Update #3 from the Lab Team

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Hi everyone,

My name is Sophia and it is now my turn to update you on the adventures of the Lab Team. Our adventures are somewhat different from those of the Ice Team, but just as exciting none-the-less.

As you may already know, each member of Dr Hawke's Lab team is investigating a different aspect of Weddell seal muscle tissue. Peter is examining muscle stem cells, Jesse is determining fibre type and intramuscular lipid content and I will be investigating myoglobin, vasculature and intramuscular adiposity. While we each have our own area of focus, at the end of the study we hope to pull our results together and not only describe what satellite cells, intramuscular fat, myoglobin and vasculature looks like in the Weddell seal, but also how each relates to one another. The various relationships among these cellular components are probably the most interesting aspect of our study. The 2005 expedition provided us with a good foundation of knowledge about the Weddell seals. It is now our job to confirm last year's findings and discover how each part affects the other. However, before we dive into the world of cellular interdependence, I would like to discuss the role of the cardiovascular system and myoglobin in the animal model.

What is the cardiovascular system and how does it work?

The cardiovascular system can be thought of as one long, continuous, intertwining channel. This channel consists of the heart that acts like a pump; the arteries take the blood away from the pump and to the working tissues; the capillary networks aid in exchange of materials; and veins carry the blood back to the heart. (Figure 1). Each of these components is equally important and plays a vital role in keeping the rest of the body functioning properly.

When oxygen (O_2) is taken into the lungs, the pulmonary vasculature takes the O_2 molecule from the alveolar sacks, found in the lungs and transports it to the heart with red blood cells. *Hemoglobin* is a small protein molecule found on red blood cells and it is responsible for carrying O_2 throughout the entire cardiovascular system. Each hemoglobin molecule transports up to four O_2 molecules around the body as if on piggy-back. When oxygen is bound to hemoglobin the complex is called *oxyhemoglobin*. Oxyhemoglobin travels from the lungs, freshly loaded with O_2, to the heart. Since every type of muscle needs oxygen in order to function, when oxyhemoglobin reaches the heart some of the oxygen it is carrying is used by the heart, while the rest is pumped into the aorta, the main artery leaving the heart and the arterial system.

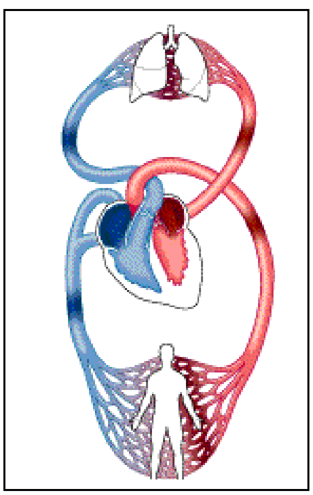

Figure 1. Cardiovascular system is broken down in to the pulmonary circuit that extends into the lungs and supplies the heart with oxygen and the systemic circuit which branches out from the heart and extends throughout the entire body. The red portions depict the blood being pumped out of the heart through the arteries and arterioles, while the blue represents the blood coming back to the heart via venous return. (1)

Arteries and arterioles have very thick walls in comparison to the other vessels in the cardiovascular system. This allows arteries to maintain their structure when blood is pumped forcefully through them from the heart. This pressure is normally measured by the family physician to see if the heart is working well. If the doctor finds an individual to have high blood pressure that means that cardiovascular system might be in danger; and since the cardiovascular system is responsible for making the rest of the body function properly, your overall health might become compromised.

As blood is transported throughout the body, it flows away from the heart to arteries and then to arterioles and finally into the capillaries. The thick walls in the arteries are well designed to withstand the high pressure created by the heart pump. Capillaries can be found around every organ and every tissue of the body. They are only one cell thick and are able to exchange oxygen freely with surrounding tissue. Capillaries usually branch out into hundreds of little vessels that surround tissues. These capillary clusters are referred to as *capillary beds*, which provide incoming oxygen to the tissue and take away any unneeded materials or wastes.

