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Anastasia S. Lopukhin Date

Assessing Inspiratory Volume in Adolescents during Physical Activity

By

Anastasia S. Lopukhin
Master of Public Health

Global Environmental Health

Roby Greenwald, PhD
Committee Chair

Paige Tolbert, PhD
Committee Member

Assessing Inspiratory Volume in Adolescents during Physical Activity

By

Anastasia S. Lopukhin

Bachelor of Science
University of Wisconsin- Madison
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Thesis Committee Chair: Roby Greenwald, PhD

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Abstract

Assessing Inspiratory Volume in Adolescents during Physical Activity

By Anastasia S. Lopukhin

Adolescents are a unique demographic for whom predicting physiological characteristics is difficult. Given their developmental differences from both adults and prepubescent children, making assumptions surrounding certain measures such as heart rate variability and minute ventilation can be challenging. Inspiratory volume is one such uncertain characteristic. At a time when ambient air pollution has become the leading environmental health risk, determining inspiratory volume can give tremendous insight into estimating inhaled pollutant dose. This study tested n=18 adolescent students from an Atlanta area high school to elucidate a relationship between heart rate and inspiratory volume at different levels of physical activity. Inspiratory volume for each breath was measured as participants increased their activity levels from rest to a running sprint on a treadmill. The resulting model that was formulated related the natural logarithm of instantaneous heart rate (lnHR) to the ratio of inspiratory volume (IV) to forced expiratory volume (FVC) in the following manner: $IV/FVC = -0.598 + 0.199\ (lnHR)$ ($p<0.001$, $R^2=0.225$). This model can be used in conjunction with ambient air pollution measures to estimate inhaled pollutant dose provided that information about the subject's FVC and heart rate during physical activity are available. This information can further be used to guide policy concerning extracurricular activities in area schools so that adolescents are protected during period of high ambient air pollution while still engaging in physical activities conducive towards promoting and maintaining good health.

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Table of Contents

Introduction

In recent years, we have seen air pollution become the world's largest environmental health risk. As of March 25, 2014 the World Health Organization estimates that approximately every one in eight people globally dies prematurely from air pollution. This translated into 7 million deaths in 2012 due to air pollution, at least 2.6 million of which were attributed to outdoor air pollution[1]. Furthermore, this is a dramatic increase from the 1.34 million deaths the WHO attributed to outdoor air pollution in 2008[2]. Ischemic heart disease, stroke, chronic obstructive pulmonary disease, lung cancer, and acute lower respiratory infections (in children) have all been associated with outdoor air pollution exposure[1]. With this rise in air pollution, we have also witnessed rising concern about, and additional research into, all the potential health affects associated with air pollution, not just mortality.

With increased pollution, more people are unwillingly exposed to something detrimental to their health. The adverse health effects of air pollution in both acute and low exposures have been documented and compiled in numerous publications[3-6]. They have found that air pollutants cause irritation of the nose and throat, bronchoconstriction and dyspnea (specifically from sulfur dioxides, nitrogen oxides and heavy metals)[6]. Lung irritation results from particulate matter penetrating into the alveolar epithelium and nitrogen oxides increase the respiratory system's susceptibility to infection[6]. These effects can cumulatively result in reduced lung function, asthma, emphysema, and lung cancer. Air pollution has also been linked to angina, myocardial infarction, tachycardia, hypertension, and anemia. Heavy metals in the air have been specifically linked to neuropathies and kidney damage, and dioxins cause liver cell damage[6]. The United Kingdom even established The Committee on the Medical Effects of Air Pollution to help guide government action on the issue.

However, much uncertainty still exists in this field of research, especially with regards to estimating the dose of air pollution an individual inhales. Therefore the applicability of this discovered information varies across situations. For example, we cannot assume that air pollution exposure would have the same effects on children as it does on adults. Regressing in age, adolescents, children, and infants have a larger surface area to volume ratio, higher ventilation rate per unit body mass than adults, and they are still undergoing critical windows of development, making their susceptibility to the absorption of potential toxins greater[7-10]. Their development, including that of their immune and defense systems[11], has also not yet

reached completion, and the uncertainty about how a given toxin may affect development is important to address in determining future health.

Adolescents are of particular concern with regard to air pollution, because they engage in many outdoor sports and extracurricular activities- increasing their exposure time. They also participate in these activities during periods in the day when pollutant levels (ozone) are highest[12]; during afternoons, weekends, and summer months. Adolescents are also less likely to report exposure related symptoms during physical activity[13]. During physical activity people breath more frequently, the airflow in and out of their lungs speeds up, and they breathe more through their mouths than noses[14]. Some predictions have been made about the extent of these changes in adults[15], but the extent of these changes in adolescents have yet to be fully explored.

An important distinction that needs to be made is that ambient air pollution refers to the levels of air pollutants in the surround area or environment. Personal dose, on the other hand, is a function of the ambient conditions, the exposure duration (i.e. how long the person is in contact with environmental air), and ventilation data. Ventilation rate and depth of breathing will affect the amount of pollutants circulating and depositing in, or reacting with, the lungs [16]. Technological advances enable us to more easily measure ambient air pollutant concentrations, and we can use models to predict how much an average human being breathes at rest. However, this information is limited because it does not take into account different geographical areas of exposures through which a person moves on a daily basis, nor does it account for changes in breathing patterns that occur as a result of varying degrees of activity. Some studies have attempted to estimate dose by actually measuring minute ventilations in humans but these are limited by interpersonal differences, as will be discussed later.

Adolescents are a very unique demographic. They are in different developmental stages, their activity patterns are different, and their physiological responses to stimuli are different from both children and adults[8]. All of these factors are important in determining to what actual dose of air pollutants adolescents are exposed during physical activity- something that is crucial for adolescents' health. Health during adolescence can set the stage for an individual's health for the rest of their life[17]. Many health policies and campaigns are directed towards adolescents to foster good health habits (i.e., physical education classes and extracurricular sports) or provide environments conducive to good health (i.e. the prohibition of marketing tobacco products to teenagers or providing healthy food choices at schools). Physical activity in

adolescence is critical to both emotional and physical health and helps ensure better health in the future[18]. The benefits of physical activity for all ages have been reported by numerous studies. For example, regular physical activity confers significant protection from chronic diseases including cardiovascular disease and diabetes mellitus and protects against future diseases[18]. Current recommendations for physical activity vary among researchers; some state that youth should exercise at a moderate to vigorous level for approximately 60 minutes daily and others suggest that adolescents should engage in aerobic activity at least three times per week for a minimum of thirty minutes per workout at an intensity of 75% of their heart rate reserve- defined as:

$$HR_{75\%}=(\text{resting HR} +0.75(\text{max HR- resting HR}) \text{ [18].}$$

Hartog et al found that for adults in Amsterdam, the health benefits conferred by cycling in an urban setting outweighed the detrimental effects of the urban air pollution[19]. This study, however, leaves questions with regards to adolescents and their vulnerability to air pollution in relation to the benefit of cardiovascular activities. If air pollution levels in a certain city are considered to be dangerously elevated and adolescents are encouraged to participate in physical activities outdoors, it is important that we find a good methodology for determining a relatively accurate inhaled dose. Through this, we will be better able to assess the potentially detrimental health affects adolescents may incur from exposure to air pollution.

The current Study on Air Pollution and Physical Activity (SAPPA) aims to explore the relationship between air pollution exposure during physical activity and subsequent health effects. Currently the study is in the process of characterizing the concentration and composition of ambient air pollutants at two Atlanta-area high schools while simultaneously determining various aspects of these athletes' breathing profiles. The two high schools in this study were chosen based on the surrounding air quality. Ambient ozone, particulate matter, and black carbon concentrations are measured at these locations to explore the synergism within pollutant mixtures. One high school is located next to a major highway (I-75/I-85) and a major railway and has characteristically high levels of ozone and particulate matter. The other high school is located between two state parks and has characteristically lower levels of particulate matter. Study participants wear monitors that track their breathing and heart rates, acceleration and activity levels, chest expansion amplitude, and record ECGs. They also undergo testing for

pulmonary function, exhaled nitric oxide, and exhaled breath condensate. Inspiratory volume however, cannot be measured directly when subjects at these schools are actually participating in practices for their extracurricular activities so instead it must be elucidated indirectly. The current research project was undertaken to address the discrepancies between measuring ambient pollutant concentrations and inhaled dose at different heart rates and activity levels.

Aims

- To estimate inhaled dose of air during periods of physical activity
- To determine the relationship between dose and heart rate

Background

Not only do adolescents differ from adults in their development, but the "adolescent" years also encompass a wide spectrum of physical and cognitive developmental changes. Therefore, we see a wide variety of body shapes, sizes, and maturations among adolescents. Children and adolescents are not simply small adults. Pertinent to air pollution, lung development cannot be easily predicted given an adolescent's height; they may grow in stature more quickly than their lungs develop. Most humans do not reach full lung development in adolescence; peak development is not reached until age 20-25, with females generally reaching their peak lung function sooner than males[20]. Also noteworthy, as children grow older, their ventilatory frequency decreases as the length of time for inhale and exhale increases[21] and breaths elongate. Just within the first three years of life, the respiratory rate decreases exponentially with increased body weight[10, 13]. Because infants have a larger surface area per unit body weight (and because they are still growing), children have a higher resting metabolic rate of oxygen consumption per unit body weight than adults[11]. Thus, infants at rest have twice the volume of air passing through their lungs than do adults at rest and so twice the amount of pollutants may pass through, and deposit in or react with, their lungs[13]. For infants, the body mass standardized minute ventilation is estimated at 400mL/min/kg while that of children is 150mL/min/kg[13].

