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Exhaled breath nitric oxide: Is there a baseline difference due to ethnicity

Sunita I. Patel

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Exhaled Breath Nitric Oxide: Is There A Baseline Difference Due To Ethnicity?

by

Sunita I. Patel, M.D.

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Public Health
Department of Environmental And Occupational Health
College of Public Health
University of South Florida

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Keywords: African descent, Caucasian, male, non-parametric, generalizability

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ABSTRACT  

The air that humans exhale contains various chemical markers whose levels have been associated with various respiratory disorders. Therefore, measurement of these markers offers a potential method of examining airway disease status. Furthermore, exhaled breath offers the advantage of being easy to collect and non-invasive. Hence, these exhaled breath markers are potentially of significant clinical use in examining airways. Therefore, examination of exhaled breath has become the subject of intense study. Current research is targeting the development of methods and parameters for looking at these markers.  

The goal of this cross-sectional pilot study was to consider the variability in the measurement of these exhaled breath markers between members of different ethnic populations. Specifically, measurements of the exhaled breath marker Nitric Oxide (NO) were compared between two ethnic groups (Caucasian men versus men of African descent). Ten healthy men in each group were studied to examine whether baseline NO measurements differed between them.
In this study, a cross-sectional design was used. The study sample consisted of young, healthy men with no history of environmental allergies, asthma, or lung diseases and no significant smoking history. A total of twenty-five men volunteered for the study, including fourteen men of Caucasian descent and eleven men of African descent. Because four men were excluded and one withdrew, ten men in each ethnic group were included in the final analysis.

The source population from which the sample was drawn included students and workers. All participants were residing in Florida at the time of study. Ideally, the target population for this study was young, healthy, working men.

Large inter-measurement variation was seen between the participants of each ethnic group. This was hypothesized to be attributed to a tri-modal distribution due to the existence of 3 populations of subjects: (1) asymptomatic with normal airways, where NO levels were under 30 parts per billion (ppb); (2) asymptomatic with airway pathology, where NO levels were over 30 ppb; and (3) asymptomatic just before the onset of an upper respiratory tract infection, where NO levels were over 60 ppb.

This pilot study did not find statistically significant evidence that there is a difference in the baseline exhaled breath NO measurements between the two ethnic groups studied. Nonetheless, in participants with NO levels under 30 ppb the mean of the African group was found to be 7.6 ppb lower than the mean of the Caucasian group when attempts were made to exclude individuals with underlying airway pathology or imminent upper respiratory tract infection.

In order to find statistical significance in the results, a power analysis using the standard deviation of 7.7 ppb that was found in this study indicates that at least thirty-two...
eligible participants with NO levels under 30 ppb would be required. Only 13 such
participants were examined in this study. Thus, at least fifty eligible participants would
be required to find significant results.

The implication is that even though statistical significance was not achieved, the
crude mean averages differed between the two groups in participants with NO levels
under 30 ppb. This implies that a larger-scale well-designed study is warranted before
NO is used in clinical settings in the diagnosis and monitoring of patients.
INTRODUCTION

Methods of Studying Airway Disease

Airway inflammation has been seen in a variety of pulmonary disorders, including asthma, chronic obstructive pulmonary disease, and bronchiectasis. Current methods to evaluate for disease or inflammation include invasive procedures such as bronchoscopy, bronchial biopsy, and bronchoalveolar lavage. Although it is not invasive, sputum induction can involve discomfort to patients. With this in mind, an alternate method of examining airway disease that is non-invasive and produces less discomfort to patients has been actively sought by researchers.

Examination of exhaled breath markers offers a possible alternative method. When people exhale, they breathe out warm, humidified air. This exhaled air contains gases, water vapor, and various volatile substances that come from the airways and the fluid that lines the airways. Several of these dissolved substances have been shown to be markers of airway disease or inflammation. Since this exhaled air is easily obtained in a non-invasive manner with little discomfort to patients, it shows promise as a method for examining the degree of airway disease and/or monitoring patient response to treatment. However, measurements of markers in exhaled breath can vary based on the parameters used to study them. For example, the equipment used, the exhalation duration, the method of acquiring samples, and the expiratory flow rate can all affect measured values.
Nitric Oxide in Exhaled Breath

One of the exhaled breath markers currently being investigated is nitric oxide (NO). NO has been found to be elevated in various disease states, such as asthma and COPD during exacerbations. Other factors have also been found to affect NO levels. (See Table 1) [11] For example, a recent study performed by Doctors Robert Haight and Robert Gordon comparing nitric oxide levels in old versus young people found increased exhaled breath NO levels in older individuals (median = 36.9 ppb) when compared to young people (median = 18.7 ppb). [17]

Genetic variation may also play a role in the amount of NO that is exhaled. An understanding of possible sources of genetic variation may be obtained by examining the mechanism of NO synthesis. (See Figures 1 and 2) “Endogenous NO is primarily synthesized from arginine via the enzyme Nitric Oxide Synthase (NOS).” (Kharitanov, 2001, p. 1694) [8]

There are 3 different forms of NOS: (1) Type I- Neuronal NOS, (2) Type II- Inducible NOS, and (3) Type III- Endothelial NOS. Types I and III are activated by “small rises in intracellular calcium to cell activation.” (Kharitanov, 2001, p. 1694) [8] Type II “has a much greater level of activity…; is independent of calcium concentration …; may be induced by inflammatory cytokines, endotoxin, and viral infections; and may show increased expression in inflammatory diseases.” (Kharitanov, 2001, p. 1694) [8]
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Definition of abbreviations: URTI = upper respiratory tract infection, ACE = angiotensin-converting enzyme.

Modified from Kharitanov SA and Barnes PJ. Exhaled Markers of Pulmonary Disease. Am J Respir Crit Care Med 2001; 163: 1693-1722. [8]
Figure 1. Nitric Oxide Synthesis

Modified from Kharitanov SA and Barnes PJ. Exhaled Markers of Pulmonary Disease. Am J Respir Crit Care Med 2001; 163: 1693-1722. [8]

Figure 2. Genetic Induction of NOS

Modified from Kharitanov SA and Barnes PJ. Exhaled Markers of Pulmonary Disease. Am J Respir Crit Care Med 2001; 163: 1693-1722. [8]
“Genetic polymorphisms of all three isoforms of NOS have been detected. Associations have been found between polymorphisms in the NOS1 gene and asthma in Caucasian populations… In patients with mild asthma, there is a significant association between the length of the AAT repeat polymorphism in intron 20 of the NOS1 gene and exhaled NO levels.” (Kharitanov, 2001, p. 1694) [8] These findings leave open the possibility that genetic variations among different ethnic groups could result in variations in baseline NO levels between different ethnic groups.

It has been assumed that baseline NO levels are the same among different ethnic groups. No prior studies have directly examined whether a difference may exist between the different ethnic groups. This study will examine whether ethnicity could be a factor that affects NO levels.

Race and Ethnicity Considerations

A race is defined as “any population that differs from other populations of its species in the frequency of one or more genes.” (Bryant, 2004) It is an arbitrary classification based on group. In comparison, an ethnic group is defined as “a group of people who share a similar cultural and regional origin, hold common norms and beliefs, and form part of a larger population, interacting with people from other segments of society. … Ethnic groups (1) share fundamental cultural and norms that distinguish them from other groups, (2) communicate and interact together, reaffirming their ethnic identity, and (3) are recognized by members and other groups as distinct.” (Bryant, 2004) [15] These qualities can create variations among people. (See Next Section on Epidemiologic Considerations)
“In conducting a study, the most valid method of ethnic assessment is self-identification.” (Bryant, 2004) In keeping with this, to assign ethnicity, participants of this study will be asked to identify their own ethnicity as a strict criterion.

It must be “recognized that historical patterns of migration, intermarriage, and distribution of genetic characteristics suggest that there are no pure races.” (Bryant, 2004) As an example, a person who may be identified as Caucasian may have one parent who is Caucasian while another parent is Hispanic. To gain insight into the possibility of mixed ethnicity, participants will also be asked to identify the birthplace of their parents and whether they identify the ethnicity of two generations of ancestors differently from themselves.

Epidemiologic Considerations

A primary consideration in any research study is internal validity. Internal validity is defined as the “extent to which the results of a study are correct for the particular group being studied. Internal validity is a prerequisite for external validity.” (Sanchez-Anguiano, 2004) Once findings have been made through research with care to preserve internal validity, a general epidemiologic challenge then becomes to provide information that can be applied to populations outside the specific one studied. [22]

External validity is defined as “the extent to which the results of a study apply to people who were not in the study.” (Sanchez-Anguiano, 2004) Many times, in an attempt to preserve internal validity, external validity may become compromised. A potential cause of loss of external validity is ethnic differences. [22]
In 1999 and 2005, the American Thoracic Society promulgated recommended practices and instruments for obtaining and measuring NO in exhaled breath. Ethnic considerations were not included in these recommendations. [3], [19]

Ethnic differences have sometimes been found to result in variations in parameter measurements based on different factors such as socio-economic status (eg. access to health care), environmental factors, and genetic variations. For instance, pulmonary spirometry test results require adjustments in people of African descent in order to portray a more accurate clinical representation when measuring their pulmonary function. [18] This suggests the possibility that other pulmonary monitoring parameters may require similar adjustments based on ethnic differences. These adjustments results from generalized differences in variables such as those listed above.

Specifically, in this study, measurement of exhaled breath levels of NO were compared between two dominant ethnic groups in American society: people of non-Hispanic Caucasian descent and people of African descent. In performing this study, the goal was to begin to provide an examination of the generalizability of exhaled breath measurements to various populations.

Research Question

Are Baseline Measurement Values of Airway Markers of Inflammation In Exhaled Breath Generalizable To Different Ethnic Populations?
Hypothesis

To study this, measurements of NO levels in exhaled breath were compared between people of Caucasian descent and people of African descent. The null hypothesis was there is no difference in baseline measurements of exhaled breath nitric oxide levels between people of Caucasian descent and people of African descent. The alternate hypothesis was that baseline measurements of markers of inflammation in exhaled breath varied among different ethnic populations.

As measurement protocols are developed, such variations, if they exist, should be considered to improve accuracy in detecting and monitoring methods of airway disease states.
Goals and Objectives

The specific objective of the proposed study was to examine whether a difference may exist in baseline clinical measurements of exhaled breath NO levels between young, healthy men in the two ethnic groups. The goals were: [1] to begin to provide an examination of the generalizability of measurements of exhaled breath markers to various populations, which may serve as the basis for a larger and more in-depth study [2] to examine whether baseline NO measurements are appropriately representative of individuals from different ethnic backgrounds, [3] to examine whether adjustment factors based on ethnicity need to be considered as protocols for measuring markers in exhaled breath become advanced for potential use in clinical settings to improve clinical accuracy in detection and monitoring of lung diseases.
Significance and Utility of Research

The study was unique since it looked at a hypothesis that had not been previously examined in measurements of exhaled breath. The examination provided insight into the possible applicability of measurements of markers in exhaled breath to others groups in the general population. In performing the study, insight was provided to researchers who are actively developing methods and parameters to use markers in exhaled breath for the detection of pulmonary disease or for monitoring treatment.
Materials and Methods

Overall Study Design

In this pilot study, the variable studied was ethnicity and the outcome of interest was levels of the marker nitric oxide in exhaled breath. A cross-sectional design was utilized where individual level data was obtained simultaneously.

