ago, his method has long been considered the gold standard with regards to validity and reliability of gas exchange measurements for decades. In principle, the methods include the serial collection and

independent analysis of expired gas collections. As the participant exercises, his/her expired gas is periodically collected in airtight bags with the use of a two-flap, one-way valve. Upon completion of each collection, the bag is removed and taken for analysis of gas composition, and another bag is placed on the one-way valve for collection of the subsequent gas collection period of the exercise test.

Despite countless studies confirming the validity and reliability of the Douglas bag technique ^[6, 16-18], gas measurement has predominately moved towards the use of automated systems. Both laboratory and portable metabolic systems are now widely used in place of Douglas bags due to the cumbersome, time-consuming procedures required for the Douglas bag technique. These new metabolic gas analysis systems have become incredibly easy to operate; however many of the systems have their flaws in comparison to the Douglas bag method. While the Douglas bag method does take a considerably longer time than these newer metabolic systems, it does provide benefits with its minimal cost and much greater control over the entire measurement process. It is important to acknowledge that a lot of trust is put into the abilities of these new automated systems, as the measurement process is not in the hands of the technician.

However, the Douglas bag method is not without its own faults. As previously mentioned, one of its disadvantages is the extremely long time it takes to complete an analysis of a full exercise tests. Another common limitation of the method is that it is highly dependent on the skill of the technician and the level of precision in which the technique is carried out. Studies have also shown problems with the bags themselves as the earlier versions have shown the diffusion of gases through the walls of the bags ^[19,20].

NITROGEN CORRECTION FACTOR

The question of whether or not the human body excretes nitrogen was unanswered at the beginning of the 20th century. It was not until famed zoologist Dr. August Krogh answered the question in 1906 that the question was definitively answered.

In 1904 the Imperial Academy of Sciences in Vienna announced that they would award the prestigious Seegan Prize for the ‘best scientific showing “whether or not free nitrogen or nitrogenous gases are excreted from the body as a normal by-product of the metabolism”’ [21]. Krogh recognized that many of the previous studies had too many sources of error and consequently conducted his own studies with the apparatus he designed and constructed himself. Rather than designing a large apparatus similar to what was used in other studies, Krogh designed an apparatus that was only large enough for small animals of no more than 50 grams. Through his small apparatus and use of ‘animals that do not eat, drink, or move about’ (butterfly pupae) he was able to eliminate many sources of error. In reference to the results of this study, Krogh says that the results “were highly satisfactory and showed no or minimal releases of free nitrogen from the pupae.”

Having reached the conclusion that the butterfly pupae released no or minimal free nitrogen, he then tested mammals (mice) to see if the same conclusion held true. Krogh was able to eliminate all known sources of error by submerging his glass apparatus in water to keep a uniform temperature and eliminate any leakage. After analyzing the resulting gas composition from his experimentation on mice, Krogh claimed, “I now feel that I have found the solution to the nitrogen question. The answer is

that animals do not excrete gaseous nitrogen in measurable quantities.” As a result of his studies, Krogh was awarded the Seegan Prize in 1906 ^[21].

The affirmation of whether or not humans excrete nitrogen was still controversial until a study by Wilmore and Costill validated the calculation of VO_2 with the Haldane transformation in 1973 ^[22]. In contrast to the early studies by Krogh, many studies claimed that humans do indeed retain and produce nitrogen ^[23-28], thus making the Haldane transformation equation (now called the N_2 correction factor) for VO_2 an inaccurate calculation. The study by Wilmore and Costill tested 6 male participants on a treadmill at 3 different submaximal workloads (4.0, 6.0, and 7.5 mph). The following variables were measured every 15 seconds of inspiration and expiration by two Parkinson-Cowan, CD-4 dry gas meters: V_E , FEO_2 , FECO_2 , RQ, and VO_2 . The results showed that the differences in minute ventilation volume of inspired to expired nitrogen were significant for 3 of the 6 participants. However the maximum difference of the measured nitrogen of expired to inspired volumes of those 3 subjects was only 1.6%. In addition, the largest difference of the 68 total determinations between expired and inspired volumes throughout the study yielded only a 2.1% error. Overall, the average difference of expired to inspired nitrogen volumes for the 6 subjects was only 0.8%. Despite the significance of the data, both the 1.6% and 2.1% differences were within the normal measurement error and perceived as insignificant in the calculation of VO_2 with the N_2 correction factor. ^[22]

The study concluded that the use of the N_2 correction factor in the calculation of VO_2 during exercise is valid and acceptable for continued use. This conclusion is based on the data that suggests humans do not retain or produce nitrogen during exercise, and in

the event that humans do indeed retain and produce nitrogen, the actual levels are so small that they do not affect the calculation of VO_2 .

AUTOMATED SYSTEMS

Automated metabolic measurement systems are complex systems that consist of many different parts. While some systems contain various optional parts, all systems have some sort of flow-sensing device. These flow-sensing devices, such as a pneumotach or turbine, produce a signal that is proportional to the gas flow. By the analysis of the gas flow, gas volume can be measured. The ParvoMedics cart used in this particular study utilizes a heated Hans-Rudolph pneumotachometer.

Other components of the automated systems are the O_2 and CO_2 gas analyzers. These gas analyzers are able to measure the FEO_2 and the FECO_2 to produce an accurate VO_2 reading. While some systems measure both the O_2 and CO_2 gases together, most systems use separate analyzers for O_2 and CO_2 analysis. The ParvoMedics cart used in this study used individual analyzers. The CO_2 analyzer for the ParvoMedics system utilizes infrared technology to analyze CO_2 content in a gas mixture. CO_2 absorbs infrared radiation and through a measurement of the resulting oscillating signals, the analyzer is able to determine the CO_2 composition of the gas samples ^[29]. The O_2 analyzer used in this study is a paramagnetic analyzer that utilizes the paramagnetic properties of O_2 . Essentially the paramagnetic properties of O_2 cause the movement of

internal pieces of the analyzers and the magnitude of movement provides an accurate and fast analysis of the O₂ content in a gas mixture [29].

While all automated metabolic measurement systems include the aforementioned components, the ParvoMedics system used in this study also includes a 3-Liter precision calibration syringe, a mixing chamber, and a calibration gas cylinder and regulator to go along with the computer software. The 3-Liter syringe allows for precise volumes to be pushed through the heated Rudolph pneumotach prior to testing to ensure proper calibration of the system. Similarly the E-cylinder gas and regulator allow for the measurement of a precise mixture of O₂ and CO₂ to calibrate the system. The mixing chamber is used in many automated systems and it simply mixes the gas sample to ensure that the sample is uniform in composition for accurate analysis.

While the fundamental principles of measuring O₂ consumption and CO₂ production have remained the same over the course of time, the evolution of computer technology has drastically altered the manner in which many physiological variables are measured. The composition of the expired gas has been historically collected and analyzed in a variety of ways depending largely on the frequency of analysis, duration of the test, and the equipment/personnel available. Common forms of expired gas composition measurement include, but are not limited to, the use of a mixing chambers, aliquots, and large collection bags.

Among these options, mixing chambers are the most commonly used in laboratories today. The expired gas analysis used in this particular study utilizes a mixing chamber that allows for continuous gas sampling. Mixing chambers consist of a plastic

box that contains plastic obstructions that deflect the expired gas, called baffles, which help to mix the expired gas. One of the advantages of a mixing chamber is that it allows for the continuous sampling of gas without the technological issues that are present during breath-to-breath sampling ^[9]. While some error may occur when the composition of gas changes rapidly, the error is non-significant by most accuracy standards.

