2008

Effect of Hemoglobin-Based Oxygen Carriers on Arterial Pressure and Vasoactivity in the Rat Mesentery

Michael Kim
Virginia Commonwealth University

EFFECT OF HEMOGLOBIN-BASED OXYGEN CARRIERS ON ARTERIAL PRESSURE AND VASOACTIVITY IN THE RAT MESENTERY

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at the Medical College of Virginia Campus, Virginia Commonwealth University

by

MICHAEL Y KIM
B.A. Biology, University of Virginia 2006

Advisor
Roland N. Pittman, Ph.D.
Professor
Department of Physiology and Biophysics

Virginia Commonwealth University
Richmond, Virginia
May 2008

Table of Contents

Table of Figures

List of Tables

Acknowledgment

I would first like to take the time to thank Dr. Pittman for graciously allowing me to complete my Master's in his lab. His help and advice have been invaluable to me. I want to thank him for taking the time to work so closely with me on my projects and for offering help whenever I needed it. Dr. Pittman was constantly making sure that my project was moving forward and was quick to help if a problem arose. He has also offered a great deal of support for a Master's student who has never even touched a rat much less complete a Master's doing rat surgeries. I would then like to thank Bjorn Song and Pete Meliagros for showing me the ins and outs of rat surgery and their constant and insightful advice that helped me in getting a better mastery of rat surgeries. When initially I was struggling just to complete the surgery, the two of them made themselves completely available to questions or problems that I may have had. I would also like to thank Helena Carvalho another lab member with whom I could not have completed my Master's in the timely manner that I did. Her past experience with working with the mesentery of the rat has been with out a doubt one of my greatest resources. When I was struggling just to get a decent image for my studies, she was more than happy to offer the help and advice I needed to correct my errors. Matt Barker was also a great help by just showing me how to use the equipment necessary to obtain the data that I needed. Alex Golub was another great help that offered technical advice when trying to get the equipment to work and his skill with the machines is like none other. His ability to make and replace a faulty screw was an invaluable resource to me.

I could not have done any of this with out the support of my parents and two brothers. They have constantly been there for me offering me the emotional support that I needed to make it through the past two years. Without them I am sure I would be nowhere near to where I am today. My girlfriend is another support in which I owe a great deal to. She has been pushing me and keeping me on track, ensuring that I never lose sight of my goals. I would also like to thank Dr. Ford for all the help he has offered me the past two years. He has been there for me to answer my questions and provide the advice that I needed to make it where I am today.

So to all, thank you for everything you have done for me and I owe all of you a great debt of gratitude.

Abstract

Hemoglobin-based oxygen carriers provide a promising future as an alternative to human blood transfusions. Hemoglobin-based oxygen carriers, HBOCs, provide a low-cost, easy to maintain, and safe solution. They require no refrigeration and are universally compatible, and the required transfusion volume is less than that of a normal transfusion. HBOCs have been known to have adverse side effects such as renal toxicity, gastrointestinal dismotility, and hypertension. Many of these problems stem from the lack of a membrane, which protects the hemoglobin from dissociating and extravasating into the blood vessel wall. Extracellular hemoglobin, like that found in HBOCs, has a greater affinity for nitric oxide, NO, than oxygen due to the lack of a protective membrane like that of a red blood cell. This NO scavenging effect has been used to explain the increase in mean arterial pressure, MAP, as well as any other smooth muscle dysfunction. NO has been accepted as the endothelial derived relaxing factor which serves to dilate or maintain the vascular tone of the arterioles. It is theorized that the drop in NO due to scavenging by hemoglobin causes an increase in MAP. In this study, using the mesentery of rats as a model to observe the microcirculation, various doses of HBOC 201, HBOC 205 (MW 400), and HBOC 205 (MW 600) were infused into the rats. Measurements of MAP and arteriolar diameter were taken in response to increasing doses of each HBOC to establish a dose-response relationship. Chemicals such as sodium nitrite, $NaNO_2$, and N_w-Nitro-L-arginine methyl ester hydrochloride, L-NAME, a NOS inhibitor, were used to chemically alter the levels of NO. The purpose was to see if modifying the levels of NO could alter the changes in MAP due to the HBOCs. MAP rose in response to the

increasing doses of HBOCs, but the arterioles failed to show any vasomotor responses. $NaNO_2$ showed an ability to reduce the increase in MAP as a result of the HBOC, but again had no affect on arteriolar diameter. L-NAME was able to reproduce changes in MAP similar to that of the HBOCs, but again had no effect on diameter. The results support the theory of NO scavenging by the HBOC leading to an increase in MAP. The lack of activity in arterioles indicates that NO may not be a major factor in controlling vascular tone in this tissue. The results support that HBOC's side effects are a result of NO scavenging, but further work is needed to better understand vasoactivity in the mesentery.

Introduction

The Cardiovascular System

Circulation

The circulatory system of our body functions to provide an adequate flow of blood for the entire body. It centers around the heart which works as a pump to move blood from the veins into the arteries. Blood enters the right atrium of the heart, is pumped to the right ventricle then goes to the lung via the pulmonary artery. In the lung, oxygen is taken up and carbon dioxide is expelled. From the lung, the blood moves into the left atrium from the pulmonary veins and then flows into the left ventricle. The left ventricle forcefully pumps the blood into the aorta from which the blood is distributed throughout the body via the vast network of arteries and capillaries. Blood delivers oxygen to the various organs of the body. The deoxygenated blood is returned to the heart by the veins. All the veins of the body feed into two major veins, the superior and inferior vena cava. The superior vena cava receives blood from the upper portion of the body and the inferior vena cava receives blood from the lower portion of the body. Both veins feed the deoxygenated blood to the right atrium of the heart. As an overview, the heart pumps oxygenated blood into the arteries, which delivers oxygen to the body by the capillaries and is returned to the heart by the veins.

The microcirculation is the location in the circulatory system where oxygen exchange takes place. As the arteries decrease in size they become arterioles. The arterioles further decrease in size becoming capillaries. The arterioles are responsible for a significant portion of the vascular resistance. This is achieved because of the cellular

make up of the walls of the arterioles. The walls are mostly made up of smooth muscle cells, which are regulated by the sympathetic nervous system and local or circulating vasoactive factors, and together the arterioles act to regulate the blood flow. The arterioles feed into the smaller capillaries, which are comprised of a thin single layer of endothelial cells with a lumen only wide enough for the passage of red blood cells in single file. The exchange of water and small solutes occurs in the capillaries. The capillaries then widen into venules, which further widen into veins that return to the heart.

Blood Composition

Blood makes up roughly 7% of the human body weight and is more of a heterogeneous than homogeneous solution. It is comprised of 55% plasma, an aqueous solution made up mainly of water, blood plasma proteins, and inorganic electrolytes. Plasma is able to transport gases, lipids, carbohydrates, hormones, waste, clotting factors, and other nutrients the body may need. The remaining 45% of the blood is composed of the following corpuscles: platelets (150,000-400,000), white blood cells (7,000-12,000), and red blood cells (4.5-5.5 million per cubic millimeter) (Nucci and Abuchowski 1998). Platelets act to help blood clot, white blood cells, or leukocytes, are part of the immune system acting to destroy pathogens, and red blood cells, or erythrocytes, contain the blood's hemoglobin that transports oxygen.

Oxygen

Cellular Requirements for Oxygen

All cells in the body need a continuous supply of oxygen to produce the necessary energy to carry out day to day functions. Adenosine triphosphate (ATP) is used by cells as a source of energy drawing from the high energy bonds of ATP. ATP can be produced both by anaerobic and aerobic metabolic pathways. Anaerobic pathways do not require the oxygen that aerobic pathways require. In addition to needing no oxygen, less energy is created by the process of glycolysis, which also produces by-products that can be harmful to the body, such as lactic acid. The aerobic pathway uses oxygen and is the much more efficient pathway of the two. Its by-products are water and carbon dioxide, which can easily be eliminated by the body. Of the two, the aerobic pathway is the much more efficient of the two and is why cellular respiration has such a high demand for oxygen.

Oxygen Transport

Oxygen can be transported in the blood by one of two ways: dissolved in the plasma or bound to hemoglobin. Dissolved oxygen plays a very small part in oxygen transport, since only about 2% of the total oxygen is carried in this way and is an inadequate supply of oxygen for the body. Hemoglobin is the other method of delivery that makes up for the other 98% of oxygen transport. Oxygen is able to bind reversibly to hemoglobin allowing it to bind oxygen when PO_2 is high and release it when PO_2 is low. Hemoglobin is transported around the body and is found encapsulated in red blood cells.

Oxygen is able to diffuse through the membrane of the red blood cell and bind to hemoglobin causing a conformational change in the hemoglobin. Hemoglobin has four sites available for oxygen binding and the conformational change causes the three other binding sites to cooperatively bind other oxygen. This results in a progressive increase in

affinity as oxygen molecules are bound one by one to the hemoglobin. This also applies in a reverse manner, as oxygen is released from its binding sites on hemoglobin and, the affinity for oxygen decreases. The cooperative binding of oxygen to hemoglobin is manifested in a sigmoidal-shaped binding curve. The curve can be shifted by various local factors such as PCO_2, pH, and temperature, which ultimately affect the rate of disassociation of oxygen from hemoglobin.

Hemoglobin

Hemoglobin is the molecule found in red blood cells that is responsible for the transport of oxygen. Each human red blood cell contains roughly 300 million molecules of tetrameric hemoglobin. The red blood cell membrane encapsulates and protects the hemoglobin from dissociation and gives the hemoglobin a life span of 120 days, which is usually the life span of the red blood cell itself (Winslow 2000). Hemoglobin is a 64 kDa tetrameric protein that consists of two α chains and two β chains. Each of the four polypeptides of the hemoglobin molecule contains a single heme group which is a single iron atom surrounded by a porphyrin structure. The heme groups are the sites of oxygen binding where a single oxygen molecule can reversibly bind to a single heme group, allowing four oxygen molecules to bind to a single hemoglobin molecule (Kim and Greenburg 2004). The average percent hematocrit in an adult human is 45% with a normal blood concentration of hemoglobin at 15 g/100 ml. Each hemoglobin molecule is capable of carrying 1.34 ml O_2/g Hb which means that the total oxygen carrying capacity of human blood is 20ml O_2/dl (Berne and Levy 1998).

Nitric Oxide

Physiological Effects

Nitric Oxide, NO, is a small, diatomic, free radical gas molecule that is lipophilic, allowing it to pass freely through cellular membranes and other tissues. NO does contain a free electron making it very reactive and unstable. Unlike most free radicals, NO has a low level of reactivity which allows it to penetrate into tissues much further without reacting than a faster reacting free radical (Lane 2002). NO itself has a wide array of physiological activities. Several actions include promoting and suppressing apoptosis; acting as both an oxidant and antioxidant; emptying of the stomach; control of blood flow; release of hormones and neurotransmitters; learning and memory; pain sensation; and protection of cells from cellular parasites. This is achieved because of the variety of NO species and the available protein targets. Nitrogen oxide is able to react with oxygen species, other free radicals, transition metals, thiols, molecular oxygen, and redox reactions to create new products that only further nitric oxide's ability to exert its effects on a larger array of targets. Several sites in which nitric oxide plays a physiological role include the brain, lungs, kidneys, GI tract, and blood flow. NO is able to bind to cytochrome c oxidase, which is located in the mitochondria. This molecule is part of the final pathway, the electron transport chain, for cellular respiration and normally uses oxygen as the electron acceptor; however NO will bind to this molecule preventing oxygen from receiving the electron. This causes a disruption in cellular respiration and decrease in oxygen consumption due to NO's ability to disrupt the electron transport chain in the mitochondria.

Vasoactivity

Vasoactivity is controlled by the sympathetic inputs to the arterioles and in tandem, local and circulating factors. One such factor that controls vasodilation is known as Endothelial Derived Relaxing Factor, EDRF. EDRF has been shown to affect the endothelium in arterioles by causing the smooth muscle to relax. It has been shown that without the endothelium, the ability of the arteriole to dilate is severely reduced (Iwatani 2008). Nitric oxide (NO) is the most likely molecular candidate for EDRF. It has been shown the EDRF activity and NO levels parallel each other leading to the conclusion that NO is in fact EDRF and a vasodilator (Palmer 1987). NO acts by activating soluble guanylate cyclase, sGC, that produces cGMP from GTP. The rise in cGMP then causes a decrease in intracellular calcium . The drop in intracellular calcium causes a dephosphorylation of myosin in the smooth muscle causing relaxation of the smooth muscle and overall vasodilation (Lane 2002). NO is also able to react with hemoglobin after which it becomes inactive (Lane 2002).

Nitric Oxide Synthase

Nitric oxide is produced by nitric oxide synthase, NOS, which has three isoforms: nNOS, iNOS, and eNOS. Each NOS is found in a different area of the body with small differences among the three. Neuronal NOS, nNOS, is found in the brain and skeletal muscle and constitutively produces low levels of NO; endothelial NOS, eNOS, is found in the endothelium and cardiac muscle and constitutively produces low levels of NO; and inducible NOS, iNOS, is found in macrophages and possibly all nucleated cells and can be induced to produce high levels of NO. All three follow a similar pathway of nitrogen oxide production. L-arginine and oxygen are reacted together in the presence of NADPH,

which acts as an electron donor, to produce L-citrulline and nitric oxide. The regulation of NOS is all calcium-dependent except for iNOS (Lane 2002). N_{ω}-Nitro-L-arginine methyl ester hydrochloride (L-NAME) is a non-selective inhibitor of NOS enzyme activity. L-NAME has been used as a potent vasoconstrictor because of its ability to reduce the action of NOS and drastically reduce the levels of NO produced by the endothelium.

Hemoglobin-Based Oxygen Carriers

History

Hemoglobin-based oxygen carriers have always been an attractive alternative to blood transfusions. Even though the current blood supply provides a source of relatively safe blood, screening is not always perfect and the occasional transmission of disease or viruses does occur. Transfusions always run the risk of unfavorable host reactions, postoperative infectious complications, and other various side effects. Human blood also has a relatively short shelf life of 42 days and requires refrigeration to remain safe for use. When human blood is required, the use of allogenic blood requires that the donor blood be matched to the recipient (Gould 1996, Spahn 1994). The source of allogenic testing and limited shelf life comes from the actual membrane of the red blood cell (RBC). Each RBC contains the durable hemoglobin protein, which is actually able to survive outside for the RBC membrane (Gould 1996).

HBOCs have proven to be very effective replacements to human blood transfusions. HBOCs do not require refrigeration, have a much longer shelf life, and are universally compatible. Patients who received HBOCs required a smaller transfusion volume due to HBOCs' increased oxygen capacity. HBOCs have been shown to improve

wound healing, enhance hepatic cell proliferation, and decrease splanchnic bacterial translocation when compared to human blood transfusions (Spahn 2001).

An unmodified hemoglobin solution is derived from outdated blood. There has been no evidence that the source of the hemoglobin is of any consequence. The RBCs are rinsed with a pyrogen-free water which causes the cells to undergo lysis. After several filtration steps, pure hemoglobin is extracted from the RBC membrane debris. Since the antigens of the RBC are found on the stroma, or membrane of the RBC, the new solution is universally compatible and is referred to as stroma free hemoglobin (SFH). When preparing solutions, colloid osmotic pressure is adjusted to ensure that the concentration is isooncotic and safe for clinical use. SFH has been shown to be able to support life in primates that lack RBCs (Spahn 2001, Gould 1996).

Side Effects of HBOCs

It has been shown that as useful as unmodified tetrameric hemoglobin is; it does have some unwanted side effects. The tetrameric hemoglobin molecule has a tendency to dissociate when outside of the RBC. This leads to the formation of dimers that causes renal complications and reduction in function; oxidative stress; and unwanted rapid clearance of the unmodified tetrameric hemoglobin. In addition there seems to be an interference with the smooth muscle that causes hypertensive and gastrointestinal side effects. It is believed that the hypertensive effects derive from the fact that hemoglobin is a NO sink leading to vasoconstriction (Spahn 2001, Gould 1996, Olson 2004). Baxter Hemoglobin Therapeutics was able to produce an HBOC known as HemAssist, which made it to Phase III clinical trials, but began exhibiting adverse side effects. HemAssist was a diaspirin cross-linked human hemoglobin, DCLHb. By 1998, Baxter stopped

production due to reports that extracellular hemoglobin was toxic and unsuitable as a blood substitute. One of the serious side effects noted was a significant spike in blood pressure after administration of the DCLHb (Olson 2004).

There are two major theories as to the cause of the vasoactivity of HBOCs. The most widely accepted theory is related to the ability of free tetramers to act as NO scavengers. When the hemoglobin tetramer is free from the RBC, it is able to extravasate into and across the vessel wall where it is able to scavenge NO. In normal human blood, the hemoglobin is encased in the RBC preventing the hemoglobin tetramer from moving into the endothelium. The sudden drop in NO due to scavenging causes a drastic reduction in vasodilator tone and results in vasoconstiction. The ability of hemoglobin to extravasate comes from the lack of the RBC membrane and the ability of the tetramer to move into the endothelium. Once in the endothelium, hemoglobin's affinity for NO is 8,000 times that of oxygen. The alternative theory relies on HBOC's increased ability to transport oxygen to the smooth muscle of the vascular wall. The idea is that the vasoconstriction comes from an autoregulatory response due to the increased oxygen delivery to the smooth muscle. So in theory, one might be able to alter the effects of vasoconstriction by altering the P50, or the affinity of the hemoglobin for oxygen. One such study showed that differences in P50 among various HBOCs did not produce a difference in total peripheral resistance (TPR) or mean arterial pressure (MAP) (Olson 2004). The NO scavenging theory is currently the most widely accepted and steps have been taken to try to curb this unwanted side effect by trying to physically alter the hemoglobin molecule to limit its interaction with NO.

Alternatives to HBOCs

Perflourocarbons, PFCs, are derived from hydrocarbons with all of the hydrogen atoms replaced with fluorine atoms. A high gas solubility, low viscosity, and biological inertness characterize this synthetic compound. PFCs are emulsified in a plasma-like aqueous fluid such as albumin to prevent the formation of embolisms and infarctions. PFCs are capable of dissolving large amounts of gases, mainly oxygen and carbon dioxide. A PFC can dissolve 20-30 times the amount of oxygen as can plasma or water. This allows them to be able to deliver an amount of oxygen that is proportional to the PO_2 of the blood.

Modification of HBOCs

A great deal of effort has been put into minimizing the side effects associated with earlier HBOCs. It is now believed that many of the toxicities come from the free tetramers of hemoglobin and their dissociation into dimers. Several theories have been proposed to help prevent these unwanted side effects by altering the hemoglobin protein itself. Several approaches try to tether the hemoglobin together or to other macromolecules. One such approach has been to create a conjugated tetramer that is a tetramer bound to a macromolecule such as polyethlyne glycol. This prevents extravasation across the microvessels by increasing the overall size of the hemoglobin molecule. Cross-linked tetramers are another approach by which the tetramer is bound together to prevent dissociation into dimers. Polymerization is another method by which tetramers are linked to each other using glutaraldehyde, forming a polymer of tetramers. The final method is encapsulation within a lipid membrane or liposome which tries to

mimic the natural membrane which surrounds the hemoglobin in a RBC. Of the four described approaches, the polymers and encapsulation proved to be the most effective in preventing extravasation (Spahn 1994, Olson 2004). The polymerized tetramer is unable to pass through the vessel wall and therefore remains in the lumen allowing NO to exert its normal effects. It has been shown that the increase in MAP is inversely proportional to the size of the hemoglobin "particle," indicating that the increased size decreases the likelihood of extravasation (Olson 2004).

Purpose of the Study

The purpose of this study was to further investigate the NO scavenging hypothesis of the pressor response to HBOC infusion. The goal of the study was to investigate whether or not NO scavenging was responsible for the vasoconstriction found when using HBOCs. For each study, the mean arterial pressure and diameters of three different sizes of arterioles from the mesentery were monitored and recorded. The animal model for the studies was the male Sprague-Dawley rat. Three different HBOCs were studied by administering increasing doses to establish a dose response relationship to observe the increase in MAP and arteriolar vasoconstriction in the mesentery. In addition, sodium nitrite was administered to chemically alter the levels of NO and observe whether MAP and arteriolar diameter would return to baseline with increasing levels of sodium nitrite following a single dose of HBOC. Finally, a single dose of L-NAME, a NOS inhibitor, was administered to mimic a dose of HBOC, followed by increasing doses of sodium nitrite. The goal of the last study was to see if L-NAME, which decreases NO production via NOS inhibition, would mimic similar effects as that of a single dose of HBOC and

would return to MAP and arteriolar diameter to baseline with increasing doses of sodium nitrite like the HBOC. It was anticipated that an increase in MAP and a decrease in arteriolar diameter would be observed with increasing doses of HBOC. The increasing sodium nitrite should replace the scavenged NO and return MAP and diameter back to baseline. If the HBOC acted by scavenging NO, then L-NAME should produce similar effects to that of the HBOC. The actual results showed the expected increase in MAP with increasing doses of HBOC, and a return to baseline following increasing doses of sodium nitrite. The L-NAME produced results with increases in MAP similar to that of the HBOC. These results indicated that NO scavenging was a plausible theory due to the response of MAP to HBOCs. The vessel diameters, however, showed little to no response throughout the studies and indicated that NO is not a major player in the vasoactivity of the arterioles of the mesentery.

Methods and Materials

Animal Preparation:

For the study, 36 male Sprague-Dawley rats with an average weight of about 300 ± 5 (SE) grams were used. They were all housed in pairs with access to food and water and put in a temperature- and light-controlled environment with 12 hours of light and 12 hours of dark.

The animals were initially anesthetized with a mixture of ketaset and acepromazine (75mg/kg of ketamine and 2.5mg/kg of acepromoazine, Henry Schein, Melville, NY) with approximately 0.1ml per 100g of body weight; additional doses were given as needed at increments of 0.1ml or 0.05ml. The site of intraperitoneal injection

was the underside of the animal with care to avoid piercing any of the major organs. The animal's neck, ventral thigh region, and ventral stomach regions were shaved using electric shears.

Surgical Procedures:

The animal was placed in the supine position on a heated pad which maintained a temperature of 37 °C. The toe pinch reflex and the eye reflex were used to ensure the fact that the animal was completely anesthetized before the surgery began. Prior to the surgery, three syringes full of heparinized saline were prepared for the cannulation. The initial incision was at the ventral side of the inner thigh. The right femoral vein and artery were located and cannulated with PE-90 tubing with a PE-10 tip and a syringe of heparinized saline. The cannula was secured with 5-0 braided surgical silk. The femoral vein was used to infuse Alfaxan (Alfazalon 10mg/ml, Abbeyvet Export LTD, North Yorkshire, England) to maintain adequate anesthesia during the procedure. The femoral artery was use to measure the mean arterial pressure and take blood samples for hematocrit, arterial blood gas measurements(ABL 705, Radiometer America Inc., Westlake, OH), and hemoglobin measurements (Radiometer OSM3, Radiometer, Crawley, West Sussex, UK). Another incision was made on the ventral side of the neck. Then PE-240 tubing was placed in the trachea of the rat and tied in place with 5-0 surgical silk. With the same incision, the left jugular vein was located and cannulated with PE-10 tubing and a syringe of heparinized saline that was used to infuse solutions. The incisions were closed using Super Glue.

Mesentery Preparation

A 1.5 inch incision was made on the ventral side of the rat from the sternum down. The skin was separated from the abdominal wall. Another incision was made into abdominal muscle and cauterized (Gemini RS-300, Roboz Surgical Instrument Co., Rockville, MD) to prevent any bleeding that might bleed onto the preparation. The animal was then placed on a thermostatic platform. The platform was custom-made to allow the animal to maintain a constant temperature via a heated plate. The animal was placed on its side with the incision of the abdomen in line with a raised clear circular pedestal. A loop of the mesentery was gently placed over the viewing window on the platform. The criterion for an acceptable loop of the mesentery was clearly visible arteries located in the membrane of the mesentery. It was kept moist using PBS and was then wrapped with Saran wrap to keep the mesentery from drying out and to prevent gas exchange with the atmosphere. The platform was then secured to the stage of an imaging microscope. During a 30-minute acclimation period, blood samples were taken from the animal, and meanwhile three arterioles of different sizes were located.

Properties and Preparation of HBOC's

Three types of HBOC's, HBOC 201, HBOC 205 (MW400), and HBOC 205 (MW 600), were used for similar experiments. All three were obtained from Biopure Corporation and are derived from purified bovine hemoglobin that is crosslinked by a reaction with glutaraldehyde in modified Ringer's lactate to form hemoglobin polymers. HBOC's are stable when stored at room temperature or at a refrigerated temperature of 2-30 °C for up to 36 months.

HBOC 201 Infusions

HBOC 201 was prepared at 5 different concentrations, 0.01 μM, 0.1 μM, 1 μM, 10 μM, and 100 μM. The desired concentrations of HBOC were calculated by taking into account both the altered plasma volume produced by the serial infusions and the current plasma concentration of HBOC 201 produced by previous injections (see checklist for equations used). For each of the first four infusions, the volume injected was 0.1ml. Dilutions of the stock HBOC 201 solution, 12.4 g/dL, were calculated and prepared. Normal saline was used to perform any of the necessary dilutions. For the final injection, 100 μM, the stock solution was used without any of the previous dilutions.

HBOC 205 (MW 400)

HBOC 205 (MW 400) was prepared at 4 different concentrations, 1μ M, 10 μM, 100 μM, and 300 μM. The desired concentrations of HBOC were calculated by taking into account both the altered plasma volume produced by the serial infusions and the current plasma concentration of HBOC 205 (MW 400) produced by previous injections (see checklist for equations used). For the first two infusions, the volume injected was 0.1 ml. Dilutions of the stock HBOC 205(MW 400) solution, 13.2 g/dL, were calculated and prepared. Normal saline was used to perform any of the necessary dilutions. For the final two injections, 100 μM and 300 μM, the stock solution was used without any of the previous dilutions.

HBOC 205 (MW 600)

HBOC 205 (MW 600) was prepared at 3 different concentrations, 10 μM, 100 μM, and 300 μM. The desired concentrations of HBOC were calculated by taking into account both the altered plasma volume produced by the serial infusions and the current plasma concentration of HBOC 205 (MW 600) produced by previous injections (see checklist for equations used). For the first infusion, the volume injected was 0.1 ml. Dilutions of the stock HBOC 205 (MW 600) solution, 13 g/dL, were calculated and prepared. Normal saline was used to perform any of the necessary dilutions. For the other two injections, 100 μM and 300 μM, the stock solution was used without any of the previous dilutions.

Solutions Prepared

Normal Saline

Normal saline was use create the solutions used for the infusions. The solution was prepared using 9.08g of NaCl in 1L of deionized water.

Heparinized Saline

Heparinized saline was used to prevent clotting in the tubing of the cannulas or near the sites of cannulations in the vein or artery. The solution was prepared using 10 ml of 1000 u/ml heparin in 1L of normal saline.

Sodium Nitrite

Sodium nitrite in the body is able to deliver extra nitric oxide to the vascular smooth muscle. It acts to reduce hypertension via vasodilation of the blood vessels.

