The Postural Affects on the Distribution of Microbubbles in the Lungs; A Model Study

by

Lauren Earthman

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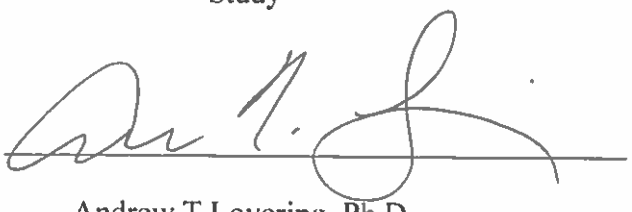
Approved: 

Andrew T Lovering, Ph.D.

Intrapulmonary arteriovenous anastomoses, (IPAVAs) are pathways located in the lungs that provide an alternate route for blood to travel though. Blood that travels through IPAVAs may not participate in gas exchange. We know that these paths exist, but their exact location in the lungs is unknown. With the use of microbubbles and macroaggregates of albumin (MAA) research was done that attempted to determine the size and location of IPAVAs. My research specifically looked at the routes that microbubbles take when they enter into the lungs and how this may relate to the routes bubbles take in the lungs when the lung is upright or supine. The results of this study suggest that when a subject is lying on their back bubbles should be distributed evenly throughout the lungs. Conversely, when a subject is sitting upright the same amount of bubbles float towards the top of the lungs as travel to the bottom, despite differences in the distribution of blood flow.

Acknowledgements

I would like to thank Professor Lovering for his immense amount of time and assistance to help me research the distribution of microbubbles in the lungs. I cannot express how thankful I am for the unique opportunity and for the guidance and knowledge that I received from him every step of the way. This has been a difficult yet rewarding journey that I will use to propel me forward in the future. I would also like to extend my gratitude to Jim Davis and Frank Petrassi for their assistance in our studies and for donating their blood, sweat and tears to my research. Special thanks to all other members of the Lovering Lab in giving me feedback and listening to my presentations. This has been an unforgettable journey and I also would like to thank my family for their constant support and love.

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Introduction:

The past couple of years I have spent time working in Dr. Lovering's Cardiopulmonary and Respiratory Physiology Lab, a research lab in the Human Physiology Department. After travelling to Bolivia with this lab following my freshman year of college I became interested in the research that they were doing. One area of interest for the Lovering Lab focuses on the effects of being at altitude on the body, as well as how different heart and lung pathologies can affect various aspects of our life such as exercise. Many of the studies and research done in the lab use human subjects in order to better understand different pulmonary and cardiac pathologies. As a result of the variation among humans there are thousands of different developmental and anatomical differences that someone can be born with or develop throughout the course of their life; many of these are not to our advantage. One of these pathways, called shunts, allows blood to pass from the arterial to venous end of a vessel without having to enter into capillary beds. More specifically, if located in the lungs they are referred to as intrapulmonary arteriovenous anastomoses, (IPAVAs) (9). These IPAVA, or shunt pathways, are distributed throughout the lung and the blood that passes through them bypasses gas exchange. Shunt pathways are known to be present in all humans, although their reason for existence remains unclear (6). These paths for the most part are not advantageous because they do not allow blood to deliver oxygen to tissues in the systemic circulation, or pick up oxygen if they are located in the lungs. IPAVAs have been shown to open in low oxygen; (hypoxic) conditions, or during exercise (5, 9). Blood flow through IPAVAs can also occur during rest (4). Our research has been trying to determine the location of these pathways as many people disagree on exactly

where the IPAVAs are located in the lungs. This controversy helped to provide some of the foundation for my research.

My thesis research focused on trying to determine where microbubbles (tiny saline/air bubbles), end up when they are injected into the body and travel to the lungs. In our lab much of the research is done using microbubbles and small, radioactively labeled particles of albumin, (MAA), because they can be safely injected into human subjects and easily detected on instruments that are readily accessible to the lab. Microbubbles are small bubbles that we create in the lab by agitating room air and saline with a stopcock and syringe, and these bubbles can be seen in the blood with the use of an ultrasound machine because bubbles are echogenic (7). MAA are tiny protein particles that can also be safely injected into humans. When these particles are able to pass through IPAVAs to the left heart they are viewed through radiographic imaging techniques in which the MAA is seen distributed throughout the body (8). The current research was inspired by a study done in the lab in which the results proposed that microbubbles and MAA travel to different areas of the lungs (6). The purpose of our work was to enhance our understanding and to better be able to predict where microbubbles end up within the lungs after they are injected into the venous blood. Our prediction was that the majority of microbubbles would float to the top of the lungs when they are injected.

Understanding the pathways that bubbles take as they travel through the lungs will help the lab to better understand where IPAVAs are located. This research sought to better understand the potential location of the IPAVAs. To do this, a simple model of the lungs was constructed that consisted of a single bifurcation and two branches to

mimic the main pulmonary artery and two subsequent vascular branches. The lung model was made out of polymer tubing and a saline solution was used to represent blood for perfusing the polymer lung. The bubbles for this study were carbon monoxide, (CO), instead of room air because CO binds to blood with a very high affinity and consequently can be used to quantify where the bubbles go. The CO content of the solutions at the end of our trials was easily quantified to determine how many CO bubbles went down each path of the bifurcation. Once an ideal setup was established the position of the tubing could be altered to represent different body positions such as upright or supine (lying on your back). The bubbles were injected into the tubing and could flow into one of two reservoir bags at the end of the system in any position. The distribution of bubbles in the upright and supine positions was measured by the amount of the CO that went through either path and ended up in the bags. The distribution between the left and right position was not significantly different, but we found that the same amount of bubbles went to the upper tubing in the upright position, even though only one third of the perfusate went to the upper tubing.

Background:

A. Lung Physiology:

The lungs are one of the most important organs of our body ensuring that our blood is well oxygenated and that carbon dioxide concentrations are strictly regulated. The lungs and the heart incessantly work together to provide our body with oxygen in order for our survival. Figure 1 shows deoxygenated blood enter into the right atrium of the heart after traveling through the body delivering oxygen to tissues, muscles and organs, and picking up carbon dioxide. The blood then travels through the right atrium into the right ventricle where it is sent to the lungs through the pulmonary arteries. The vessels leading to the lungs get increasingly smaller as they approach the lung alveoli until they become capillaries. The reasoning for the millions of small vessels versus a few larger ones serves to increase the surface area of the vessels increasing the capacity for efficient gas exchange. Once this deoxygenated blood arrives in the lungs oxygen diffuses across the walls of the capillaries. Capillaries are only wide enough to allow one red blood cell to pass through them, allowing for optimal gas exchange. Carbon dioxide diffuses across the capillary membrane into the extracellular space and oxygen is able to enter into the capillary and bind to the red blood cell. The blood continues through the capillaries and returns back to the heart where it travels through the pulmonary veins, and enters into the left atrium and then into the left ventricle. Blood exits out the aorta and this oxygenated blood is sent to the rest of the body to bring oxygen where it is needed for cellular metabolism.

