THE EFFECT OF BODY POSITION ON PULMONARY VENTILATION AND RESPIRATORY GAS EXCHANGE.

BY

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CONTENTS

ABSTRACT

This study looked at the potential use of supine gas transfer measurements using carbon monoxide and nitric oxide, and positional structured light plethysmography (SLP), in detecting the location and severity of emphysematous destruction within the lungs.

Healthy normal subjects (n=95) were compared to those with Alpha-1 antitrypsin deficiency (A1AD) (n=64), a genetic condition known to cause emphysema. There were statistically significant differences observed in the A1AD group when tested in the supine position, with decreases seen in TL_{NO}, VAeff, and TL_{NO}/TL_{CO} of 10%, 10% and 4.1% respectively. There was a significant increase in K_{co} for both the healthy controls and A1AD group of 12.6% and 6.2% respectively. There was no significant change in K_{NO} from seated to supine in any study group.

Disease severity had a significant impact on transfer measurements whilst in the supine position. A1AD subjects with airflow obstruction (OBA1) showed significant changes to TL_{CO} (-12.6%) compared to non-obstructive A1AD subjects (NOA1) (-4.0%). All subject groups showed statistically significant postural changes for K_{CO} and TL_{NO} showed significant postural changes for both groups of A1AD subjects. K_{NO} showed no significant changes between postures for any of the three cohorts.

Measurements using SLP showed that all subject groups demonstrate asynchronous, abdominally dominant breathing movements whilst supine. There was a significant decrease in ribcage contribution (RCC%) and upper ribcage contribution (URCC%) in healthy controls (-35.8%), NOA1 (-32.7%), and OBA1 (-26.0%).

These findings validate current understanding of gas transfer measurements in healthy controls and emysematous disease and measurements using Nitric Oxide/Carbon Monoxide and SLP measurements provide additional insight into patients disease location and severity and therefore, would be of value to be used as part of patient's clinical investigations.

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1.0 INTRODUCTION

1.1 Ventilation

Ventilation is the process by which air is repeatedly drawn into (inspiration) and subsequently expelled (expiration) from the lungs. This occurs due to alternating pressure differences between the alveoli and the atmosphere by the contraction and relaxation of the muscles of the respiratory system.

During tidal breathing (normal/quiet breathing) the diaphragm descends towards the abdominal cavity by about 1 - 2cm, this produces a pressure decrease of 1.4 – 4.1 cmH_2O relative to atmospheric pressure. When the body is being worked and breathing becomes more strenuous, during exercise for example, the diaphragm can fall up to 10cm. This creates a much greater pressure difference, enabling inhalation volumes of up to 3 litres. The muscles responsible for the remaining 25% of inspiratory effort are the external intercostals. Contraction of these muscles causes the ribs as a whole to be elevated, allowing the upper rib cage to elevate in synchrony with the lower rib cage.

However, this is an over simplistic view of ventilation and portrays the lungs as one unit, expanding and contracting in complete harmony. In reality the distribution of ventilation is disparate throughout the lungs. A study by Bryan et al (1964) eloquently demonstrated the inequality of ventilation distribution using radioactive xenon. They showed that in an upright human lung, the level of ventilation per unit of alveolar volume decreases from the base towards the apex. Their data is displayed in Figure 1.1. Similar studies by Dollfuss et al (1967) in standing subjects and Holland et al in 1968 in elderly subjects have since replicated these techniques and found similar findings.

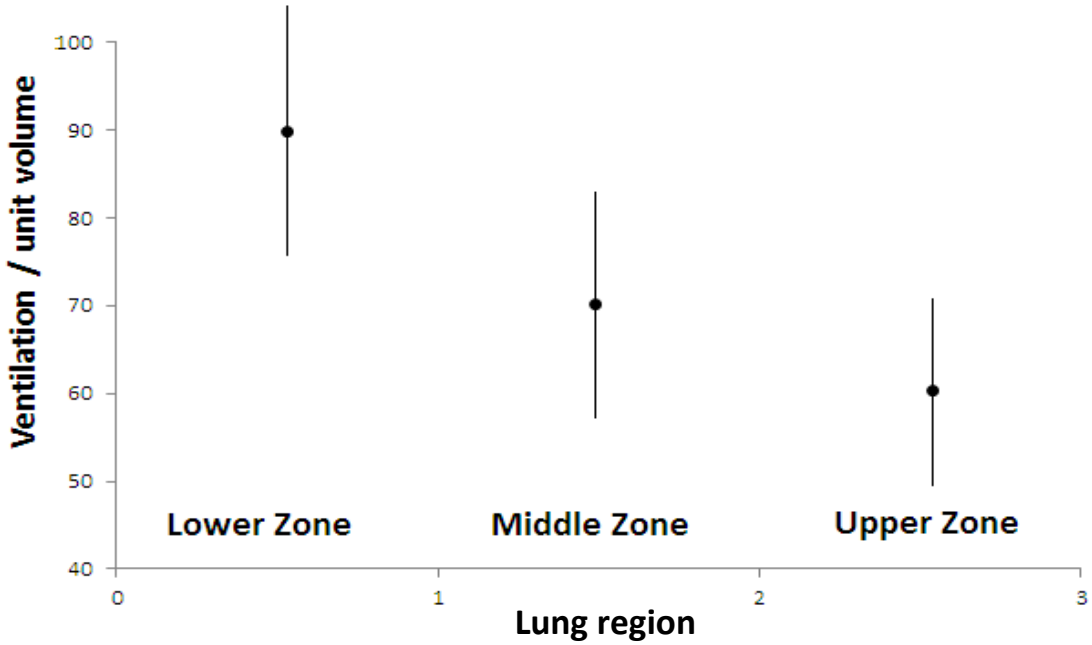

Figure 1.1 – The distribution of ventilation in an upright healthy human lung shown by mean values and standard errors. Ventilation per unit volume clearly decreases up the lung from base to apex. [Bryan et al, 1964]

Out of the original 31 subjects measured in a seated upright posture 7 were also measured in a supine position. When lying supine, the distribution of ventilation from apex (upper zone) to base (lower zone) of the lung was much more uniform, and the difference from upper to lower zones resolved as the weight of the lungs shifts to the posterior side. Additionally, an animal study by Glaister (1967) showed the inverted lung ventilates best at the apex and so the normal pattern associated with ventilation is reversed. From these studies it became apparent that the primary cause of the normal uneven distribution of ventilation is due to gravity.

In an upright posture the weight of the lungs is supported by the chest wall and the diaphragm due to gravitational effects. This causes the intrapleural pressure to be greater at the base and so the alveoli become compressed and those at the apex become distended. This theory supported Dollfuss (1967) who showed that at the start of inspiration from a position of residual volume (the amount of gas left in the lungs after full expiration (RV)) the majority of inhaled xenon administered went to the apex of the lungs.

As the inspired breath continues the concentration gradient is reversed until there was a linear increase in concentration from apex to base between 26% and 90% of the total inspired vital capacity (total amount of air inspired from a position of RV under relaxed conditions (IVC)). One can therefore, conclude once inspiration commences, the change in volume is greater at the base and so ventilation is higher, this is shown in Figure 1.2. Changing the centre of gravity by changing the posture of the study subjects, the distribution of ventilation is affected.

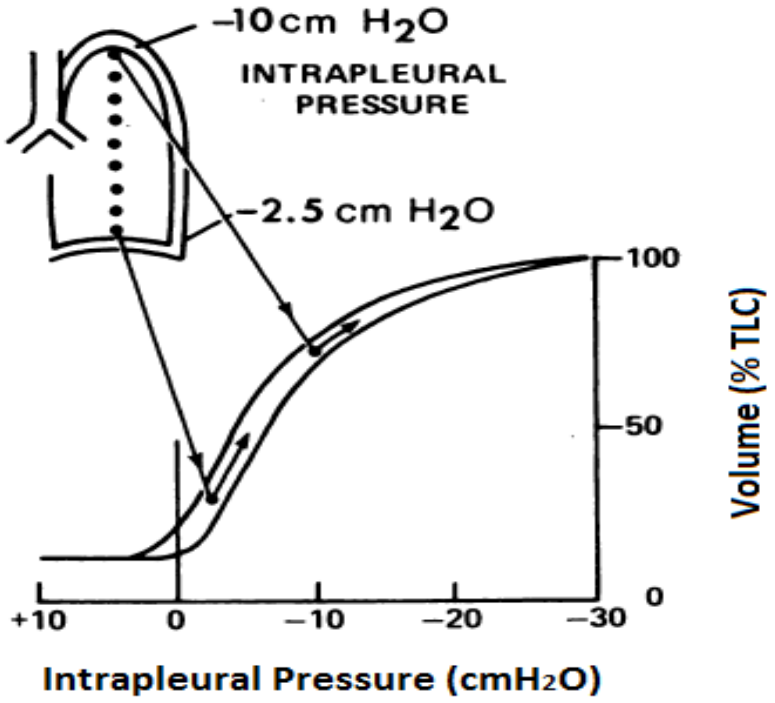

Figure 1.2 – The uneven distribution of ventilation due to intrapleural pressure. The apex and the base are at different intrapleural pressures (as displayed) and so they are at different positions on the pressure volume curve. Therefore lung units at the base have a small initial volume and a greater change in volume and so ventilation is greater at the base of the lungs. (Adapted from West, 1985)

1.2 Lung Mechanics – The Work of Breathing

Work is the product of a force and displacement. For example, when a ball is dropped, the work on the ball as it falls is equal to the weight of the ball (a force) multiplied by the distance to the ground (a displacement). In the respiratory system, displacement relates to the change in lung volume and force translates to the transpulmonary pressure required for overcoming

the elastic work of breathing. Transpulmonary pressure (P_L), denotes the pressure inside the lungs relative to that outside the lungs.

The elastic recoil of the lungs is comprised of the negative elastic recoil of lung tissue, positive elastic recoil of the chest wall and the surface tension of the alveoli.

At a static position (end of an expiratory tidal breath/Functional Residual Capacity (FRC)) the transpulmonary pressure is equal to that of the elastic recoil pressure. FRC is the resting position of the respiratory system, in this position the respiratory muscles are relaxed and the transpulmonary pressure is equal to that of the pressure of the chest wall (P_{CW}) (lung inward recoil equals that of the chest wall outward spring). If no pressure gradient exists between atmospheric pressure (P_{atm}) and alveoli pressure (P_A) the pressure of the respiratory system (P_{rs}) equals zero thus no air flow occurs. If the P_{rs} is positive then air is being drawn in and inspiration is occurring, if P_{rs} is negative then air is being drawn out of the lungs and an expiration is taking place.

Equation 1: $P_{rs} = P_A - P_{atm}$

Lung pressure and chest wall pressure are themselves determined as a result of a balance of pressures. Lung pressure is the balance of the pressure at the pleural surface (P_{PL}) and the P_A i.e.

Equation 2: $P_L = P_A - P_{PL}$

This relationship is shown in Figure 1.3.

In order to draw air into the lungs (inspiration), a difference in P_A and P_{atm} must be created. This is achieved by contracting inspiratory muscles, primarily the diaphragm. This causes flattening

of the diaphragm allowing the lung to expand, increase in volume thus decreasing the pressure to below that of the atmosphere.

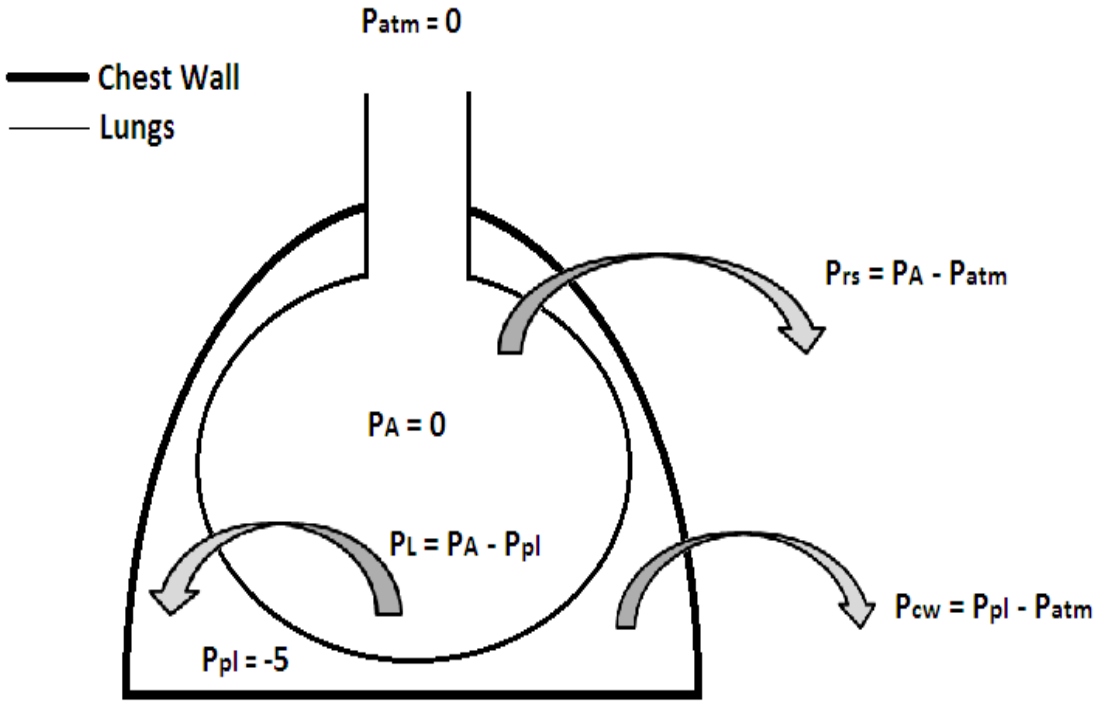

Figure 1.3 - Represents the lungs in a static position (FRC) and the relationship of alveolar (PA), intrapleural (Ppl), atmospheric (Patm) & transpulmonary pressure (P_L) in this stage of a breath cycle. All pressures are measured in units of cmH20.

As the contraction occurs the diaphragm flattens performing two functions. Primarily it will increase the vertical diameter of the thoracic cavity by increasing its depth as it flattens. Secondly it expands and elevates the lower rib cage. Diaphragm muscle fibres are arranged such that when the muscle contracts and is met by counter-pressure of the abdominal cavity, the diaphragm fibres pull the rib cage up and out. This is why on inspiration the diaphragm moves down. Both actions of the diaphragm and the chest under normal conditions are a synchronised movement and result in an increased volume within the lungs.

The value of each of the pressures mentioned above depends upon the elasticity of the lungs and is measured by compliance. Compliance is the measure of distensibility of matter and specifies the ease with which matter can be stretched or distorted. The compliance of the lungs

is often described by examining the pressure-volume characteristics of the lung under static conditions; this is often referred to as a Rahn Diagram as shown in Figure 1.4.

At low lung volumes P_{CW} becomes negative and opposes further deflation at RV. Here, the lungs are highly distensible and change in pressure results in a relatively large increase in lung volume. At higher volumes the lungs reach there elastic limit and their recoil is maximal so for an equivalent change in transpulmonary pressure, there is little change in volume.

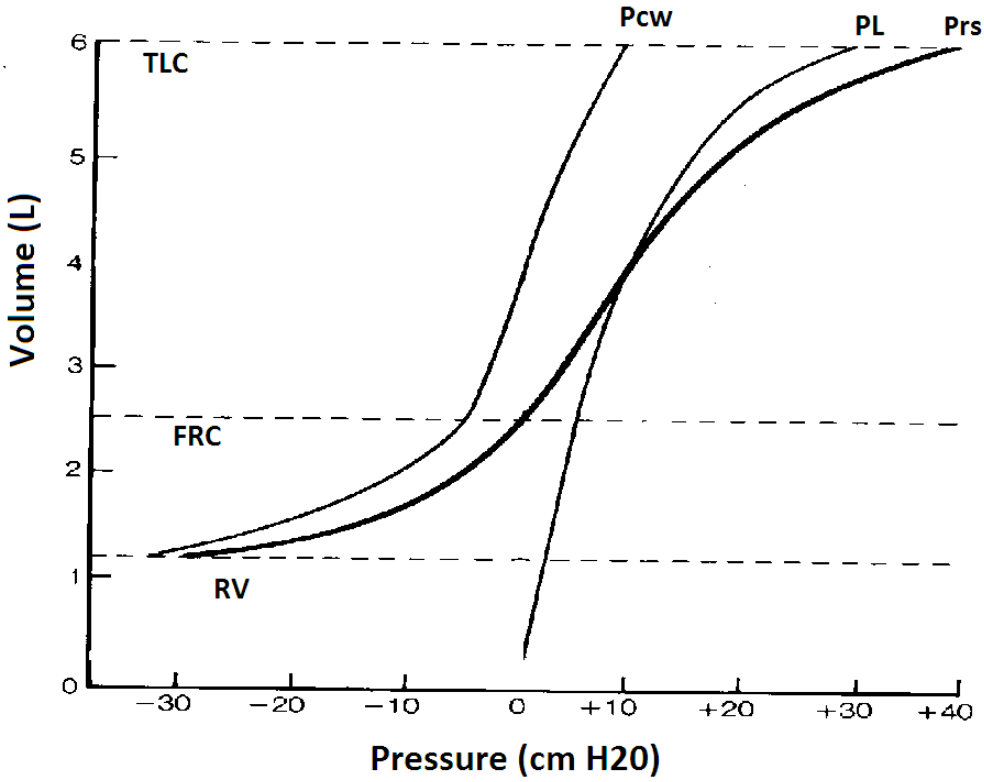

Figure 1.4 – Rahn Diagram. This Diagram describes the relative changes of P_L and Pcw with varying lung volumes (RV, FRC & TLC) and their influence on Prs. Pressure and volume have a curvilinear association so as pressure increases so does volume. [Adapted from Rahn et al, 1946]

Disease however, can have a profound effect on these mechanics. Conditions like chronic obstructive pulmonary disease (COPD) have been shown to cause asynchronous movements between the chest and abdomen and even between different regions of the chest itself.

Hoover et al (1920) originally described an inspiratory retraction of the lower intercostal spaces that occurs with severe obstructive airway disease, today known as Hoover's sign.

Gilmartin et al (1984) examined the mechanisms of this abnormality with the use of magnetometers, relating rib cage movement to the changes in P_{PL}, abdominal pressure (P_{AB}), and P_L pressures in 13 patients with COPD and hyperinflation. It was suggested these abnormal chest movements related well to increasing P_L. It was concluded this inward movement of the lower ribcage was the result of hyperinflation (a common symptom of severe COPD) causing the diaphragm to flatten. The increased volume and resulting expansion of the chest causes a greater amount of muscle tension, elevated anterior externally rotated ribs and the loss of the zone of apposition (the cylindrical aspect of the diaphragm that opposes the inner aspect of the lower chest wall). These possible mechanisms are illustrated in Figure 1.5, with the 5 points listed culminating in the lower rib cage motion directed inward on inspiration instead of outward, although the true cause remains elusive.

Hoover's sign has been reported by Priori et al (2013) to have a sensitivity and specificity for identifying airflow obstruction of 58% and 86% respectively, with up to 70% of severe sufferers displaying this inward drawing. The presence of Hoover's sign has the potential to provide valuable prognostic information in patients with COPD and supplement other clinical or functional tests.

However, Hoover's is not the only type of abnormal chest wall movement. In the same study by Gilmartin et al (1984) three types of abnormal movements were described when measuring COPD sufferers in an upright seated position. These were; (i) Lateral rib cage paradox (the most common), (ii) in-drawing of the lower sternum (Hoover's) (iii) paradoxical inspiratory motion of the abdomen.

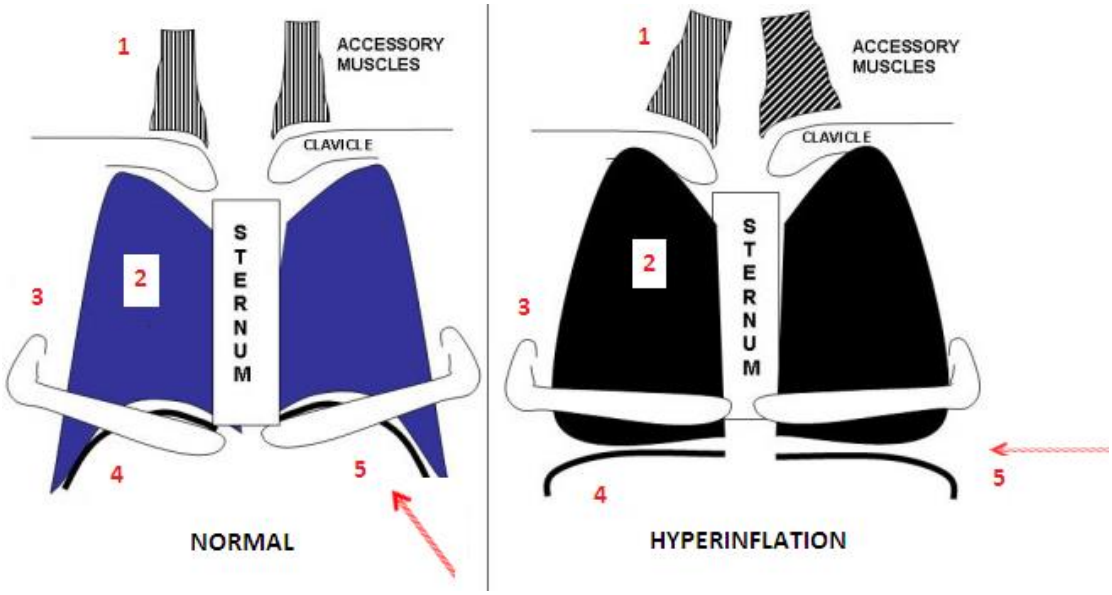

Figure 1.5 - The possible mechanism behind Hoover's sign: 1 = accessory muscles, 2 = hyper-expansion of the lungs, 3 = alteration of rib orientation to horizontal, 4 = flattened diaphragm and 5 = decreased zone of apposition. (Adapted from Johnston et al, 2008)

Gilmartin et al (1964) continued their work focusing on the more commonly seen abnormal movement of lateral rib cage paradox. Again using magnetometers it was concluded these movements were the product of a flattened diaphragm, radially positioned muscle fibres and a reduced zone of apposition; all accredited to hyperinflation and associated with worsening airways obstruction.

Paradoxical inspiratory motion of the abdomen has also been investigated further by Marini et al, (1988). They studied the weaning process from artificial, mechanical ventilation in a variety of subjects including those with severe COPD. It was noted that this abnormal movement was more frequent during loaded breathing where the workload to breathe was increased. This could be achieved by simply moving subjects into a supine position, using the ribcage as a weight which the respiratory muscles have to overcome to maintain 'normal' breathing and ventilation.

1.3 Perfusion

Perfusion means “to permeate or suffuse with a liquid, colour, or quality”. In medicine this translates to supplying an organ or tissue (in our case the lungs) with a fluid (blood) by circulating it through blood vessels or other natural channels (capillaries).

Like ventilation blood flow to the lungs is not uniformly distributed and can be clearly shown with the use of inhaled radioactive Carbon dioxide ($C^{15}O_2$). Inhaled $C^{15}O_2$ is rapidly taken up by pulmonary blood. With the use of radiation detection equipment over the chest during breath holding, the clearance rate of radioactive material from the lungs can be recorded. This clearance is proportional to the level of pulmonary perfusion in any given area of the lung as the blood flow carries the material away from the lungs.

A study by West and Dollery in 1960 used this technique in normal subjects. The clearance rate varied from about 20% a second at the base of the lung to virtually nothing at the apex and the change was almost linear with distance up the chest. These findings are shown in
Figure 1.6 and illustrates a typical distribution of blood flow in a healthy, upright human lung. This technique has since been modified and is synonymous for lung perfusion scintigraphy, commonly used in the assessment of pulmonary emboli. This test again involves the intravenous injection of radioactive material, commonly technetium macro aggregated albumin.

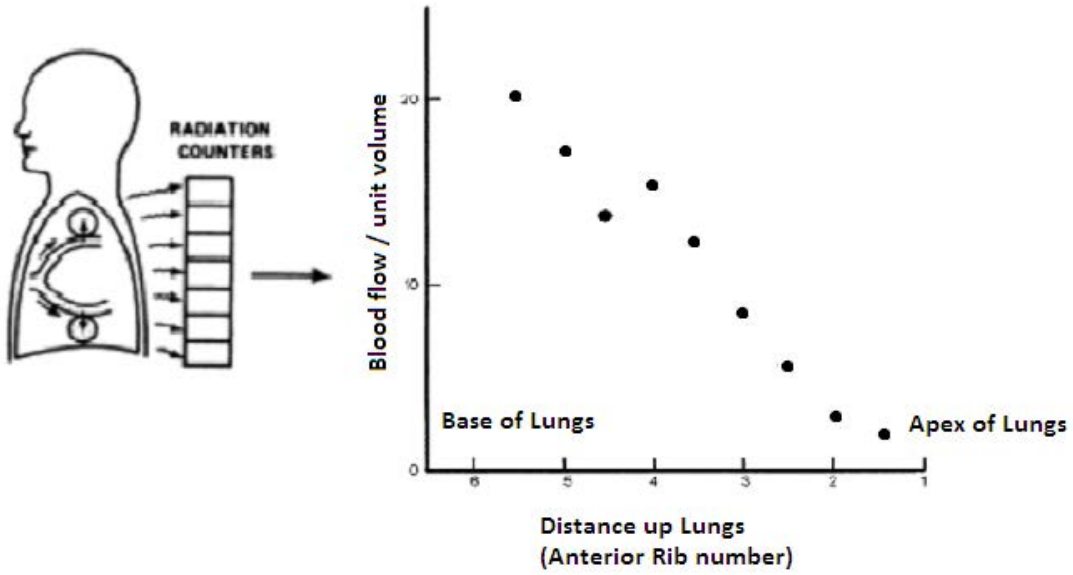

Figure 1.6 – The mean distribution of pulmonary perfusion using radioactive Carbon Dioxide ($C^{15}O_2$) in the upright human lung at the position of total lung capacity (TLC). Note the peak perfusion is located just above the base of the lungs due to the effects of gravity. (Adapted from West & Dollery, 1960)

A gamma camera or a PET scan acquires the images for the study which can be seen in figure 1.7.

This typical distribution of perfusion can be influenced in a variety of ways primarily through strenuous exercise and changes in posture. Previous investigations have been conducted to analyse the effects of posture on perfusion in both children and adults (Bhuyan et al, 1989). Their work used the same technique as described above and demonstrated greater mean perfusion of 7% (range 4.8 - 10.9%) to the lower regions of the lungs while in the upright posture, compared to the supine position in both adults and children. It was concluded that in a supine position the apex of the lungs did receive greater levels of blood flow but the base remained relatively unchanged and so the two regions achieved a state of equilibrium.

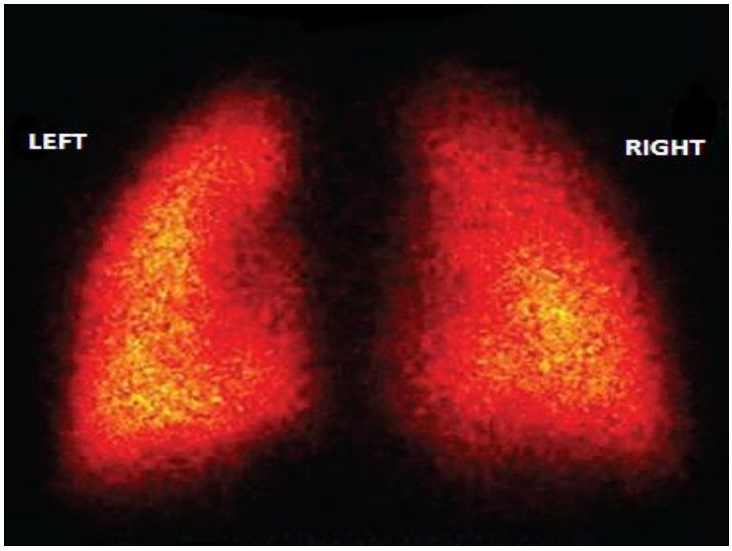

Figure 1.7 – Scintigraphic scan image using Technetium-99m (^{99m}Tc - a metastable nuclear isomer) macro aggregated albumin tracer showing normal distribution of pulmonary perfusion in an upright human lung. The lighter colour depicts areas of high perfusion at the base of the lungs compared to that of the darker coloured lower perfused areas at the apex. [Taken from Gandhi et al, 2013].

1.4 Gas Exchange

The respiratory system comprises two components; the lungs, which are responsible for ventilation (airflow) and the cardiovascular system (heart and blood vessels) that supply a constant blood flow to the lungs. These systems work in harmony to provide an effective means by which oxygen (O_2) can be absorbed into the body and carbon dioxide (CO_2) excreted from the body. This process is known as "gaseous exchange". For this process to be optimal there must be a matching of ventilation in the alveoli of the lungs to allow the gases to be inspired and expired and perfusion of the pulmonary capillaries to transport the gases either to or from working muscles. Any impairment to either ventilation e.g. bronchoconstriction from Chronic Obstructive Pulmonary Disease (COPD) or perfusion e.g. pulmonary emboli (PE) will lead to impaired gas transfer.

Each alveolar is lined with one layer of squamous epithelium cells. This one cell thick layer just 0.5 µm thick, reduces the resistance to the diffusing gas molecules allowing them to pass through the membrane easily.

The pulmonary capillaries have similar properties and are constructed of a single layer of endothelium tissue to again minimise resistance to diffusing gas molecules. This double layered membrane is referred to as the alveolar capillary membrane as illustrated in Figure 1.8.

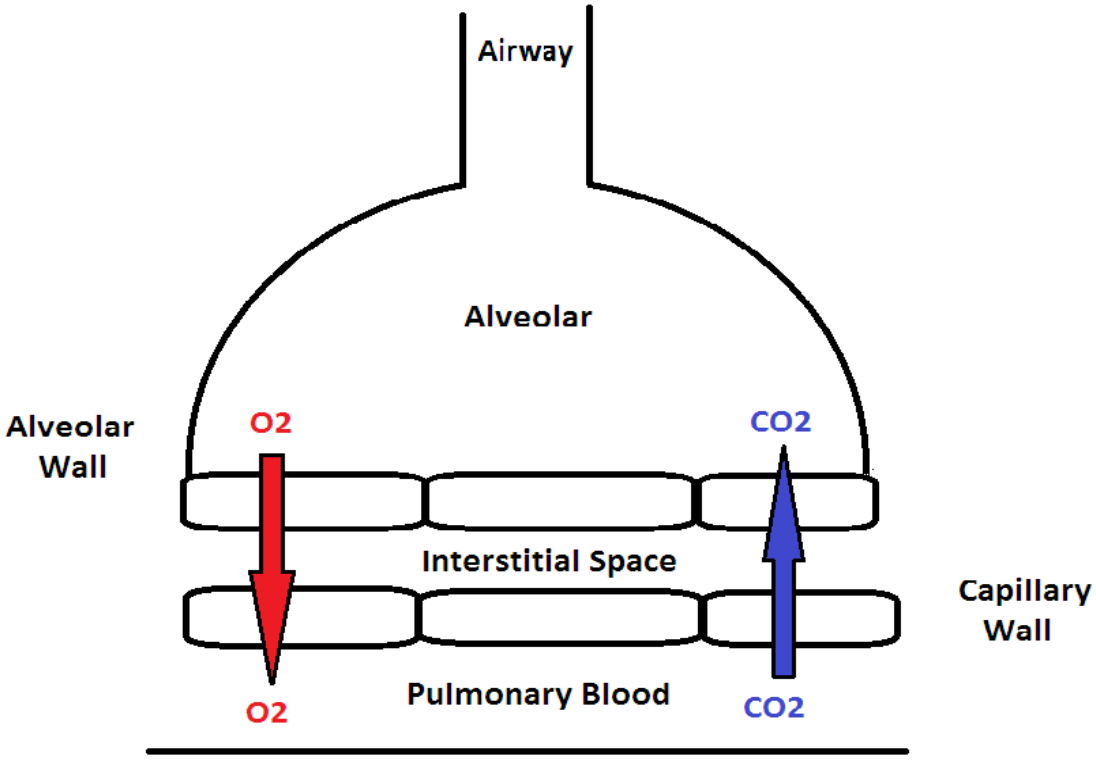

Figure 1.8 - Oxygen and carbon dioxide rapidly diffuse across the blood-gas barrier (alveolar capillary membrane) and equilibrate in normal, healthy resting individuals.

Fick's law describes the transfer of gas molecules by diffusion and shows that this is proportional to the pressure gradient (ΔP) across the membrane (in this case between that of the alveolar air and pulmonary capillary blood) and the area of membrane (A), It is inversely proportional to the thickness of the membrane (X) and is shown in equation 3 below.

$$\text{Equation 3: Mass flow of Gas} = \frac{\Delta P \text{ (alveolar-capillary)} \times A}{X}$$

As it is not possible to measure the area and the thickness in living subjects one can introduce Transfer capacity of the lungs (TL) and equation 3 can be corrected to equation 4 or 5:

Equation 4: Mass flow of Gas = TL . (P1 – P2)

OR

Equation 5: TL = Mass flow of Gas / (P1 – P2)

However, this equation is an over simplification of the whole process and additional resistive elements such as the diffusion rates through a membrane for different gases and the rate of uptake from the plasma to chemically combine with haemoglobin (Hb) need to be considered.

The theoretical principal which is still used today was originally described in 1957 by Roughton & Forster who stated that the (TL) is determined by the pulmonary diffusing membrane (D_M), the volume of pulmonary capillary blood (Vc) and the constant uptake of gas to haemoglobin (Θ).

They expressed this as shown in equation 6;

Equation 6: $1/TL = 1/D_M + 1/ \Theta Vc$

This equation is applied to the modern day techniques used to assess the lungs capacity to transfer gas and is explained in more detail later on in this thesis.