L-NAME

N_{ω}-Nitro-L-arginine methyl ester hydrochloride (L-NAME) is a non-selective inhibitor of NOS enzyme activity. A solution was made by dissolving 300 mg of L-NAME (Sigma, St. Louis, MO) in 10 ml of deionized water to arrive at a final concentration of 30 mg/ml. In the L-NAME portion of the experiments, a 5 mg/kg dose was given intravenously. The amount given was directly proportional to the body weight of the animal.

Imaging Microscope and Software

Microscopic measurements were made using an Axioplan-2 microscope (Zeiss, Thornwood, NY) equipped with Achroplan objectives. The microscope was connected to a CCD camera (Cool Snap cf, Roper Scientific, Tucson, AZ) and a computer (Dell Dimension 8250, Dell), which was used for acquiring images of the microcirculation. The automatic features of the microscope were controlled with IP Lab software (version 3.6, Scanalytics, VA). IP Lab controlled image acquisition, and the diameter measurements were made directly over the image generated. A 100-watt halogen lamp was used to transilluminate the muscle preparation. Objective 4X/NA = 0.10 (Achroplan,

Zeiss, Thornwood, NY) was used for observing and identifying the three arterioles that were to be measured. The same objective was used to measure the large and medium arterioles. The objective 20X/NA = 0.45 was used to measure the smallest arteriole. An initial and baseline image was taken before any part of the experiment began, and additional images were taken at the time of the infusion, 5 minutes after, and 10 minutes after.

Data Acquisition

Measurement of Mean Arterial Pressure

The cannula in the femoral artery was used to measure the mean arterial pressure of the animal throughout the procedure. The cannula was connected to the CyQ 103/301 acquisition and display system (CyberSense Inc., Nicholasville, KY). The system gave a continuous reading of the MAP that was recorded at regular intervals. For all of the experiments, there was an initial reading recorded when the animals was first put on the microscope platform and another baseline reading right before the first infusion that was preceded by the 30-minute acclimation period. From then on, a measurement was taken at the time of the infusion, 1 minute after the infusion, 5 minutes after the infusion, and 10 minutes after the infusion. This process was repeated for all infusions for all of the experiments. The transducer was placed at the level of the animal's heart to give an accurate blood pressure reading. The MAP was used to monitor the animal's systemic response HBOC infusion and to give the time course of MAP, as well as the dose response relationship between the infusions and the average change in MAP.

Measurement of Arteriolar Diameter

The three arterioles were chosen based on the clarity of the image. The ideal sizes for the three vessels were about 300 μm for the largest, 200 μm for the intermediate, and 40 μm for the smallest. Clarity and ease of measurement were the primary criteria when selecting the vessels to be measured. The transillumination images were captured via the camera and saved to IP Lab for later viewing and diameter measurements. The images were captured 1 minute after the infusion, 5 minutes after the infusion, and 10 minutes after the infusion. The measurements were later displayed to give the time course of diameter of the three arterioles, as well as a dose response relationship between the infusions and average change in arteriolar diameter.

Experimental Protocol

Human Serum Albumin

Human serum albumin (HSA) was used as a volume control prior to the initial experiments with HBOC's. This was to establish the response in a control group that was injected with a solution of similar osmolarity and concentration as the HBOC infusions.

HBOC

For the HBOC portion of the protocol, three series of experiments were done with

HBOC 201, HBOC 205 (MW400) and HBOC 205 (MW600). For all three, similar protocols were followed. The goal was to establish a dose-response relationship between MAP and diameter of the three arterioles as a function of HBOC concentrations. After the animal was placed on the stage of the microscope and three arterioles were located and baseline MAP measurements were taken, calculations and dilutions were preformed to prepare the necessary HBOC concentrations. HBOC infusions were prepared using the stock solution of HBOC 201, 205 (MW400), and 205(600). For HBOC-201 (n=6), five different plasma concentrations were prepared, 0.01, 0.1, 1.0, 10.0, and 100.0 μM. For HBOC-205 (MW 400) (n=6), four different plasma concentrations were prepared, 1.0, 10.0, 100.0, and 300.0 μM. For HBOC-205 (MW 600) (n=6), three different plasma concentrations were prepared, 10.0, 100.0, and 300.0 μM. After the 30-minute acclimation period, and measurements of baseline MAP and diameters were made, the infusion was administered. Each dose was separated by a 15-minute interval with measurements taken in between. MAP measurements were taken immediately after the infusion, one minute later, five minutes later, and 10 minutes later. Diameter measurements were taken one minute later, five minutes later, and 10 minutes later.

Sodium Nitrite Controls

A control group, n=2, was used to establish a baseline response for $NaNO_2$. The rats were given increasing doses of $NaNO_2$, 10 μM, 100 μM, 1 mM, and 3 mM. Doses were given at fifteen minute intervals with measurements taken in between. MAP was taken at the time of the infusion, one minute after, five minutes after, and 10 minutes after. Diameter was taken one minute after, five minutes after, and 10 minutes after the

infusion. This was repeated for all doses.

$NaNO_2$ and HBOC Time Controls

A time control was taken to observe the length of time the HBOC and $NaNO_2$ were still effective. For the HBOC time control (n=2), a single dose of 100 μM HBOC-201 was infused. MAP was taken at the time of the infusion, one minute after, and every five minutes after until a return to baseline was observed. Diameter measurements were made one minute after the infusion and every 5 minutes thereafter until a return to baseline was observed. For the sodium nitrite control (n=1), the animal was give a single does of 100 μM HBOC-201 followed by an injection of 100 μM $NaNO_2$. MAP was measured at the time of the injection, 1 minute after, and every five minutes until a decrease in the $NaNO_2$ effect was observed. Diameters were measured 1 minute after the infusion and every five minutes until the effects of the $NaNO_2$ began to decrease. Then a 1 mM infusion was given and measured by the same protocol as the 100 μM injection.

HBOC and $NaNO_2$

For this portion of the study (n=6), the animal was given an initial dose of 100 μM HBOC-201 followed by increasing doses of $NaNO_2$: 10 μM, 100 μM, 1 mM, and 3 mM. Doses were given at 15 minute intervals with measurements taken in between. MAP was taken at the time of the infusion and every five minutes there after. Diameter was taken one minute after the infusion and every 5 minutes thereafter.

Simultaneous HBOC and $NaNO_2$

For this portion of the study (n=2), a dose of 100 μM HBOC 201 was given, followed immediately by a 1mM infusion of $NaNO_2$, which was then followed by a 3 mM $NaNO_2$ injection 15 minutes after the 1 mM injection. Doses were given at 15 minute intervals with measurements taken in between. A MAP measurement was taken immediately following the $NaNO_2$ infusion then 1 minute after, 5 minutes after, and 10 minutes after. Diameter measurements were taken 1 minute after, 5 minutes after, and 10 minutes after.

L-NAME

For this portion of the study (n=6), a single 5 mg/kg dose of L-NAME was given to the rat followed by increasing doses of $NaNO_2$: 100 μM, 1 mM, and 3 mM. Doses were given in 15 minute intervals with measurements taken in between. A MAP measurement was taken immediately after all infusions then 1 minute after, 5 minutes after, and 10 minutes after. Diameter measurements were taken 1 minute after, 5 minutes after, and 10 minutes after each infusion.

Table 1. Baseline physiological variables of all animals tested.

Parameter	HBOC (n = 20)	$NaNO_2$ (n=19)
Weight (g)	287.7 ± 5.4	313.7 ± 8.2
MAP (mmHg)	92.7 ± 3.7	87.1 ± 2.3
PO_2 (mmHg)	73.8 ± 3.8	80.4 ± 3.1
PCO_2 (mmHg)	42.3 ± 1.3	40.3 ± 1.6
tHB (g/L)	15.8 ± 0.2	15.5 ± 0.3
Hct (%)	0.4 ± 0.005	0.4 ± 0.004
pH	7.37 ± 0.01	7.38 ± 0.01

This table presents the physiological baselines for the rats prior to any infusions. Baseline measurements were taken to ensure the rat was in a stable, well maintained condition. MAP was taken using the CyQ 103/301 data acquisition and display system (CyberSense Inc., Nicholasville, KY). PO_2 and PCO_2 were measured on blood samples taken from the right femoral artery using an ABL 705 (Radiometer America Inc., Westlake OH). Hemoglobin concentration, tHB, was measured by a hemoximeter configured for rats (Radiometer OSM3, Radiometer, Crawley, West Sussex, UK). Percent hematocrit, Hct, was measured by capillary tube centrifugation.

Results

HBOC

HSA

HSA was the volume control group that established a baseline for comparison with the rest of the HBOC experiments. The animals (n=2) showed little response to the increasing doses of HSA. There was no noticeable increasing trend with MAP, which stayed fairly constant and the baseline measurement. Vessel diameters of all three sizes show some fluctuations but none of them showed any significant change from baseline (*Figure 1).* The percent change indicates that MAP and diameter of all three arterioles showed no significant differences from baseline (*Figure 2).*

Dose Dependent Effects HBOC 201

The initial doses of HBOC, 0.01 μM, 0.1 μM, and 1.0 μM, showed no significant changes from baseline. MAP showed no significant rise from baseline until the 100 μM dose. There was an increase at the time of the 10 μM injection, but was not significantly different from initial baseline. The diameters of all three arterioles showed no upward or downward trend and did not deviate significantly from baseline (*Figure 3).* There was a significant percent increase from baseline for MAP with the largest dose of 100 μM, but again diameters showed no significant change from baseline. Overall, MAP only

increased at the highest doses, but there was no change for any of the diameters (*Figure 4).*

Dose Dependent Effects of HBOC 205 (MW 400)

Increasing doses of HBOC 205 (MW 400) showed significant changes with respect to MAP. From the 10 μM dose, a steady increase was observed with each increasing dose of the HBOC. The MAP percent changes show a general trend of increasing change that is statistically significant from baseline. The greatest change was observed with the largest dose of 300 μM after 5 minutes. The arteriolar diameters failed to show any sort of change or trend in response to any of the doses *(Figure 5).* The percent change of diameter for each arteriole showed no significant changes from baseline (*Figure 6).*

Dose-Dependent Effects of HBOC 205 (MW 600)

Increasing doses of HBOC 205 (MW 600) showed significant changes with respect to MAP. From the 100 μM dose, a steady increase was observed with each increasing dose of the HBOC. The MAP percent changes showed a general trend of increasing change that is statistically significant from baseline. The greatest change was observed with the second largest dose of 100 μM and remained elevated with the final dose of 300 μM. The arteriolar diameters failed to show any sort of change or trend in response to any of the doses (*Figure 7).* The percent change in diameter for each arteriole showed no significant changes from baseline (*Figure 8).*

Sodium Nitrite

$NaNO_2$ Control

The sodium nitrite control groups showed a decreasing trend with MAP, but little to no change with respect to arteriolar diameters. The decrease in MAP was a gradual drop with each increasing dose of $NaNO_2$. The lowest MAP reading was found to be at the 10 minute mark after the final dose of 3 mM (*Figure 9).* There was a general trend of a decreasing percent change with significance from baseline for the 1 mM and 3 mM doses. Arteriolar diameters showed no significant change from baseline for any of the doses (*Figure 10).*

$NaNO_2$ Time Control

This study showed how long a single dose of 100 μM and 1 mM $NaNO_2$ could last. The initial dose of 100 μM HBOC 201 showed a spike in MAP which was brought down to 117 mmHg 15 minutes after the 100 μM dose of $NaNO_2$ (*Figure 11).* At the 25 minute mark, MAP began to rise. Another dose of 1 mM brought MAP down to 105 mmHg at the 10 minute mark but then began to rise again at the 15 minute mark. Arteriolar diameters showed no change or variation with any of the doses (*Figure 12).*

HBOC 201 Time Control

The HBOC time control established the length of time that HBOC 201 from a single dose of 100 μM could exert its effects. An increase in MAP was noted immediately after the 100 μM injection, but no return to baseline was noted until the 60

minute mark. (*Figure 13).* Arteriolar diameters showed some small changes, but no definite trend was observed (*Figure 14).*

$NaNO_2$ Dose Response Effects following Single HBOC 201 Dose

There was a noticeable rise in MAP after the 100 μM dose of HBOC 201. After the initial 0.1 μM $NaNO_2$ dose, there was a decreasing trend of the MAP with increasing doses of $NaNO_2$. The largest drop in MAP was observed around the 100 μM dose. The arteriolar diameters showed no response to the HBOC or increasing doses of $NaNO_2$. The percent change of MAP showed a significant increase from baseline after the HBOC infusion. The decreasing MAP percent change as a result of the $NaNO_2$ infusion showed statistical significance compared with the initial HBOC response. Arteriolar diameters showed no statistically significant change from baseline or from the HBOC response (*Figure 15).* MAP showed an increase following the HBOC infusion and MAP decreased with increasing doses of the $NaNO_2$; however, there was no diameter response in the arterioles (*Figure 16).*

Simultaneous $NaNO_2$ and HBOC effects

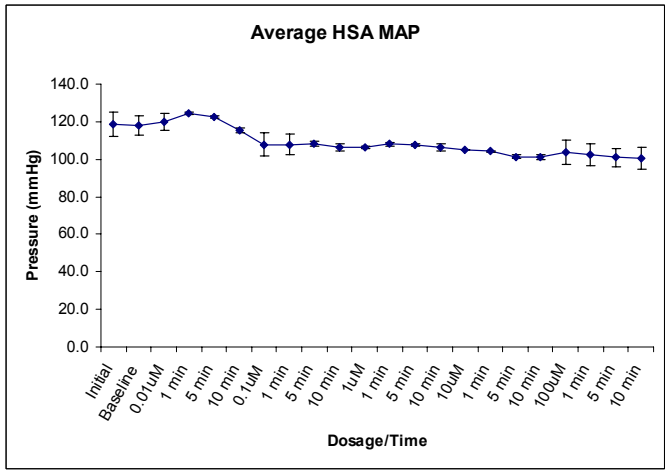
The initial dose of the HBOC showed a rise in MAP, but was quickly counteracted with the immediate 1 mM dose of $NaNO_2$. The following dose of 3 mM $NaNO_2$ showed a further decrease in MAP. The arteriolar diameters showed no response to the HBOC or any of the $NaNO_2$ doses (*Figure 17).* Percent change of MAP showed a statistically significant change from baseline after the HBOC and $NaNO_2$ simultaneous infusions, but showed a return to baseline following the increasing doses of $NaNO_2$.

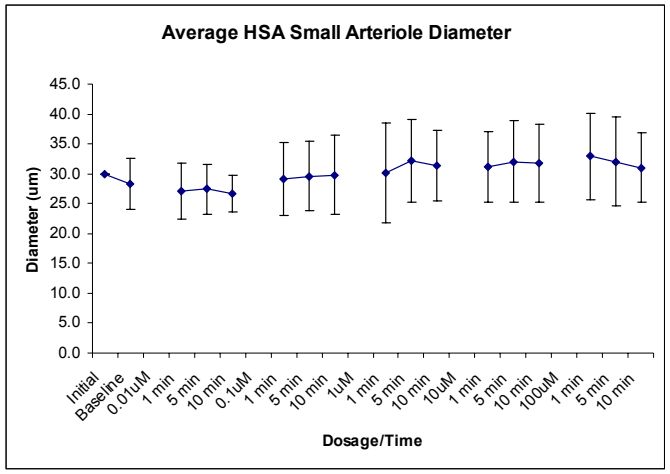
There was no statistical difference from baseline for the small arteriole, but there were statistical differences from baseline observed in the medium and large arterioles. In the medium arteriole an increase in percent change from baseline was observed after the 3 mM $NaNO_2$ dose, but a return to baseline occurred at the 10 minute marker. The large arteriole showed an increase in percent change from the baseline after the 1 mM dose, but returned to baseline after the 3 mM dose. Overall MAP showed a limited rise from the HBOC due to the presence of $NaNO_2$, but even though significant percent changes were observed in some of the arterioles, no overall trends were observed (*Figure 18).*

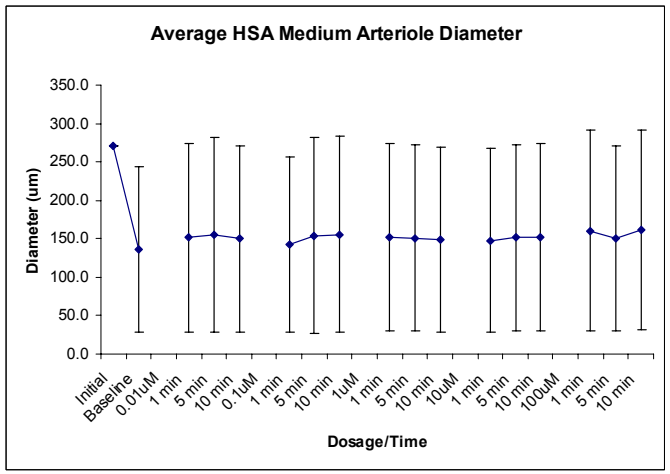
L-NAME and $NaNO_2$ Dose Response Effects

The initial dose of L-NAME showed a very quick increase in MAP, peaking at the 5 minute mark after the infusion. Increasing doses of $NaNO_2$ showed a steady decrease in MAP with a return to baseline after the 3 mM dose. Arteriolar diameters showed no significant changes or trends in response to the L-NAME or the $NaNO_2$ (*Figure 19).* The percent change of MAP showed a statistical difference from baseline with the infusion of the L-NAME and a steady drop in the increased percent change as the $NaNO_2$ was administered. A return to baseline was observed 5 minutes after the final $NaNO_2$ dose of 3 mM. There were no significant deviations from baseline observed in the diameter of any of the arterioles (*Figure 20).*

Figure 1. HSA Dose Response Curve. The upper left graph represents the change in mean arterial pressure. The upper right graph represents the change in diameter of the small arteriole. The lower left graph represents the change in diameter of the medium arteriole. The lower right graph indicates the change in diameter of the large arteriole. The error bars represent standard error.


Average HSA MAP
Pressure (mmHg)
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Initial Baseline
0.01uM 1 min 5 min 10 min
0.1uM 1 min 5 min 10 min
1uM 1 min 5 min 10 min
10uM 1 min 5 min 10 min
100uM 1 min 5 min 10 min
Dosage/Time


Average HSA Small Arteriole Diameter
Diameter (um)
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Initial Baseline
0.01uM 1 min 5 min 10 min
0.1uM 1 min 5 min 10 min
1uM 1 min 5 min 10 min
10uM 1 min 5 min 10 min
100uM 1 min 5 min 10 min
Dosage/Time


Average HSA Medium Arteriole Diameter
Diameter (um)
350.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
Initial Baseline
0.01uM 1 min 5 min 10 min
0.1uM 1 min 5 min 10 min
1uM 1 min 5 min 10 min
10uM 1 min 5 min 10 min
100uM 1 min 5 min 10 min
Dosage/Time

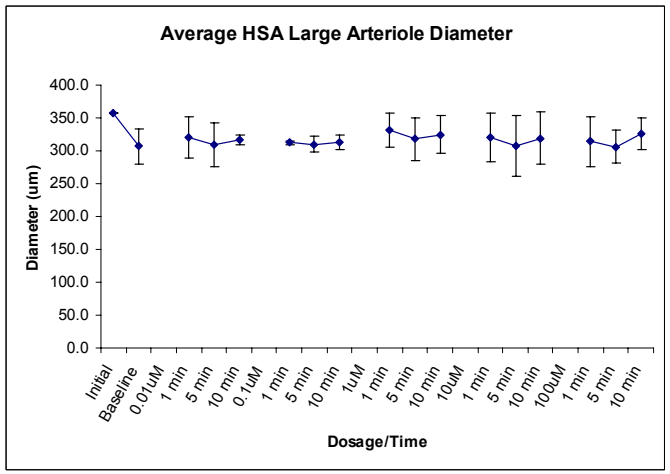
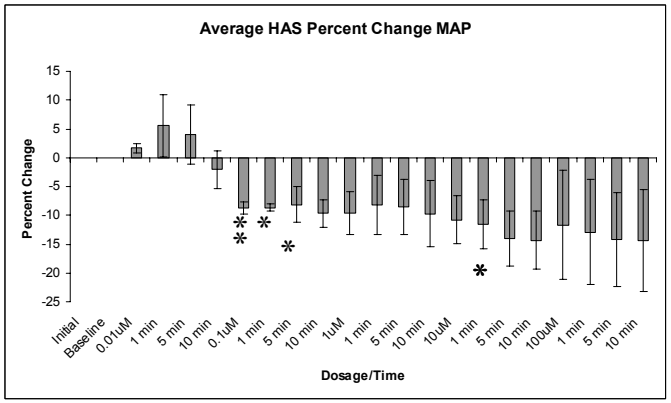
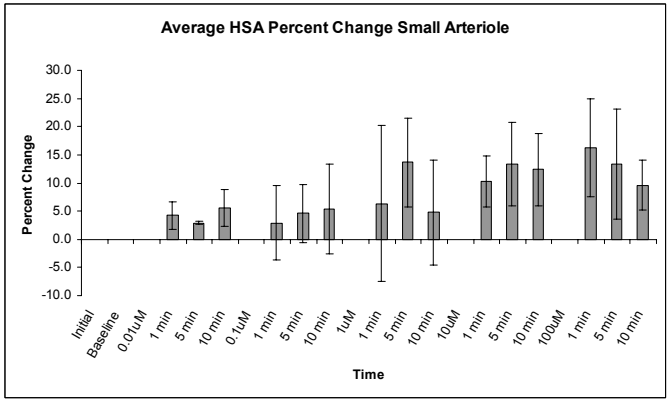
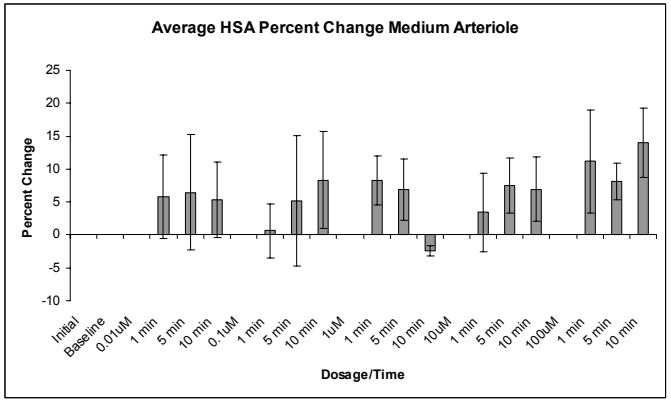

Average HSA Large Arteriole Diameter
Diameter (um)
400.0
350.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
Initial Baseline
0.01uM 1 min 5 min 10 min
0.1uM 1 min 5 min 10 min
1uM 1 min 5 min 10 min
10uM 1 min 5 min 10 min
100uM 1 min 5 min 10 min
Dosage/Time

Figure 2 HSA Percent Change. The upper left graph represents the percent change in mean arterial pressure. The upper right graph represents the percent change in diameter of the small arteriole. The lower left graph represents the percent change in diameter of the medium arteriole. The lower right graph indicates the percent change in diameter of the large arteriole. The error bars represent standard error. An (*) represents significance at $P < 0.05$. An (**) represents significance at $P < 0.01$. An (***) represents significance at $P < 0.001$.


Average HAS Percent Change MAP
Percent Change
15
10
5
0
-5
-10
-15
-20
-25
Initial Baseline
0.01uM 1 min 5 min 10 min
0.1uM 1 min 5 min 10 min
1uM 1 min 5 min 10 min
10uM 1 min 5 min 10 min
100uM 1 min 5 min 10 min
Dosage/Time


Average HSA Percent Change Small Arteriole
Percent Change
30.0
25.0
20.0
15.0
10.0
5.0
0.0
-5.0
-10.0
Initial Baseline
0.01uM 1 min 5 min 10 min
0.1uM 1 min 5 min 10 min
1uM 1 min 5 min 10 min
10uM 1 min 5 min 10 min
100uM 1 min 5 min 10 min
Time


Average HSA Percent Change Medium Arteriole
Percent Change
25
20
15
10
5
0
-5
-10
Initial Baseline
0.01uM 1 min 5 min 10 min
0.1uM 1 min 5 min 10 min
1uM 1 min 5 min 10 min
10uM 1 min 5 min 10 min
100uM 1 min 5 min 10 min
Dosage/Time

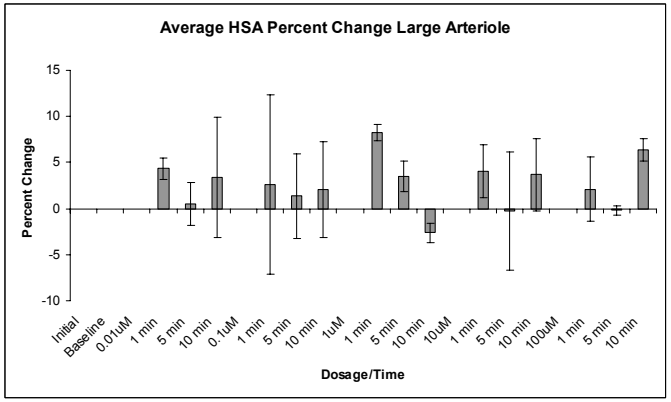
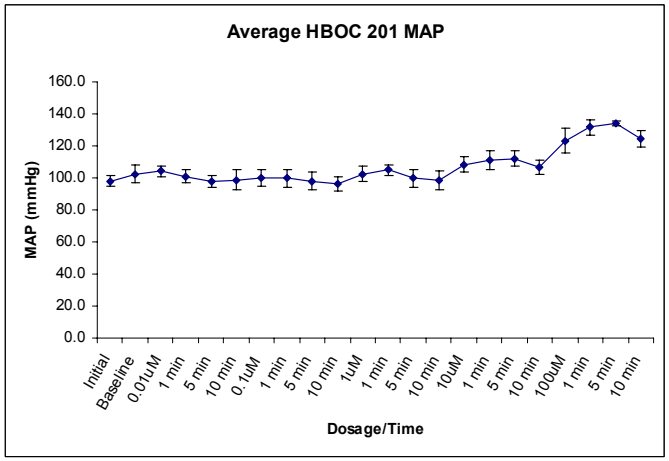
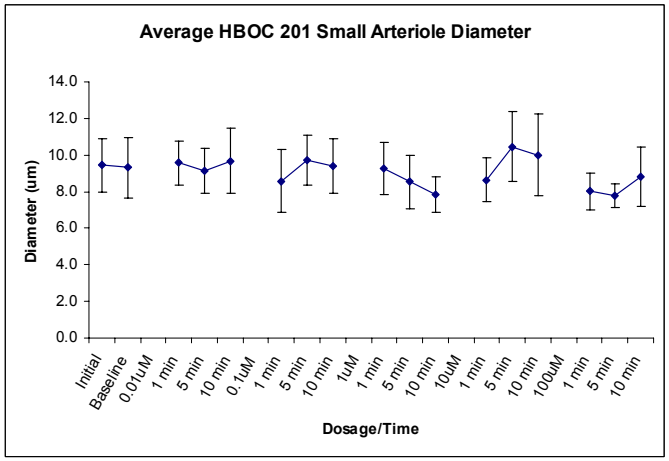
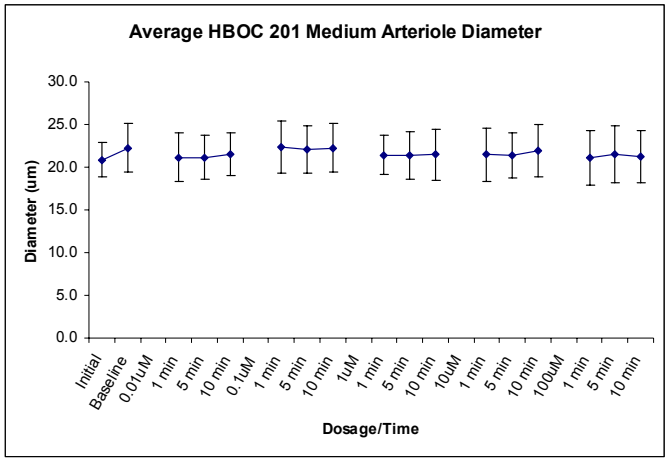

Average HSA Percent Change Large Arteriole
Percent Change
15
10
5
0
-5
-10
Initial Baseline
0.01uM 1 min 5 min 10 min
0.1uM 1 min 5 min 10 min
1uM 1 min 5 min 10 min
10uM 1 min 5 min 10 min
100uM 1 min 5 min 10 min
Dosage/Time

Figure 3. HBOC-201 Dose Response Curve. The upper left graph represents the change in mean arterial pressure. The upper right graph represents the change in diameter of the small arteriole. The lower left graph represents the change in diameter of the medium arteriole. The lower right graph indicates the change in diameter of the large arteriole. The error bars represent standard error.