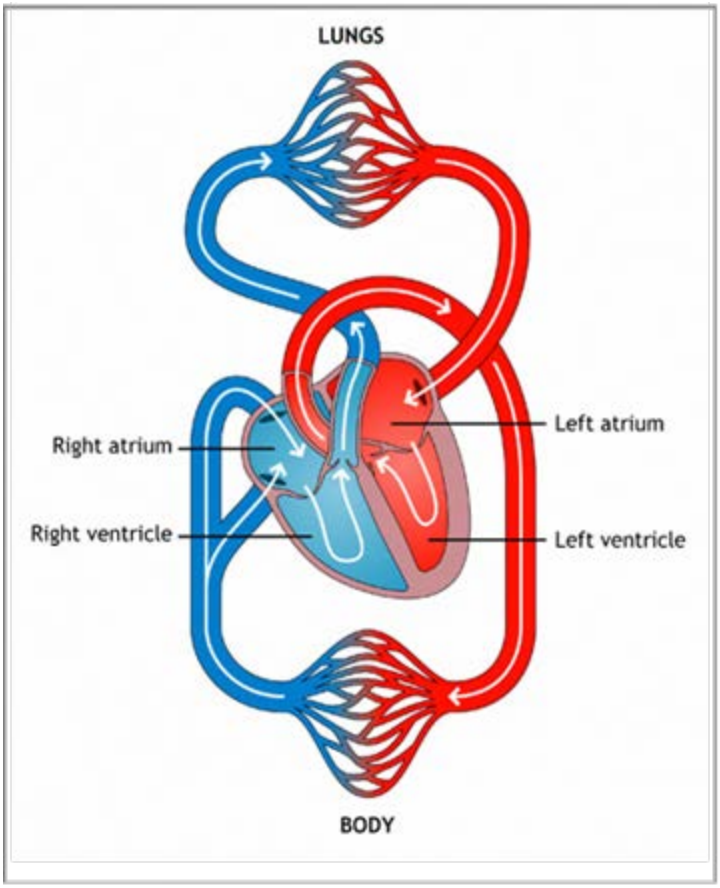

Figure 1. Lungs and Heart System

B. IPAVA vs Capillary

IPAVAs provide a route for blood, MAA and microbubbles to travel through in the lungs because some IPAVAs are thought to be larger in diameter than the capillaries in the lungs. This indicates that there is a route for particles to travel through the lungs even if they are too large for the capillaries (6). Figure 2 shows an example of an IPAVA compared to a capillary. Bubbles, in yellow, are shown entering in and not able to get through the capillaries, but are able to fit through the IPAVA shunt pathway (the top pathway of Figure 2).

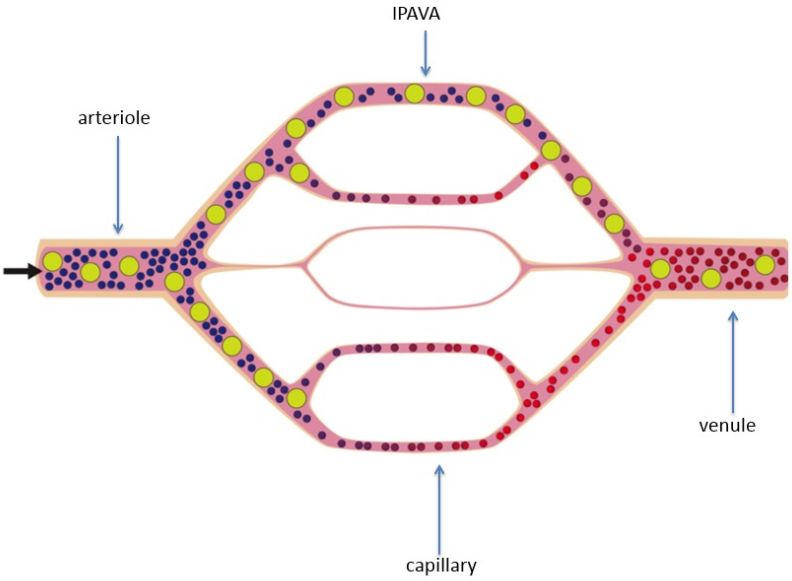

Figure 2. IPAVA and Capillaries in the Lung

In this figure the bubbles or other particles larger than a red blood cell are represented by the yellow/green, deoxygenated red blood cells = blue, oxygenated red blood cells = red.

The problem with these shunts is that when blood passes through them, it does not participate in gas exchange (9). These pathways are not advantageous to humans because they end up increasing the amount of deoxygenated blood that returns from the lungs back to the heart. In Figure 2 the blue blood entering in the arteriole side either travels through the capillaries and is oxygenated, seen at the bottom of the figure, or it travels through the IPAVA shown at the top, and does not pick up oxygen which is why the blood remains blue. This is a magnified and simplified depiction of these pathways because in the body IPAVAs and capillaries are very small and there are millions of capillaries located in the lungs. Another problem with these vessels is that they may allow for blood clots to get through the lungs and into the systemic circulation where they could block an arteriole and cause a stroke or heart attack.

C. Macroaggregates and Microbubbles

As previously mentioned, much of the research in our lab is done using microbubbles and MAA because they are relatively safe to inject into human subjects and can be used to detect blood flow through IPAVAs on instruments that the lab has access to. Microbubbles are created with a stopcock and syringes by mixing air and saline together and these bubbles can be seen with an ultrasound machine, Figure 3 shows what this would look like on an ultrasound machine (7).