Aliquot sampling techniques are quite different than mixing chamber techniques and most common to the large collection bag techniques ^[30]. This unique part of this technique is the use of a three-way valve. An aliquot, or small sample of gas, is pumped from the expired flow stream into a small container (such as a rubber bag) which is attached to each of the valves. At the discretion of the technician, the three-way valve is set so that one bag collects the expired gas, one bag is being analyzed, and one bag is being emptied by a vacuum pump – all simultaneously. The aliquot sampling technique does slightly resemble the large collection bag technique in the sense that gas is captured in a bag and that it utilizes a three-way valve as well. This technique however is set-up quite differently. Only two of the valves are open at one time. The technician sets the valves so that the subject inspires from the room air and rather than sometimes expiring into the outside air (i.e. collection bag closed), the subject is always expiring into a collection bag. While one bag is collecting the expired air, one bag is emptied into the gas analyzers, and the third is evacuated by a vacuum pump. Once the end of the collection period is reached, the bags rotate 120° and the collection process continues in the same manner.

The Douglas Bag method is a technique that utilizes the collection of expired gas in large collection bags. The Douglas Bag method is still considered the gold standard for the measurement of VO_2 , however the simplicity, ease, and speedy manner in which the new automated metabolic gas analysis systems can analyze and produce the data have made these automated systems the most common form of gas analysis today. Due to the accuracy of the Douglas Bag method, new gas analysis systems are often validated by the comparison against the Douglas Bag method. While the Douglas Bag method remains useful and extremely accurate to this day the cumbersome, time-consuming measurements it is used less often today than in the past.

ParvoMedics 'TrueMax 2400'

The automated system used in this particular study is the Parvo Medics TrueMax 2400 – a model that is now known as the ParvoMedics TrueOne® 2400 (Sandy, UT). The TrueMax 2400 system is an open circuit indirect calorimetry system designed by Dr. Pat Yeh. Bassett et al. configured two TrueMax 2400 systems in series in order to measure both the inspired and expired air simultaneously, along with a meteorological balloon. Expired gas collected in the meteorological balloons was used to determine VO_2 by the Douglas bag method. With the Douglas bag method as the criterion, the validity of the TrueMax 2400 could be determined. The study showed that the Parvo Medics TrueMax 2400 provided minimal differences in comparison to the Douglas bag method (difference between Douglas Bags and Parvomedics <1%), and while some differences were significant, they were so small that they were deemed “not physiologically significant” by the authors ^[6]. They concluded that the computerized system, whether

configured to measure inspiratory or expiratory ventilation, yielded gas exchange variables that were extremely close to those obtained by the Douglas bag method.^[6]

One aspect of this system that makes it unique is the many common features and options that most automated metabolic gas analysis systems do not have. Outside of the obvious variables (minute ventilation, O₂ uptake, CO₂ production, and heart rate (HR)), the TrueOne® 2400 systems includes a mixing chamber, an ECG module, and the ability to perform pseudo breath-by-breath (BxB) analysis, spirometry, noninvasive cardiac output, and pulse oximetry. Many of the other automated systems do not provide this broad availability of options. However, it is important to note that this system does not provide a true BxB analysis like some of the systems described below.

When discussing the ParvoMedics TrueMax 2400 it is necessary to mention the algorithms that make this system unique. Yeh questioned the linear pressure-flow relationship that had been widely accepted for decades, and consequently developed an innovative algorithm that provides the <1% error needed for research and clinical settings. Rather than the pressure-flow relationship, this algorithm-based system is based on the weighted-averaging technique of the conductance-flow relationship. Because it is difficult (if not impossible) to generate constant flows with a syringe, this weighted-averaging technique generates conductance values, and ultimately a conductance value table, based on hundreds of strokes with a calibrated syringe. By multiplying the instantaneous measured pressure by the pneumotach and the corresponding conductance value (based on hundreds of strokes from a calibrated syringe), the instantaneous flow rate can be measured, and the average minute flow rates can be determined with a mean absolute percent error of less than $\pm 0.5\%$ ^[8].

Cosmed 'Quark'

The Cosmed Quark provides many of the similar options as the TrueMax 2400 such as spirometry, however it is different in the sense that it provides only BXB analysis. The BXB analysis is done by measuring gas composition at very precise times of instantaneous expiration.

A study by Norris et al. investigated the validity of 3 different automated systems (Cosmed Quark, SensorMedics Vmax, and SensorMedics Horizon systems) by comparing the three systems against one another^[31]. The study showed what appeared to be a high correlation of VO_2 and V_E between the 3 systems, however with Bland Altman analysis, the V_E showed a higher level of disagreement than originally thought when the V_E levels climbed above 80 L/min. The conclusion of the study was that researchers must be careful when comparing various metabolic systems because the data can be skewed. This is the reason that many validation studies are validated against the proven Douglas bag method. When comparing two automated systems, the results may prove to be highly correlated, but if both of the systems are invalid in comparison to the Douglas bag method, then the results of the inter-system comparison is irrelevant with regards to validity.

Jaeger 'Oxycon Pro'

The Jaeger Oxycon Pro is a portable system that provides multiple options to the user. While it traditionally provides a true BXB analysis, it also has a mixing chamber measurement option that allows for the continuous sampling of gas for composition

analysis. As with many of the other automated systems, the Oxycon Pro provides the option of spirometry and ECG with its system as well.

A validation study was done by Rietjens et al. for the Oxycon Pro automated system^[18]. The study used the criterion of the Douglas bag method for comparison. Twelve highly trained subjects performed an incremental cycle ergometer test which consisted of 5-minute stages beginning at 95 Watts and increasing 35 Watts each stage, with the expired air being collected in the Douglas bag after minute 3 of each stage. The Oxycon Pro constantly measured minute ventilation (V_E), VO_2 , and VCO_2 during the entire test. The study revealed that there were no significant differences between the two methods for V_E , VO_2 , or VCO_2 indicating that the Oxycon Pro is valid for the measurement of those variables during both submaximal and maximal exercise.

Medical Graphics 'CPX/D'

The Medical Graphics CPX/D is similar to the Quark in the sense that it only performs BXB gas analysis. It does include the almost standard option of using the system for spirometry as well.

A study performed by Porszasz et al. compared the VO_2 values produced by the CPX/D systems to the values produced by the simultaneous Douglas bag method^[32]. Each of the 4 subjects in the study performed 23 tests at rest and during exercise. The results of the study showed the CPX/D system provided values within 5.9% of the Douglas bag values at rest and 6.6% during exercise. The VCO_2 value differences were

very similar to that of the VO_2 differences listed above, and the V_E values were “generally within the $\pm 2\%$ range”^[32].

Prieur et al. performed an interesting validation study of the CPX/D system in comparison to the Douglas bag method^[33]. This particular study evaluated the VO_2 , VCO_2 , and V_E values during submaximal exercise in both euoxic (normal oxygen level) conditions and hyperoxic (higher than normal oxygen level) conditions. The study showed no significant differences between the CPX/D values and the Douglas bag values for VO_2 , VCO_2 , and V_E values during either euoxic or hyperoxic conditions. A small ($<0.2\%$), yet statistically significant drift in the high and low FEO_2 values was seen over time, however it is unlikely that this low of a difference is physiologically significant. The conclusion of the study was that the CPX/D system is valid for assessing VO_2 , VCO_2 , and V_E in both normoxic and hyperoxic conditions during submaximal exercise. As with many other studies, this particular study did not include maximal exercise testing.

Research by Engebretson et al. also performed a validation study of the CPX/D system with the Douglas bag method as the criterion^[34]. Unlike the aforementioned studies, this study measured values from both submaximal and maximal workloads. The CPX/D system was put in series with the Douglas bag system. The results showed no significant differences between systems for VO_2 and V_E at steady state at the end of the 3 minutes of exercise, but did report significant differences ($<3.6\%$) in VCO_2 , respiratory exchange ratio (RER), FEO_2 , and FECO_2 . The authors of the study concluded that while there were statistically significant differences for many of the variables, the differences were “within the 4% standard range of most metabolic systems”^[34].