Ten men were compared in each of two ethnic groups. The men in the first group were of African descent, whereas the men in the second group were of Caucasian descent. In this examination, only healthy men aged 18-45 were studied. Health was defined as having no history of allergies, asthma, lung diseases, and no significant smoking history (defined as less than one-half pack year history with no smoking in the past 2 years). Also, since certain medications have been found to affect NO levels, those participants taking any of the medications listed in Table 1 were excluded. Finally, men with severe heart disease or coronary vessel disease were excluded as a safety precaution.

Facilities and Equipment

The participants were seen at the College of Public Health at the University of South Florida in the Breath Laboratory (MHH Room 323). The records were maintained in a secured cabinet in this room. The key to access the laboratory was distributed by the University of South Florida only to authorized personnel (obtained through the College of Public Health). The necessary equipment discussed above that was needed to perform this project was available in this room.
Participant Recruitment

Healthy subjects were asked to volunteer for the study through one or two visits at the Breath Laboratory at the College of Public Health (MHH Room 323), an air-conditioned, comfortable room. They were asked to sit in the same comfortable chair. Subjects who expressed a willingness to volunteer were given information about the study procedure, the risks, the benefits, and the alternatives to the procedure. They were then asked to demonstrate an understanding of all components discussed. They were encouraged to ask questions at any time. If they still agreed to proceed, they were asked to sign an informed consent. They were monitored by a physician throughout the visit via direct observation. Because performance of spirometry maneuvers immediately before NO measurement affects the NO levels obtained, subjects were given the option of presenting for either one or two visits based on their own convenience. Those subjects who chose to present for one visit were asked to perform NO maneuvers before spirometry was obtained. Those subject who completed the study over two visits performed the spirometry maneuvers on the first visit and NO maneuvers on the second visit. For one hour prior to the visit where NO levels were obtained, subjects were asked not to eat or drink anything and to refrain from strenuous exercise as recommended in both the 1999 and 2005 American Thoracic Society (ATS) criteria. [2], [19]

Study Subjects and Restrictions

Only normal, healthy people aged 18-45 years old who denied any history of environmental allergies, asthma, or other lung diseases were invited to participate. Also, they must not have suffered from any recent upper or lower respiratory tract infections.
The study was restricted to men only because there have been reports that the level of exhaled breath NO differs between men and women. Subjects must also have no significant smoking history as defined by less than one-half pack-year history of smoking, no smoking within the past 2 years, and no significant second-hand smoke exposure. Subjects must refrain from strenuous exercise, food, or drink for one hour prior to the test at a minimum per 1999 and 2005 ATS Criteria.

Study Questionnaire and Eligibility

During each subject’s first visit, a questionnaire was initially given that asked for medical and demographic information. For those who participated in a second visit, the subjects were asked a brief questionnaire on the second visit to ascertain the absence of changes during the interim between visits. During each visit, subjects were also given a brief exit questionnaire to help assess any possible adverse effects from the study visit itself. (See Appendices A and B)

The medical history was elicited to determine “health”, and the demographic questionnaire determined age, attained ethnic affiliation, and asked for SES variables. (See Appendix A) In this study, participants were administered a demographic questionnaire to assess ethnicity that specifically asked, “How would you classify your ethnicity?” They were also asked where they and their parents were born. This was done to assess migration patterns and differences between subjects within each ethnic group. They were asked if they would classify the ethnicity of anyone in two prior generations of their ancestors differently from themselves. Anyone who classified 50% or more of their ancestors differently from themselves would be disqualified. To consider socio-
economic status differences between the two groups (which may act as a potential confounding factor), participants were also asked for their income level and occupation.

Subjects who fulfilled the criteria of age, ethnicity, and being “healthy” then underwent brief physical examination including auscultation. Those subjects found to be healthy after questionnaire, interview, and physical examination were invited to participate in the remainder of the study. To be eligible for the study, all participants needed to show normal spirometry.

Those subjects who chose to participate in two visits performed spirometry on the first visit and the NO maneuvers on the second visit. Alternatively, those subjects who chose to participate during a single visit performed the NO maneuvers prior to spirometry. The reason for this is that performance of spirometry maneuvers could affect NO levels. Subjects who chose to participate in two visits were given a brief entry questionnaire on the second visit, and auscultation was performed again to ensure absence of change in health status during the interval between the first and second visits.

Subject Safety Considerations

Subject safety was given the utmost consideration in this study. Subjects were told they may discontinue at any time. The subjects were healthy individuals who agreed to participate in the study. The entire study was non-invasive.

Furthermore, physical examination and initial medical screening questionnaires were reviewed directly by a physician prior to any procedure being performed. Only subjects who had normal physical examination were allowed to continue in the study. Spirometry, which is commonly used in clinical practice to assess lung function, was
obtained as an eligibility criterion. To obtain spirometry, subjects blew out through a disposable filter (to prevent infection) using maximum effort after a maximal inhalation. Measurement of exhaled breath NO, which is currently still a research tool, involved tidal breathing and normal respiration through a disposable plastic filter. Therefore, it was even safer to obtain than spirometric measurements.

During each visit, subjects were also given a brief exit questionnaire to help assess any possible adverse effects from the study visit itself. For those who participated in a second visit, the subjects were asked a brief questionnaire on the second visit to ascertain the absence of changes during the interim between visits.

Participation in this study did not affect the standard care subjects would receive from their personal physicians unless [1] abnormal auscultation or spirometry was found, in which case the subject was advised to consult their personal physician, or [2] an adverse effect occurred. In the latter case, most events would only be temporary and subside within seconds or minutes after stopping the activity. Although rare, a subject could experience chest pain or other adverse event during spirometry or exhaled breath nitric oxide measurement. In that event, emergency equipment was available at all times during the study to assist the individual until appropriate emergency personnel arrives. To ensure patient safety, all subjects were directly observed by a physician who was ACLS-certified during the entire visit. The sampling procedure was completely non-invasive. Since real-time measurements of exhaled breath were made, no samples were kept beyond their immediate measurement.

For those individuals who participated in two visits, a questionnaire was administered at the start of the second visit to ensure the absence of new symptoms in the
interval between visits. Also, these participants were verbally asked if there had been any changes in their health status or medications in the interim.

Physical Examination

All subjects were assessed for cyanosis and clubbing. Also, auscultation was performed prior to any breathing tests and at the conclusion of each visit. “Cyanosis is a bluish discoloration of the skin resulting from an inadequate amount of oxygen in the blood. Cyanosis occurs when oxygen-depleted blood, which is bluish rather than red, circulates through the skin. Cyanosis can be caused by many types of severe lung or heart disease or certain malformations that produce low levels of oxygen in the blood.

Clubbing is an enlargement of the tips of the fingers or toes and a loss of the angle where the nails emerge. Finger clubbing occurs when the amount of soft tissue beneath the nailbeds increases. The reason this increase occurs is not clear, but clubbing seems to occur with some pulmonary disorders (lung cancer, lung abscess, bronchiectasis), but not with others (pneumonia, asthma, emphysema). Finger clubbing also occurs with some congenital heart diseases or, in some cases, may be inherited and not indicate any disease.” (Merck, 2005) [20] In auscultation, a stethoscope is used to listen to both heart sounds and breath sounds.

Any subject demonstrating cyanosis, clubbing, or abnormal breath sounds was immediately disqualified from the study. Auscultation of the heart was also performed for the purposes of subject safety.
Acquisition of Spirometry

Spirometry, which is a common clinical method employed in assessing lung function, was performed as part of the entry criteria into the study. The Koko spirometer was used and calibrated using a standard 3-liter syringe to ambient temperature, humidity, and barometric pressure at least once a day on days when participants were examined. The spirometer was additionally re-calibrated when at least six hours had elapsed since the prior calibration or at the discretion of the examiner. The raw FEV1 and FVC measurements obtained for each participant were automatically compared to their predicted normal values based on age, ethnicity, weight, height, and non-smoking status in determining their percent of predicted values using parameters as set forth by Crapo et al.

The spirometry parameters examined on each study participant included FEV1, FVC, FEV1/FVC ratio, and the flow-volume loop. At least three spirometric measurements were obtained on each subject, with at least one flow-volume loop showing good effort. Proper technique was ensured by evaluating the flow-volume curve and continuance of the expiratory maneuver for at least six seconds according to ATS criteria.

A noseclip was placed on the subjects’ noses during the study to prevent nasal breathing in order to obtain more accurate spirometry results. Subjects were asked to forcibly exhale for at least six seconds through a disposable single-use filter after maximal inspiration, followed by another maximal inhalation. The maneuver was demonstrated to them to help achieve consistency.
Any subject with abnormal spirometry measurements was notified of this information and advised to consult a healthcare provider and disqualified from the study. All subjects with an FEV1/FVC ratio greater than 70% of predicted, FEV1 greater than 80% of predicted, FVC greater than 70% of predicted, and a normal appearing flow-volume loop were eligible for the study by spirometric criteria. In those participants who did not meet all of these strict criteria, the overall clinical picture, including medical history and physical examination were assessed individually to determine study eligibility. When eligibility due to spirometry did not meet these strict criteria, Dr. Stuart Brooks (faculty advisor, Board Certified in Pulmonary Medicine) was consulted to determine whether the participant would be eligible for the study.

Adverse effects were unlikely to occur during this phase of the study because subjects identified themselves as being in good health, the room was temperature controlled, and this phase of the study was brief in duration. All subject were seated during the spirometry. In the event that adequate trials could not be obtained in the seated position as demonstrated by their flow-volume loops, subjects were asked to stand and instructed on safety precautions in the event of symptoms.

Nonetheless, there was the slight possibility of unusual symptoms such as lightheadedness, dizziness, chest pains, palpitations, or shortness of breath during the spirometry from overexertion in breathing. As a precaution, subjects were instructed to stop, be seated, and notify the physician examiner immediately if they experienced any symptoms at any time.
Measurement of Exhaled Breath Nitric Oxide

The Nitric Oxide Analyzer was calibrated at least on a daily basis on days where subjects were being examined. It was calibrated again when at least 6 hours had elapsed since the last calibration. Additional calibrations occurred at the judgment of the study examiner. NO analysis calibrations were made using zero NO air and air containing 45 ppm NO. The flow meter was calibrated with a 3-liter syringe.

Subjects were asked to maximally inhale ambient air and then to immediately breathe out normally through a single-use disposable filter mouthpiece attached to a flow meter at a constant flow rate of 50 milliliters per second as recommended by the ATS. The mouthpiece also provided a specific amount of resistance during exhalation to allow for velopharyngeal closure to prevent contamination of the exhaled breath NO measurements with nasal nitric oxide. (See Figures 3 and 4) Because exhaled breath NO concentrations depended on the rate of airflow, subjects exhaled at a constant airflow rate determined by the Nitric Oxide Analyzer (NOA) through biofeedback by real-time computer display of their own flow rates. The NO flow rate was equal to the product of NO concentration in the exhaled air multiplied by the airflow rate. [2], [19]

The following ATS recommendations were followed. ATS recommended an exhalation duration of at least six seconds (up to 30 seconds) to obtain at least a 3-second plateau of the measured NO level. The plateau was defined as “the first portion of the NO versus time profile where [the difference between the start of the plateau and the end of the plateau varied by no more than] 10%. …Online electronic analysis of NO profiles allowed automatic identification of valid NO plateaus according to these criteria. …Repeated reproducible exhalations were performed, resulting in three NO plateau
values that agreed within 10% of the mean value. …[The ‘average NO level’] was then calculated as the mean of these three values. At least 30 seconds of relaxed tidal breathing off the NO measurement circuit elapsed between exhalations to allow the subject to rest.” (ATS, 2005, p. 916) (See Figure 5) [2] In certain cases, where automatic computer selection chose clearly incorrect plateau levels, the most appropriate 3-second plateau that met ATS criteria was selected for the calculation of the average NO level.