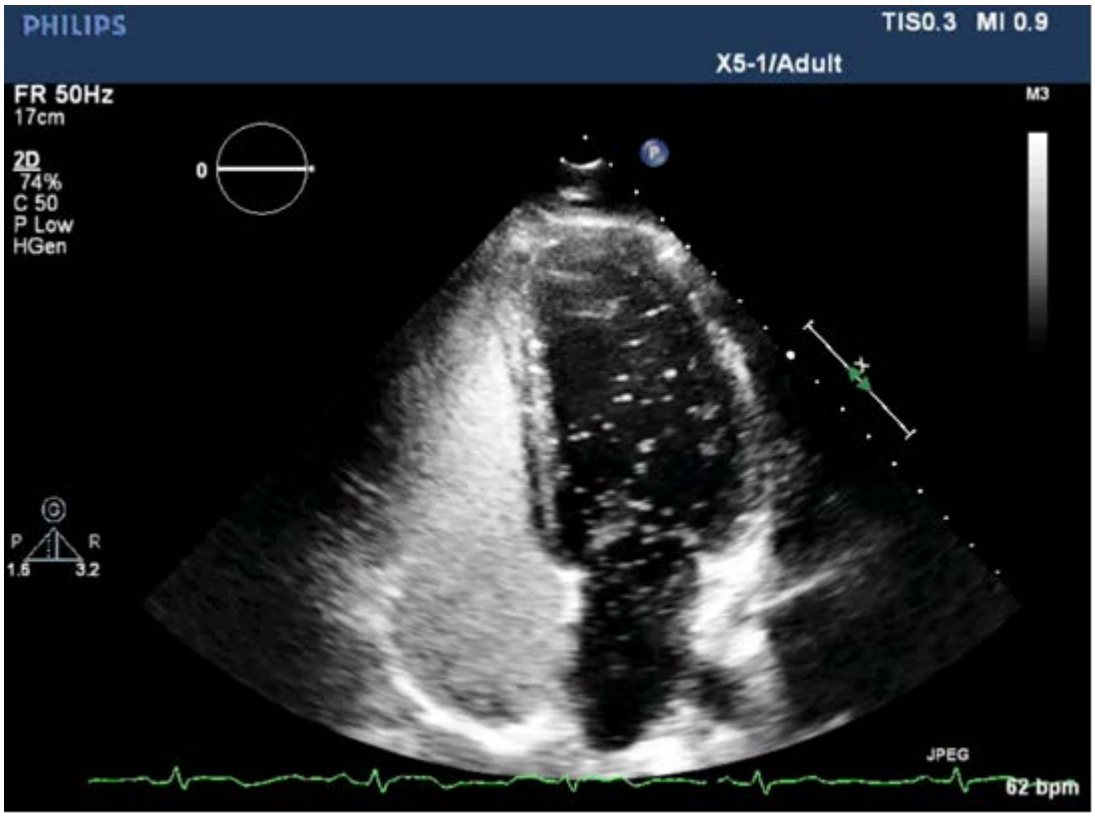

Figure 3. Bubbles in the Right and Left Heart detected with Ultrasound

Figure 3 is a four-chamber view of the heart, but it is upside down and flipped, so the white area on the left side of the figure are the bubbles entering into the right

atrium and ventricle. The smaller white spots on the right side of Figure 3 are the bubbles in the left atrium and ventricle, implying that they traveled through an IPAVA to get through the lungs and to the left heart. When bubbles are injected into the venous system they are small enough such that they will not get trapped in the body prior to reaching the heart so we will see them enter into the right atrium and ventricle of the heart. In order for the bubbles to be seen in the left heart they have to bypass the lungs through a route other than the capillaries. The capillaries are the smallest of the blood vessels with their diameter being around 10 micrometers (μm) whereas the diameter of microbubbles is closer to 60-90μm. If the injected bubbles are bigger than the capillaries and they still appear in the left atrium and ventricle, there has to be another route for bubbles to take that is large enough for them to fit through; this is one of the ways intrapulmonary shunting is tested and researched.

MAA are tiny protein particles that are also relatively safe to inject into humans, and when they are able to get through an IPAVA in the lung to the left heart we can see them with radiographic imaging distributed in the body. Figure 4 shows what this image would look like. The size of MAA ranges, but they are on average around 35μm and, similar to bubbles, they are too large to fit through capillaries; if they do pass through an IPAVA and are able to travel to the left heart we will know this because they can be detected in the body. The color differentiation that can be seen in Figure 4 indicates the concentration of the MAA in those regions. The lungs, seen with darker colors, have the highest concentration of MAA because most are trapped there. There are still some MAA that get through the IPAVAs and can be detected in the body, primarily in the legs.

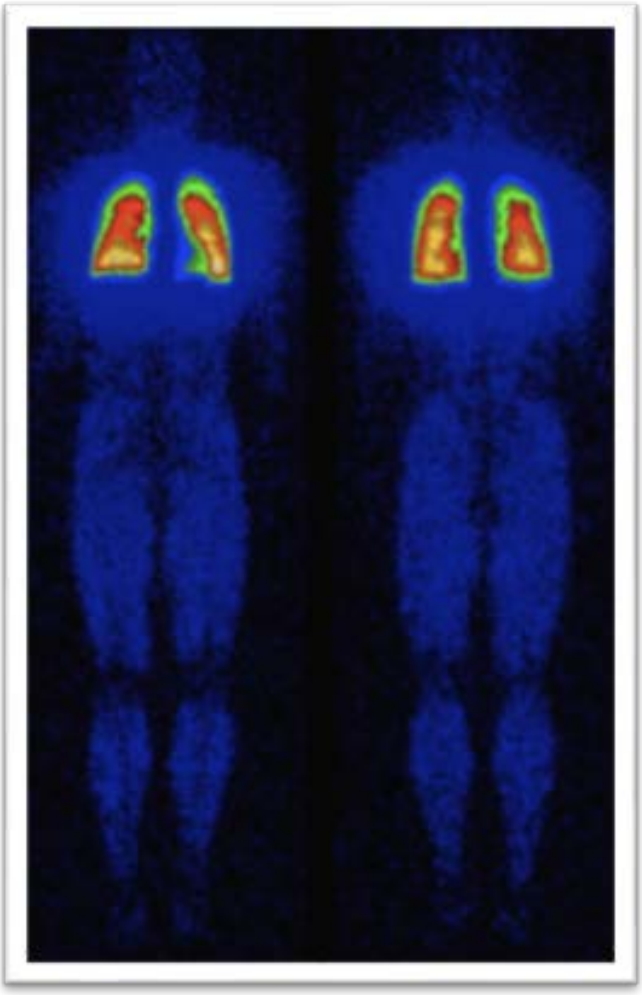

Figure 4. Distribution of MAA in Body During Exercise.

D. IPAVA in Lungs

The classical way the airways and blood vessels of the lungs are separated is by dividing them up into three different postural and architectural dependent zones; zone one, zone two and zone three (seen in Figure 5).

Zone one is the apex (top) of the lungs, zone two is the middle zone and zone three is located at the base or bottom of the lungs. Blood flow is determined by the architecture of the lung and by gravity, so when blood enters into the lungs most blood

flow occurs within zone three because it is the most inferior. It is known that the largest number of capillaries and a majority of blood flow occurs through zone three. During different stages of daily activity blood flow will also occur in zone two and with exercise it reaches zone one.