Physio-Dyne 'Max-1'

The Physio-Dyne Max-1 is similar to some of the other systems in that it has the capability of performing spirometry testing. Unlike the Quark, Oxycon Pro, and CPX/D the Max-1 does not offer BXB analysis. This system utilizes a mixing chamber to allow for the continuous sampling of gas over selected intervals of time.

Cullum et al. conducted a study in which they connected the mixing chamber of the Physio-Dyne Max-1 system in series with a Douglas bag system ^[35]. The study consisted of testing 19 males subjects over 4 different work rates from rest to maximal exercise. The results showed a near-significantly lower (3.3%) VO_2 value for the Max-1 system in comparison to the Douglas bag system, however values for VO_2 , VCO_2 , FEO_2 , or $FECO_2$ did not show significant difference in comparison to the Douglas bag system. When testing 7 subjects for the repeatability error of the systems, the Max-1 system produced only a 0.7% higher absolute error than the Douglas bag technique. The combination of these two findings led the authors to conclude that the Max-1 system is suitable for the measurement of VO_2 .

A study by Yates et al. evaluated the validity of the Max-1 in comparison to the Douglas bag system across a range of flow rates (21 to 90 L/min) ^[36]. The study showed that at the lower flow rates, the Max-1 overestimated the VO_2 values by about 3.1% when compared to the Douglas bag values, and underestimated VO_2 values by 6.1% at high flow rates. Overall the 2.9% underestimation of the Max-1 system led the authors to conclude that the Max-1 can provide accurate measurements of VO_2 , even though the ventilations and gas fractions are incorrect.

Rayfield

The Rayfield automated system was the first automated system to be validated. The validation study performed by Powers et al. utilized a dry gas meter fitted with a potentiometer to measure inspired ventilation (V_I)^[37]. The expired gas was collected in a 9.2-L mixing chamber and then analyzed by O_2 and CO_2 applied electrochemistry analyzers. The electrical signals from both the dry gas meter and applied electrochemistry analyzers were sent to a Rockwell Aim-65 microcomputer for analysis and processing. Due to normoxic conditions used in this study, it was fair to assume that there was no net nitrogen exchange^[22], so the VO_2 was calculated using the Haldane transformation of the Fick equation: expired gas was collected in a 9.2-L mixing chamber and then analyzed by O_2 and CO_2 applied electrochemistry analyzers. The electrical signals from both the dry gas meter and applied electrochemistry analyzers were sent to a Rockwell Aim-65 microcomputer for analysis and processing. Due to normoxic conditions used in this study, it was fair to assume that there was no net nitrogen exchange^[22], thus VO_2 was calculated as follows:

$$VO_2 = (V_I \times F_{IO_2}) - [(V_I \times F_{IN_2}/F_{EN_2}) \times F_{EO_2}]$$

One of the key components of this study was their measurement of a time delay from the introduction of an unknown gas to the analysis of the gas from the mixing chamber. The time delay was determined as the time it took the gas analyzers to reach a stable plateau plus the time it took to washout the mixing chamber at various flow rates. The actual testing consisted of four subjects performing 16 rest-to-exercise cycle tests at approximately 140W. In a randomized order, each participant performed 8 tests that were

analyzed using a mixing chamber and the aforementioned setup. The other 8 tests were analyzed using the Douglas bag technique.

The results of the study showed an inverse relationship between flow rate and time to a stable plateau for the gas analyzers from the mixing chamber. Powers et al. also found no significant difference between the VO_2 values from the two procedures. Thus, this study concluded that the use of an open-circuit system mixing chamber provides valid measurements of VO_2 during non-steady state exercise ^[37].

SensorMedics ‘Vmax’

The SensorMedics Vmax is one of the most widely used automated gas analysis systems in hospital settings and is similar to the Oxycon Pro in that it has the capabilities to perform true BXB analysis, but it also has a mixing chamber. Spirometry is among the many other optional tests that can be run with this system.

The validation of the SensorMedics Vmax system has been attempted in at least three studies. Similar to a previously mentioned study (see the “Cosmed ‘Quark’” section above), a study by Hiilloskorpi et al. also compared 3 metabolic systems ^[38]. The study by Hiilloskorpi et al. compared the VO_2 , V_E , and VCO_2 values of 3 separate submaximal workloads produced by the SensorMedics Vmax system, the Medikro 202 system, and the Cosmed K4 system. When comparing the results from the 3 separate workloads, the Vmax system provided significantly higher VO_2 values (0.13 L/min) than the Medikro 202 system and significantly lower VO_2 values (0.29 L/min) than the Cosmed K4. Due to the testing of only submaximal workloads, there were no data

presented on the validity at maximal workloads. Once again this study shows that researchers need to be careful when comparing various automated systems.

Another study by Storer et al. simultaneously compared the Vmax system and the Douglas bag method ^[39]. While the study only consisted of testing four submaximal steady state conditions for each of its five male participants, the results showed that the Vmax system was very accurate. The data showed only a 0.3% difference in VO_2 , a 1.8% difference in VCO_2 , and a 1.5% difference in V_E between the SensorMedics Vmax system and the gold standard Douglas bag method. The study was able to validate the accuracy of the Vmax system within the work rate range of 40-160 W, however since maximal data were not obtained, the study was unable to prove the validity of the system at maximal workloads.

PULMONARY FUNCTION TESTING

Pulmonary function tests (PFT) use spirometry to measure lung function. More specifically PFTs can measure the volume and flow that is either inhaled or exhaled by the lungs. PFTs are often used in the assessment of pulmonary impairment (i.e. asthma) in the clinical setting, but can be used in the research setting as well to determine such cases as exercise-induced bronchoconstriction (EIB), or in the case of this study, the effects of varying conditions (purple filter vs. no filter) on pulmonary function. While PFTs yield quite a few outcome measurements, this study will concentrate only on forced

vital capacity (FVC), forced expiratory volume in one second (FEV_1), peak expiratory flow (PEF), and FEV_1/FVC ratio ($FEV_1/FVC\%$).

The pulmonary function testing done in the present study used the Cosmed Pony FX. Dynamic testing was done in the LDS Hospital pulmonary laboratory to determine the accuracy of the Cosmed Pony FX ^[40, 41]. The testing was done in accordance with the standards for spirometry as outlined by the American Thoracic Society. The study utilized standard volume-time waveforms in the assessment of FVC and FEV_1 while flow-time waveforms were used to assess the PEF accuracy. The results showed that the Cosmed Pony FX meets both accuracy and precision recommendations as determined by the ATS for FVC, FEV_1 , and PEF. The accuracy and precision for FVC values were within 1.00% and 0.80% respectively, the FEV_1 values within -0.15% and 0.49%, and the PEF values within -0.75% and 0.53%. Due to various factors including height, weight, sex, age, smoking status it is difficult to provide general values for normal measurements. The Cosmed Pony used in this particular study takes all of these factors into account and provides a predicted value along with the actual value. An example of a PFT output from the Cosmed Pony spirometer is shown below in Figure 1.

While newer systems utilize either a pneumotachometer or flow turbine as mentioned earlier, past methods for spirometry testing were quite different. One example is a closed circuit spirometry system. They require an individual to breathe into a pre-filled container and the exhaled air causes an “inverted cylinder” in a water bath to rise and fall, and this allows the lung volumes to be measured. Typically, an ink pen that moved up and down with the inverted cylinder allowed the lung volumes to be recorded on special graph paper that was attached to rotating drum.

Whole body plethysmography is another way to obtain multiple lung measurements. In a traditional plethysmograph test, a participant is placed into an air-tight chamber which contains a single mouthpiece. Similar to a Hans-Rudolph valve, the valve is closed at the end of expiration. The changes in pressure and volume inside the box due to inhalation and exhalation are used to determine functional residual capacity and total lung capacity. When the subject breathes inside the air-tight plethysmograph, the changes in lung volume are measured between the compression and decompression of the thoracic gas. In addition to the changes in thoracic volume, pressure transducers measure changes in pressure at the pneumotachometer connected to the mouthpiece and the changes in pressure across the chamber walls. The combination of thoracic changes and pressure changes in a constant-volume variable pressure plethysmograph allow for the measurement of small volume changes in the lungs ^[42].