Figure 3. Nitric Oxide Flow Rate Versus Time [2], [19]

The mouthpiece apparatus was specially designed with an attached filter during inhalation to provide inhalation air at less than 5 ppb NO. Alternatively- as used in this study- ambient air was inhaled, which caused an early peak in real-time NO levels. In this case, plateau levels of NO were equivalent to when NO-free air was inhaled.

Figure 4. Flowmeter Design [2], [19]
Nitric oxide in exhaled breath was collected in a chamber in the Sievers 280i Nitric Oxide Analyzer (Boulder, CO) and measured according to recommended methods set forth by the American Thoracic Society in 1999 and 2005. The machinery contained
an ozone generator that produced ozone in the chamber containing the exhaled breath. The ozone reacted with the NO within the chamber according to the following photochemical reactions:

\[ \text{NO} + \text{O}_3 \rightarrow \text{NO}_2^* + \text{O}_2 \]
\[ \text{NO}_2^* \rightarrow \text{NO}_2 + \text{hv} \]

NO reacted with ozone to form “nitrogen dioxide in an electronically excited state, which then emitted light in the red and near-infra-red region of the electromagnetic spectrum to return to the more stable state. This red light generated from the reaction was detected by a thermoelectrically cooled, red-sensitive photomultiplier tube within the machine. The Sievers 280i Nitric Oxide Analyzer (Boulder, CO), a high-sensitivity detector for measuring NO based on a gas phase chemiluminescent reaction between nitric oxide and ozone, was used in measuring NO levels. … The Nitric Oxide Analyzer (NOA) had a sensitivity of 1 ppb NO and measured from 0.1 to 500 ppb nitric oxide within a timeframe of 500msec.” (Sievers, 1995) [14]

Since this limb of the study was not a maximal forced exhalation, subjects were even less likely to experience symptoms during this phase of the study. However, subjects were be given the same instructions as during the spirometry phase of the study.

Data Collection and Analysis

To appropriately assess the research question, a large number of eligible subjects would have been required to participate. Primarily because of the limited availability of subjects who were willing to participate and who met the criteria for entry into this study,
it had been undertaken as a pilot study. Therefore, the study was performed with the goal of examining at least twenty eligible subjects to provide insight into the question of whether a difference may exist between the two ethnic groups. A power calculation was performed which was derived using alpha= 0.05 and power=80% to show the level of difference in the means of the two groups that was needed for significance given a specific sample size. (See Appendix E) To do the power calculation, a recent study by Kharitanov et al. [9] which reported an average NO level of 17.8 with a standard deviation of 6.8 was used for the calculations.

In any study where there are two groups being compared, the participants are ideally randomly assigned to each group. However, random assignment is not possible when two distinct groups are being used such as men versus women or young versus old. That was the case in this study, where the two distinct groups were people of Caucasian descent versus people of African descent.

The mean of each of the two groups in a study sample may be compared using a Students’ t-Test, a parametric analysis, when the population studied is normally distributed. When the population is not normally distributed, a non-parametric analysis must be used. In the case where two distinct groups are being compared, non-parametric tests include the Wilcoxon Ranked-Sum Test or the Mann-Whitney U Test. Because the overall study set was not normally distributed (probably tri-modal), the Wilcoxon Rank-Sum Test was used in the statistical analysis of this data. When those individuals with extremely high NO levels were excluded, the remaining data consisted of those subjects who were asymptomatic but either did or did not have underlying airway pathology. Therefore, a probable bi-modal distribution remained, also requiring calculation using the
Wilcoxon Rank-Sum Test. When all participants with levels over 30 were also excluded, the data better resembled a normal distribution for which a Student’s t-Test could be used. However, upon statistical analysis using SAS, the average NO values obtained between the two ethnic groups were found to consist of different variances. Hence, the Satterthwaite Test was a more appropriate test to use in this case than the Student’s t-Test. (See Appendixes G and H)

Statistical calculations were performed by inputting the data into Statistical Analysis Software (SAS), which calculated the data automatically. For this study, SAS was utilized to calculate descriptive statistics such as the mean, median, variance, standard error of the mean, standard deviation, range, minimum and maximum values, skewness, and kurtosis both for each ethnic group and for all of the study subjects together. SAS was also used to calculate inferential statistics: the Satterthwaite T-Test was completed for those subjects with average NO levels below 30. The Wilcoxon Rank-Sum Test was used for statistical analysis of data that was not normally distributed. In a crude attempt to assess whether there might be a correlation between SES and NO levels, a Pearson-Product Correlation Coefficient was calculated.
Study Results

Exclusions

In response to recruitment strategies, approximately 15 to 20 men besides those who actually participated expressed an interest in being part of the study. These men were excluded on initial contact based on failure to meet all of the entry criteria. The primary reasons included not meeting the age criteria, a history of significant smoking, environmental allergies, or asthma.

Failure in proper communication was demonstrated by five men who presented to the lab for testing but did not meet the inclusion criteria. They were excluded early in the informed consent process. One Caucasian was excluded due to a history of environmental allergies. Another Caucasian male was excluded for current upper respiratory tract infection. He was invited back into the study but chose to withdraw. Two men of African descent were excluded due to a significant smoking history. A fifth person was excluded early in the questionnaire process after discovering that he was Hispanic.

No one was excluded due to abnormal auscultation. All of the men who underwent auscultation had normal cardiac examinations with S1 and S2 heart sounds with regular rate and rhythm and no murmurs, rubs, or gallops. Lung exams were clear to auscultation bilaterally with no audible wheezes, rales, or rhonchi. No cyanosis or clubbing was noted in the upper extremities.
All of the men who completed the spirometry maneuvers had FEV1/FVC ratio values above 70%. After complete participation, two Caucasian men were excluded for markedly prolonged expiratory phases on their flow-volume loops despite having FEV1, FVC, and FEV1/FVC ratio values that were above the cutoffs for exclusion from the study. Interestingly, both of these men were Caucasian and had the best FEV1/FVC ratios between 80-85%, whereas the men who were included had best FEV1/FVC ratios between 90-110%. One of these two men was found to have an average NO level under 10 parts per billion (ppb), while the other had an NO level between 40-50 ppb. A third Caucasian male who completed the study was subsequently eliminated because he reported taking oral steroids. His NO level was between 10-15 ppb. One participant of African descent who completed the study and denied any history of allergies but reported significant runny/irritated nasal passages compounded with poor spirometry results was eliminated due to both spirometry and possible concurrent URI or new-onset of allergies. His NO level was 54.5 ppb.

Questionnaire Collection

The following tables demonstrate the results of the demographic information that was collected during the study.

<table>
<thead>
<tr>
<th>TABLE 2. Number of Subjects in Each Ethnic Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethnicity</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>African</td>
</tr>
<tr>
<td>Caucasian</td>
</tr>
</tbody>
</table>
Figure 6. Participant Distribution By Age

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>19</th>
<th>20</th>
<th>21</th>
<th>22</th>
<th>23</th>
<th>24</th>
<th>31</th>
<th>33</th>
<th>34</th>
<th>36</th>
<th>39</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethnicity</td>
<td>African</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Caucasian</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Distribution By Age

Number of Participants

Age (in years)

African

Caucasian
Table 3. Sociodemographic Characteristics

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>African</th>
<th>Caucasian</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birthplace</td>
<td></td>
<td></td>
</tr>
<tr>
<td>US</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Africa</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Jamaica</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Different Ancestry By Ethnicity?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Yes- Parents Born in Haiti</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Yes-1/4 Hispanic</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Yes-Less than 1/4 Various Mixed</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Current Occupation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Para-Professional (Occupation Code=1)</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Student (Occupation Code=1)</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Professional (Occupation Code=2)</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Annual Salary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$0-$20,000</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>$20,000-$50,000</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>$50,000-$100,000</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Over $100,000</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
Two variables for socio-economic status (SES) were elicited by the demographic questionnaire. These included current income and current occupation. Because there may be an association between lower SES and asthma, and nitric oxide levels are higher in people with asthma, a Pearson-Product Moment Correlation was performed to determine if lower SES is correlated with a higher nitric oxide level. [21]

To do this, the raw data needed to be converted into numerical values to plug into the equation for the Pearson-Product Moment Correlation. Accordingly, the subject responses were classified into three groups for occupation: Student, Para-Professional, or Professional. Students and Para-Professionals each received a score of one point whereas professionals received a score of two points. Salary information, which was elicited at $10,000 intervals, was rated at one point for a salary of <$20,000; two points for $20,000-$50,000; three points for $50,000-$100,000, and four points for >$100,000. The scores from the employment data and the salary data were then multiplied together for each individual to arrive at a score representing their socio-economic status. These values were used in the statistical analysis against nitric oxide levels to better visualize the descriptive data already obtained.
Table 4. Pearson-Product Correlation Coefficient

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Mean</th>
<th>Std Dev</th>
<th>Sum</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average NO</td>
<td>20</td>
<td>33.55500</td>
<td>23.47091</td>
<td>671.10000</td>
<td>8.90000</td>
<td>94.40000</td>
</tr>
<tr>
<td>Salary Code</td>
<td>20</td>
<td>1.75000</td>
<td>0.96655</td>
<td>35.00000</td>
<td>1.00000</td>
<td>4.00000</td>
</tr>
<tr>
<td>Occupation Code</td>
<td>20</td>
<td>1.40000</td>
<td>0.50262</td>
<td>28.00000</td>
<td>1.00000</td>
<td>2.00000</td>
</tr>
<tr>
<td>SES Code Product</td>
<td>20</td>
<td>2.80000</td>
<td>2.35305</td>
<td>56.00000</td>
<td>1.00000</td>
<td>8.00000</td>
</tr>
</tbody>
</table>

Pearson Correlation Coefficients, N = 20
Prob > |r| under H0: Rho=0

<table>
<thead>
<tr>
<th></th>
<th>SalaryCode</th>
<th>OccupationCode</th>
<th>SESCodeProduct</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>-0.05156</td>
<td>-0.21656</td>
<td>-0.12463</td>
</tr>
<tr>
<td>p-value</td>
<td>0.8291</td>
<td>0.3591</td>
<td>0.6006</td>
</tr>
</tbody>
</table>

When the raw data was considered, a higher NO level was weakly inversely correlated with SES. However, because the p-value was greater than 0.05, the test was not statistically significant.

Medical Information

Following are [1] the medical problems that were reported by participants on the medical questionnaire, [2] the findings on the physical exam, and [3] the adverse events reported by participants upon completion of the study.
Table 5. Medical Information

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>African</th>
<th>Caucasian</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prior Respiratory Tract Ailments</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Reports Bronchitis &gt;5 years ago</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Once told he had child-hood asthma but never took medications for this; he has never had any symptoms</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Current Medications</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Diabetes medication</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Glucovance, Enalapril</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Maxzide, ASA</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Topical Steroids</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Tramadol</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Doxycycline for acne</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Inhalation Exposures</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Denied</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Firefighter x 5yrs</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Roomate smokes, but not in common areas</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Other Medical Conditions Reported By Subjects</td>
<td>Ethnicity</td>
<td></td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>----------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>African</td>
<td>Caucasian</td>
</tr>
<tr>
<td>None</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Acne</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Atopic Dermatitis</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Back Pain; Anxiety</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Diabetes Mellitus; Palpitations from Coffee</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>HTN</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>HTN; Diabetes</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Irregular Heart Beat</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Irregular Heart Beat when eating pepperoni</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Musculoskeletal Complaints</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Prior subdural hematoma</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>groin hernia age 4 repaired</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Physical Exam</th>
<th>Ethnicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>African</td>
</tr>
<tr>
<td>WNL</td>
<td>9</td>
</tr>
<tr>
<td>Obesity</td>
<td>1</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>African</td>
</tr>
<tr>
<td>-----------</td>
<td>---------</td>
</tr>
<tr>
<td>None</td>
<td>9</td>
</tr>
<tr>
<td>None/ got URI that night</td>
<td>1</td>
</tr>
<tr>
<td>Raspy Voice and tiredness several hours later; sick the next day</td>
<td>0</td>
</tr>
</tbody>
</table>

Spirometry Measurements

Of the twenty-four participants on whom spirometry was performed, three Caucasians were excluded: two for having a prolonged expiratory phase on spirometry, and one for taking oral steroids. A fourth male of African descent was excluded for low FEV1 and FVC values; he also reporting irritated nasal passages with rhinorrhea.