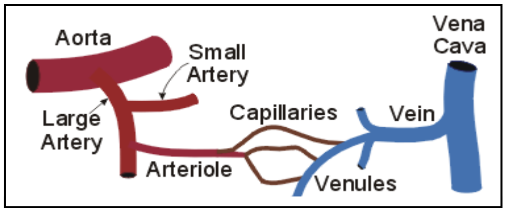

Figure 2. Schematic showing the relative size and number of each type of blood vessel in the cardiovascular system. The larger vessels like the arteries and veins are thicker in diameter and few in number, while the smaller vessels like the capillaries are very thin and can be found in abundance throughout the body (2)

How is oxygen removed from the cardiovascular system and taken up by working tissue?

When blood is pumped out of the heart it travels from a high pressure and oxygen rich environment to an area of lower pressure and oxygen depleted tissues. The exchange of oxygen happens through diffusion, a process in which something moves from an area of high concentration (O_2 in the blood) to an area of low concentration (very little O_2 in tissue). As mentioned previously, hemoglobin in the blood acts as a vehicle for O_2 transport throughout the body. When oxyhemoglobin reaches an area of low oxygen content it will release its O_2 molecules, which will travel into the cell. Once inside the cell, O_2 is picked up by myoglobin. *Myoglobin* is a protein molecule similar to hemoglobin and is primarily found

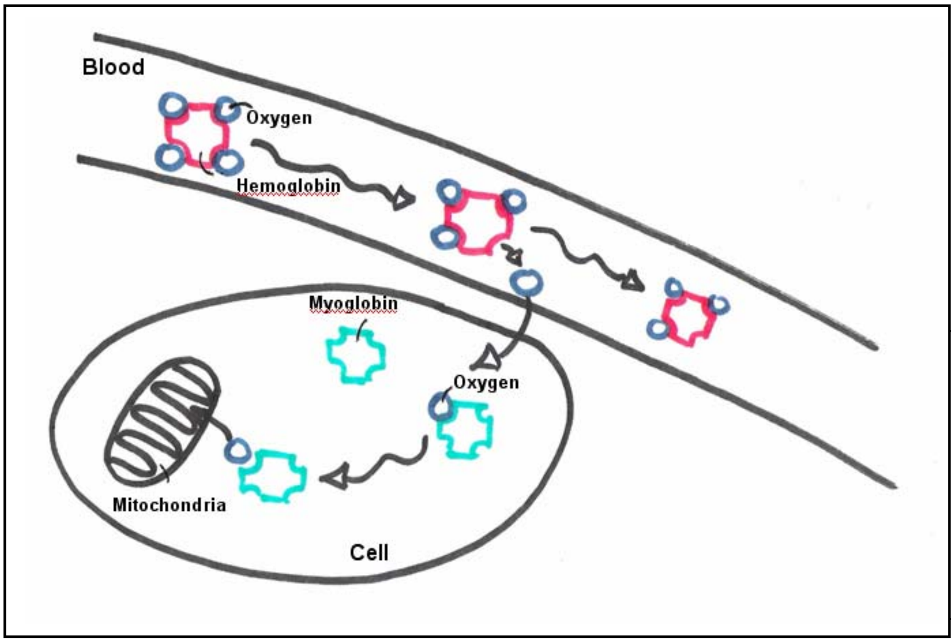

Figure 3. The fate of oxygen as it travels down the capillary. Once oxyhemoglobin reaches cells that need oxygen, it releases-its O_2 molecules. They enter the cell and attach to myoglobin that will either deliver the O_2 molecules to the mitochondria to make energy or it will hold on to it creating an oxygen reservoir.

inside striated muscles. The main responsibility of myoglobin is to pick up free floating O_2 molecules in the cell and then supply them to the mitochondria, where they are vital to the cell's energy making process. Therefore, if the muscle is active, myoglobin will continuously look for O_2 molecules in the cell and transport them to mitochondria to keep the tissue working, as seen in Figure 3. On the other hand, if the muscle is resting, myoglobin will hold on to as many oxygen molecules as it can to store for later use.