Anatomical lung differences are also important. At birth, the bronchial tree is fully developed, but the airway size will increase with age. Because of the narrower airways, an

irritation response invoked by a certain pollutant can obstruct the airways of infants to a significantly greater extent than it would in adults[11, 13]. In addition, the pulmonary airway and alveoli will continue to increase in number until age eight, after which point they will increase in size until the chest wall is fully grown[22]. At birth, infants have approximately 24 million alveoli, but by the time they've reached adulthood that number reaches approximately 600 million[11, 23]. The muscles in the chest wall and other secondary respiratory muscles are also still immature[24] which can play a roll in ventilation rates, as will be discussed later. If exposure occurs at critical windows of developmental vulnerabilities, then all of these maturation, differentiation, and growing patterns can be critically altered[23, 25]. If damaged during critical windows, the lungs may remodel in such a fashion that would ultimately increase their vulnerability to future insults[23]. The immune system is of particular concern, because disruption of its maturation can lead to deficiencies in a person's ability to fight attacks on the body later in life; making that person more susceptible to infections and damage from air pollutants due to immature anti-oxidant and detoxification capacities[13]. Last, children do not have fully-developed nasal passages, so they breathe more through their mouths; as they get older and the nasal passages grow, the ratio of nose to mouth breathing also increases[23]. This is important, because it implies that the filtering mechanisms of the nose do not protect children to the same extent that they protect adults[26] and can lead to greater particle deposition and irritation in the lower respiratory tract[13].

Adolescents also spend more time outdoors than do adults- both in terms of frequency and duration[23], playing various sports,- and engaging in physical activity[11-13]. Physical activity alters the breathing pattern in many ways. Rabler et al found that the degree of coordination between breathing and stepping in adults was enhanced with increased walking speed[27]. Carlisle et al[14] describes how physical exertion increases minute ventilation, increases airflow velocity, and increases the ratio of mouth to nose breathing. Benchetrit et al [28] suggest that each individual selects a particular breathing pattern that is characterized by particular ventilatory variables (tidal volume and expiratory duration) as well as airflow profile. Others [29, 30] go further to suggest that adults use different strategies of breathing for different exercise activities. This makes modeling and predicting their breathing profiles difficult, and determining actual exposure and dose of air pollutants becomes problematic.

To address the distinctions between children and adolescents versus adults, Boule et al underwent a characterization of breathing patters in untrained children ages six to fifteen. They

found that oxygen uptake, tidal volume, minute ventilation and the ratio of inspiration time to duration of the respiratory cycle all significantly increased ($p<0.001$)[31] when the subjects increased their physical activity from rest to maximal load. Oxygen uptake was found to positively correlate to mean inspiratory flow, and tidal volume was positively correlated to the ratio of inspiration time to duration of the respiratory cycle and tidal. Respiratory frequency and inspiratory time responded slightly differently to increasing exertion. Respiratory frequency increased and inspiratory time decreased from rest to 2/3 of the maximal workload, but then remained the same from 2/3 of the maximal workload to 100% of the maximal workload[31]. Although several of their subjects were pubescent, the study did not characterize the full age range of adolescents. Nor does it account for children trained in a specific sport.

Trained adolescents can be very conscious of how they breathe during specific sports, such as cross-country or football, in order to maximize their athletic abilities, but limited research has been done to specifically characterize this. For example, Wells et al [32] describe how elite athletes can reach maximal breathing frequencies between 40-60 breaths per minute which could bring their maximal tidal volumes up to 1.5-4.0 liters per minute to try to meet the metabolic demands of their physical exertion. This is, however, a limited characterization of breathing profiles, because not all adolescents playing school sports may be considered “elite” athletes.

Adolescent athletes can also commonly suffer from a variety of exercise induced respiratory symptoms, including bronchospasms, vocal cord dysfunction, paradoxical arytenoid motion, hyperventilation, and cardiac disease[33], some of which is hypothesized to be related to the immaturity of respiratory muscles. Bronchospans result from high ventilation rates and unfortunately lead to a 10% decrease in FEV_1 after exercise, causing reduced lung function from that otherwise predicted[34]. These variations further contribute to the imprecise nature of estimating the volume of inhaled air. Furthermore, some athletes may undergo respiratory muscle training, which would enhance maximal minute ventilation and forced vital capacity [24], effectively increasing the volume of inhaled air and thus the variability of breathing profiles within the adolescent demographic.

One historically contentious point of research in respiratory physiology has been maximum ventilatory capacity. Some studies previously concluded that no upper ventilator capacity existed in humans[35], but this notion has been recently challenged. As described by Wells et al, athletes can reach 40-60 breaths per minute, but this was also found to be a *limit* of

ventilatory capacity. This limitation was evident as well in research conducted by Boule et al when respiratory frequency and inspiratory time did not change significantly from 2/3 of the maximal workload to full maximal workload activity[31]. Harms et al[36] also states that there is increasing evidence to support the notion that the pulmonary system does not exceed the metabolic demands of exercise. Ventilatory capacity is limited by mechanical factors, ventilatory muscle function, genetics, aging, disease status, and broncodilation or bronchoconstriction [37] but the specifics are hard to definitively measure. Determining ventilatory capacity may be important in ascertaining at the very least, maximum amount of air, and subsequent pollutants, that an exercising human may inhale and consume during physical exertion. Being able to determine maximal ventilatory capacity can help determine at least the upper limit of inhaled pollutant dose.

How adolescents breathe at rest and during physical activity is an important determinant in how much air they inhale, which is subsequently indicative of how much air pollution they consumed. Specifically, the changes in minute ventilation affect total airflow in and out of the lungs. Air pollution consumption at this life stage is detrimental because development is still occurring and air pollution can negatively affect it leading to adverse health effects in adult life[38]. A cross sectional survey conducted by Second National Health and Nutrition Examination Survey (NHANES II) concluded from their analysis that there was a statistically significant negative correlation between FVC and FEV1 with the annual concentrations of total suspended particles, nitrogen oxide and ozone[39]. This gives a good representation of the national health burden attributable to air pollution. Gauderman et al have studied the effects of ambient air pollution on children and adolescents through age 18. They found that there is a correlation between decreased FEV_1 (forced expiratory volume of the first second) in 18 year olds and a lifetime exposure to a combination of air pollutants and that the decrease in FEV_1 is clinically significant [40], and not reversible in adulthood[41]. This in turn increases the risk of chronic respiratory illnesses as an adult[41]. It is not then surprising that a major clinical indicator of mortality risk in adults for a plethora of diseases is impaired lung function[42]. This group went on to explore the lung function deficit in children who spent more time outdoors than children who spent more time indoors. They found that there were statistically significant deficits in lung function growth (FEV_1, FVC, maximal midexpiratory flow, and FEF_{75}) with age when these children were exposed to PM_{10}-$PM_{2.5}$, NO2, and inorganic acid vapors. They also showed that the more time these children spent outside, the worse their lung

deficits and that both asthmatic and non asthmatic children were affected [41]. McConnell et al[43] further corroborated these findings, as will be discussed.

Particulate Matter

Particulate matter (PM) air pollution refers to a mixture of solid particles and liquid droplets. Examples of PM include black carbon, polyaromatic hydrocarbons, and diesel exhaust particles, which range in size from soot or dust to microscopic particles less than 2.5 micrometers in size. Inhalable course particles range in size from 2.5 to 10 micrometers, while those less than 2.5 micrometers are called fine particles. Particulate pollution is particularly high during smog formation; either in winter when emission builds up in cold weather or in summer when emissions interact with ozone[14]. Primary particles are those that are expelled directly into the air due to various combustion or environmental processes- these include black carbon and ultrafine organic particles. Secondary particles are those that are created through secondary environmental reactions with sulfur dioxides and nitrogen oxides[44]. The size and source of particulate matter is important, because particles that are small enough to stay suspended in the air can then be inhaled into the body, where they can travel deep enough into the lungs to interact with alveolar tissue. [45].

When exercising, one increases the velocity of airflow in the lungs, which carries pollutants deeper into the lungs[14]. Using dosemetric models Cheng et al [46] found that increasing flow rate from 7.5 to 15 $Lmin^{-1}$ would significantly increase deposition of ultrafine aerosols in the intrathoracic pulmonary region, regardless of nasal or oral breathing patterns. This has been corroborated by studies that demonstrated that the deposition of diesel exhaust particles in the alveolar region increases with increased minute ventilation[22]. These studies also showed that while keeping minute ventilation constant, if the volume of each individual breath is made bigger to compensate for decreased breathing frequency, particles will deposit deeper into the alveoli as air travels further into the lungs. This type of breathing is characteristic of the recovery period after intense activity. Furthermore, actual deposition was greater than predicted deposition and deposition was greater in younger adults and adolescents than in the lungs of those 25 years of age and older. Both of these factors can be very applicable to adolescent susceptibility to adverse health effects due to outdoor physical activity. Findings by Daigle et al further support the correlation between increased minute ventilation and particle deposition, which they found to also be applicable during exercise[47]. They found the burden

of ultra fine particle deposition was significantly greater in the alveoli due to increased particle intake and deposition from exercise[47]. This increase was greater than those predicated by models as well[47]. Also noteworthy is that asthmatics have even greater deposition of ultra fine particles than non-asthmatics[48], which adds further to the uncertainty in estimating dose. The deposition of particulate matter in the lungs can reduce their function and lead to adverse health effects. In brief, the particles that manage to deposit on the mucosal lining of the alveoli can make their way to the luminal side of the alveoli, reaching systemic circulation. The processes are complex and research continues on the precise mechanisms. However, plenty of epidemiologic studies have demonstrated the link between particulate matter and lung function. For example, the Six Cities Study of Air Pollution showed that as PM concentrations increased, so did the percentage of children with some lung function abnormality [49]. According to Sacks et al[50], compared to adults, children suffer from more respiratory episodes such as wheezing, cough and hospital admissions. Brunekreef et al have demonstrated reduced lung function in children who live near motorways and are exposed to diesel exhaust particles[51]. For children who lived with 1,000m of a motorway in their study, there was a corresponding 2.5% reduction in their FEV_1 and motor traffic density[51].

