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# Prediction Equations for Pulmonary Diffusing Capacity for Nitric Oxide in Healthy African-American Adults

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## ACCEPTANCE

This thesis, A PREDICTION EQUATION FOR PULMONARY DIFFUSING CAPACITY FOR NITRIC OXIDE IN HEALTHY AFRICAN-AMERICAN ADULTS, by Ahmad Saleh Almamary was prepared under the direction of the Master's Thesis Advisory Committee in the Department of Respiratory Therapy at Georgia State University. It is accepted by the committee in partial fulfillment of requirements for the Masters of Science degree in Respiratory Therapy at Byrdine F. Lewis School of Nursing and Health Professions at Georgia State University. The Master's Thesis Advisory Committee, as representatives of the faculty, certifies that this thesis has met all standards of excellence and scholarship as determined by the faculty.

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## DEDICATION

In the beginning, I thank God for all the blessing during the completion of this thesis and throughout my life. I dedicate this thesis to the two people who always motivated me and guided me through my whole life who made me be the man who I am today. My father is the first man that I always go to whenever I want any advice, motivation, and guidance. And my lovely mother, mom, you are the only person who I go to whenever I feel down, and when I talk to you, you gave me the strength and motivation to overcome difficulties that I face in my life. Father and mother, there is no words that will express my gratitude, and I wish you health and happiness forever. Thank you very much, my father and mother.

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Ahmad Saleh Almamary

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# Prediction equations for pulmonary diffusing capacity for nitric oxide in healthy African-American Adults

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## ABSTRACT

Pulmonary diffusing capacity for nitric oxide (DLNO) is a relatively new pulmonary function test to assess gas transfer in the lung. To date, there are no prediction equations made for healthy adult African-American (black) subjects. Thus, the purpose of this study was to create prediction equations for DLNO in this ethnic/racial group. A total of 59 healthy subjects (27 males and 32 females) were recruited to perform pulmonary function testing at Georgia State University. They were diverse in age (18-67 yr), height (140-189 cm), and body mass index (17.2-32.3 $kg/m^2$). All subjects completed single-breath maneuvers at rest inhaling 43 ± 4 ppm NO with a standard diffusion mixture. The breath-hold duration was 5.6 ± 0.6 s. Multiple linear regression predicted DLNO based on the subject's age, height, and sex. The prediction equation for DLNO (mL/min/mmHg) = $0.92 \cdot$(height in cm) $+38.8 \cdot$(sex) $- 0.012 \cdot (age^2) - 25$, where 1 = male, 0 = female for sex. About 77% of the variance in DLNO was accounted for by sex (67%), $age^2$ (7%), and height (4%). The standard error of the estimate in predicting DLNO was 16.3 mL/min/mmHg. Those with higher resting heart rates had a lower DLNO ($r = -0.28$, $p = 0.03$) but it was not included in the regression model as it did not enhance the fit. Black males had a 7-10% lower DLNO and black females had a 12-15% lower DLNO compared to matched white subjects. Black males of the same age and height had a 10% smaller alveolar volume, while black females had a 15% lower alveolar volume compared to matched white subjects. In conclusion, DLNO values and alveolar volumes are reduced in blacks compared to matched whites. The regression model presented best predicts DLNO in African-Americans below 40 years of age.

# Table of Contents

# CHAPTER I
# INTRODUCTION

Pulmonary function tests (PFTs) are essential tools in today's routine clinical assessment of respiratory function. They are used in the evaluation of patients with respiratory symptoms and for guiding the management of diagnosed lung disease. Without the guidance of PFTs, large number of patients get misdiagnosed and improperly treated. One such pulmonary function test, otherwise known as pulmonary diffusing capacity for carbon monoxide (DLCO), was established in 1957 [1]. Since 1957, the single-breath DLCO test has become the clinical standard to assess gas transfer through the alveolar-capillary membrane. Historically, prediction equations have been made for DLCO, but not DLNO. Prediction equations are necessary for deciphering between normal and abnormal gas transfer through the lung and is usually based on the sex, height, and age, and sometimes the racial backgound of the subject. These equations have been created for adults [2-7] and children [8, 9], with a wide range of racial backgrounds [10-17].

Two abstracts were published in the early 1980's examining the use of nitric oxide as a transfer gas through the alveolar-capillary membrane [18, 19]. These abstracts resulted in the first publications of DLNO in the late 1980s [20, 21]. Since, then several studies have been published demonstrating its superiority compared to the DLCO test. The chief barrier to CO uptake (~70-80%) resides within the red cell while ~25% is located in the alveolar membrane (See Figure 1 elsewhere [22]). The main advantage of using NO as a transfer gas is that the main barrier for NO uptake resides between the alveolar and red blood cell membranes (~60%) [23]. This advantage gives DLNO a better representation of gas transfer through the alveolar-capillary membrane compared to DLCO. Unlike DLCO, the DLNO is unaffected by changes in hemoglobin concentration [24], carboxyhemoglobin concentration [25], alveolar oxygen pressure

($P_AO_2$) or inspired oxugen concentration [26, 27]. DLNO is more affected by lung volume compared to the DLCO which makes the KNO (mathematically, DLNO divided by alveolar volume) a better measure than the KCO (mathematically, DLCO divided by alveolar volume) in those with restrictive lung disease [22]. Finally, the sensitivity in detecting cardipulmonary disease is better using DLNO compared to DLCO [28]. These examples demonstarate there is evidence that the DLNO test is techinically and physiologically superior than the DLCO test [28].

To date there has been few prediction equations developed for single-breath DLNO in adults [29-32] and children [33, 34]. One study has combined data from three studies to create a more accruate prediction model on nearly 500 white subjects [22]. However, no prediction equations for DLNO have been developed in the African-American population (herein known as the black population).

There are ethnic/racial differences in certain aspects of lung function. For example, 15% of the variability in vital capacity is accounted for by race/ethnicity [35]. In a black population, vital capacity (i.e. lung volume) is about 15% lower in blacks compared to age, height, and sex matched whites [11, 36], and the DLCO is also lower in blacks compared to matched whites [11, 17]. Specifically, DLCO is lower by ~2 (~6%) and ~5 (~15%) mL/min/mmHg in black, age and height matched males compared to matched white females, respectively [17]. Thus the difference in DLCO between blacks and whites stem from differences in alveolar volume and hemoglobin concentration as there is a ~6% larger hemoglobin concentration in whites compared to blacks [17]. Even though DLNO is minimally affected by hemoglobin concentration [24], the development of DLNO prediction equations for blacks do seem warrented since there are large

differences in lung volumes between the two difference ethnic/racial groups. This thesis examines DLNO in the younger, adult black population of Atlanta, GA.