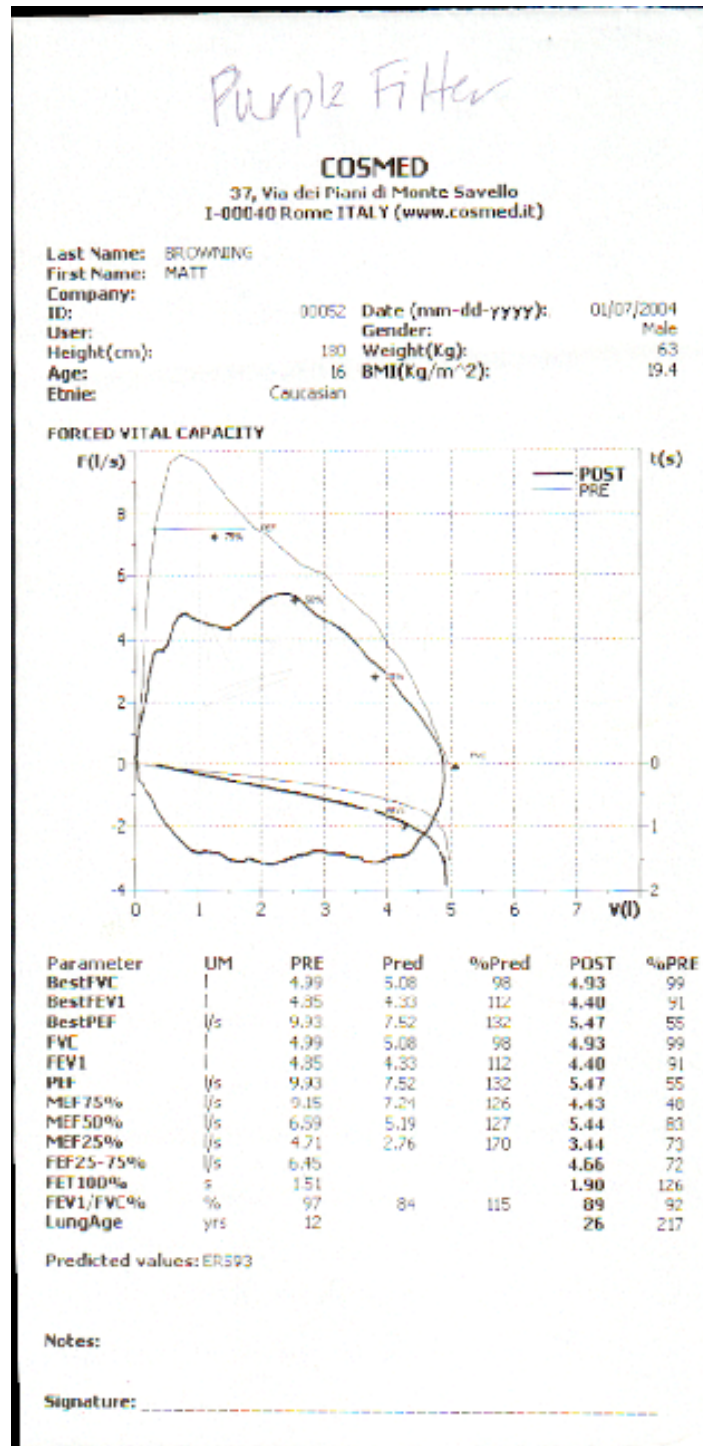


Figure 1. Pulmonary Function Test Output from Cosmed Pony FX Spirometer

*Pred = predicted value based on subject characteristics; %PRE = the percentage that the actual measured value was of the predicted value

TREADMILL VS. CYCLE ERGOMETER

Research has shown that the amount of muscle mass recruited during exercise affects the VO_{2max} measurements for each individual. Due to different amounts of muscle recruitment, various exercise modes yield differing VO_{2max} values. Treadmill locomotion and stationary cycling are the most common exercise modes used for testing. Due to the nature of the subjects recruited in this particular study (trained runners), the treadmill was chosen as the exercise mode for the study. In addition to the running background of the subjects, research has shown that treadmill tests yield significantly higher VO_{2max} values than do cycling tests. A study by McArdle et al. tested 15 male subjects on four different treadmill tests and two different cycling tests^[43]. The results showed that the cycling tests yielded VO_{2max} that were 10-11% lower than the treadmill tests.

Despite the use of trained runners in this study, it should be noted that other exercise modes can indeed elicit higher VO_{2max} values than a treadmill test if the subject is highly trained in the particular mode of exercise. Stromme et al. performed a study in which two VO_{2max} tests were performed on 37 athletes – one treadmill test and the other an exercise that simulates activity in their respective sport^[44]. In contrast to the McArdle study mentioned earlier, this study showed that the athletes elicited a higher VO_{2max} value in their sport-specific exercise than they did during the uphill treadmill test. For instance, the cyclist elicited a 5-10% higher VO_{2max} on the cycle ergometer than on the treadmill test.

ROBUSTNESS OF VO_{2max}

Research has proven that VO_{2max} can be influenced by many factors. VO_{2max} is influenced by sex, age, altitude, training, and seasonality (A study by Ingemann-Hansen et al. ^[45] even showed that subjects had a significantly higher VO_{2max} value in summer months in comparison to winter months). Considering the many factors that can influence VO_{2max} and one of the hypotheses of this study (that the purple filter reduces the VO_{2max} value), it is of critical importance to understand the day-to-day variability in VO_{2max} and the repeatability of a maximal exercise test.

Research done by Mitchell et al. ^[46] showed that the repeatability of VO_{2max} tests is very high if the researchers use rigid criteria for the attainment of true VO_{2max} values. Their results showed that duplicate trials for 15 normal male subjects produced an average of 3.06 L/min at maximal oxygen consumption for Trial 1 and an average of 3.07 L/min for Trial 2. More specifically the variance analysis of the results showed that almost all of the variance was due to inter-individual differences and that the intra-individual component was “virtually negligible.”

Another study by Amorim et al. ^[47] examined the within- and between-day repeatability of children’s oxygen uptake during submaximal treadmill walking at a self-selected pace. While this study does not examine variance at maximal exercise levels and does not have a set pace, it can be assumed that a rigid protocol would only strengthen the repeatability of the test. The study concluded that within- and between-day repeatability was verified for both VO₂ and HR for all four submaximal walking speeds.

In addition the study was in agreement with the Mitchell study ^[46] as it showed that between-day data showed no significant difference.

Similarly, studies by Mendonca et al. ^[48] and Garrard et al. ^[49] showed no between-day differences in VO_{2max} , V_{Emax} , and maximal HR. However, these two studies have shown within-subject coefficients of variation (CVs) of VO_{2max} to be 8.6% and 8.4% respectively, and V_{Emax} to be 11.0% and 12% respectively. The reality is that research on intra-individual day-to-day variation for VO_{2max} has been variable itself. Studies investigating the intra-individual day-to-day variation of VO_{2max} have reported variation as low as 3% (Miles et al. ^[50] and Taylor et al. ^[51]), others a 4-6% variation ^[52], and some as high as 10%-12% (Versteeg et al.) ^[53].