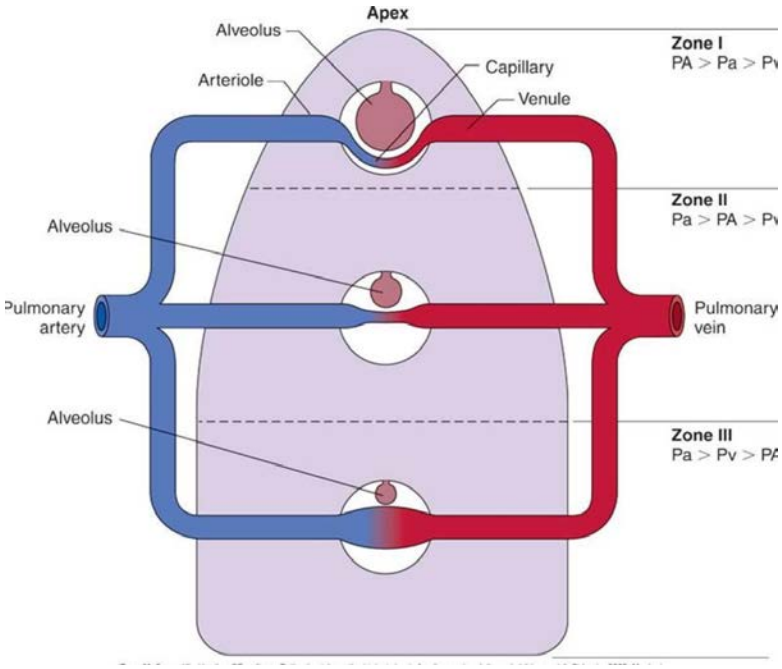

Figure 5. Zones of the Lungs in the Upright Posture

It is thought that IPAVAs within the lungs are distributed such that " a significant number of larger IPAVA are located throughout the bottom half of the lung" (6). There would be medium sized IPAVAs in the middle of the lungs (zone two in an upright subject), and the smallest IPAVA pathways, at the apex of the lungs (zone one). If this is in fact how the IPAVAs are arranged, then bubbles could circumvent zone three shunt pathways because of their affinity to float into zone one (1). We know these bubbles are small but because they are still larger than a capillary their only route through the lungs would be through zone three IPAVAs. But as we move more superiorly through the zones of the lungs the IPAVA vessels get smaller. By the time

bubbles reach zone one there is little to no blood flow and the IPAVAs would be quite small, in this case we would not detect bubbles travelling through IPAVAs. The buoyancy of bubbles would give them the inclination to rise and go through zone one but the problem is that once in zone one the bubbles will end up with no route to take because of the size of the IPAVAs (1). Accordingly, if bubbles are injected into the veins but are not seen in the left heart our thought is that they are decreasing in size until they collapse in the zone one IPAVA pathways that are too small for them to travel through.

The zone dynamics change, however, when the subject is lying in a supine position (on their back) as seen on the left in Figure 6. When subjects are lying on their back zone three is still the most inferior, but now zone three would include large, medium and small IPAVAs from the top middle and bottom of the lung. When lying down the bubbles may still have the tendency to float but now when bubbles go to the anterior surface of the lungs in addition to small pathways, they have the opportunity to go through the medium and large IPAVAs that were previously near the bottom of the lung when in the upright position. These IPAVAs that used to be in the bottom of the lungs are predicted to be larger in diameter and can accommodate the size of the bubbles so this is one possible explanation as to why bubbles are showing up back in the left heart (6).

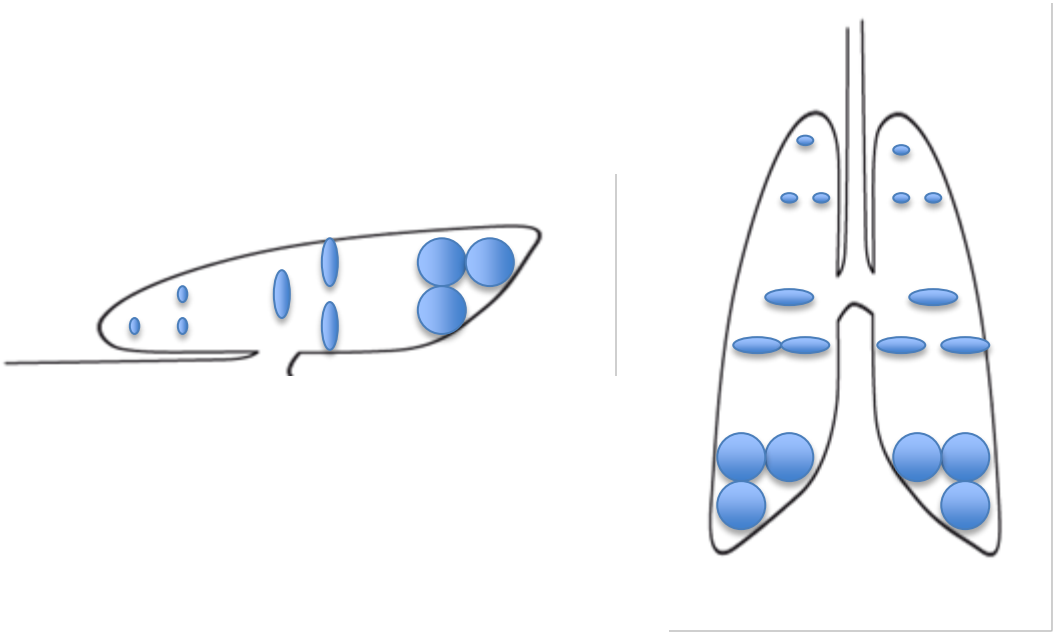

Figure 6. Hypothesized Supine and Upright lung IPAVA distribution.

E. Previous Studies

Though we know that these shunts do exist, their exact locations and size are not well understood. The current research examined the pathway that microbubbles take when they enter into the lungs with a simplified, single bifurcation lung model. By comparing the data that I gathered in my research with preexisting data collected by J. Duke and J. Elliott in the Lovering lab, some of the questions that we have about these IPAVAs may be better understood (4,6).

Since bubbles are filled with air and buoyant, they have the tendency to rise in a fluid suspension (1). A study completed by Duke et. al. had results consistent with the hypothesis that if bubbles enter the lungs and can either go to a superior (upper) pathway or inferior (lower), most bubbles will choose to take the upper pathway (6). In that study bubbles were injected once into a subject sitting upright, and with the ultrasound machine they saw bubbles enter into the right heart, but did not see bubbles

show up again in the left heart. It is thought that these bubbles could have been trapped in the apex of the lungs where the shunt pathways that would allow them through were either not present or too small. This same procedure was done injecting bubbles in a supine subject and the lab observed the presence of bubbles in the left side of the heart with this posture change. This would indicate that in the supine position the bubbles are given the option to travel through the larger pathways that would be included at the top of the lungs along the anterior surface.