*Statement of the Problem*

Age, sex, height, and ethnicity/race predict several lung function variables. Height is the greatest predictor of lung volume since about 60% of the variance in vital capacity is shared by height [29]. Sex also contributes to a difference in lung volumes as women have about 600 mL lower alveolar volume (VA) compared to height and age matched males [29]. Moreover, as mentioned previously, there are also ethnic/racial differences in lung volumes [36] and DLCO [11, 17]. The reasons for differences in lung volumes between white and blacks are complex, because race is a social as well as a biological construct [37]. Even after adjusting for anthropometric, socioeconomic, and nutritional variables, there remains a large unexplained portion of the differences in vital capacity between blacks and whites [37].

As there are no prediction equations developed for DLNO in blacks, the development of these prediction equations is warranted given the ethnic/racial differences in DLCO. Imagine if a prediction equation for diffusing capacity for whites was used in a black population. There could be a misdiagnosis of a pathophysiology in the black population since more blacks would be below the lower limit of normal for diffusing capacity due to their lower alveolar volumes compared to the white population. Thus, a black individual with normal diffusing capacity would be misdiagnosed due to the use of an incorrect prediction equation meant for whites. That's why ethnic/racial specific equations should be made for DLNO. Furthermore, a 2017 European Respiratory Society (ERS) Task Force document suggests that reference values are

lacking in non-white populations and should be pursued [22]. Thus, the major goal of this study was to develop prediction equations for DLNO in a black population.

### *Purpose of the study*

The purpose of this study is to develop prediction equations for DLNO and KNO in the black population. Prediction equations for DLNO in the black population are essential so that there is a reduced chance for misdiagnosis of lung disease.

### *Research questions*

1. What is the prediction equation for both DLNO and KNO in a relatively healthy black population living in Atlanta, GA?
2. What is the percent difference in DLNO and VA between blacks and whites matched for age, height, and sex?

### *Significance of the Study*

Prediction equations have been developed for all sorts of lung function tests. These equations are necessary to guide pulmonologists in the diagnosis and management of lung disease, so that their interpretation of a patient's lung function can be made more accurately. The "predicted" value of a certain lung function test for a patient's age, height, sex, and race are compared against the patient's measured value. Creation of such race specific equations will allow for better accuracy in determining lung disease, and these values will assist the pulmonologist to formulate the proper care plan. By understanding what the lower limit of normal (LLN) is for DLNO for a black person of given height, age, and sex, we can understand what are the values below which a pathophysiology exists in the group so as to promote better lung health in this population. If abnormal, then appropriate treatment can be determined. Thus, the development of prediction

equations for DLNO and KCO in African-Americans are essential to provide accurate predicted values.

*Definitions of words or terms*

ATS – American Thoracic Society

CO – Carbon Monoxide

ΘCO – specific conductance in the blood for CO [mL CO · (mL blood/min/mmHg)]

COHb – carboxyhemoglobin

COPD - Chronic Obstructive Pulmonary Disease

DL – Diffusing Capacity of the Lung

DLCO – Diffusing Capacity of the lung for carbon monoxide (mL/min/mmHg)

DLNO – Diffusing Capacity of the lung for nitric oxide (mL/min/mmHg)

DMCO – Alveolar-capillary membrane diffusing capacity for CO

ERS – European Respiratory Society

$FEV_1$ – Forced expiratory volume in 1 second

FVC – Forced vital capacity

FRC – Functional residual capacity

GSU – Georgia State University

Hb – Hemoglobin

KCO – Logarithmic change in CO concentration per unit time and unit pressure (mL STPD/min/mmHg/L) and mathematically equivalent to DLCO divided by alveolar volume

NO – Nitric Oxide

ΘNO – Specific conductance in the blood for NO [mL NO · (mL blood/min/mmHg)]

KNO – Logarithmic change in NO concentration per unit time and unit pressure mL STPD/min/mmHg/L and mathematically equivalent to DLNO divided by alveolar volume

LLN – Lower limit of normal, taken as the $2.5^{th}$ percentile

PFTs – Pulmonary Function Tests

VA – Alveolar volume (L)

Vc – Pulmonary capillary blood volume (mL)

TLC – Total lung capacity (L)

ULN – Upper limit of normal, taken as the $97.5^{th}$ percentile

### *Assumptions*

It was assumed that at least 100 healthy black male and female subjects from the Atlanta, GA area can be recruited. The subjects will vary age, sex and height. It is also assumed that each subject will give their best effort during the tests. Furthermore, it was assumed that each subject was truthful in reporting that they were healthy non-smokers with no symptoms of cardiopulmonary disease. Finally, it was assumed that the equipment used to measure DLNO is functioning adequately.

### *Limitations*

The study findings might be affected due to the geographical location of the data collection. The data was collected at one location, Georgia State University (GSU), in Atlanta, GA, and that could be a limitation to the study as the results may not be representative of the entire population of African-Americans. It was also difficult to recruit older subjects (>50 years old) as most of the subjects that attend GSU are younger (< 29 years old).

# CHAPTER II
# REVIEW OF THE LITERATURE

The diffusing capacity of the lung (DL) is the measure of the flow of inhaled gas from the alveoli to the blood per unit difference in pressure. The total DL is made up of two conductances: the diffusing capacity of the alveolar-capillary membrane (DM) and the pulmonary capillary volume (Vc) multiplied the specific conductance in the blood for CO (ΘCO). Roughton and Forster (1957) demonstrated that the resistance to total oxygen diffusion derived from two resistances in series: membrane resistance and red cell resistance such that $1/DLCO = 1/DMCO + 1/(\Theta CO \cdot Vc)$ where DLCO is the pulmonary diffusing capacity for carbon monoxide in mL/min/mmHg, DMCO is the alveolar-capillary membrane diffusing capacity for CO in mL/min/mmHg, ΘCO is specific conductance in the blood for CO in mL CO·(mL blood/min/mmHg), and Vc is the pulmonary capillary blood volume in mL [38].

In order to obtain DMCO and Vc, a subject inspires a diffusion mixture at two different oxygen concentrations and then plot the 1/DLCO results on a graph. For example, the first mixture would be 0.3% CO, 21% $O_2$, 10% He, balance $N_2$, and the subject would calculate DLCO from that inspired mixture. Then the subject would perform another test where he/she would inspire a second mixture containing 0.3% CO, 60% $O_2$, 10% He, Balance $N_2$ and the subject would calculate DLCO from that mixture. Then the results are plotted: The y-axis would be the inverse of DLCO (1/DLCO), and the x-axis would be 1/ΘCO for each mixture. The slope of the two points would be 1/Vc and the y-intercept would be 1/DMCO. Then the inverse of 1/DMCO and 1/Vc would be the DMCO and Vc, respectively.