Gauderman et al further elaborated on these findings as shown by a 0.9% per year deficit in the growth rate of FEV_1 among children exposed to PM_{10} they found in one of their studies[41]. This deficit in growth resulted in children in their cohort not achieving maximal lung function, and this reduced lung function was not reversed in later years. This means that these children would live through adulthood with an impaired respiratory system. It should be noted that this deficit is much greater than the 0.2% deficit in lung growth incurred by children from passive smoking exposure. A follow up study on this same cohort of children was conducted by Avol et al [52]and tracked down children who had moved to different communities with either lower or high pollutant concentrations. They found that for children who moved to communities with significantly better air quality, lung function was improved, suggesting that some of the lost lung function may be regained *if* children are taken to cleaner air environments. Kulkarni et al have also found that as more carbon accumulated in the airway macrophages of children, their FEV_1, FEF_{25-75}, and FVC decreases in a dose dependent manner[53]. Specifically, for every increase in ambient PM_{10} by $1\mu g^3$, carbon content of airway macrophages increased by $.01\mu m^2$. Every 1 μm^2 in macrophage carbon context was then association with a 17% decrease in FEV_1, a

12.9% decrease in FVC, a 34.7% decrease in FEF_{25-75} [53]. These results are indicative that more exposure leads to greater and greater decreases in lung function.

Ultrafine particulate matter can be considered even more of a concern, because it can evade macrophages[5]. These particles are those less than 100 nanometers, so they contribute very little to overall mass. However, they can be very high in number[5]. In studies conducted on animal models, researchers found that inhaled ultrafine particles largely evade macrophages, getting into the interstitium and inducing a greater inflammatory response per a given mass than larger particles[5].

Studies have also suggested that $PM_{2.5}$ generated from outdoor sources could be more harmful per unit mass than particles generated from indoor sources; these outdoor particles are associated with asthma in children, decreased lung function in children with asthma, hospital admissions and mortality[54]. Diesel exhaust particles (DEP), particles generated mainly from automobile combustion processes that occur outdoors, have been linked to immune deficits in the lungs. The DEPs impair the ability of effector cells to grow properly, particularly with regard to their receptors, and thus the cells cannot respond as well to bacteria and virus incursions into the lungs[55]. This in turn impedes the ability of the body to combat respiratory infections. This difference in particulate matter toxicity is an important consideration given that children spend more time outdoors, their activity levels are higher, and they have a greater minute ventilation per unit body weight than do adults. All these factors can increase the PM dose per surface area of their lungs and lead to proportionately greater adverse effects[50]. Lastly, Rundell et al [56] looked specifically at the effects of high and low PM on lung function during exercise, finding a statistically significant dose-dependence inverse relationship between the two at high levels of PM. Specifically, when PM levels were high during exercise, the actual chemistry of the lungs was altered. Exhaled nitric oxide and total nitrate decreased post exercise, suppressing lung function[56]. Their theoretical conclusion from these results was that lipid peroxidation was occurring due to the high PM levels[56]. This further supports the notion that exposure to air pollution during exercise can have unintended negative consequences.

Ozone

Ozone is chemical air pollutant produced by the reactions between nitrogen oxides (NOx), volatile organic compounds (VOCs) and sunlight. The VOCs and NOxs generally come from motor vehicle, power plants, and industrial processes. Ozone forms in urban environments when temperatures are high and sunlight is intense but can also be transported into rural areas by weather patterns. It generally peaks in the late afternoon when traffic is highest, and it is a major contributor to what we call "smog". The diurnal pattern exhibited by ozone peaks at the time of day when adolescents are engaged in outdoor activities- mid to late afternoon- making it of particular interest to this project.

A study conducted by Tolbert et al demonstrated emergency room visits for asthma was 1.04 ($p<0.05$) for every 20ppb increase in ozone over an 8-hour period[57]. Emergency room visits may sometimes be necessary following ozone exposure, because ozone can trigger many respiratory problems in humans such as difficulty breathing, shortness of breathe and pain when breathing, cough and/or sore throat, and inflammation of the airways[58]. It can increase the frequency of asthma, emphysema, and chronic bronchitis, and make the lungs more susceptible to infection[58]. Studies have been done on newborns infants through fourteen year olds, as well as adults, showing how ozone absorption increases with age [59], but little is known about ozone absorption in adolescents.

One study conducted on young males by McDonnell at al [60] found a dose-dependent inverse relationship between ozone levels and lung function when exercising and at rest. When ozone levels were greater than or equal to 0.24ppm during periods of exercise, the subjects experienced changes in respiratory frequency, tidal volume, airway resistance, pain on inspiration, and shortness of breath[60]. This 0.24ppm ozone level also produced deficits in FVC, FEV_1 and FEF_{25-75} and these results occurred at levels lower than had previously been described to be detrimental[60]. Adams et al also conducted a study on adults, which demonstrated that at high ventilation rates ozone exposure produced a dose dependent reduction in lung function. We can conclude from this that increased physical exertion leads to increased acute adverse health effects from ozone[61]. Schelege et al further supports this suggestion with findings that show that it is the *rate* at which a dose of ozone is administered to adults, i.e. how quickly one breaths ozone polluted air in, that determines the dose dependent response in lung function impairment and not the cumulative amount of ozone administered. When someone breathes more quickly during higher levels of physical activity, they would increase the rate at which they

consume ozone and thus activity intensity is an important factor in determining the amount of pulmonary function decline[62].

McConnell et al [43]conducted cohort studies in various California communities looking at the relative risk of asthma associated with ozone exposure amongst children playing sports. They found that in communities where ozone concentrations were high the relative risk of developing asthma was 3.3 (95%CI 1.9-5.8) amongst children playing three or more sports compared to children who did not play sports[43]. The more sports the children played, the higher the risk of asthma. Children who lived in communities with low ozone exposure, however, were not at a statistically significant risk of acquiring ozone-associated asthma [43].

The cumulative effect of not only particulate matter and ozone, but also of other pollutants such as nitrogen oxides and sulfur dioxides, can chronically stress child and adolescent lungs. The processes that control and mediate oxidative stress, inflammation, and radical capture in response to airborne pollutants are very complex and at times interplay with one another to enhance the reactions [63]. Delving into these process is outside the scope of this research project, but the importance of these processes on lung tissue damage, decreased ventilatory capacity, increased airway reactivity, decreased macrophage clearance and altered immune system function[63] should be noted as reasons for further study into estimating inhaled dose.

Previous Minute Ventilation Studies

The Health Effects Institute sponsored a pair of studies to determine a feasible way of measuring minute ventilation in freely moving subjects[64]. One group estimated ventilation by measuring body surface displacement with elastic bands that used changes in electrical signals to indicate breathing depths and frequencies.. The displacement method was highly dependent on the type of activity being conducted and had difficulties elucidating a relationship between body displacement and ventilation at low levels of activity. The group also found a source of error in that brief increases in ventilation did not necessarily correspond to brief increases in heart rate. Though the group stated that the displacement data was comparable to the spirometry data, our preliminary data indicated that breathing amplitude is still not a good predictor of minute ventilation.

The second group collected plethysmograph data and plotted it against heart rate data obtained from monitors worn by the subjects during different types of activities in the laboratory setting[65]. They then predicted ventilation rates in the field from the ventilation-heart rate curves they calibrated for each individual. They obtained good fits from a linear regression of the log transformed ventilation and heart rate data, but there was a lot of inter individual variation, as with the first study. The curves they created depended on subject specific traits, such as size and fitness (i.e. heart rate), so applying the results to application of the findings to larger epidemiology studies would require calibration of each individual.

Zuurbier et al[66] looked at differences in minute ventilation between different types of commuters- i.e. pedestrians, cyclists, car passengers. While heart rate was measured during commutes, the minute ventilations corresponding to the heart rates were measured while the participant was on a bicycle ergonometer in the lab in 15-second intervals. This group also did a regression analysis on of the log transformed minute ventilation compared to heart rate. The differences in slope were not dramatic but the differences between intercepts were substantial. Overall, the minute ventilations while commuting via bike were found to be twice as large as those while riding the bus or in a car. The results showed lots of variation between subjects just as with the previously mentioned studies.

Our preliminary data (**Graphs 1, 2, 3**) also sees this pattern of different slopes and intercepts for each subject, either for comparing minute ventilation against heart rate or for comparing percent span of tidal volume against heart rate. The changes in minute ventilation with heart rate also very quite dramatically between individuals if you are to simply follow the two variables over time on the graph. These studies indicate that in order to be able to generalize the relationship between activity level and ventilation, the relationship cannot be dependent on subject specific traits. The specific traits and inter-individual differences introduce too much uncertainty into larger population level estimates. This research project aims to find a more appropriate and generalizable method for estimating inspiratory volumes at various levels of activity for adolescent populations participating in extracurricular and sports activities.

Research Methods and Design

Previously within SAPPA, tidal volume was estimated (and thus corresponding inhaled dose) from equations based on height. In this sub-study, we collected actual tidal volume and inspiratory volumes measurements in high school students. Because it is not possible to measure inspiratory volume while the participants are engaged in their extracurricular activities- it could only be done at rest at the two main locations- we recruited a subset of similar adolescents from a third high school and performed the measurements on them instead. These measurements were collected at various activity levels to measure inspiratory volume as a function of heart rate. This relationship will then be applied to all subjects in the main SAPPA study to estimate inhaled dose.