The following participants were entered into the study after approval was received from Dr. Brooks. One morbidly obese man (>300 pounds) of African descent who had low FEV1 and FVC values had a normal flow-volume loop and normal FEV1/FVC ratio. His low FEV1 and FVC values were attributed to his increased girth pushing against the diaphragm, creating limitations in total lung capacity. One man of African descent who was 79 inches tall had normally appearing flow-volume loops and normal FEV1/FVC ratios but low individual FEV1 and FVC readings. Because spirometry is less accurate at the extremes of height, he was included in the study. Two men with normally appearing flow-volume loops, normal FEV1/FVC ratios, and with FEV1 and FVC trials very close.
to 80% were kept in the study after discussion with Dr. Brooks (some degree of operator error was involved in each of these two cases).

Exhaled Breath Nitric Oxide Measurements

The data obtained during this study are shown in the figure below:

![Figure 7. Average NO Level Of Each Subject By Ethnicity](image)

(Note: Each [ ] Or [ ] Represents One Participant)

Visualization of the overall results in Figure 7 show that the data obtained was not normally distributed for either group. Inspection of the distribution of values for African descent reveals that the data may have contained three or four modes. The statistical analysis of the data is presented in Table 6.

<table>
<thead>
<tr>
<th></th>
<th>Overall</th>
<th>African</th>
<th>Caucasian</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>20</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Sum Observations</td>
<td>671.1</td>
<td>356.6</td>
<td>314.5</td>
</tr>
</tbody>
</table>
The above data showed that the mean of the African group was slightly higher than the mean of the Caucasian group. The medians differed by only 1.8 ppb. The African group had a greater variance whereas the Caucasian group showed less variance in data with significantly greater kurtosis (peakedness of the curve).
Table 7. Wilcoxon Scores (Rank Sums) for Variable Average NO Classified by Variable Ethnicity

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>N</th>
<th>Sum of Scores</th>
<th>Expected Under H0</th>
<th>Std Dev Under H0</th>
<th>Mean Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>African</td>
<td>10</td>
<td>104.50</td>
<td>105.0</td>
<td>13.223782</td>
<td>10.450</td>
</tr>
<tr>
<td>Caucasian</td>
<td>10</td>
<td>105.50</td>
<td>105.0</td>
<td>13.223782</td>
<td>10.550</td>
</tr>
</tbody>
</table>

_Average scores were used for ties._

**Wilcoxon Two-Sample Test**

<table>
<thead>
<tr>
<th>Statistic</th>
<th>104.5000</th>
</tr>
</thead>
</table>

**Normal Approximation**

<table>
<thead>
<tr>
<th>Z</th>
<th>0.0000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two-Sided Pr &gt;</td>
<td></td>
</tr>
</tbody>
</table>

**t Approximation**

| Two-Sided Pr > | |Z| | 1.0000 |

_Z includes a continuity correction of 0.5._

To show a significant result, the two-tailed z-score calculated by the Wilcoxon-Rank Sum analysis would need to be at least 1.96 (alpha=0.05%). Thus non-parametric statistical analysis using Wilcoxon-Rank Sum failed to reject the null hypothesis that there is no difference between the means of the two ethnic groups.

When the extreme values above fifty were removed from the data set, the following non-normally distributed results were obtained:
<table>
<thead>
<tr>
<th></th>
<th>Overall</th>
<th>African</th>
<th>Caucasian</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>17</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td><strong>Sum Observations</strong></td>
<td>420.8</td>
<td>180.2</td>
<td>240.6</td>
</tr>
<tr>
<td><strong>Median</strong></td>
<td>25.90000</td>
<td>20.85000</td>
<td>25.90000</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>24.752941</td>
<td>22.525</td>
<td>26.733333</td>
</tr>
<tr>
<td><strong>Lower 95% CL for Mean</strong></td>
<td>19.8197848</td>
<td>11.6675971</td>
<td>22.7272157</td>
</tr>
<tr>
<td><strong>Upper 95% CL for Mean</strong></td>
<td>29.6860976</td>
<td>33.3824029</td>
<td>30.7394510</td>
</tr>
<tr>
<td><strong>Std Deviation</strong></td>
<td>9.59473278</td>
<td>12.986999</td>
<td>5.21176554</td>
</tr>
<tr>
<td><strong>Variance</strong></td>
<td>92.0588971</td>
<td>168.662143</td>
<td>27.1625</td>
</tr>
<tr>
<td><strong>Std Error Mean</strong></td>
<td>2.32706451</td>
<td>4.59159753</td>
<td>1.73725518</td>
</tr>
<tr>
<td><strong>Skewness</strong></td>
<td>-0.2552595</td>
<td>0.25915577</td>
<td>0.5170626</td>
</tr>
<tr>
<td><strong>Kurtosis</strong></td>
<td>-0.8535283</td>
<td>-2.0829279</td>
<td>-1.1213304</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>30.70000</td>
<td>30.70000</td>
<td>14.50000</td>
</tr>
<tr>
<td><strong>Minimum</strong></td>
<td>8.9</td>
<td>8.9</td>
<td>21.0</td>
</tr>
<tr>
<td><strong>Maximum</strong></td>
<td>39.6</td>
<td>39.6</td>
<td>35.5</td>
</tr>
</tbody>
</table>
Again, the above data showed a significantly different variance between the two groups. This time, however, the mean of the African group is somewhat lower than the mean of the Caucasian group. The medians differed between the two groups by 5.05 ppb.

Table 9. Wilcoxon Scores (Rank Sums) for Variable Average NO Classified by Variable Ethnicity When Extreme Values Are Excluded

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>N</th>
<th>Sum of Scores</th>
<th>Expected Under H0</th>
<th>Std Dev Under H0</th>
<th>Mean Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>African</td>
<td>8</td>
<td>65.50</td>
<td>72.0</td>
<td>10.385935</td>
<td>8.187500</td>
</tr>
<tr>
<td>Caucasian</td>
<td>9</td>
<td>87.50</td>
<td>81.0</td>
<td>10.385935</td>
<td>9.722222</td>
</tr>
</tbody>
</table>

Average scores were used for ties.

Wilcoxon Two-Sample Test

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Statistic</td>
<td>65.5000</td>
</tr>
</tbody>
</table>

Normal Approximation

<table>
<thead>
<tr>
<th>Z</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z</td>
<td>-0.5777</td>
</tr>
</tbody>
</table>

One-Sided Pr < Z | 0.2817 |
Two-Sided Pr > |Z| | 0.5635 |

t Approximation

<table>
<thead>
<tr>
<th>One-Sided Pr &lt; Z</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>One-Sided Pr &lt; Z</td>
<td>0.2858</td>
</tr>
</tbody>
</table>

Two-Sided Pr > |Z| | 0.5715 |

Z includes a continuity correction of 0.5.
Non-parametric analysis by the Wilcoxon Rank-Sum test failed to find a statistical difference between the means of the two ethnic groups since the two-sided p-value is greater than 0.05. It must be kept in mind that this was a pilot study. Because of the small sample size, there was less reliability in the statistical analysis. A significantly larger sample size would have been needed to determine whether or not a truly significant difference exists.

When the data was re-calculated using only those values below 30 in a crude attempt to exclude those asymptomatic participants with underlying airway pathology, the following data was obtained:

<table>
<thead>
<tr>
<th></th>
<th>Overall</th>
<th>African</th>
<th>Caucasian</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>13</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td><strong>Sum Observations</strong></td>
<td>274.7</td>
<td>102.2</td>
<td>172.5</td>
</tr>
<tr>
<td><strong>Median</strong></td>
<td>22.50000</td>
<td>12.60000</td>
<td>22.90000</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>21.1307692</td>
<td>17.0333333</td>
<td>24.6428571</td>
</tr>
<tr>
<td><strong>Lower 95% CL for Mean</strong></td>
<td>16.4543913</td>
<td>7.0101150</td>
<td>21.3635183</td>
</tr>
<tr>
<td><strong>Upper 95% CL for Mean</strong></td>
<td>25.8071471</td>
<td>27.0565517</td>
<td>27.9221960</td>
</tr>
<tr>
<td><strong>Std Deviation</strong></td>
<td>7.73858133</td>
<td>9.55105579</td>
<td>3.54582249</td>
</tr>
<tr>
<td><strong>Variance</strong></td>
<td>59.885641</td>
<td>91.2226667</td>
<td>12.5728571</td>
</tr>
<tr>
<td><strong>Std Error Mean</strong></td>
<td>2.14629629</td>
<td>3.8992022</td>
<td>1.34019493</td>
</tr>
</tbody>
</table>
Table 10. Statistical Analysis of Average NO Levels When Excluding Levels over 30 ppb

<table>
<thead>
<tr>
<th></th>
<th>-0.4877608</th>
<th>0.8640237</th>
<th>0.61145295</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skewness</td>
<td>-0.4877608</td>
<td>0.8640237</td>
<td>0.61145295</td>
</tr>
<tr>
<td>Kurtosis</td>
<td>-1.2966622</td>
<td>-1.8466342</td>
<td>-1.6296301</td>
</tr>
<tr>
<td>Range</td>
<td>20.80000</td>
<td>20.80000</td>
<td>8.70000</td>
</tr>
<tr>
<td>Minimum</td>
<td>8.9000000</td>
<td>8.9</td>
<td>21.0</td>
</tr>
<tr>
<td>Maximum</td>
<td>29.7000000</td>
<td>29.7</td>
<td>29.7</td>
</tr>
</tbody>
</table>

Table 11. t-Test of Average NO Levels When Excluding Average NO Values Over 30

| Variable      | Method     | Variances | DF   | t Value | Pr > |t|   |
|---------------|------------|-----------|------|---------|------|----|
| Average_NO    | Pooled     | Equal     | 11   | -1.97   | 0.0748 |
| Average_NO    | Satterthwaite | Unequal   | 6.18 | -1.85   | 0.1131 |

This time, the means of the two groups differed by 7.6 ppb, whereas the medians differed by 10.3 ppb. Although the two groups still had different variances, the difference in the variances between the two ethnic groups was not as great as before. Again, the statistical analysis (Satterthwaite Test for normally distributed data with different variances) failed to show a difference between the two means of the ethnic groups as the p-value was greater than 0.05. However, the mean of the African group had dropped even more than the decline in the mean of the Caucasian group so that there was an even greater difference between the means of the two groups in this small pilot study.
Using the $\mu$ and $\sigma$ data obtained from the normally-distributed participants in this study with average NO levels under 30 ppb, the power of the analysis of was calculated to be 36.5%.