As one can see, calculating DMCO and Vc takes time. One would need at least two tests at the 21% $O_2$ concentration to obtain an average DLCO at that concentration, then another two

tests at the 60% $O_2$ concentration to obtain an average at that concentration. Thus, these 4 tests would take about 16 minutes of time and there would be a small build-up of CO in the blood.

However, in the 1980's, researchers determined that by inhaling a small amount of NO together with CO in a single-breath maneuver, one could obtain DLCO and pulmonary diffusing capacity for nitric oxide (DLNO) in a single-maneuver, and then interpolate DMCO and Vc from that one maneuver [20]. Since that discovery in the 1980's, there has been much debate on whether DMCO and Vc could be determined from this modified Roughton-Forster technique as there are a lot of considerations that need addressing [39, 40]. Nevertheless, the latest DLNO standardization document published in 2017 has said that it is possible to obtain DMCO and Vc using modified Roughton-Forster technique [22].

One important fact about DLNO is that unlike the DLCO, the main barrier for NO resides between the alveolar and red blood cell membranes [23]. Thus, DLNO better represents gas transfer through the alveolar-capillary membrane compared to DLCO. This is evident in Figure 1 of a recent ERS Task Force document on DLNO that demonstrates that only ~25% of the resistance to CO diffusion is located in the alveolar-capillary membrane, while ~60% of the resistance NO diffusion is located in the alveolar-capillary membrane [22]. Since DLNO is a better index of gas transfer though the alveolar-capillary membrane compared to DLCO, a measurement of DLNO should be technically superior compared to DLCO in evaluating pulmonary disease.

DLNO is considered to be a better test compared to the DLCO for several reasons. The first reason is DLNO is unlike the DLCO, it is a better represents gas transfer through the alveolar capillary membrane[22]. Also, unlike the DLCO, the DLNO is unaffected by the subject's hemoglobin concentration [24], carboxyhemoglobin concentration [25] and partial

pressure of inspired oxygen ($PO_2$) [26, 41]. However, DLNO is more affected by lung volumes changes compared to DLCO [22], thus the DLNO test would be better to examine restrictive lung disease that the DLCO test. There is also evidence to support that DLNO has better sensitivity to detect cardiopulmonary disease compared to DLCO [28], and as such, it is about time that clinicians use this test routinely [42, 43].

Nevertheless, in order for DLNO to gain use worldwide, prediction equations need to be created. With the DLCO, predictions have been created been created for adults [2-7] and children [8, 9], and over a wide range of racial backgrounds [10-17], no such racially-adjusted equation has been developed for DLNO. To date there has been few prediction equations developed for single-breath DLNO in adults [29-32] and children [33, 34]. A recent ERS Task Force combined data from three studies to create a more accruate model on nearly 500 white subjects rangfing from 18 to 93 years old [22]. However, there are no studies to date that has created prediction equations for DLNO in the black population. The main goal of this study is to develop prediction equations for DLNO in the black population.

Table 1 shows the few studies on prediction equations for DLNO in adults and children. Nearly all subjects in these studies were performed on the white population from Europe or in Canada. All studies in Table 1 use a combination of age, height and sex in their models to predict DLNO. As Table 1 shows, the models have a relatively good fit in predicting DLNO from height, age and sex. That is ~70% of the variance in DLNO can be predicted by the models in Table 1.

Van der lee *et al.* (2007) carried out a study to develop reference DLNO [30]. The authors selected healthy volunteers from local hospital staff. The inclusion criteria included negative history of smoking, no complaints related to respiratory system and negative history

about use of any medication. Individuals with serious respiratory or chronic illnesses such as diabetes mellitus were excluded. The study enrolled 124 individuals (59 females and 65 males) who were undergone plethysmography to determine static and dynamic blood volumes. The authors concluded that DLNO is most closely related to membrane diffusion capacity thus indicating preferential use of DLNO over DLCO. Aguilaniu *et al.* (2008) calculated reference values for transfer of NO and CO in two European cities; Grenoble (300-m altitude) and Bordeaux (30 m altitude) [31]. In the present study 303 individuals were recruited in the range of 18 to 94 years who underwent single breath technique to measure true diffusion capacity, pulmonary capillary blood volume and diffusion capacity for CO and NO. The authors concluded that the diffusion capacity was affected by age, sex, height and where the test was carried out. Zavorsky *et al.* (2008) attempted to develop reference values for DLNO in adult white population. The study sample comprised of 130 (66 males and 64 females) individuals from young to old age who performed single breath holding maneuvers for a duration of 5 seconds [29]. Only 20 of these subjects (15%) were $\geq$ 60 years old. All subjects were subjected to spirometry before participation in the study and 96% of them did not have any signs of pulmonary disease a displayed by spirometry. They showed that DLNO was 5x larger than the DLCO, with height, sex, and age as significant predictors of DLNO (adjusted $R^2 = 0.70$). If alveolar volume was used instead of height, the model had a higher adjusted $R^2$.

The most comprehensive model for predicting DLNO was developed using nearly 500 white subjects ranging from 18 to 93 years old [22]. It was a model based on data from three previous studies [29-31]. Height explained 45% of the model, followed by age$^2$ (13%), followed by sex (11%). The full model accounted for 69% of the variance in DLNO. The LLN (2.5$^{th}$ percentile) and ULN (97.5$^{th}$ percentile) was developed and presented as an on-line supplement

[22]. The model demonstrates that the LLN and ULN for DLNO are ± 39.2 mL/min/mmHg below and above the predicted values, respectively [22]. For DLCO, the LLN and ULN for were ± 8.2 mL/min/mmHg below and above the predicted values, respectively [22].

While it is recommended that a 10 seconds breath-hold time for DLNO tests be used for better gas mixing [22], a 4-6 seconds breath-hold time can be acceptable if using a less sensitive NO electrochemical cell [22]. In the comprehensive prediction model for DLNO [22], the breath-hold time was ~6 seconds, and the inspired oxygen and NO concentration was 19.5% (SD 0.7%), and 35 (12) ppm, respectively [22].

In Table 2, the prediction equations for DLCO are presented for comparison to DLNO. Table 2 demonstrates that DLNO and DLCO follow each other closely. There is an approximate 90% shared variance between DLNO and DLCO [22], with the DLNO being 4.6 to 4.8x larger than the DLCO in healthy white subjects [22]. In subjects with various cardiopulmonary diseases, the percentage shared variance between DLNO and DLCO drops to 62% [28]. Furthermore, when there is a mean reduction in DLCO compared to healthy controls, DLNO is reduced similarly [28]. Finally, DLNO is more affected by lung volume changes than the DLCO. For a 50% reduction is alveolar volume, DLCO is only reduced by ~25%, while, DLNO is reduced by ~40% [22]. These results suggest, DLNO is a better indicator of gas transfer in those with restrictive lung disease.