Participants' tidal volume at rest was tested first to ensure an accurate measurement before any kind of exertion was made. Then pulmonary function (to determine forced expiratory volume and forced vital capacity, etc.) was tested. Minute ventilation during varying levels of physical activity was tested last. The participants wore a monitor throughout all of these tests to obtain vital signs, such as heart rate, breathing rate, acceleration, and activity level. Height measurements were taken at the time of testing to ensure more accurate pulmonary test results

Subject recruitment

As approved by the Emory Institutional Review Board (IRB0055533), subjects were recruited from a local Atlanta area high school. Liaisons within the school informed subjects of the study, and researchers subsequently spoke with and distributed consent forms to interested students. Attempts were made to recruit an equal number of male and female participants, participants who played a wide variety of sports, and those students who did not play extracurricular sports. Informed consent was obtained from all participants, and for those participants under the age of 18, their parents' consent was also obtained. Each recruited participant was given a written questionnaire to fill out prior to testing, which inquired about basic demographic information, domiciliary address, extracurricular activities, asthma status, allergies, and potential smoking status. After all testing was complete participants were compensated $5 for their time and effort.

Capturing Vital Signs

After consent and questionnaires were obtained, subjects were fitted with ZEPHYR BIOHARNESSes. These bioharnesses are chest straps with integrated sensors, which capture an extensive amount of vital and physiological measurements at one-second intervals. The ones we were most interested in were heart rate, heart rate variability, breathing rate, breathing rate variability, activity level, ECG amplitude, and core body temperature. Participants wore these bioharnesses until they had completed all other tests.

Tidal Volume at Rest

Tidal volume- defined as the volume of a single breath taken *at rest*- is measured via a Wright respirometer. The participants wore nose clips while breathing into tubing connected to the Wright respirometer. The Wright respirometer is designed to measure how much air is exhaled during each breath but is not affected by inhales. Researchers recorded three measurements of the volume of at least five consecutive breaths The timing of the three measurements were conducted without the participants' knowledge to avoid the participants significantly altering their resting breathing pattern in response to testing.

Pulmonary Function Test

Using an EasyOne, NDD Medical Technologies spirometer we conducted a pulmonary function test on each participant in accordance with the American Thoracic Society guidelines. The following information from the spirometry data assess lung function:

- Forced vital capacity: the total volume of air that can be forcibly exhaled
- Forced expiratory volume in 1 second (FEV_1): the volume of air that can be forcibly exhaled in the first second of exhalation:
- The ratio of FEV_1 to FVC
- Forced expiratory flow, 25-75% (FEF_{25-75}): the average flow rate during the two interior quartiles of exhalation
- Peak expiratory flow (PEF): the maximum volumetric flow rate achieved during exhalation

Inspiratory Volume while Active

Inspiratory volume- defined as the volume of air inhaled per breath- was measured using a modified TRAINING MASK 2.0. The mask covers the participants' nose and mouth and is strapped across the back and top of the head to keep it in place (image in Appendix). It was modified to provide minimal resistance while still capturing all airflow out of the mouth and nose. The mask was connected via tubing to the Wright respirometer and a camera captured video of the respirometer so that data on each breath could be recorded after testing. Technically, this measured expiratory volume, but we are assuming that inspiratory volume and expiratory volume are equivalent provided that there are no leaks between the face and mask or in the tubing.

The participants were asked to first stand on the treadmill breathing as normally as possible for 1-2 minutes, then walk for 1-2 minutes. After this, the participants were made to run at different levels of exertion. Moderate activity was considered to be 50-70% of their maximal heart rate and vigorous activity was considered to be 70-80% of their maximal heart rate.

The bioharnesses were configured to show heart rate in real time on a laptop, and the display would change colors (green, yellow, red) depending on what preset heart rate threshold was programmed into the software. To determine heart rate we used the following commonly accepted equation based on age:

$$HR_{MAX} = 208\text{-}0.7(age)\ [67]$$

Using this equation we then programed 65% of maximal heart rate and 75% of maximal heart rate to be thresholds on the display for moderate and vigorous respectively.
We increased the speed of the treadmill from walking to running at a steady pace (over the course of 1-2 minutes) until the participants reached 65% of their HR_{max}. The participants would run at this level of exertion for another 1-2 minutes, enabling us to look at averages in inspiratory volume at a given heart rate. After this plateau, we would rapidly increase the treadmill to stimulate a sprint, increasing the level of the participants' level of exertion until they surpassed their 75% HR_{max} threshold. Once they crossed this threshold we would not change the speed of the treadmill but would keep the participants running for approximately 30 second to 1 minute at this level. After they had completed this last plateau, the participants' heart rate was brought back down, below 60% HR_{max}. They were steadily but quickly returned to a brisk walk

for 1-2 minutes after which they were asked to stand still until their heart rate had dropped below 60% HR_{max}. At this point testing was concluded for that participant.

Statistical Analysis

All information pertaining to each participant was identified only by ID numbers, which were assigned based on order of participation.

Data was collected on the following variables:

- From the questionnaires:
  - Birthdate (age)
  - Height
  - Weight, participant estimated
  - Gender
  - Race/ethnicity
  - Home address
  - Healthcare worker diagnosed allergies
  - Healthcare worker diagnosed asthma,
  - Asthma medication use,
  - Tobacco use
  - Tobacco exposure in the home.
- From the Zephyr Bioharness (all at 1s intervals)
  - Heart rate (HR)
  - Breathing rate (BR)
  - Activity value (as defined by the Bioharness: sum of vector magnitude additions)
  - Breathing rate amplitude
  - ECG amplitude
  - Heart rate variability (HRV)
  - Core temperature
- From spirometry
  - Forced vital capacity (FVC)
  - Forced expiratory volume in the first second (FEV_1)
- From testing
  - Tidal volume (TV): volume of a breath at rest
  - Inspiratory volume (IV): volume of a breath while active
  - Activity (i.e. standing, walking, running, sprint, cool down)

Maximum heart rate was calculated based on age as follows:

$$HR_{MAX} = 208 - 0.7(age)$$ [67]

Microsoft Excel was used to generate:

- % TV span: (IV – TV)/(FVC – TV)
- %HR span: $(HR_{Instantaneous} - HR_{resting})/(HR_{max} - HR_{resting})$
- Minute ventilation (MV): total volume inhaled over 60 seconds
- Average heart rate: heart rate averaged over 60 seconds
- Predicted body weight (PBW) in kilograms based on the following equations [68]
  - Males: PBW(kg)= 50 + 0.91*(Height(cm) – 152.4)
  - Females: PBW(kg)= 45.5 + 0.91*(Height(cm) – 152.4)
- Predicted tidal volume[47]:
  - TV_{pred} = PBW(kg)*0.7L/kg

Only one estimated weight was missing from the data set. Data on average heart rate, minute ventilation, %TV span, %HR span, inspiratory volume and activity data was analyzed only for the period of time that the participant was on the treadmill and breathing into the Wright respirometer. Data on resting heart rate was determined from the period of time in which the participant was undergoing testing for tidal volume.

All remaining analyses were conducted in SAS for statistical analysis 9.3.

- Paired t-tests were conducted to evaluate the statistical differences between predicted tidal volume and measured tidal volume and between participant estimated body weight and predicted body weight.
- Regression analysis was used to build the models relating heart rate to inspiratory volume. Variables were assessed for their colinearity and only those free of colinearity were included in the model. The standard p value of <0.05 was used as a threshold for statistical significance.

Results

Study sample

A total of 18 high school participants were recruited to participate in the study, 7 females and 11 males. Permission for sampling and recruiting at the school was obtained from the administration and sampling sessions were conducted at the school, in the weight room facility, on a school owned treadmill. All sampling sessions occurred either during the students lunch hours, immediately after school, or after their sports practice. **Table 1** displays

characteristics obtained through the questionnaires. This sample participated in a plethora of activities: track, cross-country, dance, swing dancing, choir, tennis, basketball, lacrosse, golf, ultimate Frisbee, weight training, soccer, swimming, and baseball. All but one participant engaged in at least one activity that was held outdoors.

Difference between activities

The data pertaining to the increase in heart rate (from standing to sprint) and the data pertaining to decreasing heart rate (the cool down) followed distinctly different patterns; while the data for increasing heart rate followed a curvilinear tread ($p<0.0001$), no statistically significant tread was found for the data for decreasing heart rate ($R^2=0$). Therefore, further data analysis was split, dividing up the increasing and decreasing heart rate portions of the test.

Predicted body weight and participant estimated body weight

No statistically significant difference was observed ($p<0.05$) between predicted body weight and participant estimated body weight. This justifies our approach for using predicted tidal volume in subsequent analyses.

Predicted tidal volume and measured tidal volume

No statistically significant difference was found between predicted tidal volume and measured tidal volume at the $p<0.05$ level. The two variables were also not statistically correlated at the $p<0.05$ level. The scatterplot in **Graph 4** depicts these two variables. The lack of statistical difference justifies our use of the predictive tidal volume when building the models.

Description of collected data

The scatterplot of the data looking at inspiratory volume and heart rate show that the values generally plateau: IV does not continue to increase with increasing HR. Males, on average, tended to have greater inspiratory volumes at any given heart (this is shown with red color coding). See **Graph 5**.