For example, if you are helping your mom and dad with the dishes, the myoglobin in the muscles of your arms and hands will continuously pick up O_2 molecules from incoming hemoglobin and donate them to the working mitochondria to create energy and make your arms move. While the myoglobin in the muscles that are not active, like your leg muscles, will remain inactive and continue to store O_2 molecules that it picked up earlier until they are needed.

Why is myoglobin so important when studying the Weddell Seal?

There are several reasons why we are interested in studying myoglobin in Weddell seals. First and foremost, myoglobin acts as an oxygen reservoir in muscle. As Peter mentioned in the first update, adult Weddell seals have the amazing ability to hold their breath for 80-90 minutes while seeking prey and avoiding predators underwater. Their muscles are not all that different from ours; they too need to continuously supply oxygen to working tissue. If oxygen is not delivered in time the cells will become ischemic (oxygen starved) and will start to die. So why is it that Weddell seals can exercise for so long without coming up for air while maintaining the integrity of their muscles? In addition, what changes occur in the seal as it matures from a land bound pup to a modest diver juvenile and to an elite swimming adult?

My hypothesis is that changes in myoglobin amount and distribution, capillary density and intramuscular fat as the Weddell seal grows from a pup to a juvenile to an adult contribute to this amazing development.

Pup/Non diver | Juvenile/Novice diver | Adult/Elite diver

Figure 4. The Weddell seal undergoes significant physiological changes as it matures from a pup to a juvenile to an adult.

What are the possible relationships between myoglobin content and distribution, capillary density and intramuscular fat?

1. One of my goals is to look at the changes in intramuscular content and distribution of myoglobin in Weddell seals as they age. I hypothesize that myoglobin content will increase with age for two reasons. First, studies have shown that myoglobin tends to transiently increase hours after muscle injury and during

hypoxic stress (1); and second we know that as the seal grows, it starts to venture out into the water for longer periods of time. The more time it spends underwater the longer his/her muscles are deprived of oxygen and are under hypoxic stress. It is possible that the longer swimming times are causing for a permanent increase in myoglobin content. This larger oxygen reservoir might be one of the reasons why adult seals are able to swim for more prolonged periods each time.

2. Another common finding is an increase in capillary beds around exercising muscles. I hypothesize that there will be an increase in capillary density as the Weddell seals evolve from non-divers to an elite divers. The more the seals swim, the more they use their muscles. I suspect a proportional increase in capillary density around the swimming muscles as the seal's swimming ability and duration increases. The reason behind this hypothesis is the fact that extra capillary beds will create extra access for the cardiovascular system. Therefore there will be more oxygen being delivered to the muscles that are used most during exercise allowing it to swim longer.

3. Previous research has also shown that intramuscular lipids aid in O_2 transport within muscle, much like myoglobin. We know intramuscular fat stores change as the seal matures from a pup to a juvenile to an adult. It is possible that this is another factor that is helping Weddell seals become expert divers. I hypothesize that as the seal grows, the extra fat stores are helping myoglobin become more efficient. Therefore, the extra intramuscular fat the seal is gaining will allow him/her to stay under water longer and avoid ischemia.

4. I will also be looking at how myoglobin concentration relates to intramuscular fat distribution. Since Jesse's experiments will determine how much fat is within each of the different fibre types as the seal grows, we will be able to pull our findings together and determine (1) how fibre types change as the seal matures; (2) how intramuscular fat changes as the seal matures and whether the changes coincide with fibre type changes; (3) how myoglobin content distribution changes and if it is related to intramuscular fat changes and fibre type changes; (4) how capillary density changes and if those changes are proportional to intramuscular fat gain.

How can we determine if these hypothesized interdependent relationships really exist?