Vital capacity (i.e. lung volume) is about 15% lower in blacks compared to age, height, and sex matched whites [11, 36]. The DLCO is also lower in matched whites [11, 17]. These predictors demonstate the need for racial specific equations for DLNO. Studies demonstrate that DLCO is lower by ~2 (~6%) and ~5 (~15%) mL/min/mmHg in black, age and height matched males compared to matched white females, respectively [17]. Thus the difference between blacks

and whites is mostly from lung volume differences, but also from hemoglobin differences as there is a ~6% larger hemoglobin concentration in whites compared to blacks [17]. Previous work has shown that DLNO is minimally affected by hemoglobin concentration [24], so the hemoglobin differences between blacks and whites should be inconsequential in the development of DLNO prediction equations for blacks. However, DLNO prediction equations are warrented since lung volume differences must be accounted for between the two ethnic/racial groups.

# CHAPTER III
# METHODOLOGY

Healthy black male and female non-smoking adults from Georgia State University (GSU) were recruited by word of mouth and through the posting of flyers to participate in one testing session involving the measurement of various lung parameters including DLNO and DLCO at the Petit Science Center (room 457) at GSU. This study was performed in conjunction with another study examining the six-minute walk test in this same ethnic/racial group and was approved by the Georgia State University ethics board (IRB #H16120, Reference # 335588). The inclusion and exclusion criteria was as follows:

*Inclusion criteria:*

- Apparently healthy, black, non-smoking and non-pregnant individuals' ≥ 18 years of age, with a body mass index (BMI) ranging from 17.0 to 34.9 kg/m$^2$. Non-smoking will be defined as never smoked or quit smoking > 6 months previously.
- These subjects did not have cardiopulmonary disease or they will be absent of major signs/symptoms suggestive of cardiopulmonary disease [44].

*Exclusion criteria:*

- Individuals who were less than 18 years of age, who are not black, or have a BMI < 17.0 or ≥ 35.0 kg/m$^2$, or are currently smoking or ceased smoking within the previous six months, or who are pregnant.
- Have cardiopulmonary disease, or presence of major signs/symptoms suggestive of cardiopulmonary disease [44],
- Have chest or abdominal pain or any cause, oral or facial pain exacerbated by a mouthpiece, stress incontinence, dementia or in a state of confusion [45].

*Procedures*

The study was conducted at Room 457 of the Petit Science Center and the procedures lasted approximately 1.75 hours per subject. Subjects signed an informed consent form to participate in the study. Subjects filled out a questionnaire about their date of birth, sex, as well as physical activity readiness questionnaire (PAR-Q) and a health questionnaire. Then height, weight, waist and hip circumference was measured. After those parameters were obtained, heart rate was measured via a POLAR A300 heart rate monitor (Polar Electro Oy, Kempele, Finland) during the pulmonary function tests. The average heart rate recorded during these lung function tests (i.e. 20-30 minutes) was used for data analyses.

Several pulmonary function tests were performed: spirometry, slow vital capacity, total lung capacity, pulmonary diffusing capacity, maximal inspiratory (MIP) and expiratory pressures (MEP). The procedures, rules, and established safety for conducting these lung function test are described elsewhere [46-50]. The diffusing capacity test involved subjects inspiring about 4 to 6 liters of a standard diffusion gas mixture that is used for diagnostic purposes including a small amount of NO (i.e. 0.3% CO, 21% $O_2$, 10% He, 40-60 ppm NO, Balance $N_2$). This gas is inhaled once for 5 to 10 seconds at total lung capacity, and then exhaled.

The Hyp'Air discrete lung diffusion system (Medisoft Inc., Dinant, Belgium) was used for the assessment of DLNO and DLCO using the 5 second breath-hold maneuver. The Medisoft / Morgan Scientific Bodybox (Plethsymograph, model 5550) was used for the assessment of spirometry, TLC, and DLCO using the 10 s breath-hold maneuver. The best values for spirometry were reported [46], and the mean value for DLNO and DLCO was reported when the two highest DLCO values varied by not more than 3 mL/min/mmHg and the two highest DLNO

values did not vary by more than 17 mL/min/mmHg [22]. For the measurement of TLC from the body box, three values for FRC that agreed within 5% was obtained, and the mean reported [48].

All data were manually recorded on specially formatted collection sheets which was stored at a secure location in PSC 457. This lab was locked with access granted only to the faculty advisor and student researchers. Electronic backup of information was provided by the investigators on excel spreadsheets on password protected computers, locked inside PSC 457. Subjects were paid $30 for their participation. The funding for this study came from the Jerome M. Sullivan Research Fund from the American Respiratory Care Foundation.

### *Calculation of DLNO, DLCO, DMCO, and Vc*

The DLNO and DLCO was calculated using the recommended guidelines from the ATS and ERS [22, 51]. The DMCO and Vc were estimated by using the ΘCO formula from Guénard and colleagues [27], the ΘNO of 4.5 mL NO·(mL blood/min/mmHg) from Carlson and Comroe [52], the DMNO/DMCO ratio of 1.97, a standardized women's hemoglobin concentration of 13.4 g/dL, a standardized men's hemoglobin concentration of 14.6 g/dL, and a $P_AO_2$ of 100 mmHg, as recommended by the ERS Task Force [22].

### *Statistical analyses*

Spirometry values (FVC, $FEV_1$, $FEV_1$/FVC ratio) was compared to predicted values for the black population [36]. Total lung capacity was compared to the predicted values for a white population [3]. The mean predicted values were compared to the mean measured values via a paired *t*-test. In prediction studies, the number of subjects should be sufficiently large because the larger the sample, the more likely it will be to represent the population. It is known that there is a direct relationship between the correlation and the ratio of the number of variables in the model ($k$) to the number of participants in the model ($n$), such that $(k - 1) \div (n - 1)$ [53]. Thus, if

a study has 40 participants and 30 variables, the $R^2$ would be 0.74 based on chance alone and the results would be meaningless. Thus, it is recommended that there be at least a 10:1 participant to variable ratio to avoid this error [53]. Furthermore, another formula used: $n \geq 50 + 8 \cdot k$ also predicts the number of subjects needed to develop a reliable equation [54]. So in this case, where there are five potential predictors for DLNO (age, age$^2$, the interaction term age·age$^2$, sex, weight, height), at least 60 to 98 subjects would be needed.