<table>
<thead>
<tr>
<th>$\mu_1$</th>
<th>$\mu_2$</th>
<th>$\sigma$</th>
<th>$\alpha$</th>
<th>NCP</th>
<th>Critical Value</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.0333</td>
<td>24.6429</td>
<td>7.73858</td>
<td>0.05</td>
<td>3.12391</td>
<td>4.84434</td>
<td>0.36483</td>
</tr>
</tbody>
</table>

Because $\beta = 1 - \text{Power}$, beta (or the probability of making a type II error) was calculated to be equal to 63.5%.
Discussion

Findings

To reiterate, the question of interest has been whether a difference exists in the average NO levels between two ethnic groups: Caucasians and people of African descent. First, information on demographics, socio-economic status, and medical information was obtained and described. The descriptions were summarized as: [1] There was more age variability in the Caucasian group when compared to the African group; [2] all Caucasians were born in the United States, whereas four of the people in the African group were born outside the United States; [3] two men in the African group and one man in the Caucasian group report having different ancestry; [4] five Caucasians worked in professional occupations, whereas only three of the African men worked in professional capacities; [5] most participants reported annual incomes under $50,000.

Next, in a crude attempt to examine whether a correlation may exist between socio-economic status (SES) and average NO levels, individuals were given numerical assignments for their stated occupation and income responses, which were then multiplied together and used to calculate a Pearson Product Correlation Coefficient (r). The analysis resulted in a weakly negative r-value, indicating that there may be a weak inverse relationship between SES and NO levels. However, as expected with the small sample size studied, the analysis did not yield a statistically significant result. The average NO levels automatically determined by the NOA for each of the participants were then plotted to visualize the variability in NOA-determined average NO levels.
obtained. (See Figure 7) Inspection of this figure pointed to a non-normal distribution of the data with possible tri-modal distribution. One mode centered around extreme NOA-determined average NO values over 60 ppb. A second mode included individuals with normal airways. A possible third mode could include asymptomatic individuals with underlying airway pathology with NO levels between 30-40 ppb.

Therefore, the “average NO level” data was analyzed in 3 ways: [1] non-parametric analysis of the average NO levels for all 20 eligible participants, [2] non-parametric analysis of the average NO levels excluding participants with extreme NO levels over 70 ppb, and [3] parametric analysis of the average NO levels excluding participants with average NO levels over 30 ppb.

In this pilot study, none of the statistical analyses showed significance. Nonetheless, when attempts were made to exclude individuals with average NO levels over 30 ppb so that the data becomes normally distributed, the mean and median of the average NO levels for the African group was 7.6 ppb and 10.3 ppb lower than the mean and median for the Caucasian group, respectively. This indicated the possibility that a statistically significant difference could be found in this analysis group when a larger sample size is studied. The power and β of the study for the group of participants with NO levels under 30 ppb were calculated to be 36.5% and 63.5%, respectively. Thus, there is a high likelihood of committing a Type II error in this group. Therefore, in participants with NO levels under 30 ppb, the high Type II error rate indicates a high probability of missing a true difference between the two means if one really exists.

In order to find statistical significance in the results, a power analysis was performed. It indicated that at least thirty-two eligible participants with NO levels under
30 would be required. (See appendix E). At least sixty eligible participants would be required to obtain a study sample of sixteen in each group. The number of willing participants needed would be substantial given the strict criteria of the study where anyone with a known history of smoking, environmental allergies, asthma, or other lung diseases was excluded. Even then, those participants who were asymptomatic but who demonstrated either [1]abnormalities after spirometric testing (for example, prolonged expiratory phase), or [2]high NO levels possible indicative of underlying airway pathology or an imminent upper respiratory tract infection would also need to be excluded.

Of the three participants who were excluded due to clinically abnormal spirometry, two had high NO levels, whereas one had a low NO level. Thus, the presence of abnormal spirometry did not absolutely predict a high NO level in this tiny number of excluded participants for spirometry criteria. As may be expected, one participant taking oral steroids had a low NO level.

Implications

The implication gleaned from this study was that statistical significance was not achieved due to the small sample size. However, the crude mean of the average NO levels in the normally-distributed male participants (with average NO levels under 30 ppb) of African descent was found to be 7.6 ppb lower than that for their Caucasian counterparts. This implies that a larger well-designed study may find a statistically significant difference and provides a basis for performing a more extensive study including both men and women and examining varying ethnic backgrounds (for example,
Alaskan native, Pacific islander, etc.) in addition to those studied here prior to the use of NO in clinical settings to in the diagnosis or monitoring of patients.

Future Directions

After investigators of a concurrent capsaicin study mentioned that certain asymptomatic participants in their study with extremely high NO levels became ill with an upper respiratory tract infection within the next several days of testing, three participants with NO levels over sixty were asked to notify the investigator of this study if they became ill within the next week. Two of the three study participants reported the onset of an upper respiratory tract infection within the next two days. The third participant had not yet made contact with the investigator. This generated the hypothesis that NO levels may be extremely high in normal individuals up to a few days prior to the onset of an upper respiratory tract infection. Because the current study was not designed to investigate this hypothesis, this conclusion would need to be further investigated in a properly designed and controlled study to examine whether or not this is the case.

Other hypotheses generated by this pilot study include a possible weak inverse correlation between SES and average NO level and a possible tri-modal distribution of NO levels. These would also need to be investigated in larger, well-controlled studies designed to specifically examine these questions.

Study Limitations

As a pilot study with only 20 eligible participants, the entire study was of limited value. Nonetheless, the goal here had been to provide a cursory look at the question of
whether a difference may exist in baseline nitric oxide levels in members of different ethnicities.

Limitations of the study could effect on its validity. In this study, calculation of the sample size was based on the mean and standard deviation as measured in a prior study. [22] However, it was unknown whether these were the true population values for these parameters. The values measured for mean and standard deviation of average NO levels in this study varied significantly from the prior values. This could affect the accuracy of the measurement.

Errors due to chance may have occurred due to small sampling size. “The stronger the association is in nature, the easier it is to find with a small sample size. The closer the true association is to the null value, the larger sample size you need to find it to be statistically significant.” (Sanchez-Anguiano, 2004, unit 6) [22] Therefore the results of this study showed that it was highly unlikely that there was a strong association between exhaled breath NO levels and ethnicity.

When “the sample is too small, a study is more likely to miss an association, increasing the likelihood of a Type II error.” (Sanchez-Anguiano, 2004, unit 6) [22] A Type II error may have been committed in this study where a weaker association could still be present and would not be detected by this study.

Subjects were asked to participate based on the eligibility criteria of no history of allergies, asthma, or lung diseases and no significant smoking history. Participants with NO values over 30 may have had imminent upper respiratory tract infection or underlying airway pathology despite being asymptomatic. With this in mind, the data was also calculated when the participants with levels over 30 ppb were excluded. In
doing this, the sample size was reduced even more, further increasing the likelihood of error due to chance.

Another way the results of the study may have been limited was through bias. There were numerous biases that may have occurred during the study. An example included prevalence-incidence bias, where participants who were “mild or silent cases” were missed by the entry criteria and allowed to participate. [22] Thus, they were misclassified. Other biases may have included observer bias because no blinding occurred during the study. Self-selection bias was present since volunteers were used. [22] Also, a susceptibility bias was present since all subjects were students or workers, creating a healthy worker effect. Thus, the study could not be generalized to the general population. [22]

Unknown confounders may also have affected the study. In this study, one participant reported taking enalapril on a regular basis. The investigator had inadvertently overlooked that this medication has been found to raise exhaled breath NO levels (See Table 1). However, because the participant’s average NO level was below 10 ppb, his NO level off the medication would have been even lower than the measured value. In that case, the difference between the means of the two ethnic groups would have increased slightly, giving more power to the study. In essence, the effect of the oversight was in the direction closer to the null hypothesis. Because the power of the study was so low, it did not dramatically impact the results of the study.

Methods of controlling for confounders included restriction, randomization, and matching. [22] Multiple restrictions were used in the study design to attempt to control for confounders. Although randomization is a powerful method of controlling for
confounders, it could not be used in this study because two intact groups—Caucasians and Africans—were being compared. Matching would also have been beneficial. However, it would have been “difficult and tedious. Once a factor had been matched, it could not be studied as a risk factor for disease.” Because the matching would have been by factors of socio-economic status, their relationship to NO levels could not have been examined if participants were matched based on these factors. (Sanchez-Anguiano, 2004, unit 8) [22]

In performing statistical analyses where two distinct groups were compared, three underlying assumptions were made: (1)Assumption of Normality, (2)Assumption of Homogeneity of Variance, and (3)Assumption of Independence. [23]

Statistical analyses are most robust to violations of the Normality Assumption. Still, this was addressed by using a non-parametric Wilcoxon Ranked-Sum Test for analysis of data that was not normally distributed. [23]

Statistical analyses are moderately robust to the Homogeneity of Variance Assumption. In this study, the two groups had different variances, so this assumption was violated. However, performance of a Satterthwaite analysis attempted to account for violations of homogeneity of variance in the group with NO levels under 30 ppb. Nonetheless, the Satterthwaite analysis is less reliable for small sample sizes. [23]

Statistical analyses are the least robust to the Assumption of Independence, the most serious of the violations. In this study, care was taken not to allow people who were genetically related to each other participate in the study. Everyone participating in the study had some association to the University of South Florida (USF). Two participants, one Caucasian male and one African male, were significant others of USF
student/employees. Otherwise, everyone in the study was directly a student or employee of USF.
Conclusion

Given the small sample size (due to difficulty in subject recruitment), the limited population that the study could be generalized to, and the various other limitations listed above, this pilot study is limited in its value. Despite this, a raw difference between the means and medians of the average NO levels for the two ethnic groups was noted when only those participants with average NO levels under 30 ppb were included. Therefore, differences in baseline NO levels between members of different ethnic groups requires further study prior to the institution of NO measurements in clinical settings for patient care.
References


18. ACOEM position paper on spirometry


Appendices
Appendix A: Initial Medical and Ethnicity Questionnaires

Subject number:_______

Exhaled Breath Study Questionnaire

Today’s Date ______________________________________

Gender:               Male           Female        (circle one)

What is your date of birth? ______________________

How old are you? _____________________________ years old.

Where were you born? ____________________________________________

How would you classify your ethnicity?

(Please check one of the following):

______Caucasian

______African American

______ Other- Please explain: ________________________________________

Are any of your biological parents or grandparents from a different ethnicity than your stated ethnicity above?      YES         NO      (circle one)

Where was your mother born? _____________________________________

Where was your father born? ______________________________________
What is your current occupation?

_________ Student

_________ Other- Please Specify _______________________________________________
_____________________________________________________________________________

What is your current annual salary? Please check one of the following:

(Please note that this information is used solely to further subdivide the subject population. Like all of the other personal information obtained for this study, this information will be kept strictly confidential.)

_________ $0 to $10,000 per year  __________ $60,000 to $70,000 per year

_________ $10,000 to $20,000 per year  __________ $70,000 to $80,000 per year

_________ $20,000 to $30,000 per year  __________ $80,000 to $90,000 per year

_________ $30,000 to $40,000 per year  __________ $90,000 to $100,000 per year

_________ $40,000 to $50,000 per year  __________ More than $100,000 per year
$50,000 to $60,000 per year

Other- please explain: __________________________________________________________

Do you currently have, or have you ever had any of the following conditions listed below? (Please circle YES or NO)

YES  NO  CHEST PAIN, PALPITATIONS, IRREGULAR HEART BEAT, OR HEART DISEASE?

YES  NO  HIGH BLOOD PRESSURE?

YES  NO  ASTHMA, BRONCHITIS, EMPHYSEMA, OR OTHER LUNG OR BREATHING DISORDERS?

YES  NO  DIFFICULT OR HEAVY BREATHING

YES  NO  A LARGE AMOUNT OF PHLEGM PRODUCTION

YES  NO  PREGNANCY?