Average HBOC 201 MAP
MAP (mmHg)
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Initial Baseline
0.01uM
0.1uM
1uM
10uM
100uM
1 min
5 min
10 min
Dosage/Time


Average HBOC 201 Small Arteriole Diameter
Diameter (um)
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Initial Baseline
0.01uM
0.1uM
1uM
10uM
100uM
1 min
5 min
10 min
Dosage/Time


Average HBOC 201 Medium Arteriole Diameter
Diameter (um)
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Initial Baseline
0.01uM
0.1uM
1uM
10uM
100uM
1 min
5 min
10 min
Dosage/Time

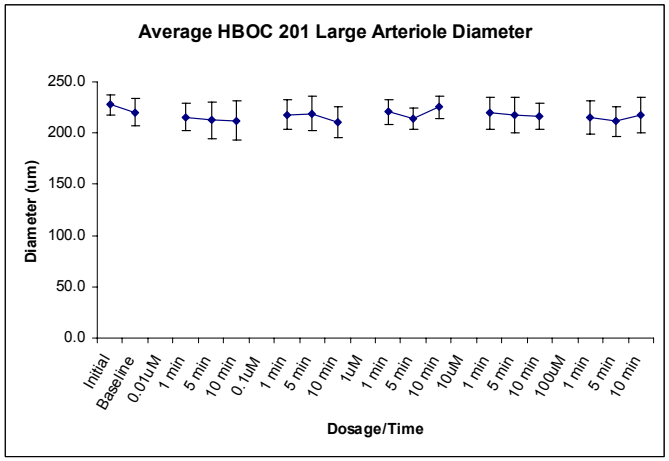
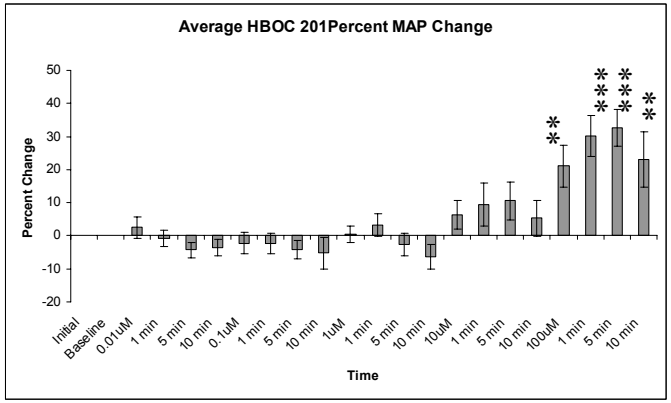
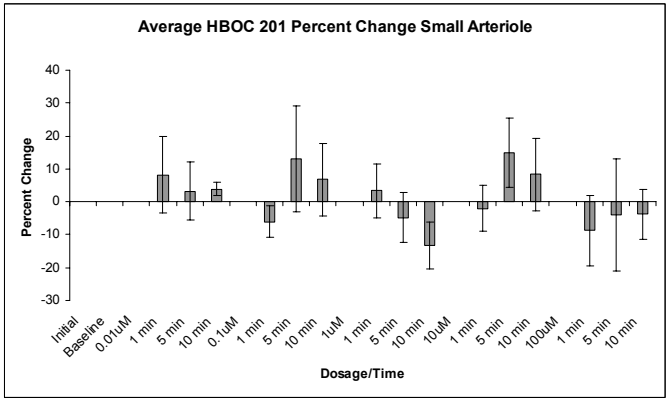
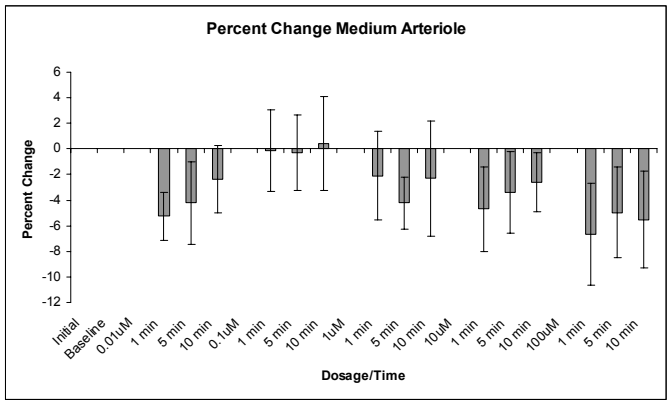

Average HBOC 201 Large Arteriole Diameter
Diameter (um)
250.0
200.0
150.0
100.0
50.0
0.0
Initial Baseline
0.01uM
0.1uM
1uM
10uM
100uM
1 min
5 min
10 min
Dosage/Time

Figure 4. HBOC-201 Percent Change. The upper left graph represents the percent change in mean arterial pressure. The upper right graph represents the percent change in diameter of the small arteriole. The lower left graph represents the percent change in diameter of the medium arteriole. The lower right graph indicates the percent change in diameter of the large arteriole. The error bars represent standard error. An (*) represents significance at $P < 0.05$. An (**) represents significance at $P < 0.01$. An (***) represents significance at $P < 0.001$.


Average HBOC 201Percent MAP Change
Percent Change
50
40
30
20
10
0
-10
-20
**
**
***
***
**
Initial Baseline
0.01uM 1 min 5 min 10 min
0.1uM 1 min 5 min 10 min
1uM 1 min 5 min 10 min
10uM 1 min 5 min 10 min
100uM 1 min 5 min 10 min
Time


Average HBOC 201 Percent Change Small Arteriole
Percent Change
40
30
20
10
0
-10
-20
-30
Initial Baseline
0.01uM 1 min 5 min 10 min
0.1uM 1 min 5 min 10 min
1uM 1 min 5 min 10 min
10uM 1 min 5 min 10 min
100uM 1 min 5 min 10 min
Dosage/Time


Percent Change Medium Arteriole
Percent Change
6
4
2
0
-2
-4
-6
-8
-10
-12
Initial Baseline
0.01uM 1 min 5 min 10 min
0.1uM 1 min 5 min 10 min
1uM 1 min 5 min 10 min
10uM 1 min 5 min 10 min
100uM 1 min 5 min 10 min
Dosage/Time

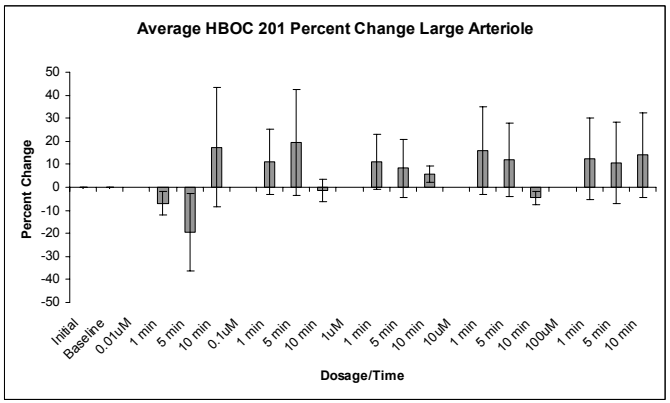
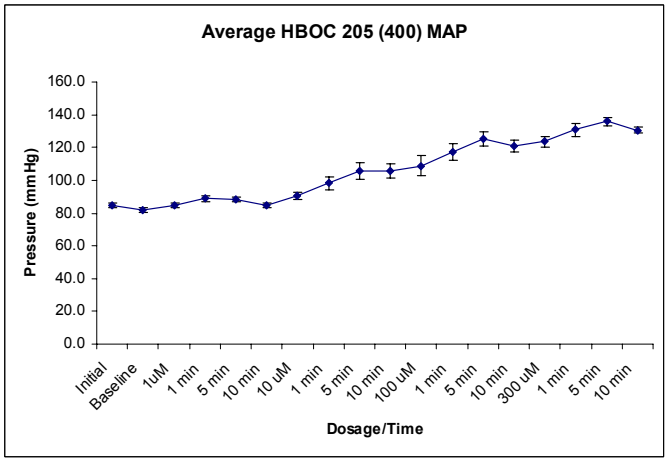
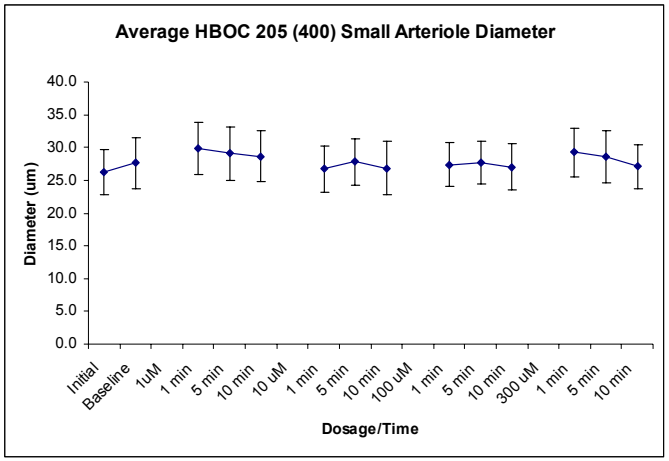
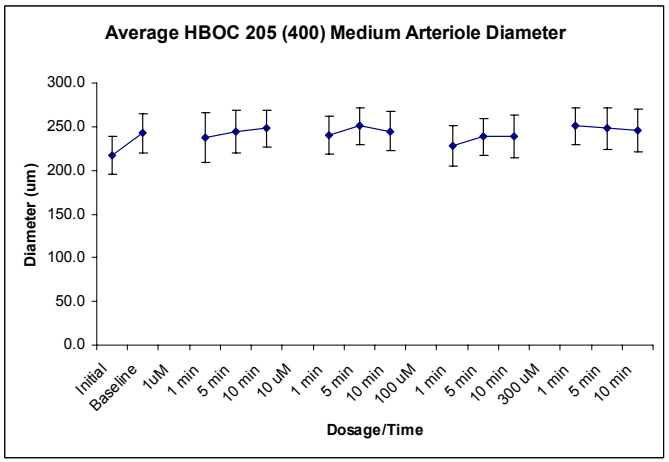

Average HBOC 201 Percent Change Large Arteriole
Percent Change
50
40
30
20
10
0
-10
-20
-30
-40
-50
Initial Baseline
0.01uM 1 min 5 min 10 min
0.1uM 1 min 5 min 10 min
1uM 1 min 5 min 10 min
10uM 1 min 5 min 10 min
100uM 1 min 5 min 10 min
Dosage/Time

Figure 5. HBOC-205 (MW 400) Dose Response Curve. The upper left graph represents the change in mean arterial pressure. The upper right graph represents the change in diameter of the small arteriole. The lower left graph represents the change in diameter of the medium arteriole. The lower right graph indicates the change in diameter of the large arteriole. The error bars represent standard error.


Average HBOC 205 (400) MAP
Pressure (mmHg)
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Initial Baseline
1uM
1 min
5 min
10 min
10 uM
1 min
5 min
10 min
100 uM
1 min
5 min
10 min
300 uM
1 min
5 min
10 min
Dosage/Time


Average HBOC 205 (400) Small Arteriole Diameter
Diameter (um)
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Initial Baseline
1uM
1 min
5 min
10 min
10 uM
1 min
5 min
10 min
100 uM
1 min
5 min
10 min
300 uM
1 min
5 min
10 min
Dosage/Time


Average HBOC 205 (400) Medium Arteriole Diameter
Diameter (um)
300.0
250.0
200.0
150.0
100.0
50.0
0.0
Initial Baseline
1uM
1 min
5 min
10 min
10 uM
1 min
5 min
10 min
100 uM
1 min
5 min
10 min
300 uM
1 min
5 min
10 min
Dosage/Time

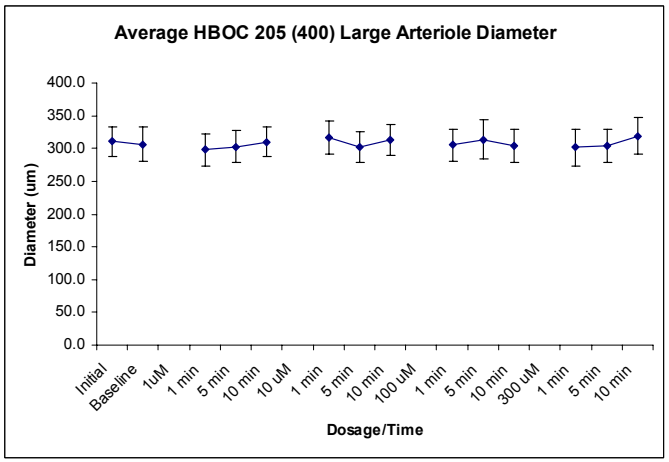
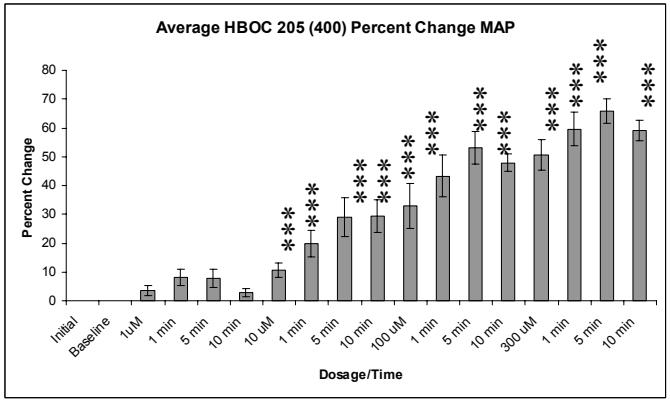
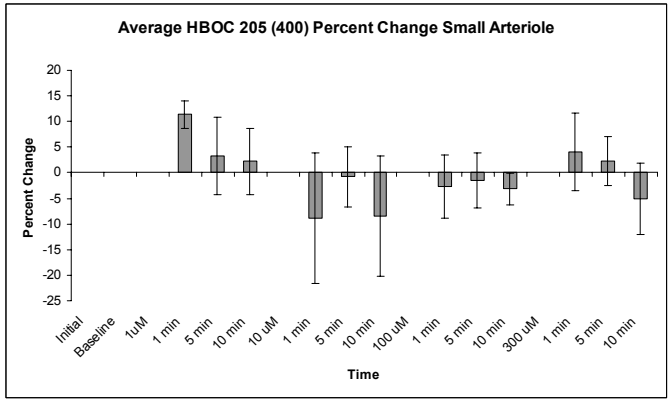
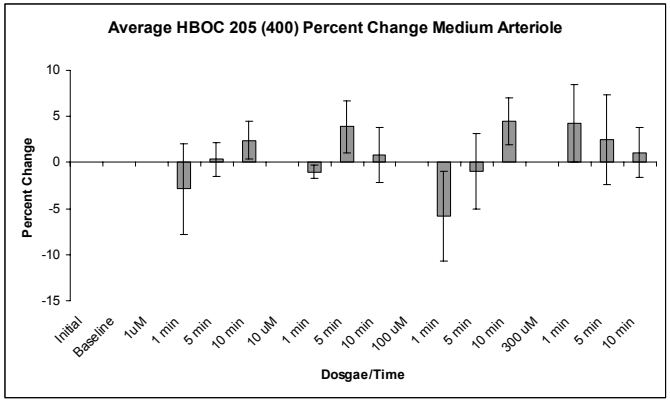

Average HBOC 205 (400) Large Arteriole Diameter
Diameter (um)
400.0
350.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
Initial Baseline
1uM
1 min
5 min
10 min
10 uM
1 min
5 min
10 min
100 uM
1 min
5 min
10 min
300 uM
1 min
5 min
10 min
Dosage/Time

Figure 6. HBOC-205 (MW 400) Percent Change. The upper left graph represents the percent change in mean arterial pressure. The upper right graph represents the percent change in diameter of the small arteriole. The lower left graph represents the percent change in diameter of the medium arteriole. The lower right graph indicates the percent change in diameter of the large arteriole. The error bars represent standard error. An (*) represents significance at $P < 0.05$. An (**) represents significance at $P < 0.01$. An (***) represents significance at $P < 0.001$.


Average HBOC 205 (400) Percent Change MAP
Percent Change
Dosage/Time
Initial
Baseline
1uM
1 min
5 min
10 min
10 uM
1 min
5 min
10 min
100 uM
1 min
5 min
10 min
300 uM
1 min
5 min
10 min


Average HBOC 205 (400) Percent Change Small Arteriole
Percent Change
Time
Initial
Baseline
1uM
1 min
5 min
10 min
10 uM
1 min
5 min
10 min
100 uM
1 min
5 min
10 min
300 uM
1 min
5 min
10 min


Average HBOC 205 (400) Percent Change Medium Arteriole
Percent Change
Dosgae/Time
Initial
Baseline
1uM
1 min
5 min
10 min
10 uM
1 min
5 min
10 min
100 uM
1 min
5 min
10 min
300 uM
1 min
5 min
10 min

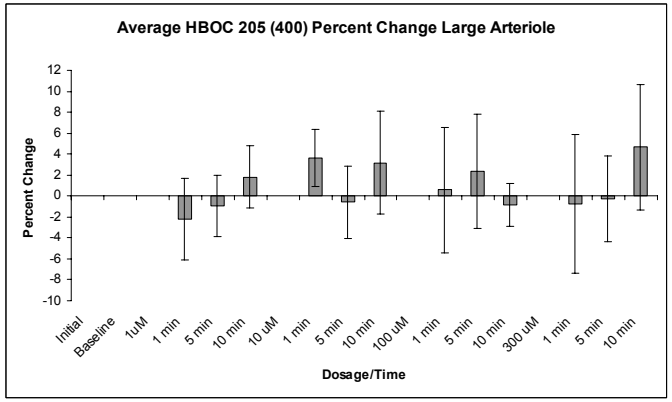
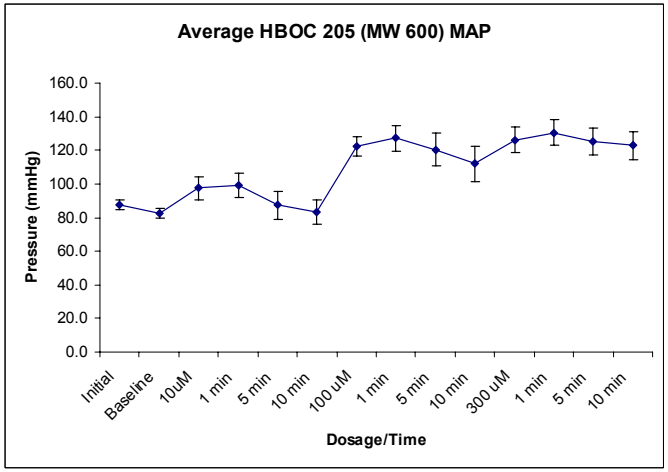
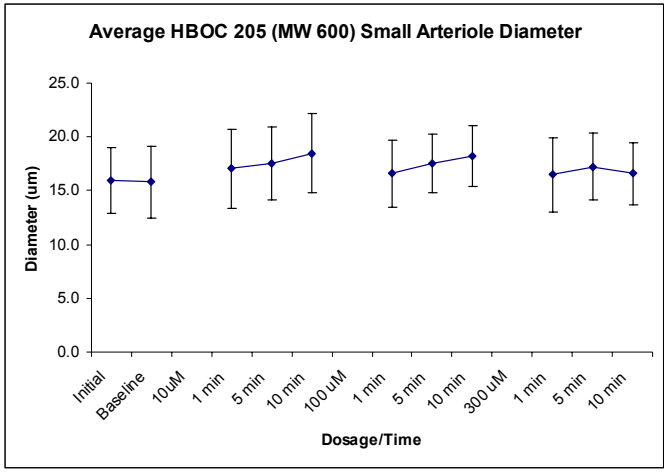
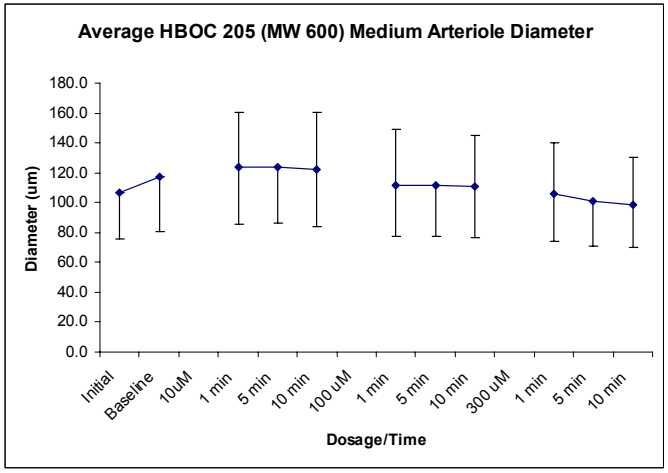

Average HBOC 205 (400) Percent Change Large Arteriole
Percent Change
Dosage/Time
Initial
Baseline
1uM
1 min
5 min
10 min
10 uM
1 min
5 min
10 min
100 uM
1 min
5 min
10 min
300 uM
1 min
5 min
10 min

Figure 7. HBOC-205 (MW 600) Dose Response Curve. The upper left graph represents the change in mean arterial pressure. The upper right graph represents the change in diameter of the small arteriole. The lower left graph represents the change in diameter of the medium arteriole. The lower right graph indicates the change in diameter of the large arteriole. The error bars represent standard error.


Average HBOC 205 (MW 600) MAP
Pressure (mmHg)
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Initial
Baseline
10uM
1 min
5 min
10 min
100 uM
1 min
5 min
10 min
300 uM
1 min
5 min
10 min
Dosage/Time


Average HBOC 205 (MW 600) Small Arteriole Diameter
Diameter (um)
25.0
20.0
15.0
10.0
5.0
0.0
Initial
Baseline
10uM
1 min
5 min
10 min
100 uM
1 min
5 min
10 min
300 uM
1 min
5 min
10 min
Dosage/Time


Average HBOC 205 (MW 600) Medium Arteriole Diameter
Diameter (um)
180.0
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Initial
Baseline
10uM
1 min
5 min
10 min
100 uM
1 min
5 min
10 min
300 uM
1 min
5 min
10 min
Dosage/Time

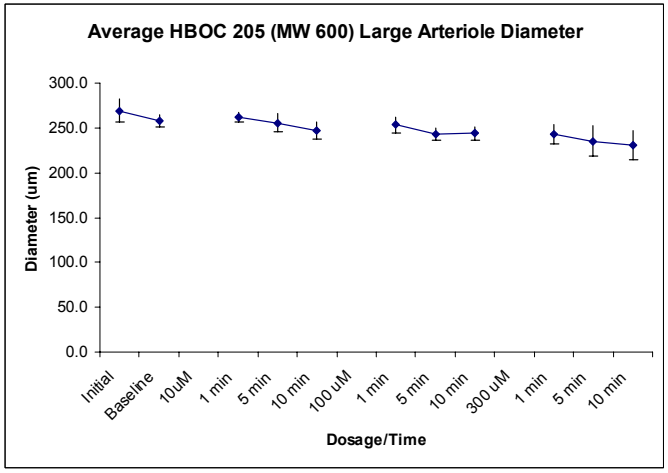
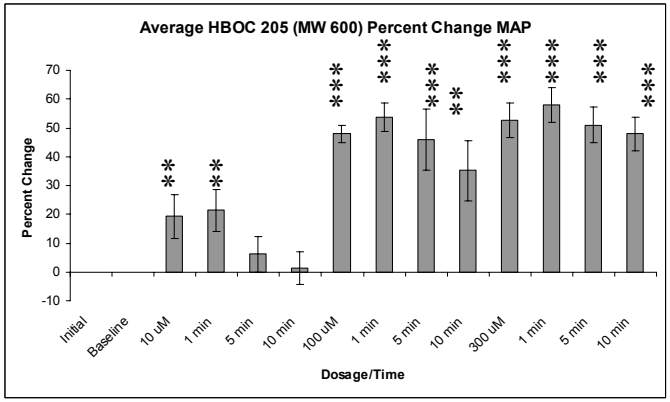
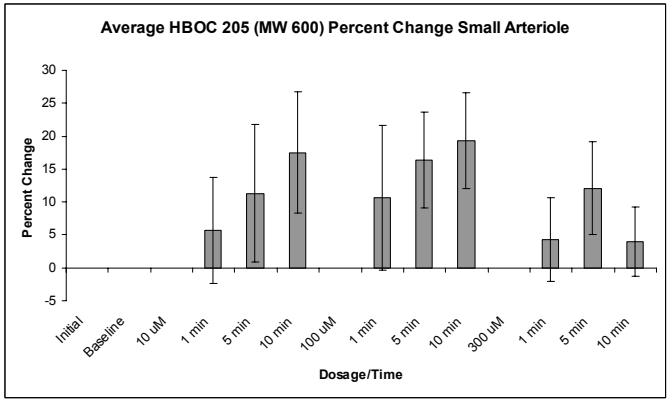
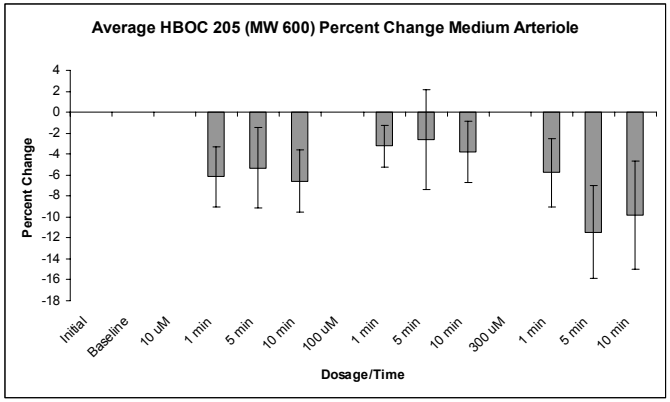

Average HBOC 205 (MW 600) Large Arteriole Diameter
Diameter (um)
300.0
250.0
200.0
150.0
100.0
50.0
0.0
Initial
Baseline
10uM
1 min
5 min
10 min
100 uM
1 min
5 min
10 min
300 uM
1 min
5 min
10 min
Dosage/Time

Figure 8. HBOC-205 (MW 600) Percent Change. The upper left graph represents the percent change in mean arterial pressure. The upper right graph represents the percent change in diameter of the small arteriole. The lower left graph represents the percent change in diameter of the medium arteriole. The lower right graph indicates the percent change in diameter of the large arteriole. The error bars represent standard error. An (*) represents significance at $P < 0.05$. An (**) represents significance at $P < 0.01$. An (***) represents significance at $P < 0.001$.