A multiple linear regression using the stepwise procedure was conducted to determine which independent variable DLNO (age, age$^2$, the interaction term age·age$^2$, sex, weight, height) were predictors of DLNO (first dependent variable), KNO, (second dependent variable) and alveolar volume (third main dependent variable). Since regression is very sensitive to extreme cases, outliers were removed. Any data point that exceeded the chi-square criteria (standard deviation of the residuals > 3.0) was eliminated. Then, linearity was analyzed by creating a scatterplot matrix of the variables age, age$^2$, DLNO, KNO, weight, and height. Another plot was created between the standardized residuals (y-axis) and standardized predicts (x-axis) to see if the values will be consistently spread out, which would indicate normality and homoscedasticity. The lower limit of normal (LLN) will be calculated by multiplying the standard error of the estimate by 2.0 and then subtracting that number from the prediction[1]. This value would represent the 2.5$^{th}$ percentile according to *t*-tables. Any patient that has a value below LLN was considered a true pathophysiological abnormality in diffusing capacity. The upper limit of normal (ULN) was calculated by multiplying the standard error of the estimate by 2.0 and adding it to the prediction[1]. This value would represent the 97.5$^{th}$ percentile according to *t*-tables. Any patient that has a value above the ULN would indicate a clinically meaningful increase in

[1] When the Degrees of Freedom is 60, the z-score is not ± 1.96 for the 2.5$^{th}$ and 97.5$^{th}$ percentiles, it is ± 2.00.

diffusing capacity above predicted, which signifies genetically superior alveolar-capillary membrane diffusion capability. A Type I probability level of 0.05 will be used. Statistical software utilized for this project will be IBM SPSS Statistics Version 21.0, Chicago, IL.

# CHAPTER IV
# RESULTS

Fifty-nine adult black subjects were recruited from GSU over a period of six months in 2016 (Table 3). All the fifty-nine subjects (32 females, 27 males) completed all tests. The subjects ranged from 18 to 67 years of age and the mean resting heart rate was 80 (SD 10) beats/min. The breakdown of the age categories was as follows: 18 to 29 years old (17 males and 18 females), 30 to 39 years old (7 males and 4 females), 40 to 49 years old (2 males and 7 females), 50 to 59 years old (1 male and 1 female), 60-69 years old (0 male and 2 females) with a BMI ranging from 17 to 32 $kg/m^2$. Only 22% of the subjects were $\geq$ 40 years old. All the subjects claimed to be healthy with no previous cardiac or pulmonary issues. Most subjects had normal spirometric function for their race. However, five subjects (8%) of the subjects did have mild obstructive issues as determined by the $FEV_1/FVC$ being below the LLN (Table 4).

The DLNO using the five second breath-hold ($DLNO_{5s}$) ranged from 71 to 205 mL/min/mmHg (Table 5). The DLCO using the five second breath-hold ($DLCO_{5s}$) and 10 second breath-hold ($DLCO_{10s}$) were similar and ranged from about 16 to 45 mL/min/mmHg (Table 5). The inspired NO and oxygen concentration from the $DLNO_{5s}$ was 43 (4) ppm, and 19.7 (0.12) %, respectively (Table 6). The mean $DLCO_{5s}$ values were not statistically different compared to the mean $DLCO_{10s}$ values (Table 7); although VA was statistically larger, by 150 mL (3%) in the $DLCO_{5s}$ test (Table 7). The $DLNO_{5s}$ was about 5x larger than the $DLCO_{5s}$ (Table 2).

Prediction equations for DLNO and the components of DLCO are presented in Table 8. About 77% of the variance in DLNO was accounted for by sex (67%), $age^2$ (7%), and height

(4%). Interestingly, both DLNO and DLCO negatively correlated with resting heart rate (Figure 1), but the heart rate was not included in any of the models due to its poor predictive value.

As expected, the prediction equations in the black population (Table 8) yielded lower predicted DLNO, KNO and VA compared to the ERS prediction equations [22]. When compared to whites of the same age and height, black males had a 7-10% lower DLNO, and black females had a 12-15% lower DLNO (Table 9). Black males of the same age and height had a 10% smaller alveolar volume, while black females had a 15% lower alveolar volume compared to matched whites (Table 9).

# CHAPTER V
# DISCUSSION

The purpose of this study was to develop prediction equations for DLNO and KNO in healthy adult black subjects. The development of prediction equations in this ethnic/racial group was a response to a recent ERS Task Force document citing its need [22]. To date, there are no published papers on prediction equations for DLNO in this ethnic/racial group. These are the first predictions equations ever developed for the black population.

Overall, data collection was performed adequately. Mean inspired volumes were 94% of the FVC during the $DLCO_{10s}$ and 89% of the FVC for the $DLNO_{5s}$ test. The mean VA measured from $DLCO_{10s}$ and $DLCO_{5s}$ was 91% and 88% of the measured TLC from the Bodybox, respectively.

The data demonstrates that black males have a $DLNO_{5s}$ that is 34 mL/min/mmHg higher than black females. This is a similar difference between the sexes in white subjects [22]. For every one cm increase in height, $DLNO_{5s}$ increases similarly in the white (0.8 mL/min/mmHg per cm) [22] and black population (0.9 mL/min/mmHg per cm) (Table 8). However, only 59 subjects were studied, and most of the subjects were < 40 years of age, so the comparison of slopes between ethnic/racial groups are only accurate up to 40 years of age.

Studies demonstrate that $DLCO_{10s}$ is lower by ~2 (~6%) and ~5 (~15%) mL/min/mmHg in black, age and height matched males compared to matched white females, respectively [17], due to mostly, differences in lung volumes between the two ethnic/racial groups. We also found that the predicted DLNO in the male black population was 7% to 10% lower compared to the predicted value for the age and height matched white male population. In the female black population, DLNO was 12 to 15% compared to the predicted value for the age and height matched white female population. And these differences was largely due to a 10% (male) to 15%

(female) smaller predicted alveolar volume in blacks compared to whites. This shows that if a prediction equation for DLNO for whites was used in a black population, there would be a misdiagnosis of poor gas transfer in the lung in 12 to 15% of the black population, and that is because of the lower alveolar volumes in blacks compared to sex, age, and height matched whites. That's why ethnic/racial specific equations must be made for DLNO.

In the future, it will be interesting to see the comparisons of DLNO prediction equations with other racial/ethnic groups including mixed-race groups. As one can see, there there is a lot of work that still needs to be done.

*Limitations*

We were only able to obtain data on 59 subjects due to several factors. First, there was great difficulty in finding subjects ≥ 40 years of age. Second, the HYP'Air pulmonary function system needed repair and thus we were not able to collect data for two months. However, we are confident that the DLNO prediction equation works well in subjects < 40 years of age as ~80% of the subjects were below 40 years of age.