1.5 Chronic Obstructive Pulmonary Disease (COPD)

The Department of Health (DOH) released national statistics in 2011 showing COPD is the leading cause of respiratory related fatalities worldwide and is the fifth biggest killer in the UK. This equates to 25,000 deaths a year with an estimated 3.2 million suffers in the UK alone. However, this figure could be much higher as many sufferers have yet to be diagnosed. This produces a significant cost to not just the National Health Service (NHS) but the UK economy as a whole. In 2010 the cost of COPD to the NHS was estimated at £800 million a year, this

resulted in a loss of £2.7 billion to the UK economy through sick leave, hospital appointments and admissions. [DOH, 2011].

Due to its complexity, COPD is very difficult to detect in its early stages as symptoms only present themselves once the condition has reached an advanced stage several years after its initial development.

This in part explains why the prevalence of COPD increases with age which is illustrated in Figure 1.9 which is taken from The National Institute for Health and Care Excellence (NICE) costing report published in 2011. These figures continue to rise yearly and clearly work needs to be done to address this ever worsening situation.

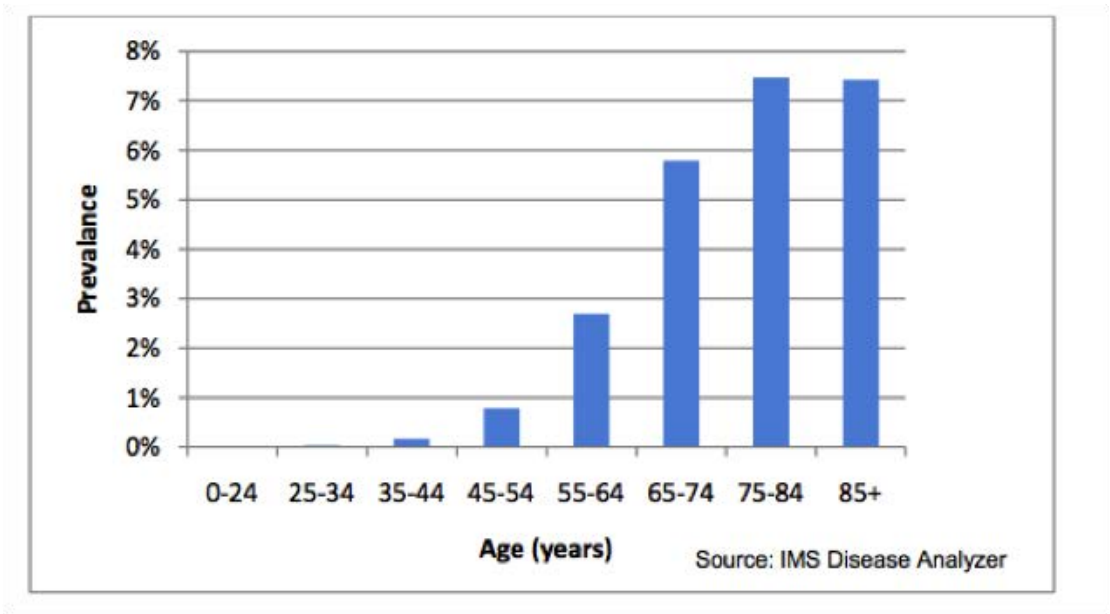

Figure 1.9 - The Estimated Prevalence of COPD by Age. The graph shows how the prevalence of COPD increases with age. [Taken from the NICE costing report, 2011]

1.5.1 Pathophysiology of COPD

Chronic obstructive pulmonary disease (COPD) is a disorder that is comprised of a number of different disease pathologies including emphysema (the destruction of lung tissue) chronic bronchitis (increased mucous secretions) and bronchiolitis (small airways disease). Emphysema

can be caused a number of different ways, the commonest is through smoking and makes up 86% of all cases [DOH, 2011].

It is known that there is the presence of neutrophils and alveolar macrophages in the small airways and in increased number in smokers. These cells produce the enzymes, protease and elastase that break down elastin fibres in the walls of the alveolar.

A serum antiprotease protein produced by the liver called alpha-1 antitrypsin inhibits these enzymes, preventing them destroying the connective tissues in the walls of the alveolar. Prolonged exposure to cigarette smoke deactivates the alpha-1 antitrypsin molecule allowing the protease and elastase enzymes to attack the connective tissue in the alveolar causing the breakdown of their walls. Neutrophils release these enzymes to travel through the lungs in search of foreign organisms to destroy. This destruction and break down of tissues causes the airways to become 'floppy'.

The rings of cartilage continue to hold and support the upper airways but, as the lower airways do not have this feature they collapse under the external pressure on the airway during expiration, often referred to as dynamic compression. This is the reason why emphysema initially affects the small airways. It is the dynamic compression that causes the patient to become hyper inflated and present signs of gas trapping.

As the patient inspires the flow of air causes a positive pressure inside the airway, which holds the walls open enabling a relatively normal breath in. However, during expiration the opposite effects are true; the flow of air leaving the airway along with the smooth muscles surrounding the airways contracting causes this dynamic compression. As the small airways collapse, gas is unable to escape and the airways only open once inspiration begins again filling the lungs with air. This process continues as the disease progresses and as more air is trapped in the lung the patient develops a "barrel chest" as they become hyper-inflated. The air that is

trapped in the lungs becomes depleted of oxygen causing areas of the lung to become poorly ventilated. This hyperinflation of the lungs causes the alveolar tissue to be overstretched; this along with the loss of elasticity due to the destruction of elastin causes the tissue to tear. The tearing of these walls creates the large spaces seen in an emphysematous lung, reducing its surface area that plays a critical role in the efficiency in gaseous exchange.

As the lungs become more and more inefficient at exchanging O_2 and CO_2 many of the vessels around the unventilated areas of the lungs start to vaso-constrict. This is an attempt to compensate for the low blood oxygen saturation by allowing more blood to travel to the ventilated areas. The constriction puts more pressure on the vessels causing pulmonary hypertension, which in turn results in the strain and enlargement on the right side of the heart resulting in oedema. This usually can be seen in the patient's feet and ankles and explains why many emphysematous patients present these symptoms.

However, it is not just cigarette smoke that has this effect, many occupational exposures to industrial dusts and air pollution can also have the same effect. There is also a genetic disorder causing a deficiency in the alpha-1 protein.

1.6 Alpha 1-Antitrypsin Deficiency (A1AD)

A1AD is a rare hereditary genetic disorder that is linked with a susceptibility to develop early onset, rapidly progressive emphysema. This association was first described in 1963 by Laurell and Eriksson who noted that patients were presenting clinical symptoms of severe early onset basal panacinar emphysema much younger than is typically seen and was not determined by the severity of smoking history. They reported 5 cases, the oldest subject being 42 years of age which was deemed a young cohort for typical presentation of emphysema. Three of the subjects were female, which for the time was again unusual as significant smoking histories

were generally associated with males and so had the highest prevalence. Upon medical consultation it was discovered one subject had a significant family history of emphysema. Their observations that these individuals were susceptible to a severe form of hereditary emphysema led to an influx of new work in this area, leading to a major breakthrough in understanding of the role of protease-antiprotease imbalance in the pathogenesis of COPD.

We now know from previous work (Senior et al, 1977 & Kao et al, 1988) that this is a result of reduced levels of serum alpha1-antitrypsin, produced in the liver.

The Alpha-1-antitrypsin (AAT) molecule is a 52 kilodalton single chain glycoprotein with a 394 amino acid sequence; its primary task is to inhibit serine proteinase. The protein is encoded on the SERPINA1 gene and consists of 7 exons on the long arm of chromosome 14. The function of the protein is determined by a methionine amino acid at position 358. This gives the protein its specificity for binding with serine proteinases, primarily neutrophil elastase. This binding results in the inactivation of both proteins and forms of a stable complex preventing the breakdown of tissue fibres (Matheson et al, 1986).

At normal serum levels it inhibits specific proteolytic enzymes such as trypsin and leukocyte elastase that are secreted by neutrophils and macrophages during bacterial induced inflammation to destroy said bacteria. Unless sufficient levels of antitrypsin are present in the blood and lungs the neutrophils responding to inflammation will go unchallenged, resulting in elevated levels of elastase secretion, causing the breakdown of lung tissue elastin typically seen in emphysematous changes (Seersholm et al, 1998).

Several mechanisms are recognised as being related to deficiency, these include total absence of the gene, frame shift mutations that lead to premature stop codons as well as point mutations that may lead to no production or production of abnormal AAT phenotypes (Dickens et al, 2011).

It is thought over 80 variations of A1AD exist, each resulting in different levels of serum AAT. The most common phenotypes diagnosed are;

- PiMM: 20-48ug (100% normal)
- PiMZ: 17-33ug (80% of normal serum level of A1AD)
- PiSS: 15-33ug (60% of normal serum level of A1AD)
- PiSZ: 8-16ug (40% of normal serum level of A1AD)
- PiZZ: 7-2.5ug (10-15% severe alpha 1-antitrypsin deficiency)

Patients with A1AD with a PiZZ phenotype will have severely reduced levels of AAT in blood, classically below 20% of 'normal' levels. Approximately one third of these patients will develop basal emphysema as opposed to the central emphysema typically seen in smokers. However, some subjects have exhibited a greater involvement of the apex of the lungs (Parr et al, 2004 & 2006) with the use of lung densitometry calculations, derived from High Resolution Computed Tomography (HRCT) as shown in Figure 1.10.

By calculating the volume of space (as a voxel index (VI)) throughout different lung regions one can show the distribution of emphysema as the condition destroys lung tissue, increasing the amount of space. HRCT scans are still currently being used to determine those regions of the lungs affected by emphysematous change and are the gold standard diagnostic tool in diagnosing COPD.

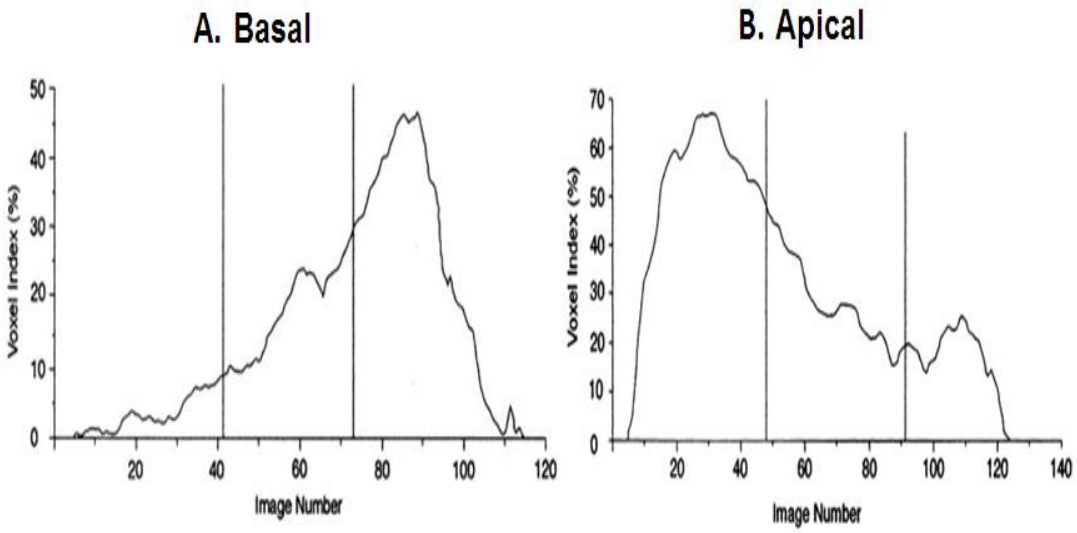

Figure 1.10 - Examples of VI (%) distribution profiles, indicating the patterns of emphysema distribution used for subject grouping. (A) Basal. (B) Apical. The vertical black lines indicate the level of the upper (aortic arch) and lower zone (inferior pulmonary veins) images at the left and right, respectively. The graphs clearly show that cohort (A) with basal emphysema had higher VI (%) in the lower third of the lungs compared with cohort (B) with apical emphysema having higher VI (%) within either the middle or upper third of the lung. [Taken from Parr et al, 2004]

However, these scans are costly, expose patients to radiation, and are in high demand leading to lengthy wait for patients before receiving an appointment. This thesis investigates other diagnostic measurements which may produce results quicker, simpler and more cost effective than imaging scans, and can easily be incorporated into a patient's routine lung function tests.

1.7 Measurements of Lung Function

1.7.1 Single breath gas transfer (diffusion) of the lungs using Carbon Monoxide (TL_{CO}, K_{CO}, VAeff)

The primary task of the lungs is to exchange gas between the pulmonary blood and the atmospheric air. This function can be measured with the use of single-breath gas transfer manoeuvres using carbon monoxide (CO). This complex series of interactions can often but inappropriately is referred to as 'diffusion capacity', primarily in the US. As described above the process of gaseous exchange is complex and involves many more factors than just simply diffusion, therefore in this paper the process will be correctly named 'Transfer'.

The use of CO can be traced back to the discovery of its affinity to Hb was significantly greater than that of O2. This allows very small concentrations of CO to be used as there is effectively none present in human blood (apart from small amounts in those who smoke). By using these small concentrations any potential back pressure of carboxyhaemoglobin (COHb) caused by repeated measurements can be regarded as non-significant and so can be ignored. The need for arterial blood sampling to estimate the partial pressure of CO (PC_O) was no longer needed allowing the simplification of a standardised technique and its calculations.

There are a number of other reasons why CO is used instead of O_2;

- The rate of reaction of CO with haemoglobin is linearly related to that of O_2.
- O_2 and CO bind to the same site on the haemoglobin.
- Both oxyhaemoglobin (O2Hb) and COHb are both affected by similar factors such as temperature, pH etc.

However, it was Marie Krogh who first pioneered the single breath transfer measurement in 1915 and published her findings using the term 'The Krogh Factor' as the principle of gas transfer. This method was abandoned until the 1950s with the introduction of the infra-red carbon monoxide analyser.

In 1957 Ogilvie et al developed and published a standardised technique for a single breath TL_{CO}, K_{CO} and VAeff commonly used today, which was based on Krogh's first publication.

The journey of CO from inspired air to pulmonary blood typically encounters two resistances. The first is the diffusing capacity of the membrane (D_M) and is expressed as $1/D_M$. Although this membrane barrier is 0.5μm thick it still presents a resistance which the CO molecules have to overcome. The second resistance is encountered as the CO molecules attempt to bind within the red blood cell. This resistant is comprised of the rate of reaction of gas (in this case CO) with erythrocytes, expressed as θ and the capillary volume in contact with inhaled CO expressed as

1/Vc. This gives us all the components to construct Roughton & Forster's equation; $1/TL = 1/D_M + 1/\theta.Vc$ as discussed in the previous section 1.4.

The measurement of gas transfer across the lungs (TL) provides valuable information on both structural and functional dimensions such as lung volumes, membrane thickness and capillary blood volume, level of ventilation, level of perfusion, Haemoglobin (Hb) levels, blood transit time and the amount of functioning capillary bed in contact with ventilated alveoli. This information is invaluable as it can indicate the presence of certain types of pulmonary, vascular and parenchyma disorders.

TL_{CO} is the total transfer of the lungs and describes the rate of transfer of carbon monoxide between the alveoli and the red blood cells in the alveolar capillaries. This parameter is comprised of the K_{CO} (the transfer factor coefficient) relating to the uptake of carbon monoxide per litre of alveolar volume and VAeff which is the volume of gas in the alveoli of the lungs measured during the single breath gas transfer test.

1.7.2 The effect of posture on gas transfer

During standard TL_{CO} K_{CO} measurements subjects are normally required to sit in an upright position. This results in a hydrostatic redistribution of pulmonary blood flow such that the base of the lungs receives the most blood flow resulting in the majority of gas transfer occurring basally. If, however, the same tests are performed with subjects in a supine position, the apex is more perfused, leading to an increase in total gas transfer.

Previous studies have shown some degree of heterogeneity between dependent (reliant on pulmonary perfusion to perform gaseous exchange) and non-dependent lung regions. An overall increase of 15% was shown in TL_{CO} due to the changes in Vc. Although one might not have expected any modification in diffusing capacity in normal subjects from upright to supine,

these findings shown in figure 1.11 shows that there is a measurable difference (Peces-Barba *et al*, 2004). This difference, although relatively small, could affect transfer factor in patients with diffuse alveolar disease such as chronic obstructive pulmonary disease (COPD).

Further work (Stam et al, 1991) recruited 37 healthy normal volunteers to perform seated and supine gas transfer measurements quoting TL_{CO} and K_{CO}. They found a significant increase in both TL_{CO} and Kco from a position of seated to supine in normal subjects less than 50 years of age when an inspiratory volume between 50-100% of TLC was achieved.

In those subjects greater than 50 years old the increase in gas transfer measurements between body positions was not as great and was shown to be non-significant.

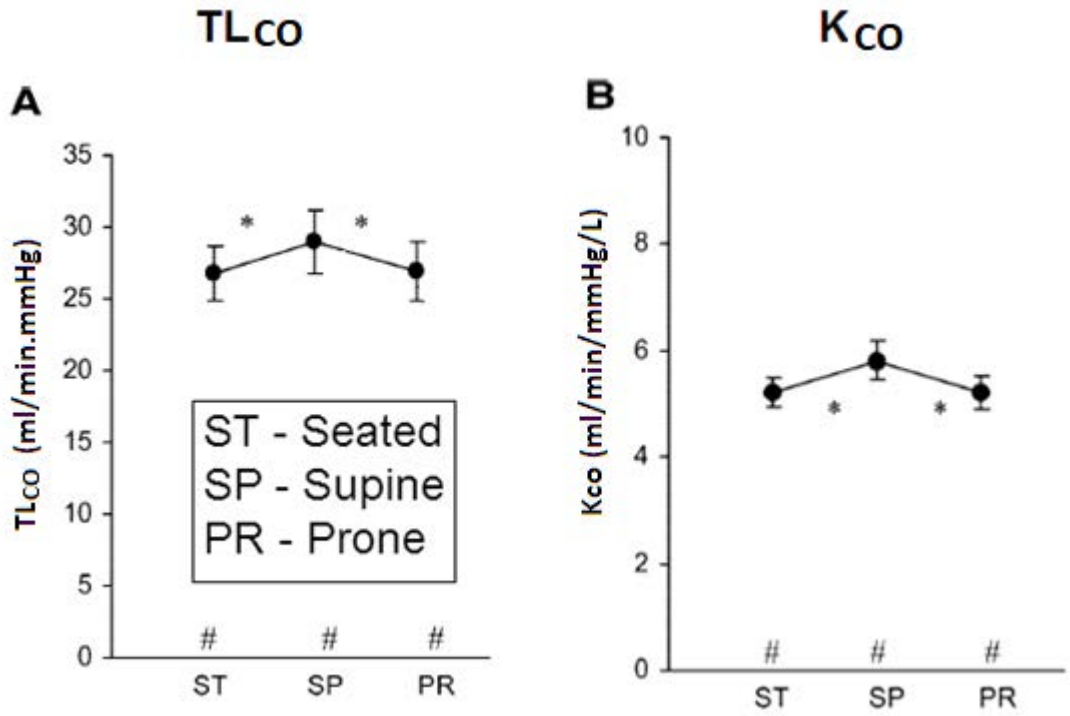

Figure 1.11 – An example of the effects of posture on gas transfer measurements. 14 subjects performed a minimum of two manoeuvres in accordance with ATS standards. Values shown are means ± SE. A significant increase in both TLco and Kco can be seen as subjects moved from an upright seated position to a supine position [Adapted form Peces-Barba et al, 2004]

The study also found that a volume variation in VAeff of 20% caused a significant difference in the response to posture. An overestimation occurred in a sitting position and an

underestimation occurred in TL_{CO} measurements in a supine position. It has been well documented that a supine position increases the work load of breathing and can result in a fall in alveolar volume of up to 50% in the more severe patients. This discovery was essential to keep in mind when applying the technique to those suffering from COPD.

Since CO is effectively competing with O2 for the binding sites of haemoglobin if TL_{CO} was measured at two different O2 tensions the relationship 1/TL vs 1/ Θ could be used to estimate the different components of D_M from the intercept and the Vc from the slope.

It is then possible to distinguish which of the two components is the cause of any abnormality in TL_{CO}. However, practically the results tend to vary significantly because of the measurements sensitivity to changes in pulmonary capillary blood volume. However, there may be an easier, more clinically viable alternative.

1.7.3 Single breath gas transfer using Nitric Oxide (TL_{NO}, K_{NO})

By introducing the gas nitric oxide (NO) two new parameters can be obtained, the pulmonary capillary perfusion using NO (K_{NO}) and the total transfer of the lungs using NO (TL_{NO}). NO has been cited to have 400x the affinity for Hb than CO. It achieves this reacting directly with the oxygen of O2Hb rather than competing directly with oxygen for the Haem sites of Hb to form a nitrate plus a deoxygenated form of Hb called methaemoglobin (metHb).

Equation 7: $NO + Hb(Fe^{2+})O2$ R $metHb(Fe^{3+}) + NO3$

CO does not react with O2 but competes with oxygen for the Fe++ site on the haem ring:

Equation 8: $CO + Hb(Fe{+}{+})O2$ R $Hb\ (Fe{+}{+})CO + Hb(Fe{+}{+})O2$

Thus the rate of diffusion for NO is independent of Vc and solely dependent upon the ability of D_M (Guenard et al, 1987).

Aguilaniu et al, (2008) recruited 303 healthy subjects (142 Females; 161 Males) all without evidence of airflow obstruction (FEV1/FVC% >0.70). They concluded TL_{NO} predominantly measures the diffusion pathway from alveolus to capillary plasma independently of haemoglobin as seen when using CO due to the negligible resistance to the red blood cells. Another similar study conducted in the same year also came to the same conclusion.

Nowak (2008)

By using the same Roughton-Forster transfer equation discussed earlier, we can substitute the membrane diffusing capacity D_M for TL_{NO} (Borland, 1991). By measuring TL_{NO} and TL_{CO} simultaneously this ratio is a truer reflection to D_M/Vc and therefore a more representative measurement of the transfer of gas across the lungs. Van der Lee (2007)

This eliminates the variability seen when measuring TL_{CO} at two different O2 tensions as mentioned in the previous chapter.

Hughes et al, (2015) re-composed the original Roughton-Forster equation to the following;

Equation 9: $TL_{NO}/TL_{CO} = \alpha(1+D_M,co/\Theta co.Vc)$

where α (=1.97) is the ratio NO and CO diffusing capacity in plasma. As both α and Θ are a constant this equation exemplifies the dependence on D_M/Vc and one could argue the TL_{NO}/TL_{CO} is a truer reflection of the D_M/Vc ratio.

TL_{NO}/K_{NO} used with the TL_{CO} tool has been validated in other clinical situations in healthy controls and in disease groups but never in A1AD groups. A body of work was produced that looked at 124 healthy subjects (59 Females; 65 Males) in the age range of 25-55 years old. Van der Lee et al (2007). They found an averaged TL_{CO}/TL_{NO} ratio of 4.45, which was very similar to a study by Aguilaniu (2008) which reported a TL_{CO}/TL_{NO} ratio of 4.80.

The small difference that was seen between the two studies are likely to be due to methodological differences as well as differences in the populations studied. The study by Van Der Lee et al. used lower inspiratory NO fractions (7–9 ppm) with long breath-holding periods, leading to NO concentrations in the range 200 ppb. Altitude could also contribute to the slight discrepancies in the results, these factors must be considered when interpreting these results When the ratio was analysed against age, the study by Van der Lee (2007) reported an increase of 0.33% per year compared to no change that was reported by Aguilaniu.

The individual parameters of TL_{NO} and TL_{CO} were also looked at separately. Again, in the same paper produced by Van der Lee et al, (2007) both parameters were compared at varying lung volumes. From VA max to 50% max it was shown TL_{CO} decrease by 29% compared to a 49% in TL_{NO}. This can be explained when observing K_{CO} which increase with VC/VA, in other words as VA decreases K_{CO} increases, which compensates TL_{CO}. This indicates TL_{NO} relates more to the surface area of the membrane and K_{NO} related more to the thickness of membrane and so could provide further valuable information into membrane characteristics which would potentially aid diagnosis. These findings correlate well with Stam et al (1991) which was performed in the absence of NO gas, one could conclude from this that the presence of NO has no impact on the TL_{CO} measurement. This could be a significant finding for the development of the TL_{CO}/TL_{NO} measurement as its common knowledge NO has vasodilating properties which have exploited in cardiac medicine for some time.

1.7.4 Structured Light Plethysmography

The movements of the chest wall and abdomen allow atmospheric air to flow in and out of the lungs creating an exchange of oxygen and carbon dioxide from the pulmonary blood. This flow of air is achieved via passive physical diffusion by creating a pressure gradient between the

atmosphere and the lungs, forcing air to move from high pressure to low thus creating the motion of breathing.

The flow of air is traditionally measured using a pneumotachograph which is a device that detects pressure changes across a fixed resistance via pressure transducers. This change in pressure is directly proportional to the flow of air and is calculated using equation 10 below:

$$\textbf{Equation 10: Flow} = \frac{(P1 - P2)}{\text{Resistance}}$$

The total volume of air in the thoracic cavity can currently be measured via a technique known as body plethysmography. This device is comprised of an air tight chamber of known volume (approximately 500 litres) and a pneumotachograph. By implementing Boyles law which states at a constant temperature, the pressure of a fixed mass of gas multiplied by its volume will remain constant;

$$\textbf{Equation 11: } (PV = k \textit{ or } P_1.V_1 = P_2.V_2).$$

Hence, if a gas in a closed container is compressed at a constant temperature, the volume of the gas will be reduced in proportion to the pressure applied to it.

Structured Light Plethysmography (SLP) is based on the analysis during breathing of the trajectory of a series of light grids at which they intersect. The concept of structured light analysis has been around for many years and is more commonly used in engineering to measure 3D shapes. Zhang (2002)

When applied on the thoracic-abdominal surface of the patient, a computing unit can accurately measure, not only the movement of the whole thoracic-abdominal wall but also the variations of the many compartments. Figure 1.12 shows the thorax (green) and the abdomen (blue), this allows the relative contribution of movement of these areas to analyse.

Movement of the thorax is often referred to as RCC or rib cage contribution which will be one of the parameters analysed as will the upper rib cage contribution (URCC). This could indicate which areas are moving and therefore ventilating better than others and indicate which areas of the lungs are most affected but emphysema.

The timing of the movement of these areas can also be analysed using the SLP device to determine if two regions are moving in symmetry and is known as the phase angle. The Phase Angle (PA) provides a measure of synchronicity between two regions (e.g. Thorax and Abdomen or Upper and Lower Thorax). A phase of 0 (or 360 degrees) means both regions move perfectly in synchrony with each other and a phase of 180 means one region moves in complete paradox to the other. Respiratory phase angle may offer an indication of disease severity in COPD.

This study will explore the phase angle (synchronicity of movement) of the rib cage vs the abdomen (PARA), the upper rib cage vs lower rib cage (PAUL) and the upper rib cage vs abdomen (PAUA).

This could potentially lead to the detection, for example, of asymmetries in the action of respiratory muscles that result in paradoxical breathing caused by COPD as show by Gilmartin et al (1984) as discussed earlier in chapter 1.2. Usher-Smith et al (2009)

This is a new and exciting innovative examination instrument. It is non-invasive, accurate, and easy to use. Unlike traditional plethysmography technologies, is not affected by humidity and temperature variations and can be used to easily perform measurements for extended periods of time.

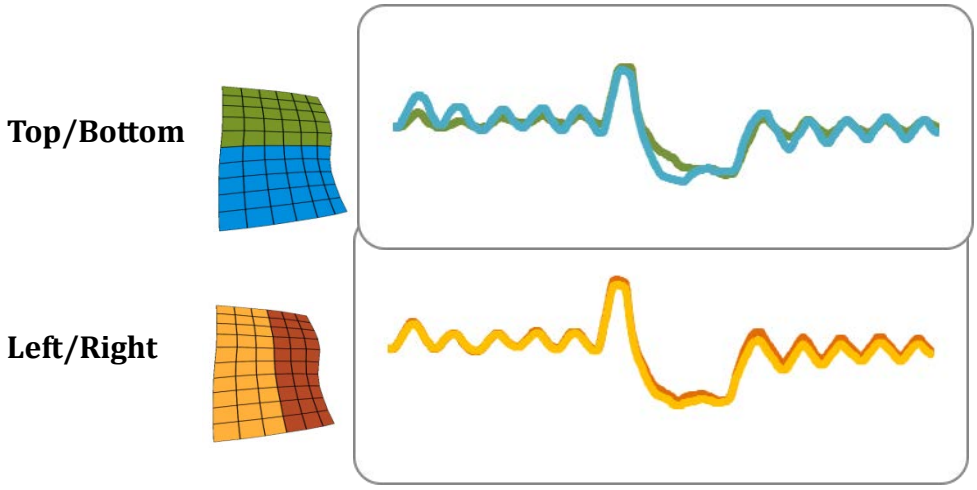

Figure 1.12 – Data Output from SLP. Left image shows the different regions of the thoracic cavity being analysed, right image showing the relative movements of those areas.

1.8 Hypothesis and Aims

1.8.1 Hypothesis

This study was designed to determine whether a measurable change in TL_{CO}/TL_{NO} and SLP from a sitting to a supine position can be observed in patients with A1AD and if so, whether this might be a useful diagnostic and monitoring approach to the assessment of lung disease.

We hypothesise that no clinically significant changes in TL_{NO} or K_{NO} should be observed as no changes in membrane are expected to occur with change in body position. However, expected changes in VA particularly in our A1AD cohort may have an impact on these parameters. TL_{CO} and Kco should increase with perfusion, as these parameters are dependent on pulmonary capillary blood volume. SLP should show differences in breathing patterns from sitting to supine as the diaphragm has a greater role in ventilation when in a supine position. Detectable differences in regional movements were also expected as the emphysematous changes cause the destruction of elastin thus diminishing the lungs ability to expand and contract. Both measurements of positional gas transfer and SLP should therefore, provide valuable and reliable information regarding how various lung regions are affected over time, whilst also

providing information on their functionality.

1.8.2 Aims of the study.

I. To establish the optimum time for sufficient perfusion of the apices of the lungs, resulting in an increase in gas transfer measurements. The duration for which a subject should be placed in the supine position before a stable and representative measure of gas transfer can be made, is not known.

II. To investigate the effect of breathing patterns in healthy normal controls, to analyse the results that both techniques produce under normal physiological conditions and to validate against current understanding.

III. To measure gas transfer by TL_{CO} and TL_{NO} and breathing patterns by SLP in different body positions in patients with A1AD, a disease known to have a differential effect on the lung apices and bases.

IV. Basal and apical disease states in both the sitting and supine positions to see if there are noticeable differences that may save the need for repeated CT scans to detect apical/basal involvement.

2.0 METHODS AND SUBJECTS

2.1 Subjects

95 subjects with a confirmed diagnosis of Alpha-1 Antitrypsin Deficiency (A1AD) and 53 healthy adults were recruited and studied both in seated and supine postures.

All subjects recruited onto the current study had their height, weight, age and gender documented before testing took place. Prior to any measures being performed, each subject had an explanation of all the tests to be performed, their date of birth verified, lists of current medications reviewed (both respiratory and non-respiratory) and any documented or current infection status recorded.

All subjects gave written, informed and witnessed consent and have the approval of the West Midlands - South Birmingham ethics committee and University Hospitals Birmingham NHS Foundation Trusts R&D department.

All participants that entered into the current study were negative for all exclusion criteria, which are listed below;

- Any known cardiovascular disease
- Unstable cardiovascular status (within 6 weeks)
- Any known pulmonary disease
- Any blood defects
- Neuromuscular disease or any other disease/condition effecting muscle strength
- Current chest infection (at time of study).
- Haemoptysis of unknown origin (within 6 weeks)
- Pneumothorax (within 6 weeks)
- Thoracic, abdominal, or cerebral aneurysms

- Recent surgery of thorax or abdomen (within 6 weeks)
- Recent eye surgery (within 8 weeks)
- Presence of an acute disease that might interfere with test performance (e.g. nausea or vomiting)

In addition the healthy control cohort also had the following exclusion criteria;

- Spirometry measurements outside of normal standard residuals (± 1.64).
- Significant smoking history (>20 pack years)
- Current smoker

2.1.1 Alpha 1 Antitrypsin deficient (A1AD) subjects

All 95 A1AD subjects were recruited to the current study from the Antitrypsin Deficiency Assessment and Programme for Treatment (ADAPT), which is the UK register A1AD sufferers. All attended the department as part of their annual routine review and so was able to perform tests for the current study on the same day.

Spirometry, Lung Volumes via Helium Dilution, Body Plethysmography, Single Breath Gas transfer using Carbon Monoxide (TL_{CO}), Ear Lobe Capillary Sample (ELCS) and High-resolution computed tomography (HRCT) were conducted prior to the study measurements.

Out of the 95 subjects 89 performed Structured Light Plethysmography (SLP) in both sitting and supine postures (6 unable to perform due to technical reasons) and 64 additionally performed Single Breath Gas Transfer Using Nitric Oxide (TL_{NO}) in both sitting and supine postures.

2.1.2 Healthy Control Subjects

Our non-obstructive healthy control cohort was recruited mainly from various hospital departments including the Lung Function and Sleep Department, ADAPT, Clinical Trials and Pathology Laboratories. Spirometry was performed on all subjects and deemed non-obstructive if their Forced Expiratory Volume after 1 Second (FEV1) was above - 1.64 Standard Residuals (SR).

The A1AD and control subjects were well matched for height, weight and BMI but showed a slight discrepancy in age which could cause a decrease in values of gas transfer. (See Table 2.1).