Average HBOC 205 (MW 600) Percent Change MAP
Percent Change
70
60
50
40
30
20
10
0
-10
**
**
**
***
***
***
**
***
***
***
Initial
Baseline
10 uM
1 min
5 min
10 min
100 uM
1 min
5 min
10 min
300 uM
1 min
5 min
10 min
Dosage/Time


Average HBOC 205 (MW 600) Percent Change Small Arteriole
Percent Change
30
25
20
15
10
5
0
-5
Initial
Baseline
10 uM
1 min
5 min
10 min
100 uM
1 min
5 min
10 min
300 uM
1 min
5 min
10 min
Dosage/Time


Average HBOC 205 (MW 600) Percent Change Medium Arteriole
Percent Change
4
2
0
-2
-4
-6
-8
-10
-12
-14
-16
-18
Initial
Baseline
10 uM
1 min
5 min
10 min
100 uM
1 min
5 min
10 min
300 uM
1 min
5 min
10 min
Dosage/Time

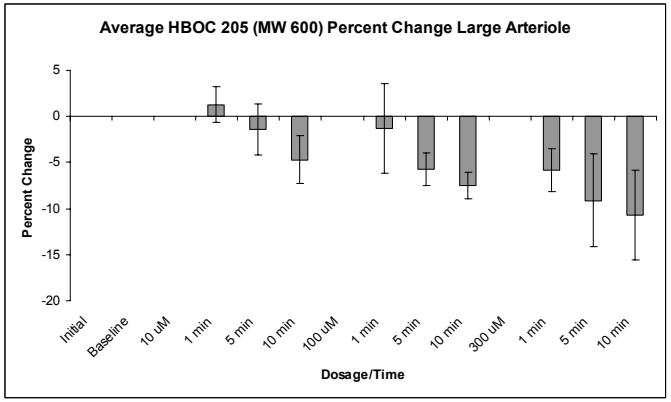
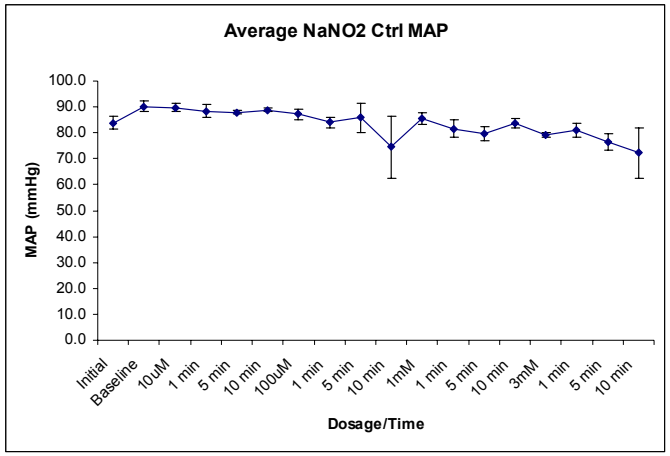
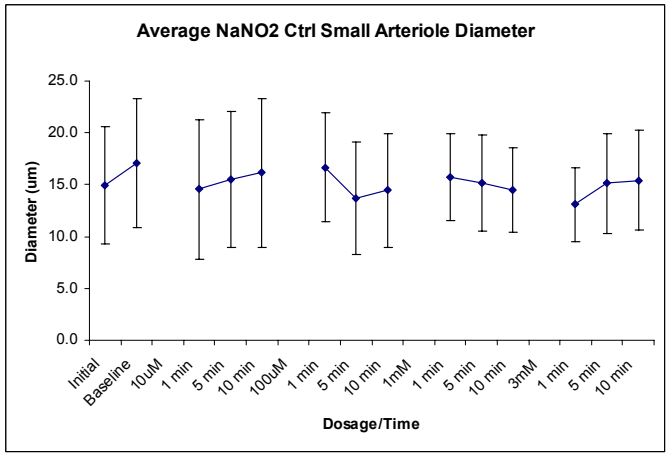
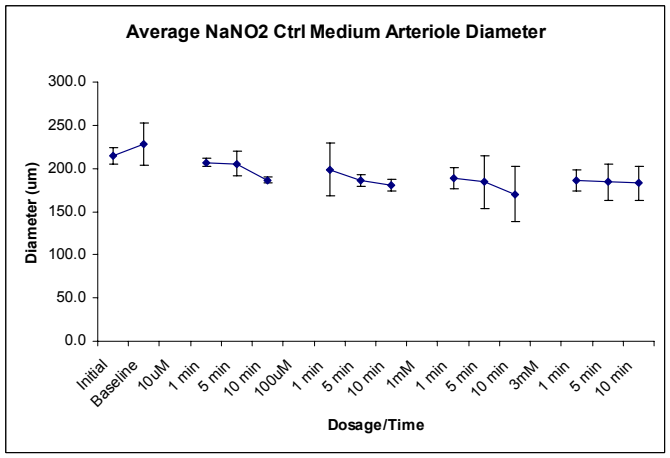

Average HBOC 205 (MW 600) Percent Change Large Arteriole
Percent Change
5
0
-5
-10
-15
-20
Initial
Baseline
10 uM
1 min
5 min
10 min
100 uM
1 min
5 min
10 min
300 uM
1 min
5 min
10 min
Dosage/Time

Figure 9. $NaNO_2$ Control Dose Response Curve. The upper left graph represents the change in mean arterial pressure. The upper right graph represents the change in diameter of the small arteriole. The lower left graph represents the change in diameter of the medium arteriole. The lower right graph indicates the change in diameter of the large arteriole. The error bars represent standard error.


Average NaNO2 Ctrl MAP
MAP (mmHg)
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Initial
Baseline
10uM
1 min
5 min
10 min
100uM
1 min
5 min
10 min
1mM
1 min
5 min
10 min
3mM
1 min
5 min
10 min
Dosage/Time


Average NaNO2 Ctrl Small Arteriole Diameter
Diameter (um)
25.0
20.0
15.0
10.0
5.0
0.0
Initial
Baseline
10uM
1 min
5 min
10 min
100uM
1 min
5 min
10 min
1mM
1 min
5 min
10 min
3mM
1 min
5 min
10 min
Dosage/Time


Average NaNO2 Ctrl Medium Arteriole Diameter
Diameter (um)
300.0
250.0
200.0
150.0
100.0
50.0
0.0
Initial
Baseline
10uM
1 min
5 min
10 min
100uM
1 min
5 min
10 min
1mM
1 min
5 min
10 min
3mM
1 min
5 min
10 min
Dosage/Time

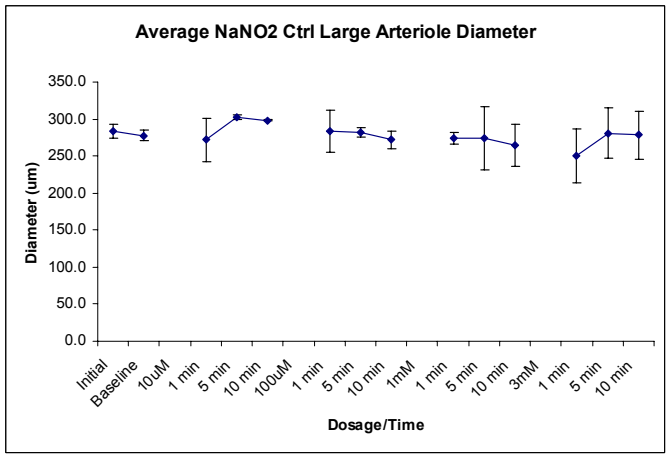
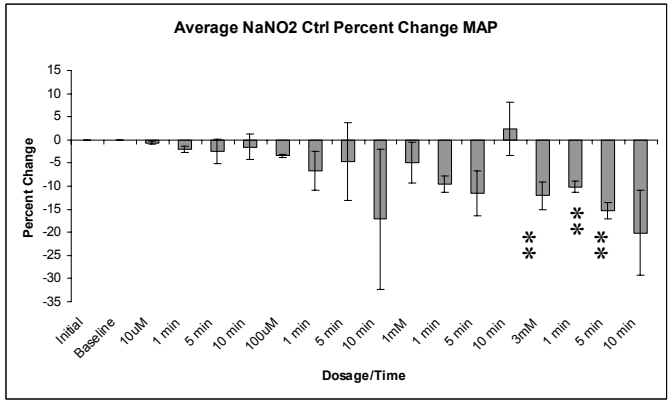
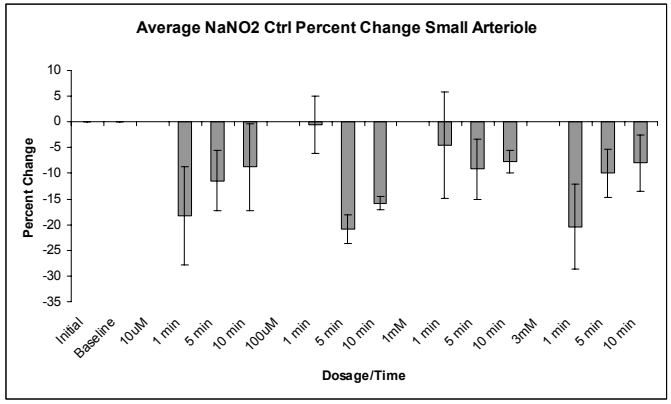
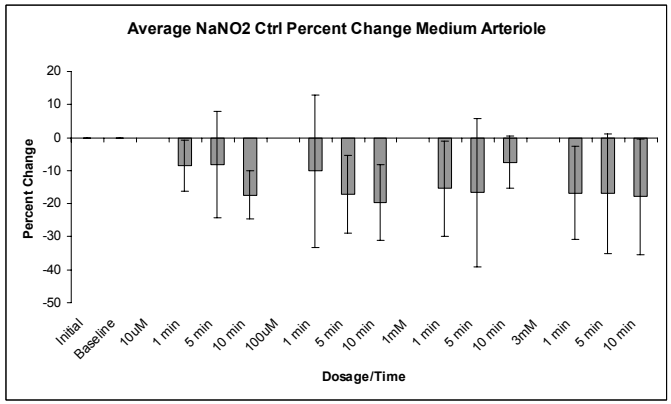

Average NaNO2 Ctrl Large Arteriole Diameter
Diameter (um)
350.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
Initial
Baseline
10uM
1 min
5 min
10 min
100uM
1 min
5 min
10 min
1mM
1 min
5 min
10 min
3mM
1 min
5 min
10 min
Dosage/Time

Figure 10. $NaNO_2$ Control Percent Change. The upper left graph represents the percent change in mean arterial pressure. The upper right graph represents the percent change in diameter of the small arteriole. The lower left graph represents the percent change in diameter of the medium arteriole. The lower right graph indicates the percent change in diameter of the large arteriole. The error bars represent standard error. An (*) represents significance at $P < 0.05$. An (**) represents significance at $P < 0.01$. An (***) represents significance at $P < 0.001$.


Average NaNO2 Ctrl Percent Change MAP
Percent Change
15
10
5
0
-5
-10
-15
-20
-25
-30
-35
**
**
**
Initial
Baseline
10uM
1 min
5 min
10 min
100uM
1 min
5 min
10 min
1mM
1 min
5 min
10 min
3mM
1 min
5 min
10 min
Dosage/Time


Average NaNO2 Ctrl Percent Change Small Arteriole
Percent Change
10
5
0
-5
-10
-15
-20
-25
-30
-35
Initial
Baseline
10uM
1 min
5 min
10 min
100uM
1 min
5 min
10 min
1mM
1 min
5 min
10 min
3mM
1 min
5 min
10 min
Dosage/Time


Average NaNO2 Ctrl Percent Change Medium Arteriole
Percent Change
20
10
0
-10
-20
-30
-40
-50
Initial
Baseline
10uM
1 min
5 min
10 min
100uM
1 min
5 min
10 min
1mM
1 min
5 min
10 min
3mM
1 min
5 min
10 min
Dosage/Time

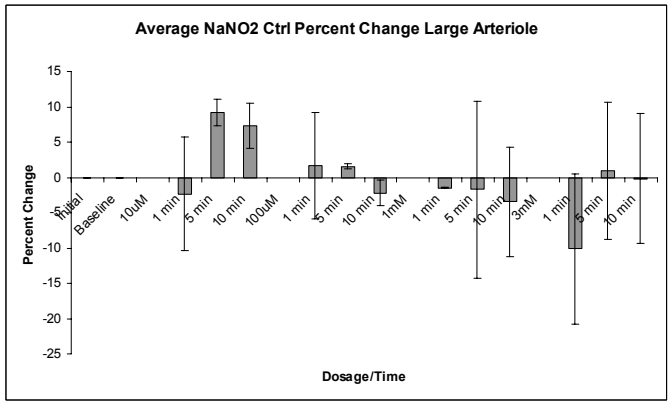
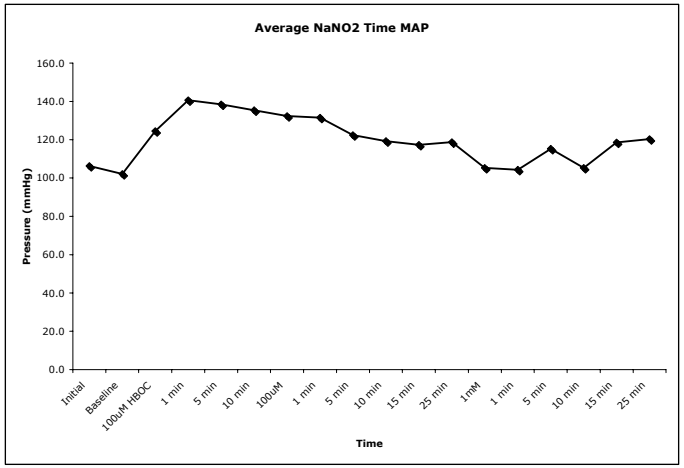
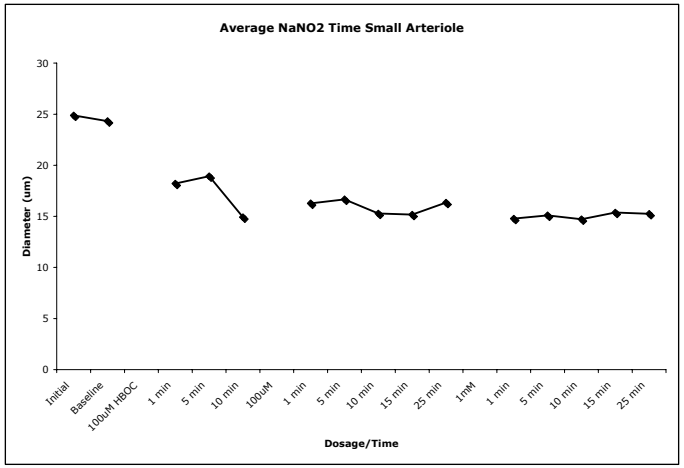
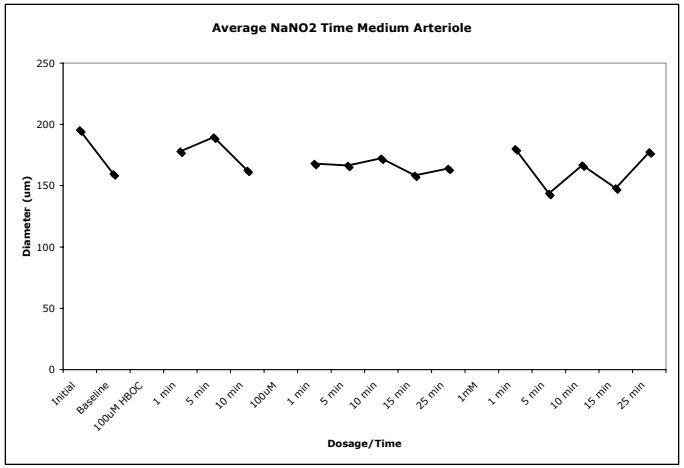

Average NaNO2 Ctrl Percent Change Large Arteriole
Percent Change
15
10
5
0
-5
-10
-15
-20
-25
Initial
Baseline
10uM
1 min
5 min
10 min
100uM
1 min
5 min
10 min
1mM
1 min
5 min
10 min
3mM
1 min
5 min
10 min
Dosage/Time

Figure 11. $NaNO_2$ Time Control Dose Response Curve. The upper left graph represents the change in mean arterial pressure. The upper right graph represents the change in diameter of the small arteriole. The lower left graph represents the change in diameter of the medium arteriole. The lower right graph indicates the change in diameter of the large arteriole. The error bars represent standard error.


Average NaNO2 Time MAP
Pressure (mmHg)
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Initial
Baseline
100uM HBOC
1 min
5 min
10 min
100uM
1 min
5 min
10 min
15 min
25 min
1mM
1 min
5 min
10 min
15 min
25 min
Time


Average NaNO2 Time Small Arteriole
Diameter (um)
30
25
20
15
10
5
0
Initial
Baseline
100uM HBOC
1 min
5 min
10 min
100uM
1 min
5 min
10 min
15 min
25 min
1mM
1 min
5 min
10 min
15 min
25 min
Dosage/Time


Average NaNO2 Time Medium Arteriole
Diameter (um)
250
200
150
100
50
0
Initial
Baseline
100uM HBOC
1 min
5 min
10 min
100uM
1 min
5 min
10 min
15 min
25 min
1mM
1 min
5 min
10 min
15 min
25 min
Dosage/Time

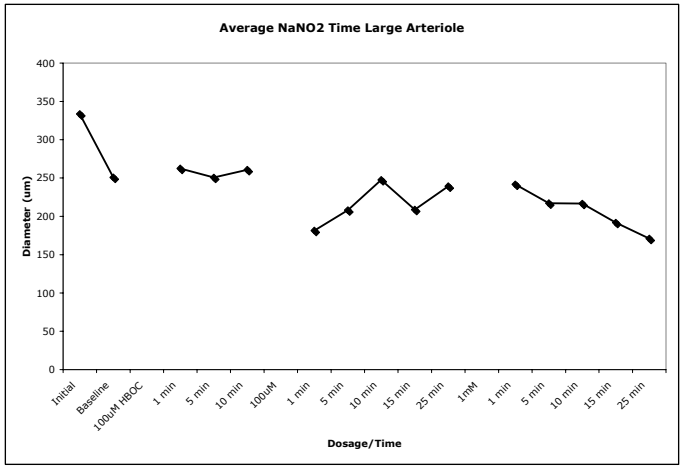
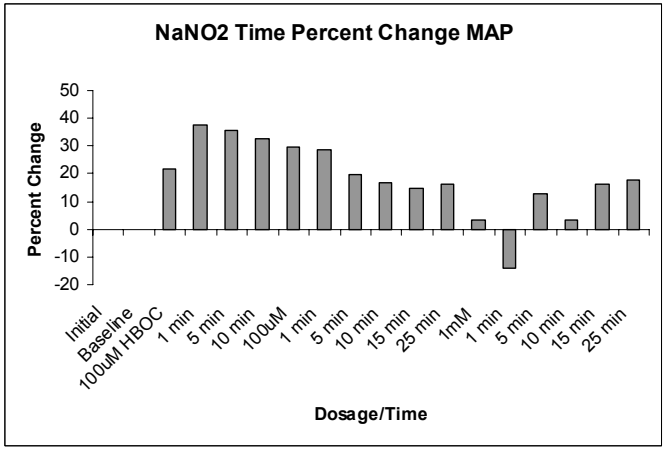
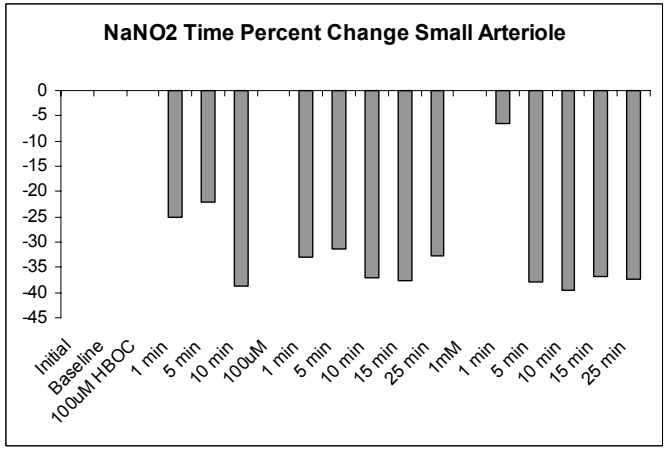
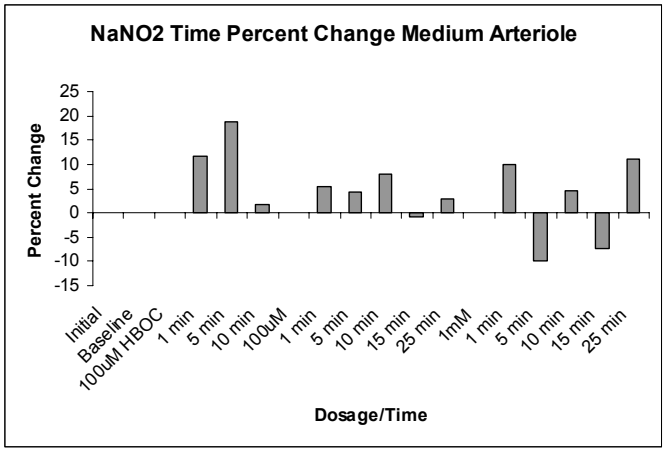

Average NaNO2 Time Large Arteriole
Diameter (um)
400
350
300
250
200
150
100
50
0
Initial
Baseline
100uM HBOC
1 min
5 min
10 min
100uM
1 min
5 min
10 min
15 min
25 min
1mM
1 min
5 min
10 min
15 min
25 min
Dosage/Time

Figure 12. $NaNO_2$ Time Control Percent Change. The upper left graph represents the percent change in mean arterial pressure. The upper right graph represents the percent change in diameter of the small arteriole. The lower left graph represents the percent change in diameter of the medium arteriole. The lower right graph indicates the percent change in diameter of the large arteriole. The error bars represent standard error. An (*) represents significance at $P < 0.05$. An (**) represents significance at $P < 0.01$. An (***) represents significance at $P < 0.001$.


NaNO2 Time Percent Change MAP
Percent Change
50
40
30
20
10
0
-10
-20
Initial Baseline
100uM HBOC
1 min
5 min
10 min
100uM
1 min
5 min
10 min
15 min
25 min
1mM
1 min
5 min
10 min
15 min
25 min
Dosage/Time


NaNO2 Time Percent Change Small Arteriole
0
-5
-10
-15
-20
-25
-30
-35
-40
-45
Initial Baseline
100uM HBOC
1 min
5 min
10 min
100uM
1 min
5 min
10 min
15 min
25 min
1mM
1 min
5 min
10 min
15 min
25 min


NaNO2 Time Percent Change Medium Arteriole
Percent Change
25
20
15
10
5
0
-5
-10
-15
Initial Baseline
100uM HBOC
1 min
5 min
10 min
100uM
1 min
5 min
10 min
15 min
25 min
1mM
1 min
5 min
10 min
15 min
25 min
Dosage/Time

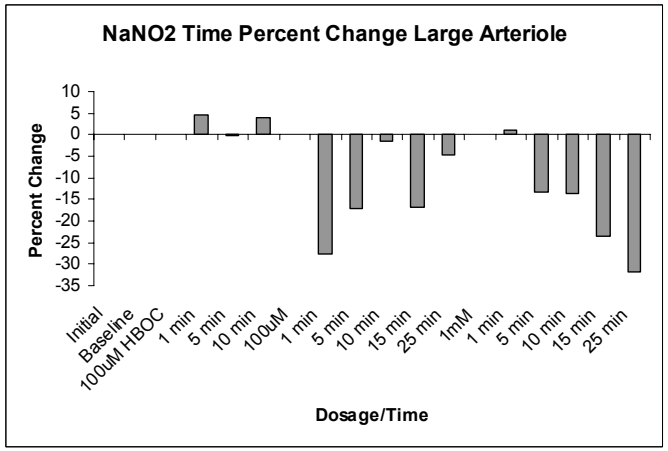
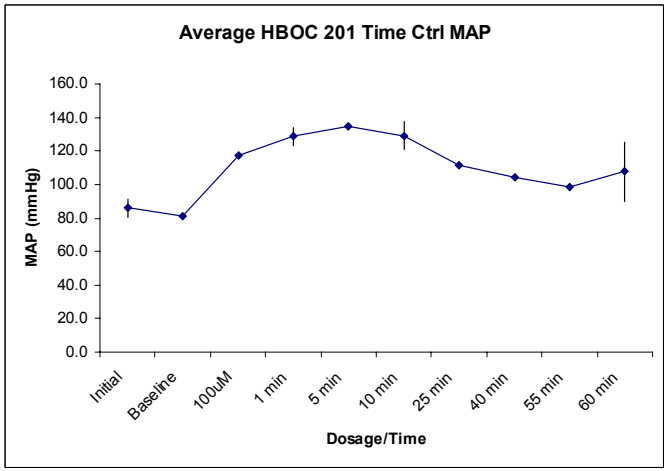
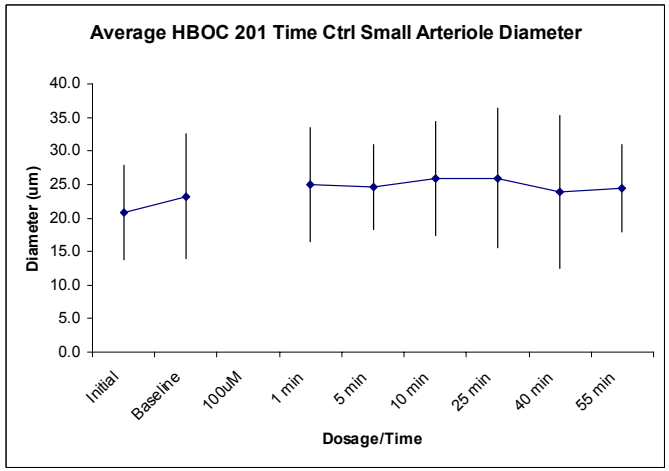
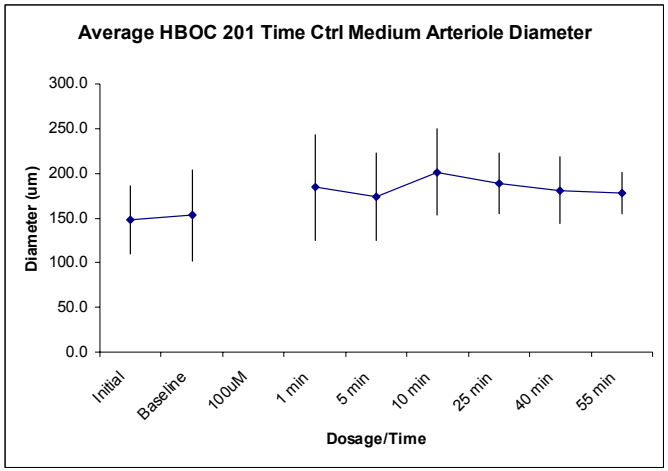

NaNO2 Time Percent Change Large Arteriole
Percent Change
10
5
0
-5
-10
-15
-20
-25
-30
-35
Initial Baseline
100uM HBOC
1 min
5 min
10 min
100uM
1 min
5 min
10 min
15 min
25 min
1mM
1 min
5 min
10 min
15 min
25 min
Dosage/Time

Figure 13. HBOC-201 Time Control Dose Response Curve. The upper left graph represents the change in mean arterial pressure. The upper right graph represents the change in diameter of the small arteriole. The lower left graph represents the change in diameter of the medium arteriole. The lower right graph indicates the change in diameter of the large arteriole. The error bars represent standard error.