*Conclusion*

The development of racially-adjusted prediction equations for DLNO can help prevent misdiagnosis of lung disease. Racially-adjusted prediction equations for DLNO allows clinicians to make a more accurate interpretation of a patient's lung function which will lead to a better diagnosis and management of lung illnesses. In this study, about 77% of the variance in DLNO was accounted for by sex (67%), age$^2$ (7%), and height (4%). Also, the prediction equations in the black population yielded lower predicted DLNO, KNO, and VA compared to the prediction equations developed for white subjects [22]. Black males had a 7-10% lower DLNO, and black females had a 12-15% lower DLNO compared to matched white subjects. Black males of the

same age and height had a 10% smaller alveolar volume, while black females had a 15% lower alveolar volume compared to matched whites. Heart rate was negatively associated with DLNO and DLCO. Finally, the regression model presented predicted DLNO in black subjects, but only the model was only accurate in those below 40 years of age. The addition of older black subjects to this model in the future will help make this prediction equation more useful.

# LIST OF TABLES

Table 1. $DLNO_{5s}$ prediction equations for white subjects.

| Study | Prediction equation for $DLNO_{5s}$ | BHT | Number of subjects | $R^2$ | SEE |
|---|---|---|---|---|---|
| **Adults (White subjects)** | | | | | |
| van der Lee *et al.* (2007) (mmoL/min/kPa) Females | 0.5347· (height in cm) – 0.077·(age) – 48.28 | 10s | 59 F | -- | 5.22 |
| van der Lee *et al.* (2007) (mmoL/min/kPa) Males | 0.5984· (height in cm) – 0.25·(age) – 44.20 | 10s | 65 M | -- | 6.39 |
| Aguilaniu *et al.* (2008) (mL/min/mmHg) >59 years old | 1.365· (height in cm) – 0.90 · (age) + 27.35·(Sex) – 54.69 | 4 s | 59 F<br>45 M | 0.72 | -- |
| Aguilaniu *et al.* (2008) (mL/min/mmHg) ≤ 59 years old | 1.365· (height in cm) – 0.88 · (age) + 27.35·(Sex) – 54.69 | 4 s | 59 F<br>45 M | 0.72 | -- |
| Zavorsky *et al.* (2008) (mL/min/mmHg) | 1.17· (height in cm) – 1.21 · (age) + 31.81·(Sex) – 20.1 | 5s | 64 F<br>66 M | 0.70 | 20.2 |
| Zavorsky *et al.* (2017) (mL/min/mmHg) | 0.81· (height in cm) – 0.010·($age^2$) + 34.4 · (sex) + 9.7 | 6s | 242 F<br>248 M | 0.69 | 20.0 |
| **Children (white subjects)** | | | | | |
| Thomas *et al.* (2014) (mmoL/min/kPa) | Exp [1.3145 + 0.0214·(age) – 0.0058·(sex) + 0.0119· (height in cm) – $1.2893 \cdot 10^{-8}$ · (height in $cm)^3$ + $2.7070 \cdot 10^{-8}$·(Sex) · (height in $cm)^3$] | 5s | 312 | -- | -- |

BHT = breath-hold time (s); SEE = standard error of the estimate; Sex (1 = male, 0 = female).
Note: There were prediction equations developed by Rouatbi et al. (2010) for North-African adults where DmCO and Vc were estimated based on DLCO and DLNO [32]. However, no actual prediction equations for DLNO were found in that article. As such, Rouatbi's paper using for North-African adults is not included in this table.

Table 2. $DLCO_{5s}$ prediction equations for white subjects.

| Study | Prediction equation for $DLCO_{5s}$ | BHT | Number of subjects | $R^2$ | SEE |
|---|---|---|---|---|---|
| **Adults (White)** | | | | | |
| van der lee et al. (2007) (mmoL/min/kPa) Females | 10.51· (height in m) – 0.030·(age) – 7.43 | 10s | 59 F | -- | 1.37 |
| van der lee et al. (2007) (mmoL/min/kPa) Males | 12.02· (height in m) – 0.074·(age) – 6.88 | 10s | 65 M | -- | 1.74 |
| Aguilaniu et al. (2008) (mL/min/mmHg) >59 years old | 29.291· (height in m) – 0.262 · (age) + 5.044·(Sex) – 12.954 | 4 s | 59 F 45 M | 0.73 | -- |
| Aguilaniu et al. (2008) (mL/min/mmHg) ≤ 59 years old | 29.291· (height in m) – 0.161 · (age) + 5.044·(Sex) – 12.954 | 4 s | 59 F 45 M | 0.73 | -- |
| Zavorsky et al. (2008) (mL/min/mmHg) | 0.214· (height in cm) – 0.242 · (age) + 5.94·(Sex) – 1.3 | 5s | 64 F 66 M | 0.69 | 4.0 |
| Zavorsky et al. (2017) (mL/min/mmHg) | 0.23· (height in cm) – 0.002·($age^2$) + 6.0 · (sex) – 8.5 | 6s | 242 F 248 M | 0.68 | 4.2 |
| **Children (white)** | | | | | |
| Thomas et al. (2014) (mmoL/min/kPa) | Exp [0.9440 + 0.0205·(age) + 0.0908·(sex) + $1.6233 \cdot 10^{-7}$ · (height in cm)$^{-3}$] | 5s | 312 | -- | -- |

BHT – breath-hold time (s); Sex (1 = male, 0 = female). Note: There were prediction equations developed by Rouatbi et al. (2014) for North-African children where DmCO and Vc were estimated based on DLCO and DLNO [34]. However, no actual prediction equations for DLNO were found in that article. As such, Rouatbi's paper using North African children is not included in this table.

Table 3. Anthropometric characteristics of the subjects including age.

| | **Males (n = 27)** | **Females (n = 32)** | **Combined (n = 59)** |
|---|---|---|---|
| **Age (years)** | 28 (10) [18 to 55] | 32 (14) [20 to 67] | 30 (12) [18 to 67] |
| **Weight (kg)** | 78.6 (11.1) [61 to 102.4] | 68.3 (13.9) [47.8 to 95.7] | 73.0 (13.6) [47.8 to 102.4] |
| **Height (cm)** | 176.2 (6.7) [163.1 to 189.4] | 163.2 (6.7) [140.2 to 180.0] | 169 (10) [140 to 189] |
| **Body mass index (kg/m$^2$)** | 25.3 (2.9) [18.8 to 30.6] | 25.3 (4.4) [17.2 to 32.3] | 25.3 (3.8) [17.2 to 32.3] |
| **Waist Circumference (cm)** | 83.7 (8) [66 to 100] | 82.3 (11) [65 to105] | 82.9 (9.7) [65 to 105] |
| **Hip Circumference (cm)** | 101.8 (6.9) [90.5 to 119.0] | 102.5 (9.6) [87.6 to 119.0] | 102.2 (8.4) [87.6 to 119] |
| **WHR** | 0.82 (0.04) [0.72 to 0.90] | 0.80 (0.06) [0.69 to 0.96] | 0.81 (0.05) [0.69 to 0.96] |

Mean (SD). Brackets represent the range. Body mass index (BMI) is calculated weight (Kg) divided by height$^2$ (meters).