Table 2.1: Anthropometric Data

		CONTROLS		A1AD
MALES	n	24		56
	HT	1.8 ± 0.1		1.8 ± 0.1
	WT	79.2 ± 13.3		80.6 ± 15.2
	BMI	25.2 ± 3.6		25.2 ± 3.9
	Age	43.3 ± 22.4		58.1 ± 10.4
FEMALES	n	29		39
	HT	1.6 ± 0.1		1.6 ± 0.1
	WT	69.2 ± 14.3		74.2 ± 16.5
	BMI	26 ± 5.8		28.1 ± 6.2
	Age	42.5 ± 15.4		54.4 ± 11.6
TOTAL	n	53		95
	HT	1.7 ± 0.2		1.7 ± 0.2
	WT	72.5 ± 16.6		77.4 ± 17.7
	BMI	25.2 ± 5.7		26.2 ± 5.6
	Age	42.5 ± 18.4		56.2 ± 11.9

All values presented as mean values ± 1 SD. HT = Height in metres, WT = Weight in Kilograms. BMI = body mass index ($kg.m^2$) Age in years.

2.2 Study protocols

All measurements were conducted at the Lung Function and Sleep Department, Queen Elizabeth Hospital, Birmingham.

The Lung Function Tests (LFT's) performed were predominantly on equipment operating in accordance with the American Thoracic Society (ATS)/European Respiratory Society (ERS) 2005 standards using a Jaeger Master Screen Pro lung function system (Jaeger Ltd, Hochberg, Germany) with a flanged mouthpiece to reduce possible leaks at the mouth. Any deviation from these standards will be outlined and justified throughout this chapter.

The tests performed are listed in Table 2.2.

Table 2.2: Tests performed

	CONTROLS		A1AD
SPIROMETRY	✓		✓
HELIUM DILUTION	✖		✓
BODY PLETHYSMOGRAPHY	✖		✓
HRCT SCAN	✖		✓
EAR LOBE CAPILLARY SAMPLE	✖		✓
SEATED TL_{CO}/TL_{NO}	✓		✓
SUPINE TL_{CO}/TL_{NO}	✓		✓
SEATED/SUPINE SLP	✓		✓

HRCT scan = High Resolution Computer Tomography, TL_{CO} = Transfer factor of the lungs using Carbon Monoxide, TL_{NO} = Transfer factor of the lungs using Nitric Oxide, SLP = Structured Light Plethysmography.

Alpha-1 Anti-Trypsin Deficiency subjects had CT scans measured within two years prior to the study, which is held on their hospital records. All testing took place between the hours of 09:00 – 17:00 with the ambient temperature and pressure recorded.

The order of testing remained the same throughout with the same physiologist performing the tests on each participant. The same equipment was also used throughout the study to eliminate the possibility of variations between equipment.

2.2.1 Calibration & Quality Control

Equipment within the Lung Function Department is regularly calibrated/verified and monitored within a quality control (QC) program to ensure quality patient results are generated which are both reliable and reproducible.

All calibration/verification QC records are recorded and stored within the department for future reference.

A quality control program can employ both biological quality control methods and physical quality control methods.

Physical quality control is performed daily with the use of calibration syringes; a standard 3 litre syringe is used. Syringes are calibrated within +/- 1.5% of the measured value biannually.

Physical quality control is repeated after cleaning and reassembling the equipment, at the start of each spirometry session, whenever temperature fluctuates by >4^{o}C, after every ten patients in a busy clinic or if there are any doubts about sequential values in a stable subject.

Biological quality control is performed by healthy staff members of the Lung Function and Sleep department on a weekly basis. Staff test themselves on a Monday and Wednesday morning to ensure that the measurements obtained, fall within their reference range for that piece of equipment. The reference range is determined by statistical analysis of 10 measurements made over a 10 day period. In cases where biological quality control measurements are outside the appropriate reference range, the equipment is then thoroughly examined for any issues which may be causing inaccurate measurements and any required rectifications are carried out. A second staff member can perform QC and if this too, is outside their reference range, a senior member of staff is informed. This equipment is then taken out of use and the relevant company is called to arrange for an engineer to visit or it is sent to medical engineering for repair. An example of serial results is shown in Figure 2.1.

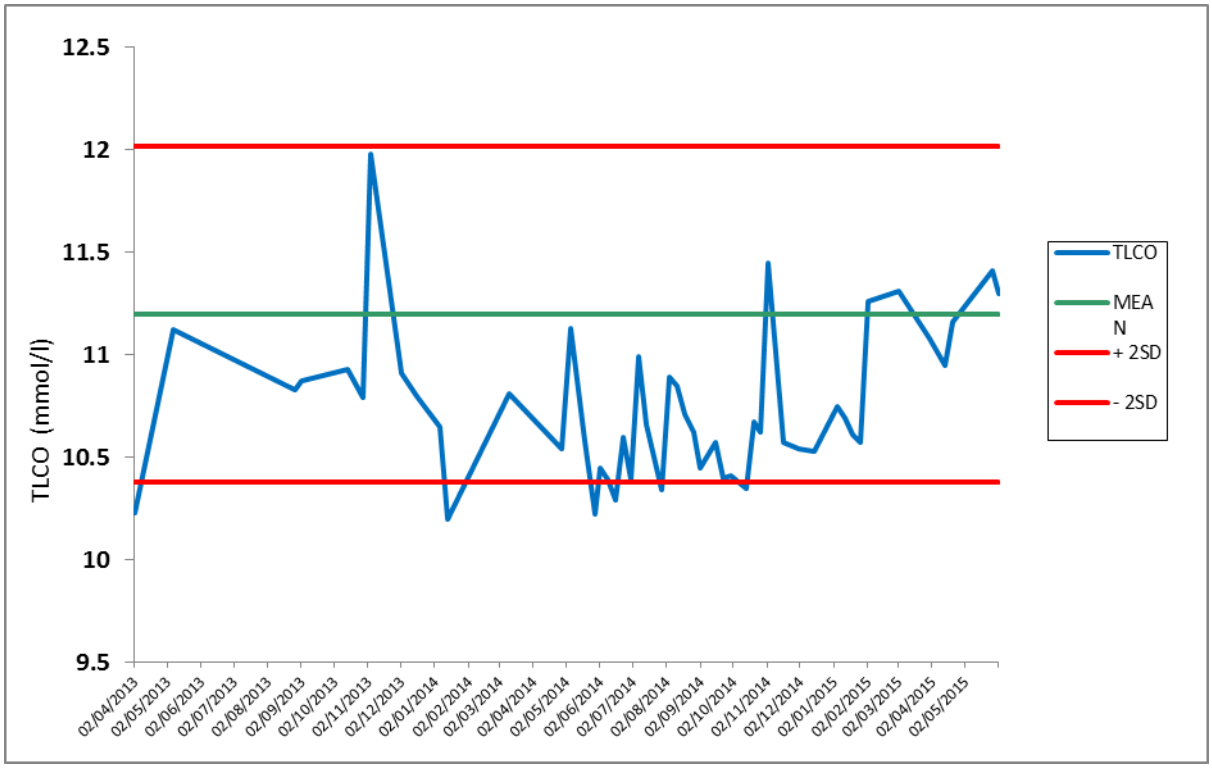

Figure 2.1: A Levey-Jennings graph to show serial TL_{CO} data performed bi-weekly as part of the departmental Biological QC program. The graph enables the user to establish whether the results obtain fall within a ± 2SD range of the mean value.

2.2.2 Spirometry

All subjects performed spirometry using the BTS/ARTP 1994 standards to either confirm the absence of disease or to confirm the severity of a known respiratory disease, in the current study this will be emphysema as a result of A1AD.

Relaxed VC's were the first measurements to be conducted. Each subject was instructed to breathe tidally through the mouthpiece for a minimum of three breaths, then encouraged to inhale maximally to TLC followed by an exhalation at a comfortable speed to RV then return to tidal breathing. A minimum of three manoeuvres within 100ml or 5% (which ever was greater) were performed with the highest value being quoted.

Once this was achieved, each subject proceeded to perform forced spirometry. (FEV1 and FVC's) Subjects were again instructed to breathe tidally through the mouthpiece for a minimum

of three breaths. Before inhaling maximally (to total lung capacity) and rapidly expiring with a forced single breath manoeuvre, keeping the glottis open and relaxed.

Subjects were encouraged, whilst maintaining an upright posture, to maintain a continual blow until all air could was expelled. The end of the test, which defines FVC and VC, occurs when less than 0.05 litres has been expired over a period of 2 seconds or for clinical reasons, the operator had to terminate the procedure.

This was repeated for a minimum of three manoeuvres to achieve three FEV1 within 100ml for smaller volumes or 5% and FVC within 100ml for smaller volumes and 5% and two PEF within 20L/min (BTS/ARTP 1994).

The current study applies several rejection criteria for the performance of spirometric measurements. These are listed below;

- A leak at the mouth
- An obstructed mouthpiece due to tongue or false teeth
- A poorly co-ordinated start to the manoeuvre
- A cough within the first second of the manoeuvre or a later cough if it is deemed to have interfered with the blow
- Early termination of the blow
- The subject did not inspire to TLC
- The expiratory effort was sub maximal
- The maximal expiration should last at least six seconds or until there has been no volume change for at least one second (Miller et al, 2005).

2.2.3 Lung volumes using Helium Dilution

Initially, subjects breathed tidally through an open circuit via the mouthpiece and using nose pegs until a stable and tidal breathing cycle was achieved. Once a visually stable tidal breathing had been established the subjects connected to the circuit at the end of a tidal breath out (the position of FRC) and instructed to steadily exhale to RV. Once they could no longer exhale, they returned to tidal breathing ensuring they kept a tight seal. The subjects continued to breathe tidally until equilibrium was achieved. This was indicated by a helium concentration varying less than 0.02% within 30 seconds. If equilibrium was not reached after 10 minutes the test was stopped and the final He concentration noted. If oxygen was added to the system the operator waited until the helium concentration had fully stabilised before the FRC was recorded. The subject was then instructed to perform a relaxed VC manoeuvre. Manual calculations could then be performed to obtain RV and TLC and the results were recorded.

(J. Wanger et al, 2005)

2.2.4 Lung volumes via Whole Body Plethysmography

Subjects were instructed to sit in an upright position with the chin perpendicular to the trunk of the body and both feet positioned on the floor in front of the seat. The subject was seated inside the cabin and the door was sealed. The operator then established that the intercom was working by ensuring that the patient could hear them. The plethysmograph was then vented to ensure the pressure within the cabin stabilised. Once the mouthpiece and nose pegs were in place, the subjects were then instructed to place their hands flat onto the checks to minimise volume changes in the buccal cavity and to start breathing tidally. The shutter was activated at the end of a tidal expiration, temporarily occluding the flow of air. Subjects were then

encouraged to continue making efforts against the shutter for three breath cycles to allow the pressure at the mouth within the body box to be recorded.

The shutter was then released, and the subject was instructed to perform a relaxed EVC manoeuvre. (J. Wanger et al, 2005)

A minimum of three reproducible results, including Thoracic Gas Volume (TGV) and Expiratory Vital Capacity (EVC) measurements, were obtained within 5%. The mean of at least 2 consistent sets of results (within 5%) were then reported.

2.2.5 Single breath Gas Transfer using Carbon monoxide (TL_{CO}).

Each parameter was obtained with the device Masterscreen PFT PRO (Jaeger; Hochberg, Germany) via the single-breath method used as standard within the department. The following settings were used for both cohorts: Washout volume – 900ml, Sample volume - 600ml (settings may be changed to a minimum washout volume of 600ml, if VC is <1.5L). Any changes to the settings were noted on the worksheet.

The nature of the test was explained to the subject, followed by a demonstration which showed them any manoeuvres required to perform the test. Subjects were then sat in an upright position looking straight ahead with their feet flat on the floor and hands on the arm rests of the chair. Nose pegs were placed on all subjects and they were instructed to breathe tidally through the open circuit via a flanged mouthpiece. After a minimal of five tidal breaths, subjects were then instructed to exhale fully until they achieved residual volume (RV) at which point, a demand valve was activated which enabled a certificated gas mix of 0.28% CO, 9% He, 19.05% O2, balanced in synthetic air (BOC Limited, Surrey, UK) to be inspired.

Next, the subjects inhaled maximally to at least 90% of their biggest VC, obtained by performing the initial spirometry as described above. This should be achieved in at least 1.5-2secs in our healthy normal cohort. In subjects with FEV_1/FVC <50%, the inspire time should be achieved in at least 4secs with a total volume inspired >95%.

Once the subject achieved full inspiration they were required to hold their breath for about 10 seconds. Subjects were encouraged to relax against the shutter and instructed not to breathe in or out against it. The breath-hold time was reduced depending on the subject's dyspnoea, but was never set to less than 6 seconds. Mouth pressure must not exceed 40.8 cmH_2O.
Finally, once the 10 seconds of breath holding had elapsed, each subject exhaled as far as possible. The initial portion of gas exhaled was discarded (washout) as this was from the anatomical and instrument dead space (BTS/ARTP 1994).

Anatomical dead space is defined as the portion of the lungs used to conduct air or gas to the alveoli, no gaseous exchange takes place here therefore this volume must be subtracted from the final results. Instrumental dead space is the volume of gas within the system, from the source of the gas (in this case the gas cylinder) and subject's mouth. The system used in the current study has a dead space of 240ml which must be accounted for otherwise TL_{CO} will be overestimated (J West, 2011).

The second portion (sample) is assumed to be representative of typical alveolar gas. If the subject was dyspnoeic, exhaling to just beyond sample was sufficient. On completion of the manoeuvre, the subject was disconnected from the circuit and then required to remain seated for the duration of 4 minutes to allow the gases to wash out of the lungs (N. MacIntyre, 2005).

A maximum of 3 efforts were made with the highest two values within 5% being quoted for TL_{CO}, K_{CO}, and VAeff, as per departmental protocol. The need for this is partially reflected in the occasional differences between the first and the two subsequently determined values often witnessed during routine gas transfer measurements. To take into account the time requirements of clinical practice, repeated measurements would be performed at 4-min intervals.

All subjects had their transfer factor re-measured after 10, 15, 20, 25 & 30 minutes of lying supine respectively (see results section). The mouthpiece assembly has been rotated 180 degrees to allow these measurements to be performed in a supine position. Only one measurement for each time category was performed due to the total number of measurements being performed. This should minimise the possibility of creating a backpressure of CO due to gas retention by haemoglobin.

2.2.6 Single breath Gas transfer using Carbon Monoxide (TL_{CO}) and Nitric Oxide (TL_{NO})

By introducing the NO test gas to the standard gas mix used for standalone TL_{CO} measurements (as described in the previous chapter) it permits the parameters of TL_{NO} and K_{NO} to be measured simultaneously with TL_{CO} and K_{CO}. This allows alveolar membrane (D_M) and pulmonary capillary volume (Vc) component differences. (H. Guenard et al, 1987)

All gases were prepared in a 7 litre reservoir bag which the subjects inspired from when instructed. This had to be performed immediately after all gases are present in the bag to ensure no reactions between substances took place.

Like the previous measurement of TL_{CO} subjects breathed tidally through the mouthpiece with nose pegs on. They were then instructed to exhale until they achieved residual volume (RV) at which point the equipment system opened and connected to the reservoir bag which enabled the subject to inspire a gas mix of 50 ppm NO (*INO Therapeutics, Lindingo, Sweden)*, 0.28% CO, 9% He, 19.05% O2 balanced in synthetic air (BOC Limited, Surrey. UK). Subjects again were encouraged to inspire fully to total lung capacity (TLC) and hold their breath for 10 seconds in a relaxed state allowing the CO and NO to diffuse into the pulmonary blood. Once the time had elapsed the subjects were again instructed to exhale fully**.**

Once more like the measurements for standalone TL_{CO} a maximum of 3 efforts were made with the highest 2 TL_{NO}, K_{NO} and VAeff measurements, within 5% quoted as per departmental protocol. All subjects were then moved into a supine position and their transfer factor re-measured after 15 minutes, which was determined as the optimal time from Study 1 of the current thesis which is described below.

2.2.7 Structured Light Plethysmography (SLP)

This took place between TL_{NO}/TL_{CO} manoeuvres and required passive tidal breathing from the subjects. They all wore tightly fitting, plain white, slightly elasticated; Nike® sports tops to wear during the measurement of SLP. This ensured maximal accuracy of the measurement by eliminating differences in material, colour and texture that may have altered the reflection of light produced by the SLP device. Once the top was in place, subjects were then instructed to sit in an upright position, place their hands upon the armrest of the chair and look straight ahead. The operator aligned the projected light grid onto the participant's chest and abdomen. A blue cross in the centre of the grid aligned with each subject's xiphoid process

(located at the inferior end of the sternum as show below in figure 2.2. This ensured a standardised placement of the grid on all subjects in both postures and is demonstrated in the illustration below. (Levai et al, 2011).

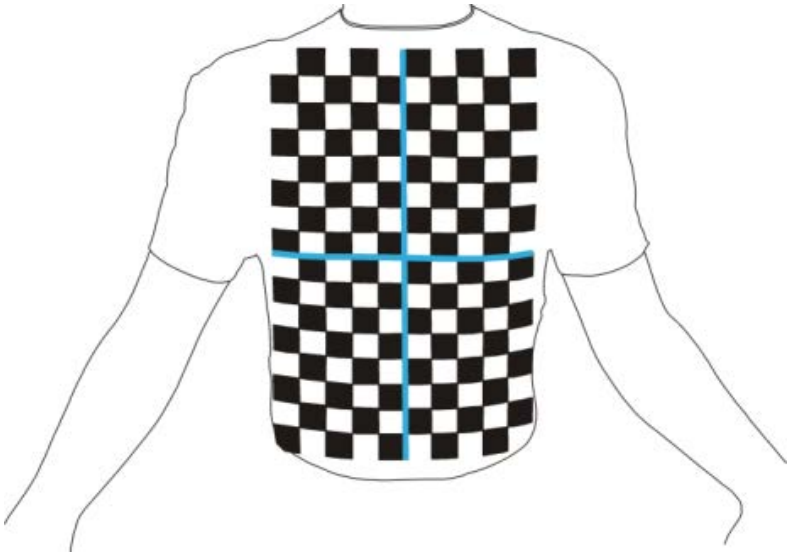

Figure 2.2 - A diagram to show the ideal placement of the light grid produced by the SLP device with the blue cross being placed directly onto the xiphoid process. (Taken from the Thora 3Di instruction manual).

Once the cross was aligned in the correct position each subject was advised to remain relaxed, still, avoid talking and breathe tidal. SLP was then recorded for 5 minutes (approximately 50 simultaneous tidal breaths) while the data was captured. The movement-time trace was displayed in real time as the subject's thorax and abdomen expanded and contracted. The system processed the data which permitted the quality of the measurement to be determined in real time by a simple colour coding system. Once the data was collected in the sitting position the same process was then carried out in the supine position. (Levai et al, 2011)

2.3 Statistical analysis

Statistical analysis was conducted using SPSS statistical program (version 16.0, Chicago, USA) or GraphPad Prism (version 5.03, La Jolla, USA). The Initial analysis was performed using a Kolmogorov-Smirnov test to establish if data groups were normally distributed.

The distribution of data dictated what statistical tests could be performed. Parametric tests for normally distributed data or non-parametric tests if the data was not normally distributed. Group comparisons were then reliant on the number of data groups being compared and if the data groups were from different patients (unpaired) or separate data from the same patients. (paired; e.g. pre and post-intervention)

Finally, the use of a one-tailed or two-tailed test. If a group's results were predicted to be different, such as an increase from baseline following an intervention such as lying supine, the test was one-tailed. In occurrences where the outcome could not be predicted due to lack of previous data, a two-tailed test was used. This resulted in the "p" value being twice the value of a one-tailed test ensuring the probability of a chance outcome to double.

Data that were normally distributed, either a student's t-test (paired or unpaired as described above) or an ANOVA test (if 3 or more groups were compared) followed by Tukey's test to show which groups were significantly different from each other. Data found to be not normally distributed, either a Mann-Whitney U test (paired or unpaired as described above) or a Kruskal-Wallis test (if 3 or more groups were compared) followed by Dunn's test to show which groups were significantly different from each other, were performed.

In the current thesis, a p value of 0.05 or less ($p \leq 0.05$) was classified as statistically significant.

To make certain the trails were sufficiently powered, a power calculation shown below in Equation 12 was executed to generate sample size were performed using the following formula; **Equation 12: $n = 1+2C \times (s / D)$**

D is the smallest difference detected, **s** represents the standard deviation of the observations and C = 7.85 to provide an 80% power of detecting a difference at the 5% level of significance (Snedecor & Cochran 1989).

3.0 RESULTS

3.1 STUDY 1 - The change in TLco & Kco from sitting to supine postures in healthy controls.

We were interested in using the change in gas transfer from sitting to supine in patients with A1AD, but were unable to find any published studies showing how long a subject should be supine before a stable representative measurement could be made. Therefore, we designed and conducted a study to establish the optimum time for the apex of the lungs to be sufficiently perfused, leading to an increase in the gas transfer measurements. We performed this study on 14 healthy subjects (11F:3M; Ages: 22-51 years).

The results are shown in Table 3.1 and Figure 3.1.

Table 3.1 - Summary of gas transfer data using CO from a seated to supine posture in 14 healthy subjects.

	Baseline (seated)	+ 10mins (supine)	+15mins (supine)	+20mins (supine)	+25mins (supine)	+30mins (supine)
TLco	8.73 (0.48)	+1.16 (0.17)	+0.76 (0.19)	+0.78 (0.18)	+0.61 (0.19)	+0.74 (0.18)
% Change		+14%	+9%	+10%	+8%	+9%
Kco	1.57 (0.04)	***+0.32 (0.04)****	***+0.24 (0.04)****	+0.24 (0.04)	+0.23 (0.3)	***+0.24 (0.04)****
% Change		***+ 21%****	***+15%****	+15%	+14%	***+15%****
VAeff	5.55 (0.33)	-0.25 (0.04)	-0.29 (0.04)	-0.24 (0.03)	-0.27 (0.07)	-0.27 (0.05)
% Change		-6%	-6%	-5%	-5%	-6%

Values shown as Mean (± SEM), time fractions expressed as absolute change from the baseline. TLco in mmol/kPa/min; Kco in mmol/kPa/min/L; VAeff in Litres.. * p ≤ 0.05 indicating a significant change from baseline measurements.

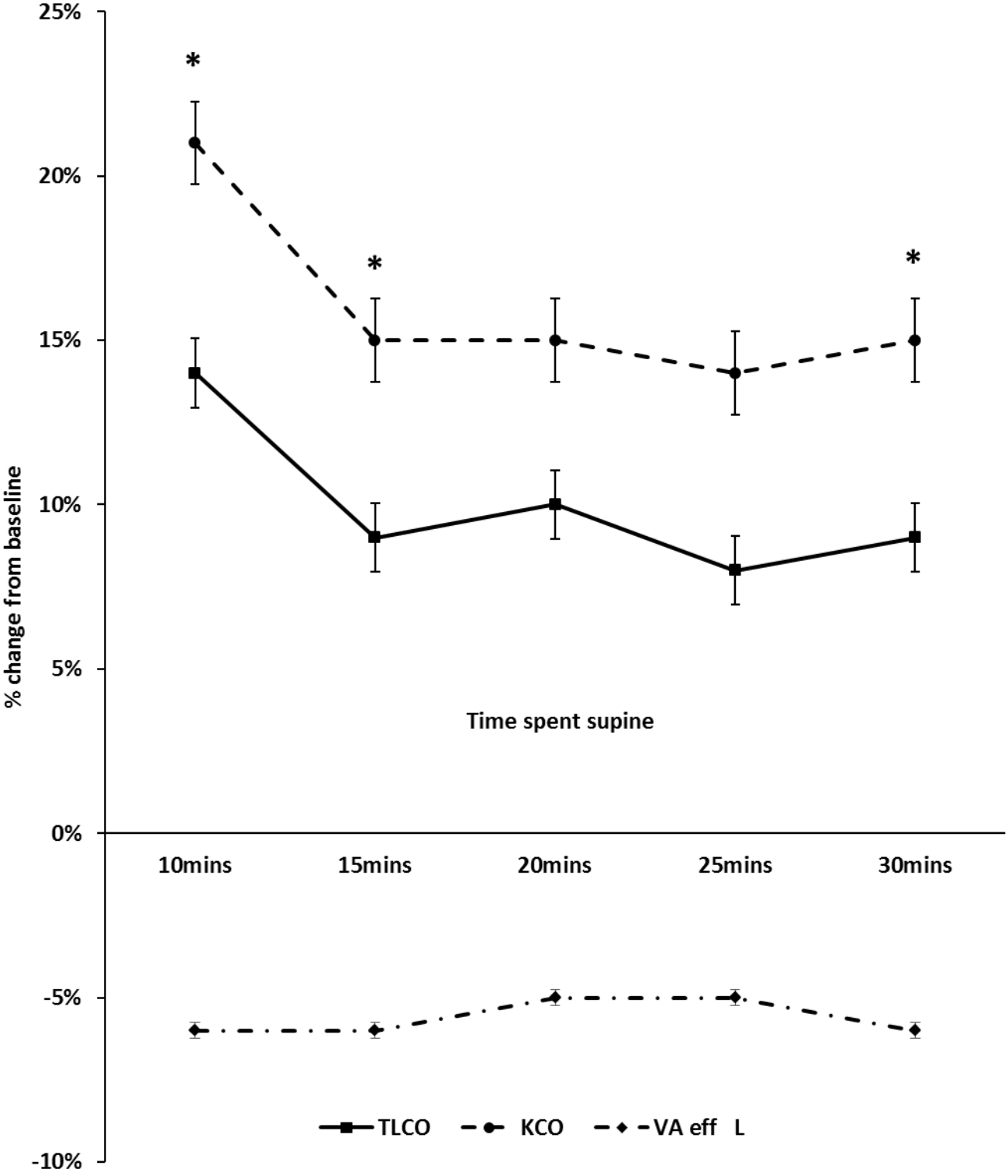

Figure 3.1 - A line graph summarising the mean percentage change of single breath gas transfer manoeuvre using CO from a seated to supine position in healthy subjects. Values shown as Mean ± 1 SEM of the percentage change from baseline. TLco in mmol/kPa/min; Kco in mmol/kPa/min/L; VAeff in Litres. % change from baseline. * p ≤ 0.05 indicating a significant change from baseline measurements.

3.1.1 STUDY 1 DISCUSSION

This study has shown that both TL_{CO} and K_{CO} had a peak increase after 10 minutes of lying supine which can be explained by an accommodation through an accommodation through an increase in pulmonary perfusion taking place immediately in a supine position. The variance in blood flow from the apex to the base of the upright human lung has been well described. Blood flow increases down the lungs as intravascular pressure increases, an effect of the hydrostatic pressure building from apex to base. This creates a positive transmural pressure; pulmonary capillaries are open reducing blood flow resistance allowing more recruitment and distension of blood vessels at the base allowing good perfusion to the region, hence the rise in both TL_{CO} and K_{CO} together. (Lewis et al, 1960)

In the sitting position intravascular pressure decreases with distance up the lung, so there will be a point at which the pressure inside the pulmonary capillaries becomes inferior to alveolar pressure thus creating a negative transmural pressure. The blood vessels collapse under the alveloar pressure and no blood will flow, as seen in the apex of the upright human lung effectivly causing 'alveolar dead space' (ventilated but not perfused), (West et al, 1964).

However, once supine the apex of the lungs have been shown to have greater levels of perfusion as blood shifts from the systemic system into the pulmonary system (Stam et al, 1991). This hydrostatic redistribution of blood increases intravascular pressure at the apex of the lungs. Alveolar pressure remains constant creating a positive transmural pressure opening the vessels. This reduces blood flow resistance allowing perfusion to occur at this region.

The stablisation of TL_{CO} & K_{CO} by 15 minutes from baseline was chosen as the optimal time for testing gas transfer in a supine position and so agrees with one previous published study (Verbanck et al, 2009).

However, earlier studies looking at postural change demonstated a variety of stablisation times. Some groups have taken measurements around 5 minutes (Peces-Barba et al, 2004 & Rohdin et al, 2003) but the current study shows that measurements taken before 15 minutes are transient and are unsuitable for making these supine gas transfer estimations. Other studies have taken measurements at 30 minutes (Terzano et al, 2009) which this current study shows the measurements remaining stable for this period of time so results should not be affected. In order to keep the study time to a minimal (for ethical and practical reasons) 15 minutes was used for all further investigations.

Whilst TL_{co} changes showed no statistically significant differences for either 10 or 15 minutes supine and correlates well with previous work (Verbanck et al, 2009), the change can be considered clinically significant because it falls outside the usual 5% variability of the test and subject as discussed previously in section 2.2.4 of the methodology. Similarly K_{CO} showed both statistically and clinically significant increases for both time frames and again agrees with previous work (Peces-Barba et al, 2004).

The expected decreases in VAeff by 5% from sitting to supine across the entire time frame confirmed the previous findings from studies by Behrakis (1983), Svanberg (1957) and Moreno (1961) However, this was not deemed clinically significant as this was within the 5% limits. This explains the different responses between TL_{CO} and K_{CO} as TL_{CO} is dependent on VAeff measurements, so that decreases in VAeff although slight could be inhibiting any increase in TL_{CO} due to relative perfusion increases at the alveolar level. This observation is discussed later in the patient studies (studies 3, 4 & 5).

3.2 STUDY 2 -The change in TL_{CO}, TL_{NO}, K_{CO} , K_{NO} & their ratios from sitting to supine in 32 healthy subjects.

Tables 3.2 and 3.3 show the anthropometric and spirometric data from the 32 healthy control subjects respectively. Gender and age were better matched than in study 1 which may be an important difference when comparing the results with study 2. Again, all spirometric data showed all healthy controls presented no evidence of underlying airflow obstruction.

Table 3.2 - Anthropometric Data of 32 healthy control subjects.

Sex	Age (years)	Height (m)	Weight (kg)	BMI
15M/17F	32.2 (9.40)	1.70 (0.12)	74.4 (13.6)	25.6 (4.36)

Values shown as Mean ± 1 SD.

Table 3.3 – Spirometry data from 32 healthy control subjects.

	FEV1 (L)	FEV1 (% Pred)	FEV1 (SR)	FEV1/FVC (%)	FEV1/FVC (% Pred)	FEV1/FVC (SR)
MEAN	4.10	114.7	1.10	79.1	84.3	-0.40
± 1 SD	1.14	4.31	0.31	6.30	6.86	0.85

The results in Table 3.4 and Figure 3.2 show that TL_{CO} and K_{CO} increased and stablised by 6.1% and 12.6% respectively by 15 minutes and agreed well with study 1. The VAeff again decreaseed by 5% from sitting to supine as it did in the previous study.

The same table and figure also indicated TL_{NO} and K_{NO} did not alter within the first 15 minutes of being supine, however K_{NO} measurements made post 20 minutes did show significant changes peaking after 25 minutes by 10.3%.

Table 3.4 - Summary of gas transfer data using CO & NO from sitting to supine posture in 32 healthy subjects over a 30 minute period.

	Sitting		+10mins	+15mins	+20mins	+25mins	+30mins
TL_{CO}	MEAN	9.21	9.73	9.76	9.57	9.55	9.47
	SEM	0.42	0.45	0.45	0.44	0.47	0.47
	AB Change		0.53	0.56	0.36	0.35	0.27
	%change		5.7%	6.1%	3.9%	3.8%	2.9%
TL_{NO}	MEAN	35.91	35.95	35.85	37.62	37.81	37.25
	SEM	1.58	1.81	1.69	1.93	1.86	1.92
	AB Change		0.04	-0.06	1.71	1.90	1.34
	%change		0.1%	-0.2%	4.8%	5.3%	3.7%
TL_{NO}/TL_{CO}	MEAN	3.92	3.69	3.69	3.92	3.97	3.94
	SEM	0.06	0.07	0.08	0.07	0.06	0.06
	AB Change		-0.23	-0.23	0.00	0.04	0.01
	%change		-6.0%	-6.3%	-0.1%	1.1%	0.3%
K_{CO}	MEAN	1.56	***1.75***	***1.75***	1.72	1.70	1.67
	SEM	0.03	***0.04***	***0.04***	0.04	0.04	0.04
	AB Change		***0.20****	***0.20****	0.16	0.15	0.12
	%change		***12.8%****	***12.6%****	10.4%	9.3%	7.6%
K_{NO}	MEAN	6.10	6.34	6.34	***6.64***	***6.72***	***6.56***
	SEM	0.07	0.10	0.10	***0.11***	***0.15***	***0.13***
	AB Change		0.25	0.24	***0.55****	***0.63****	***0.46****
	%change		4.0%	3.9%	***9.0%****	***10.3%****	***7.6%****
K_{NO}/K_{CO}	Mean	3.89	3.66	3.66	3.90	3.98	3.95
	SEM	0.07	0.07	0.07	0.06	0.07	0.06
	AB Change		-0.24	-0.23	0.01	0.09	0.06
	%change		-6.0%	-6.0%	0.3%	2.3%	1.4%
VAeff	Mean	5.95	5.66	5.65	5.64	5.66	5.65
	SEM	0.24	0.27	0.25	0.25	0.27	0.27
	AB Change		-0.29	-0.30	-0.31	-0.30	-0.30
	%change		-5%	-5%	-5%	-5%	-5%

Values shown as Mean with Standard Error of the Mean ± SEM, Absolute change (AB Change) & percentage change (%change). TL_{CO} & TL_{NO} in mmol/kPa/min; K_{CO} & K_{NO} in mmol/kPa/min/L; VAeff in Litres. The mean sitting value is from two consistent values within 5%. * $p \leq 0.05$ indicating a significant change from baseline measurements.