This same study was done by injecting MAA into subjects. MAA are protein particles that range in size, but many of them are too large to fit through the capillaries. However, the MAA are smaller than bubbles and are solid, unlike bubbles. This means that MAA do not float and may not take the same path as bubbles in the lungs because gravity will draw the MAA downward, whereas the bubbles may be able to float. When MAA were injected into the veins of an upright subject, nearly twice the amount of MAA was quantified as getting through the IPAVA compared to when the subject was lying on their back (6). The size of the MAA allows them to fit through the medium and large IPAVAs located in both zone two and zone three as seen previously in Figure 6. With an upright subject the force of gravity may draw MAA to the bottom of the lungs exposing them to the pathways in zone two and three that they can fit through; in this case we would expect a large amount of MAA to be seen in the body. In the experimental study when this was done, twice the amount of MAA appeared in the body when compared to the same subject lying supine (6). When the subject is supine, the MAA still travel inferiorly due to gravity but now will also be dispersed among some of the IPAVAs from zone one. These pathways are too small and end up trapping

some of the particles, decreasing the amount sent out to the body. By changing posture from upright to supine and injecting MAA in both conditions, less was seen in the body when someone was lying supine as compared to upright.

F. Summary & Proposal

Ultimately, this research attempted to reconcile data from previous studies using microbubbles and MAA. With the use of a simple lung model, the present study attempted to 1) understand what happens when bubbles enter into the lungs, 2) where bubbles may be getting trapped, and 3) why bubbles are seen in the left heart. Although I was not using actual human lungs, by creating a simple model of them I was able to quantify the pathway that bubbles take to see if there was a preferential direction the bubbles travelled to when are given the option of either going superiorly or inferiorly, and how this might change with posture. My hope is that with the results of my research, in the future, the lab will be able to make predictions of what is happening to the bubbles when the body is either upright or supine. Thus, the data that I have collected can be used to better analyze and understand some of the preexisting data the lab has collected.

Methods:

A. Developing the Lung Model

After weeks of various arrangements we developed the “lung” design that would be used for future studies, the entire setup is shown below in Figure 8, whereas the schematic version of the setup is seen in Figure 7. There was not much previous work that we could use to create this idea, however Calderon et al. and Chang et. al both had previously used branching models similar to ours that were helpful in designing our setup (1,3). In Figure 7, point A represents the beaker that was our pressure column and was full of the saline that flowed through the system. Connected to that was a 60 centimeter (cm) plastic polymer tube, indicated as B. This tube had a bifurcation at the end, indicated by C, to provide two different paths from the perfusate system into the reservoir bags at the distal end (E and F). Each reservoir bag was arbitrarily given a color, blue or purple and this was how we were able to indicate which bag was on the top vs. bottom, or left vs. right. We labeled the bags as blue and purple because we repeated every different positional trial in the opposite configuration; if blue was on top for the first trial, it would be on bottom for the second trial in an upright study. We did the same thing for supine study, alternating left and right. The upright study was repeated four times, and the supine study was repeated three times.

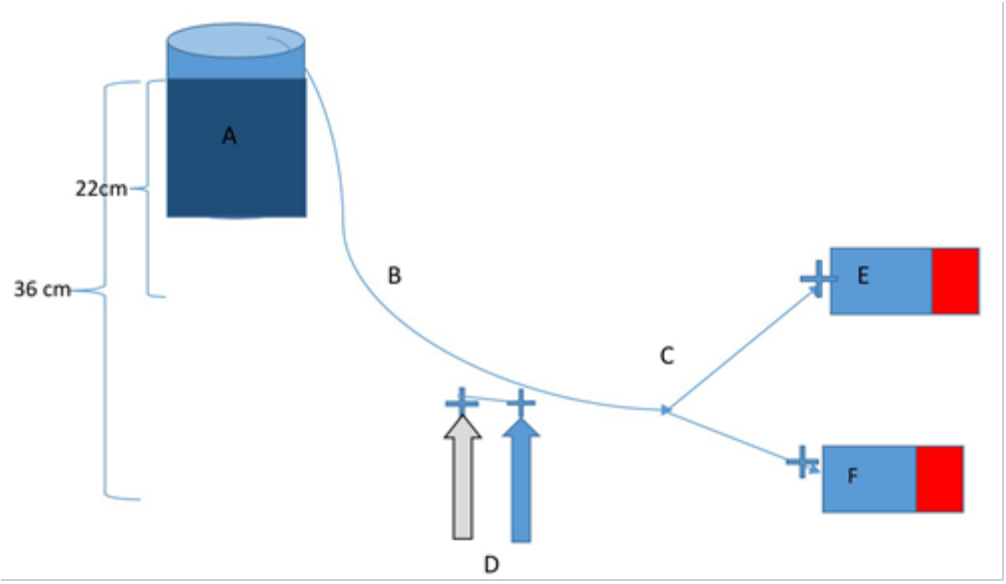

Figure 7. Schematic of Lung Model

Continuing along the polymer tubing just proximal to the bifurcation, indicated by letter D were two syringes attached to a stopcock, this was where the CO was injected into the lung model system through an intravenous (IV) catheter as it is done in humans. What we sought to test was the distribution of bubbles within a system that simulated bubbles flowing through pulmonary arteries in the lungs. To represent a person sitting in an upright position we altered the model lung bifurcation at the end to be in the vertical plane as seen by the different heights of the two reservoir bags in my diagram. To represent a supine subject the bifurcation was placed in the horizontal plane with both branches at the same level. The top branch of our bifurcation in the tubing represented superior portions of the lungs whereas the lower branching of the tubing represented a branch that was going to the inferior part of the lungs.

B. Development of Perfusion System:

The process that we came up with to perform our studies began with an immense amount of trial and error because there was no baseline procedure to start

with. We began with a few different ideas about how to set up a system that could flow similarly to the way blood flows through the lungs. The first version was to use a motorized pump that pushed water through tubing and stimulated blood flowing through the system. This did not work because the flow through the pump did not have a high enough power to push the saline or water at a rate and pressure that would be similar to what happens in the body. The second attempt, the one we ultimately used, to deliver perfusion and a constant flow to the system was to create a pressure column by placing a beaker with saline on a shelf that was between 22 and 36 cm above the bifurcation of the tubing of the lung model. The same level of saline was maintained in the pressure column over the course of each trial so that our flow would be consistent throughout all of our trials. Though the flow rate that we stimulated does not accurately represent the lungs in the body, the pressure was similar to what would be measured in humans.