Average HBOC 201 Time Ctrl MAP
MAP (mmHg)
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Initial
Baseline
100uM
1 min
5 min
10 min
25 min
40 min
55 min
60 min
Dosage/Time


Average HBOC 201 Time Ctrl Small Arteriole Diameter
Diameter (um)
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Initial
Baseline
100uM
1 min
5 min
10 min
25 min
40 min
55 min
Dosage/Time


Average HBOC 201 Time Ctrl Medium Arteriole Diameter
Diameter (um)
300.0
250.0
200.0
150.0
100.0
50.0
0.0
Initial
Baseline
100uM
1 min
5 min
10 min
25 min
40 min
55 min
Dosage/Time

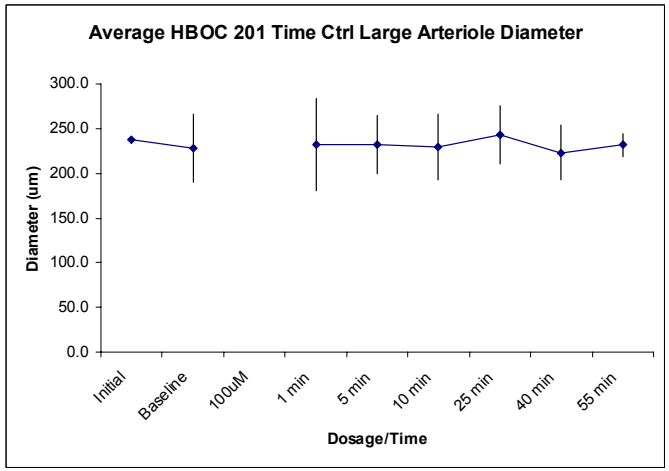
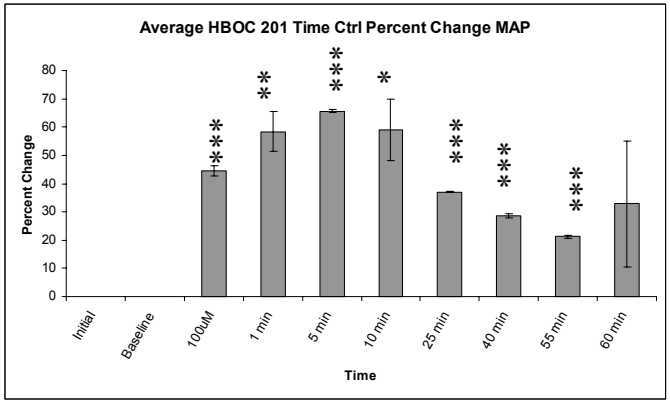
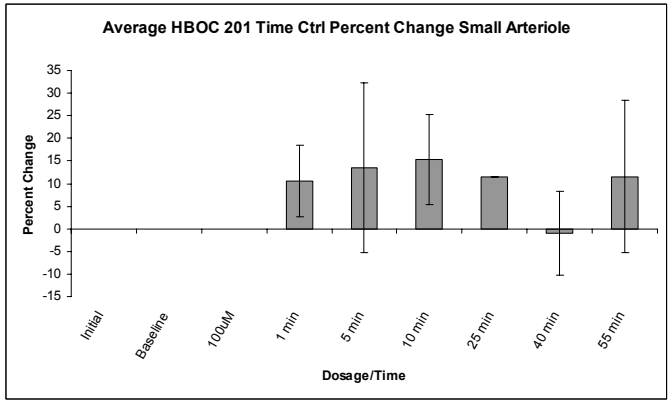
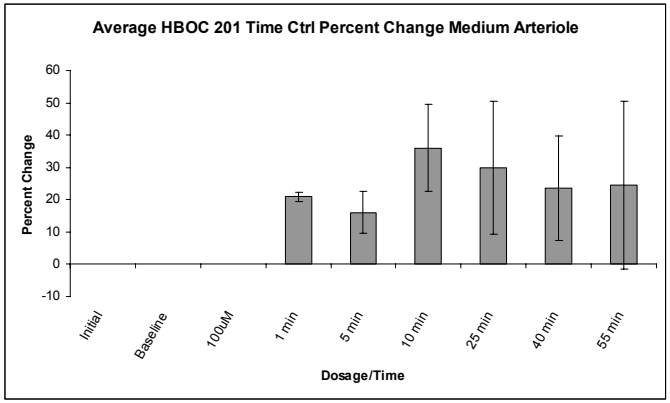

Average HBOC 201 Time Ctrl Large Arteriole Diameter
Diameter (um)
300.0
250.0
200.0
150.0
100.0
50.0
0.0
Initial
Baseline
100uM
1 min
5 min
10 min
25 min
40 min
55 min
Dosage/Time

Figure 14. HBOC-201 Time Control Percent Change. The upper left graph represents the percent change in mean arterial pressure. The upper right graph represents the percent change in diameter of the small arteriole. The lower left graph represents the percent change in diameter of the medium arteriole. The lower right graph indicates the percent change in diameter of the large arteriole. The error bars represent standard error. An (*) represents significance at $P < 0.05$. An (**) represents significance at $P < 0.01$. An (***) represents significance at $P < 0.001$.


Average HBOC 201 Time Ctrl Percent Change MAP
Percent Change
80
70
60
50
40
30
20
10
0
Initial
Baseline
100uM
1 min
5 min
10 min
25 min
40 min
55 min
60 min
Time


Average HBOC 201 Time Ctrl Percent Change Small Arteriole
Percent Change
35
30
25
20
15
10
5
0
-5
-10
-15
Initial
Baseline
100uM
1 min
5 min
10 min
25 min
40 min
55 min
Dosage/Time


Average HBOC 201 Time Ctrl Percent Change Medium Arteriole
Percent Change
60
50
40
30
20
10
0
-10
Initial
Baseline
100uM
1 min
5 min
10 min
25 min
40 min
55 min
Dosage/Time

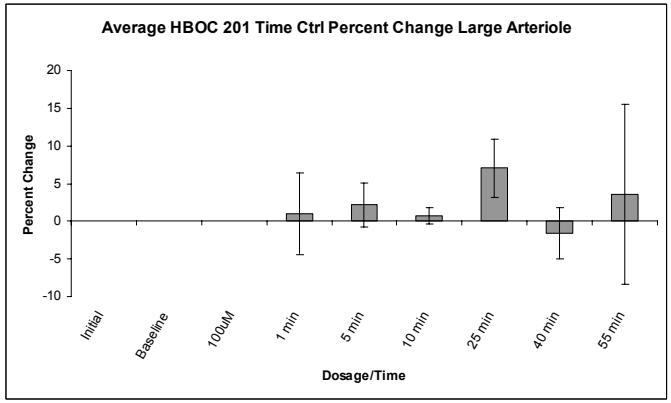
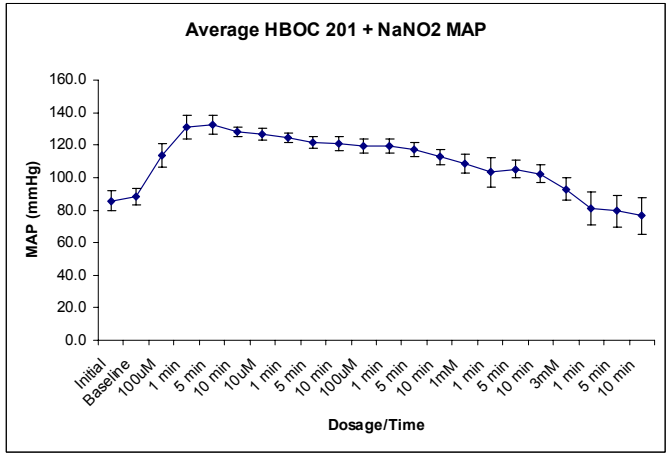
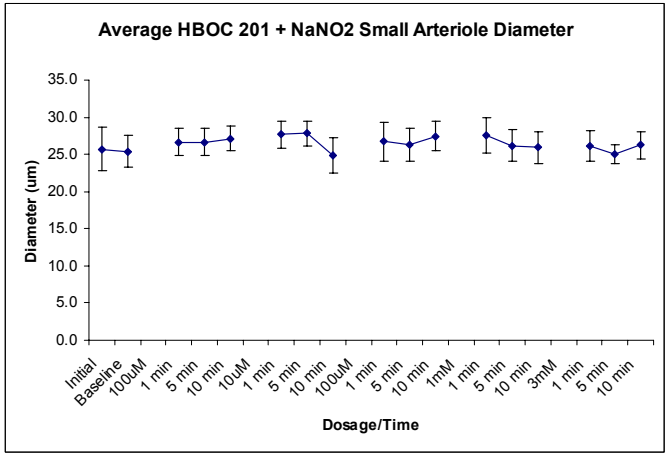
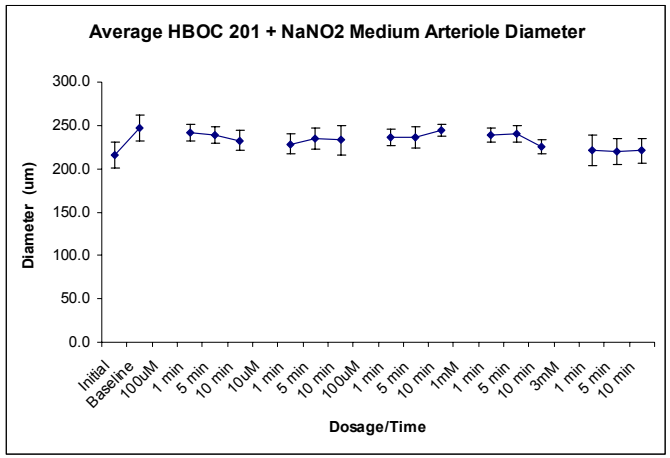

Average HBOC 201 Time Ctrl Percent Change Large Arteriole
Percent Change
20
15
10
5
0
-5
-10
Initial
Baseline
100uM
1 min
5 min
10 min
25 min
40 min
55 min
Dosage/Time

Figure 15. HBOC-201 + $NaNO_2$ Dose Response Curve. The upper left graph represents the change in mean arterial pressure. The upper right graph represents the change in diameter of the small arteriole. The lower left graph represents the change in diameter of the medium arteriole. The lower right graph indicates the change in diameter of the large arteriole. The error bars represent standard error.


Average HBOC 201 + NaNO2 MAP
MAP (mmHg)
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Initial Baseline
100uM
1 min
5 min
10 min
10uM
1 min
5 min
10 min
100uM
1 min
5 min
10 min
1mM
1 min
5 min
10 min
3mM
1 min
5 min
10 min
Dosage/Time


Average HBOC 201 + NaNO2 Small Arteriole Diameter
Diameter (um)
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Initial Baseline
100uM
1 min
5 min
10 min
10uM
1 min
5 min
10 min
100uM
1 min
5 min
10 min
1mM
1 min
5 min
10 min
3mM
1 min
5 min
10 min
Dosage/Time


Average HBOC 201 + NaNO2 Medium Arteriole Diameter
Diameter (um)
300.0
250.0
200.0
150.0
100.0
50.0
0.0
Initial Baseline
100uM
1 min
5 min
10 min
10uM
1 min
5 min
10 min
100uM
1 min
5 min
10 min
1mM
1 min
5 min
10 min
3mM
1 min
5 min
10 min
Dosage/Time

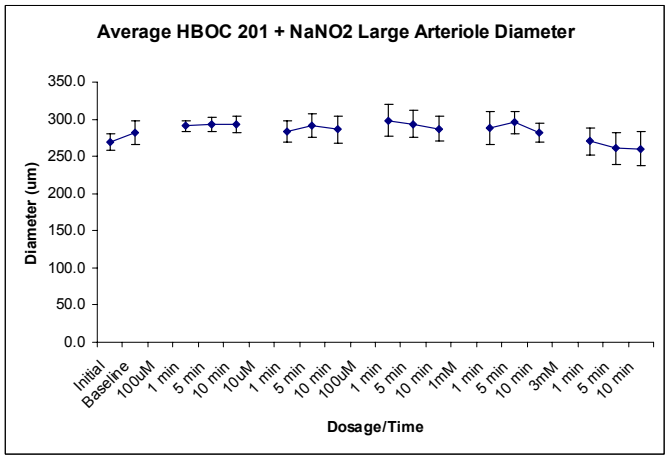
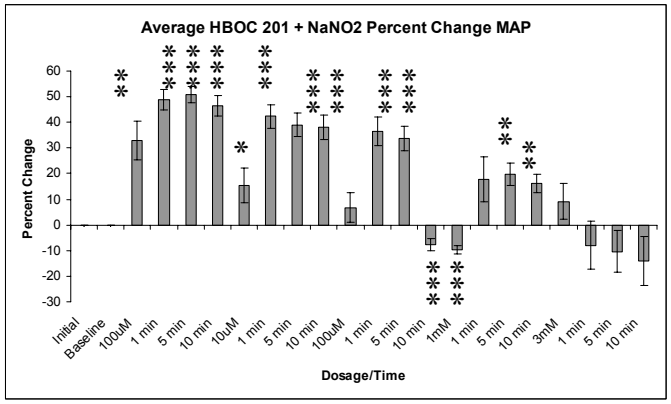
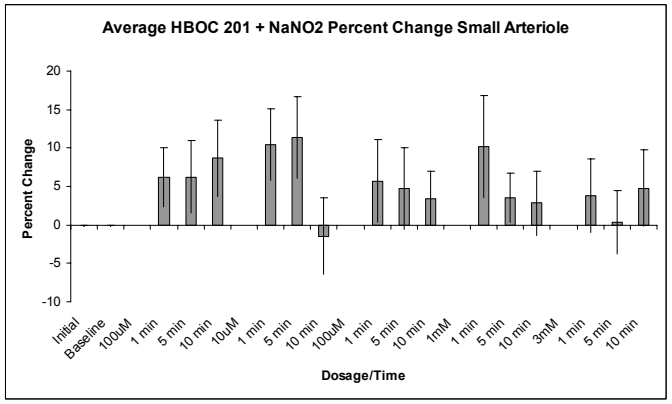
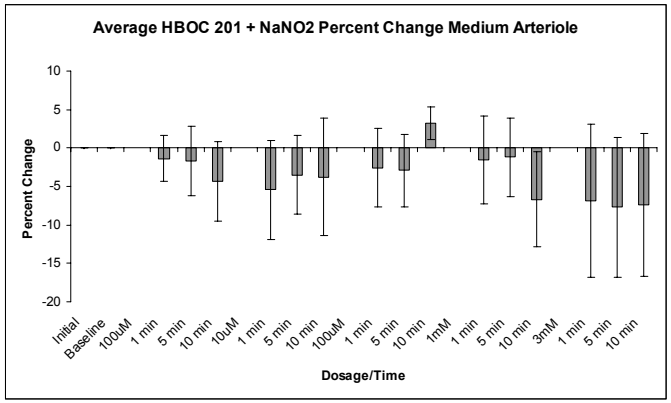

Average HBOC 201 + NaNO2 Large Arteriole Diameter
Diameter (um)
350.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
Initial Baseline
100uM
1 min
5 min
10 min
10uM
1 min
5 min
10 min
100uM
1 min
5 min
10 min
1mM
1 min
5 min
10 min
3mM
1 min
5 min
10 min
Dosage/Time

Figure 16. HBOC-201 + $NaNO_2$ Percent Change. The upper left graph represents the percent change in mean arterial pressure. The upper right graph represents the percent change in diameter of the small arteriole. The lower left graph represents the percent change in diameter of the medium arteriole. The lower right graph indicates the percent change in diameter of the large arteriole. The error bars represent standard error. An (*) represents significance at $P < 0.05$. An (**) represents significance at $P < 0.01$. An (***) represents significance at $P < 0.001$.


Average HBOC 201 + NaNO2 Percent Change MAP
Percent Change
60
50
40
30
20
10
0
-10
-20
-30
Initial Baseline 100uM 1 min 5 min 10 min 10uM 1 min 5 min 10 min 100uM 1 min 5 min 10 min 1mM 1 min 5 min 10 min 3mM 1 min 5 min 10 min
Dosage/Time


Average HBOC 201 + NaNO2 Percent Change Small Arteriole
Percent Change
20
15
10
5
0
-5
-10
Initial Baseline 100uM 1 min 5 min 10 min 10uM 1 min 5 min 10 min 100uM 1 min 5 min 10 min 1mM 1 min 5 min 10 min 3mM 1 min 5 min 10 min
Dosage/Time


Average HBOC 201 + NaNO2 Percent Change Medium Arteriole
Percent Change
10
5
0
-5
-10
-15
-20
Initial Baseline 100uM 1 min 5 min 10 min 10uM 1 min 5 min 10 min 100uM 1 min 5 min 10 min 1mM 1 min 5 min 10 min 3mM 1 min 5 min 10 min
Dosage/Time

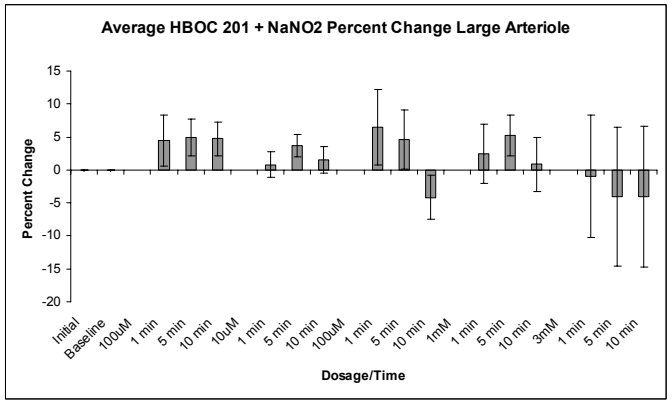
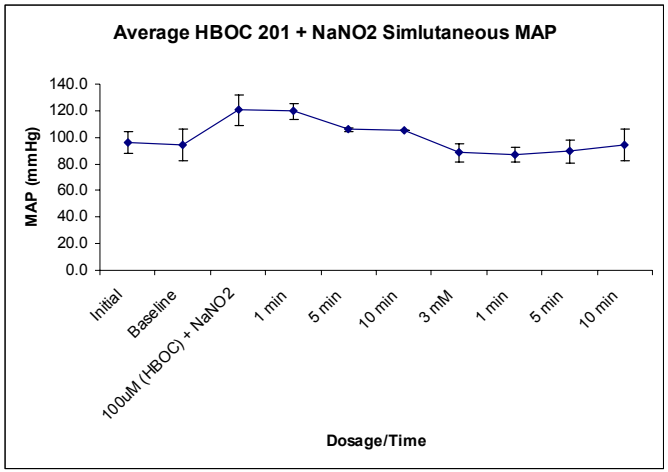
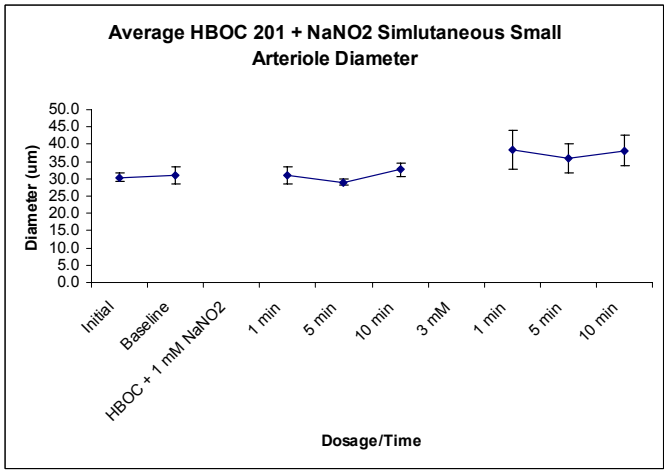
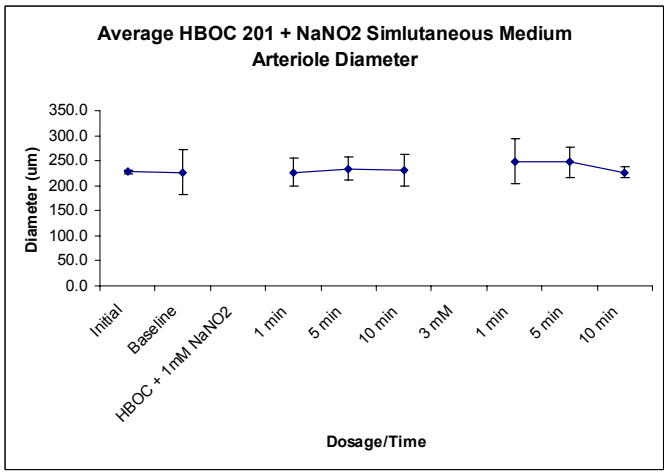

Average HBOC 201 + NaNO2 Percent Change Large Arteriole
Percent Change
15
10
5
0
-5
-10
-15
-20
Initial Baseline 100uM 1 min 5 min 10 min 10uM 1 min 5 min 10 min 100uM 1 min 5 min 10 min 1mM 1 min 5 min 10 min 3mM 1 min 5 min 10 min
Dosage/Time

Figure 17. HBOC-201 + $NaNO_2$ Simultaneous Dose Response Curve. The upper left graph represents the change in mean arterial pressure. The upper right graph represents the change in diameter of the small arteriole. The lower left graph represents the change in diameter of the medium arteriole. The lower right graph indicates the change in diameter of the large arteriole. The error bars represent standard error.


Average HBOC 201 + NaNO2 Simlutaneous MAP
MAP (mmHg)
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Initial
Baseline
100uM (HBOC) + NaNO2
1 min
5 min
10 min
3 mM
1 min
5 min
10 min
Dosage/Time


Average HBOC 201 + NaNO2 Simlutaneous Small Arteriole Diameter
Diameter (um)
50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Initial
Baseline
HBOC + 1 mM NaNO2
1 min
5 min
10 min
3 mM
1 min
5 min
10 min
Dosage/Time


Average HBOC 201 + NaNO2 Simlutaneous Medium Arteriole Diameter
Diameter (um)
350.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
Initial
Baseline
HBOC + 1mM NaNO2
1 min
5 min
10 min
3 mM
1 min
5 min
10 min
Dosage/Time

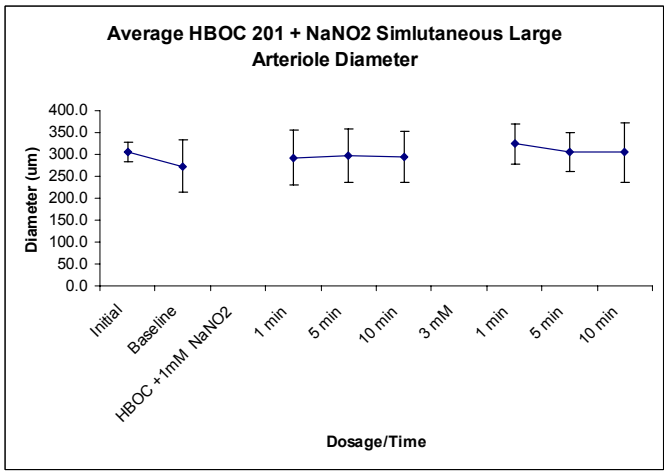
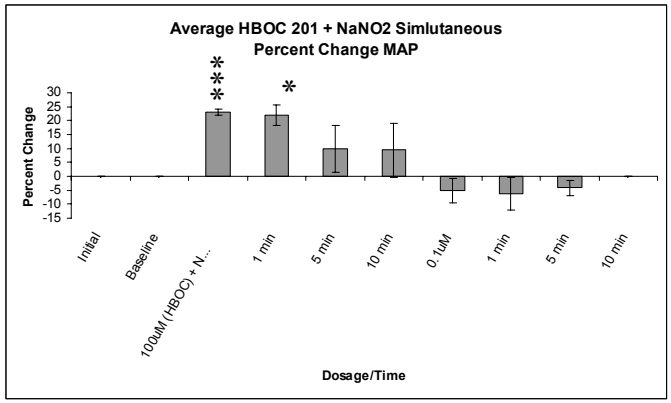
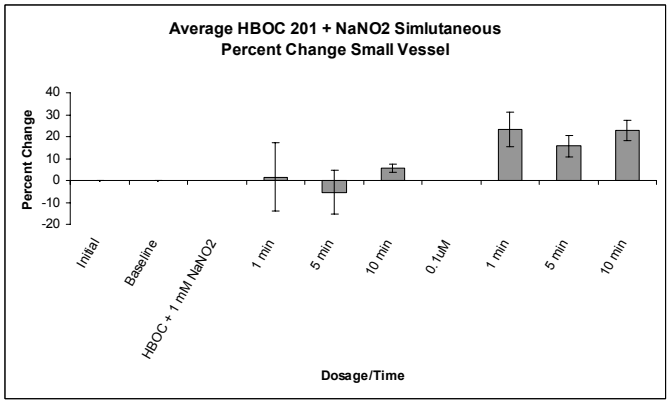
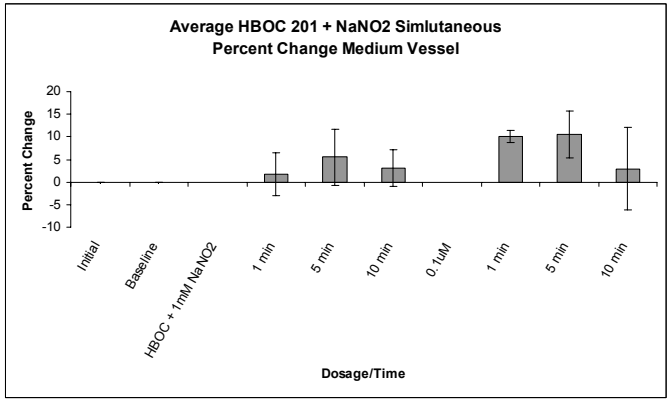

Average HBOC 201 + NaNO2 Simlutaneous Large Arteriole Diameter
Diameter (um)
400.0
350.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
Initial
Baseline
HBOC +1mM NaNO2
1 min
5 min
10 min
3 mM
1 min
5 min
10 min
Dosage/Time

Figure 18. HBOC-201 + $NaNO_2$ Simultaneous Percent Change. The upper left graph represents the percent change in mean arterial pressure. The upper right graph represents the percent change in diameter of the small arteriole. The lower left graph represents the percent change in diameter of the medium arteriole. The lower right graph indicates the percent change in diameter of the large arteriole. The error bars represent standard error. An (*) represents significance at $P < 0.05$. An (**) represents significance at $P < 0.01$. An (***) represents significance at $P < 0.001$.