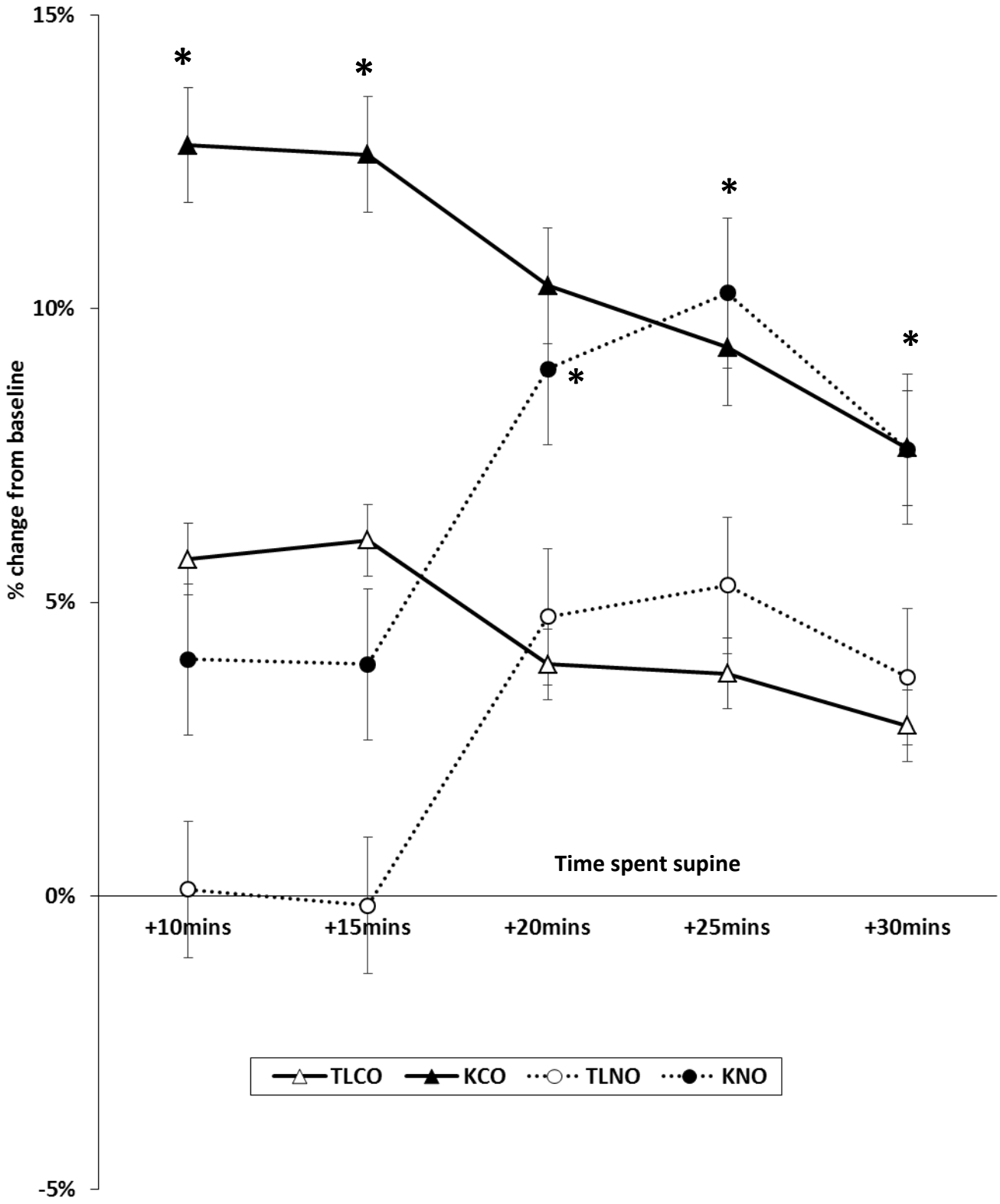

Figure 3.2 - A line graph summarising the mean percentage change of single breath gas transfer manoeuvre using CO & NO from a seated to supine position in healthy subjects. Values shown as Mean ± SEM of the % change from baseline. TL_{CO} in mmol/kPa/min; TL_{NO} in mmol/kPa/min; K_{CO} in mmol/kPa/min/L; K_{NO} in mmol/kPa/min/L. Stabilisation can be seen for all parameters between 10 and 15 mins only, suggesting this as the most stable time frame. * $p \leq 0.05$ indicating a significant change from baseline measurements.

Finally, the ratios of TL_{NO}/TL_{CO} and K_{NO}/K_{CO} were analysed over the same time course. Both TL_{NO}/TL_{CO} and K_{NO}/K_{CO} showed a 6.3% and 6.0% decline respectivally within the first 10 minutes of being supine and remained stable until 15 minutes. However no changes throughout the 30 minute time course were deemed significant. Both ratios then returned to baseline by 20 minutes, this is illustrated in Figure 3.3.

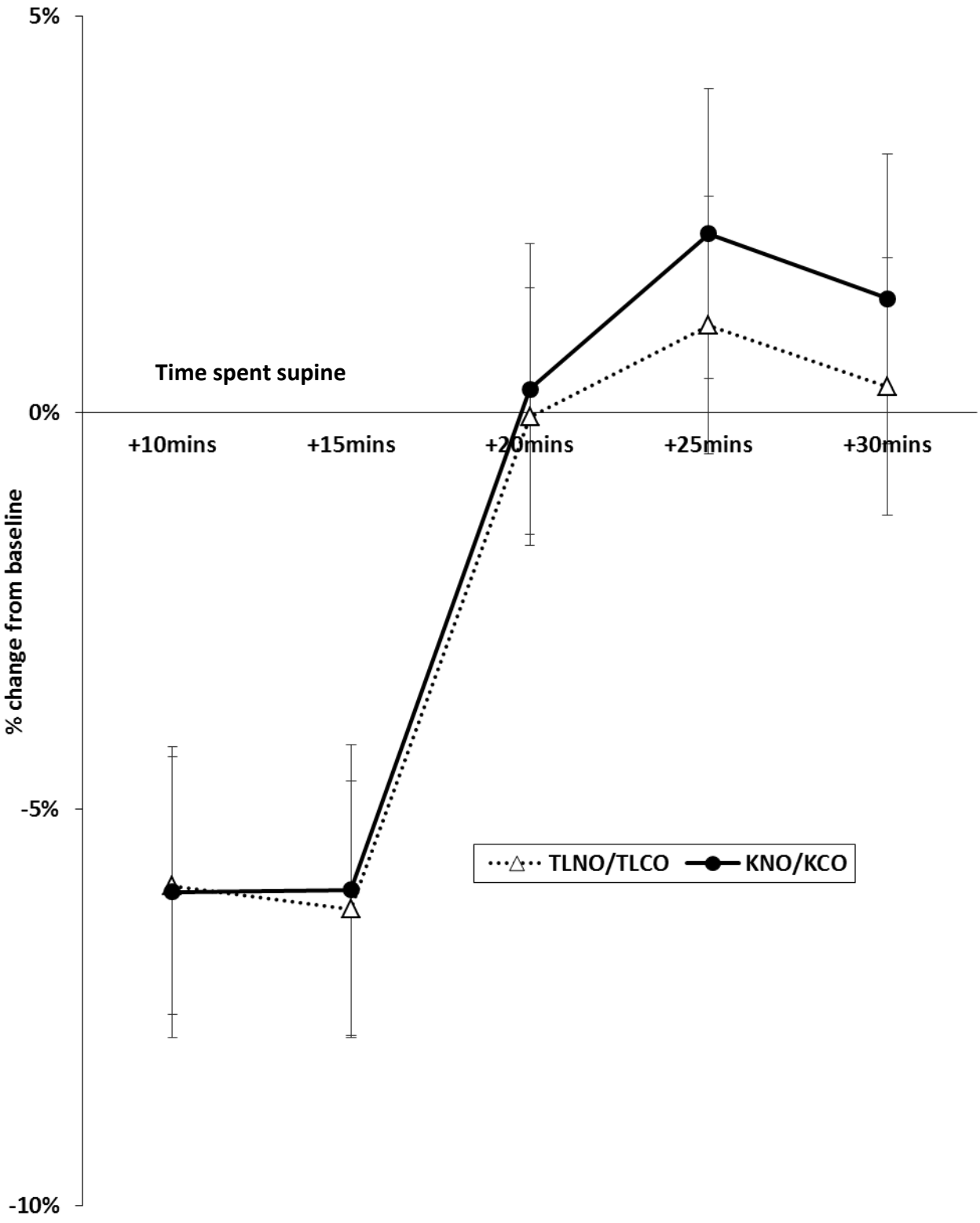

Figure 3.3 - A line graph summarising the mean percentage change of single breath gas transfer manoeuvre ratios using CO & NO from a seated to supine position in healthy subjects. Values shown as Mean ± SEM for the % change from baseline. Stablisation can be seen for all parameters between 10 and 15 mins, suggesting the most stable time frame.

3.2.1 STUDY 2 DISCUSSION

Having established the natural changes that occurred from sitting to supine in TL_{CO}, it was necessary to validate the changes taking place in TL_{NO} and K_{NO} measurements. Recruitment of healthy subjects was increased and the same sitting-supine technique was effectively used using the transfer factor for Nitric Oxide (TL_{NO}/K_{NO}) which when measured simultaneously with carbon monoxide (TL_{CO}/K_{CO}) can be used to effectively estimate alveolar membrane (D_M) and pulmonary capillary volume (V_C) component changes.

The results showed that TL_{CO} and K_{CO} increased and stablised by 15 minutes when subjects were supine and this correlated well with the previous study. Unexpectantly, the measurements of TL_{CO} and K_{CO} taken post 15 minutes both began to return to the baseline readings. There are several possible explanations. It could indicate that the presence of NO changing the physiological processes of CO transfer into the pulmonary circulation causing some form of "compensation". NO is known to be a potent vasodilator and could change blood flow by altering the resistance. It has previously been shown that the presence of NO has no significant influence on gas transfer using CO (Zavorsky et al, 2006) as NO and CO do not interact with each other (Borland et al, 1989) (as discussed in chapter 1.7.3). However, because this current study was not designed to investigate this, it is a theoretical possibility that a reaction between them may have occurred since both gases were held within the same reservoir bag before being inspired. NO is known to react with oxygen (O2), oxidising to NO_2 when in contact with air. It is stored in a gas cylinder and released just before use for this reason. This reaction however, is slow consequently mixing the NO with air in the inspiratory bag does not instantly lead to substantial NO_2 production. NO does react with certain plastics and connections to and from the dispensing bag, unless made of polytetrafluoroethylene.

This raises technical concerns over the technique and the reliability of the measurements being obtained. Further work is needed to confirm this.

An alternative explanation may be due to the differences in the subject group. The same 14 subjects were recruited to both studies with an additional 18 subjects recruited to study 2. These new recruits were mainly males which meant the ratio of males to females changed from 3/11 to 15/17 respectively. These findings could indicate that males and females respond differently to changes in posture due to differences in body size as previously advocated (Kilbride et al, 2003). However, further analysis has shown no differences were apparent between sexes. The mean age of the group did not alter greatly (36.5 years old to 32.2 years old) however, since pulmonary artery pressure can increase with age, blood flow may still decrease towards the apex of the lungs so significantly higher levels of pulmonary blood will still reach this area. This may explain why some subjects displayed minimal changes in gas transfer when altering their posture. If their resting blood pressure was elevated significantly, more blood will be flowing through the apex of the lungs which contribute less overall to diffuson, therefore limiting the changes able to take place under the changes in posture. Further research is clearly required to investigate a full explanation of these findings, which we were unable to do with the current study design.

As in the previous study using CO only, the VAeff again decreased by 5% from sitting to supine. VAeff therefore had no influence over the slight differences in response between the two studies. The likely explanation of the variation is caused either by the equipment or subject variability and from the findings it is likely that since lung volume, age differences, alveolar-capillary membrane thickness are no different, there may be a technique/equipment issue.

TL_{NO} and K_{NO} did not alter within the first 15 minutes of being supine and so supports the initial hypothesis.

However, both TL_{NO} and K_{NO} measurements made beyond 20 minutes did show an increase, peaking after 25 minutes. K_{NO} response to posture was significant suggesting that the lung parenchyma is altering after 20 minutes under the effects of gravity on vasodilation/perfusion. Previous studies (Van der Lee et al, 2007) also showed slight increase in K_{NO}. This could be caused by the matching of ventilation and perfusion changes from a total lung capacity (TLC) level of inspiration to a lower one. Inspiration to TLC recruits all of the lungs but doesn't cause increases in perfusion. Even in a supine posture where perfusion is increased and more evenly distributed there are still areas such as the anterior side that are still relatively less perfused (West et al, 1997). The likely variable factor lies in changing capillary blood volume which may show altered vascular tone in response to NO exposure over time.

For the purposes of methodology, since measurements made using CO and CO/NO gases increased and stablised by 15 minutes the decision was made that stability of all measurements were reached between 10 and 15 minutes once supine and was applied to the subsequent studies.

3.3 STUDY 3 - The change in TL_{CO}, TL_{NO}, K_{CO}, K_{NO} from sitting to 15 minutes supine in 62 subjects with A1AD.

The data from Study 2 established the most stable and therefore, optimal time to obtain single breath gas transfer measurements using both CO & NO was 15 minutes post supine. This finding was then applied to the methodology of study 3 when testing those with A1AD. In order to minimise the potential for back pressure and fatigue of the subjects, a maximum of 4 manoeuvers were performed; 2 baseline measurements within 5% out of a maximum of 3 efforts, then one measurement obtained after the 15 minute time frame set.

The anthropometric data and spirometric data for the A1AD cohort are shown in Table 3.5 and 3.6 respectively.

Table 3.5 – Anthropometric Data of 62 A1AD subjects.

	Age	Sex	pk/yrs	Height (m)	Weight (kg)	BMI
MEAN	56.5	36M:26F	25.1	1.72	79.5	27.0
± 1 SD	10.0		21.9	0.10	17.1	5.8

Table 3.6 – Spirometry data from 62 A1AD subjects.

	FEV1 (L)	FEV1 (%)	FEV1 (SR)	FEV1/FVC	FEV1/FVC (SR)
MEAN	1.90	62.3	-2.50	45.7	-4.62
± 1 SD	0.98	29.1	2.02	18.7	2.64

Table 3.7 shows all gas transfer parameters for both postures are shown and compared to those of the healthy cohort. (*) indicates those chages that showed positive statistical difference ($p \leq 0.05$).

Table 3.7 – Summary of gas transfer data using CO & NO from a seated to 15 minute supine posture in 62 A1AD subjects and compared to previous data for healthy controls.

		HEALTHY		A1AD	
		Sitting	15mins supine	Sitting	15mins supine
TL_{CO}	MEAN	9.21	9.76	4.84	4.64
	SEM	0.42	0.45	0.25	0.28
	Change		0.55		-0.20
	%change		6.2		-5.8
TL_{NO}	MEAN	35.91	35.85	18.62	***16.84***
	SEM	1.58	1.69	0.98	***0.93***
	Change		-0.06		***1.78****
	%change		-0.3		***-10.0****
TL_{NO}/TL_{CO}	MEAN	3.92	3.69	3.84	***3.68***
	SEM	0.06	0.08	0.04	***0.05***
	Change		-0.23		***-0.16****
	%change		-6.0		***-4.1****
K_{CO}	MEAN	1.56	***1.75***	0.90	***0.96***
	SEM	0.03	***0.04***	0.04	***0.05***
	Change		***0.2****		***0.06****
	%change		***12.6****		***6.2****
K_{NO}	MEAN	6.10	6.34	3.46	3.46
	SEM	0.07	0.10	0.15	0.14
	Change		0.24		0.00
	%change		4.0		0.2
K_{NO}/K_{CO}	MEAN	3.95	3.66	3.85	3.68
	SEM	0.06	0.07	0.04	0.05
	Change		-0.30		-0.17
	%change		-7.5		-4.4
VAeff	MEAN	5.88	5.65	5.38	***4.85***
	SEM	0.24	0.25	0.15	***0.16***
	Change		-0.23		***-0.54****
	%change		-4.1		***-10.0****

Values shown as Mean with Standard error of mean ± SEM, Absolute change (AB Change) & percentage change (%change). TL_{CO} & TL_{NO} in mmol/kPa/min; K_{CO} & K_{NO} in mmol/kPa/min/L; VAeff in Litres. The mean sitting value is from two consistent values within 5%. * $p \leq 0.05$ indicating a significant change from baseline measurements.

The change in TL_{CO} in the A1AD group contradicted that of the healthy cohort by actually decreasing by 5.8% compared to an increase of 6.2%. Neither change was deemed statistically or clinically significant but a comparison of the two cohorts showed an obvious difference. It was noted that the VAeff decreased by 10% which is more than twice that observed with the healthy subjects.

K_{CO} like the healthy group increased in the A1AD group by 6.2% however, this level of reponse was more than half when compared to the healthy group's 12.6% improvement.

TL_{NO} also decreased in the A1AD group by 10%, again conflicting with what was seen in the healthy control group which showed no chage (0.3%).

Analysis of K_{NO} showed no change from baseline (0.2%), again like $K_{CO,}$ it is not effected by volumetric changes. This indicates the change in both TL_{CO} and TL_{NO} are based on volumetric changes caused by changes in posture in the A1AD cohort.

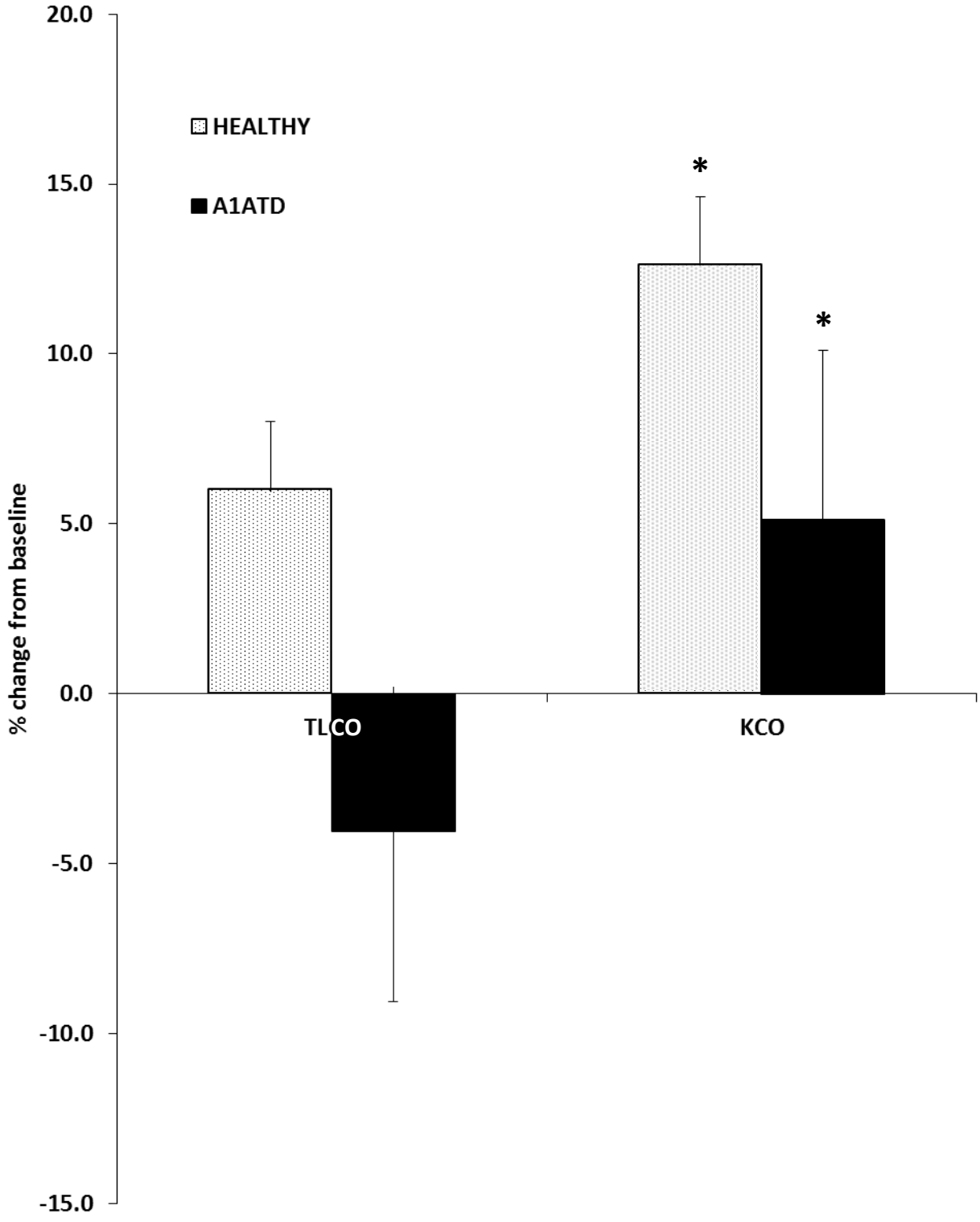

Figure 3.4 - A bar graph summarising the mean percentage change of single breath gas transfer manoeuvre using CO from a seated to supine position in healthy subjects compared to those with A1AD. A difference in response to postural change can be seen between the two cohorts. TLco in mmol/kPa/min; Kco in mmol/kPa/min/L. * p ≤ 0.05 indicating a significant change from baseline measurements.

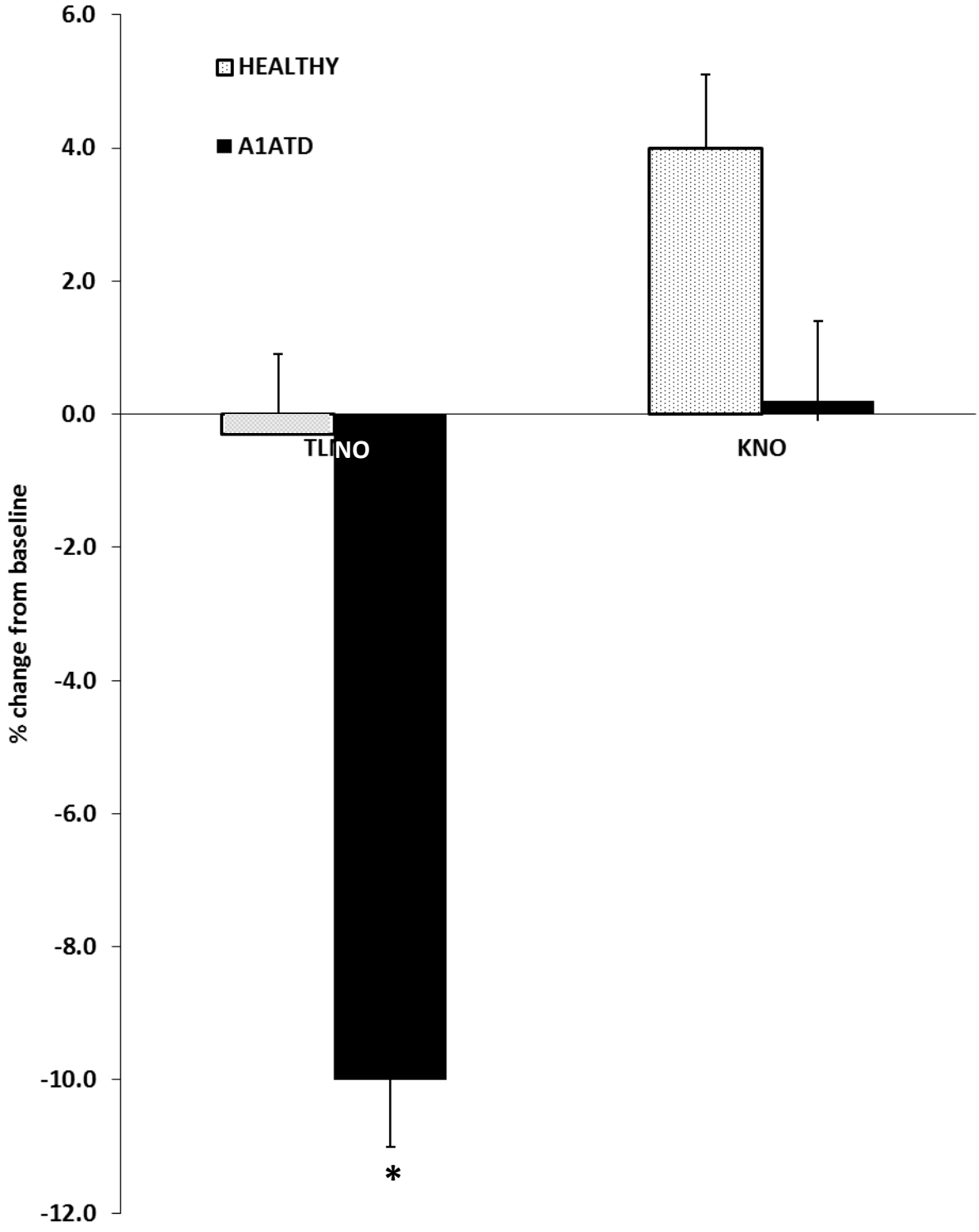

Figure 3.5 - A bar graph summarising the mean percentage change of single breath gas transfer manoeuvre using NO from a seated to supine position in healthy subjects compared to those with A1AD. A clear difference in response to postural change can be seen between cohorts. TL_{NO} in mmol/kPa/min; K_{NO} in mmol/kPa/min/L. * $p \leq 0.05$ indicating a significant change from baseline measurements.

TL_{NO}/TL_{CO} decreased by 4.1 % in A1AD group which and was deemed statistically significant. This was similar to the change of -6.0% in our healthy group which interestingly, was not deemed statistically significant presumably influenced by number of subjects in each cohort. The values themselves were very similar and would not be classed as showing clinically relavents, thus supporting previous work that emphysematous change shown using HRCT scans has little effect on the TL_{NO}/TL_{CO} ratio. (Van der Lee et al 2009).

K_{NO}/K_{CO} like TL_{NO}/TL_{CO} decreased from a sitting to supine posture in both A1AD and healthy cohorts by 4.4% and 7.5% respectively. Neither showed statistical significant nor clinical relevant and again supports work that emphysematous changes have little effect on gas transfer measurements using NO and so no difference in postural change is witnessed between our two groups, illustrated in Figure 3.6.

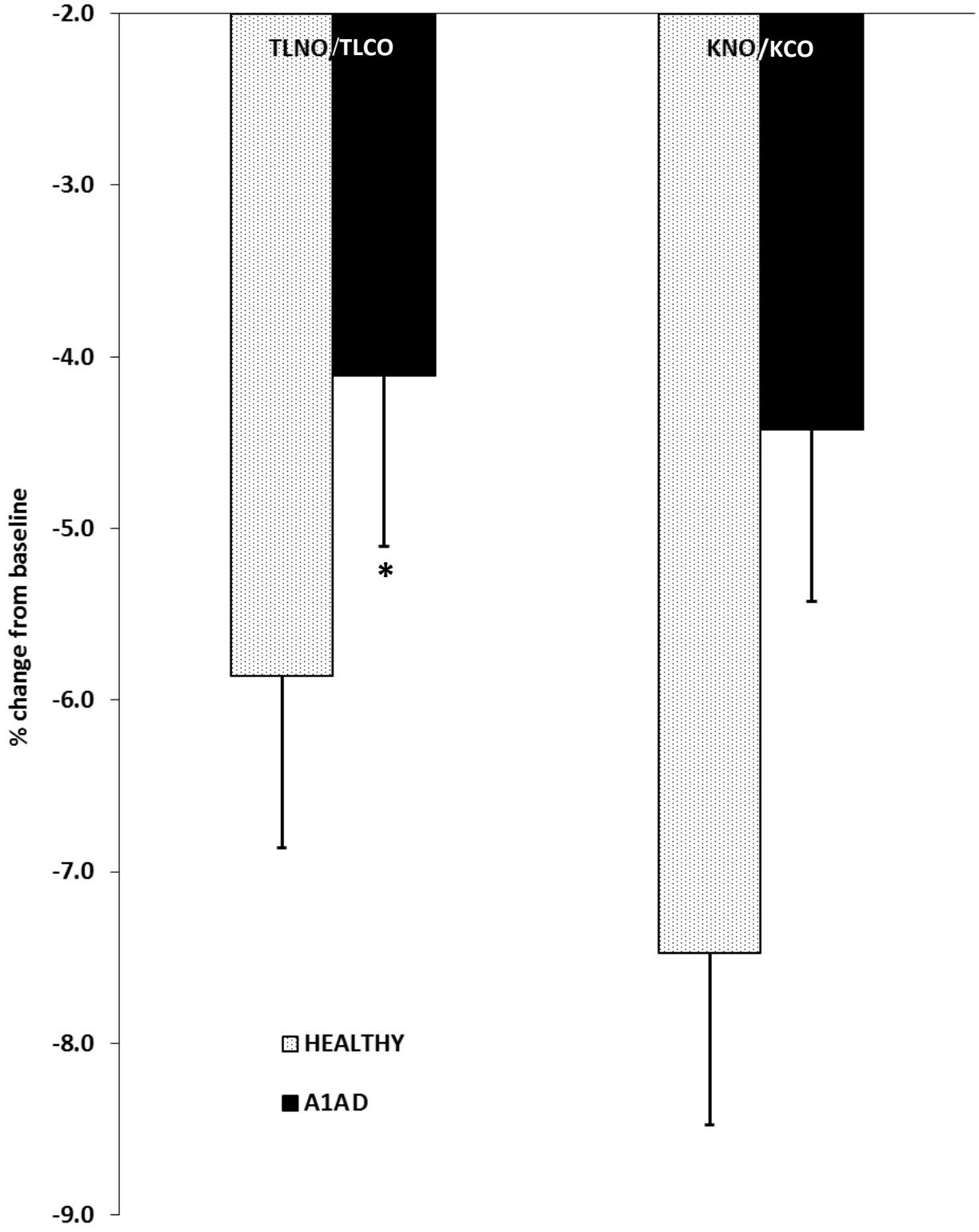

Figure 3.6 - A bar graph summarising the mean percentage change of single breath gas transfer manoeuvre using CO & NO from a seated to supine position in healthy subjects compared to those with A1AD. A difference in response to postural change can be seen between cohorts. TL_{NO}/TL_{CO} in mmol/kPa/min; K_{NO}/K_{CO} in mmol/kPa/min/L. * $p \leq 0.05$ indicating a significant change from baseline measurements.

3.3.1 STUDY 3 DISCUSSION

The standardised 15 minute supine methodology was used for the patient groups. The change in $TL_{CO,}$ which is the product of alveolar/capillary membrane function (K_{CO}) and surface area of lung tissue (VAeff) actually showed a decrease in the A1AD group after 15 minutes supine and again disagreed with our hypothosis. This conflicted with the increase seen in the healthy volunteers. Neither change was statistically or clinically significant but comparison of the trends in the two groups showed a difference suggesting different processes taking place. It was noted that the VAeff decreased in the A1AD subjects by 10% which is more than twice that of the healthy subjects ,which had been suggested in Study 2. This could explain the decrease in TL_{CO} from sitting to supine postures as the total transfer of gas is dependant on the surface area of the diffusing membrane. However, previous work (Stam et al, 1991) found that a volume variation in VAeff of 20% causes a significant difference in response to posture and suggests other factors need to be considered. The respiratory mechanical changes caused by A1AD, underlying changes in respiratory compliance and recoil due to the pathophysiological processes should be considered. If this is the case then it should be reflected in a reduced response of K_{CO}.

$K_{CO,}$ which is the rate of depletion of CO from alveolar gas to pulmonary capillary blood during the breath hold and reflects the transfer factor per unit alveolar volume showed a diminished response to postural change in the A1AD group when compared to the healthy group. This is likely to be the effect of emphysematous destruction of the alveoli in the A1AD group causing decreased surface area and therefore, reducing the diffusing membranes ability to transfer gas. This negates any increasing perfusion, thus reversing the natural increase seen in TL_{CO} and reducing the response in K_{CO} when supine.

This reduced response in K_{CO} when compared to the healthy controls is not affected by volume changes and so is more likley to be the effect of emphysematous destruction of the lungs in the A1AD group by limiting the diffusing membranes ability to transfer gas so increasing levels of perfusion becomes less effective.

$TL_{NO,}$ a truer measure of the diffusing membrane (D_M) and more specifically surface area of alveoli also decreased in the A1AD group. This again contradicted what was seen in the healthy control group who showed no change. This decrease could again be caused by the decrease in VAeff in the supine position. As discussed previously in section 1.7.3 TL_{NO} is more susceptable to changes in volume than TL_{CO} (Van der Lee et al, 2007).

K_{CO} is known to increase as VAeff decreases (MacIntyre et al, 2005) while K_{NO} remains comparatively constant. As previously discussed (section 1.7.1 of the introduction) TL_{CO} is the product of the estimated alveolar volume (VAeff) and the transfer coefficient (K_{CO}), in other words both K_{CO} and K_{NO} are directly measured during the test procedure and multiplied by VAeff to obtain TL_{CO} or TL_{NO}. Therefore the volumetric effect on K_{CO} and K_{NO} parameters highlights why TL_{NO} is more sensitive to these changes in volume.

K_{NO} relates to D_M as TL_{NO} does however, due to K_{NO}'s independency to lung volume it is a better estimate of the thickness of the alveolar membrane. This showed no change from baseline and reinforces the sensitivity of NO. Logically, change in membrane thickness is unlikely to occur as K_{CO} and K_{NO} are effected by changes in membrane thickness so these findings suggest that a loss of surface area has occurred when subjects are supine but membrane thickness has remained constant. This maybe interpreted as TL_{CO} and TL_{NO} are based on volumetric changes caused by changes in posture in the A1AD group with alveolar capillary thickness remaining constant as indicated by the unchanged K_{NO}.