Regression model relating inspiratory volume with heart rate

Tidal volume, FVC, and FEV are all inherently related to one another so only one of these lung function terms (FVC) was used in the model to avoid colinearity. The different heart rate variables are also inherently interrelated so only one of the variables (HR) was used as well. Age, height, weight, gender, and race/ethnicity are all incorporated into FVC, because these variables were all derived from NHANES data. After performing a simple linear regression relating inspiratory volume to heart rate, the following model provided the best fit to our data:

$$\text{InspiratoryVolume/FVC} = \ln(\text{HeartRate});$$

$$\text{IV/FVC} = -0.598 + 0.199 \ln(\text{HR})$$

This model accounts for data pertaining to the portion of the testing that increased heart rate- from standing to sprinting. The descriptive statistics of the model are presented in **Table 2**. The goodness of fit was nominal ($R^2 = 0.225$), however the model and all of its variables were statistically significant. The residuals for this model were all centered around zero (see **Graph 7**) and a majority of the data points all fall within two standard deviations of the error (RMSE= 0.1027). The distribution of the IVC/FVC data we collected closely followed a normal distribution (**Graph 11**). The normality of the data is presented in **Table 3**. The distribution of heart rate on the other hand, did not follow a normal distribution.

The following is another possible model:

$$\text{InspiratoryVolume/TV}_{\text{predicted}} = \ln(\text{HeartRate});$$

$$\text{IV/TV}_{\text{pred}} = -5.364 + 1.772 \ln(\text{HR})$$

The descriptive statistics of the model are presented in **Table 4**. The goodness of fit was nominal ($R^2 = 0.242$), however the model and all of its variables were statistically significant. The residuals for this model were all centered around zero (see **Graph 9**) and most, though arguably less than for the previous model, data points fall within two standard deviations of the error (RMSE= 0.8712). The distribution of the $\text{IV/TV}_{\text{predicted}}$ data we collected closely followed a normal distribution (**Graph 11**). Data on this normality is presented in **Table 5**. The distribution of heart

rate is the same as with the previous model. Predicted tidal volume is solely a function of height so we are left with a model that is based on two easily measurable variables, heart rate and height. These models are very similar in fit because FVC is also correlated to height (following NHANES methods). The first model has a lower goodness of fit but more precise error while the second model has a higher goodness of fit but less precise error.

Discussion

The purpose of this study was to assess the relationship between level of physical exertion and inspiratory volume in adolescents. Previous research has looked minute ventilations but these measures have introduced too much variability to produce generalizable models. This novel approach to measuring the volume of each breath has reduced that uncertainty. The major relationship elucidated in this study was that heart rate increases more quickly than does inspiratory volume in a curvilinear manner. The model built from the collected data shows that the best fit for the heart rate data is produced from a natural logarithmic transformation of the heart rate. Simply taking inspiratory volume at every breath did not provide a good relationship to heart rate- the ratio of the inspiratory volume to the subjects specific forced vital capacity showed a better relationship to heart rate. Given that every person has a slightly different forced vital capacity this ratio serves as a proper equalizer across subjects with regard to change in the volume of air inhaled. The issue of differences between individuals still exists in this data set. However, because inspiratory volumes account for the volume of every breath whereas minute ventilations are averaged over the span of sixty seconds, the inter-individual differences can be dealt with more precisely in future research. This model is statistically significant but it may be advisable to utilize the upper limit of the confidence intervals to err on the side of caution with regards to how much air pollution maturing adolescents could potentially consume.

The major noticeable issue with the data is that the HR data points do not follow a normal distribution. This is most likely due to the manner in which data was collected. The participants ran for a greater amount of time than they were standing or walking (see Methods section), contributing to more data points on the higher end of the heart rate range. The low

goodness of fit most likely results from this uneven distribution. The confidence intervals associated generated by the model provide valuable information with regards to the upper limits of air pollution an adolescent may consume when engaging in physical activity. See **Graph 6.** A potential way to address the non-normality of the data would be to find where heart rates reach a relative plateau within a given exertion level (i.e. standing, walking, running, sprint), and calculate a pre-specified number of points for each individual so that all subjects have the same number of data points.

One limitation of this research study is the composition of adolescents who participated potentially leading to selection bias. At this study location adolescents who already where in good physical fitness, or where on a sports team, were much more willing to participate in the study. Also, older students were more likely to be proactive about their participation than younger students who tended to be more timid about being judged on their physical characteristics. However, the majority of students at the school were part of at least one sports team or an active extracurricular activity such as dance and choir. Therefore, the sample may have been more representative of the school's population, reducing internal selection bias. The matter of generalizability then arises. This school is in an affluent Atlanta neighborhood and students there are of a middle- to high-socioeconomic status (SES). Confounding could potentially be an issue if SES does indeed affect lung and physical health. Previous literature asserts that health outcomes from air pollution are indeed modified by SES. In this study, the concern is whether or not SES modifies the relationship between inspiratory volume and heart rate. One scenario is that with high SES the adolescents have had fewer environmental factors acting as barriers to their development so they have healthier, stronger lungs than those of lower SES. When they are then exposed to pollutants, their body may be able to recover more efficiently and effectively and so the relationship between inspiratory volume and heart rate would not be altered as much for high SES adolescents. Another scenario may occur when adolescents of high SES, who are normally exposed to low levels of air pollutants, participant in some sports activity in an area of high air pollutants. Given the health of their lungs they could potentially inhale more air pollutants that they normally would. This larger acute exposure may then be more detrimental to them and the body would have to compensate for reduced oxygen delivery but potentially increasing heart rate. Conclusions cannot yet be made as to the specific effect SES may have on the relationship between inspiratory volume and heart rate.

However, SES may not be a contributing factor in this study because the distribution of IV/FVC data shows a normal distribution. FVC data generated by the spirometer is compiled from the National Health and Nutrition Examination Survey (NHANES) conducted by the Center for Disease Control and Prevention. NHANES data is considered to be representative of the national population so we could argue that our data is sufficiently representative as well.

The three other potential sources of error are systematic and pertain to the method of data collection. First, it is likely that wearing the mask during exercise inherently altered the participants' breathing patterns. Simply being conscious of the fact that your breathing is being monitored can cause one to alter their breathing pattern from their autonomic breathing pattern. Second, although the Wright respirometer measures expiratory volume we are making the assumption that inspiratory volume and expiratory volume are equal and using the Wright respirometer data to represent inspiratory volume. The participants were told, with great emphasis, to immediately inform the researcher if the mask was leaking, however, various factors could affect the seal of the mask to the face. The seal of the mask depended on the participants' face shape and how tightly we could strap the mask onto the participants' heads. Also, as their level of physical exertion increased, the participants began to sweat more, which caused the mark to occasionally slip and need readjustment. The participants were periodically asked how the mask was fitting to ensure that minimal leaking was occurring and thus leakage problems were avoided for the majority of the participants. The last source of error could potentially be attributed to the resistance provided by the tubing. The shortest possible amount of tubing was used for the participants and it is expected that if there was some error due to the tubing, that the error would be relatively equal for all participants.

Although we see a general pattern among the subjects in our study the issue of asthmatics still persists. One participant who had exercise induced asthma had prominent fluctuations in her heart rate that are not easily explained. When this participant first started to transition from walking to running their heart rate surpassed 85% of their HR_{max}, yet when they were brought quickly to a sprint, their heart rate barely surpassed 75% HR_{max}. These fluctuations in heart rate could cause physiological responses demanding more oxygen that would alter breathing patterns at the lower spectrum of physical exertion, while not affecting breathing patterns at higher levels of exertion. Therefore further research may be required on asthmatic, and exercise induced asthmatic adolescents with regard to their breathing profiles.

Data for the cool down phase does not appear to follow a predictive enough pattern to be able to fit it to a significant statistical model. **Graph 10** illustrates this lack of pattern with a scatterplot of the data. Therefore, we are only addressing data pertaining to the increasing of heart rate and not the decreasing heart rates (after the sprint) in our models. Further research may provide more data points for the cool down period in this study population. From this, elucidating the relationship between inspiratory volume and heart rate when the breathing rate decreasing more quickly than heart rate may be more feasible.

Conclusion

In a time of growing concern about air pollution and it detrimental health effects on the world population, the ability to determine more specific exposures can provide insightful information into the specific detriments individuals face. Using our generalized regression model, it is possible to elucidate an inspiratory volume given a certain heart rate and thus estimate dose of air an adolescent inhales. This information can be used in conjunction with ambient air pollution data to determine how much pollution is consumed during physical activity. For a demographic that is still developing, these estimates can inform future health outcomes. For the Atlanta area specifically, this information is valuable for creating protective school policies on dangerously high air pollution days and educating the public on strategies to maintain their health.