Average HBOC 201 + NaNO2 Simlutaneous
Percent Change MAP
***
*
Percent Change
30
25
20
15
10
5
0
-5
-10
-15
Initial
Baseline
100uM (HBOC) + N...
1 min
5 min
10 min
0.1uM
1 min
5 min
10 min
Dosage/Time


Average HBOC 201 + NaNO2 Simlutaneous
Percent Change Small Vessel
Percent Change
40
30
20
10
0
-10
-20
Initial
Baseline
HBOC + 1 mM NaNO2
1 min
5 min
10 min
0.1uM
1 min
5 min
10 min
Dosage/Time


Average HBOC 201 + NaNO2 Simlutaneous
Percent Change Medium Vessel
Percent Change
20
15
10
5
0
-5
-10
Initial
Baseline
HBOC + 1mM NaNO2
1 min
5 min
10 min
0.1uM
1 min
5 min
10 min
Dosage/Time

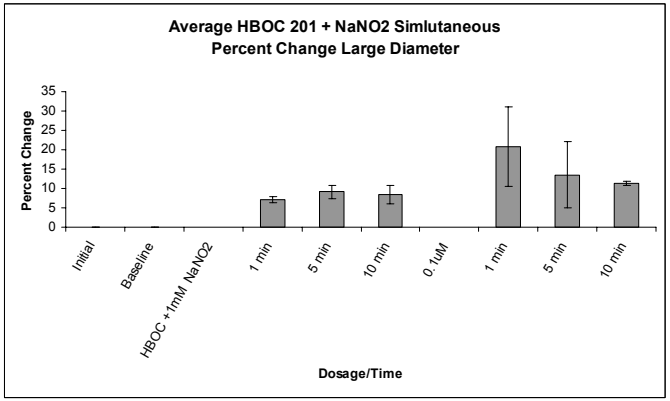
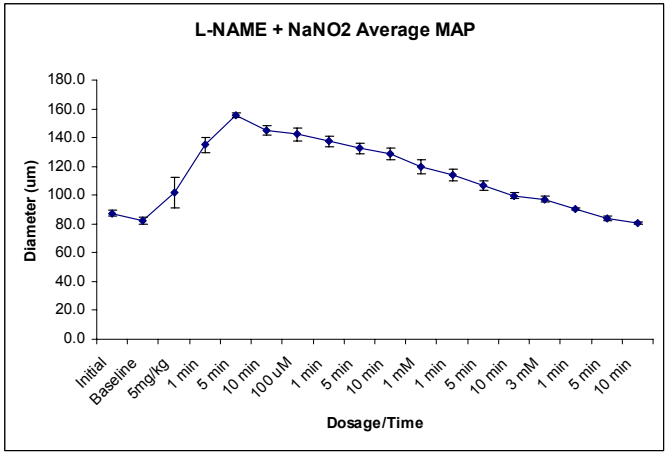
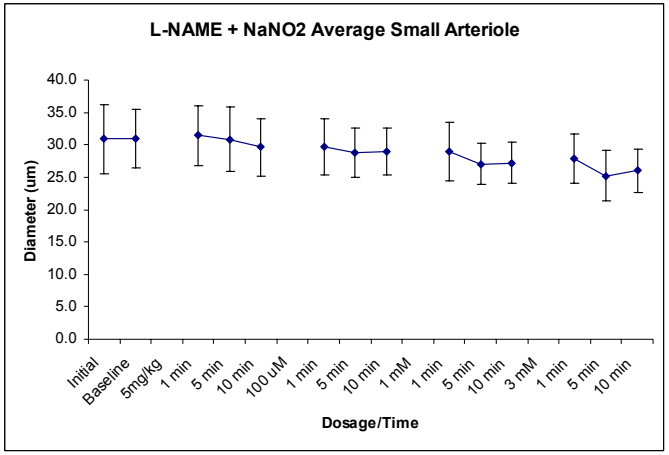
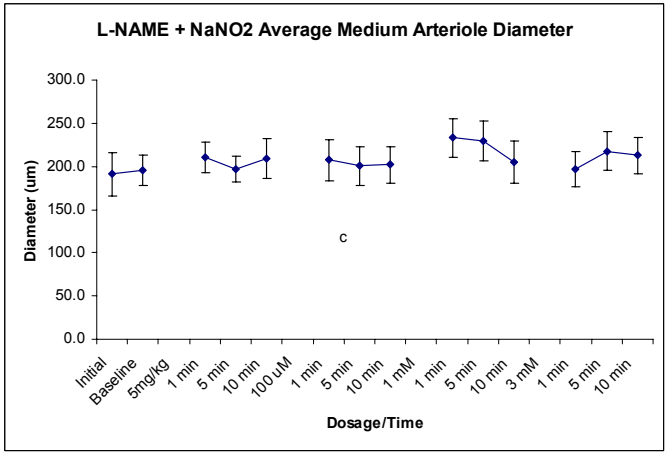

Average HBOC 201 + NaNO2 Simlutaneous
Percent Change Large Diameter
Percent Change
35
30
25
20
15
10
5
0
Initial
Baseline
HBOC +1mM NaNO2
1 min
5 min
10 min
0.1uM
1 min
5 min
10 min
Dosage/Time

Figure 19. L-NAME + $NaNO_2$ Dose Response Curve. The upper left graph represents the change in mean arterial pressure. The upper right graph represents the change in diameter of the small arteriole. The lower left graph represents the change in diameter of the medium arteriole. The lower right graph indicates the change in diameter of the large arteriole. The error bars represent standard error.


L-NAME + NaNO2 Average MAP
Diameter (um)
180.0
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Initial
Baseline
5mg/kg
1 min
5 min
10 min
100 uM
1 min
5 min
10 min
1 mM
1 min
5 min
10 min
3 mM
1 min
5 min
10 min
Dosage/Time


L-NAME + NaNO2 Average Small Arteriole
Diameter (um)
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Initial
Baseline
5mg/kg
1 min
5 min
10 min
100 uM
1 min
5 min
10 min
1 mM
1 min
5 min
10 min
3 mM
1 min
5 min
10 min
Dosage/Time


L-NAME + NaNO2 Average Medium Arteriole Diameter
Diameter (um)
300.0
250.0
200.0
150.0
100.0
50.0
0.0
c
Initial
Baseline
5mg/kg
1 min
5 min
10 min
100 uM
1 min
5 min
10 min
1 mM
1 min
5 min
10 min
3 mM
1 min
5 min
10 min
Dosage/Time

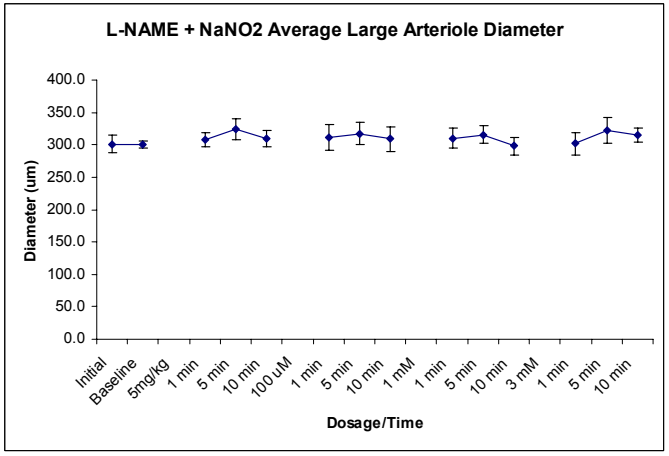
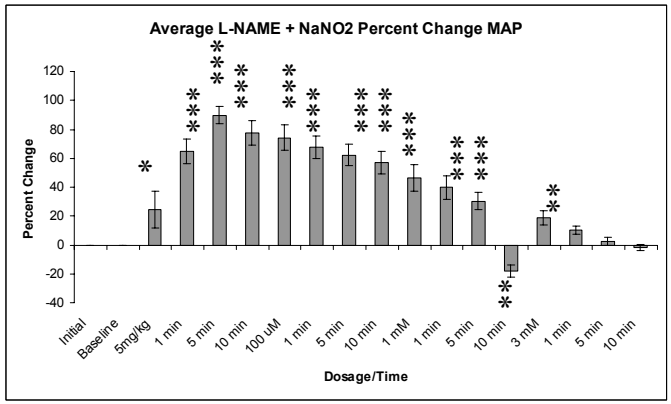
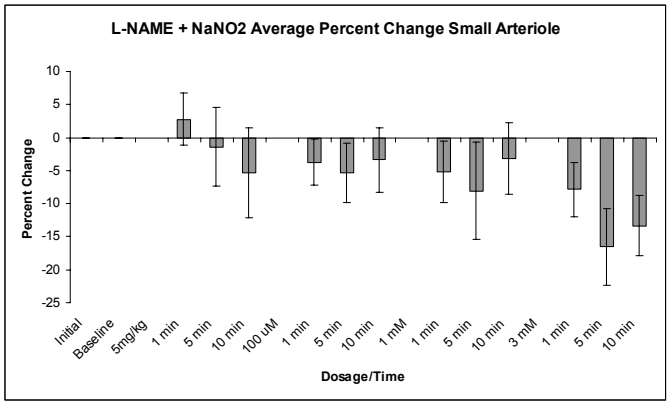
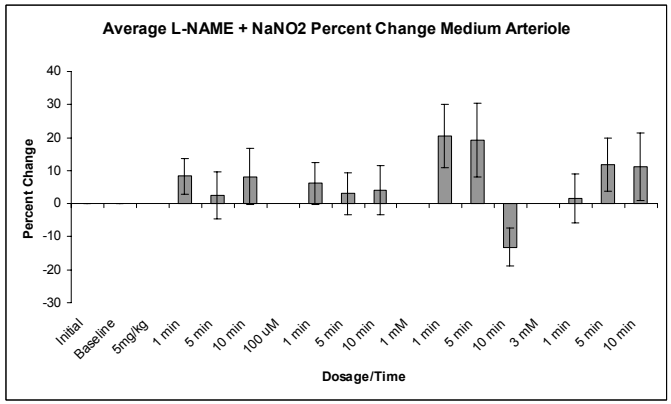
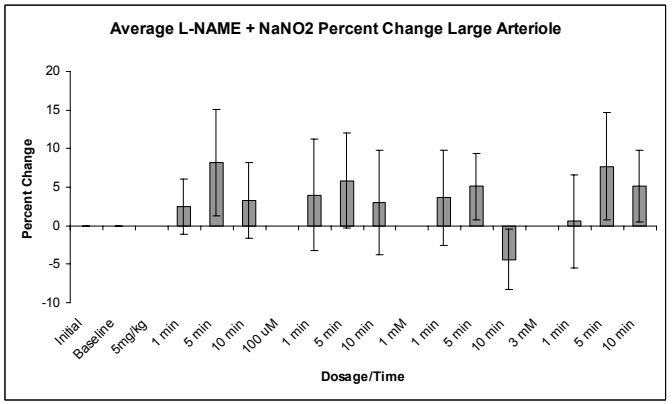

L-NAME + NaNO2 Average Large Arteriole Diameter
Diameter (um)
400.0
350.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
Initial
Baseline
5mg/kg
1 min
5 min
10 min
100 uM
1 min
5 min
10 min
1 mM
1 min
5 min
10 min
3 mM
1 min
5 min
10 min
Dosage/Time

Figure 20. L-NAME + $NaNO_2$ Percent Change. The upper left graph represents the percent change in mean arterial pressure. The upper right graph represents the percent change in diameter of the small arteriole. The lower left graph represents the percent change in diameter of the medium arteriole. The lower right graph indicates the percent change in diameter of the large arteriole. The error bars represent standard error. An (*) represents significance at $P < 0.05$. An (**) represents significance at $P < 0.01$. An (***) represents significance at $P < 0.001$.


Average L-NAME + NaNO2 Percent Change MAP
Percent Change
Dosage/Time
Initial
Baseline
5mg/kg
1 min
5 min
10 min
100 uM
1 mM
3 mM


L-NAME + NaNO2 Average Percent Change Small Arteriole
Percent Change
Dosage/Time
Initial
Baseline
5mg/kg
1 min
5 min
10 min
100 uM
1 mM
3 mM


Average L-NAME + NaNO2 Percent Change Medium Arteriole
Percent Change
Dosage/Time
Initial
Baseline
5mg/kg
1 min
5 min
10 min
100 uM
1 mM
3 mM


Average L-NAME + NaNO2 Percent Change Large Arteriole
Percent Change
Dosage/Time
Initial
Baseline
5mg/kg
1 min
5 min
10 min
100 uM
1 mM
3 mM

Discussion

HBOC

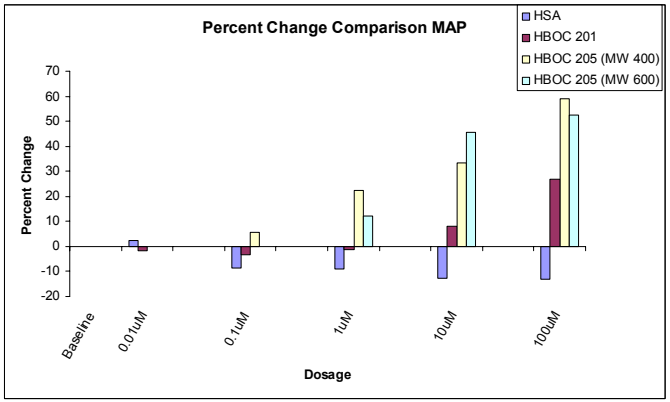
Dose-Dependent Effects of the HBOCs

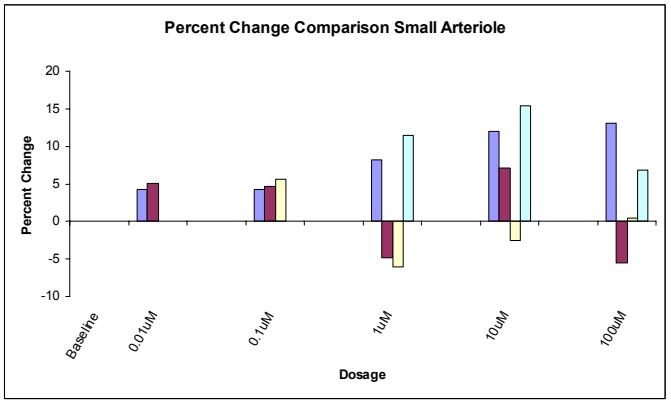
The results of the three HBOCs showed that MAP increased for nearly all of the HBOCs beginning at the 10 μM dose. Using HSA as a baseline comparison, it can be seen that for HBOC 201, a rising trend was noticed at the 10 μM dose, but was not significant until after the 100 μM dose. HBOC 205 (MW400) and HBOC 205 (MW 600) showed significant increases from baseline following the 10 μM injection and steadily increased with each dose up to 300 μM. These results confirm earlier observations that HBOC produces a hypertensive effect. Diameters of all three arterioles failed to show any sort of response that might indicate a vasoactive effect from any of the three HBOCs. (*Figure 21).*

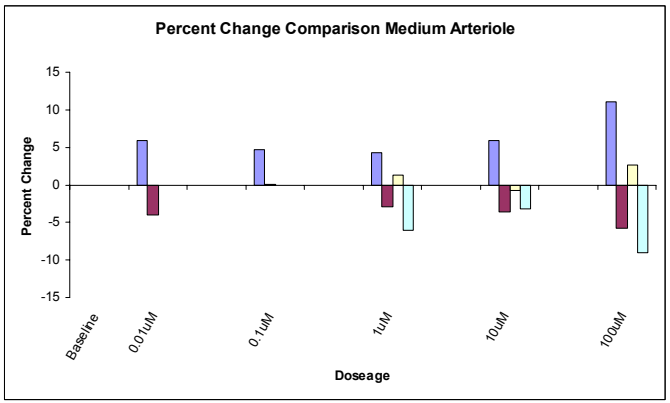
Comparison to Previous Literature

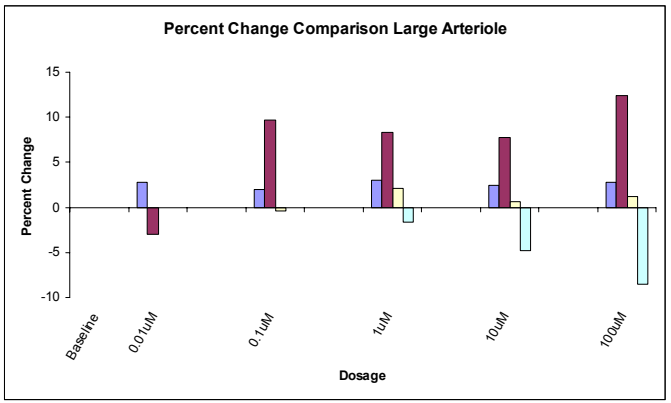
Based on previous literature, studies showed that HBOC has a hypertensive effect due to vasoconstriction. During a phase II clinical trial a group of 13 patients were administered small doses of HBOC 201. Measurements of HR, MAP, RAP and MPAP were monitored continuously during the study. Results showed an increase in mean arterial pressure which was due to an increase in systemic vascular resistance (Kasper 1996). Other models have shown that HBOCs induce systemic vasoconstriction by

Figure 21. HBOC 201, 205 (MW 400), and 205 (MW 600) Percent Change Comparison. The upper left graph represents the change in mean arterial pressure. The upper right graph represents the change in diameter of the small arteriole. The lower left graph represents the change in diameter of the medium arteriole. The lower right graph indicates the change in diameter of the large arteriole. The error bars represent standard error.


Percent Change Comparison MAP
HSA
HBOC 201
HBOC 205 (MW 400)
HBOC 205 (MW 600)
Percent Change
70
60
50
40
30
20
10
0
-10
-20
Baseline
0.01uM
0.1uM
1uM
10uM
100uM
Dosage


Percent Change Comparison Small Arteriole
Percent Change
20
15
10
5
0
-5
-10
Baseline
0.01uM
0.1uM
1uM
10uM
100uM
Dosage


Percent Change Comparison Medium Arteriole
Percent Change
15
10
5
0
-5
-10
-15
Baseline
0.01uM
0.1uM
1uM
10uM
100uM
Doseage


Percent Change Comparison Large Arteriole
Percent Change
15
10
5
0
-5
-10
Baseline
0.01uM
0.1uM
1uM
10uM
100uM
Dosage

increasing the activity of vasoconstrictor molecules or decreasing the activity of the vasodilator molecules in the endothelial cells of the vascular wall (Hare 2006, Spahn 2001, Surg 1996). Hare carried out studies on rats that were given doses of HBOCs to study their MAP response. Results showed an increase in MAP after the HBOC infusion confirming the HBOC's hypertensive effect. Another study done by Meliagros, observed MAP and arterial diameter in the spinotrapezius muscle of rats in response to increasing doses of HBOCs. Using the same three HBOCs and same increasing doses, MAP and diameters were recorded at the same time points as this study. Similar effects were seen with MAP, but vasoconstriction was observed for all three HBOCs in the spinotrapezius. Other studies have found a lack of vasoactivity in the arterioles as well. Many *in vivo* studies have shown little evidence for vasoconstriction as well (Spahn 1994).

Sodium Nitrite

Dose-Dependent Effects of $NaNO_2$ following HBOC 201 Infusion

The results indicated that sodium nitrite was effective at decreasing MAP following the administration of HBOC-201. After the initial dose of the HBOC, increasing doses of sodium nitrite were able to return MAP back to baseline by the end of the experiment. These results indicate that the mechanism of vasoactivity of the HBOC is due to the NO scavenging effects of the HBOC. The return to baseline with increasing doses of $NaNO_2$ indicates that NO that was scavenged by the HBOC was restored allowing a decrease in vascoconstriction. If the HBOC caused an increase in MAP by an alternative mechanism, such as increased oxygen delivery, the doses of $NaNO_2$ should

have had no effect on MAP after the HBOC 201 dose. The simultaneous infusion of the HBOC and $NaNO_2$ showed that the $NaNO_2$ was able to prevent MAP from rising as high as it would have if just the HBOC had been administered alone. There was a brief rise in MAP after the HBOC infusion, but immediately after the $NaNO_2$ was administered, the rise in MAP was inhibited and there was a gradual decline to baseline. This shows that the $NaNO_2$ was able to prevent the HBOC from scavenging the NO in the endothelium of the arteriolar wall. Studies with $NaNO_2$ confirm the theory that NO scavenging is the mechanism by which the HBOC causes vasoconstriction. Even though changes in MAP were observed with the $NaNO_2$ doses, no changes in arteriolar diameters were observed. There was no vasoconstriction observed with the administration of HBOC 201 or a vasodilation with the administration of $NaNO_2$.

L-NAME and $NaNO_2$ Effects

Because L-NAME is a NOS inhibitor, it should be able to decrease NO levels by the inhibiting the synthesis of NO. The sudden inhibition of NOS causes a drop in NO level leading to vasoconstriction and an increase in MAP. Although L-NAME does not directly affect levels of NO, it should have similar effects to scavenging of the NO by HBOC-201. The studies with L-NAME showed that L-NAME had very similar effects to that of the HBOC. The increase in MAP caused by L-NAME was similar to that of the increase from the HBOC. After the L-NAME injection, sodium nitrite was able to return MAP back to baseline. Because L-NAME has such similar effects to HBOC-201, it would indicate that HBOC-201 does in fact cause an increase in MAP due to its NO scavenging effects. The fact that L-NAME caused similar effects to HBOC-201 further

supports the theory that increased oxygen transport is not the mechanism of HBOC-induced vasoconstriction.

No vasoactivity was observed in the mesentery as a result of the L-NAME infusion or the following $NaNO_2$ doses. Looking at the results, it would seem that NO does not seem to play a major role in regulating vascular tone of the mesenteric arteries. Infusion of $NaNO_2$ or L-NAME had no effect on constricting or dilating any of the arterioles observed. One explanation for this is that the mesentery does not produce a significant amount of NO in the first place. Another possible explanation would be to assume the mesentery is not very sensitive to levels of NO. These two explanations actually help explain each other. If the mesentery does not produce a high level NO in the first place, it could be assumed that the mesentery therefore would not need to be sensitive to levels of NO leaving it unresponsive to any concentrations of circulating NO. Although NO is a potent vasodilator, there are several other factors such as prostacylcin that might play a role in the mesentery (Feletou 2006). Additionally, it is possible that the preparation of the mesentery rendered it unresponsive to any vasodilators or vasoconstrictors.

Comparisons to Previous Literature

Several studies have been done to show that the mechanism of vasoconstriction by HBOCs is in fact due to NO scavenging. Studies done by Olson looked at the pathway by which extracellular HbO_2 is able to cause vasoconstriction. Olson looked at the effects of extracellular hemoglobin and its NO scavenging effects. Looking at the side effects of many HBOCs, hypertension and gastrointestinal dysmotility, it seems that HBOCs are

potent NO scavengers since the side effects can be explained by decreasing levels of NO. Observing the cardiac output in addition to the blood pressure showed that the rise in MAP was proportional to TPR. This would indicate that a systemic vasoconstricion was occurring to cause the increase in MAP. Extracellular hemoglobin is free to extravasate into the endothelial wall scavenging the NO and causing vasoconstriction (Olson 2004). A study done by Hare showed that an increased molecular weight of the hemoglobin decreased its vasoactive effects. The increase in molecular weight prevents extravasation into the endothelial wall, which would decrease extracellular hemoglobin's vasoactive effects. By not being able to extravasate, the hemoglobin was unable to scavenge NO. A decrease in MAP rise because of a larger hemoglobin molecule supports the theory that HBOCs produce an increase in MAP by acting as a NO sink (Hare 2006, Gould 1996).

Few studies have been done to observe the NO activity of the mesentery *in vivo* or *in vitro*. One study done examined the concentration of NO found in the mesentery. Tschudi observed direct in situ measurements of NO in the mesentery of rats and found that the level of NO in the mesentery compared to that of the aorta was drastically lower. In addition, it was observed that the NOS activity of the mesentery was reduced compared to that of the aorta (Tschudi 1996). A study done by Mitchell looked specifically at the mesenteric arteries and their response to varying levels of NO. She used LPS to induce NOS and observe the response of the mesentery to other vasoconstrictors. What she found was that the increased activity of NOS did not show a reduced responsiveness to vasoconstrictor agents (Mitchell 1993). These studies support the idea that NO may not be a major factor in regulating the vascular tone of the mesenteric arteries.

Further Studies

Further studies observing mesenteric vasoactivity would be of great benefit. It would be useful to know which factors vary vascular tone in the mesentery. Also it could show that the mesentery preparation responds to vasoactive factors. This could be done by topically applying other vasoconstrictors and other vasodilators that do not alter NO levels, such as papaverine. It would also be of some interest if NO electrodes could be used to establish a baseline for *in vivo* measurements of NO and see if the HBOC is causing a drop in NO.

Conclusion

Even though no vasoactivity was observed in the mesentery, the MAP results support the theory of NO scavenging. Just because the mesentery did not respond to the HBOCs, $NaNO_2$, or L-NAME does not rule out vasoconstriction at other sites, such as skeletal muscle. The increase in MAP points to a systemic vasoconstriction, but the diameters of arterioles in the mesentery indicate that NO will not affect local vascular tone in the mesentery. Looking together at the results of the various HBOCs, $NaNO_2$, and L-NAME, it would seem that NO scavenging is the major factor in the vasoconstrictor side effect of HBOCs, but there are remaining studies to look at mesenteric vasoactivity.

Bibliography

Buerk D. (2007). "Nitric Oxide Regulation of Microvascular Oxygen." Antioxidants & Redox Signaling 9; 829-841.

Dewhirst M, Klitzman B, Braun R, Brizel D, Haroon Z, Secomb T. (2000). "Review of Methods Used to Study Oxygen Transport at the Microcirculatory Level." International Journal of Cancer 90; 237-255.

Feletou M, Vanhoutte P. (2006). "Endothelium-Derived Hyperpolarizing Factor Where Are we Now?" Arterioscler Thromb Vasc. Biol. 26:1215-1225.

Gould S, Moss G. (1996). "Clinical Development of Human Polymerized Hemoglobin as a Blood Substitute." World Journal of Surgery 20; 1200-1207.

Fitzpatrick C, Savage S, Kerby J, Clouse W, Kashyap V. (2004). "Resuscitation with a Blood Substitute Causes Vasoconstriction Without Nitric Oxide Scavenging in a Model of Arterial Hemmorhage." Journal of American College of Surgeons 199; 693-701.

Hare G, Harrington A, Liu E, Wang J, Baker A, Mazer D. (2006). "Effect of Oxygen Affinity and Molecular Weight of HBOCs on Cerebral Oxygenation and Blood Pressure in Rats." Cardiothoracic Anesthesia, Respiration and Airway 53; 1030-1038.

Iwatini Y, Kosugi K, Isobe-Oku S, Atagi S, Kitamura Y, Kawasaki H. (2008). "Endothelium Removal Augments Endothelium-Indpendent Vasodilation in Rat Mesenteric Vascular Bed." British Journal of Pharmocology: 1-9.