Both subject groups showed a small decrease to postural change for TL_{NO}/TL_{CO} ratio but for different causes. The healthy controls fall in the TL_{NO}/TL_{CO} ratio associates with an increase pulmonary capillary blood volume (Vc) (increase TL_{CO}) versus stable D_M (no change in TL_{NO}) due to pulmonary capillary recruitment, which occurs in a supine position as discussed above. Subjects with A1AD however showed greater decreases in D_M (decreased TL_{NO}) due to loss of surface area, as seen in VAeff. This reversal of the natural effect of increasing perfusion once supine can be seen by a decrease in TL_{CO} thus decreasing the TL_{NO}/TL_{CO} ratio.

3.4 STUDY 4 - The change in TL_{CO}, TL_{NO}, K_{CO}, K_{NO} from sitting to 15 minutes supine in subjects with A1AD when seperated from those with airflow obstruction and those without.

The 62 A1AD subjects were then separated into two different cohorts, those with airflow obstruction (FEV1/FVC >-1.64 SR) (n=51) and those without (n=11). This analysis enables the separation of those subjects with a more advanced disease condition. This was one of the previous limitations from work intitially discussed.

Tables 3.8 and 3.9 show the anthropometric and spirometric data respectively for these two new cohorts and Table 3.10 shows the data collected for these subjects with significant differences highlighted.

A small discrepancy in age exists between our new cohorts by 9 years as was expected, as the severity of emphysematous change increases with age.

A large difference can be seen in spirometric data proving a difference in severity between the two groups.

Table 3.8 - Anthropometric data of subject with obstructive airways (OBA1) & non-obstructive airways (NOA1) with A1AD.

	Age	GENDER	pk/yr	Height (m)	Weight (kg)	BMI
NOA1	49.0 (15.3)	7M:4F	17.5 (15.2)	1.73 (0.2)	78.8 (15.7)	26.3 (3.4)
OBA1	58.1 (9.2)	29M:22F	25.9 (21.8)	1.71 (0.1)	79.6 (16.3)	27.2 (5.5)

Values shown as Mean ± 1 SD

Table 3.9 - Spirometry data of subject with obstructive airways (OBA1) & non-obstructive airways (NOA1) with A1AD.

	FEV1 (L)	FEV1 (%)	FEV1 (SR)	FEV1/FVC	FEV1/FVC (SR)
NOA1	3.49 (0.75)	106.0 (13.7)	0.42 (0.94)	76.7 (6.96)	0.32 (0.80)
OBA1	1.56 (0.62)	52.8 (20.6)	-3.13 (1.5)	39.0 (12.0)	-5.54 (1.66)

Values shown as Mean ± 1 SD

Table 3.10 - Summary of gas transfer data using CO & NO from sitting to 15 minute supine posture in healthy, non-obstructive (NOA1) & obstructive (OBA1) subjects with A1AD.

		HEALTHY		NOA1		OBA1	
		Sitting	Supine (15mins)	Sitting	Supine (15mins)	Sitting	Supine (15mins)
TL_{CO}	MEDIAN	8.64	9.22	7.20	7.49	***4.11***	***3.59***
	IQR	0.42	0.45	0.53	0.67	***0.22***	***0.23***
	AB Change		0.58		0.29		***-0.52****
	%change		6.7		4.0		***-12.6****
TL_{NO}	MEDIAN	33.9	33.9	***27.7***	***26.5***	***15.5***	***14.2***
	IQR	1.58	1.69	***1.99***	***1.93***	***0.88***	***0.83***
	AB Change		0.00		***-1.20****		***-1.3****
	%change		0.0		***-4.3****		***-8.4****
TL_{NO}/ TL_{CO}	MEDIAN	3.91	3.63	***3.77***	***3.61***	***3.84***	***3.64***
	IQR	0.06	0.08	***0.09***	***0.15***	***0.05***	***0.05***
	AB Change		-0.28		***-0.16****		***-0.20****
	%change		-7.2		***-4.2****		***-5.2****
K_{CO}	MEDIAN	***1.56***	***1.81***	***1.25***	***1.43***	***0.84***	***0.87***
	IQR	***0.03***	***0.04***	***0.10***	***0.13***	***0.03***	***0.04***
	AB Change		***0.25****		***0.18****		***0.03****
	%change		***16.0****		***14.4****		***3.6****
K_{NO}	MEDIAN	6.16	6.30	4.87	5.05	3.14	3.07
	IQR	0.07	0.10	0.35	0.34	0.12	0.12
	AB Change		0.14		0.18		-0.07
	%change		2.3		3.7		-2.2
K_{NO}/ K_{CO}	MEDIAN	3.91	3.62	***3.77***	***3.61***	***3.85***	***3.65***
	IQR	0.06	0.07	***0.09***	***0.15***	***0.05***	***0.05***
	AB Change		-0.29		***-0.16****		***-0.20****
	%change		-7.5		***-4.2 ****		***-5.2****
VAeff	MEDIAN	5.51	5.33	***5.63***	***5.22***	***5.00***	***4.64***
	IQR	0.24	0.25	***0.25***	***0.26***	***0.18***	***0.17***
	AB Change		-0.18		***-0.41****		***-0.36****
	%change		-3.3		***-7.3****		***-7.2****

Values shown as Medians with Inter-Quatile Range (IQR), Absolute change (AB Change) & percentage change (%change). TL_{CO} & TL_{NO} in mmol/kPa/min; K_{CO} & K_{NO} in mmol/kPa/min/L; VAeff in Litres. The median sitting value is the mean from two consistent values within 5%.
*** $p \leq 0.05$ indicating a significant change from baseline measurements.**

TL_{CO} showed no significant change (4.0%) from sitting to supine positions for the non obstructive A1AD cohort and so agreed with our healthy cohort (6.7%). Statistical analysis also proved no significant differences exist between these two groups. The same cannot be said for those displaying airflow obstruction who showed a significant change of -12.6% from seated measurements so this group was responsible for the drop in TL_{CO} in the previous analysis. Significant differences were seen in the level of percentage change between those with airflow obstruction and the other two groups.

All three groups showed statistically significant postural changes for K_{CO}; healthy controls showed 16.0% increase, non-obstructive A1AD subjects 14.4% increase and obstructive A1AD subjects 3.6% increase. Although the three groups showed statistical differences the 3.6% change demonstrated by the obstructive group would not be deemed clinically relevent. This is supported when we compared the three groups against each other. The percentage change between our healthy controls and non-obstructive subjects showed no difference however, significant differences were shown when compared to those with airways obstruction.

Both TL_{CO} and K_{CO} analysis are displyed in Figure 3.7 and 3.8 respectively with significant differences indicated. Figure 3.7 and 3.8 shows the healthy controls displayed the largest values in both TL_{CO} and K_{CO} when compared to the two A1AD groups as would be expected. Although the NOA1 group showed no airflow obstruction these reduced gas transfer parameters suggests emphysematus destruction of lung tissue had taken place. The OBA1 subgroup showed the smallest values suggesting the most amount of destruction to lung tissue has taken place in this group and the least amout of spread as the ability of the lungs to transfer gas diminishes.

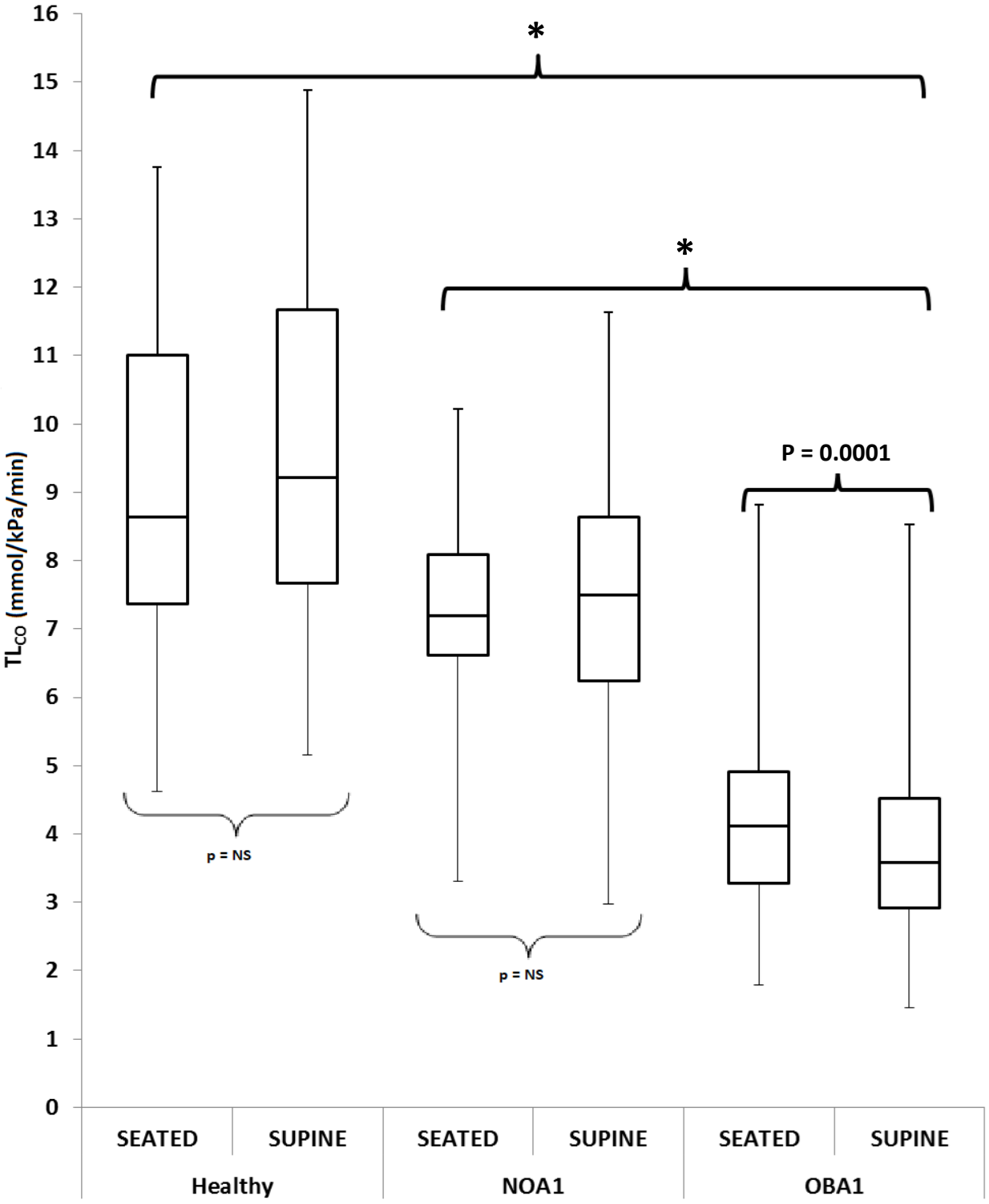

Figure 3.7 - A box & whisker plot summarising the median change, IQR and the extreames of TL_{CO} data from a seated to supine position in healthy subjects compared to those with and without airflow obstruction. A difference in response to postural change can be seen in the obstructive cohort. Differences in the level of change between the healthy cohort vs obstructive and the non-obstructive vs obstructive cohorts were also seen. TL_{CO} in mmol/kPa/min. * $p \leq 0.05$ indicating a significant change between cohorts. p = NS indicating non-significant change. There were no outliers.

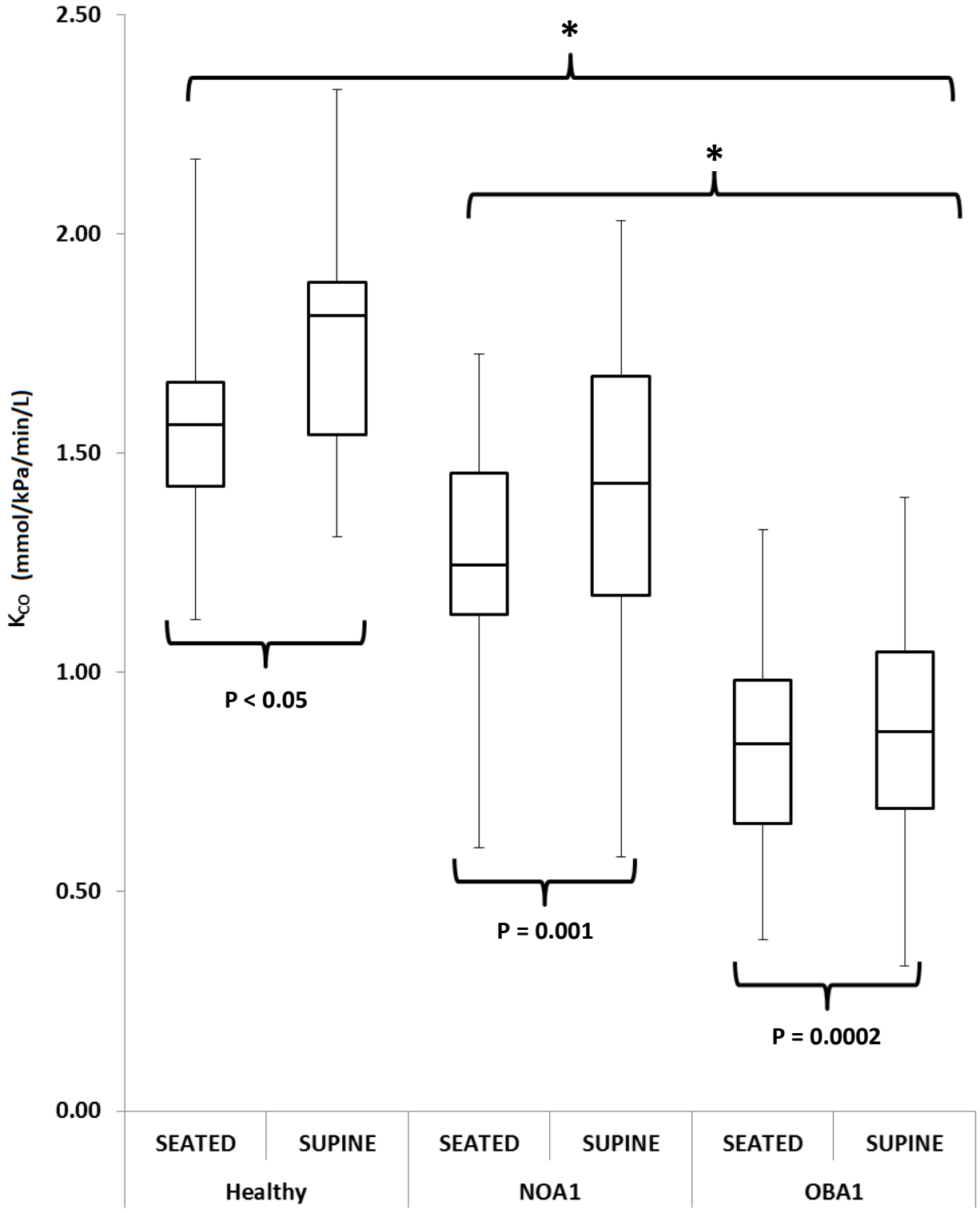

Figure 3.8 - A box & whisker plot summarising the median change, IQR and the extreames of K_{CO} data obtained from a seated to supine position in healthy subjects compared to those with and without airflow obstruction. A clear difference in response to postural change can be seen in both obstructive & non-obstuctive cohorts. Differences in the level of change between the healthy vs non-obstructive cohorts and the healthy vs obstructive cohorts. K_{CO} in mmol/kPa/min/L. * $p \leq 0.05$ indicating a significant change between cohorts. There were no outliers.

Analysis of TL_{NO} showed significant postural changes for both groups of A1AD subjects with the non-obstructives showing 4.3% and obstructive showing 8.4% decreases.

Significant differences in TL_{NO} were seen between the healthy controls and both NOA1 and OBA1 cohorts.

K_{NO} showed no significant changes between postures for any of the three cohorts.

The healthy control group showed a change of 2.3 %, the NOA1 group showed a change of 3.7% and the OBA1 showed a -2.2% change. Interestingly differences in K_{NO} were also seen between healthy controls and those with airflow obstruction which could only be caused by differences in the D_M resulting from emphysematous changes taking place due to A1AD.

These observations are shown in Figure 3.9 and 3.10 with significant differences indicated.

Figures 3.9 and 3.10, like figures 3.7 and 3.8, showed that the healthy controls displayed the largest values in both TL_{NO} and K_{NO} when compared to the two A1AD groups again as would be expected for the same reasons as TL_{CO} and K_{CO}. The OBA1 subgroup again showed the smallest values suggesting the most amount of destruction to lung tissue has taken place in this group. However for K_{NO} the spread of data was very similar to that of the healthy controls, the reasons for this are unknown at present.

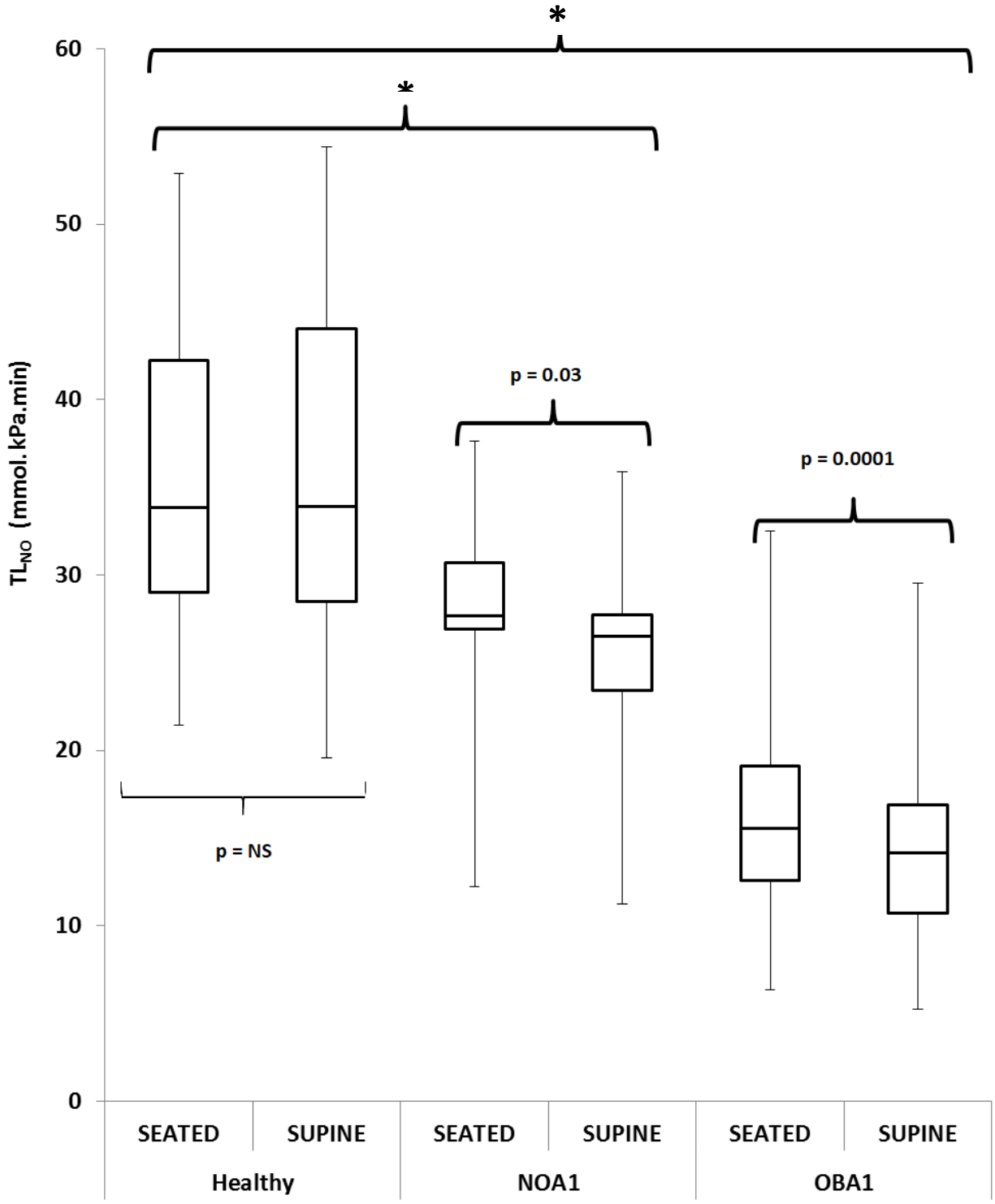

Figure 3.9 - A box & whisker plot summarising the median change, IQR and extreames of TL_{NO} data obtained from a seated to supine position in healthy subjects compared to those with and without airflow obstruction. A difference in response to postural change can be seen in both obstructive & non-obstuctive cohorts. Differences in the level of change between the healthy vs non-obstructive cohorts and the healthy vs obstructive cohorts were also witnessed. TL_{NO} in mmol.kPa.min;. * p ≤ 0.05 indicating a significant change between cohorts. p = NS indicating non-significant change. There were no outliers.

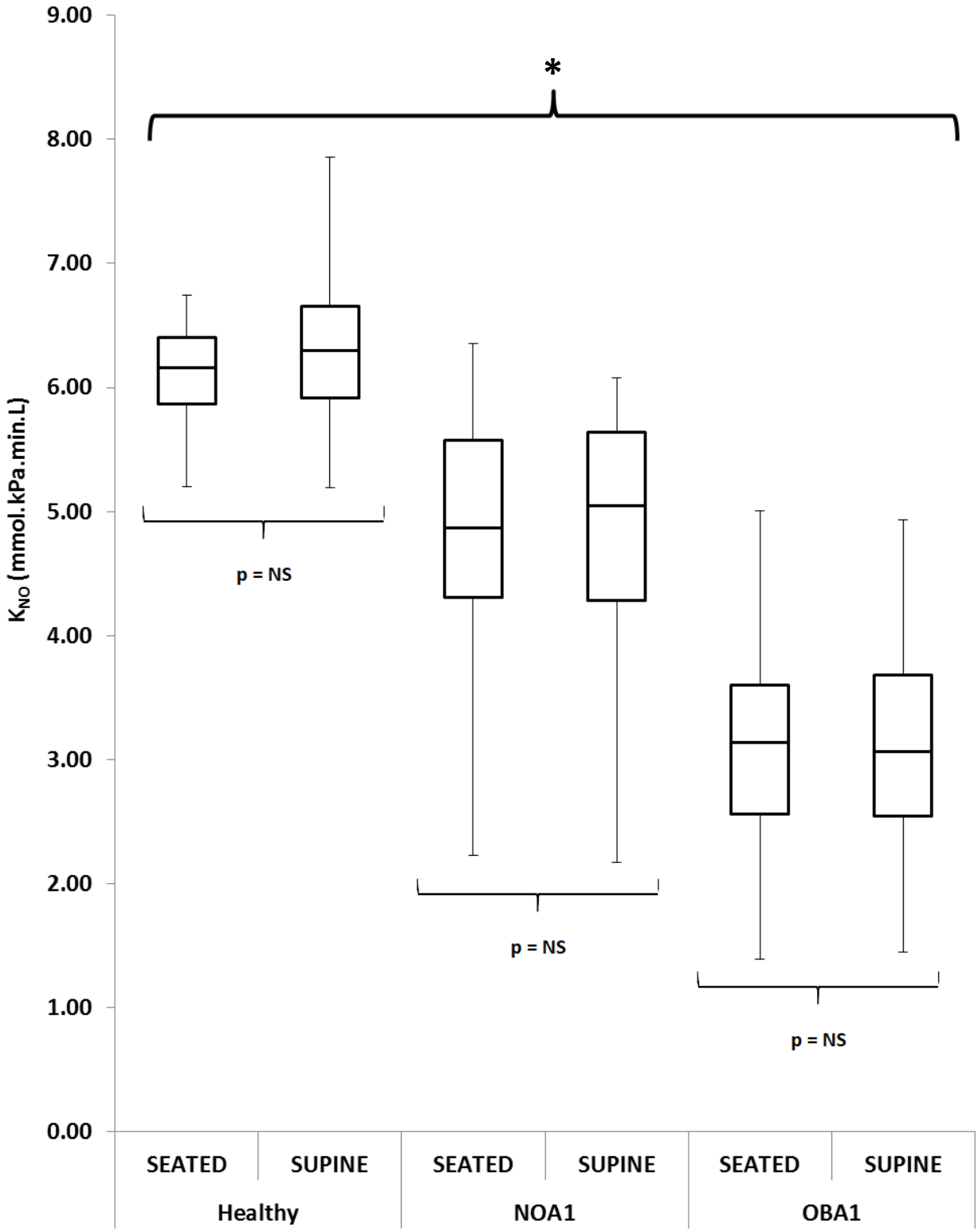

Figure 3.10 - A box & whisker plot summarising the median change, IQR and extreames of K_{NO} data from a seated to supine position in healthy subjects compared to those with and without airflow obstruction. No difference in response to postural change can be seen in any cohort. However, a significant difference in the level of change between healthy vs obstructive cohorts was observed. K_{NO} in mmol.kPa.min.L;. * $p \leq 0.05$ indicating a significant between cohorts. p = NS indicating non-significant change. There were no outliers.

Both groups of A1AD showed significant postural change for TL_{NO}/TL_{CO}; the non-obstructed subjects by – 4.2% and those with airflow obstruction changing -5.2%. Even though both A1AD groups showed a mathamatical difference this would not be seen as clinically relevent as both fall within the 5% variability of the test.

No difference was seen when comparing the healthy control group and either A1AD group. However a significant difference between postures did exist when comparing the A1AD group showing no air flow obstruction to the A1AD group that did show airflow obstruction. This will be explained further in chapter 3.4.1.

A similar outcome can be seen for K_{NO}/K_{CO} with both non-obstructive & obstructive A1AD groups demonstrating significant postural changes by -4.3% and -5.2% respectivally and so show that the two ratios agree well. Again both A1AD groups showed a statistical difference however, this would not be seen as clinically relevent as both fall within the 5% variability of the test. The healthy control group displayed no difference between NOA1 group but did show a difference from those in OBA1 group.

These data and statistical differences are shown in Figure 3.11 and 3.12 respectively

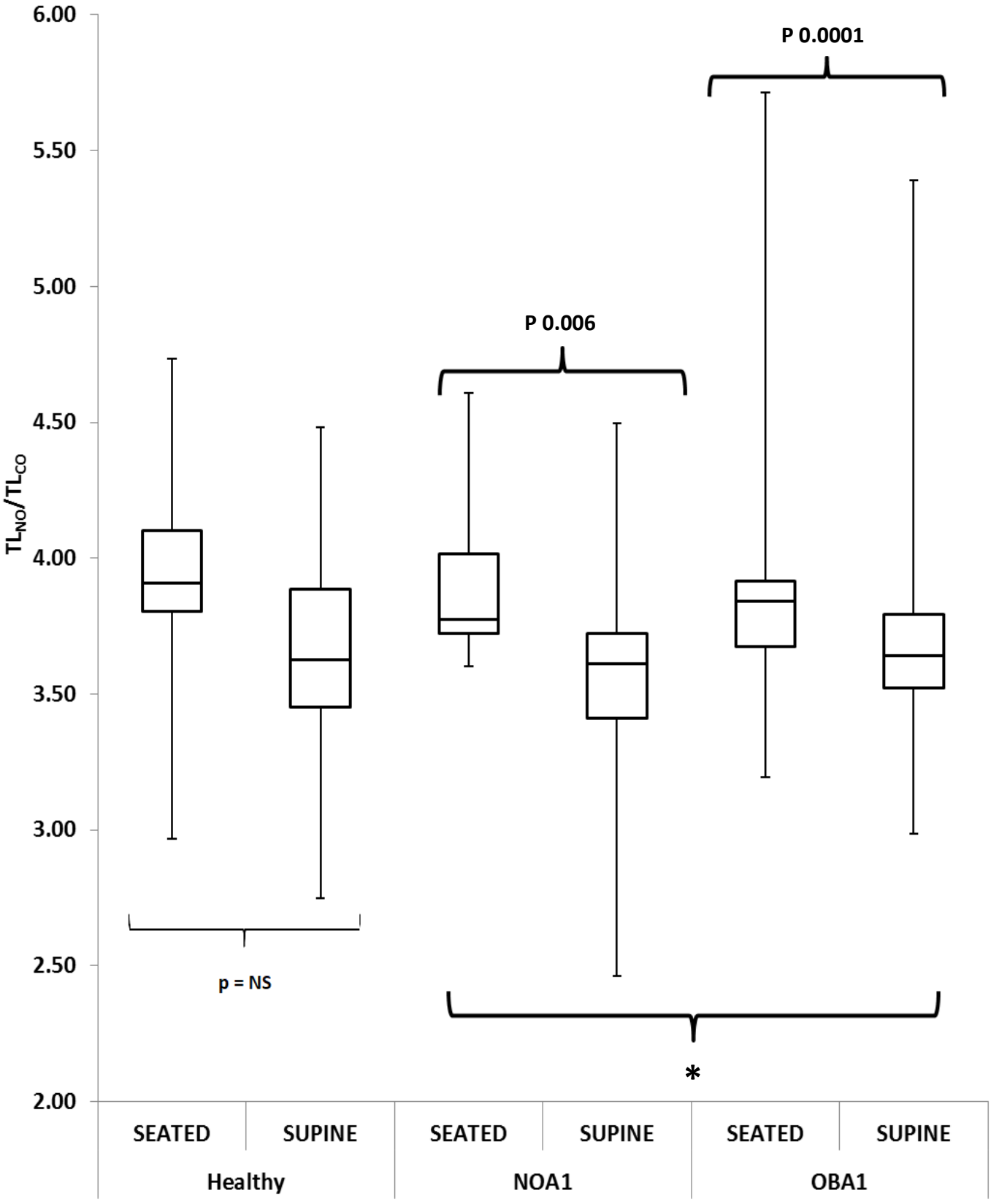

Figure 3.11 - - A box & whisker plot summarising the median change, IQR and the extreames of TL_{NO}/TL_{CO} data from a seated to supine position in healthy subjects compared to those with and without airflow obstruction. Differences in response to postural change can be seen in both A1AD groups. A significant difference in the level of change between non-obstructive vs obstructive cohorts was also observed. * p ≤ 0.05 indicating a significant between cohorts. p = NS indicating non-significant change. There were no outliers.

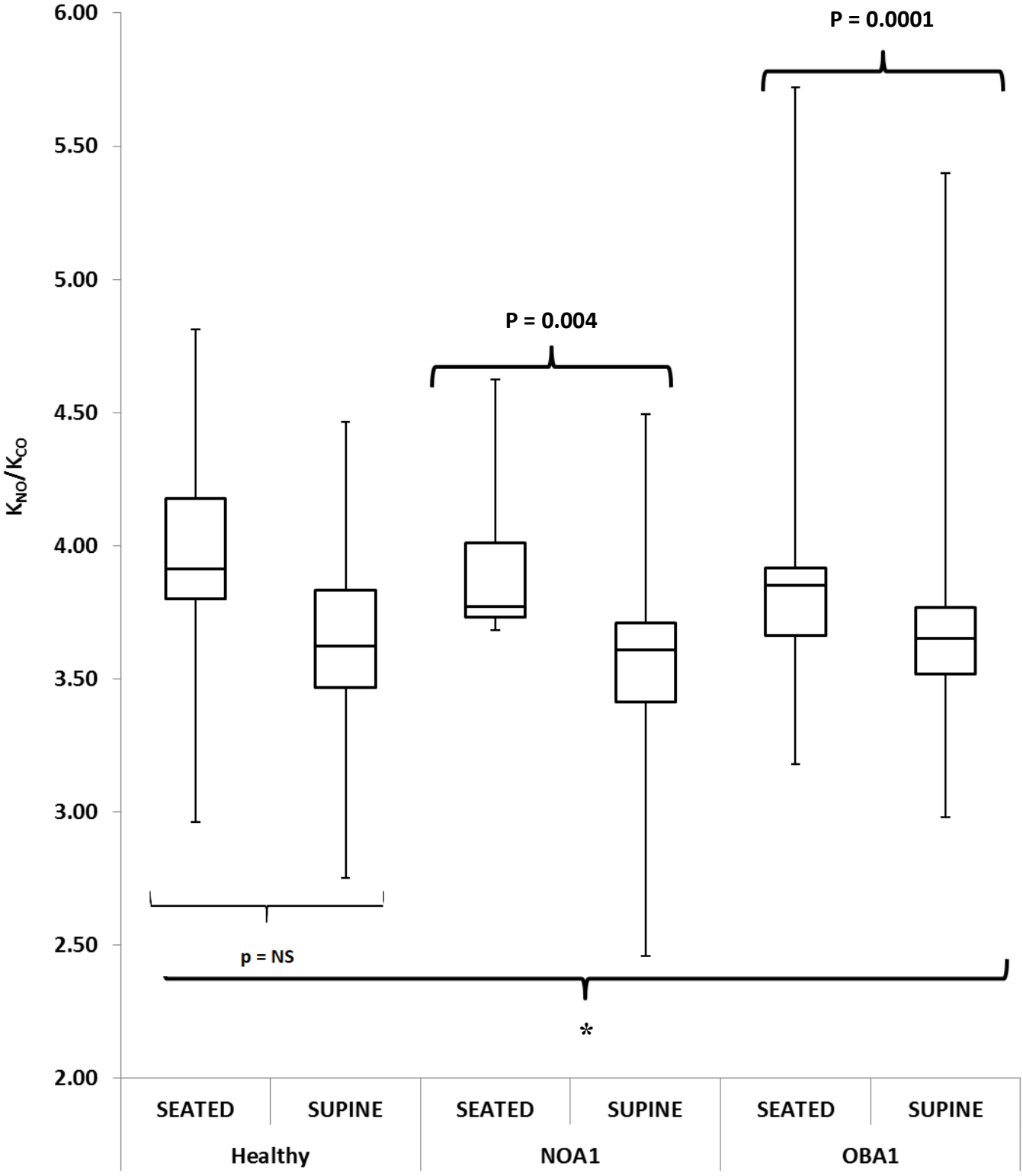

Figure 3.12 - - A box & whisker plot summarising the median change, IQR and the extreames of K_{NO}/K_{CO} data from a seated to supine position in healthy subjects compared to those with and without airflow obstruction. Differences in response to postural change can be seen in both A1AD groups. A significant difference in the level of change between healthy vs obstructive cohorts was also observed. * p ≤ 0.05 indicating a significant between cohorts. p = NS indicating non-significant change. There were no outliers.