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Tables and Figures

Table 1: Study Sample Characteristics

Gender	n	%
Male	11	61
Female	7	39
Race/Ethnicity		
White	13	72.2
African American	1	5.56
Asian	1	5.56
Hispanic	1	5.56
Biracial	2	11.1
Age		
14	1	5.56
15	2	11.1
16	2	11.1
17	9	50
18	4	22.2

	No	Yes	Don't know
Tobacco use	18	0	0
Tobacco exposure in home	19	1	0
Healthcare worker diagnosed allergies	15	2	1
Healthcare worker diagnosed asthma	14	4	0
Still asthmatic	NA	3	1
Episode of asthma	2	2	0
Take an asthma medication	2	2	0
Inhaler in the past 3 months	2	2	0

Table 2: Univariate Regression: IV/FVC=ln(HR)

Variable	DF	Coefficient	Type II Sum of Square	Mean Square	F Value	Pr>F
Intercept	1	-0.5984	4.4097	4.4097	418.37	<.0001
ln(HR)	1	0.1991	11.3857	11.3857	1080.21	<.0001

Table 3: Descriptive Statistics for IV/FVC

N	5295	Sum Weights	5295
Mean	0.36776	Sum Observations	1947.31085
Median	0.36491	Range	0.79137
Std Deviation	0.11494	Variance	0.01321
Skewness	0.04876	Kurtosis	-0.03868
Uncorrected SS	786.10197	Corrected SS	69.95097
Coeff Variation	31.25615	Std Error Mean	0.00157

Table 4: Univariate Regression: IV/TV_{pred}=ln(HR)

Variable	DF	Coefficient	Type II Sum of Square	Mean Square	F Value	Pr>F
Intercept	1	-5.364	354.377	354.377	466.89	<.0001
ln(HR)	1	1.772	901.419	901.419	1187.62	<.0001

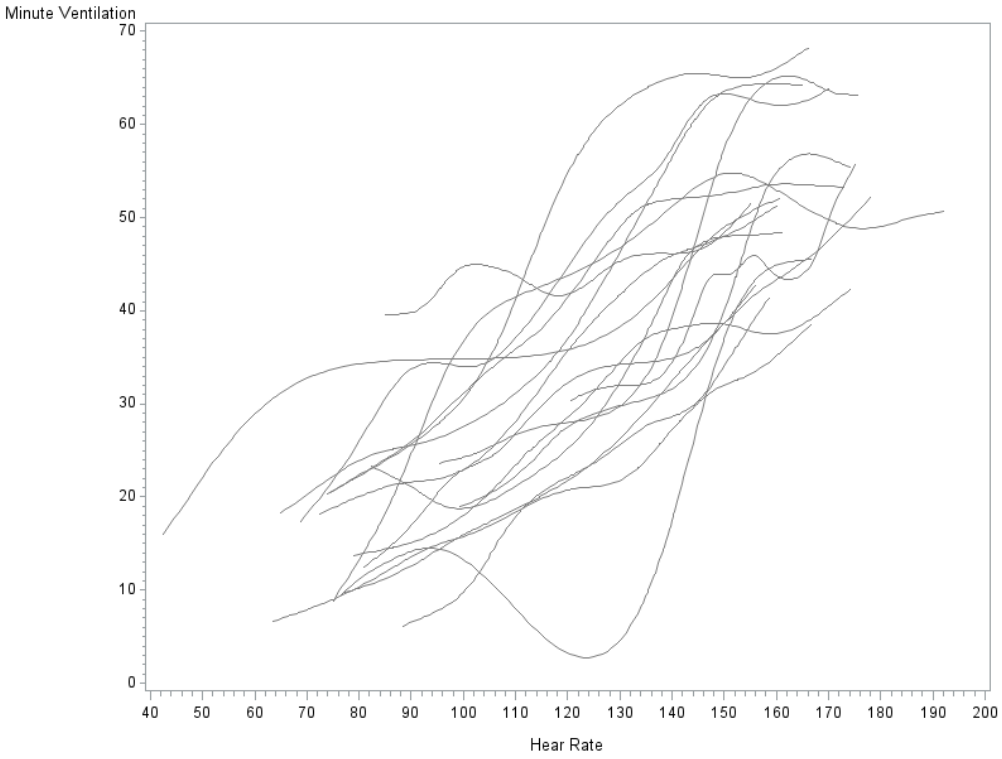
Table 5: Descriptive Statistics for IV/TV_{pred}

N	5295	Sum Weights	5295
Mean	3.23644	Sum Observations	17136.98980
Median	3.19311	Range	7.22001
Std Deviation	0.98023	Variance	0.96086
Skewness	0.10017	Kurtosis	0.24836
Uncorrected SS	60549.76970	Corrected SS	5086.80120
Coeff Variation	30.28738	Std Error Mean	0.01347

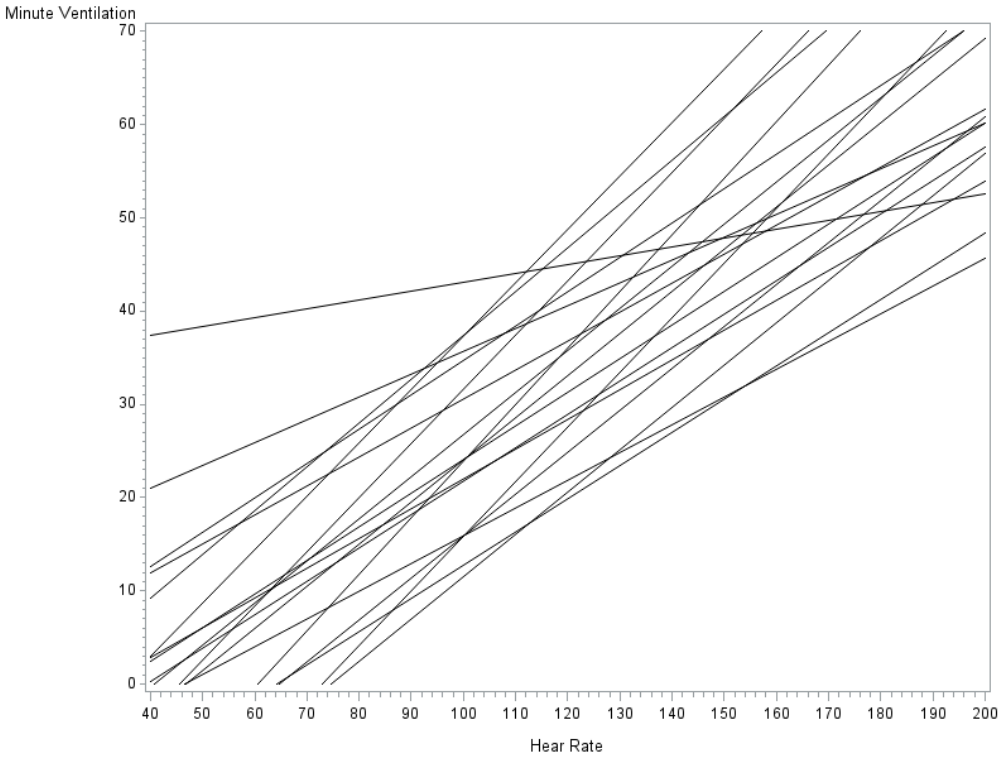
Table 6: Descriptive Statistics for HR

N	5295	Sum Weights	5295
Mean	134.06794	Sum Observations	709889.78500
Median	141.00000	Range	151.50000
Std Deviation	29.85719	Variance	891.45226
Skewness	-0.67941	Kurtosis	-0.17539
Uncorrected SS	99892815.20000	Corrected SS	4719348.31000
Coeff Variation	22.27019	Std Error Mean	0.00158

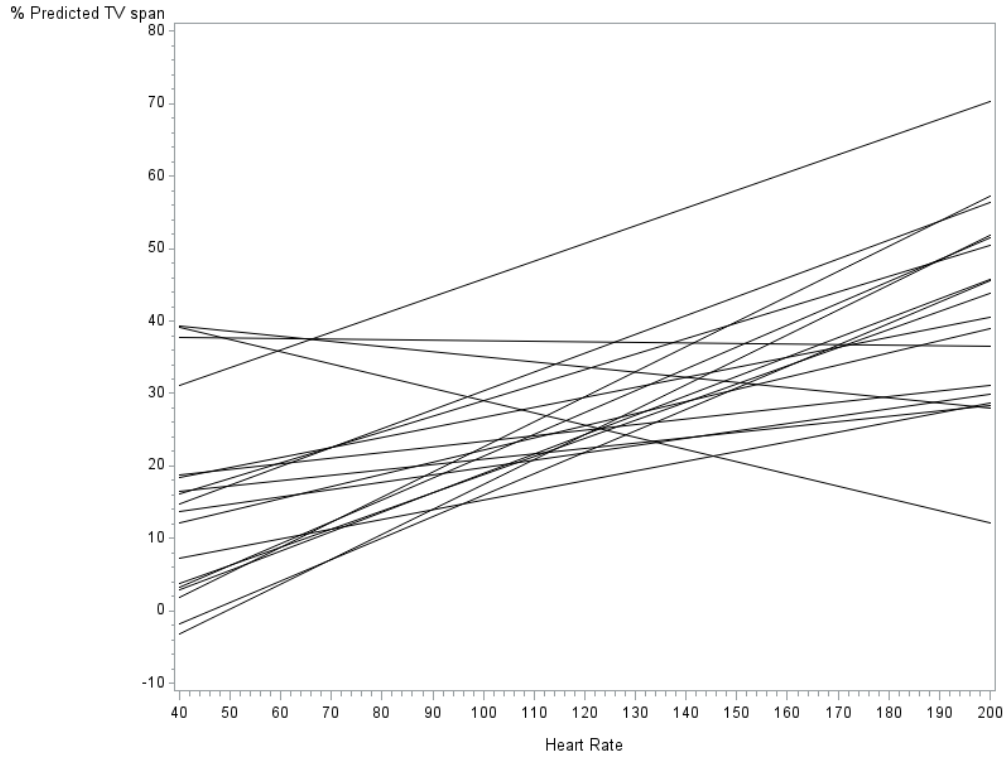
Graph 1: Minute ventilation vs. Heart Rate, Individual scatterplot patterns