The procedures I will use to investigate the hypotheses include: Oil-Red-O staining to look at intramuscular fat droplets, Lead ATPase staining to identify vasculature, and Myoglobin immunohistochemistry (IHC) to determine myoglobin distribution. The goal is to do these procedures on serial muscle sections. Since cryostat (explained last week by Jesse) makes incredibly thin slices (8µm) of the muscle fibres, serial slices look identical to one another. Therefore, if one section is stained with Oil-Red-O and the other with Myoglobin IHC, I will be able to determine where the fat droplets are inside the muscle, where the myoglobin is inside the muscle, and how the two relate to one another. This type of overlaying procedure can also be done with the Lead ATPase stain. The combination of this stain with the other two will clearly show the vascular development in muscle tissue and how it relates or is affected by myoglobin and intramuscular fat distribution.

I will overlay the three differently stained muscle tissues for Weddell seal pups, juveniles and adults to see if this relationship changes with age and diving ability. If you are interested in a detailed description of how staining procedures work please refer back to update #1 and update #2.

After performing different staining procedures, muscle cross-sections need to be analyzed with a microscope. Figure 5 depicts the real-life user interface that we use to control our microscope; we can manipulate anything from lens changes to exposure time through the touch of a button.

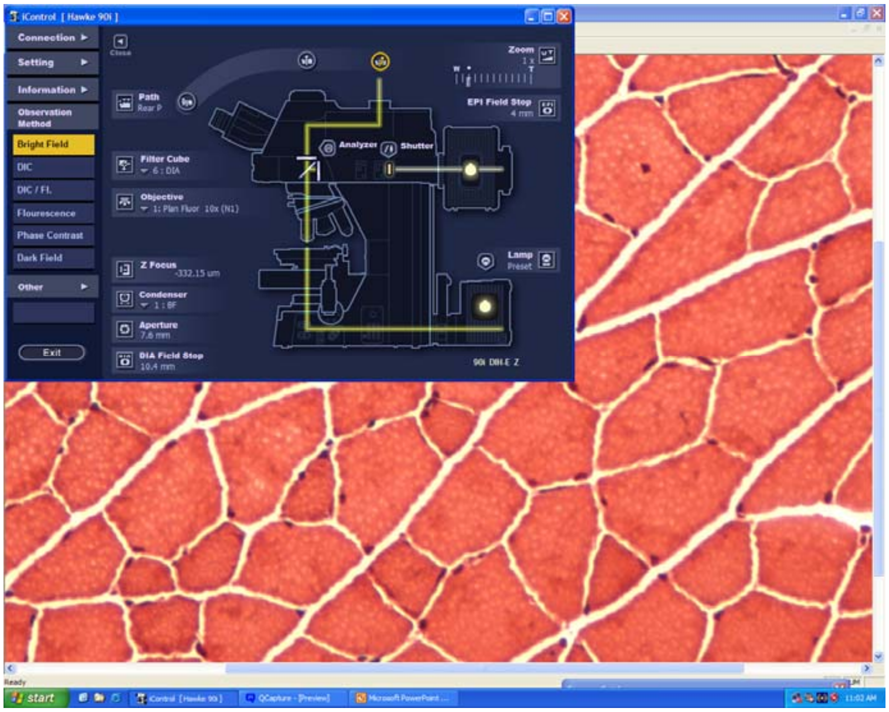

Figure 5. Computer software that allows the user to analyze muscle cross section. Once the slide is placed under the microscope lens the user controls the entire system using computer soft ware. The top left corner shows the microscope software, which allows the user to photograph cells. The rest of the screen shows a muscle cross-section that is currently being worked on by the user.

As you can see in Figure 6, when the two serial cross-sections are placed on top of one another, we are able to see the interactions between the two colors giving the slide a purple appearance. Don't be fooled by how easy this looks! It is very difficult and time consuming! While Figure 6 is only a demonstration of how two serial sections dyed with different stains interact, the same method will be used when Jesse, Peter and I start staining Weddell seal muscle sections for different cellular constituents and looking for patterns in their interactions.