CHAPTER III

MATERIALS AND METHODS

ABSTRACT

Purpose: To determine the effects of a purple bacteriological filter (PF) on the measured maximal oxygen uptake (VO_{2max}) during a maximal treadmill test as well as its effect on the expiratory flow measurements during a pulmonary function test. **Methods:** Male ($n=7$) and female ($n=7$) runners (18-35 years old) completed two continuous graded exercise tests (GXTs) followed by pulmonary function tests (PFT); one with a purple filter (PF) and one with no filter (NF). The GXT consisted of running at a constant speed on a treadmill while gradually increasing the grade until volitional exhaustion was reached. Following each of the GXTs two pulmonary function tests were performed under the same filter conditions used in the GXT. Testing was conducted over two consecutive days, and the order of the testing was randomized. **Results:** Mean values for maximal ventilation ($V_{E_{max}}$), VO_{2max} , and test duration were larger for the NF condition, however peak expiratory flow (PEF) was the only variable that proved to be statistically significant ($p<0.05$). Under the NF condition, $V_{E_{max}}$ was 4.33 ± 8.93 L/min higher and VO_{2max} was 1.1 ± 2.7 ml/kg/min higher, compared to the PF condition. There was a significant interaction between order of testing and $V_{E_{max}}$ ($p<0.001$), and between order of testing and VO_{2max} ($p<0.015$). Mean values of FVC, FEV1, and FEV1/FVC% were 0.04 ± 0.11 L, 0.06 ± 0.25 L, and 0.54 ± 4.57 % higher in the NF conditions, but were

insignificant. PEF values increased significantly by 0.41 ± 0.63 L/sec, from PF to NF conditions ($P < 0.031$). **Conclusion:** The PF does not appear to have a large effect on VO_{2max} , FVC, FEV₁, and FEV₁/FVC% values. However, PEF was significantly affected and V_{Emax} trended towards significance with a near-significant p-value of 0.051. When including order in the analysis, there does appear to be an order effect as participants increased VO_{2max} and V_{Emax} values from day 1 to day 2 regardless of condition order. Overall, VO_{2max} and V_{Emax} were not greatly affected by the presence of the purple bacteriological filter inserted between the expired gas hose and pneumotachometer.

INTRODUCTION

Maximal oxygen intake (VO_{2max}) is defined as the highest rate at which oxygen can be taken in and consumed by the body, during strenuous, dynamic exercise ^[1]. VO_{2max} is widely accepted as a measure of aerobic fitness and has been linked to numerous health and performance outcomes. In addition, VO_{2max} is viewed as an important determinant of endurance athletic performance. Medical personnel use VO_{2max} for diagnostic purposes, and health fitness professionals often use it as the basis for prescribing exercise.

The ParvoMedics TrueOne 2400 is one of the most widely used computerized metabolic measurement systems in exercise physiology laboratories. One of the standard accessories of this system is a purple, disposable bacteriological filter (Creative BioTech Clear Advantage Pulmonary Function Filter, Kansas City, MO). With the Parvomedics system, ventilation is usually measured on the expired side of the equipment. The filter is placed between the expired air hose and the Hans-Rudolph pneumotachometer that adjoins the mixing chamber. The purpose of the filter is serve as a bacterial shield ^[54]. However, given that there is a one-way flow of air from the person to the mixing chamber there is no need for a bacteriological filter located 1.3 meters downstream from the Hans Rudolph breathing valve. The Hans Rudolph valve only allows for the participant to inspire room air, therefore the bacteriological filter is unnecessary for purposes of a bacterial shield during an exercise test.

The purple filter is designed for use with pulmonary function testing, including measurement of functional residual capacity (FRC) and forced expiratory volume (FEV_1). It is possible that the condensation of the expired air could saturate the filter and impede the airflow across the filter. However, the effects of the purple filter during maximal exercise testing have not previously been examined, to our knowledge. An accurate VO_{2max} value is critical to the field of exercise physiology.

Thus, the primary purpose of this study is to test the effects of the purple filter on during a maximal treadmill test. Specifically, we wanted to measure the differences in physiological variables (VO_{2max} and V_{Emax}) with and without the purple filter. It has been commonly accepted that the filter does not alter the measured VO_{2max} values, but pilot studies conducted at the University of Tennessee indicate that the use of the purple filter leads to a 7-9% decrease in measured VO_{2max} values. A secondary purpose of this study is to test the effects of the purple filter on expiratory flow measurements during pulmonary function testing. Thus, we measured the differences in FVC, FEV_1 , peak expiratory flow rates, and $FEV_1/FVC\%$. Overall, it is hypothesized that the purple filter will decrease VO_{2max} and V_E , due to increased breathing resistance during exhalation.

METHODS

Participants

Fourteen runners (7 men, 7 women) were recruited from Knoxville, TN to participate in this study. Eligibility criteria included: 18-35 years of age, running at least 10 miles per week on average over the past 6 months, and low risk for cardiovascular disease as defined by ACSM/AHA ^[55]. Prior to participating in the study, participants signed an informed consent form that was approved by the university's Institutional Review Board (Appendix A). All testing was completed in the Applied Physiology Laboratory in the Health, Physical Education, and Recreation Building on the University of Tennessee campus.

Exercise Protocol

Pre-screening:

All participants were screened prior to the exercise with the questionnaire shown in Appendix B. The questionnaire is a modified version of the American College of Sports Medicine (ACSM) risk stratification for coronary artery disease risk factors and the ACSM/American Heart Association (AHA) facility pre-participation screening questionnaire.

Laboratory Visit #1:

Prior to the beginning of testing, the participants had the nature of the study explained to them (including risks and benefits), and were given an opportunity to ask questions about any aspects of the study that were unclear to them. They then signed an informed consent form approved by the University of Tennessee IRB.

Researchers then obtained information on age, sex, height, weight, and body fat percentage. Height was measured using a stadiometer, and weight was measured using a Tanita BC 418. Height, weight, and body fat percentage were measured without shoes. Standardized height and weight measurements were needed to make accurate VO_{2max} comparisons between and within participants. Participants also underwent bio-electrical impedance analysis (BIA) to test body fat. The Tanita BC 418 was also used to determine percent body fat. The participants were asked to stand barefoot on a scale and grasp two handles containing sensors and an electric current (which cannot be felt) was circulated through the participant's body. The impedance to flow of electric current as it travels through the body was measured.

Each participant then changed into athletic apparel and running shoes, and performed a maximal graded exercise test (GXT) on a treadmill. Participants were randomly assigned to one of two conditions (with a purple filter or without) on day 1. The participant was instructed to perform a 5-minute warm-up on the treadmill at a self-selected pace to get the muscles warmed up as well as get the participant acclimated for treadmill running. They were then given a 5-minute rest before the exercise test began. Participants were then asked to run on the treadmill at a speed equivalent to their

approximate 5-km race pace. For all tests, the subject straddled the treadmill belt while the speed was brought up to the pre-determined setting. Once treadmill speed was reached, the subject was instructed to start running, at which point the test began. Treadmill speed remained constant while the grade was increased by 1.0% each minute until the participant attained volitional exhaustion, or requested to stop the test.

During the GXT, each participant breathed through a mouthpiece and a nose clip was used to prevent nasal breathing. A Hans Rudolph non-rebreathing valve was connected to the mouthpiece allowed the participant to inhale room air and exhale expired gas into an attached tube leading to a metabolic cart. The percentage of carbon dioxide and oxygen in the expired air was analyzed using a metabolic cart (ParvoMedics TrueMax 2400), and data was collected in 15-second epochs. The volume of expired air was measured using a Hans Rudolph 3813 heated pneumotachometer. A 3.0-liter calibration syringe was used to calibrate the pneumotachometer within 10 minutes before the start of testing. Multiple strokes of the calibration syringe were used to assess the pneumotachometer resistance across different flow rates. Gas calibration was also performed within 10 minutes of the start of testing. Standard gas with known concentrations was used to compare to the real gas concentration and allow the computer to obtain conversion factors. In addition, the participant wore a heart rate monitor that consisted of a receiving unit attached to the handle of the treadmill that received heart rate information transmitted by a thin strap with electrodes worn around the participant's chest.

The highest value of $\dot{V}O_2$, \dot{V}_E , respiratory exchange ratio (R), and heart rate (HR) measured over a 15-second period were used to determine maximal oxygen uptake. The

participants were told that they should give a maximal effort, but could terminate the test at any time. Once the test was ended, the participants performed a 5-minute cool down session.