Table 4. Spirometric results of the subjects.

| | **Mean value** | **% predicted** | ***p*-value** |
|---|---|---|---|
| **SVC (L)** | 4.16 (0.94) | -- | -- |
| **FVC (L)** | 4.20 (0.91) | 111 (11) | 0.000 |
| **$FEV_1$ (L)** | 3.48 (0.72) | 109 (13) | 0.000 |
| **$FEV_1$/FVC** | 0.83 (0.07) | 98 (7) | 0.014 |
| **TLC (L)** | 5.82 (1.24) | 99 (11) | 0.529 |

5 of 59 subjects (~8%) demonstrated a mild obstructive pattern based on being below the LLN for $FEV_1$/FVC. The % predicted for FVC, $FEV_1$, and $FEV_1$/FVC was from The Global Lung Function Initiative prediction equations for blacks [36]. The % predicted TLC was from white prediction equations from Verbanck and colleagues [3].

Table 5. Pulmonary diffusing capacity results.

| | **Males (n = 27)** | **Females (n = 32)** | **Combined (n = 59)** |
|---|---|---|---|
| **Hyp'Air (5 s BHT)** | | | |
| **DLNO (mL/min/mmHg)** | 165 (23) [120 to 205] | 110 (162) [71 to 138] | 135 (34) [71 to 205] |
| **DLCO (mL/min/mmHg)** | 32.9 (5.0) [23.1 to 43.9] | 21.4 (2.6) [16.6 to 27.0] | 26.6 (7) [16.6 to 43.9] |
| **DMCO (mL/min/mmHg)** | 169 (29) [127 to 238] | 107 (26) [54 to 192] | 136 (42) [54 to 238] |
| **Vc (mL)** | 73 (13) [50 to 103] | 52 (7) [44 to 69] | 62 (14) [44 to 103] |
| **VA(L)** | 6.2 (0.9) [5.8 to 7.9] | 4.5 (0.7) [3.1 to 6.3] | 5.3 (1.2) [3.1 to 7.9] |
| **DmCO/VA ratio (mL/min/mmHg/L)** | 27.4 (4.5) [19.8 to 35.2] | 23.8 (5.1) [16.7 to 36.7] | 25.5 (5.1) [16.7 to 36.7] |
| **KCO(mL/min/mmHg/L)** | 5.3 (0.8) [3.5 to 6.7] | 4.8 (0.6) [3.6 to 6.0] | 5.0(0.8) [3.5 to 6.7] |
| **KNO(mL/min/mmHg/L)** | 26.7 (3.8) [18.6 to 32.9] | 24.6 (3.3) [18.3 to 29.6] | 25.5 (3.6) [18.3 to 32.9] |
| **DLNO/DLCO ratio** | 5.0 (0.3) [4.3 to 5.5] | 5.1 (0.4) [4.3 to 6.3] | 5.1 (0.4) [4.3 to 6.3] |
| **Bodybox (10 s BHT)** | | | |
| **DLCO (mL/min/mmHg)** | 32.6 (4.0) [26.6 to 44.6] | 21.5 (2.3) [16.4 to 26.8] | 26.6 (6.4) [16.4 to 44.6] |
| **VA (L)** | 6.0 (0.8) [4.5 to 8.4] | 4.4 (0.7) [3.1 to 5.9] | 5.1 (1.1) [3.1 to 8.4] |
| **KCO(mL/min/mmHg/L)** | 5.5 (0.8) [3.4 to 7.4] | 5.0 (0.8) [3.4 to 6.8] | 5.2 (0.8) [3.4 to 7.4] |
| **TLC (L)** | 6.8 (1.0) [5.0 to 9.3] | 5.0 (0.7) [4.0 to 7.0] | 5.8 (1.2) [4.0 to 9.3] |

Mean (SD). Brackets represent the range.

Table 6. Inhaled and exhaled gases including breath-hold time (BHT) for both PFT machines (Hyp'Air and BodyBox).

| | $O_2$ | | CO | | He | | NO | | BHT |
|---|---|---|---|---|---|---|---|---|---|
| | ***Inspire*** | ***Expire*** | ***Inspire*** | ***Expire*** | ***Inspire*** | ***Expire*** | ***Inspire*** | ***Expire*** | |
| **Hyp'Air** | 19.7 (0.12) | 16.8 (0.54) | 0.30 (0.00) | 0.10 (0.02) | 9.5 (0.6) | 6.0 (0.6) | 43 (4) | 4 (1.0) | 5.6 (0.6) |
| **BodyBox** | 20.7 (2.4) | 17.7 (0.6) | 0.30 (0.01) | 0.10 (0.01) | 10 (0.1) | 7.0 (0.6) | -- | -- | 10.3 (0.5) |

Mean (SD)

Table 7. The differences in DLCO between the two PFT systems using two different breath-hold times.

| | **BodyBox (10s BHT)** | **Hyp'Air (5s BHT)** | Δ | **P value** |
|---|---|---|---|---|
| **VA (L)** | 5.1 (1.1) | 5.3 (1.2) | -0.15 (0.4) | 0.004 |
| **DLCO (L)** | 26.6 (6.4) | 26.6 (7.0) | -0.1 (3.1) | 0.868 |
| **Inspired volume (L)** | 3.95 (0.86) | 3.72 (0.84) | 0.24 (0.28) | 0.000 |

Mean (SD). BHT = breath-hold time.

Table 8. Predictive equations for healthy black adults using a 5 s breath-hold (Hyp'Air PFT system) for DLNO and DLCO, and 10 s breath-hold (Bodybox) for DLCO.

| | Height (cm) | $Age^2$ | Sex | Constant | Adjusted $R^2$ | SEE | LLN and ULN |
|---|---|---|---|---|---|---|---|
| **Hyp'Air (5s BHT)** | | | | | | | |
| **DLNO (mL/min/mmHg)** | 0.92 | – 0.012 | 38.8 | – 25.2 | 0.77 | 16.3 | ± 32.6 |
| **DLCO (mL/min/mmHg)** | -- | – 0.002 | 11.0 | 23.3 | 0.72 | 3.7 | ± 7.4 |
| **DMCO (mL/min/mmHg)** | 1.66 | – 0.016 | 35.6 | – 143.9 | 0.71 | 22.3 | ± 44.6 |
| **Vc (mL)** | -- | – 0.003 | 19.7 | 56.3 | 0.55 | 9.6 | ± 19.2 |
| **VA (L)** | 0.056 | -- | 1.0 | – 4.61 | 0.69 | 0.64 | ± 1.28 |
| **Vc/VA (mL/L)** | -0.11 | – 0.001 | 1.32 | 30.3 | 0.26 | 1.7 | ± 3.4 |
| **DMCO/VA (mL/min/mmHg/L)** | 1.32 | – 0.04 | -- | 6.9 | 0.41 | 4.0 | ± 8 |
| **KCO (mL/min/mmHg/L)** | – 0.034 | – 0.00033 | 0.87 | 10.8 | 0.51 | 0.6 | ± 1.2 |
| **KNO (mL/min/mmHg/L)** | -- | – 0.00264 | -- | 28.31 | 0.43 | 2.8 | ± 5.5 |
| **BodyBox (10s BHT)** | | | | | | | |
| **DLCO (mL/min/mmHg)** | -- | -- | 11.14 | 21.46 | 0.75 | 3.2 | ± 6.4 |
| **VA (L)** | 0.06 | -- | 0.89 | -5.41 | 0.72 | 0.59 | ± 1.18 |
| **KCO (mL/min/mmHg/L)** | -- | – 0.000394 | -- | 5.62 | 0.176 | 0.74 | ± 1.48 |