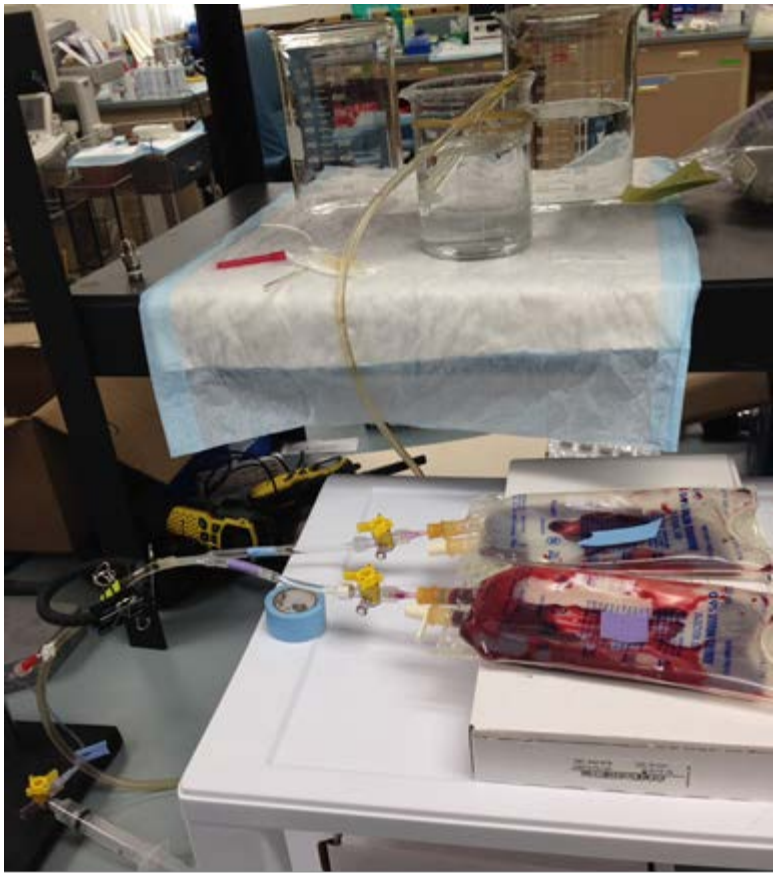

Figure 8. Picture of the actual lung model

C. Quantification of Microbubbles:

We used CO bound to hemoglobin (Hb) to determine the amount of bubbles that went into either one of the reservoir bags because CO binds to hemoglobin with a much higher affinity than oxygen. We pre-injected blood into the reservoir bags and then when we injected CO so we could measure how much was bound to Hb. We were able to obtain and quantify this value because we had an airtight system with reservoir bags that had blood in them attached to the end of the two branches of the bifurcation. We injected CO proximal to the bifurcation, seen at letter D in Figure 7, and we measured how much CO gas went to either bag based on how much CO was bound to the Hb in

each bag. By drawing a small sample from each reservoir bag we used a OSM3 radiometer (co-oximeter), to measure the total hemoglobin (tHb); met hemoglobin (metHb); Hb oxygen saturation (HbO_2%); Hb carbon monoxide saturation (HbCO%); and oxygen content. The important CO value that comes from the co-oximeter is the HbCO because this value helped to calculate the overall carbon monoxide content in the solution in the reservoir bags.

We consulted a researcher, Ben Ryan and his colleagues at University of Colorado, Boulder because they were interested in the research we were doing and are experts in using CO to measure blood volume. They directed us to use the oxygen content equation to quantify the amount of CO that was present and bound to the hemoglobin in our solution. This equation, shown below, takes into account the maximal binding capacity of CO to hemoglobin, 1.39 mL of CO are able to bind per gram of Hb (1.39mL CO/g Hb). It also accounts for the total amount of hemoglobin that is in the blood/saline (perfusate) that we are measuring at this time as seen by the tHb. The HbCO is the percent of hemoglobin bound to CO.

Carbon Monoxide Content Equation: (1.39mL CO/gHb)(tHb)(HbCO)

By using this equation we are able to quantify how much CO gas went to each bag while also taking into account how much perfusate was in the bag.

D. Process of the Experimental Trials:

On the morning or one of the days leading up to a trial venous blood was obtained and stored in the blood refrigerator until it was needed for the study. The day of a trial the first thing to do was to ensure that the tubing we used was cleaned out from

the previous trial and that the entire system was running properly. In order to do this, saline was removed from two different saline bags and poured into a beaker that would be used as the pressure column for the trials. With the two saline bags emptied one was arbitrarily labeled as blue and another was labeled as purple. This was done in order to keep track of which bag was on top or bottom and also to make sure that each bag was used in each condition the same number of times. The beaker was filled to 400mL, which was 22cm above the surface that the bags were on while the system was being set up. The bifurcated tubing remained attached to the distal end of the tubing of the system and there were two different stopcocks placed at the end of each bifurcation. To start the flow of the saline through the system one of the stopcocks was closed off and a syringe was attached to the other stopcock. The syringe was attached in the compressed position and once attached, air was drawn through the syringe to get the saline to flow over the pressure column. Once this was done on one end of the bifurcation this same process was done on the opposite end of the bifurcation while closing off the previous stopcock. By drawing saline into the end of each branch of the bifurcation this ensured that the saline was able to flow through both of them when necessary. Afterwards a needle was attached to the stopcock on both branches and the stopcocks were both turned to open flow. This allowed the saline to openly flow through the system and through both bifurcating branches; this also ensured that there were no issues with either of the tubes prior to commencing the study.

The next step was to get the blood out of the fridge where it was stored in vials. Depending on how many studies were being done that day, the proportional amount of blood was emptied from the vials into the beaker and all the blood was pooled. A small

sample of the blood (about 0.2mL) was taken from the beaker and brought to the co-oximeter to get the baseline total hemoglobin; this allowed us to calculate how much we could dilute the perfusate. It also measured a baseline value of the CO bound to hemoglobin. After the blood measurements were made in duplicate or triplicate with the co-oximeter we calculated out how much we could dilute the blood with saline and still get a reading of a tHb of greater than 3.2 which is the minimum detectable value, when injecting the blood/saline mix from the study. In order to get a reading that was high enough for the co-oximeter we accounted for a 1:4 dilution of blood to saline. The blood was injected to the reservoir bags prior to the trial and after running the study the saline combined with the blood in the reservoir bag. We were able to predict about how much saline would flow into each bag in either the upright or supine position from data obtained during development trials. When doing an upright study we typically injected 30 mLs of blood into the bottom reservoir bag, and 20 mLs of blood into the top empty reservoir bag.