Two pulmonary function tests were performed beginning five minutes after each maximal GXT. For the exercise test condition requiring the purple filter, the PFTs employed the moist filter that had been used during the exercise test. For the “no purple filter” condition, two separate PFTs without the purple filter were conducted following the maximal GXT. The PFTs were performed after the VO_{2max} test because it was hypothesized that the condensation of the expired gases would saturate the filter and result in increased resistance during expiration. Performing PFTs after VO_{2max} testing allowed for the measurement of pulmonary function with the condition of the filter used at the end of VO_{2max} testing.

Laboratory Visit #2:

This visit used the same measurement methods to determine oxygen consumption, height, weight, and body composition. The alternate condition of that used in the first exercise test (Visit #1) was used in the second laboratory visit. This visit took place at approximately the same time of day as visit #1.

All laboratory visits ended with an approximate 5-minute cool-down at a self-selected pace.

Statistics

Paired samples t-tests were used to compare the two conditions regardless of the order in which they were performed. When including order in the analysis, a repeated measures ANOVA was used to compare the two conditions.

RESULTS

Descriptive statistics of participants are described in Table 1 below. There were 14 participants in this study – 7 male and 7 female. The average age of the males and females was almost identical as the females had a mean age of only 0.14 years older than the males. All subjects met the inclusion criteria of a body mass index (BMI) less than 30. The females were also 10cm shorter than the males on average, and weighed 8.37kg less than the males. The females had a 6.42% higher body fat than the males as well. Due to the inclusion criteria, the total group consisted of fit and seemingly healthy individuals with an average BMI of 22.05.

Table 1. Physiological Characteristics of Participants (n=14; 7 males and 7 females)

Variables	Total Group (n=14)	Males (n=7)	Females (n=7)
Age (y)	23.07	23	23.14
Height (cm)	174.85	179.85	169.85
Weight (kg)	66.75	75.94	57.57
Percent Body Fat	13.14	9.93	16.35

*Values are mean; y = years; cm = centimeters; kg = kilograms

Maximal oxygen uptake (VO_{2max})

Under the no purple filter condition, VO_{2max} was 1.11 ± 2.66 ml/kg/min higher than under the purple filter condition. This difference was not statistically significant, with a

p-value of 0.144. When controlling for order, there was a significant interaction effect with a p-value of 0.015. The group that used the purple filter on day 1 (Group 1) had a higher VO_{2max} (by 2.51 ± 2.14 ml/kg/min) on day 2, with a p-value of 0.013. The group that used no filter on day 1 (Group 2) had a non-significant increase in VO_{2max} (by 0.77 ± 2.15 ml/kg/min) on day 2. This interaction can be seen below in Figure 2.

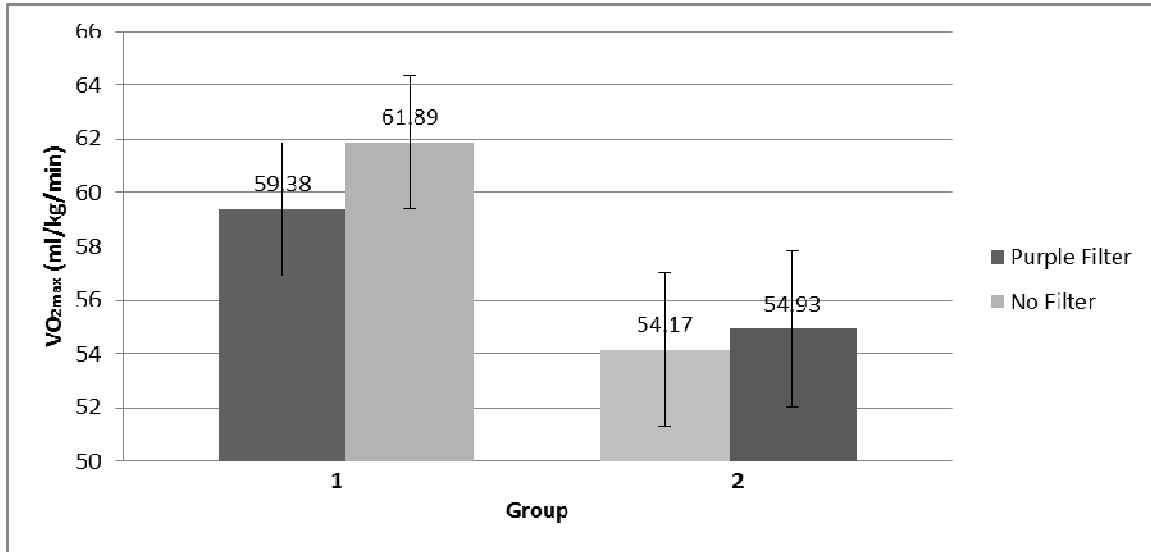


Figure 2. Graph showing the interaction between purple filter effect and order effect on VO_{2max} during a maximal treadmill test.

* VO_{2max} = maximal oxygen uptake (ml/kg/min); Group 1 = group that used the purple filter on day 1 and no filter on day 2; Group 2 = group that used no filter condition on day 1 and purple filter on day 2

Maximal ventilation (V_{Emax})

Maximal ventilation was higher under the no purple filter condition (by 4.33 ± 8.93 L·min⁻¹) than the purple filter condition, but the difference was not statistically significant ($p=0.093$). When controlling for order, the group that used the purple filter on day 1

(Group 1) had a higher $V_{E_{max}}$ on day 2, with mean values of $122.95 \pm 9.20 \text{ L}\cdot\text{min}^{-1}$ and $133.06 \pm 9.09 \text{ L}\cdot\text{min}^{-1}$ respectively ($p=0.001$). Those who used no filter condition on day 1 (Group 2) had a non-significant increase from day 1 to day 2, with mean values of $114.38 \pm 10.50 \text{ L}\cdot\text{min}^{-1}$ and $117.76 \pm 10.62 \text{ L}\cdot\text{min}^{-1}$ respectively ($p=0.241$). This interaction can be seen below in Figure 3.

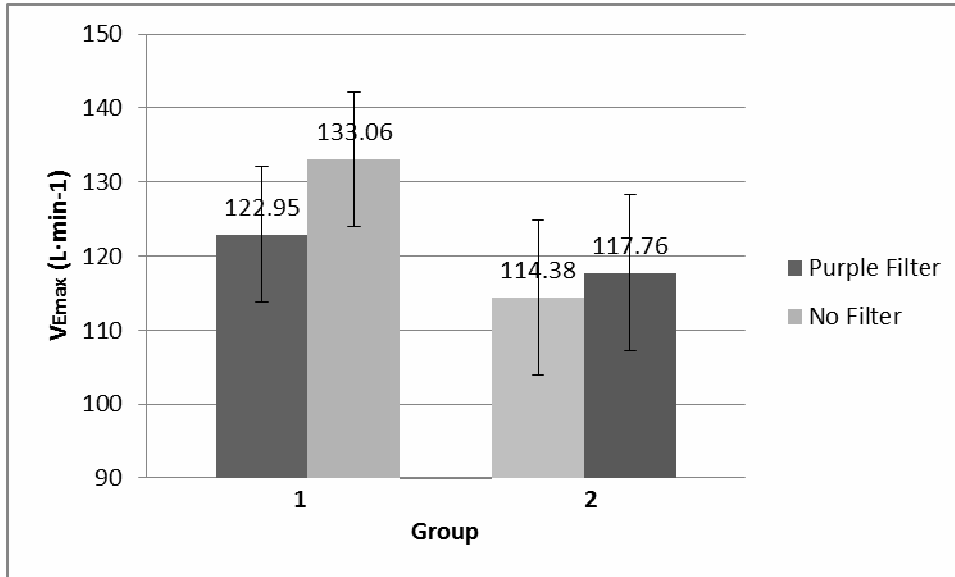


Figure 3. Graph showing the interaction between purple filter effect and order effect on $V_{E_{max}}$ during a maximal treadmill test.