IF YOU HAVE CIRCLED YES TO ANY OF THE ABOVE QUESTIONS, PLEASE EXPLAIN: __________________________________________________________
Do you have any health problems or past medical history of health problems that you have seen a physician for? Please list them below.

1. __________________________________________________________

2. ________________________________________________________

3. ________________________________________________________

4. ________________________________________________________

5. ________________________________________________________

Are you taking any medications? If so please list them below. (Including over the counter medications)

1. __________________________________________________________________

2. __________________________________________________________________

3. __________________________________________________________________
At this point in time, to what degree do you note the following symptoms?

(a) Phlegm production:
___NONE   ___VERY LITTLE   ___MODERATE AMOUNT   ___VERY MUCH

(b) Runny or irritated nose or nasal passages:
___NONE   ___VERY LITTLE   ___MODERATE AMOUNT   ___VERY MUCH

(c) Throat irritation or burning sensation:
___NONE   ___VERY LITTLE   ___MODERATE AMOUNT   ___VERY MUCH

(d) Sensation of a “weight” or tightness of the chest:
___NONE   ___VERY LITTLE   ___MODERATE AMOUNT   ___VERY MUCH
(e) Feeling of chest pain, burning, or tightness:

___NONE   ___VERY LITTLE   ___MODERATE AMOUNT   ___VERY MUCH

If you have ever smoked, please answer the following:

How many packs per day did you smoke? ________________________

For how many years did you smoke? ____________________________

When did you stop smoking? ________________________________

On what date were you last ill? ______________________________

What illness did you have? ________________________________

Please circle YES or NO to the following questions:

Are you exposed to second hand smoke at home or at work?
YES or NO

Were you or are you exposed to any gases, dusts, or fumes at your job?

YES or NO

If so, please explain: ____________________________________________

_____________________________________________________________________

Do you ever wheeze or become short of breath?

YES or NO

This is the end of this questionnaire. Thank you for taking the time to fill it out.

Special thanks to Dr. Gwyn Crump for allowing the use of his questionnaire in this study.
Second Visit Initial Study Questionaire

SUBJECT NUMBER: __________________________________________

Are you currently experiencing any of the following symptoms?
(Please Circle YES or NO)

(1) Difficult or heavy breathing? YES  NO

(2) Feeling of chest pain, tightness, pressure, or burning?
YES   NO

(3) Any new symptoms that were not present during your first visit here? YES  NO

(4) Any other unusual symptoms? YES  NO
Appendix B: Exit Questionnaire

Post-Study Questionnaire

SUBJECT NUMBER: ________________________________________

Are you currently experiencing any of the following symptoms?
(Please Circle YES or NO)

(1) Difficult or heavy breathing? YES NO

(2) Feeling of chest pain, tightness, pressure, or burning? YES NO

(3) Any new symptoms that were not present when you arrived here today? YES NO

(4) Any other unusual symptoms? YES NO

Special Thanks To Dr. Gwyn Crump for developing the majority of this questionnaire!
Appendix C: Recruitment Flyer

You Are Invited To Participate!

What?
- A $20.00 total honorarium will be given to each volunteer who completes the study for valuable time spent participating.
- This study only makes measurements of the normal air breathed out by subjects. The tests will measure lung function & a constituent of the air people breathe out called Nitric Oxide.
- This research study is non-invasive, meaning you will not be given any drugs or subjected to invasive procedures such as needlesticks as part of the study.

Who?
- Healthy men with normal lungs who have never smoked and have no allergies.
- Candidates must be 18-45 yrs old & either Caucasian or people of African descent.

Why?
- The study is designed to examine whether a certain marker found in the air we normally breathe out is different in normal young, healthy people of African descent as compared to Caucasians. Your participation in this study may help to determine whether
adjustments should be made in measurement of this marker in people of African descent to help more accurately portray when disease is or is not occurring. Hopefully, the results will benefit science.

When?

The study will take about two hours to complete. You may choose to participate either:

During two visits which will last for approximately one hour each, or

During a single visit which will last approximately two hours.

Where?

USF College of Public Health - Call (813) 391-9385

If you may be interested or have any questions, please call (813) 391-9385 or e-mail us at spatel5@hsc.usf.edu
Informed Consent for an Adult

University of South Florida

Information for People Being Asked to Take Part in Research Studies

IRB Study # 103323

Doctors and researchers at University of South Florida (USF) study diseases and other health problems people have. We try to find better ways to help treat these health problems. To do this, we need the help of people who agree to take part in a research study.

Title of Research Study: Exhaled Breath Nitric Oxide: Is There a Baseline Difference Due to Ethnicity?

Persons in Charge of Study: Sunita I. Patel, MD; Stuart M. Brooks, MD

Where the study will be done: University of South Florida, College of Public Health, MHH Room 323

Who is paying for the study: Sunshine Education Research Center

Should you take part in this study?

This form tells you about this research study. You can decide if you want to take part in it. You do not have to take part. Reading this form should help you decide if you want
to take part in the study. If, at any time, you have any questions feel free to ask the person explaining this study to you.

Before you decide:

Read this form.

Talk about this study with the study doctor or the person explaining the study. You can have someone with you when you talk about the study.

Find out what the study is about.

This form explains:

The purpose of this research study.

What will happen during this study and what you will need to do.

The potential benefits of being in this study, if any.

The risks of having problems because you are in this study.

The answers to any questions you might have.

You can ask questions:

You may have questions this form does not answer. If you do, ask the study doctor as you go along.

You don’t have to guess at things you don’t understand. Ask the people doing the study to explain things in a way you can understand.

After you read this form, you can:

Take your time to think about the information that has been provided to you.

Have a friend or family member read the form.

Talk it over with your regular doctor.
It’s up to you:

If you choose to be in the study, then you can sign the form.

If you do not want to take part in this study, you do not sign the form.

Why is this research being done?

The purpose of this study is to find out if there is a difference between Africans and Caucasians in the amount of a certain substance in the air we breathe out called nitric oxide. This research could eventually lead to development of more accurate baseline measurements of this and other markers in exhaled breath in Africans or individuals of other ethnicities, such as Asians, Alaskan Natives, Hispanics, etc.

The air we breathe out is currently the subject of intense research. There are certain substances in this air whose levels are affected by specific diseases associated with the lungs. In this study, one of these substances in the breath is called nitric oxide. Your breath will be measured by having you breath out into a single-use, clean, disposable tube that has never been used before. Each tube is discarded after you have used it. Two ethnic groups are being examined in this study because, in the past, ethnic differences have sometimes been found to affect certain clinical measurements.

Why are you being asked to take part?

You must be a healthy person of a certain ethnicity and age group to be eligible for the study. You will not be eligible if you have had any recent upper or lower respiratory tract diseases, heart or blood vessel disease, or hernias. You may have had a recent respiratory tract disease if you have had any recent fever, chills, coughing, shortness of breath or difficulty breathing, nasal congestion, runny nose, sinus congestion or headache, colored phlegm or (green, yellow, brown, black, or red), crackling or whistling sounds heard
when you breathe, diagnosis by a doctor of nose/sinus/lung infections, or recent treatment with antibiotics for any of these symptoms. You may not be eligible if you have any other diseases or health problems. We are asking you to take part in this study because you are a healthy young Caucasian or African adult between 18-45 year of age. We want to find out if there is a difference in the exhaled breath measurements between healthy Caucasians and Africans.

Your regular medical treatment will not actually be part of the research study. In fact you will not be eligible for the study if you suffer from any significant heart or lung disease or other significant medical problems.

How long will you be asked to stay in the study?

You will be asked to spend a total of about 2 hours in this study.

You may choose to complete this study on two one-hour visits at least twelve hours apart. On each visit, this hour is used to complete the screening questionnaires, perform a limited non-invasive physical examination, and for breathing tests. Alternatively, you may choose to complete the study in a single 2-hour visit. If you select this option, the same procedures will be performed, but in a different order.

How often will you need to come for study visits?

A study visit is one you have with the study doctor. This visit is different than the visits you make with your regular doctor. You will need to come for up to two study visits in all.

At these study visits, the doctor will obtain a questionnaire both before and after testing, perform a brief physical exam consisting primarily of listening to your heart and lungs using a stethoscope, and perform the breathing tests.
How many other people will take part?

About 50 people will take part in this study at USF.

Will the medical treatment you get from your regular doctor change if you take part in this study?

The kind of treatment you now get from your regular doctor will not change because you take part in this study. You will keep seeing your regular doctor. Your regular doctor will give you the same kind of treatment you would get anyway, whether you take part in the study or not.

If you need it, you can

Use other medicines prescribed that will help your disease.

Get any surgery you need.

You will need to talk to the study doctor about any surgery you have planned.

Talk to your regular doctor about the treatments you may need.

If you have an emergency, you can get emergency care.

Other procedures can be used for measuring airway disease. These include bronchoalveolar lavage/bronchial biopsy (which are invasive), or sputum induction (which can be less comfortable). These procedures, which can be ordered by your regular doctor if needed clinically, will not be performed here.

What other choices do you have if you decide not to take part in this study?

An alternative of being part of this research study is to not participate in this study. There will be no changes in your life. You will continue with your regular life style and are instructed to visit your personal physician for any respiratory or non-respiratory problem
you were being followed for before the study. The same is true if you decide to participate in the study.

If you decide you do not want to take part in this study that is okay. There are other choices such as chest X-ray/CT, bronchoalveolar lavage, biopsy, or sputum induction that are considered to be the current standard of clinical care for diagnosing airway disease.

How do you get started?

You must be of a certain ethnicity and age group to be eligible for the study. You must also be healthy to participate in this study. Volunteers must be between ages 18 and 45.

If you decide to participate in this study, you will be required to review this informed consent, discuss the study and your possible participation with the study doctor and decide whether you are interested in participating. If you decide to take part in this study, you will need to sign this consent form. This informed consent must be signed before any study-related test or procedure can be done. After signing this informed consent, screening tests will be completed to help determine if you meet the requirements to be in the study. You will be asked to fill out questionnaires, undergo a limited physical examination and perform a limited breathing test called spirometry.

Screening tests are tests done to see if you are able to be in the study. In order to qualify, you must record a normal health history. You must record a negative respiratory questionnaire response to treatment for any lung, nasal, or sinus diseases. You must not be suffering from any major cardiovascular condition.

There are different tests performed. These tests are considered non-invasive and do not involve drawing blood or inserting tubes into your throat or nose.
We will do these screening tests:

1. **Questionnaires**: You will be asked to fill out questionnaires about your health, ethnicity, and socioeconomic status. They will include medical questionnaires. A post-study symptom questionnaire will also be given at the end of each visit.

2. **Physical Examination**: Listening with a stethoscope to your chest and abdomen.

3. **Spirometry**: This is a common, frequently used and accepted medical test for evaluating how your lungs are working. Abnormal spirometry may indicate that you have a lung disease such as emphysema, chronic bronchitis, asthma, and other diseases. The test involves taking in a deep breath and then blowing out into a tube as hard and as fast as you can. The spirometer measures the amount and speed with which you forcibly exhale your deep breath. You will be notified if your spirometry test results are abnormal and advised to see your personal physician.

The test results will come back right away. At the end of each of these screening tests, you and the research team will decide whether or not you should be in the study.

What will you need to do to get ready for this study?

You will need to refrain from strenuous physical exercise (such as running, jogging, working out with exercise equipment, participating in sports, or doing any heavy physical labor), eating, or drinking for a minimum of one hour prior to the visit.

You must be of a certain ethnicity and age group to be eligible for the study. You must also be healthy to participate in this study. Volunteers must be between ages 18 and 45.