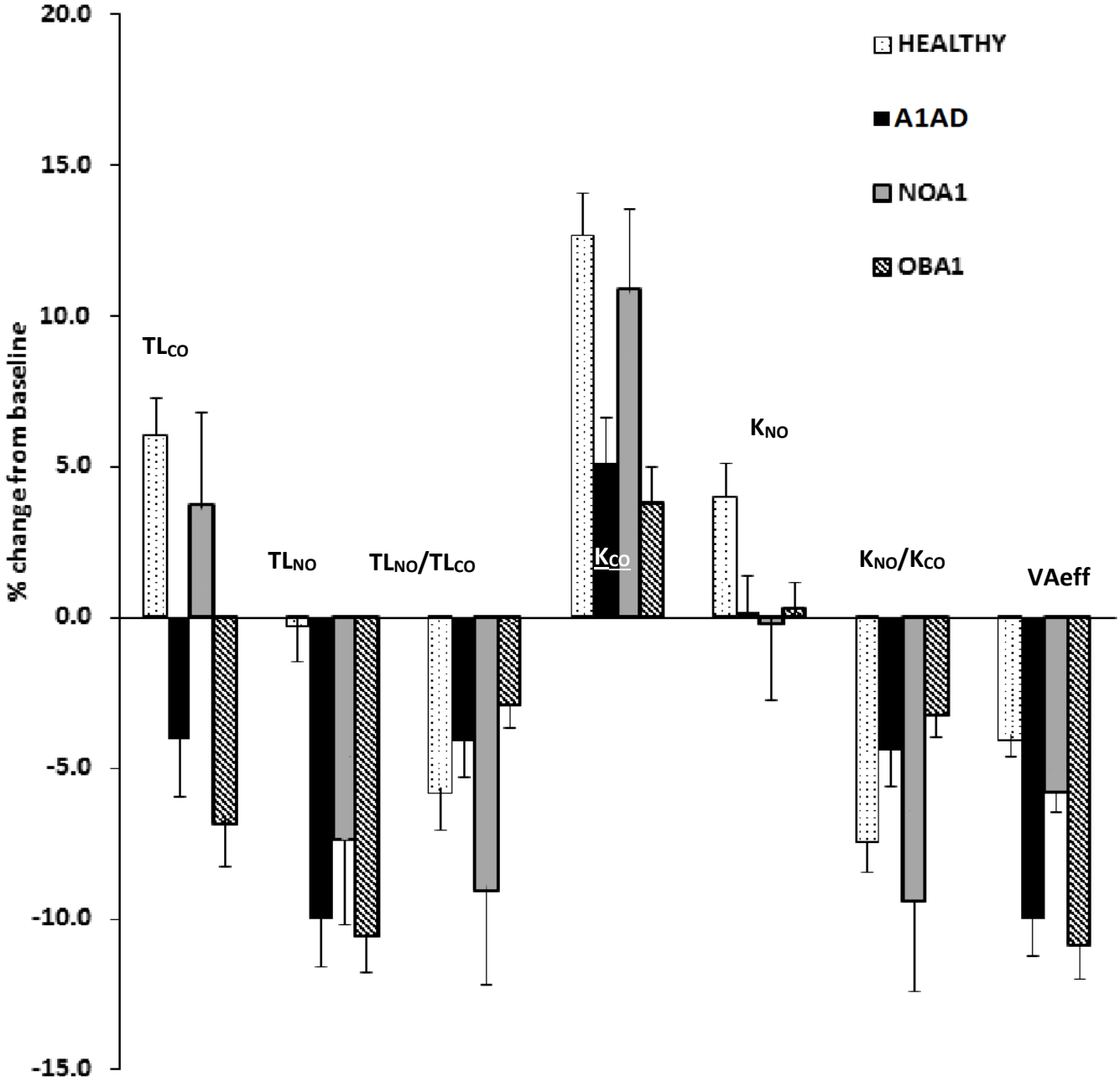

Figure 3.13 – A bar graph summarising the median postural change for all gas transfer parameters measured for our two cohorts (healthy controls & all A1AD subjects) and two subgroups of the A1AD cohort (NOA1 & OBA1).

3.4.1 STUDY 4 DISCUSSION

Since not all A1AD patients exhibited airways obstruction & emphysema, it was decided to analyse the A1AD patient once split into those with and without airways obstruction in accordance with ATS/ERS guidlines.

The sitting to supine TL_{CO} in the non-obstructed A1AD subjects were similar to that of the healthy subjects as both showed no significant change which contradicts the initial hypothesis. The explanation of the lack of response to postural change is the result of decreased lung volumes which was shown by decreases in VAeff (see study 3).

Although previous studies (Stam et al, 1991) advised changes of 20% in VAeff can significantly affect the reponse in TL_{CO} these current findings suggest much smaller changes in lung volumes are required.

Those displaying airflow obstruction (OBA1) however, showed significant decreases in TL_{CO} when supine. This can give an explanation primarily for the volumetric changes seen in the supine postion and describe the decrease seen in VAeff.

The change in VAeff in both the healthy (NOA1) and the obstructed A1AD patients (OBA1) however was consistent so other factors must explain the significant decrease in TL_{CO} with the obstructive patients. Stam, (1991) suggested the postural increases seen in gas transfer parameters diminish with increasing age and become non significant above 50 years due to natural degredation of lung tissue. The OBA1 patients showed a mean age of 58 years compared to 49 years of the healthy A1AD patients and 32 years of the healthy control group. This suggests these variations are related to age.

Conversely, a more probable explanation would be differences in disease severity causing greater damage to the alveolar/capillary membrane which would greatly limit the response to increased perfusion.

Patients with severe airways obstruction often have severe emphysema as well. This is indicated by both differences in spirometric data and baseline gas transfer between the two A1AD groups and would explain the significant differences seen in the size of TL_{CO} change between those with airflow obstruction and those without.

All three groups showed statistically significant postural changes for K_{CO} showing improvements in alveolar/capillary membrane per unit of lung volume ; although the change demonstrated by the obstructive group was not be deemed clinically relevant as this falls within the expected 5% variability of the measurement. This contradiction between healthy controls and obstructed patients may indicate a significant physiological process not previously observed.

When the three groups are compared with each other, the percentage change between the healthy controls and non-obstructive subjects showed no difference, but significant differences were shown when compared to those patients with airways obstruction. This discrepancy could be the result of poor test gas mixing due to airflow limitation caused by airway narrowing. This would prevent the whole lung unit being ventilated with the test gas severely effecting the results obtained.

These studies confirm that K_{CO} is not greatly influenced by changes in volume as TL_{CO} is and provides a more reliable reflection of the diffusing membrane (D_M). The lack of response in OBA1 group can also be interpreted as greater emphysematous destruction of lung parenchyma which decreases the level of gas transfer able to occur because of reduced surface area. This destruction is assumed since they have more severe airflow obstruction shown on their spirometric data and lower K_{CO} values in their baseline measurements.

Analysis of TL_{NO} (D_M) showed significant postural decreases for both groups of A1AD subjects with and without airflow obstruction. This again disproved the initial hypothesis which stated no changes in TL_{NO} and therefore no change in D_M was expected.

The same volumetric reductions were accountable for the unexpected changes in TL_{NO} seen in both groups and strongly agree with previous studies (Borland et al 1989 & Van der Lee et al, 2007). Significant differences in TL_{NO} and VAeff were seen between the healthy controls and both A1AD groups supporting this hypothesis. The reduction in lung volume may cause a reduction in surface area and increase the relative membrane thickness at alveolar level (Glenet et al, 2007). It is difficult to explain a mechanism for increasing membrane thickness unless pulmonary hypertension and localised oedema is the possible mechanism. However, this information was not available for these studies and might be areas to look at in future research. Hughes, (2013) suggests TL_{NO} reflects alveolar surface area due to its sensitivity to changes in lung volume while K_{NO} is more representative of membrane thickness. Using this model it appears a greater reduction in surface area rather than an increase in membrane thickness occurs in the supine condition as suggested in Study 3. If K_{NO} does reflect membrane thickness as Hughes suggests no significant changes between postures for any of the three subject groups were seen and so supports the initial hypothesis stated no changes to alveolar membrane should take place.

Interestingly, differences in the size of change in K_{NO} were observed between healthy controls and those with airflow obstruction which could only be caused by differences in the D_M. This could again be a result from greater emphysematous changes taking place due to A1AD.

All three subject groups showed significant postural change for TL_{NO}/TL_{CO} which refects the true D_M/Vc ratio. When Vc increases (TL_{CO}) and D_M (TL_{NO}) remains stable (as seen in the healthy control group) the TL_{NO}/TL_{CO} ratio decreases suggesting that the pulmonary capillary blood volume (perfusion) has increased while the alveolar/capillary membrane has remained stable. The healthy A1AD patients showed a lesser increase in TL_{CO} suggesting some increase in Vc has occurred but the response is not as prominent.

The relativly small decrease in TL_{NO} (D_M) suggests a volumetric effect once in a a supine position reducing the surface area of the diffusing membrane explaining the limited response of the TL_{CO} (Vc) parameter (Hughes 2013) .

The A1AD patients with airways obstruction showed a decrease in TL_{NO}/TL_{CO} ratio thus a decrease in D_M/Vc. This was the result of a disproportional decrease to both TL_{CO} (Vc) and TL_{NO} (D_M).

Both groups of A1AD patients showed a similar decrease in VAeff therefore volumetric changes had no influence, which seems to contrast Hughes suggestion the TL_{NO} represents surface area as discussed previously. However, variation in alveolar/capillary membrane integrity is more likely, signifying A1AD patients with airways obstruction have more severe emphysematous damage to their lung parenchyma.

Even though both A1AD groups showed a mathematical difference this may not be clinically relevent as both fall within the 5% variability of the test, however it suggests a possible pathophysiological difference. No difference was seen when comparing the healthy control group and either A1AD group but a difference did exist between the obstructive and non-obstructive subjects.

A similar outcome can be seen for K_{NO}/K_{CO} which represents the same as the TL_{NO}/TL_{CO} ratio as decribed above but is independent of VA. Both non-obstructive & obstructive A1AD patients demonsted significant postural change, showing that these ratios agreed well. However, whilst both A1AD groups showed a statistical difference, this would not be seen as clinically relevent as both fall within the 5% variability of the test. The healthy control group displayed no difference from the non-obstructive patients but did show a difference from those with airflow obstruction who are more likely to have exhibited greater emphysematous changes.

Looking more specifically at emphysematous change via high resolution computed tomography (HRCT) should provide more information and confirm this theory.

3.5 STUDY 5 - The change in TL_{CO}, TL_{NO}, K_{CO}, K_{NO} from sitting to 15 minutes supine in 43 subjects with A1AD when separated from those with apical, basal & global emphysematous changes

43 of the A1AD subjects with CT data and successfully performed both seated and supine gas transfer were then again separated into three different cohorts. Those with global emphysemtous change (n=10), those with emphysematous changes predominantly at the base of the lungs (n=28) and those changes predominantly at the apex of the lungs (n=5).

19 subjects unfortunately, had no CT data available or were unable to successfully complete the measurements and were therefore, excluded from this study.

Tables 3.11 and 3.12 show the anthropometric and spirometric data for these three new groups. The main difference that exists between the groups are the number of subjects in each group. As one third of A1AD will develop basal emphysematous changes this was a true reflection on the population.

Table 3.11 - Anthropometric data of those subjects displaying Basal, Apical & Global emphysematous changes as a result of A1AD.

	Age (Yrs)	Sex	Smoking (pk/yrs)	Height (m)	Weight (kg)	BMI
Basal	58.0 (8.8)	15M:13F	24.6 (23.1)	1.70 (0.10)	78.8 (16.9)	27.5 (7.1)
Apical	60.2 (9.7)	3M:2F	32.5 (27.3)	1.74 (0.13)	87.1 (15.9)	28.7 (3.5)
Global	56.6 (8.1)	9M:1F	32.6 (26.3)	1.74 (0.09)	76.3 (14.7)	25.2 (4.0)

Values shown as Mean ± 1 SD

Table 3.12 - Spirometry data of those subjects displaying Basal & Apical emphysematous changes as a result of A1AD.

	FEV1 (L)	FEV1 (%)	FEV1 (SR)	FEV1/FVC (%)	FEV1/FVC (SR)
Basal	1.59 (0.80)	54.0 (23.4)	-3.00 (1.6)	39.5 (12.7)	-5.52 (1.87)
Apical	1.60 (0.39)	53.6 (16.6)	-3.13 (1.21)	38.7 (12.5)	-5.52 (1.55)
Global	1.76 (0.92)	54.9 (31.1)	-3.03 (2.01)	38.6 (18.7)	-5.41 (2.51)

Values shown as Mean ± 1 SD

All groups are well matched for age and BMI, a small discrepancy exists in gender in our global cohort and smoking histories between the apical cohort. However, no significant discrepancy can be seen in spirometric data and so one could argue the revelance of these differences.

Table 3.13 shows the data collected for these subjects with statistically significant differences highlighted.

Table 3.13 - **Summary of gas transfer data using CO & NO from a seated to 15 minute supine posture in healthy, apical emphysematous change, basal emphysematous change & global emphysematous change subjects with A1AD**.

		HEALTHY		APICAL		BASAL		GLOBAL	
		Sitting	Supine (15mins)	Sitting	Supine (15mins)	Sitting	Supine (15mins)	Sitting	Supine (15mins)
TL_{CO}	MEAN	9.21	9.76	3.83	3.62	***4.44***	***4.18***	***4.26***	***3.88***
	SEM	0.42	0.45	0.44	0.40	***0.28***	***0.29***	***0.62***	***0.67***
	AB Change		0.55		-0.21		***-0.26****		***-0.38****
	%change		6.0		-5.5		***-5.9****		***-8.9****
TL_{NO}	MEAN	35.91	35.85	15.87	14.50	***16.56***	***15.06***	***16.44***	***14.57***
	SEM	1.58	1.69	1.89	1.76	***1.04***	***1.04***	***2.41***	***2.49***
	AB Change		-0.06		-1.37		***-1.50****		***-1.88****
	%change		-0.3		-8.6		***-9.1****		***-11.4****
TL_{NO}/ TL_{CO}	MEAN	***3.92***	***3.69***	4.14	3.99	***3.73***	***3.61***	3.84	3.77
	SEM	***0.06***	***0.08***	0.10	0.17	***0.04***	***0.05***	0.10	0.13
	AB Change		***-0.23****		-0.15		***-0.12****		-0.07
	%change		***-5.9****		-3.6		***-3.2****		-1.8
K_{CO}	MEAN	***1.56***	***1.75***	0.72	0.78	***0.87***	***0.92***	0.71	0.73
	SEM	***0.03***	***0.04***	0.10	0.13	***0.04***	***0.04***	0.09	0.10
	AB Change		***0.19****		0.06		***0.05****		0.02
	%change		***12.2****		8.3		***5.7****		1.4
K_{NO}	MEAN	6.1	6.34	3.01	3.07	3.25	3.28	2.73	2.71
	SEM	0.07	0.1	0.47	0.50	0.13	0.13	0.34	0.36
	AB Change		0.24		0.06		0.03		-0.02
	%change		3.9		2.0		0.9		-1.2
K_{NO}/ K_{CO}	MEAN	***3.95***	***3.66***	4.15	3.97	***3.74***	***3.60***	3.84	3.76
	SEM	***0.06***	***0.07***	0.10	0.17	***0.04***	***0.05***	0.10	0.12
	AB Change		***-0.31****		-0.18		***-0.14****		-0.07
	%change		***-7.8****		-4.3		***-3.7****		-1.9
VAeff	MEAN	5.88	5.65	5.49	4.97	***5.13***	***4.57***	***5.97***	***5.24***
	SEM	0.24	0.25	0.65	0.67	***0.25***	***0.24***	***0.35***	***0.37***
	AB Change		-0.23		-0.52		***-0.57****		***-0.72****
	%change		-3.9		-9.5		***-11.1****		***-12.1****

Values shown as Mean with Standard error of mean ± SEM, Absolute change (AB Change) & percentage change (%change). TL_{CO} & TL_{NO} in mmol/kPa/min; K_{CO} & K_{NO} in mmol/kPa/min/L; VAeff in Litres. The mean sitting value is from two consistent values within 5%. * $p \leq 0.05$ indicating a significant change from baseline measurements.

No statistical or clinical significant differences were seen for the apical group for any parameter between the two postures. This was due to the small numbers in this group and also accounts for the large error bars seen in the graph on page 99.

The basal and global groups however did show some interesting changes. For TL_{CO} a significant decrease of 5.9% and 8.9% respectively was seen once in a supine posture. TL_{NO} decreased further by 9.1% and 11.4%. Like the previous study these changes were influenced by the volumetric changes seen with VAeff decreasing by 11.1% and 12.1% for the basal and global group and deemed to be both statistically and clinically significant. These changes in TL_{CO}, TL_{NO} and VAeff are presented in Figure 3.14.

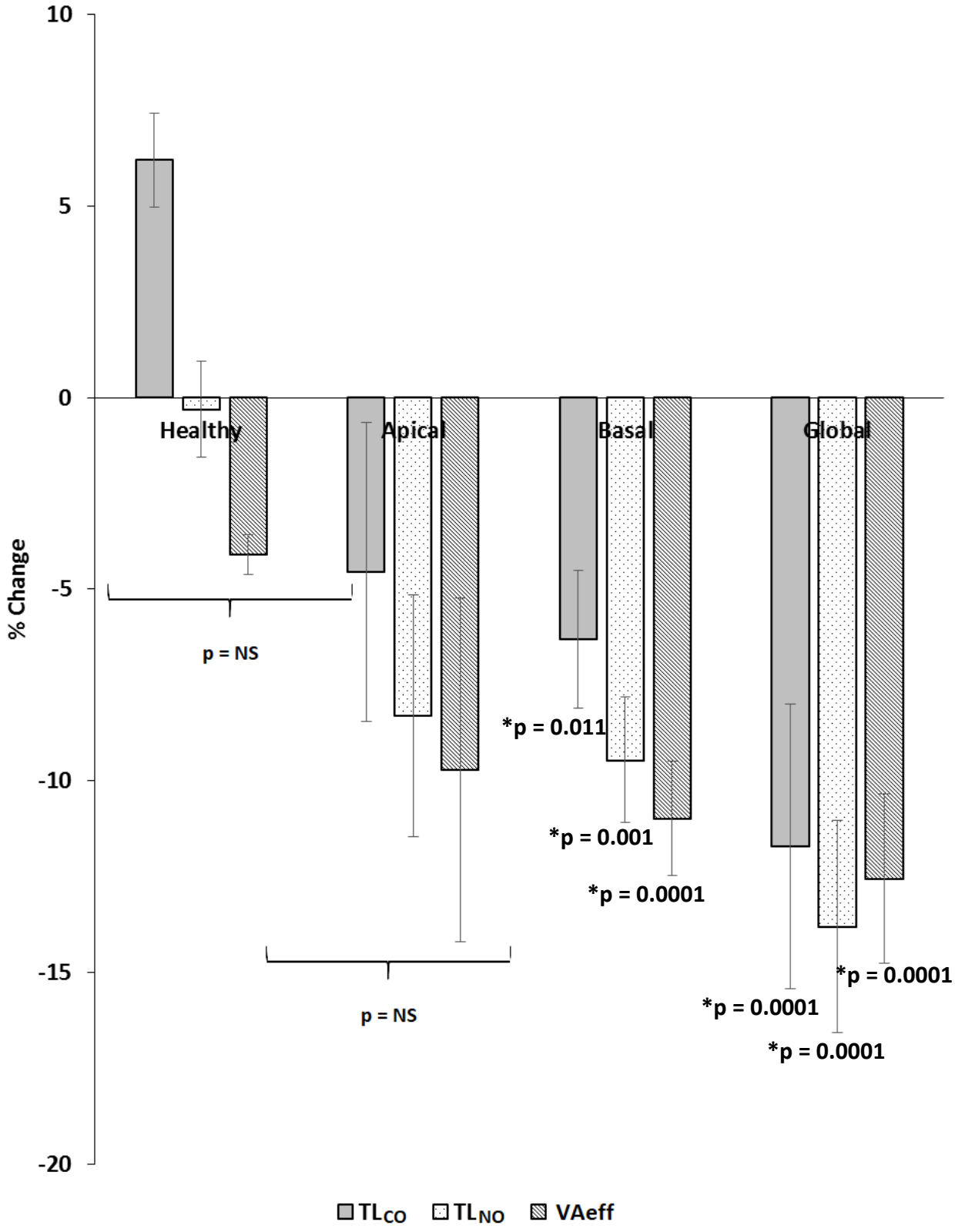

Figure 3.14 – A bar graph summarising the mean and standard error of the mean ± SEM percentage change of TL_{CO}, TL_{NO} & VAeff from a seated to supine position in healthy subjects compared to those presenting with Apical, Basal & Global emphysematus change. TL_{CO} and TL_{NO} in mmol/kPa/min, VAeff in L. p = NS indicates no significant change

K_{CO} and K_{NO} are not affected by volume changes and therefore, are likely to be the most physiologically viable parameters that fit with our hypothesis.

In the basal group K_{CO} showed a statistically significant increase of 5.7% once subjects were supine. This was due to increases in perfusion. K_{NO} which is solely dependent on membrane function (more specifically membrane thickness) showed an inconsequential increase of 0.9% as membrane thickness does not alter with postural changes.

Those with global emphysematous changes did not show any significant differences. K_{CO} showed an increase of 1.4% and K_{NO} decreases 1.2%. This lack of response could be influenced by their disease pathology as emphysematous change has taken place throughout the lung and therefore, membrane destruction can be seen. The pulmonary blood cannot be redistributed to 'healthier' regions of the lung and so has little effect on the parameters being measured. Although these results agree with the hypothesis of the study, statistically no clinical relevance has been shown and the data can be seen in Figure 3.15.

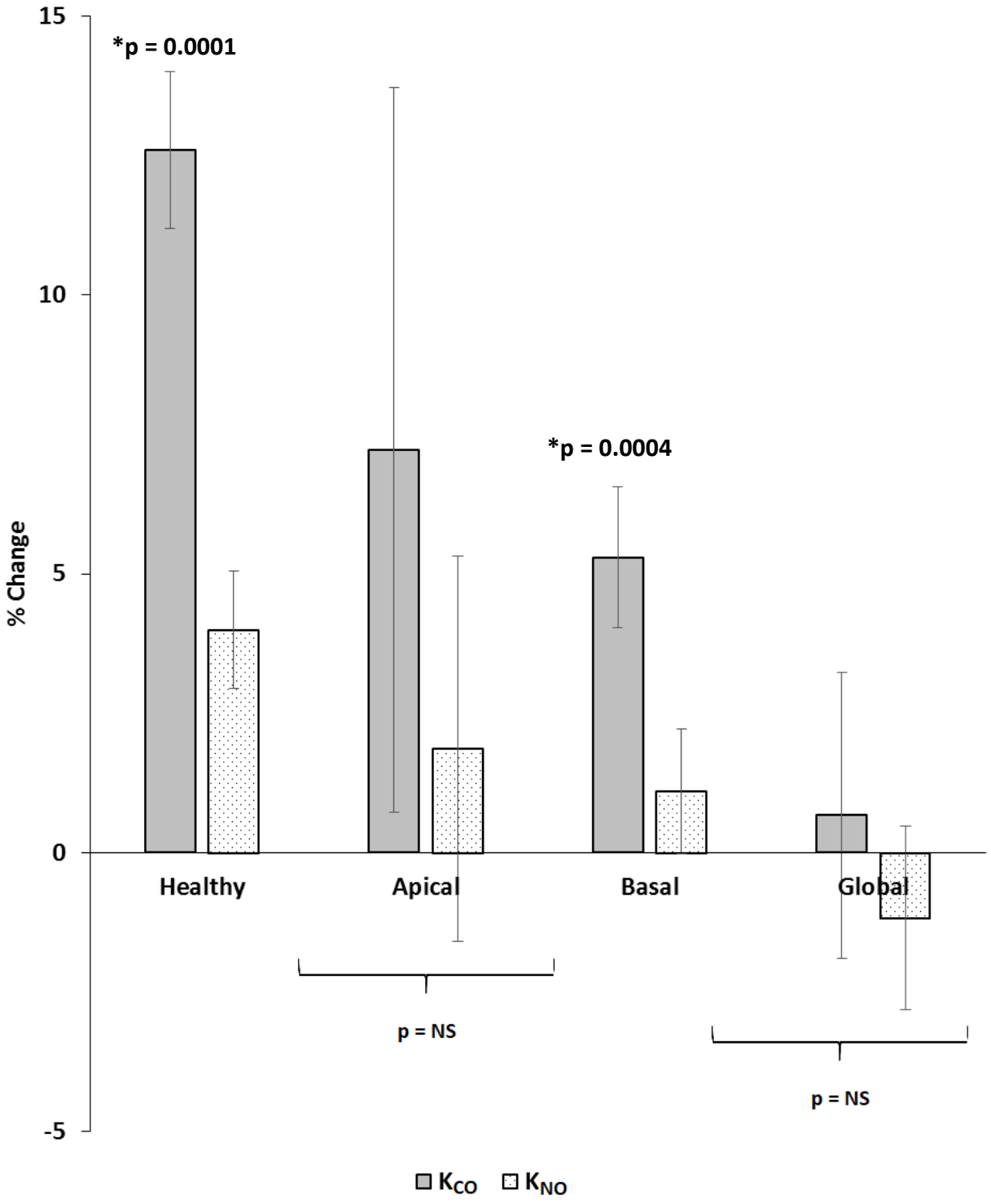

Figure 3.15 – A bar graph summarising the mean and standard error of the mean ± SEM percentage change of K_{CO} & K_{NO} from a seated to supine position in healthy subjects compared to those presenting with Apical, Basal & Global emphysematus change. K_{CO} and K_{NO} in K_{CO} & K_{NO} in mmol/kPa/min/L. p = NS indicates no significant change

When the TL_{NO}/TL_{CO} ratio was analysed for both basal and apical groups, a significant mathematical change was shown but as the percentage change was a mere -3.3% and -1.8% respectively, this was clinically not significant and fell well within the variability of the measurement.

The ratio of K_{NO}/K_{CO} in the basal group therefore, displayed a decrease of 3.9% showing mathematical significance but nothing more. Those with global emphysema showed no significant change with a decrease in mean value by 1.9%. These findings can be seen in Figure 3.16.

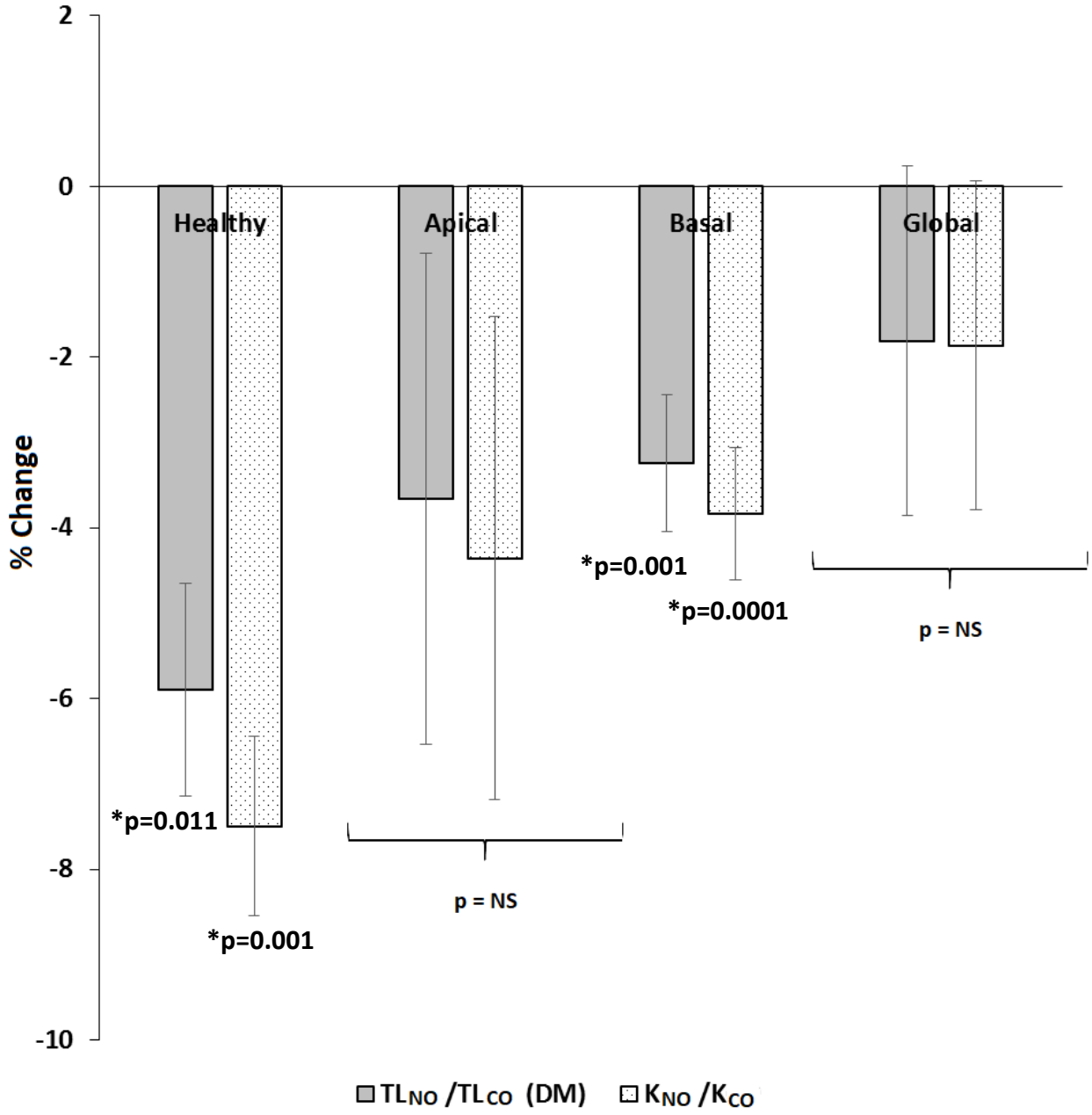

Figure 3.16 - A bar graph summarising the mean and standard error of the mean ± SEM percentage change of TL_{NO} /TL_{CO}, & K_{NO}/K_{CO} from a seated to supine position in healthy subjects compared to those presenting with Apical, Basal & Global emphysematous change. p = NS indicates no significant change

3.5.1 STUDY 5 DISCUSSION

Table 3.14 – Summary of trends in gas transfer measurements using CO & NO in A1AD subject groups once supine.

	TL_{NO}/TL_{CO} (D_M/Vc)	TL_{CO} (Vc)	TL_{NO} (D_M) (Surface area)	VAeff	K_{CO} (Vc)	K_{NO} (D_M) (Membrane thickness)	K_{NO}/K_{CO} (D_M/Vc)
Healthy Controls	↓↓	↑	↔	↔	↑↑	↔	↓↓
Apical A1AD	↓	↓	↓	↓	↑	↔	↓
Basal A1AD	↓↓	↓↓	↓↓	↓↓	↑↑	↔	↓
Global A1AD	↔	↓↓	↓↓	↓↓	↔	↔	↔

Approximately two thirds of A1AD patients will develop basal emphysema as opposed to the central emphysema more typically seen with smoking related damage. However, with the use of lung densitometry calculations, derived from High Resolution Computed Tomography (HRCT) a small number of subjects have displayed a greater involvement of the apex of the lungs (Parr et al, 2004) which can present functional differences. For this reason the A1AD patients in this study were separated into those with predominantly apical, basal and global distribution of disease. No previous work could be found that has studied postural induced changes to single breath gas transfer using Nitric oxide on basal/apical emphysematous patients.

Classically apical involvement impairs gas transfer and subjects with basal changes typically show greater airway impairment. The current study supports this to a degree with apical subjects displaying the lowest baseline results. However, all three subject groups had very similar levels of airway impairment as shown in their spirometric data. This was fortunate as the current study focuses on changes to gas transfer and any differences in airway integrity may have questioned the validity of results.

Unfortunately, there is insufficient data to show a statistically significant pattern when comparing apical to basal patients in this current study. Subsequently no significant differences were seen for the patients with apical emphysema for any parameter between the two postures due to the small numbers in this subgroup, nevertheless some interesting trends can be inferred. The exact occurrence of apical emphysema in A1AD patients is not known but it is known to be in the minority (Parr et al, 2006) so these current findings may truly reflect what happens in A1AD.

The sitting to supine change in TL_{CO} showed a decrease in all three of the A1AD patient groups which contrast the increases seen in the healthy control group. Like study 4 these changes correlated well with volumetric decreases when supine which was represented by a decrease in VAeff. The smallest change for both TL_{CO} and VAeff was seen in the apical patients, the largest in those with global (non-apical) emphysema. This relates to the size of the affected area in the lungs; the apex of the lungs is the smallest total area so produces the smallest volume changes. However, subjects presenting with global emphysema did appear to have a disproportional decrease in TL_{CO} strongly correlating to the A1AD subjects with airways obstruction in the previous study, suggesting differences in disease severity causing greater damage to the alveolar/capillary membrane which would greatly limit the response to increased perfusion.