* $V_{E_{max}}$ = maximal expired ventilation ($\text{L}\cdot\text{min}^{-1}$); Group 1 = group that used purple filter on day 1 and no filter condition on day 2; Group 2 = group that used no filter on day 1 and purple filter on day 2

Test Duration

The test duration was longer under the no purple filter conditions (8.55 ± 1.56 minutes) than under the purple filter condition (8.16 ± 1.21 minutes), which bordered on statistical significance ($p=0.051$). When including order in the analysis, there was no significant filter effect on test duration.

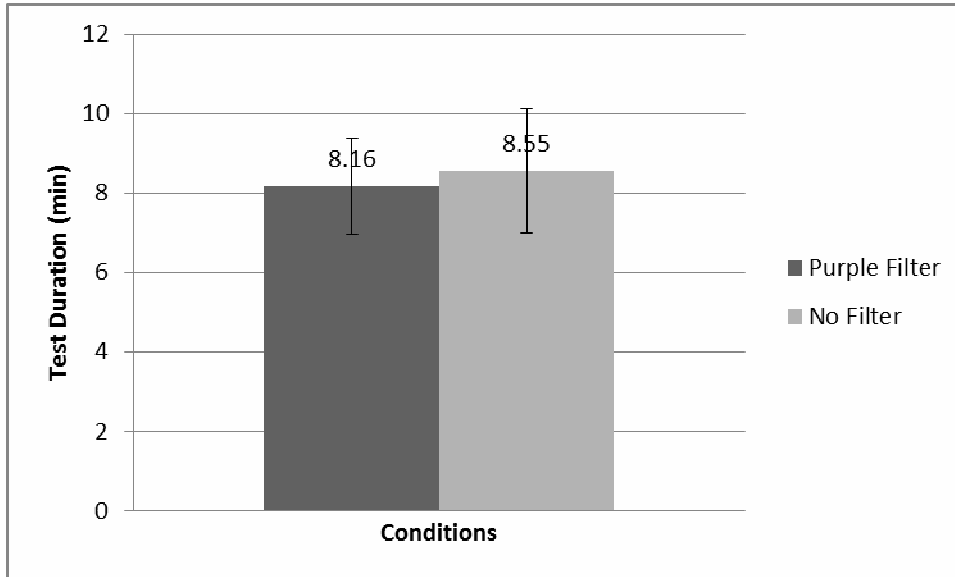


Figure 4. Effects of a purple microbial filter on test duration, during a maximal treadmill test.

Pulmonary Function Test

PEF was $8.82 \pm 2.02 \text{ L}\cdot\text{sec}^{-1}$ when using the purple filter, and $9.23 \pm 2.28 \text{ L}\cdot\text{sec}^{-1}$ when using no filter. This increase ($0.41 \pm 0.63 \text{ L}\cdot\text{sec}^{-1}$) was statistically significant ($p=0.031$). The presence of the purple filter did not have a statistically significant effect on FVC, FEV1, and FEV ($p=0.215$, 0.427 , and 0.668 respectively). When including

order in the analysis, the only statistically significant pulmonary function variable (p=0.045).

DISCUSSION

This study is the first to assess the influence of the bacteriological filter on physiological variables during maximal exercise testing. The main findings of the study were the near-significant increases in $V_{E\max}$ ($p=0.093$) and test duration ($p=0.051$), the non-significant increase in $VO_{2\max}$ ($p=0.144$), and the significant increase in PEF ($p=0.031$) when using a saturated purple filter.

The accurate measurement of V_E is critical in order to obtain precise VO_2 values. Traditionally, V_E is measured immediately downstream from the purple filter with the ParvoMedics TrueMax 2400 system^[56]. If the values of V_E are altered due to the presence of the filter, $VO_{2\max}$ values could be affected. Differences in V_E were not expected at submaximal levels but at higher ventilations characteristic of maximal exercise, we had hypothesized that an increase in V_E could be present^[57]. The ParvoMedics TrueMax 2400 system is one of the most widely used computerized metabolic measurement systems, so if use of the filter caused a decrease in physiological values ($VO_{2\max}$, $V_{E\max}$, in particular), then it would be important to know this for both research and clinical purposes.

With regards to the PFT findings, the PEF showed a significant decrease ($p=0.031$) while the other variables were non-significant. Considering the saturation of the filter and that studies have shown that the resistance across the pneumotach increases as the flow rates increase, this result was not unexpected^[8, 56]. We had also hypothesized

that the FEV₁ values would decrease significantly due to the saturation of the filter but this did not occur.

One of the main limitations to this study was the small sample size. This influenced data analysis, and may not accurately allow for the effects of the filter to be seen. The variability is very high, which can cause the significance to hide if it does indeed exist. When looking at the two groups (those who performed the filter condition first and those that performed the no filter condition first), the two groups have 8 and 6 people respectively. Since the number of participants was clearly a limiting factor for the study, future research should include a larger sample size. Based on the p-values and the differences seen in VO_{2max}, V_{Emax}, and test duration, a sample size of at least 41 people would be necessary to see significance in all three variables. Individually, a sample size of 30 would be required to detect significance in V_{Emax}, a sample size of 41 for VO_{2max}, and 29 for test duration.

An interesting result of this study was that the order of the conditions does have a significant effect on VO_{2max} and V_{Emax}. This results in the inability to determine the true effect of the bacteriological filter. Based on our results, V_{Emax}, VO_{2max}, and test duration all trended toward statistical significance, but the lack the power to detect significance due to the small sample size should be considered. However, when including order in the analysis, a significant interaction was seen between order of the condition and both V_{Emax} and VO_{2max}, which was an unexpected finding of the study. Figure 2 illustrates the V_{Emax} interaction when including order in the analysis. For participants who performed with the purple filter on day 1 and no filter on day 2 (designated as Group 1), a significant increase in V_{Emax} from was observed (122.95±28.74 L·min⁻¹ to 133.06±29.26 L·min⁻¹).

This finding supports the original hypothesis. However, the participants who performed with no filter on day 1 and filter on day 2 (designated as Group 2) also saw a small, non-significant increase in $V_{E_{max}}$ ($114.38 \pm 19.71 \text{ L}\cdot\text{min}^{-1}$ to $117.76 \pm 21.64 \text{ L}\cdot\text{min}^{-1}$).

A similar trend was seen when analyzing the VO_{2max} values and taking into account the order effect. Interestingly, an increase was seen from day 1 to day 2 in both groups, but group 1 (which used a purple filter on day 1) saw a much larger increase to day 2 than did group 2 (which used no filter on day 1). Figure 1 illustrates the VO_{2max} interaction when controlling for order. As seen with $V_{E_{max}}$ data, there is almost no change for Group 2, yet a relatively large increase was seen for Group 1.

Having seen similar trends for both $V_{E_{max}}$ and VO_{2max} when controlling for order brings into question a practice effect. Future research should address whether the practice effect could be eliminated by inserting a familiarization trial. Previous studies have examined the impact of a familiarization period^[58] on maximal treadmill testing. A study by Midgley et al. used a familiarization period as part of their study which investigated the reproducibility of VO_{2max} of 22 competitive distance runners^[58]. They found that there were no significant differences between the within-subject variance of VO_{2max} ($p=0.6$).

One way to assess the influence of the practice effect would be to include more groups in the study design. Future studies should not only increase the number of participants, but they should also include 4 groups as opposed to the 2 used in this study. The four groups should include a group that tests with a filter both days, a group that tests without a filter both days, and the two groups that were used in this particular study. If a practice effect were present, having additional groups would allow for the extent of the

effect to be determined. The current study's design did not allow for the practice effect to be quantified, so it is difficult to conclude that the purple filter has a significant effect on these measurement values. In conclusion, the presence of a purple filter did not appear to greatly impact $V_{E_{max}}$, VO_{2max} and test duration during a maximal treadmill test. In addition, the order of testing has an impact on $V_{E_{max}}$ and VO_{2max} ; this may partially obscure the impact of the purple filter. Future studies should take this into consideration.