If you decide to participate in this study, you will be required to review this informed
consent, discuss the study and your possible participation with the study doctor and decide whether you are interested in participating. This informed consent must be signed before any study-related test or procedure can be done. After signing this informed consent, medical tests will be completed to help determine if you meet the requirements to be in the study. You will be asked to fill out a questionnaire, undergo a physical examination and perform a breathing test. In order to qualify, you must record a negative respiratory questionnaire response to treatment for respiratory diseases, including asthma, emphysema, chronic bronchitis, sinusitis and interstitial lung disease. You must not be suffering from any major cardiovascular condition.

There are different tests performed. These tests are considered non-invasive and do not involve drawing blood or inserting tubes into your throat or nose.

What will happen during this study?

Only normal, healthy, non-smoking subjects without second hand smoke exposure or history of heart/lung/other diseases ages 18-45 years old will be invited to participate. You will be asked to verify that they have not had any signs of recent upper or lower respiratory tract infections. This would include any recent fever, chills, coughing, shortness of breath or difficulty breathing, nasal congestion, runny nose, sinus congestion or headache, colored phlegm or (green, yellow, brown, black, or red), crackling or whistling sounds heard when you breathe, diagnosis by a doctor of nose/sinus/lung infections, or recent treatment with antibiotics for any of these symptoms. At a minimum of 1 hour prior to each visit, subjects will be instructed not to eat or drink or participate in strenuous exercise.

If you decide to participate in two one-hour visits:
On the first visit, you will be asked to volunteer for the study. If you volunteer, you will be given information about the study procedure, the risks, the benefits, and the alternatives to the procedure. Then you will be asked if you have any questions and demonstrate an understanding of everything discussed. If you still agree to proceed, you will be asked to sign an informed consent. You will be taken to the Breath Laboratory at the College of Public Health (MHH Room 323)- an air-conditioned, comfortable room, and sit in a comfortable chair.

You will be asked to complete a symptom questionnaire, medical history form to determine “health”, and a short questionnaire to determine age and ethnicity. Those who fulfill the criteria of age, ethnicity, and being “healthy” will be asked to participate in the study. You will then undergo brief physical examination including auscultation. You will be directly watched by a physician throughout the remainder of the study via direct observation.

Limited spirometry (including FEV1, FVC, and FEV1/FVC ratio), which is part of a common method done in hospitals and clinics to check lung function, will be done to help verify that your breathing is normal. A loose-fitting device resembling a clothespin will be placed on the nose during the study to obtain better spirometry results. You will be asked to forcibly exhale into a disposable single-use tube using maximum effort after maximal inhalation. Anyone who has abnormal spirometry measurements will be notified of this information and advised to consult a healthcare provider and disqualified for the remainder of the study.

Adverse effects are extremely uncommon during this phase of the study because subjects are young/healthy individuals, the room is temperature controlled, you will be seated
during the spirometry, and this phase of the study is brief in duration. Nonetheless, there is the remote possibility of unusual symptoms such as lightheadedness, dizziness, chest pains, palpitations, or shortness of breath during the spirometry from overexertion in breathing. As a precaution, you will be instructed to notify the physician examiner immediately if you experience any symptoms at any time. Following this, auscultation will be performed again and you will be given another brief symptom questionnaire to make sure you have no adverse effects from spirometry.

On the second visit, which will occur at least 12 hours after the first visit, you will be asked if there have been any changes in your health status since the first visit. You will be given the same safety and autonomy instructions as during the spirometry phase of the study. After physical exam (primarily auscultation), you will be asked to rinse out your mouth with tap water to prevent contamination of the sample.

Next, the actual study exhaled breath testing, which is strictly part of the research, will be performed to measure the amount of nitric oxide in the breath. You will be asked to breathe out through a disposable plastic mouthpiece connected to a filter at a constant rate of flow after a maximal inspiration (which is where you take as deep a breath in as you can to fill your lungs with as much air as possible). Following this, the investigator will again perform a brief physical examination using a stethoscope to listen to your heart and lungs and you will be given another brief symptom questionnaire to make sure you have no adverse effects from the second visit.

Some study visits may be longer or shorter than one hour depending primarily on both the equipment and subject variability in breathing.

At your last visit, you will be asked to verify the absence of symptoms prior to leaving.
If you decide to participate in a single two-hour visit:

The same procedures described above for two one-hour visits will be performed. However, The study exhaled breath testing, which is strictly part of the research, will be completed prior to spirometry. Also, examination with a stethoscope will be performed at the beginning of the visit, after the study exhaled breath testing, and at the end of the visit.

We will study two groups of people:

People in one group will be Caucasian. People in the other group will be of African background.

Sometimes people of different ethnicities may exhale different levels of markers that we can measure in the breath. We want to examine whether levels of the marker nitric oxide are different between Caucasians and Africans at baseline.

Right now, we do not know for sure if the measurement of nitric oxide in your breath will accurately measure lung disease. We are doing this study to find out if the measurement of nitric oxide in your breath is different based on your ethnicity.

If the measurement is not accurate, you may still have airway or lung disease that is undetected by the study. You should consult your own physician regardless of the study results if you have any concerns or experience any symptoms at any time.

During this study, here is what you will need to do:

Subjects will be instructed to refrain from eating, drinking, or strenuous exercise for at least one hour prior to the visit.

Will you be paid for taking part in this study?
We will pay you for the time you volunteer while being in this study. You will receive a total of twenty dollars for completion of the study.

If you select to participate in two one-hour visits, you will receive five dollars for completing the first visit and fifteen dollars for completing the second visit.

If you select to participate in a single two-hour visit, you will receive twenty dollars if you complete the entire study.

What will it cost you to take part in this study?

The study will pay the costs of limited physical exam (primarily auscultation), limited spirometry, and the experimental nitric oxide measurement.

You will not have to pay the fees for tests in this study that are not a part of regular medical care for your disease/condition.

Those subjects that do not have a parking permit for on-campus parking will have to pay for any parking costs incurred as required from the University of South Florida by taking part in this study.

You will have to pay for your regular care or any other costs. Your insurance plan should cover your regular costs. Your insurance plan will not have to pay for study costs of limited physical exam, limited spirometry, or exhaled breath nitric oxide measurement.

What are the potential benefits if you take part in this study?

We don’t know if you will get any health benefits by taking part in this study. We do not know if nitric oxide measurement will help in diagnosing airway or lung diseases. That is why we are doing this study. This research study should help us learn whether nitric oxide measurement will help in diagnosing airway or lung diseases.
Since a medical questionnaire, limited physical exam, and limited spirometry will be performed, overt abnormalities detected by your examiner will be relayed to you. You will be instructed to consult your regular doctor regarding these findings.

No matter what, we will learn more about exhaled breath nitric oxide levels in Africans compared to Caucasians. We will learn more about whether differences do or do not exist. What we learn may help others who are actively studying nitric oxide levels in exhaled breath for potential use in clinical settings.

What are the risks if you take part in this study?

The treatment might not help.

Right now we do not know for sure if the measurements are consistent. Because of this, you will have to consult your regular physician if you are experiencing any shortness of breath, other symptoms, or have any concerns about disease status. If you do, your condition/disease may get worse.

There may be adverse effects.

You may have problems because of the procedure used in this study. These problems are called adverse effects. Some adverse effects are just a bother. Others could harm you. There may be some adverse effects that we don’t know about yet.

Here are the known adverse effects that could happen with this study:

This study poses minimal risk to you. Spirometry is a standard, routine physical test commonly performed in hospitals and some clinics. It is a noninvasive and relatively safe procedure and involves the requirement for maximal expiratory effort. Thus, there is a minimal risk that you may breathe too hard during the forced expiratory maneuvers. This could result in: [1]“hyperventilation”, where too much carbon dioxide is blown off
in a short period of time or [2] excessive physical exertion. Immediate effects you could experience from “hyperventilation” or excessive physical exertion may include lightheadedness, dizziness, chest pain or pressure, rapid heart beat, or shortness of breath. These usually resolve upon discontinuing the procedure. You are instructed to immediately notify the examiner if any symptoms result. A determination by the examiner of whether or not to continue with the study will be made on an individual basis. At any time, both you and the examiner have the right to stop the study for any reason. If you were to continue despite these symptoms, loss of consciousness or cardiopulmonary arrest could result in rare cases, requiring emergency treatment and/or hospitalization. In such rare cases, permanent effects could result.

Patients with history of any type of hernia are excluded from the study since they could potentially aggravate the hernia using maximal expiratory effort.

The procedure for collecting exhaled breath nitric oxide is very similar to the procedure for spirometry, except that the subject is asked to breathe out at a slow regular rate instead of the forced maximal exhalation required in spirometry. Thus, it poses even less risk and discomfort to the subject than spirometry.

Private medical information will be collected during the study to find out your health status. The health information is being collected only as a guide for participation in the study. The questionnaires used in the study must remain confidential. To maintain your confidentiality, the questionnaires and recorded data from the study will be kept in a locked file cabinet. Access to the material will only be made to the investigators of the study. Also, as an additional measure to ensure confidentiality, each person will be given a subject number. This will be the only identifier listed on the questionnaires to link the
confidential information to the subject. The master list linking subjects to their subject numbers will also be kept in the locked cabinet, but separately.

During the study, the safety of the normal subjects will be maintained by: [1] the presence of a physician at all times of testing; [2] careful direct physician observation and monitoring of subjects during testing, and [3] instruction of subjects to immediately discontinue the exercise and notify the physician should they experience any shortness of breath, chest pain or pressure, dizziness, lightheadedness, or other unusual symptoms.

No medications will be given during the study, but emergency equipment will be present, and a physician will be in attendance for every study patient.

If you have any of these or any other problems, notify your study doctor immediately. If these side effects bother or worry you, or if you have other problems, call your study doctor at (813) 974-7545. If you have an emergency, go to the closest emergency room or clinic for treatment.

You may also have problems from the medical treatment you would usually get.

We may need to stop your treatment. If we find that the breathing tests are causing adverse events, we will stop the procedure. Early stopping criteria include:

1. For individual subjects:

The following criteria will be used to discontinue participation in the study:

[1] You wish to withdraw from the study.

[2] You do not meet the criteria for inclusion into the study.

[3] You demonstrate abnormalities on spirometry, physical examination, or monitoring through the study.
[4] You complain of chest pain or pressure, palpitations, or tachypnea.

[5] If you experience other symptoms, they will be assessed by a doctor and assisted in any way needed. A decision will be made whether you may continue to participate in the study provided that your safety has not been compromised. In this case, the decision to continue would require mutual willingness on your part to continue the study.

[6] The safety of the researcher is compromised (for example, a hostile subject).

2. For the study:

The study will be terminated if the health or safety of the volunteers or examiners becomes jeopardized by an unforeseen event.

Is there any risk to your unborn children if you take part in this study?

Pregnant women will not be allowed to participate in the study. There are no adverse effects for men with partners of childbearing age.

If you are a woman

You are excluded from the study if you are or may be pregnant or breastfeeding due to the study protocol, since the study results may be affected. If you are pregnant and you inadvertently take part in this study, the risk of adverse effects is still minimal, especially if you are in the first trimester of pregnancy. Nonetheless, it could be that your unborn children may have problems now or in the future.

Tell one of the study doctors right away if:

You are pregnant.

You get pregnant.

You are breastfeeding.
If you are a woman

If you take part in this study, you must use a good birth control method, like oral contraceptives or condoms.

What if you get sick or hurt while you are in the study?

If you need emergency care:

Go to your nearest hospital or emergency room right away. Call 911 for help. It is important that you tell the doctors at the hospital or emergency room that you are participating in a research study. If possible, take a copy of this consent form with you when you go. You should know that the USF does not provide emergency care.