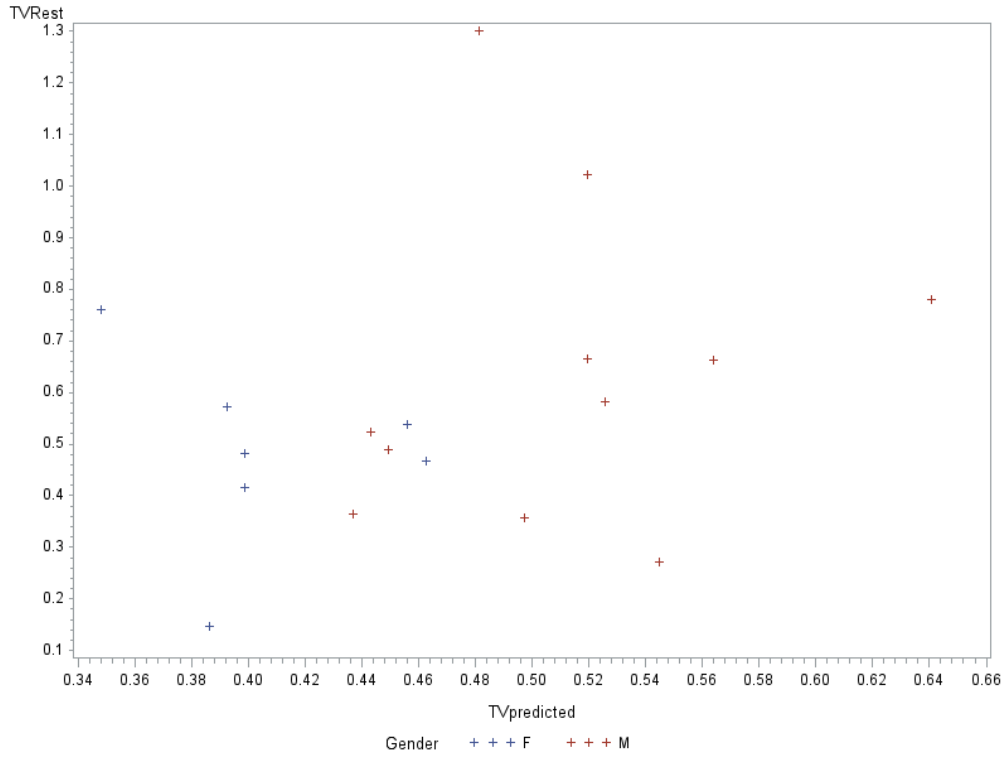
Graph 2: Minute ventilation vs. Heart Rate, Individual best fit lines



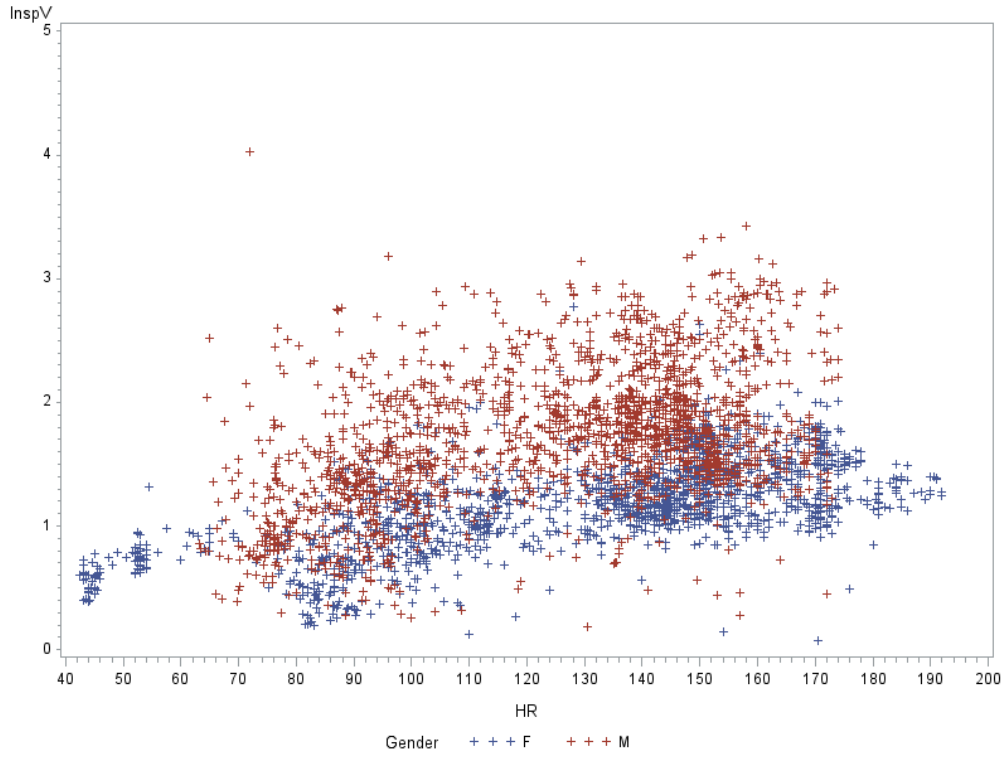
Graph 3: %Tidal Volume Span vs. Heart Rate, Individual best fit lines



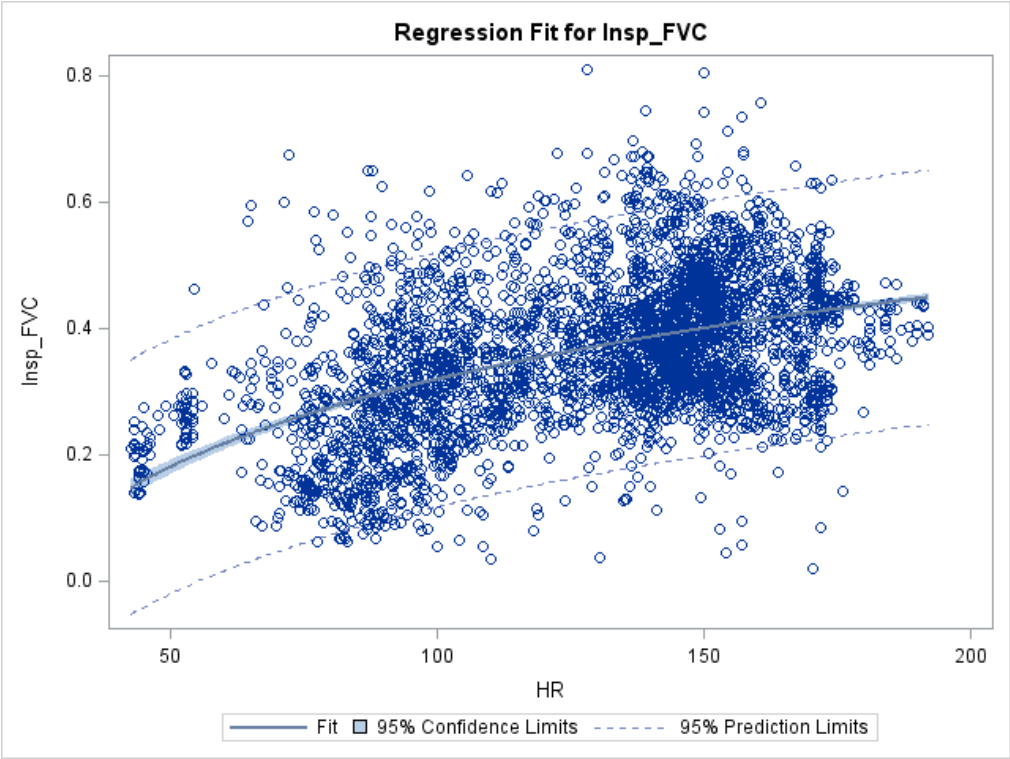
Graph 4: Comparing Predicted and Measured Tidal Volumes



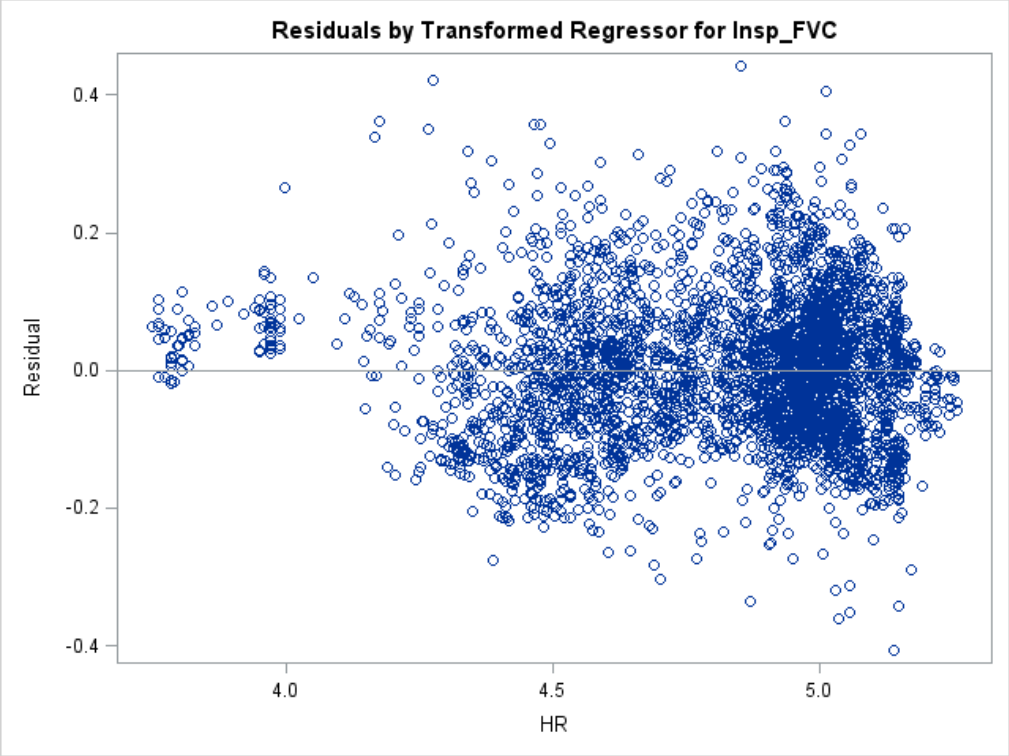
Graph 5: Scatterplot of Collected Data comparing IV and HR by Gender (increasing activity)