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APPENDIX A

INFORMED CONSENT FORM

TITLE: The Effect of a Purple Filter on Measurement of VO_{2max}

Investigators: Chris Bach, B.S.

David R. Bassett, Jr., Ph.D.

Address:

Kinesiology, Recreation, and Sport Studies

322 HPER Building

University of Tennessee

1914 Andy Holt Ave., Knoxville, TN 37996-2700

Phone: (865) 974-5091

PURPOSE

You are invited to participate in a research study. The purpose of this study is to measure your maximal oxygen uptake under two conditions: with and without a purple filter. You will be asked to participate in maximal exercise testing on two separate days.

PROCEDURES

You will be asked to come to the Applied Physiology Laboratory in the Health, Physical Education & Recreation (HPER) building on two separate days. Prior to your participation, you will be screened to determine whether you have any risk factors that would prevent you from

taking part in this study. Prior to the exercise test to determine your aerobic fitness, a researcher will measure your height, weight and body fat. Prior to the maximal exercise test, you will be given the opportunity to warm-up for 10 minutes at a self-selected pace. Following these measurements you will undergo a maximal exercise test on a treadmill. This involves running on a treadmill at a constant speed, with a gradually increasing incline until exhaustion is reached. Typically this test lasts 8-15 minutes. You will then undergo two lung function tests to determine your ability to quickly and forcefully exhale. The second visit will consist of the same protocol as the first laboratory visit, with this visit occurring within 24-48 hours of the previous visit. The one difference between the two tests will be whether or not the purple filter is used. As a research participant, you will not know which trial you are performing on a particular day, in order to prevent any psychological effects of the experimental condition.

During both tests, you will breathe through a mouthpiece and wear a nose clip and heart rate monitor. The mouthpiece allows us to collect your exhaled air and determine how much oxygen you use during exercise. In addition, the heart rate monitor will be worn around your chest. You will run at your indicated 5k race pace for the entire duration of the test with the treadmill incline increasing by 1% each minute until volitional exhaustion.

The total time commitment will be approximately 2 hours. The two visits will occur within 24-48 hours of each other and at the same time of day.

Participant Initials _____

BENEFITS OF PARTICIPATION

By participating in this study, you will learn your current aerobic fitness level (VO_{2max}), body mass index, and percent body fat. The VO_{2max} results from each of the conditions will be given to the participant after the conclusion of their final test.

RISKS OF PARTICIPATION

Risks are similar to those experienced during a typical vigorous workout. These risks include muscle soreness, dizziness, headache, leg discomfort, gastrointestinal discomfort, and a very rare possibility of a cardiac event. Sudden cardiac death during exercise among 18 to 35 year old persons could occur, but this is very rare. Researchers collecting data will be CPR/AED certified. Falling while running is the most likely injury. During running, one person will spot you. During the test, you may stop for any reason by signaling the researchers, or jumping off and straddling the treadmill belt. If the primary researcher feels it is unsafe for you to continue a test, he will terminate the test immediately. The University of Tennessee does not "automatically" reimburse subjects for medical claims or other compensation. If physical injury is suffered in the course of research, or for more information, please notify Chris Bach at (865) 974-5091 or cbach1@utk.edu.

CONFIDENTIALITY

The information in the study records will be kept confidential. Participants will be identified by assigned numbers only. Only the researchers will have access to your test results. All

information will eventually be used for a research report, however, your private information will not be presented in the report.

CONTACT INFORMATION

If you have questions or concerns at any time during the course of the testing procedures or after completion of the testing procedures, you may contact Dr. David Bassett at (865) 974-8766. If you have questions concerning your rights as a participant, contact Ms. Brenda Lawson with the Compliance Section of the Office of Research at (865) 974-3466.

PARTICIPATION

Your participation in this study is strictly voluntary. You have the option to withdraw from the study at any time without penalty and without loss of benefits to which you are otherwise entitled. If you withdraw from the study, then all data will be given to you or destroyed.

CONSENT

By signing this informed consent form, I am indicating that I have read and understood this document and have received a copy of it for my personal records. I have been given the opportunity to ask questions about the research study. By signing this form I indicate that I agree to serve as a participant in this research study.

Participant's name

Participant's signature

Date

Investigator's signature

Date

APPENDIX B

Screening Questionnaire:

1. Have any of the following occurred with your father or first-degree male relative before age 55, or with your mother or first-degree female relative before the age of 65: Myocardial infarction, coronary revascularization, or sudden death?

_____Yes _____No

2. Do you currently smoke, or quit smoking within the last 6 months?

_____Yes _____No

3. Has a doctor ever told you that you have hypertension, systolic blood pressure \geq 140mmHg, diastolic blood pressure \geq 90mmHg, or do you currently take antihypertensive medications?

_____Yes _____No

4. Has a doctor ever told you that you have hyperlipidemia (“High cholesterol”) defined as LDL > 130 mg/dL, or been told you have HDL < 40 mg/dL, or are on lipid-lowering medication? If you have only been told total serum cholesterol, has it been >200mg/dL?

_____Yes _____No

5. Has a doctor ever told you that you have diabetes, or impaired fasting glucose defined as fasting glucose ≥ 100 mg/dL measured on two separate occasions?

_____ Yes _____ No

6. What is your height and weight?

Height (inches): /0.254 = Height (meters):

Weight (lb.): /2.2 = Weight (kg):

Height (meters)*Height (meters) = [Height (meters)]²:

Weight(kg) / [Height(meters)]² = BMI:

Is the participant's BMI > 30

_____ Yes _____ No

7. During exercise, do you experience chest discomfort, unreasonable breathlessness, dizziness, fainting, or blackouts?

_____ Yes _____ No

8. Do you take heart medications?

_____ Yes _____ No

If the participant answered “no” to all questions, OR answered “yes” only to question 1 and “no” to all other questions (indicating that the participant has no risk factors other than family history), read the following text:

“According to your screening, we have determined that you are eligible to participate in the study. We can set up a time with you in which you will be able to commit 30 minutes to an hour of your time for your first testing and 30 minutes to an hour of your time for another testing period within 24-48 hours of the first one.”

If the participant answered “yes” to any question, except question 1, read the following test:

“According to your screening, we have determined that it might be unsafe for you to participate in this study given the presence of risk factors. Thank you for taking the time to contact us.”

Participant name

Time and date of first meeting

Date

VITA

Christopher William Bach was born on September 22nd, 1987 to Jim and Mary Bach. In June of 2006 he graduated from the Baylor School in Chattanooga, TN as a four-sport athlete. He began his undergraduate education at the University of Tennessee – Knoxville in August 2006 and began his major in Biochemistry, Cellular, and Molecular Biology. After graduating with his Bachelor of Science, he had aspirations of attending medical school to become an orthopedic surgeon. In order to gain an advantage on fellow med school applicants, he then enrolled in the Kinesiology Master of Science program at the University of Tennessee with a concentration in Sports Medicine/Biomechanics. While taking classes for Biomechanics, he chose his elective classes to be in the field of Exercise Physiology. It wasn't long before he realized that he loved exercise physiology and that was where his true passion lied. In August of 2012, he will graduate with a Master of Science degree in Kinesiology with a concentration in exercise physiology from the University of Tennessee. Chris will continue his graduate education in the fall of 2012 as a doctoral candidate at Florida State University.

While enrolled in school at Tennessee, Chris also worked in the athletic department as a student worker and eventual graduate assistant. His time in the athletic department solidified and strengthened his love for the University of Tennessee. Tennessee athletics are one of his biggest passions and people often say that Chris' blood runs orange. He remains certain that the Vols will return to dominance, where they belong, under the leadership and vision of Derek Dooley. Go Vols!