Call the study doctors as soon as you can. They will need to know that you are hurt or ill.

Call Dr. Sunita Patel, MD or Dr. Stuart Brooks, MD at (813) 974-7545.

If it is NOT an emergency, and you get hurt or sick while you are taking part in this study:

Go to your regular doctor. It is important that you tell your regular doctor that you are participating in a research study. If possible, take a copy of this consent form with you when you go.

The USF Medical Clinics may not be able to give the kind of help you need. You may need to get help somewhere else.

If you are harmed while taking part in the study:

The state of Florida enjoys what is called "sovereign immunity." This means that you usually cannot sue the state of Florida. However, the state has waived sovereign immunity (agreed to be sued) in certain situations. One of those situations is if a state employee, such as your study doctor or other USF employee, is negligent in doing his or
her job in a way that harms you during the study. The money that you might recover from the state of Florida is limited in amount.

You can also call the USF Self Insurance Programs (SIP) at 1-813-974-8008 if you think:

You were harmed because you took part in this study.

Someone from the study did something wrong that caused you harm, or didn’t do something they should have done.

Ask the SIP to look into what happened.

What will we do to keep your study records private?

Federal law says we must keep your study records private. Private medical information will be collected during the study to ascertain the health status of the subjects. The health information is being collected only as a parameter for participation in the study. It is obtained after discussion with the subjects that the study is only open to healthy subjects. Thus, the questionnaires in the study must remain confidential. We will keep the records of this study private by keeping them in a locked file cabinet. Access to the material will only be made to the investigators of the study. Also, as an additional measure to ensure confidentiality, each subject will be given a subject number. This will be the only identifier listed on the questionnaires to link the confidential information to the subject. The master list linking subjects to their subject numbers will also be kept in the locked cabinet, but separately.

However, certain people may need to see your study records. By law, anyone who looks at your records must keep them completely confidential. The only people who will be allowed to see these records are:
The medical staff who are taking care of you.

Certain government and university people who need to know more about the study. For example, individuals who provide oversight on this study may need to look at your records. These include the University of South Florida Institutional Review Board (IRB) and the staff that work for the IRB. Other individuals who work for USF that provide other kinds of oversight may also need to look at your records. Other individuals who may look at your records include: the Florida Department of Health, people from the Food and Drug Administration (FDA), the United States Department of Health and Human Services, and the United States Department of Labor. This is done to make sure that we are doing the study in the right way. They also need to make sure that we are protecting your rights and your safety.

People at the company who paid for this study at the Sunshine Education and Research Center (funded by the National Institute of Occupational Safety and Health, a governmental department under the United States Department of Labor may look at the study records and pertinent portions of your medical records to make sure the study is done in the right way.

We may publish what we find out from this study. If we do, we will not let anyone know your name. We will not publish anything else that would let people know who you are.

What happens if you decide not to take part in this study?

You should only take part in this study if you want to take part.

If you decide not to take part:

You will not be in trouble or lose any rights you normally have.

You will still have the same health care benefits.
You can still get your regular treatments from your regular doctor.

What if you join the study and decide you want to stop later on?

You can decide after signing this informed consent document that you no longer want to take part in this study. If you decide you want to stop taking part in the study, tell the study staff as soon as you can. If you decide to stop, you can continue getting care from your regular doctor.

Are there reasons we might take you out of the study later on?

Even if you want to stay in the study, there may be reasons we will need to take you out of it. You may be taken out of this study if:

We find out it is not safe for you to stay in the study. For example, your health may worsen. Or we may find out that the device used to measure spirometry or exhaled breath nitric oxide might harm you. Then you may be taken out of the study.

You are not coming for your study visits when scheduled.

You can get the answers to your questions.

If you have any questions about this study, call Dr. Sunita Patel at (813) 974-7545.

If you have questions about your rights as a person who is taking part in a study, call the Division of Research Compliance of the University of South Florida at (813) 974-9343.

Signatures for Consent to Take Part in this Research Study

It is up to you to decide whether you want to take part in this study. If you want to take part, please read the statements below and sign the form if the statements are true.

I freely give my consent to take part in this study. I understand that this I am agreeing to take part in research. I have received a copy of this consent form to take with me.
I choose to participate in the study as: (Please select only one option)

○ Two visits which will last approximately one hour per visit.

○ A single visit which will last approximately two hours.

____________________________________________     ___________
Signature of Person Taking Part in Study   Date

____________________________________________
Printed Name of Person Taking Part in Study

____________________________________________     ___________
Signature of Witness   Date

____________________________________________
Printed Name of Witness

Statement of Person Obtaining Informed Consent

I have carefully explained to the person taking part in the study what he or she can expect.

I hereby certify that when this person signs this form, to the best of my knowledge, he or she understands:

What the study is about.

What needs to be done.

What the potential benefits might be.
What the known risks might be.

I also certify that he or she does not have any problems that could make it hard to understand what it means to take part in this study. This person speaks the language that was used to explain this study.

This person reads well enough to understand this form or, if not, this person is able to hear and understand when the form is read to him or her.

This person does not have a medical problem that makes it hard to understand what is being explained and can, therefore, give informed consent.

This person is not taking drugs that make it hard to understand what is being explained and can, therefore, give informed consent.

____________________________________________     ___________
Signature of Person Obtaining Informed Consent  Date

__________________________________________________
Printed Name of Person Obtaining Informed Consent

____________________________________________     ___________
Signature of Witness  Date

__________________________________________________
Printed Name of Witness]
Appendix E: Power Analysis

Power Calculation: Using power = 80% and $\alpha = 0.05$, the following formula was derived:

$$a = 2.8 \sqrt{2\sigma^2/n}$$

where

$a =$ level of difference required for significance,

$\sigma =$ standard deviation of the population,

$n =$ sample size.

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Appendix F: SAS Program Used

*Read an Excel spreadsheet and import it into SAS as filename=hospital when correcting for the confounders;
PROC IMPORT DATAFILE = 'F:\thesis_excel_data_file.xls' OUT = hospital;
PROC PRINT DATA = hospital;
TITLE 'RawData';
RUN;

*Create a new SAS file=sorted that sorts the data by Ethnicity;
PROC SORT DATA = hospital OUT = sorted;
   BY Ethnicity Average_NO;
PROC PRINT DATA = sorted;
RUN;

*Create a new SAS file=ranges that sorts the data by Average_NO;
PROC SORT DATA = hospital OUT = ranges;
   BY Average_NO;
PROC PRINT DATA = ranges;
RUN;

*Initiate export of the data to HTML file;
ODS HTML BODY = "F:\sorted";
   CONTENTS = "F:\sorted";
   PAGE = "F:\sorted";
FRAME = "F:\sorted";

*Creates a report of total number of subjects in Each Ethnic Group;
PROC FREQ DATA = sorted;
    TABLES Ethnicity;
    TITLE 'Number of Subjects in Each Ethnic Group';
RUN;

*Creates a report of Age by ethnicity;
PROC TABULATE DATA = sorted;
    CLASS Age Ethnicity;
    TABLE Age, Ethnicity;
    TITLE 'Age By Ethnicity';
RUN;

*Creates a report of birthplace by ethnicity;
PROC TABULATE DATA = sorted;
    CLASS Birthplace Ethnicity;
    TABLE Birthplace, Ethnicity;
    TITLE 'Birthplace By Ethnicity';
RUN;

*Creates a report of AncestralDifference by ethnicity;
PROC TABULATE DATA = sorted;
    CLASS AncestralDifference Ethnicity;
    TABLE AncestralDifference, Ethnicity;
    TITLE 'Different Ancestry By Ethnicity';
RUN;

*Creates a report of CurrentOccupation by ethnicity;
PROC TABULATE DATA = sorted;
    CLASS CurrentOccupation Ethnicity;
    TABLE CurrentOccupation, Ethnicity;
    TITLE 'Current Occupation By Ethnicity';
RUN;

*Creates a report of AnnualSalary by ethnicity;
PROC TABULATE DATA = sorted;
    CLASS AnnualSalary Ethnicity;
    TABLE AnnualSalary, Ethnicity;
    TITLE 'Annual Salary By Ethnicity';
RUN;

*Creates a report of RespiratoryTractConditions by ethnicity;
PROC TABULATE DATA = sorted;
    CLASS RespiratoryTractConditions Ethnicity;
    TABLE RespiratoryTractConditions, Ethnicity;
    TITLE 'Respiratory Tract Conditions By Ethnicity';
RUN;

*Creates a report of Inhalation Exposures by ethnicity;
PROC TABULATE DATA = sorted;
    CLASS InhalationExposures Ethnicity;
    TABLE InhalationExposures, Ethnicity;
TITLE 'Inhalation Exposures By Ethnicity';

RUN;

*Creates a report of OtherMedicalConditions by ethnicity;

PROC TABULATE DATA = sorted;
   CLASS OtherMedicalConditions Ethnicity;
   TABLE OtherMedicalConditions, Ethnicity;
   TITLE 'OtherMedicalConditions By Ethnicity';

RUN;

*Creates a report of CurrentMedications by ethnicity;

PROC TABULATE DATA = sorted;
   CLASS CurrentMedications Ethnicity;
   TABLE CurrentMedications, Ethnicity;
   TITLE 'CurrentMedications By Ethnicity';

RUN;

*Creates a report of PhysicalExam by ethnicity;

PROC TABULATE DATA = sorted;
   CLASS PhysicalExam Ethnicity;
   TABLE PhysicalExam, Ethnicity;
   TITLE 'Physical Exam By Ethnicity';

RUN;

*Creates a report of AdverseEvents by ethnicity;

PROC TABULATE DATA = sorted;
   CLASS AdverseEvents Ethnicity;
PROC UNIVARIATE DATA = sorted mu0=33.5 ALPHA= .05;
   VAR Average_NO;
   TITLE 'Statistical Summary Overall';
RUN;

PROC MEANS DATA = sorted MIN MAX ALPHA=.05 CLM;
   VAR Average_NO;
   TITLE 'Means*Summary Overall';
RUN;

PROC UNIVARIATE DATA = sorted mu0=33.5 ALPHA .05;
   VAR Average_NO;
   BY Ethnicity;
   TITLE 'Statistical Summary By Ethnicity';
RUN;

PROC MEANS DATA = sorted MIN MAX ALPHA=.05 CLM;
VAR Average_NO;

BY Ethnicity;

TITLE 'Means*Summary By Ethnicity';

RUN;

*Create a GRAPH of Average_NO versus ethnicity ADDED ALPHA;

PROC PLOT DATA = sorted;

    PLOT Average_NO * Ethnicity;

    TITLE 'Statistical Summary';

RUN;

*Correlate SES data to NO levels;

PROC CORR DATA = sorted;

    VAR SalaryCode OccupationCode SESCodeProduct;

    WITH Average_NO;

    TITLE 'Correlate SES Data to NO Levels';

RUN;

*Perform a student’s T-Test on the normally distributed values under 30;

proc ttest data=sorted;

    var average_no;

    class ethnicity;

RUN;

*Calculate Wilcoxon Ranked-Sum;

proc npar1way data = sorted wilcoxon;

    class ethnicity;
Var Average_NO;
run;

data twosample;
Mu1=17.0333333; Mu2=24.6428571; StDev=7.73858133;
N1=6; N2=7; Alpha=0.05;
NCP = (Mu2-Mu1)**2/((StDev**2)*
(1/N1 + 1/N2));
CriticalValue = FINV(1-Alpha, 1,
N1+N2-2, 0);
Power = SDF('F', CriticalValue, 1, N1+N2-2, NCP);
proc print data=twosample;
run;
*Closes the HTML function;
ODS HTML CLOSE;