We injected this amount of blood into their respective bags, 20 mL for the top bag and 30 mL for the bottom bag and attached each of these bags to the needle that came off the bifurcation of the tubing. Each bag was also labeled either blue or purple as a way to keep the bags separate and know which was on top or bottom when we made measurements at the end. The top bag was set at 22cm below the pressure head, and the bottom bag was 36 cm below the pressure column.

The next thing was to obtain carbon monoxide. This had to be done very carefully and in a fume hood because a leakage of CO can cause major health problems and can even lead to death because of its affinity for Hb. The CO was obtained by

someone in the lab who knew how to safely work with the gas and then brought back to us to be used in our system. We set up a stopcock apparatus that had two syringes, one with 1.5mL of CO gas in it, and another with about 3 mL saline that was injected proximally to the bifurcation of our tubing. The two were mixed together with a two-way stopcock apparatus. Once the blood/saline bags were attached, the CO bubbles were ready and the pressure head beaker was filled to 400 mL the study was ready to begin. Dr. Lovering and I each had a stopcock leading towards one of the bifurcations and on my count we turned them so they were open and saline could begin flowing into the bags. Once we turned the stopcocks a stopwatch was started, and the saline flowed for one minute, then without changing saline flow, the CO/bubbles mixture was injected into the tubing and the system continued to flow for another minute. After the bubbles were injected the tubing was monitored to ensure that none of the bubbles obstructed the tubing and prevented saline from flowing into the bags or blocked other bubbles from also getting into the bags. While all of this was happening the pressure column was being monitored and continuously refilled to make sure that the level of saline in the beaker remained at 400 mL over the duration of the trial.

At the conclusion of the two minutes both Dr. Lovering and I turned the stopcocks attached to the reservoir bags off to stop the flow. Once the flow in the bags stopped, both the bags were disconnected from the tubing system. The bags were then manually shaken for another five minutes to ensure the maximum amount of binding of the CO and hemoglobin. At the end of the five minutes of manual, vigorous mixing, a small blood sample (0.5mL) was removed from each of the bags and measure in triplicate again in the co-oximeter. While one person was measuring and recording the

values in a table from the machine, the other person was calculating the flow rate of the saline into each of the bags. The way this was done was by pouring all of the perfusate (blood and saline mixture) into a graduated cylinder and recording the total volume. This volume told us the total volume of liquid that was in the bag at the end of the trial. In order to quantify our flow rate, we subtracted the amount of blood that started out in the bags from the total volume. This new value was then divided in half to get the flow of saline per minute since our study ran for two minutes:

Flow Rate (mL/min): [Total Volume in Reservoir Bag (mL) - Injected Blood Volume (mL)]/2 mins.

We used this flow to understand how much saline was getting into each bag for the trials and because the flow rates remained fairly constant when the tubing was in the vertical position we predicted how much perfusate would end up in the reservoir bags for future studies. This information helped us determine how much blood to add in the bag prior to the beginning of the next trial.

When we ran our trials to represent our subject lying supine, the overall process was the same except for a few minor changes outlined below. In the supine position, both of the saline bags were on the same level to representing a subject lying on their back so the bifurcations in their lungs was on the same plane. In doing so, we set both of the saline bags at the 22cm and had them lying adjacent to each other to ensure an equivalent pressure and flow into both of the reservoir bags. Also unique to the supine positioning, the same amount of blood was injected into both of the bags prior to the study because with them at the same level we expected the same flow rate into both bags. We followed the same steps of taking blood and measuring the tHb prior to

running any of the trials but typically we injected 30 mLs of blood in both of the bags. All of the previous precautions were taken prior to running the study, (checking the flow is normal, removing all the saline from the bags, making sure the pressure column was correct, obtaining CO, etc.). Once everything was set up Dr. Lovering and I turned the stopcock to initiate flow and started a timer. The process after here was the same as the upright day where the CO/saline bubbles were injected at the one minute mark, the 400mL pressure column was monitored to be kept constant and the tubing was monitored to make sure none of the bubbles are obstructing flow into either of the bags. At the end of the two minutes we turned the stopcocks off to prevent further flow, removed the bags and perform the same manual-shaking maneuver for five minutes. The samples were removed at the end of the five minutes and measured with the co-oximeter while the total volume of perfusate was measured for each bag and recorded, as above.

Our methods were repeated in order to get values that were statistically significant with enough support for our hypothesis within the amount of time we had. We repeated the upright/vertical trials where the bags were on top of each other four different times, and we did the horizontal/supine trials three different times.

Results:

Our values were consistent with what we predicted would happen; the flow to the top bags would be less, whereas in the bottom bag the flow would be greater, both values of flow can be seen in Table 1. Since we do not have the same ratio of blood and saline for every trial we had to calculate out this value according to each bag for each trial. The same amount of CO bubbles went to both the top and bottom reservoir bags/bifurcations of the apparatus, but because there is less blood in the top bag there is more CO per unit of volume going to the top reservoir bags. This suggests that bubbles are still floating as we predicted because bubbles are traveling against the perfusion gradient.

TABLE 1. Condition	tHb g/dL	CaCO mL CO/dL	Perfusate Total mL/min	CaCO/ Perfusate mL CO/mL/min
Purple Top	4.24	0.279385552	70.5	0.0040
Purple Bottom	3.7	0.32370638	121.1	0.0027
Blue Top	3.53	0.2968574	72.8	0.0041
Blue Bottom	3.61	0.334848133	113	0.0030
Purple Left	3.9	0.396882947	85.3	0.0047
Purple Right	3.93	0.395785473	85.3	0.0046
Blue Left	3.86	0.360310736	82.3	0.0044
Blue Right	3.9	0.363041613	84.6	0.0043
Top Averages	3.885	0.288121476	71.65	0.004020314
Bottom Averages	3.655	0.329277256	117.05	0.002818154
Left Averages	3.715	0.346870174	79.05	0.004365251
Right Averages	3.77	0.365316803	99.15	0.003801591

Table 1. Results of Bubble/Saline Data

Discussion:

The study that was done in Dr. Lovering's research lab that sparked this thesis project was trying to piece together some of the results that they observed in another study that took them by surprise. In that study one of the postdoctoral fellows was testing how posture affects blood flow through IPAVA using microbubbles and macroaggregates of albumin to detect the blood flow (6). Our data shows that there were no differences in the CaCO (carbon monoxide content) between the top and bottom pathways of our lung model. However, the total perfusate volume indicates that there was about 1.5x as much fluid going to the bottom pathway, yet there was still the same amount of CO. This indicates that bubbles are able to float to an area that is not as highly perfused, specifically, they do not have to go with the flow.