Changes to the pulmonary vascular network in COPD have been well documented as far back the 1960's (Liebow, 1959) and more recently reviewed using modern scanning technology (Peinado et al , 2008). Pulmonary vascular remodelling is highly prevalent in patients with emphysema and is recognised as an effect of hypoxia induced vasoconstriction resulting in the muscularisation of pulmonary blood vessels. Arterioles become muscularised and stiffen after a prolonged period of significant hypoxia.

This is a response by lung vasculature to balance pulmonary blood flow (perfusion) to ventilation and to protect the lung capillaries against flooding due to increased pulmonary arterial pressure as discussed previously. This hypoxia-induced pulmonary vessel muscularisation can be explained by vascular smooth muscle cell hypertrophy and hyperplasia as these muscles contract under the effects of prolonged hypoxia. Global emphysematous patients in the current study could have experienced high degrees of pulmonary vessel muscularisation which would effect changes to perfusion once in a supine position influencing changes to TL_{CO} but not TL_{NO}. This was more apparent during the analysis of K_{CO}.

TL_{NO} change also followed this pattern, albeit in a more amplified way due to the increased sensitivity to changes in lung volume as discussed previously. These findings again contrast the healthy controls which showed no change.

The three A1AD groups and the healthy controls showed decreases in the TL_{NO}/TL_{CO} ratio, indicating increases to capillary blood volume. Whilst these decreases were non-significant, it still suggests changes occurring under the effects posture.

The healthy controls showed a decrease in TL_{NO}/TL_{CO} (D_M/Vc) ratio caused by increases in TL_{CO} (Vc) with TL_{NO} (D_M) remaining stable suggesting increases in pulmonary capillary blood volume. The apical patients displayed disproportionate decreases (weighted toward TL_{NO} - D_M) in both parameters causing TL_{NO}/TL_{CO} ratio to decrease. This is due to either damage to the alveolar/capillary membrane limiting the response to increased perfusion, decreases in alveolar surface area changes once supine; or a mixture of both. The basal patients followed a very similar pattern to the apical group and suggest the same processes occurring.

Patients with global emphysematous changes showed a proportionate decrease in both TL_{CO} (Vc) and TL_{NO} (D_M) therefore the TL_{NO}/TL_{CO} (D_M/Vc) ratio showed minimal response to posture.

D_M response to posture correlated well with that of VAeff, Vc changes were more pronounced in patients with global emphysema and could be the result of vascular changes described above. The slight variation in response to VAeff should also be considered. The likelihood is all factors described above are influencing TL_{CO}'s response to the effects of posture and would explain at least in part the results observed here.

The analyses of both K_{CO} and K_{NO} have shown in the previous studies to simplify the discussion of postural change by eliminating the effects of volume. Both the healthy control group and patients with basal emphysema showed significant increase in K_{CO} once in a supine position. Potentially those subjects with apical emphysema also showed significant changes however, due to their small numbers this cannot be confirmed with statistical analysis. Both apical and basal patients had a smaller response in K_{CO} when compared to the healthy control group confirming the effects of emphysematous destruction on alveolar tissue (as described above) diminishing the response to increases in perfusion. However, these responses although reduced still occur and suggests levels of perfusion increase and hence, increases in pulmonary capillary blood under the effects of posture. Those displaying global emphysematous change showed no postural change to K_{CO}. As this group of patients showed no obvious difference in baseline gas transfer compared to the basal and apical patients the likely explanation would be these patients experiencing vascular remodelling. This combined with the homozygous destruction of alveolar membrane could explain why K_{CO} remained stable once supine in this group.

K_{NO} showed no significant change in all four subject groups showing that K_{NO} is a true mark of alveolar membrane function and is not affected by either changes to perfusion or lung volume, thus agreeing with the current study's hypothesis.

The ratio of K_{NO}/K_{CO} again followed the pattern of the TL_{NO}/TL_{CO} ratio showing that both ratios show the same physiological changes taking place with changes in posture.

3.6 STUDY 6 – The change in Structured Light Plethysmography (SLP) parameters from sitting to supine in 53 healthy controls (CON) and subjects with A1AD seperated into 73 with airflow obstruction (OBA1) and 20 without (NOA1).

93 of the A1AD subjects successfully performed SLP in both sitting and supine postures were separated into two different cohorts, those displaying airflow obstruction via spirometric data (n=73), those with no airflow obstruction (n=20). 2 subjects were unable to successfully obtain SLP data due to technique difficulties and so were exculded from this study.

Table 3.15 and 3.16 show the anthropometric and spirometric data for all cohorts respectively. All spirometric data showed all healthy controls presented no evidence of underlying airflow obstruction.

Table 3.15 - Anthropometric data of obstructive & non-obstructive subjects with A1AD.

	Age	GENDER	pk/yr	Height (m)	Weight (kg)	BMI
CON	42.8 (19.4)	24M:29F	0.9 (0.9)	1.69 (0.12)	73.5 (13.5)	25.7 (4.4)
NOA1	51.9 (15.7)	12M:8F	8.8 (7.8)	1.73 (0.24)	77.5 (16.9)	25.7 (3.1)
OBA1	58.1 (9.8)	43M:30F	18.5 (13.5)	1.71 (0.11)	78.1 (15.8)	26.6 (4.4)

Values shown as Mean with ± 1 SD

Table 3.16 - Spirometry data of obstructive & non-obstructive subjects with A1AD.

	FEV1 (L)	FEV1 (%)	FEV1 (SR)	FEV1/FVC	FEV1/FVC (SR)
CON	3.50 (0.99)	103.7 (4.9)	0.8 (0.2)	79.7 (6.3)	-0.1 (0.8)
NOA1	3.44 (0.76)	106.0 (13.4)	0.46 (0.90)	76.2 (6.9)	-0.33 (0.93)
OBA1	1.54 (0.65)	52.4 (19.7)	-3.17 (1.50)	39.0 (12.1)	-5.54 (1.59)

Values shown as Mean with ± 1 SD

Table 3.17 shows the data collected for these subjects with statistically significant differences highlighted.

Table 3.17 – Summary of SLP sitting vs. supine data in healthy controls, non-obstructive A1AD subjects (NOA1) and obstructive A1AD subjects (OBA1).

		HEALTHY		NOA1		OBA1	
		Sitting	Supine (15mins)	Sitting	Supine (15mins)	Sitting	Supine (15mins)
RCC (%)	MEDIAN	53	34	52	35	50	37
	IQR	10.5	17.3	16.3	29.3	14.0	21.5
	AB Change		-19*		-17*		-13*
	%change		-35.8*		-32.7*		-26.0*
URCC (%)	MEDIAN	28.5	18.5	27.5	21	26	20.5
	IQR	10.25	10.5	11.5	16	8	11.5
	AB Change		-10.0*		-6.50*		-5.5*
	%change		-35.1*		-23.6*		-21.2*
PARA (°)	MEDIAN	4.75	10.45	6.25	14.10	6.75	15.85
	IQR	4.78	9.10	4.45	22.6	4.60	13.4
	AB Change		5.70*		7.90*		9.10*
	%change		120*		126*		135*
PAUL (°)	MEDIAN	2.95	4.85	3.05	6.00	3.15	9.75
	IQR	2.28	5.80	5.13	5.50	2.88	7.58
	AB Change		1.90*		3.00*		6.6*
	%change		64.4*		96.7*		209*
PAUA (°)	MEDIAN	5.95	11.5	7.35	12.8	8.00	14.9
	IQR	4.23	10.5	6.95	16.8	5.00	14.2
	AB Change		5.5*		5.4*		6.9*
	%change		92.4*		73.5*		85.6*

Values shown as Median with Inter Quatile Range (IQR), Absolute change (AB Change) & percentage change (%change). Ribcage contribution (RCC) & upper ribcage contribution (URCC) values are shown as percentage. Phase angle of ribcage vs abdomen (PARA); upper ribcage vs lower ribcage (PAUL); & upper ribcage vs abdomen (PAUA) are shown as angle degrees (°). The median sitting value is from two consistent values within 5%. * $p \leq 0.05$ indicating a significant change from baseline measurements.

All three subject groups showed significant decreases in RCC when moved into a supine position however Figure 3.17 shows that some subject in both the NOA1 & OBA1 group showed significant increases thus generating a wider spread of data. These changes decrease as airflow obstruction increase with those with significant airflow obstruction (OBA1) showing the smallest percentage change. These results are displayed graphically in Figure 3.17.

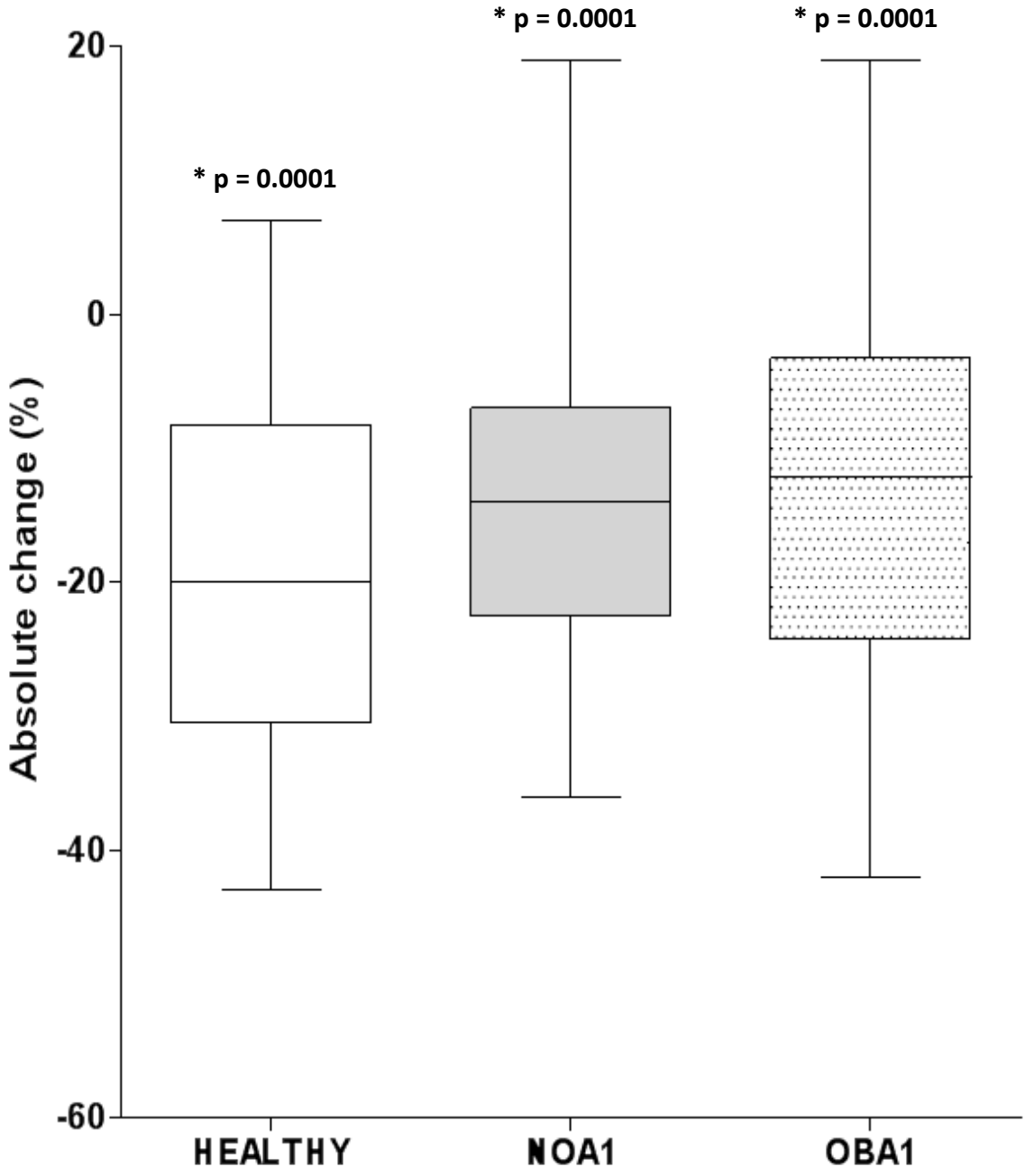

Figure 3.17 - A box & whisker plot summarising the absolute median change, IQR & extremes of data of the ribcage contribution (RCC) from a seated to supine position in healthy subjects compared to those with A1AD and presenting with (NOA1) and those with out (OBA1) airflow obstruction. RCC shown as a percentage (%). There were no outliers.

Similar results were seen when analysing URCC, all three subject groups showed statistical significant decreases when in a supine posture. The spread of data was not as vast as seen for RCC which could indicate less artifact is produced during data collection as a smaller area of the chest is being analysed. Again those subjects with airflow obstruction showed the smallest change from baseline results as seen in Figure 3.18.

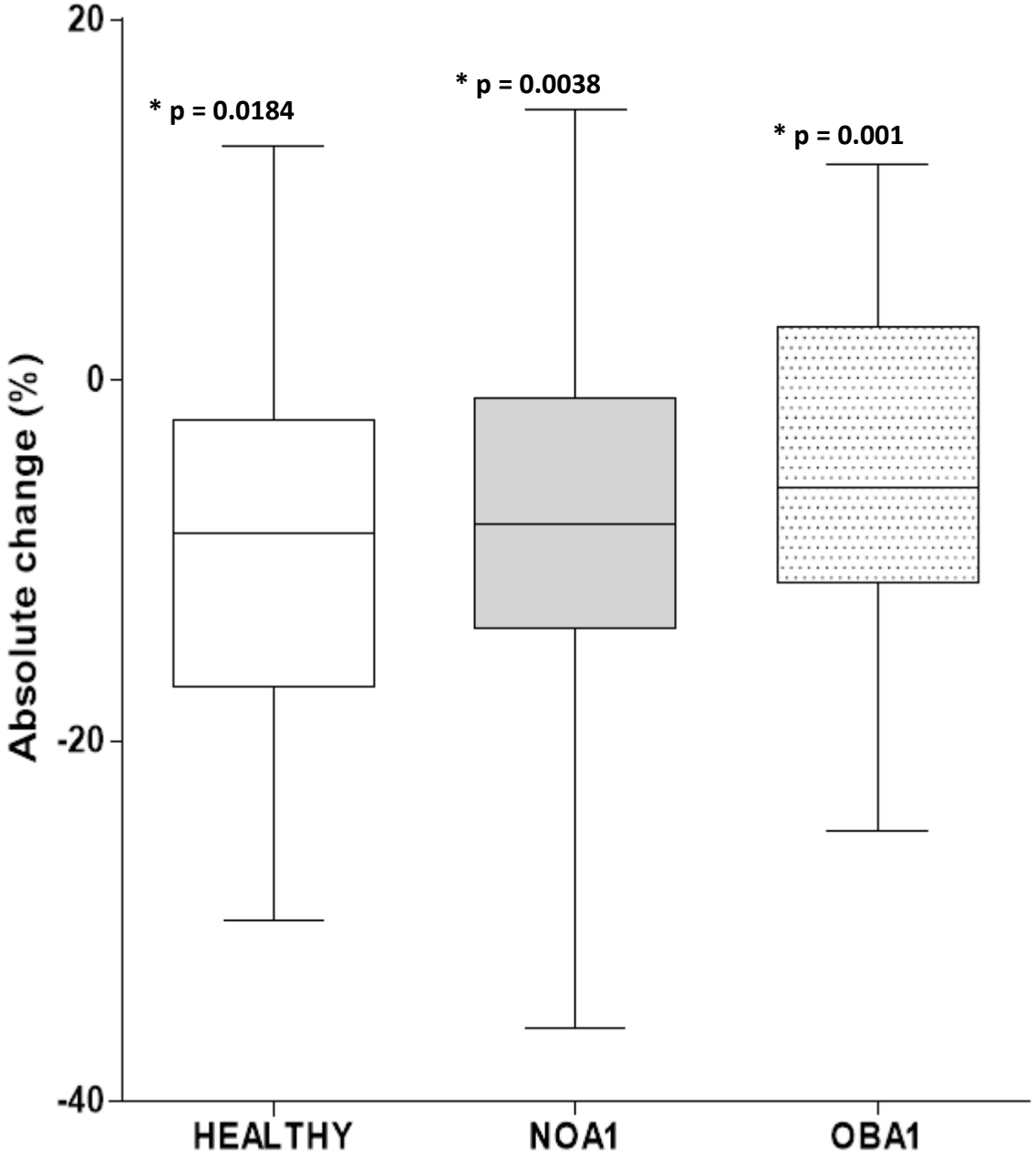

Figure 3.18 – A box & whisker plot summarising the absolute median change, IQR & extremes of data for upper rib cage contribution (URCC) from a seated to supine position in healthy subjects compared to those with A1AD and presenting without (NOA1) and those with airflow obstruction (OBA1). URCC shown as a percentage (%). There were no outliers.

Upon analysis of phase angle (in this case PARA) all three subject groups showed significant increases to posture which again appeared to be influenced by the presence of airflow obstruction. Those subjects with significant airflow obstruction (OBA1) showed the greatest change once supine but also showed the greatest range of data with some subjects showing both significant increases and decreases. These subjects are still becoming more asynchronus in breathing but the abdomen moves first rather than the chest, reasons for this are unclear.

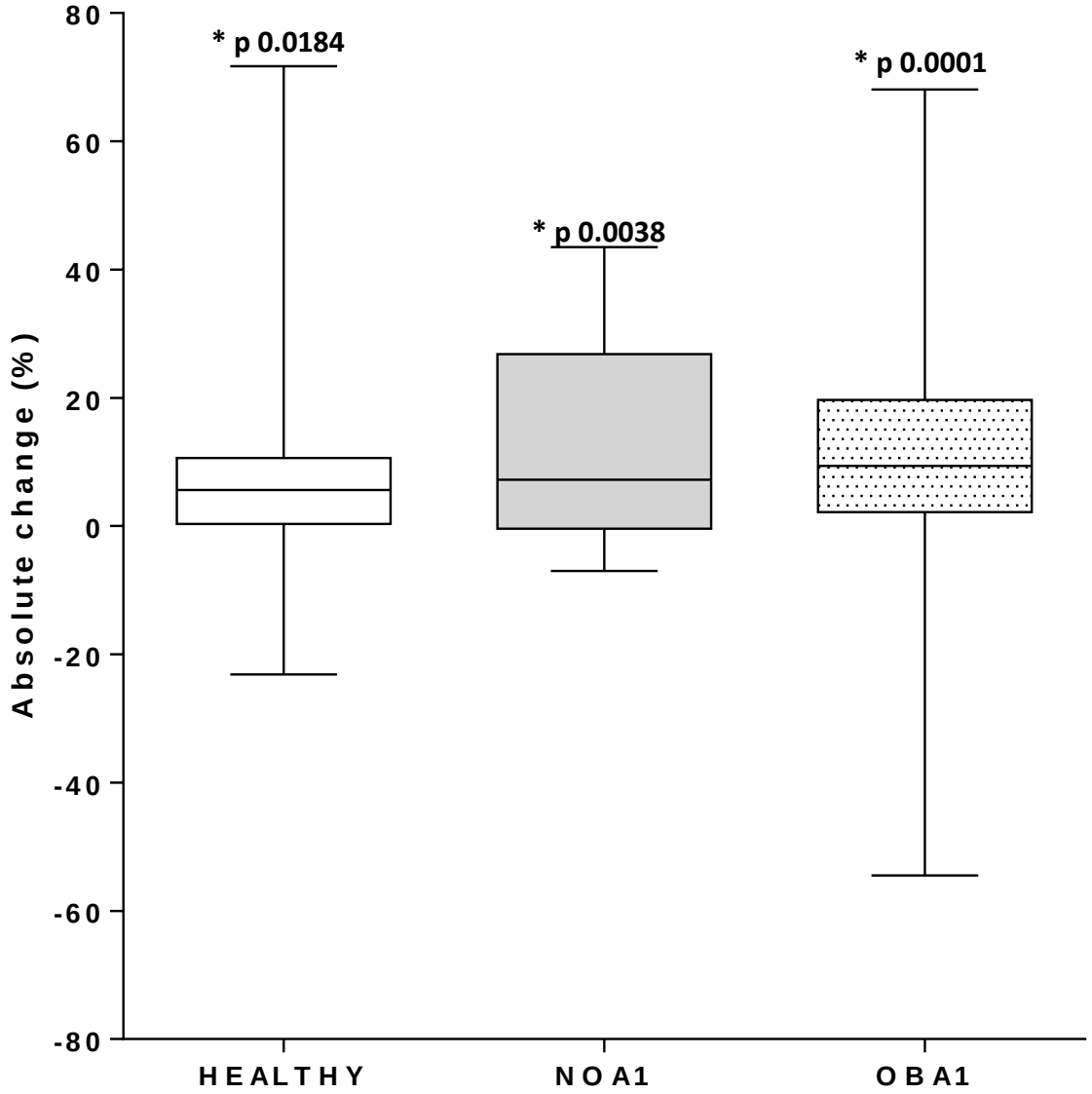

Figure 3.19 – A box & whisker plot summarising the absolute median change, IQR & extremes of data for phase angle of ribcage vs abdomen (PARA) from a seated to supine position in healthy subjects compared to those with A1AD and presenting without (NOA1) and those with airflow obstruction (OBA1). PARA shown as arch degrees (°). There were no outliers.

Analysis of the phase angle of the upper vs the lower ribcage (PAUL) showed significant increase in all three subject groups. Again those with airflow obstruction (OBA1) exhibited the greatest change, indicting measurements of PAUL relate to the presence of airflow obstruction. A minority of healthy controls showed a decrease once supine indicating the lower ribcage moves first. These changes are displayed in Figure 3.20.

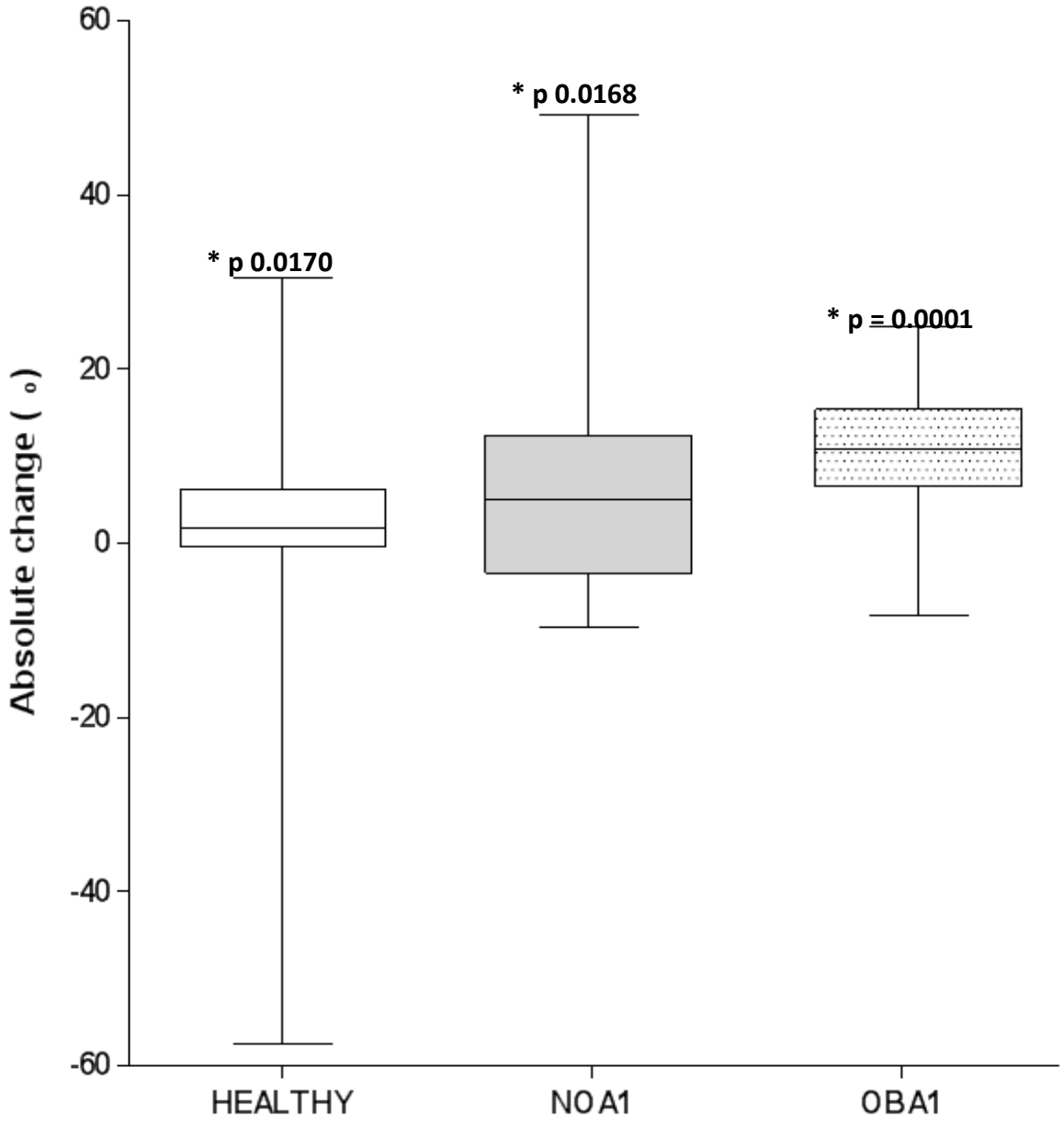

Figure 3.20 - A box & whisker plot summarising the absolute median change, IQR & extremes of data for upper ribcage vs lower ribcage (PAUL) from a seated to supine position in healthy subjects compared to those with A1AD and presenting without airflow obstruction (NOA1) and those with (OBA1). PAUL shown as arch degrees (°).

The final analysis of study 6 was of the phase angle of the upper ribcage and the abdomen (PAUA). The three subject groups showed significant increases once supine, however these increases did not relate to airflow obstruction. The healthy controls showed greater changes to those subjects with A1AD but normal spirometry (NOA1). These findings are displayed in Figure 3.21.

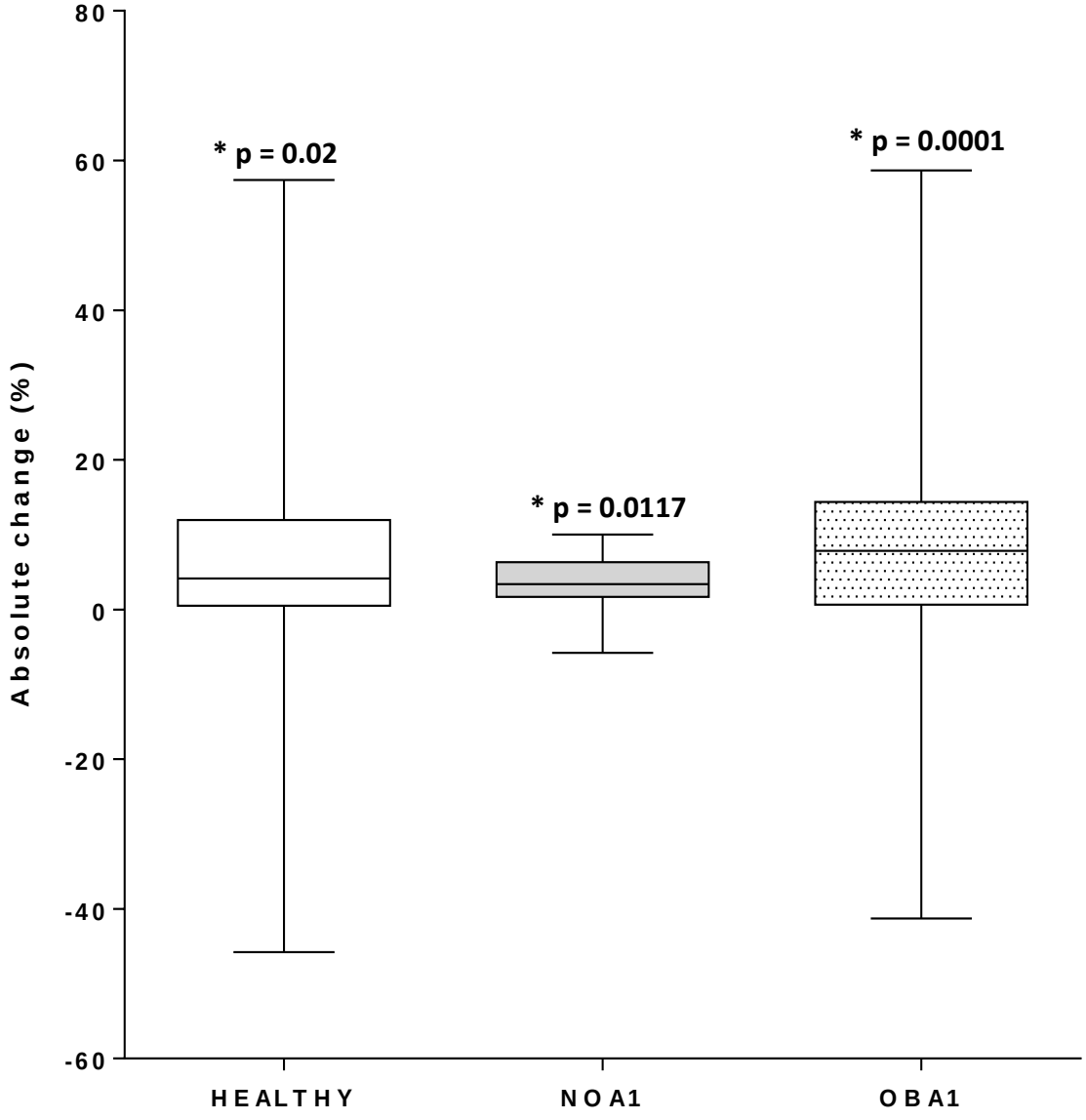

Figure 3.21- A box & whisker plot summarising the absolute median change, IQR & extremes of data for upper ribcage vs abdomen (PAUA) from a seated to supine position in healthy subjects compared to those with A1AD and presenting without airflow obstruction (NOA1) and those with (OBA1). PAUA shown as arch degrees (°).

3.6.1 STUDY 6 DISCUSSION

By using the TL_{NO}/TL_{CO} gas transfer technique in different postures, these studies have established that changes in lung volume under the effects of mechanical alterations to the respiratory system take place and are dependent on severity of the condition and possibly the region of lung involved. The use of SLP provides more information on these mechanical alterations in respiratory mechanics taking place. The A1AD subjects were again separated into those with airflow obstruction and those without.

The ribcage contribution (RCC%) and upper ribcage contribution (URCC%) both significantly decreased in the healthy control group and both A1AD patient groups showing that all subjects become abdominally dominant once in a supine position. This agrees with current understanding that being in a supine postion isolates the diaphragm, reducing activity of accessory muscles located in the upper chest so the action of breathing becomes more dependent on the action of the abdomen (Laroche et al, 1988). This phenomenon is commonally used in the assessment of respiratory muscle function, by lying the patient in a supine position to induce diaphragmatic isolation one can analyse if this causes a significant reduction in vital capacity (VC) when compared to the volume obtain whilst sitting upright (Allen et al, 1985). The quantity of change does not appear to relate with disease severity as expected signifying regional contribution is more dependent on actual muscle strength (not measured in the current study).

Phase angle is a measurement of the synchronicity of movement between two regions. For the current study three parameters analysing phase angle had been created; PARA (phase angle between ribcage and abdomen), PAUL (phase angle between the upper and lower chest) and PAUA (phase angle between upper chest and abdomen) which have all be previously decribed in chapter 1.7.4. (Gilmartin et al, 1984).

The healthy controls and both groups of A1AD patients all showed significant changes in PARA indicating that all subjects develop more asynchronous breathing movements once in a supine position. These changes become more pronounced with increasing severity of airways obstruction. This finding partly agrees with previous work (Marini et al, 1988). It's thought these changes are more frequent during loaded breathing where the work of breathing is increased. This can be achieved by simply moving subjects into a supine position, using the ribcage as a weight which the respiratory muscles have to overcome to maintain 'normal' breathing and ventilation. Airways obstruction can add further to the breathing load.

Significant changes were not expected to occur in the healthy control group and disagree with previous work (Priori et al, 2013) as it was thought these subjects should have sufficient muscle strength to maintain synchronized breathing. The main difference from the Priori et al study and the current study is the technology used. Priori and team used optoelectronic equipment (OPE) that requires the use of light reflecting spheres and several high definition cameras which may explain the differences in results. The OPE also seemed to produce large variation especially when measurements were taken in a supine position as seen in by their SD values. These large SD values could disguise significant differences present within their control subjects. Other variations exist such as the length of recording and when data collection commenced, all subjects in the current study were supine for 5 minutes before SLP data recordings were started compared to the immediate data collection in the other study. Priori's subjects were all young in comparison to the current study as all were recruited from their research department. The current participants were a mix of staff, patients and relatives which gave a spread of ages and body habitus.

The upper ribcage (PAUL) also showed significant increases when supine in all subject groups again confirming all subjects developed more asynchronous breathing, more specifically lateral rib cage paradox (Hoover's sign). The amount of change correlates with worsening airways obstruction, the smallest postural change was seen in the healthy control group and the largest was seen in those patients with airflow obstruction. This agrees closely with previous studies in COPD (Gilmartin et al, 1984) who concluded these bi-lateral rib cage movements were the result of a flattened diaphragm, outwardly positioned muscle fibres and a reduced zone of apposition; all attributed to hyperinflation and related to worsening airways obstruction. However, others (Priori et al, 2013) have stated that patients with severe COPD appear to be less asynchronous in a supine position. Again the use of OPE appears to produce a different outcome to other methods of measuring movement (magnetometry & SLP) so care must be taken when comparing measurements made using different equipment techniques.

Finally, relative upper chest monitoring (PAUA) again demonstrated that all subject groups become more asynchronous when in a supine position showing all three parameters relating to the synchronicity of chest and/or abdominal movements agree.