Kasper S, Walter, M, Grune, F, Bischoff A, Erasim H, Buzello W. (1996)"Effects of a Hemoglobin-Based Oxygen Carrier (HBOC-201) on Hemodynamics and Oxygen Transport in Patients Undergoing Preoperative Hemodilution for Elective Abdominal Aortic Surgery." Anesth Anag 83; 921-927

Lane P, Gross S. (2002) "Nitric Oxide: Promiscuous and Duplicitous." Science and Medicine; 96-107.

Mitchell J, Kohlhass K, Sorrentino, Warner T, Murad F, Vane J. (1993). "Induction by Endotoxin of Nitric Oxide Synthase in the Rat Mesentery: Lack of Effect on the Action of Vasonconstrictors." British Journal of Pharmacology 109; 265-270.

Moncada S, Higgs A. (1993). "The L-Arginine Nitric Oxide Pathway." The New England Journal of Medicine 329; 2002-2012.

Olson J, Foley E, Robbe C, Tsai A, Doyle M, Lemon D. (2004). "Serial Review: Biodmedical Implications for Hemoglobin Interactions with Nitric Oxide." Free Radical Biology & Medicine 36; 685-697.

Palmer R, Ferrige A, Moncada S. (1987). "Nitric Oxide Release Accounts for the Biological Activity of Endothelium Derived Relaxing Factor." Nature 327; 524-525.

Pittman R. (2005). "Oxygen Transport and Exchange in the Mircocirculation." Mircocirculation 12; 59-70.

Sonveaux P, Lobysheva I, Feron O, McMahon T. (2007). "Transport and Peripheral Bioactivities of Nitrogen Oxides Carried by Red Blood Cell Hemoglobin: Role in Oxygen Delivery." Physiology 22; 91-112.

Spahn, D. Leone B, Reves J, Pasch T. (1994). "Cardiovascular and Coronary Physiology of Acute Isovolemic Hemodilution: A Review of Nonoxygen-Carrying and Oxygen-Carrying Solutions." Anesth Analg 78; 1000-1021.

Spahn D, Pasch T. (2001). "Physiological Properties of Blood Substitutes." News Physiological Sciences 16; 38-41.

Tsai A, Cabrales P, Manjula B, Acharya S, Winslow R, Intaglietta M. (2006)"Dissociation of Local Nitric Oxide Concentrations and Vasconstriction in the Presence of Cel-Free Hemoglobin Oxygen Carriers." Blood 108; 3603-3610.

Tschudi M, Mesaros S, Luscher T, Malinski T. (1996). "Direct In-Situ Measurements of Nitric Oxide in Mesenteric Resistance Arteries." Hypertension 27; 32-35.

APPENDIX

APPENDIX I – Data Experimental Protocol

Michael Kim (_____)

Date: _____ **Card #:_____** **Rat Weight:_____**

Ketamine (_____/_____)

Alfaxin (_____) **Syringe #:_____** **Amount: _____-_____**

Start Time: _____

Femoral Vein: _____

Femoral Artery: _____

Tracheotomy: _____

Jugular Vein: _____

Scope: _____

Notes:

	MAP	S	M	L
Initial				
Baseline				
0.01 uM				
1 min				
5 min				
10 min				
0.1 uM				
1 min				
5 min				
10 min				
1 uM				
1 min				
5 min				
10 min				
10 uM				
1 min				
5 min				
10 min				

100 uM				
1 min				
5 min				
10 min				

APPENDIX II – Physiological Baselines

	Weight	MAP	PO2	PCO2	tHB	Hct	pH
HAS 1	260	112	66.2	49.5	15.4	0.39	7.325
HAS 2	297.5	123.4	91.2	37	16	0.39	7.327
HBOC 1	291	117.8	106	53.1		0.44	7.278
HBOC 2	283	123	93.2	44.1		0.42	7.357
HBOC 3	278	95.4	69.6	42.6	14	0.4	7.395
HBOC 4	297	85.7	59	37	16.9	0.42	7.398
HBOC 5	315	109.5	60.1	41.1	17.2	0.45	7.396
HBOC 6	315						
HBOC 6-1	244	91.5	79.6	41.4	16.3	0.43	7.401
HBOC 6-2	248	86.5	70.2	36.5	16.3	0.38	7.438
HBOC 6-3	259	85.4	42.5	33.6	16	0.39	7.419
HBOC 6-4	299	86.5	87.8	38	15.6	0.38	7.411
HBOC 6-5	295	80.8	70.2	38.4	17.1	0.42	7.382
HBOC 6-6	257	70.8	58.6	46.2	15.4	0.39	7.34
HBOC 4-1	323	81.4	53.6	35.8	17.1	0.39	7.434
HBOC 4-2	323	81.9	92.8	44.5	15.4	0.38	7.37
HBOC 4-3	276	82.8	82.9	46	15.2	0.38	7.367
HBOC 4-4	292	77.1	89.4	42	13.9	0.39	7.386
HBOC 4-5	290	88.5	65.3	49.6	15.4	0.39	7.362
HBOC 4-6	312	80.9	63.6	48	15.6	0.39	7.279
Average	287.725	92.68	73.779	42.337	15.812	0.4011	7.37184211
Std. Error	5.4	3.7	3.8	1.3	0.2	0.005	0.01
NO Ctrl 1	278	92.1	88.7	37.8	13.7	0.4	7.403
NO Ctrl 2	330	88.3	78.7	36.5	16.3	0.39	7.414
HBOC Time 1	308	81.5	49.6	28.8	16.6	0.4	7.506
HBOC Time 2	311	80.9	62.2	44.9	16.1	0.39	7.423
NO Time	347	102.1			15	0.4	
HBOC/NO 1	279	84.8	76.1	50.1	15.3	0.41	7.319
HBOC/NO 2	297	80.8	78.9	37.8	14.5	0.39	7.355
HBOC/NO 3	299	81.3	89.3	32.6	15.9	0.4	7.452
HBOC/NO 4	297	112.9	84.7	36.8	14.5	0.42	7.424
HBOC/NO 5	300	90.1	73.7	49.9	14.3	0.39	7.369
HBOC/NO 6	378	80.4	75	42.6	15.7	0.41	7.371
HBOC+NO 1	347	103.7	82.8	36.2	16.2	0.44	7.427
HBOC+NO 2	343	82.1	101	43.4	17	0.4	7.337
LNAME 1	342	81.5	81.7	33.3	16	0.41	7.344
LNAME 2	240	88.6			16.8	0.38	
LNAME 3	335	79	66.6	47.6	15.7	0.38	7.353

LNAME 4	347	89.1	90.7	48.2	16	0.4	7.37
LNAME 5	332	73.4	93	42.8	16.8	0.38	7.365
LNAME 6	251	81.9	94.2	35.8	12.3	0.38	7.388
Average	313.7	87.1	80.4	40.3	15.5	0.4	7.38
Std. Error	8.2	2.3	3.1	1.6	0.3	0.0035	0.01

APPENDIX III - Data

Average MAP
Human Albumin Serum Ctrl

	#1 MAP	#2 MAP	Average MAP	Std. Error MAP
Initial	112.0	125.0	118.5	6.5
Baseline	112.6	123.4	118.0	5.4
0.01uM	115.3	124.5	119.9	4.6
1 min	125.0	123.6	124.3	0.7
5 min	123.0	122.1	122.6	0.45
10 min	114.0	116.9	115.5	1.45
0.1uM	101.6	113.9	107.8	6.15
1 min	102.2	113.5	107.9	5.65
5 min	107.0	109.5	108.3	1.25
10 min	104.4	108.5	106.5	2.05
1uM	105.9	107.0	106.5	0.55
1 min	109.1	106.9	108.0	1.1
5 min	108.3	107.0	107.7	0.65
10 min	108.1	104.4	106.3	1.85
10uM	105.1	104.9	105.0	0.1
1 min	104.3	103.9	104.1	0.2
5 min	102.2	100.3	101.3	0.95
10 min	102.1	99.5	100.8	1.3
100uM	110.1	97.4	103.8	6.35
1 min	108.3	96.3	102.3	6
5 min	105.8	95.8	100.8	5
10 min	106.4	94.7	100.6	5.85

Average
Large
Human Albumin Serum Ctrl

	#1 S	#2 S	Average S	Std. Error S
Initial	29.85		29.9	2.8E-308
Baseline	32.51	24.07	28.3	4.22
0.01uM				

1 min	31.79	22.36	27.1	4.715
5 min	31.62	23.31	27.5	4.155
10 min	29.8	23.64	26.7	3.08
0.1uM				
1 min	35.29	22.95	29.1	6.17
5 min	35.41	23.76	29.6	5.825
10 min	36.48	23.17	29.8	6.655
1uM				
1 min	38.39	21.8	30.1	8.295
5 min	39.14	25.16	32.2	6.99
10 min	37.36	25.52	31.4	5.92
10uM				
1 min	37.12	25.31	31.2	5.905
5 min	38.89	25.21	32.1	6.84
10 min	38.33	25.26	31.8	6.535
100uM				
1 min	40.21	25.58	32.9	7.315
5 min	39.58	24.56	32.1	7.51
10 min	36.87	25.15	31.0	5.86

Average Medium
Human Albumin Serum Ctrl

	#1 M	#2 M	Average M	Std. Error M
Initial	271.42		271.4	2.8E-308
Baseline	244.51	28.74	136.6	107.885
0.01uM				
1 min	274.2	28.58	151.4	122.81
5 min	281.77	28.09	154.9	126.84
10 min	271.5	28.62	150.1	121.44
0.1uM				
1 min	256.09	27.75	141.9	114.17
5 min	281.4	27.39	154.4	127.005
10 min	282.79	29.01	155.9	126.89
1uM				
1 min	273.65	30.03	151.8	121.81
5 min	272.77	29.37	151.1	121.7
10 min	269.66	29.12	149.4	120.27
10uM				
1 min	267.52	28	147.8	119.76
5 min	273.06	29.71	151.4	121.675
10 min	273.32	29.35	151.3	121.985
100uM				
1 min	290.86	29.71	160.3	130.575
5 min	271.33	30.27	150.8	120.53
10 min	291.67	31.23	161.5	130.22

Average
Large
Human Albumin Serum Ctrl

	#1 L	#2 L	Average L	Std. Error L
Initial	357.79		357.8	2.8E-308
Baseline	333.51	280.47	307.0	26.52
0.01uM				
1 min	351.91	289.43	320.7	31.24
5 min	342.9	275.44	309.2	33.73
10 min	323.21	308.42	315.8	7.395
0.1uM				
1 min	309.61	315.09	312.4	2.74
5 min	322.66	297.23	309.9	12.715
10 min	323.21	300.95	312.1	11.13
1uM				
1 min	358.15	306.02	332.1	26.065
5 min	350.53	285.7	318.1	32.415
10 min	353.02	295.78	324.4	28.62
10uM				
1 min	356.72	283.67	320.2	36.525
5 min	354.19	261.65	307.9	46.27
10 min	358.94	279.66	319.3	39.64
100uM				
1 min	352.08	276.56	314.3	37.76
5 min	331.05	281.37	306.2	24.84
10 min	350.62	301.81	326.2	24.405

Average MAP
HBOC 201

	#1 MAP	#2 MAP	#3 MAP	#4 MAP	#5 MAP	#6 MAP	Average MAP	Std. Error MAP
Initial	103.5	97.9	105.1	86.6	88.9	105	97.8	3.377738
Baseline	107.8	123.0	95.4	85.7	92.9	109.5	102.4	5.543971
0.01uM	115.8	111.4	98.1	92.1	103.8	104.1	104.2	3.512778
1 min	110.0	115.3	97.3	90.1	95.3	98.5	101.1	3.901659
5 min	95.5	113.6	96	88.8	89.3	102.5	97.6	3.800475
10 min	101.3	126.0	95.6	87.1	83.5	99.6	98.9	6.131707
0.1uM	101.3	123.0	104.6	89	84.5	97.5	100.0	5.535788
1 min	95.5	125.0	102.1	90.1	86.7	99.5	99.8	5.551601
5 min	90.4	121.0	90.5	89.3	88.3	108.6	98.0	5.553222
10 min	83.5	118.0	90.1	95.6	95.2	95.6	96.3	4.744658
1uM	111.5	118.7	95.1	95.3	88.6	105.3	102.4	4.658499

1 min	104.5	120.3	98.1	102.3	95.3	108.6	104.9	3.633891
5 min	98.6	120.8	105	88.1	80.3	105.3	99.7	5.821765
10 min	87.5	123.7	103.5	90.7	83.6	103.6	98.8	6.026257
10uM	119.5	125.4	110.5	100.7	95.6	98.5	108.4	4.95901
1 min	122.6	123.4	128.5	98.7	98.7	95.6	111.3	6.147994
5 min	124.5	120.9	122.7	100.7	105.7	98.7	112.2	4.809643
10 min	121.5	118.5	96.3	105.6	104.3	95.4	106.9	4.475017
100uM	153.5	135.6	105.3	119.6	105.3	120.3	123.3	7.60266
1 min	142.7	137.4	138.5	128.7	110.3	132.8	131.7	4.71838
5 min	136.5	138.1	135.6	127.5	130.5	135.6	134.0	1.660054
10 min	126.5	137.1	130.6	125.3	125.5	100.3	124.2	5.121095

Average
Small
HBOC 201

	#1 S	#2 S	#3 S	#4 S	#5 S	#6 S	Average S	Std. Dev S
Initial	8.88	11.49	9.6	15.18	5.79	5.65	9.4	1.473946
Baseline	10.49	9.88	9.925	15.73	4.16	5.65	9.3	1.665632
0.01uM								
1 min	7.42	10.11	9.874	14.54	5.97	9.46	9.6	1.192744
5 min	7.67	11.26	9.98	13.57	5.59	6.68	9.1	1.233404
10 min	11.23	9.88	10.83	16.52	4.42	5.25	9.7	1.805941
0.1uM								
1 min	8.7	8.73	8.81	15.98	4.74	4.53	8.6	1.693103
5 min	9.54	8.36	10.81	15.64	7.98	5.96	9.7	1.357468
10 min	8.81	8.73	11.122	15.78	6.58	5.45	9.4	1.505775
1uM								
1 min	8.31	9.88	8.87	15.65	5.64	7.2	9.3	1.409717
5 min	6.47	9.66	9.525	14.6	4.91	6.07	8.5	1.443502
10 min	6.13	9.67	9.887	10.12	4.46	6.93	7.9	0.964538
10uM								
1 min	8.98	9.66	9.972	12.68	5.37	5.14	8.6	1.185952
5 min	7.71	11.03	10.28	19.35	6.31	8.09	10.5	1.913037
10 min	6.22	10.81	10.18	20.35	5.69	6.79	10.0	2.243356
100uM								
1 min	5.98	9.2	8.15	11.71	4.58	8.5	8.0	1.020637
5 min	5.48	9.88	8.968	8.19	6.67	7.61	7.8	0.646481
10 min	6.39	9.2	10.57	15.64	4.39	6.76	8.8	1.627806

Average Medium
HBOC 201

	#1 M	#2 M	#3 M	#4 M	#5 M	#6 M	Average M	Std. Dev M
Initial	12.44	24.67	22.64	26.22	18.22	21.06	20.9	2.035671
Baseline	11.68	29.93	22.76	29.07	18.19	22.07	22.3	2.792783

0.01uM								
1 min	11.45	28.7	21.1	29.22	15.92	20.69	21.2	2.850608
5 min	12.92	29.12	20.92	27.68	16.01	20.23	21.1	2.58981
10 min	12.1	26.88	21.9	29.34	19.02	20	21.5	2.498805
0.1uM								
1 min	10.81	26.87	23.33	32.5	18.28	22.53	22.4	3.023101
5 min	12.56	28.61	20.37	31.09	18.71	21.15	22.1	2.766449
10 min	12.31	26.67	21.53	33.07	18.9	21.15	22.3	2.87727
1uM								
1 min	13.29	26.43	22.46	28.11	17.31	20.92	21.4	2.266986
5 min	11.4	29.22	23.46	27.5	16.01	20.69	21.4	2.781845
10 min	11.36	29.08	23.25	29.35	14.4	21.38	21.5	3.029741
10uM								
1 min	10.92	28.31	22.5	31.23	15.03	20.92	21.5	3.142087
5 min	11.63	26.74	22.8	29.98	18.74	18.62	21.4	2.672994
10 min	10.28	29.62	23.46	29.66	17.91	20.69	21.9	3.025724
100uM								
1 min	9.98	31.06	21.47	29.05	17.88	17.24	21.1	3.222123
5 min	10.11	31.9	22.67	29.28	16.71	18.75	21.6	3.317964
10 min	10.5	27.25	23.88	29.95	14.57	21.61	21.3	3.049099

Average
Large
HBOC 201

	#1 L	#2 L	#3 L	#4 L	#5 L	#6 L	Average L	Std. Error L
Initial	223.59	273.15	228.05	228.05	209.56	203.97	227.7	9.958258
Baseline	182.91	279.23	227.25	227.25	205.39	200.86	220.5	13.62276
0.01uM								
1 min	173.91	274	220.49	220.49	198.91	206.84	215.8	13.60337
5 min	145.98	272.05	230.17	230.17	184.51	212.36	212.5	17.68146
10 min	140.64	276.06	220.29	220.29	235.02	179.94	212.0	19.05355
0.1uM								
1 min	175.78	272.2	229.56	229.56	221.95	178.57	217.9	14.78483
5 min	169.91	266.79	238.44	238.44	232.65	169.36	219.3	16.43124
10 min	160.97	275.48	210.59	210.59	209.82	197.36	210.8	15.10873
1uM								
1 min	185.91	273.61	221.12	221.12	219.87	202.35	220.7	12.03823
5 min	183.9	258.96	210.59	210.59	219.84	201.43	214.2	10.23473
10 min	198.62	276.58	224.86	224.86	224.65	201.58	225.2	11.40882
10uM								
1 min	162.76	281.48	221.47	221.47	227.92	202.07	219.5	15.75162
5 min	153.95	285.58	220.74	220.74	223.94	200.07	217.5	17.35873
10 min	183.54	275.3	218.56	218.56	201.49	200.87	216.4	12.93807
100uM								
1 min	169.5	269.31	230.53	230.53	225.78	167.48	215.5	16.20176
5 min	152.57	251.6	225.24	225.24	225.37	188.89	211.5	14.33169
10 min	157.41	282.5	227.98	227.98	226.84	184.36	217.8	17.55017

Average Map
HBOC 205
MW 400

	#1 MAP	#2 MAP	#3 MAP	#4 MAP	#5 MAP	#6 MAP	Average MAP	Std. Error MAP
Initial	80.1	89.9	89.3	85.3	83.1	80.7	84.7	1.715355
Baseline	81.4	81.9	82.8	77.1	88.5	80.9	82.1	1.510188
1uM	83.4	89.3	82.0	84.1	89.3	81.2	84.9	1.457262
1 min	91.4	95.4	83.9	88.3	90.1	83.1	88.7	1.903506
5 min	92.1	91.1	84.6	91.3	87.3	83.1	88.3	1.559861
10 min	81.2	87.3	83.9	83.0	90.3	81.1	84.5	1.489221
10 uM	93.4	98.7	89.3	84.5	93.6	85.3	90.8	2.230097
1 min	102.3	112.6	101.3	89.1	96.5	87.7	98.3	3.784684
5 min	118.3	117.8	115.9	96.7	96.0	89.9	105.8	5.272297
10 min	122.1	109.3	112.1	99.6	99.8	93.0	106.0	4.303906
100 uM	134.7	113.8	110.1	100.8	98.3	95.4	108.9	5.919727
1 min	137.3	125.3	120.4	110.3	101.4	109.4	117.3	5.283661
5 min	140.1	133.5	130.5	115.1	118.5	115.7	125.6	4.302454
10 min	129.1	131.4	125.6	116.7	111.2	112.8	121.1	3.543037
300 uM	134.1	132.3	124.3	120.8	115.4	114.0	123.5	3.433115
1 min	148.1	135.6	131.3	125.7	124.8	119.4	130.8	4.14145
5 min	138.9	134.8	145.3	135.0	131.2	129.8	135.8	2.299662
10 min	128.1	129.7	138.3	131.2	129.8	125.4	130.4	1.771895

Average Small Vessel Diameter
HBOC 205
MW 400

	#1 S	#2 S	#3 S	#4 S	#5 S	#6 S	Average S	Std. Error S
Initial	20.23	30.29	33.06	33.06	28.95	12.04	26.3	3.437661
Baseline	26.58	25.56	36.06	36.06	31.29	10.13	27.6	3.946142
1uM								
1 min	28.98	29.69	37.38	37.38	34.26	11.68	29.9	3.93179
5 min	22.6	27.23	39.07	39.07	33.11	13.29	29.1	4.125434
10 min	23.18	27.42	37.38	37.38	33.46	13.21	28.7	3.853079
10 uM								
1 min	18.83	28.98	32.64	32.64	34.36	12.8	26.7	3.603645
5 min	23.89	28.76	34.07	34.07	33.62	12.46	27.8	3.483402
10 min	18.9	27.93	35.1	35.1	33.19	11.04	26.9	4.044096
100 uM								
1 min	23.4	26.78	33.86	33.86	33.19	13.05	27.4	3.36295
5 min	24.3	29.32	33.67	33.67	32.1	12.85	27.7	3.293097
10 min	21.76	29.03	33.91	33.91	31.87	11.78	27.0	3.570382
300 uM								
1 min	22.86	30.03	37.5	37.5	33.1	14.5	29.2	3.696622

5 min	24.39	30.42	36.47	36.47	33.03	10.89	28.6	3.992403
10 min	22.58	28.89	33.53	33.53	31.72	12.45	27.1	3.377818

Average Medium Vessel Diameter
HBOC 205
MW 400

	#1 M	#2 M	#3 M	#4 M	#5 M	#6 M	Average M	Std. Error M
Initial	161.63	301.8	223.26	248.86	207.52	162.53	217.6	21.88288
Baseline	167.46	306.51	277.58	278.65	222.26	201.84	242.4	21.85446
1uM								
1 min	165.15	307.58	286.82	293.54	226.99	147.17	237.9	28.27708
5 min	162.79	304.81	302.17	282.02	219.6	195.17	244.4	24.56341
10 min	163.96	300.61	276.13	295.58	227.08	223.62	247.8	21.53876
10 uM								
1 min	162.89	294.7	278.22	280.84	221.24	201.78	239.9	21.50923
5 min	172.14	295.37	304.44	276.21	224.13	231.41	250.6	20.64288
10 min	155.09	285.59	276.77	302.97	225.5	220.41	244.4	22.42312
100 uM								
1 min	140.53	231.78	294.86	285.4	218.89	198.5	228.3	23.36872
5 min	165.49	244.37	294.83	296.78	229.14	201.48	238.7	21.12852
10 min	163.01	247.06	314.07	301.87	221.34	187.45	239.1	24.76338
300 uM								
1 min	179.58	257.8	315.84	295.74	238.88	215.48	250.6	20.62387
5 min	168.31	256.4	331.01	289.45	243.17	198.47	247.8	24.15653
10 min	155.5	286.78	300.57	298.42	231.81	202.78	246.0	24.25443

Average Large Vessel Diameter
HBOC 205
MW 400

	#1 L	#2 L	#3 L	#4 L	#5 L	#6 L	Average L	Std. Error L
Initial	208.59	353.89	355.7	339.08	296	314.05	311.2	22.61854
Baseline	218.89	351.34	366.6	359.83	245.47	296.9	306.5	25.79345
1uM								
1 min	202.33	359.14	314.17	357.8	278.15	278.42	298.3	24.1558
5 min	215.88	360.18	347.08	353.94	271.73	266.28	302.5	24.3051
10 min	217.48	360.67	340.61	358.74	282.45	300.16	310.0	22.58007
10 uM								
1 min	222.5	368.59	375.94	361.8	284.95	285.46	316.5	25.22793
5 min	209.6	303.41	363.8	362.08	275.22	304.09	303.0	23.58228
10 min	213.2	329.6	377.94	352.24	311.8	296.44	313.5	23.28557
100 uM								
1 min	197.3	303.67	361.75	358.71	314.8	297.84	305.7	24.39326
5 min	189.03	331.79	401.49	364.84	306.19	289.64	313.8	29.90397
10 min	204.14	289.89	383.2	351.24	295.14	297.43	303.5	25.03036

300 uM								
1 min	200.38	261.04	395.8	356.28	302.11	295.41	301.8	28.16167
5 min	205.79	312.41	379.94	349.54	288.94	288.04	304.1	24.52085
10 min	208.64	300.41	404.6	384.25	315.6	301.14	319.1	28.54255

Average MAP

	MW
HBOC 205	600

	#1 MAP	#2 MAP	#3 MAP	#4 MAP	#5 MAP	#6 MAP	Average MAP	Std. Dev MAP
Initial	98.8	84.4	89.1	88.9	89.8	75.3	87.7	3.139365
Baseline	91.5	86.5	85.4	80.5	80.8	70.8	82.6	2.88183
10uM	112.5		83.2	89.8	116.1	85.3	97.4	7.012517
1 min	123.5		81.5	94.1	106.7	90.5	99.3	7.285987
5 min	117.5		78.5	81.5	88.6	70.5	87.3	8.084083
10 min	112.6		77.8	76.3	79.1	70.6	83.3	7.472175
100 uM	146.5	126.5	126.9	110.6	118.7	105.3	122.4	5.953174
1 min	160.2	129.5	128.3	111.7	126.7	107.3	127.3	7.598176
5 min	154.6	83.4	124.6	130.4	125.5	104.3	120.5	9.901975
10 min	153.5	79.5	116.1	118.5	115.8	88.6	112.0	10.64512
300 uM	161	120.5	116.5	124.7	131.8	103.5	126.3	7.929719
1 min	163.2	134.5	113.8	131.8	132.2	108.5	130.7	7.858315
5 min	154.5	140.6	107.3	121.4	125.3	101.5	125.1	8.148906
10 min	155.3	140.3	117.8	113.5	110.3	99.5	122.8	8.511029

Average Small

	MW
HBOC 205	600

	#1 S	#2 S	#3 S	#4 S	#5 S	#6 S	Average S	Std. Dev S
Initial	6.25	13.84	26.98	10.04	18.51	20.17	16.0	3.052276
Baseline	7.48	11.73	29.16	8.68	20.14	17.6	15.8	3.35185
10uM								
1 min	7.31		27.71	10.81	17.38	21.97	17.0	3.686157
5 min	7.35		26.71	12.78	19.49	21.58	17.6	3.396306
10 min	8.87		30.3	13.15	20.02	20.1	18.5	3.643275
100 uM								
1 min	9.67	9.06	27.76	12.51	17.9	22.67	16.6	3.080503
5 min	10.09	13.89	27.17	11.87	20.91	21.55	17.6	2.715009
10 min	10.08	14.92	27.34	11.87	21.58	23.34	18.2	2.817472
300 uM								
1 min	6.19	11.84	29.17	10.72	19.7	21.36	16.5	3.443876
5 min	8.07	12.87	28.18	11.94	19.63	22.72	17.2	3.091395
10 min	9.48	10.87	27.16	11.66	18.46	21.82	16.6	2.88761

Average Medium
HBOC 205 MW 600

	#1 M	#2 M	#3 M	#4 M	#5 M	#6 M	Average M	Std. Error M
Initial	21.62	44.84	168.72	47.31	175.95	180.56	106.5	30.92439
Baseline	19.7	44.84	186.14	45.14	202.18	207.44	117.6	36.53839
10uM								
1 min	16.85		172.74	46.66	185.41	198.67	124.1	38.20011
5 min	17.09		183.37	48.17	174.34	197.67	124.1	37.85897
10 min	18.02		165.78	45.54	173.93	206.87	122.0	37.73215
100 uM								
1 min	19.73	42.99	171.15	46.76	184.02	203.41	111.3	33.94662
5 min	22.47	36.79	168.13	48.2	185.67	205.9	111.2	34.22171
10 min	19.39	42.26	159.08	47.75	186.71	209.36	110.8	34.07647
300 uM								
1 min	20.65	41.85	166.99	45.91	167.64	192.73	106.0	31.65017
5 min	16.46	40.89	172.79	46.62	181.6	146.37	100.8	30.2356
10 min	20.13	39.54	171.5	47.81	160.6	152.16	98.6	28.43257

Average
Large

MW
HBOC 205 600

	#1 L	#2 L	#3 L	#4 L	#5 L	#6 L	Average L	Std. Error L
Initial	276.58	248.09	243.13	331.45	256.41	260.16	269.3	13.29166
Baseline	283.95	250.33	238.72	268.48	259.88	245.15	257.8	6.787408
10uM								
1 min	279.32		256.41	271.3	250.89	252.73	262.1	5.599112
5 min	281.45		251.55	277.68	234.82	232.12	255.5	10.38085
10 min	284.5		243.13	235.61	244.49	227.18	247.0	9.874436
100 uM								
1 min	266.47	236.06	292.76	241.1	244.54	239.07	253.3	9.047692
5 min	272.5	241.1	227.59	247.22	225.3	243.56	242.9	6.934578
10 min	276.66	253.53	226.58	244.05	235.11	228.79	244.1	7.683847
300 uM								
1 min	288.8	253.53	214.52	244.57	233.72	223.56	243.1	10.77968
5 min	285.14	243.1	216.87	261.47	240.62	163.58	235.1	17.06975
10 min	275.12	248.56	218.54	248.09	233.12	161.58	230.8	15.83847

Average MAP Arterial
NaNO2 Ctrl

#1 MAP	#2 MAP	Average MAP	Std.