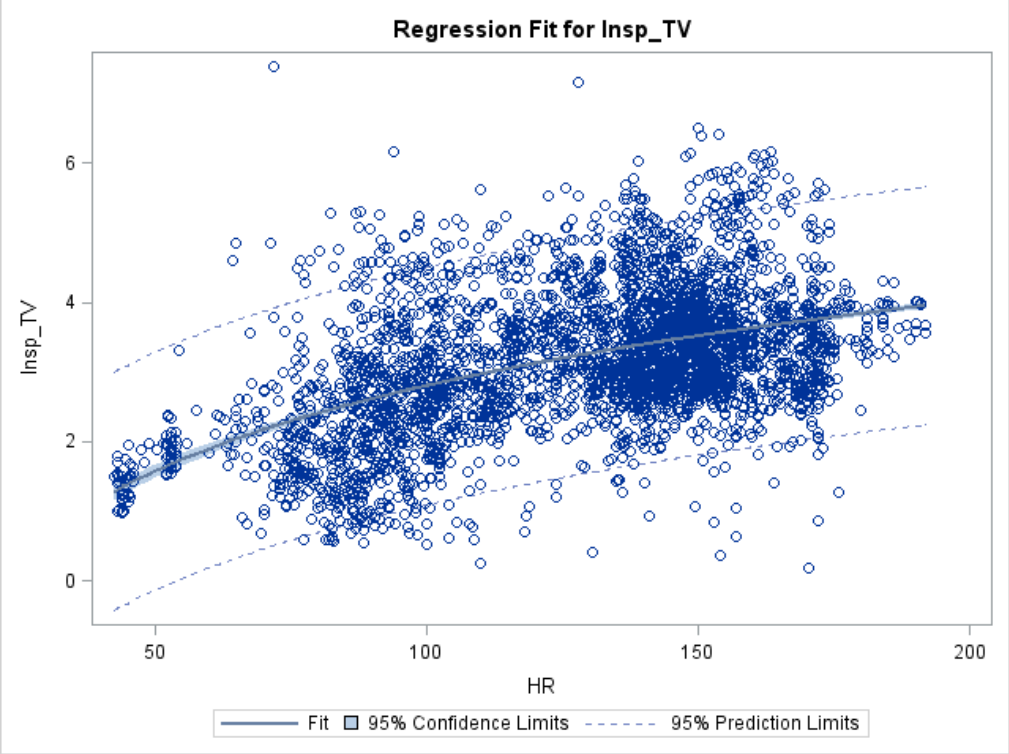
Graph 6: Scatterplot and model of IV/FVC and ln(HR)



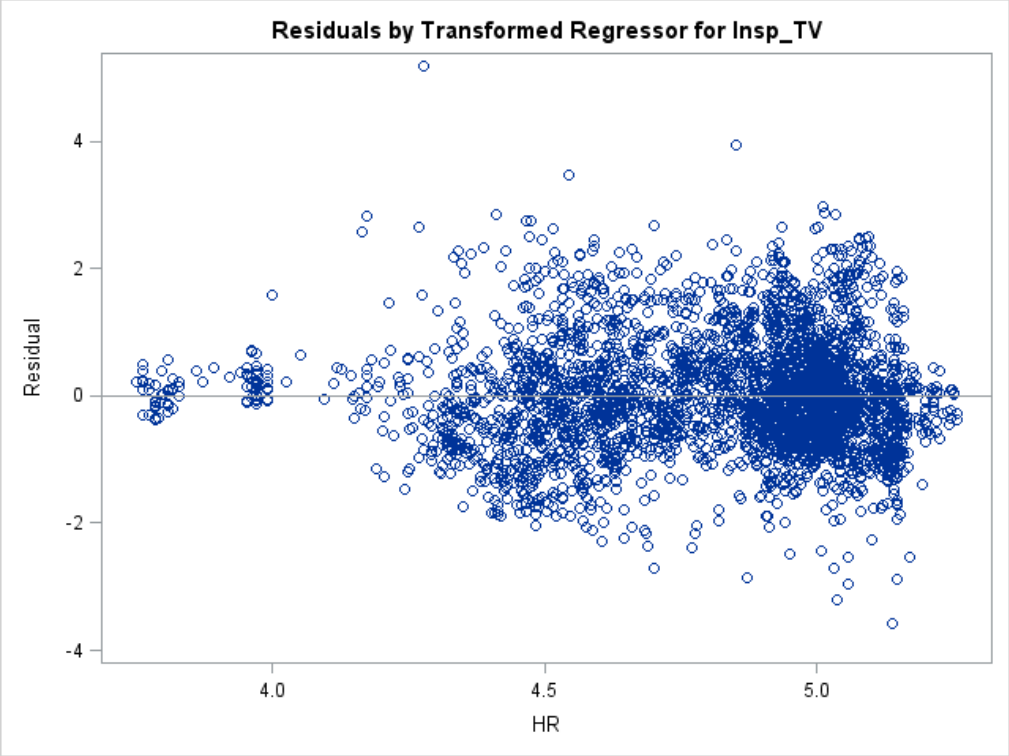
Graph 7: Residuals for IV/FVC=ln(HR); √MSE= 0.1027



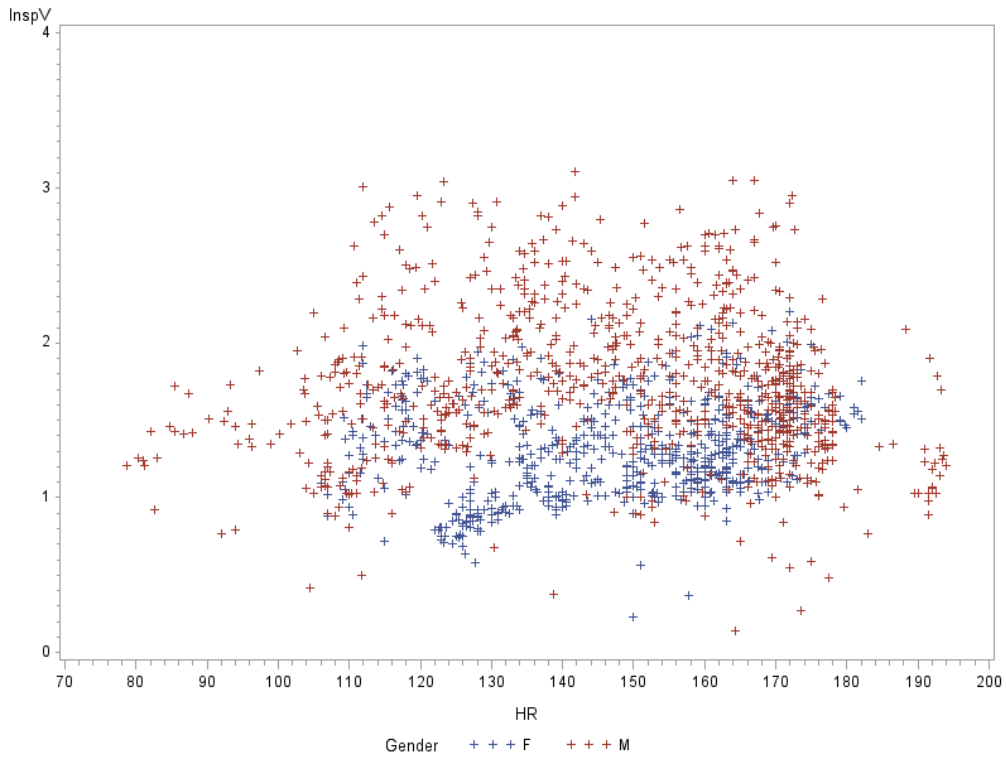
Graph 8: Graph 6: Scatterplot and model of IV/TV_{pred} and ln(HR)



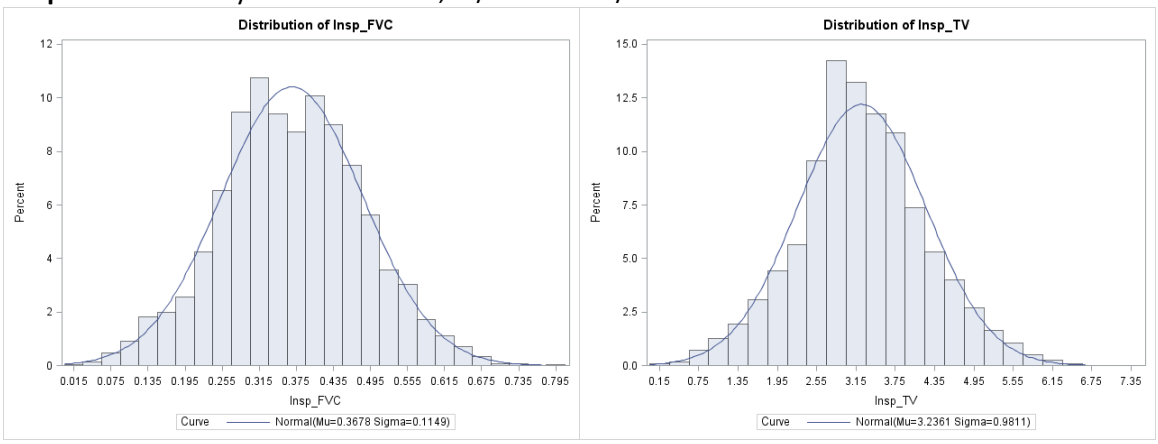
Graph 9: Residuals for IV/TV=ln(HR); √MSE= 0.8712



Graph 10: Scatterplot of Collected Data comparing IV and HR by Gender (decreasing activity)



Graph 11: Normality of Distributions, IV/FVC and IV/TV



Appendix A

TRAINING MASK 2.0 ®