Bubbles can be injected in the venous system and seen in the right heart using echocardiography, and this is also how we would see the bubbles in the left heart- were they to get through the pulmonary system (7). The bubbles are too large to fit through the capillaries in the pulmonary system, as seen in Figure 4 so if bubbles are seen in the left heart they would have to be going through an alternate route. Macroaggregates of albumin are solid, radioactive particles and these can be seen with radiographic imaging techniques (8). When the macroaggregates are injected in the venous system, they pass through the pulmonary system and reach the systemic circulation so their concentration can be quantified by the distribution of radioactivity throughout the body, or trapped in the lungs. Similar to the saline/CO bubbles that we injected into the model lung system, the researchers in this study injected an air bubble mixture into human subjects. This is a relatively safe process because the size of these bubbles is so small that there is almost

no need to worry about them getting through the lungs and into the systemic circulation. The bubbles have a matter of seconds before bubble dissolution will occur and this allows for the safe practice of injecting bubbles without worrying about posing a serious medical threat. When the micro-bubbles were injected into the subjects seated upright there was no detection of bubbles appearing in the left side of the heart after being seen first in the right heart. In contrast, when bubbles were injected in a subject lying supine there were bubbles returning to the left heart from the lungs which is indicative of them passing through an IPAVA (4, 6). With the MAA component of this previous study, when injected into a subject sitting upright there was a larger presence of MAA distributed throughout the body compared to when the subject was lying supine. These results are consistent with the hypothesis that perhaps, the largest IPAVA pathways are located in the base of the lungs (6). The base is the area that is going to have the highest perfusion rate simply because of gravitational and architectural determinants of flow, so it would be reasonable that the shunt pathways follow that same pattern.

In my study, the total amount of CO that went to the top vs. bottom bifurcation of the lung apparatus setup was consistent, in a way, with what we expected. What we found was that nearly the same amount of CO went to both the superior and inferior branches of the tubing. But with only 1/3 of the perfusion going to the superior portion of the lung model this indicates that the bubbles are being directed to the superior portion by more than just the perfusate flow. We understand this because if the bubbles simply followed the perfusion then they would end up distributed throughout the lung identical to perfusate. The buoyancy of the bubbles would be a reason for why they are still able to reach the superior portion of the tubing regardless of where the perfusate

flow is going. If this is the pattern that we witnessed with the lung model, we believe that this could also be what is happening in vivo in humans. This would explain why we saw bubbles appear in the left heart only when the subject was lying supine. The bubbles would have the tendency to float and when the subject is supine there are IPAVA shunt pathways that would be at the anterior surface of the lungs and would allow for these bubbles to pass through and return to the left heart. If the subject is sitting upright, if the bubbles have the tendency to float and if the shunt pathways at the top of the lungs are too small or non-existent then bubbles would not get through the lungs. This would explain why when the subject is sitting upright no bubbles appear back in the left heart. Accordingly, because bubbles cannot fit through the capillaries in the top of the lungs, they end up getting caught in the apex of the lungs that has small or no IPAVAs and end up diffusing air out of them until they collapse from surface tension.

Limitations:

As with any research there are some limitations that we encountered within the overall process of our trials. We intended on doing the same procedures with MAA as we did with the bubbles, but there just was not enough time because designing our apparatus took more time than we predicted. Also, the cost of MAA increased in the past few years, making this research very expensive. There also were some issues with recovering the total amount of CO that went to each bag. Although we manually agitated the bags for five minutes after each trial, the full amount of CO that was injected was not recovered. This meant that it could have been getting trapped in either the tubing prior to the bifurcation, or some of it was in the bag but not bound to the red blood cells. Either of these options would give a lower reading to the CO content of either bag. However, percent recovery was the same for either top and bottom or right and left so this did not prevent us from accurately determining where the CO went.

Conclusion:

The research that we have compiled the past few months has brought a conclusion that was expected but instead of all the CO going to the upper path as predicted, only half of it does. These data support the idea that bubbles are floating because only one third of the perfusate flow travelled to the upper bifurcation, yet half of the gas went to the upper bifurcation. This work helps to indicate why there are more bubbles that appear in the left heart when a subject is lying on their back compared to being upright. The amount of the difference of bubbles is not as large as we predicted but is enough to support our hypothesis and provides a basis for further research in this area of study.

Glossary:

1. IPAVA: intrapulmonary arteriovenous anastomoses
2. MAA: macroaggreagates of albumin, small albumin protein particles
3. OSM3: co-oximeter machine used to measure blood hemoglobin values
4. Intra-cardiac shunt: pathway within the heart that allows blood to bypass the lungs and go directly into the left atrium of the heart
5. PFO: patent foramen ovale; one type of intra-cardiac shunt that is a small hole between the right and left atrium
6. Ductus Arteriosus: extra-cardiac shunt that allows blood to flow from the pulmonary artery into the aorta allowing blood to flow directly into the body and not have to go through the lungs first
7. HbCO: quantity of hemoglobin that is bound to carbon monoxide
8. tHb: total hemoglobin quantity within the a sample of blood

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Figure 1. Blood Circulation, http://www.rch.org.au/kidsinfo/fact_sheets/Heart_problems_in_children/, May 11, 2015.

Figure 2. IPAVA and Capillaries in the Lungs. Lovering, A. T., Duke, J. W. and Elliott, J. E. (2015), *Intrapulmonary arteriovenous anastomoses in humans – response to exercise and the environment*. The Journal of Physiology, 593: 510. doi: 10.1113/jphysiol.2014.275495. Figure B.

Figure 3. Bubbles in the Right and Left Heart detected with Ultrasound. Lovering, Andrew T., and Randall D. Goodman. *Detection of intracardiac and intrapulmonary shunts at rest and during exercise using saline contrast echocardiography*. INTECH Open Access Publisher, 2012. 169. Figure 4.

Figure 4. Distribution of MAA in Body During Exercise. Lovering, Andrew T., et al. *Transpulmonary passage of 99mTc macroaggregated albumin in healthy humans at rest and during maximal exercise*. Journal of Applied Physiology, 106.6 (2009): 1989. Figure 1.

Figure 5. Lung Zones. http://www.gopixpic.com/400/lungsrespiration/http:%7C%7Cuvahealth*com%7CPlone%7Cebsco_images%7C7654jpg/, May 11, 2014.

Figure 6. Hypothesized Supine and Upright lung IPAVA distribution. Hundley, David., Earthman, Lauren. 2015.

Figure 7. Schematic of Lung Model. Earthman, Lauren.

Figure 8. Picture of the actual lung model. Earthman, Lauren.