				Error
Initial	81.3	86.4	83.9	2.55
Baseline	92.1	88.3	90.2	1.9
10uM	91.2	88.1	89.7	1.55
1 min	90.8	86	88.4	2.4
5 min	87.3	88.5	87.9	0.6
10 min	88.2	89.4	88.8	0.6
100uM	89.3	85	87.2	2.15
1 min	82.1	86.1	84.1	2
5 min	80.1	91.5	85.8	5.7
10 min	62.4	86.5	74.5	12.05
1mM	83.4	87.9	85.7	2.25
1 min	85	78.3	81.7	3.35
5 min	77	82.4	79.7	2.7
10 min	82	85.4	83.7	1.7
3mM	78.3	80.3	79.3	1
1 min	83.9	78.3	81.1	2.8
5 min	79.6	73.3	76.5	3.15
10 min	82	62.4	72.2	9.8

Average Small Arterial
NaNO2 Ctrl

	#1	#2	Average	Std.
	S	S	S	Error
Initial	9.26	20.56	14.9	5.65
Baseline	10.85	23.34	17.1	6.245
10uM				
1 min	7.82	21.3	14.6	6.74
5 min	8.97	22.04	15.5	6.535
10 min	8.98	23.26	16.1	7.14
100uM				
1 min	11.4	21.9	16.7	5.25
5 min	8.28	19.13	13.7	5.425
10 min	8.99	19.96	14.5	5.485
1mM				
1 min	11.49	19.86	15.7	4.185
5 min	10.49	19.84	15.2	4.675
10 min	10.41	18.57	14.5	4.08
3mM				
1 min	9.53	16.66	13.1	3.565
5 min	10.28	19.93	15.1	4.825
10 min	10.58	20.2	15.4	4.81

Average Medium Arterial
NaNO2 Ctrl

	#1	#2	Average	
	M	M	M	Std. Error
Initial	223.67	205.6	214.6	9.035
Baseline	252.05	203.3	227.7	24.375
10uM				
1 min	211.33	201.73	206.5	4.8
5 min	190.94	219.57	205.3	14.315
10 min	190.06	182.69	186.4	3.685
100uM				
1 min	168.42	229.29	198.9	30.435
5 min	178.93	192.43	185.7	6.75
10 min	173.85	186.78	180.3	6.465
1mM				
1 min	176.82	201.19	189.0	12.185
5 min	153.49	215.15	184.3	30.83
10 min	138.33	202.05	170.2	31.86
3mM				
1 min	174.22	198.02	186.1	11.9
5 min	163.4	205.65	184.5	21.125
10 min	162.77	202.7	182.7	19.965

Average Large Arterial
NaNO2 Ctrl

	#1	#2	Average	
	L	L	L	Std. Error
Initial	274.46	293.18	283.8	9.36
Baseline	270.26	285.29	277.8	7.515
10uM				
1 min	242.09	301.52	271.8	29.715
5 min	300.08	306.15	303.1	3.035
10 min	298.54	297.15	297.8	0.695
100uM				
1 min	254.55	311.47	283.0	28.46
5 min	275.66	288.62	282.1	6.48
10 min	259.3	284.24	271.8	12.47
1mM				
1 min	266.28	281.19	273.7	7.455
5 min	231.81	316.23	274.0	42.21
10 min	236.05	293.49	264.8	28.72
3mM				
1 min	214	286.88	250.4	36.44
5 min	246.58	315.5	281.0	34.46
10 min	244.9	311.19	278.0	33.145

NaNO2 #1 2/7/2008

Time Response

	Card #	212320		
	Wt.	348		
	MAP	S	M	L
Initial	106.3	24.88	195.44	333.63
Baseline	102.1	24.33	159.51	250.87
100uM HBOC	124.5			
1 min	140.6	18.21	177.98	262.58
5 min	138.5	18.93	189.51	250.61
10 min	135.4	14.91	162.45	260.86
100uM	132.4			
1 min	131.5	16.28	167.99	181.53
5 min	122.4	16.67	166.48	208.01
10 min	119.3	15.28	172.35	247.53
15 min	117.3	15.17	158.37	208.84
25 min	118.7	16.34	163.99	239.22
1mM	105.3			
1 min	104.3	14.77	180	241.74
5 min	115.3	15.1	143.59	217.18
10 min	105.3	14.72	166.75	216.73
15 min	118.6	15.38	148	191.72
25 min	120.3	15.25	177.41	171.03

Average MAP
HBOC 201
Time Ctrl

	#1 MAP	#2 MAP	Average MAP	Std. Dev MAP	Average ∆ MAP
Initial	80.5	91.3	85.9	5.4	85.9
Baseline	81.5	80.9	81.2	0.3	81.2
100uM	116.3	118.3	117.3	1	36.1
1 min	123.4	133.8	128.6	5.2	47.4
5 min	135.4	133.6	134.5	0.9	53.3
10 min	120.8	137.3	129.1	8.25	47.9
25 min	111.8	110.7	111.3	0.55	30.1
40 min	105.4	103.5	104.5	0.95	23.3
55 min	98.3	98.5	98.4	0.1	17.2
60 min	90.0	125.5	107.8	17.75	26.6

Average Small Arteriole
HBOC 201 Time Ctrl

	#1	#2	Average	Std. Dev	Average

	S	S	S	S	Δ S
Initial	27.87	13.8	20.8	7.035	20.8
Baseline	32.61	13.89	23.3	9.36	23.3
100uM					
1 min	33.48	16.46	25.0	8.51	1.7
5 min	30.86	18.36	24.6	6.25	1.4
10 min	34.38	17.39	25.9	8.495	2.6
25 min	36.38	15.48	25.9	10.45	2.7
40 min	35.34	12.46	23.9	11.44	0.7
55 min	30.87	17.83	24.4	6.52	1.1

Average Medium Arteriole
HBOC 201 Time Ctrl

	#1 M	#2 M	Average M	Std. Dev M
Initial	186.31	109.99	148.2	38.16
Baseline	203.3	102.41	152.9	50.445
100uM				
1 min	243.17	125.16	184.2	59.005
5 min	222.58	125.54	174.1	48.52
10 min	249.25	153.02	201.1	48.115
25 min	222.36	154.11	188.2	34.125
40 min	218.51	143.23	180.9	37.64
55 min	200.49	154.23	177.4	23.13

Average Large Arteriole
HBOC 201 Time Ctrl

	#1 L	#2 L	Average L	Std. Dev L
Initial	240.11	234.02	237.1	3.045
Baseline	266.64	189.38	228.0	38.63
100uM				
1 min	283.84	181.01	232.4	51.415
5 min	264.76	198.89	231.8	32.935
10 min	265.53	192.93	229.2	36.3
25 min	275.18	210.03	242.6	32.575
40 min	253.38	192.93	223.2	30.225
55 min	244.29	218.84	231.6	12.725

Average MAP
HBOC 201 + NaNO2

	#1 MAP	#2 MAP	#3 MAP	#4 MAP	#5 MAP	#6 MAP	Average MAP	Std. Error
Initial	84.2	81.5	78.3	113.3	86.4	70.1	85.6	6.000648
Baseline	84.8	80.8	81.3	112.9	90.1	80.4	88.4	5.125974
0.01uM	104.3	90.1	130.8	125.3	130.9	101.3	113.8	7.122379
1 min	110.5	129.5	124.8	158.9	142.9	120.3	131.2	7.060017
5 min	120.9	134.1	123.6	160.3	132.8	123.5	132.5	5.972865
10 min	119.5	124.5	125.4	142.7	130.1	126.7	128.2	3.231692
0.1uM	116.5	120.4	123.5	138.7	129.3	132.2	126.8	3.341424
1 min	114.3	124.8	122.7	135.3	128.1	121.5	124.5	2.863302
5 min	109.3	115.3	121.6	133.1	127.7	123.1	121.7	3.47682
10 min	104.3	115.1	120.3	133.1	132.3	120.3	120.9	4.429898
1uM	101.2	113.1	120.2	130.1	130.1	121.3	119.3	4.487514
1 min	102.3	113.1	119.5	129.1	128.3	124.1	119.4	4.193249
5 min	99.4	112.3	120.1	130.3	124.2	116.3	117.1	4.361269
10 min	92.4	110.7	118.3	127.1	118.3	109.8	112.8	4.811629
10uM	83.1	108.3	115.3	121.2	116.3	107.3	108.6	5.522585
1 min	60.1	105.1	114.2	124.3	111.3	105.4	103.4	9.1244
5 min	85.1	99.3	110.3	125.8	105.3	105.3	105.2	5.444289
10 min	84.3	96.3	105.3	124.6	103.8	98.3	102.1	5.429242
100uM	64.3	87.1	100.3	112.3	98.5	95.4	93.0	6.633974
1 min	35.1	86.3	91	107.0	78.4	88.8	81.1	9.964002
5 min	36.3	81.8	81	108.3	82.9	85.4	79.3	9.573067
10 min	26.1	78.3	82	113.1	79.0	80.3	76.5	11.45119

Average Small HBOC 201 + NaNO2

	#1 S	#2 S	#3 S	#4 S	#5 S	#6 S	Average S	Std. Error
Initial	23.22	32.72	32.74	28.98	22.15	14.31	25.7	2.933055
Baseline	22.56	31.98	25.74	29.43	25.46	17.23	25.4	2.11716
0.01uM								
1 min	22.43	33.85	27.05	28.69	25.95	21.99	26.7	1.790484
5 min	28.46	32.81	25.6	29.53	22.95	20.48	26.6	1.847311
10 min	28.3	30.81	27.42	31.35	23.44	21.55	27.1	1.607845
0.1uM								
1 min	27.48	34.97	26.21	29.89	24.91	22.48	27.7	1.778939
5 min	30.92	31.45	27.42	28.3	28.83	20.17	27.8	1.660473
10 min	21.42	33.84	27.32	27.43	19.02	20.08	24.9	2.324393
1uM								
1 min	25.4	37.13	24.37	31.75	20.46	21.15	26.7	2.652959
5 min	24.76	33	30.87	28.61	20.26	20.46	26.3	2.191389
10 min	24.44	34.14	28.54	31.88	24.21	21.65	27.5	1.989661
10uM								
1 min	27.73	36.46	29.62	29.3	19.93	22.64	27.6	2.372877
5 min	25.43	32.18	29.42	29.6	22.7	17.85	26.2	2.162207

10 min	25.05	34.8	23.07	29.63	22.57	20.37	25.9	2.187257
100uM								
1 min	23.81	34.47	23.7	30.03	22.44	21.92	26.1	2.060462
5 min	24.44	26.05	25	29.26	25.2	20.11	25.0	1.20437
10 min	23.27	34.12	24.39	28.61	24.13	22.76	26.2	1.793627

Average Medium
HBOC 201 + NaNO2

	#1	#2	#3	#4	#5	#6	Average	Std.
	M	M	M	M	M	M	M	Error
Initial	259.22	167.23	208.69	252.11	226.23	182.74	216.0	15.07174
Baseline	278	233.22	292.74	260.06	196.51	219.7	246.7	14.95282
0.01uM								
1 min	260.68	212.8	271.3	246.62	214.64	241.96	241.3	9.707937
5 min	277.62	218.51	225.59	247.52	222.3	243.1	239.1	9.054271
10 min	277.47	225.62	206.28	233.01	200	254.28	232.8	11.95484
0.1uM								
1 min	276.86	209.8	200.29	212.56	238.28	233.6	228.6	11.34299
5 min	287.25	210.75	218.72	227.36	213.41	252.03	234.9	12.10736
10 min	282.94	200.08	206.31	209.83	208.47	291.37	233.2	17.16153
1uM								
1 min	277.27	243.06	219.67	218.65	220.82	239.61	236.5	9.242351
5 min	268.9	242.01	255.36	199.22	200.38	255.05	236.8	12.21169
10 min	269.88	246.2	253.1	219.02	242.01	236.54	244.5	6.927665
10uM								
1 min	243.49	271.46	244.46	211.44	231.92	228.09	238.5	8.227369
5 min	268.83	270.48	238.66	222.83	228.37	212.4	240.3	9.922046
10 min	253.16	206.44	206.44	223.92	244.31	218.3	225.4	7.957731
100uM								
1 min	169.58	261.67	160.43	246.67	242.85	246.6	221.3	18.03513
5 min	172.26	225.24	178.38	243.67	243.75	255.89	219.9	14.66413
10 min	168.26	243.05	189.79	219.72	241.33	261.5	220.6	14.48683

Average
Large
HBOC 201 + NaNO2

	#1	#2	#3	#4	#5	#6	Average	Std.
	L	L	L	L	L	L	L	Error
Initial	272.59	262.26	238.72	309.02	290.77	240.36	269.0	11.35923
Baseline	346.67	268.75	309.12	267.39	234.06	264.53	281.8	16.23761
0.01uM								
1 min	290.08	307.74	310.5	288.84	261.57	287.34	291.0	7.177533
5 min	317.54	308.89	309.5	281.88	252.76	289.81	293.4	9.796424
10 min	334.14	304.88	293.81	293.62	250.47	282.01	293.2	11.20136
0.1uM								

1 min	329.12	251.53	326.29	267.13	245.43	280.62	283.4	14.89935
5 min	362.29	278.75	310.89	256.75	259.74	281.05	291.6	16.20204
10 min	358.43	260.64	326.7	249.87	255.47	266.23	286.2	18.41533
1uM								
1 min	363.2	365.07	273.68	256.74	263.87	266.26	298.1	20.98845
5 min	359.4	338.99	284.41	256.75	266.78	254.38	293.5	18.33908
10 min	357.3	309.14	278.34	249.87	243.38	285.5	287.3	17.1177
10uM								
1 min	372.25	336.38	279.29	246.15	241.7	255.57	288.6	21.95413
5 min	346.72	328.78	307.17	270.07	250.07	269.55	295.4	15.54685
10 min	295.86	311.31	306.72	238.15	251.01	286.99	281.7	12.34196
100uM								
1 min	185.41	273.29	260.65	306.38	305.86	289.02	270.1	18.46154
5 min	185.25	289.5	213.22	282.56	326.05	266.91	260.6	21.263
10 min	184.09	303.5	200.68	263.18	314.22	295.8	260.2	22.66027

Average MAP
HBOC 201 + NaNO2 Simultaneous

	#1 MAP	#2 MAP	Average MAP	Std. Error
Initial	104.6	87.8	96.2	8.4
Baseline	105.7	82.1	93.9	11.8
100uM (HBOC) + NaNO2	131.6	109.3	120.5	11.15
1 min	125.6	113.4	119.5	6.1
5 min	107.3	104.5	105.9	1.4
10 min	105.3	105.6	105.5	0.15
3 mM	95.4	81.3	88.4	7.05
1 min	92.5	81.6	87.1	5.45
5 min	98.3	80.4	89.4	8.95
10 min	105.7	82.1	93.9	11.8

Average MAP
HBOC 201 + NaNO2 Simultaneous

	#1 S	#2 S	Average S	Std. Error
Initial	31.74	29.14	30.4	1.3
Baseline	33.36	28.57	31.0	2.395
HBOC + 1 mM NaNO2				
1 min	28.66	33.44	31.1	2.39
5 min	28.16	29.91	29.0	0.875
10 min	34.68	30.68	32.7	2
3 mM				
1 min	43.84	32.9	38.4	5.47
5 min	40.22	31.65	35.9	4.285

10 min	42.53	33.73	38.1	4.4

Average Medium Arteriole
HBOC 201 + NaNO2 Simultaneous

	#1	#2	Average	Std.
	M	M	M	Error
Initial	222.72	231.97	227.3	4.625
Baseline	272.43	181.29	226.9	45.57
HBOC + 1mM NaNO2				
1 min	255.22	199.18	227.2	28.02
5 min	257.76	210.95	234.4	23.405
10 min	261.55	199.68	230.6	30.935
3 mM				
1 min	293.44	203.97	248.7	44.735
5 min	276.99	216.51	246.8	30.24
10 min	237.13	215.3	226.2	10.915

Average Large Vessels
HBOC 201 + NaNO2 Simultaneous

	#1	#2	Average	Std.
	L	L	L	Error
Initial	327.42	282.57	305.0	22.425
Baseline	333.36	212.91	273.1	60.225
HBOC +1mM NaNO2				
1 min	354.22	229.91	292.1	62.155
5 min	358.07	236.05	297.1	61.01
10 min	353.94	236.09	295.0	58.925
3 mM				
1 min	368.53	279.08	323.8	44.725
5 min	350.27	259.76	305.0	45.255
10 min	372.81	236.05	304.4	68.38

Average MAP
L-NAMEAND NaNO2

	#1	#2	#3	#4	#5	#6	Average	Std.
	MAP	MAP	MAP	MAP	MAP	MAP	MAP	Error
Initial	88.5	94.9	77.1	87.9	88.1	88.3	87.5	2.345729
Baseline	81.5	88.6	79.0	89.1	73.4	81.9	82.3	2.427722
5mg/kg	152.5	104.5	83.0	92.3	88.1	91.3	102.0	10.51867
1 min	157.7	136.5	123.5	123.8	137.3	131.3	135.0	5.145251
5 min	155.3	149.5	154.8	163.4	157.3	152.7	155.5	1.915028

10 min	140.1	132.5	148.5	145.4	153.7	149.3	144.9	3.09197
100 uM	123.1	148.8	146.8	135.7	151.1	149.1	142.4	4.465696
1 min	119.8	142.5	142.3	131.7	142.1	145.3	137.3	3.986178
5 min	117.3	135.8	139.1	125.8	135.3	141.7	132.5	3.754375
10 min	115.1	130.5	137.4	118.3	130.8	138.5	128.4	3.966835
1 mM	103.1	115.3	129.1	110.3	128.1	131.3	119.5	4.749012
1 min	105.1	110.2	121.8	101.3	120.2	125.7	114.1	4.045965
5 min	100.9	109.1	108.4	95.1	107.9	118.3	106.6	3.229078
10 min	98.9	102.3	98.7	92.1	104.8	101.4	99.7	1.780075
3 mM	98.7	98.3	95.4	89.1	100.8	100.7	97.2	1.802899
1 min	89.3	95.2	88.5	88.7	88.3	91.9	90.3	1.116069
5 min	80.5	92.3	81.3	84.3	84.0	80.3	83.8	1.843261
10 min	80.1	79.3	78.5	85.1	78.8	81.2	80.5	1.002331

Average Small Vessel Diameter
L-NAME + NaNO2

	#1	#2	#3	#4	#5	#6	Average	
	S	S	S	S	S	S	S	Std. Error
Initial	11.27	28.66	36.93	51.38	26.58	30.38	30.9	5.369891
Baseline	12.22	30.89	40.03	44.16	26.01	32.38	30.9	4.597882
5mg/kg						35.31		
1 min	13.17	31.46	36.09	47.86	29.96	30.16	31.5	4.578429
5 min	10.08	33.76	37.03	46.02	30.93	27.29	30.9	4.901912
10 min	8.75	33	33.08	41.42	30.76	30.76	29.6	4.475085
100 uM								
1 min	11.5	32.1	33.43	43.87	27.64	29.27	29.6	4.305442
5 min	11.72	29.7	34.92	39.29	29.88	27.42	28.8	3.843171
10 min	14.08	32.97	34.25	38.26	23.52	30.83	29.0	3.582622
1 mM								
1 min	12.7	28.54	32.64	46.55	27.21	26.27	29.0	4.463964
5 min	15.38	30.47	32.06	36.59	22.17	25.51	27.0	3.110094
10 min	13.98	31.18	31.5	35.73	23.89	26.99	27.2	3.124415
3 mM								
1 min	12.45	31.06	30.27	41.11	25.12	27.58	27.9	3.815031
5 min	11.5	28.58	25.67	40.17	24.12	21.45	25.2	3.823834
10 min	12.84	28.46	28.77	37.02	21.72	27.05	26.0	3.30539

Average Medium Vessel Diameter
L-NAME + NaNO2

	#1	#2	#3	#4	#5	#6	Average	
	M	M	M	M	M	M	M	Std. Error
Initial	175.13	301.52	133.65	213.95	160.47	160.2	190.8	24.60541
Baseline	153.4	258.95	150.04	231.89	182.08	196.13	195.4	17.67258
5mg/kg								

1 min	208.2	260.64	133.77	242.94	207.11	206.98	209.9	17.77747
5 min	202.27	231.54	137.6	207.75	229.29	169.89	196.4	14.87735
10 min	190.08	289.68	136.29	157.36	239.3	241.89	209.1	23.67871
100 uM								
1 min	165.62	299.59	141.87	177.26	214.19	243.86	207.1	23.67976
5 min	172.35	283.84	127.4	174.76	223.15	221.98	200.6	22.14988
10 min	183.6	264.7	119.63	177.48	222.53	242.99	201.8	21.41446
1 mM								
1 min	253.7	297.7	135.84	233.94	230.36	245.47	232.8	21.76988
5 min	262.63	293.7	126.25	225.94	252.33	216.52	229.6	23.51038
10 min	268.84	279.82	118.74	175.62	207.58	180.07	205.1	24.90357
3 mM								
1 min	201.41	254.5	119.85	221.14	224.87	159.05	196.8	20.06429
5 min	216.83	281.51	116.24	228.93	234.71	226.1	217.4	22.25012
10 min	239.26	270.25	123.84	183.72	237.13	223.55	213.0	21.18767

Average Large Vessel Diameter
L-NAME + NaNO2

	#1	#2	#3	#4	#5	#6	Average	
	L	L	L	L	L	L	L	Std. Error
Initial	331.88	325.43	281.43	323.3	248.06	296.66	301.1	13.23135
Baseline	290.56	315.65	298.07	315.3	282.64	300.9	300.5	5.392613
5mg/kg								
1 min	304.11	351.98	262.29	308.67	313.21	305.88	307.7	11.64801
5 min	380.39	344.56	286.14	270.58	349.55	311.96	323.9	17.01693
10 min	316	336.05	283.6	263.5	333.06	323.88	309.3	11.96618
100 uM								
1 min	367.96	353.6	246.86	267.19	328.76	304.87	311.5	19.53092
5 min	365.41	363.92	251	293.88	312.76	318.6	317.6	17.75196
10 min	373	341.27	250.31	270.59	313	303.59	308.6	18.34996
1 mM								
1 min	341.24	348.89	285.6	244.14	325.52	316.83	310.4	16.02911
5 min	332.49	374.4	294.8	282.56	289.77	320.03	315.7	14.08755
10 min	312.11	340.21	293.23	252.56	266.33	323.5	298.0	13.81009
3 mM								
1 min	345.33	348.32	295.67	253.81	305.88	259.83	301.5	16.50967
5 min	354.36	350.49	284.41	256.67	306.68	384.08	322.8	19.68488
10 min	353.99	331.04	298.18	276.47	312.86	318.48	315.2	10.8957

Average Large Vessel Diameter
L-NAME + NaNO2

	#1	#2	#3	#4	#5	#6	Average	
	L	L	L	L	L	L	L	Std. Error
Initial	331.88	325.43	281.43	323.3	248.06	296.66	301.1	13.23135
Baseline	290.56	315.65	298.07	315.3	282.64	300.9	300.5	5.392613
5mg/kg								
1 min	304.11	351.98	262.29	308.67	313.21	305.88	307.7	11.64801
5 min	380.39	344.56	286.14	270.58	349.55	311.96	323.9	17.01693
10 min	316	336.05	283.6	263.5	333.06	323.88	309.3	11.96618
100 uM								
1 min	367.96	353.6	246.86	267.19	328.76	304.87	311.5	19.53092
5 min	365.41	363.92	251	293.88	312.76	318.6	317.6	17.75196
10 min	373	341.27	250.31	270.59	313	303.59	308.6	18.34996
1 mM								
1 min	341.24	348.89	285.6	244.14	325.52	316.83	310.4	16.02911
5 min	332.49	374.4	294.8	282.56	289.77	320.03	315.7	14.08755
10 min	312.11	340.21	293.23	252.56	266.33	323.5	298.0	13.81009
3 mM								
1 min	345.33	348.32	295.67	253.81	305.88	259.83	301.5	16.50967
5 min	354.36	350.49	284.41	256.67	306.68	384.08	322.8	19.68488
10 min	353.99	331.04	298.18	276.47	312.86	318.48	315.2	10.8957

Vita

Michael Youn-Il Kim was born on November 5, 1983 in Silver Springs, Maryland. He was raised in McLean Virginia and graduated from Langley High School, McLean, Virginia in 2002. He received his Bachelor of Arts in Biology and a minor in Sociology from the University of Virginia, Charlottesville, Virginia in 2006. Michael was enrolled in graduate studies at the Medical College of Virginia in 2006, Richmond, Virginia. He plans on one day pursuing a career in medicine.