SEE = standard error of the estimate. Age is in years; height is in cm. Sex = 1 for males, 0 for females.

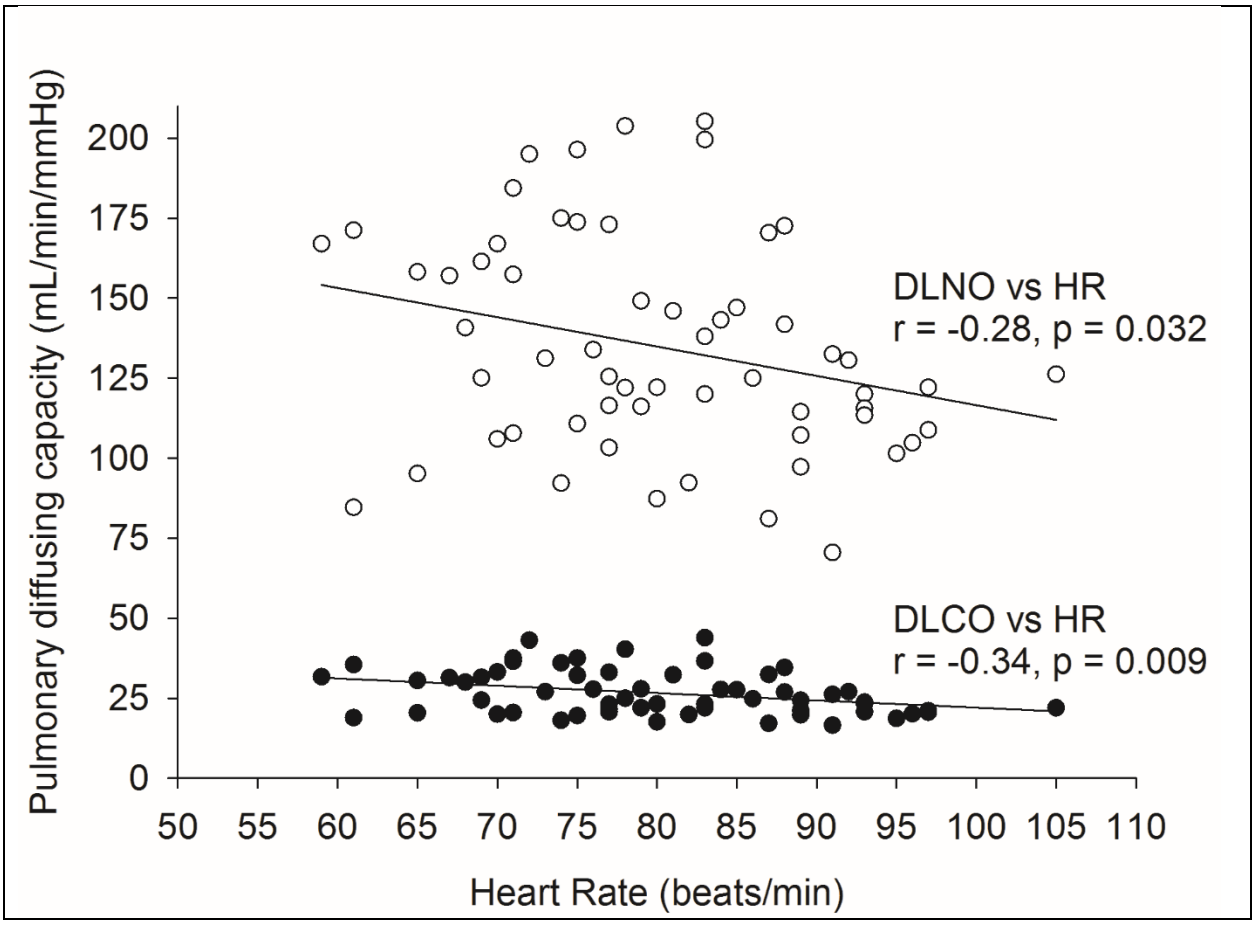
Table 9. Difference in predicted DLNO, KNO, and VA between blacks and whites of the same age.

| | 25 years of age and 175 cm tall | | 45 years of age and 175 cm tall | |
|---|---|---|---|---|
| | **DLNO (males)** | **DLNO (females)** | **DLNO (males)** | **DLNO (females)** |
| **Whites** | 180 | 145 | 166 | 131 |
| **Blacks** | 167 | 128 | 150 | 112 |
| **Difference** | 13 | 17 | 14 | 19 |
| **% Difference** | 7% | 12% | 10% | 15% |
| | | | | |
| | **25 years of age and 175 cm tall** | | **45 years of age and 175 cm tall** | |
| | **VA (males)** | **VA (females)** | **VA (males)** | **VA (females)** |
| **Whites** | 6.9 | 6.1 | 6.9 | 6.1 |
| **Blacks** | 6.2 | 5.2 | 6.2 | 5.2 |
| **Difference** | 0.7 | 0.9 | 0.7 | 0.9 |
| **% Difference** | 10% | 15% | 10% | 15% |
| | | | | |
| | **25 years of age and 175 cm tall** | | **45 years of age and 175 cm tall** | |
| | **KNO (males)** | **KNO (females)** | **KNO (males)** | **KNO (females)** |
| **Whites** | 25.5 | 25.5 | 23.6 | 23.6 |
| **Blacks** | 26.7 | 26.7 | 23.0 | 23.0 |
| **Difference** | 1.2 | 1.2 | 0.6 | 0.6 |
| **% Difference** | 5% | 5% | 3% | 3% |

For blacks, the prediction equations used were from Table 8. For whites, the prediction equations used were from the ERS Task Force document [22]. DLNO is measured in mL/min/mmHg, VA is measured in L, and KNO is measured in ml/min/mmHg/L.

## LIST OF FIGURES

Figure 1. The association between resting heart rate (HR) and diffusing capacity.



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