However PAUA does not seem to relate to the severity of COPD as the healthy controls appear to have the greatest postural change. This appears to be due to the two A1AD groups showing more asynchronous movements when seated thus reducing the amount of change seen once supine.

3.7 STUDY 7 – The change in Structured Light Plethysmography (SLP) parameters from seated to supine in healthy controls (CON) and subjects with A1AD when seperated from those with Basal, Apical and Global emphysematous changes.

48 of the A1AD subjects with CT data successfully performed SLP in both sitting and supine postures were then again separated in to three different cohorts, those with global emphysematous change (n=12), those with emphysematous changes predominantly at the base of the lungs (n=32) and those changes predominantly at the apex of the lungs (n=4). 47 subjects unfortunately had no CT data available or were unable to successfully obtain SLP data due to technique difficulties and so were exculded from this study.

Table 3.18 and 3.19 show the anthropometric and spirometric data for these three new cohorts. Table 3.20 shows the data collected for these subjects with significant differences highlighted. The main difference that exists between the groups are the number of subjects in each group. Out of 43 with the majority of subject displaying basal emphysema. As one third of A1AD will develop basal emphysematous changes we felt this was a true reflection on the population.

Table 3.18 - Anthropometric data of those subjects displaying Basal, Apical & Global emphysematous changes as a result of A1AD.

	Age (Yrs)	Sex	Smoking (pk/yrs)	Height (m)	Weight (kg)	BMI
Basal	59.0 (8.9)	20M:12F	16.5 (15.5)	1.73 (0.13)	81.2 (17.2)	27.3 (7.0)
Apical	58.3 (9.4)	2M:2F	32.4 (31.4)	1.70 (0.10)	85.9 (15.3)	28.9 (3.5)
Global	58.5 (8.3)	10M:2F	19.5 (18.5)	1.70 (0.10)	73.7 (14.1)	24.3 (3.7)

Values shown as Mean ± 1 SD

Table 3.19 - Spirometry data of those subjects displaying Basal & Apical emphysematous changes as a result of A1AD.

	FEV1 (L)	FEV1 (%)	FEV1 (SR)	FEV1/FVC (%)	FEV1/FVC (SR)
Basal	1.90 (0.82)	60.1 (24.1)	-2.7 (1.31)	42.9 (13.0)	-5.0 (1.7)
Apical	1.60 (0.39)	53.6 (16.6)	-3.2 (1.22)	37.2 (12.1)	-5.8 (1.8)
Global	1.50 (0.80)	47.9 (15.8)	-3.5 (2.19)	36.3 (15.3)	-5.7 (1.6)

Values shown as Mean ± 1 SD

All groups are well matched for age and BMI, a small discrepancy exists in gender in our basal and global cohort and in smoking histories between the apical cohort. However, no significant discrepancy can be seen in spirometric data and so again, one could argue the relevance of these differences. No signifcant differences were seen in the subjects with apical disease due to small numbers (n=4) therefore, little conclusion can be made from these findings.

Table 3.20 - Summary of SLP seated vs. supine data in healthy controls and A1AD subjects displaying Basal, Apical and Global emphysematous changes based on CT data.

		HEALTHY		APICAL		BASAL		GLOBAL	
		Sitting	Supine (15mins)	Sitting	Supine (15mins)	Sitting	Supine (15mins)	Sitting	Supine (15mins)
RCC (%)	MEDIAN	***53***	***34***	45	20	***50***	***36***	49	41
	IQR	***10.5***	***17.3***	5.0	2.8	***15.0***	***23.0***	9.7	12.8
	AB Change		***-19****		-25		***-14****		-8
	%change		***-35.8****		-55.0		***-28.0****		-16.3
URCC (%)	MEDIAN	***28.5***	***18.5***	23.5	9.50	***27***	***20.5***	26	24
	IQR	***10.3***	***10.5***	2.0	4.8	***10***	***13.3***	8.5	5.8
	AB Change		***-10.0****		-14.0		***-6.50****		-2
	%change		***-35.1****		-59.5		***-24.1****		-7.6
PARA (°)	MEDIAN	***4.75***	***10.5***	4.15	18.3	***7.1***	***16.25***	6.55	11.3
	IQR	***4.78***	***9.10***	3.4	4.4	***4.20***	***12.8***	5.70	23.1
	AB Change		***5.70****		14.2		***9.20****		4.80
	%change		***120****		340		***130****		72.5
PAUL (°)	MEDIAN	***2.95***	***4.85***	5.45	12.8	***4.5***	***9.40***	2.65	9.55
	IQR	***2.28***	***5.80***	4.0	8.9	***3.60***	***8.10***	2.03	7.00
	AB Change		***1.90****		7.35		***4.90****		6.9
	%change		***64.4****		135		***109****		260
PAUA (°)	MEDIAN	***5.95***	***11.5***	7.65	16.7	***8***	***14.5***	8.05	8.7
	IQR	***4.23***	***10.5***	10.50	11.9	***5.20***	***12.4***	5.33	13.4
	AB Change		***5.5****		9.05		***6.5****		0.65
	%change		***92.4****		118		***81.3****		8.1

Values shown as Median with Inter Quatile Range (IQR), Absolute change (AB Change) & percentage change (%change). RCC & URCC values are shown as percentage contribution (%), PARA, PAUL & PAUA are shown as angle degrees (°). The median sitting value is from two consistent values within 5%. * p ≤ 0.05 indicating a significant change from baseline measurements.

Both the healthy control group and subjects with basal emphysematous changes showed significant decreases in measurements of RCC. Those subjects with global lung changes showed no such significance once supine indicating a difference in mechanics upon changes to posture. These findings are displayed in Figure 3.22.

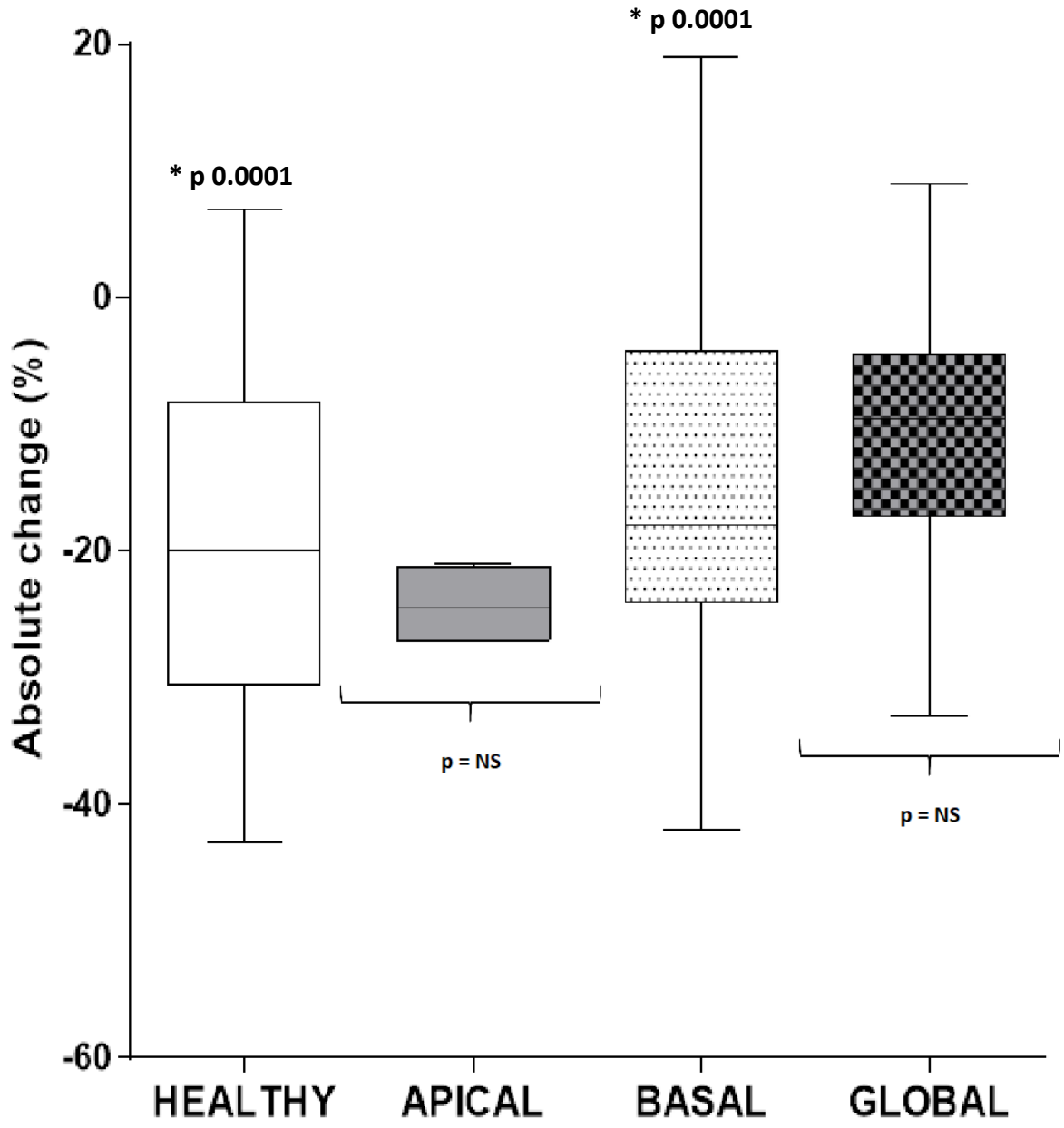

Figure 3.22 - A box & whisker plot summarising the absolute median change, IQR & extremes for data for rib cage contribution (RCC) from a seated to supine position in healthy subjects compared to those presenting with Apical, Basal & Global emphysematous change. RCC shown as a percentage (%). p = NS indicates no significant change from seated to supine measurements There were no outliers.

Similar findings were obtained when measuring URCC with both the healthy controls and those A1AD subjects with basal lung involvement showing significant decreases once supine. Those with global emphysema showed little change to posture again indicating differences in lung mechanics. These findings are illustrated in Figure 3.23.

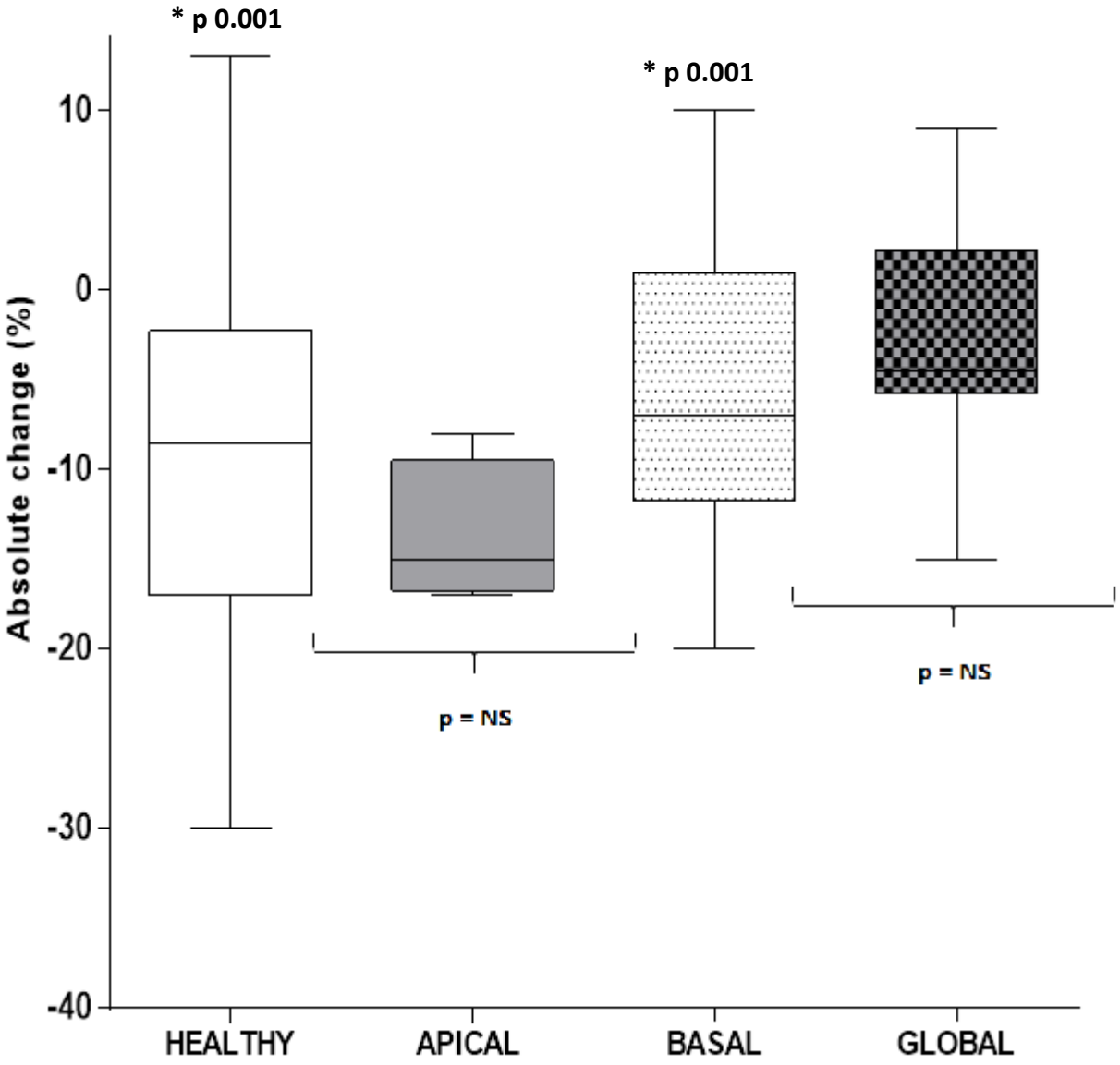

Figure 3.23 – A box & whisker plot summarising the absolute median change, IQR & extremes for upper rib cage contribution (URCC) data from a seated to supine position in healthy subjects compared to those presenting with Apical, Basal & Global emphysematous change. RCC shown as a percentage (%). p = NS indicates no significant change from seated to supine measurements There were no outliers.

Both the healthy control group and subjects with basal emphysematous changes showed significant decreases in measurements of PARA. Those subjects with global lung changes showed no such significance once supine indicating a difference in mechanics upon changes to posture. These findings are displayed in Figure 3.24.

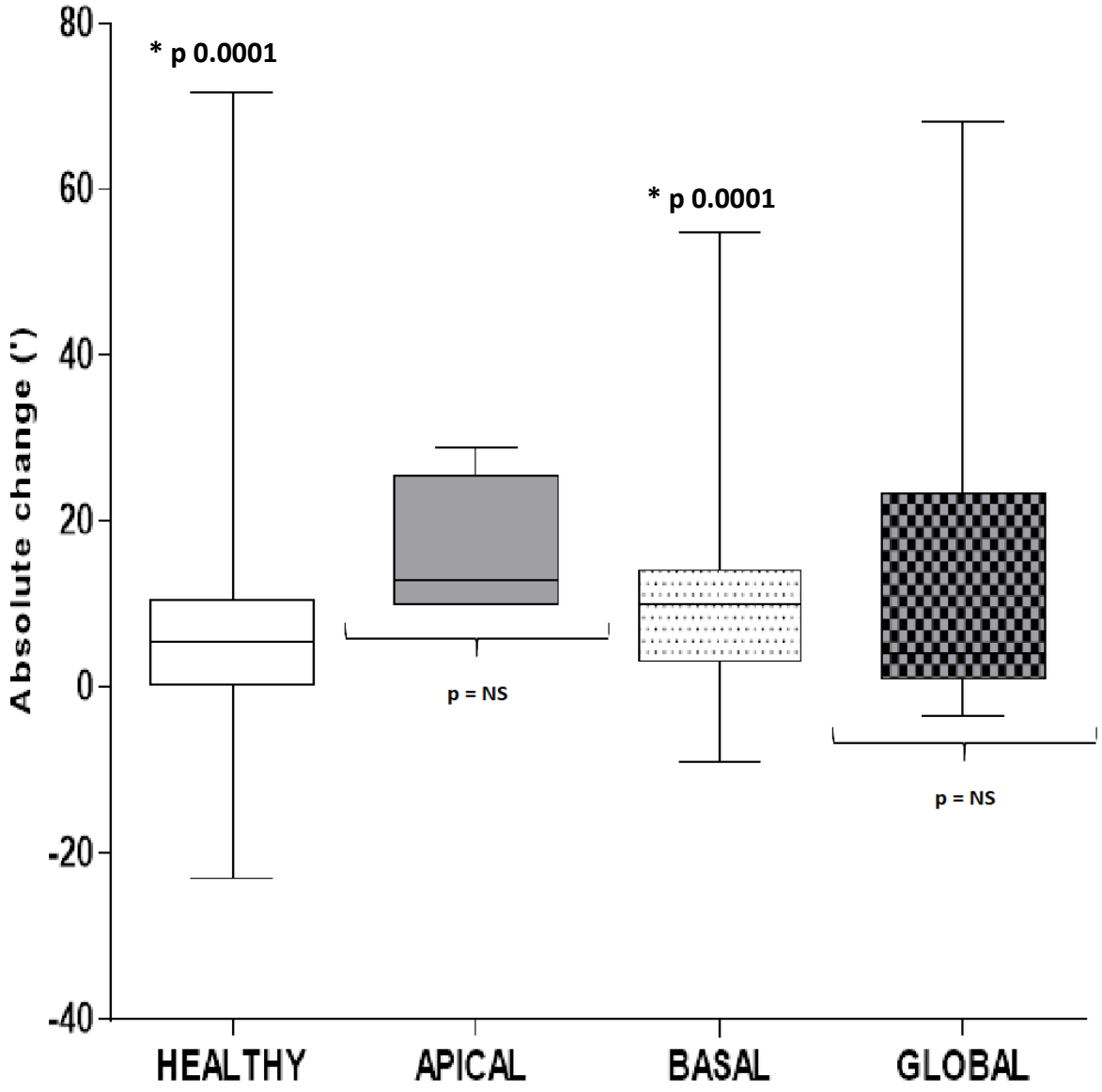

Figure 3.24 - A box & whisker plot summarising the absolute median change, IQR & extremes for phase angle data of the ribcage vs abdomen (PARA) from a seated to supine position in healthy subjects compared to those presenting with Apical, Basal & Global emphysematous change. PARA shown as arch degrees (°). p = NS indicates no significant change from seated to supine measurements There were no outliers.

Again, similar findings were obtained when measuring PAUL with both the healthy controls and those A1AD subjects with basal lung involvement showing significant decreases once supine. Those with global emphysema showed little change to posture again indicating differences in lung mechanics. These findings are illustrated in Figure 3.25.

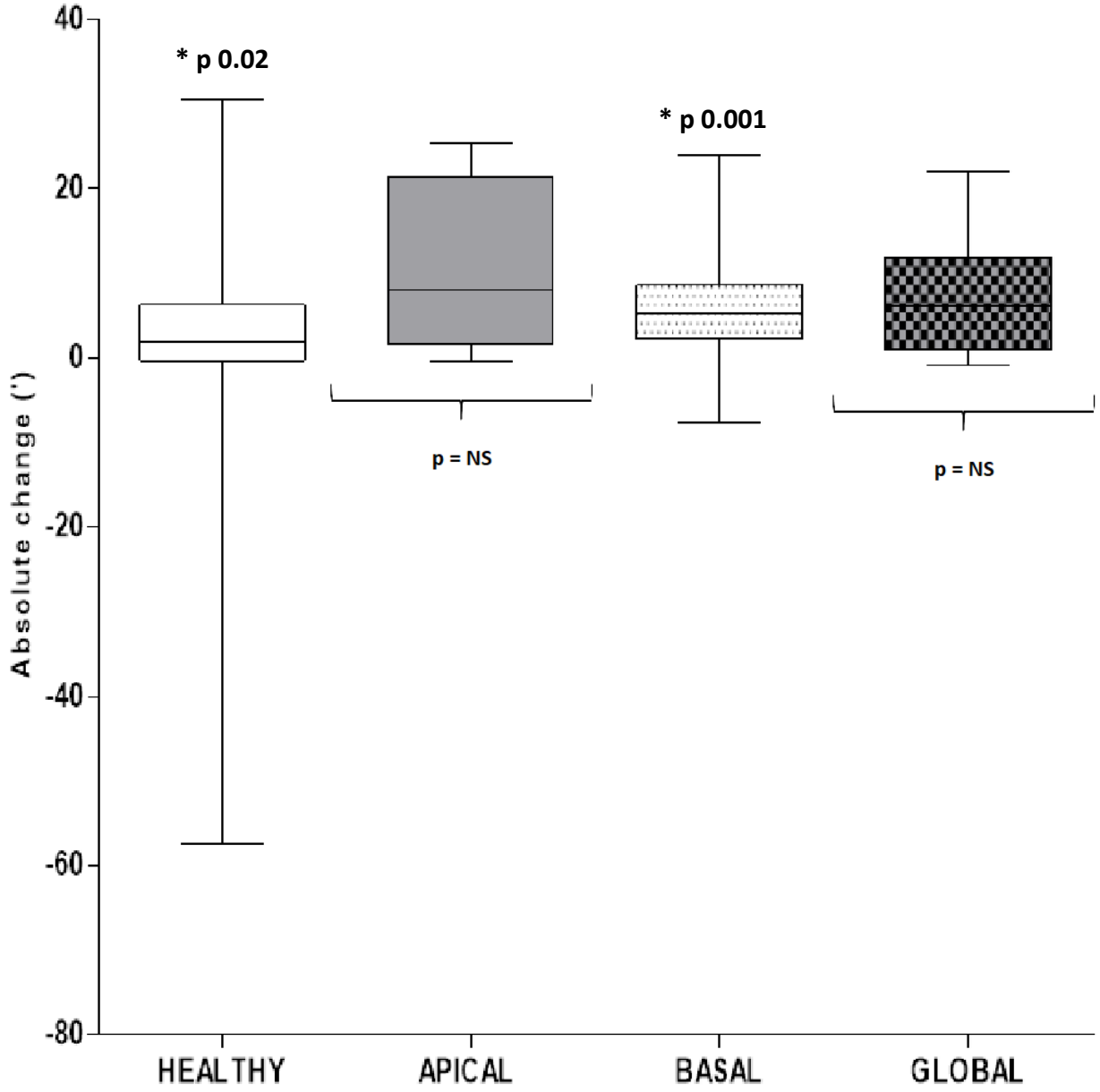

Figure 3.25 – A box & whisker plot summarising the absolute median change, IQR & extremes for phase angle of the upper ribcage vs lower ribcage (PAUL) from a seated to supine position in healthy subjects compared to those presenting with Apical, Basal & Global emphysematous change. PAUL shown as arch degrees (°). p = NS indicates no significant change from seated to supine measurements There were no outliers.

The final analysis of the phase angle was of the upper ribcage and the abdomen (PAUA). The heathy controls and basal subject groups showed significant increases once supine. The healthy controls showed greater changes to those subjects with A1AD but normal spirometry (NOA1). These findings are displayed in Figure 3.26.

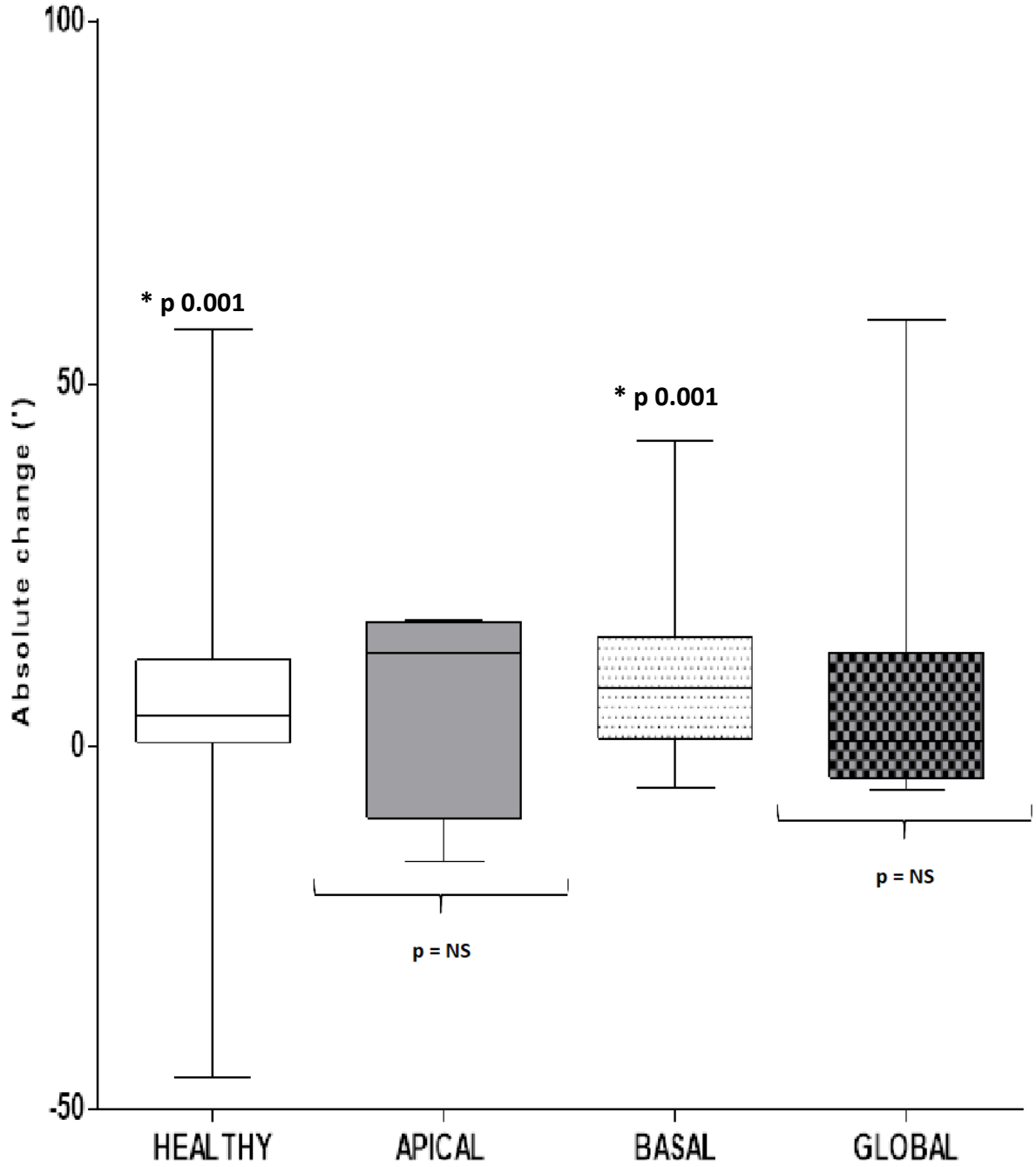

Figure 3.26 - A box & whisker plot summarising the absolute median change, IQR & extremes for phase angle of the upper ribcage vs abdomen (PAUA) from a seated to supine position in healthy subjects compared to those presenting with Apical, Basal & Global emphysematous change. PAUA shown as arch degrees (°). p = NS indicates no significant change from seated to supine measurements There were no outliers.

3.7.1 STUDY 7 DISCUSSION

The previous study (study 6) has shown that differences do exsist in thorax/abdominal movements between healthy subjects and patients with varying grades of airways obstruction as a result of emphysematous damage. However these differences do not necesserally relate with severity of airflow obstruction. The A1AD patients were again separated into those with basal, apical and global distributions of emphysema to establish if differences in regional emphysema cause differences in chest movement which aid in the diagnosis. No previous work of respiratory movements have been conducted on regional distributions of emphysema so the following results may provide an insight into mechanical differences present.

Due to the small number of "apical" patients (n = 4) recruited to this study no statistically significant differences where seen for any parameter measured, however small differences were obtained and therefore the implications of these trends are discussed but their limitations must be appreciated.

The ribcage contirbution (RCC%) and upper ribcage contribution (URCC%) both significantly decreased in the healthy control group and patients with basal & apical emphysema showing abdominal dominance once in a supine position as would be predicted physiologically. The global emphysematous group also showed a small decrease but was not significant. This result may reflect the increasing volume (hyperinflation) and therefore decreasing compliance, of the ribcage as hyperinflation develops due to the onset of emphysema.

The healthy controls and the three groups of A1AD patients all showed significant changes in chest wall/abdominal synchronicity (PARA) when supine indicating that the subjects develop more asynchronous breathing movements due to the altered mechanics of being in a supine position and this agrees with accepted wisdom.

Those with apical disease showed a trend towards the biggest change.

The basal and healthy groups showed similar changes with the global group showing the least change in paradoxical movement. Previous work has proposed rib cage abdominal paradox predominantly relates to increases in respiratory load rather than muscle fatigue (Tobin et al, 1987). In supine position the patients weight is the primary load due to the effects of gravity and the subjects BMI correlate well with rig cage-abdominal paradox. The global emphysema patient group were the lightest (weight = 73.7kg) and had the smallest change in breathing pattern, the heaviest group were the apical patients (weight = 85.9kg) and had the largest change.

One could conclude that a more homogenous distribution of lung disease produces more homogenous movements of the chest reducing asynchronous movements. These changes become more prononuced with increasing severity of emphysema as those subjects with airways obstruction developed the greatest change. These findings partly agree with previous work as discussed in section 1.2 of the introduction (Marini et al, 1988). It's thought these changes are more frequent during loaded breathing where the workload to breathe has been increased. This can be achieved by simply moving subjects into a supine position, using the ribcage as a weight which the respiratory muscles have to overcome to maintain 'normal' breathing and ventilation. Significant changes were not expected to occur in the healthy control group and disagree with previous work (Priori et al, 2013) as it was thought these subjects should have sufficient muscle strength to maintain synchronized breathing. The main difference from the referred study and the current study is the technology used. Priori and team used optoelectronic equipment (OPE) that requires the use of light reflecting spheres and several high definition cameras which may explain the differences in results. The OPE also seemed to produced large deviations especially measurements taken in a supine position. These large SD values could disguise significant difference present within their control subjects.

The upper vs lower ribcage measures (PAUL) also showed significant increases in all subject groups again confirming all subjects become more asynchronous in the movement of breathing and more specifically lateral rib cage paradox. The extent of change correlates with worsening airways disease. This agrees closely with previous studies (Gilmartin et al, 1984). This indicates respiratory mechanics may have a role to in TL_{NO}/TL_{CO} results.

The upper ribcage vs abdomen measures (PAUA) again demonstrated that all subject groups become more asynchronous when in a supine. Paradoxical inspiratory motion of the abdomen has been investigated in 1988 by a group led by Marini. It was noted that this abnormal movement is more frequent during loaded breathing where the workload to breathe has been increased. Does the mechanical changes in ventilation/perfusion relationships in different areas of the lung?

This conclusion is speculative, but warrants further investigation in an appropriately powered study.

4.0 CONCLUSIONS

The studies within this thesis have provided both novel information and confirmed current understanding regarding physiological and mechanical changes that take place when subjects move into a supine posture.

The first aim of the current study was to establish the optimum time for sufficient perfusion of the apices of the lungs, resulting in an increase in gas transfer measurements. The duration for which a subject should be placed in the supine position before a stable and representative measure of gas transfer can be made, was not known. The current study has shown all healthy control subjects showed increased gas transfer measurements (TL_{CO} & K_{CO}) demonstrating increases in pulmonary perfusion immediately after lying supine, with an optimal effect after 15 minutes. This could then be applied to subsequent investigations.

Both measurements in gas transfer and SLP showed that the healthy control cohort showed a reduction in VAeff of 5% once in a supine position. This was surprising as it was thought that healthy controls would have sufficient muscle strength to maintain alveolar volume. SLP showed that all subjects including the healthy controls became more abdominally dominant when in a supine position. This agrees with current understanding as isolation of the diaphragm takes place once supine, what wasn't expected was the healthy controls showing greater movements of the chest compared to abdomen so disagreed with our current understanding. All though this is a disagreement is still answers question two of aims.

Patients with A1AD did not respond in the same way; many patients showed significant decreases in lung volume once supine, reducing lung surface area resulting in decreases in $TL_{CO,}$ which was exacerbated in TL_{NO}. This related with disease severity as those displaying significant

airways obstruction and global emphysematous destruction had the greatest drop in lung volumes thus the largest decrease in gas transfer once supine.

$K_{CO,}$ which is not effected by changes to lung volume provided more reliable information on pulmonary perfusion. Heathly control subjects showed superior changes in K_{CO} when compared to those with A1AD as perfusion increased. These changes deminished as disease serverity increased, those showing significant airways obstruction and global emphysematous destruction exhibited the smallest response to posture. This suggests K_{CO} is a true reflection of the volume of pulmonary capillary blood (Vc) and would be a useful measurement in montoring disease progression in A1AD.

K_{NO} showed no response to changes to perfusion for any subject group tested, confirming its independance to Vc irrespective of disease. Therefore the parameter of K_{NO} is a true representation of the diffusing membrane (D_M) more specfically the thickness of the membrane. By performing CO and NO gas transfer measurements simultaneously K_{NO}/K_{CO} ratio is weighted towards the ratio of $D_M/V_{C.}$ Again this has shown to potentially be a useful measurement in monitoring A1AD.

The SLP device also showed all subjects become more asynchronous in the breathing movements once in a supine position and is affected by the severity of the disease rather than its location. Measurements using SLP have confirmed all subjects become more abdominantly dominant once in a supine postion which appears to be influenced by disease location rather than severity.

These studies have shown that simultaneous gas transfer measurements using Nitric Oxide/Carbon Monoxide and SLP measurements provide additional insight into patients disease location and severity and therefore, would be of value to be used as part of patients clinical investigations. It is not felt that these techniques will ever replace the need for HRCT scans but would be a useful addition for screening and monitoring of A1AD.

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