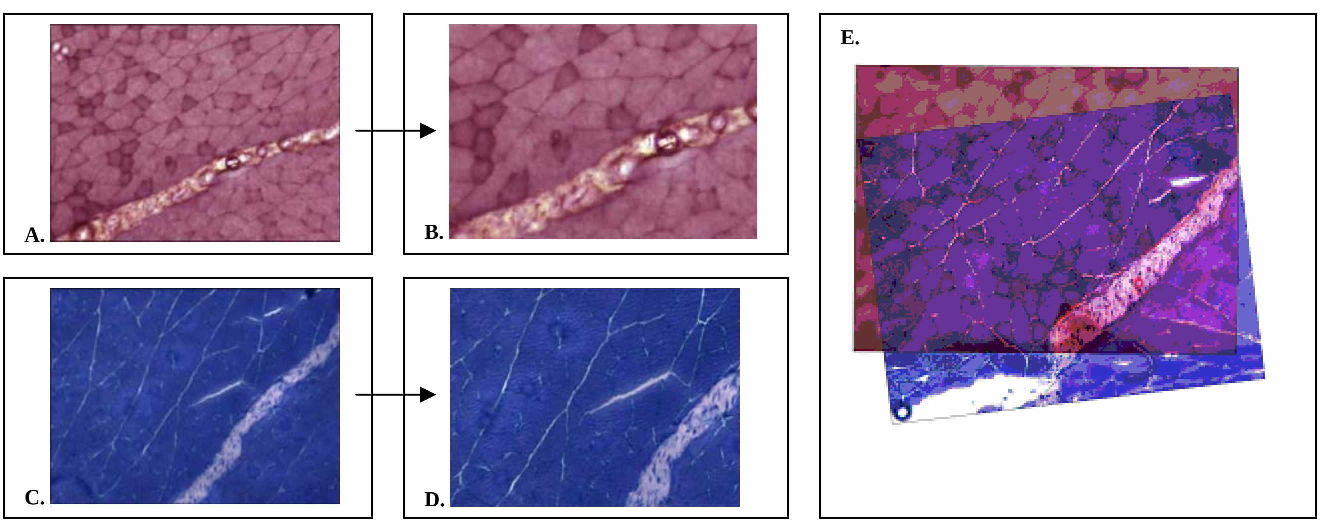

Figure 6. Example of how a muscle overlay would be performed. **A** Mouse gastrocneimus muscle (calf muscle) cross-section stained with Hematoxylin; **B.** magnified image of Figure A; **C.** Mouse gastrocneimus muscle (calf muscle) cross-section stained with Toluidine blue; **D.** magnified image of Figure C; **E** an overlay of two serial sections using hematoxylin and Toluidine blue to get a combined effect.

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Over the next several weeks I will continue to prepare mouse gastrocnemius serial cross-sections. I am going to learn and perfect my Oil-Red-O staining and Lead ATPase staining technique on the mouse sections and hopefully graduate to using seal tissue.

If you have any questions for the Lab Team feel free to email myself, Peter, Jesse or Dr. Hawke and we will gladly answer your questions.

Challenge Question #1

Myoglobin molecules from which muscle groups will donate O_2 molecules to mitochondria when you are:

a) Running
b) Playing basketball
c) Eating

Challenge Question #2

What are some of the difficulties Peter, Jesse and I may encounter when attempting to combine serial muscle cross-sections?

If you would like to learn more about last year's expedition in Antarctica please refer to the archives on the Polar Science website

Figure References

Figure 1: Klabunde, Richard E. *Cardiovascular Physiology Concepts*. Retrieved October 20, 2006 from http://www.phschool.com/science/biology_place/biocoach/cardio2/intro.html

Figure 2: Addison Wesley Longman. *BioCoach Activity*. Retrieved October 20, 2006 from http://www.cvphysiology.com/Blood%20Pressure/BP019.htm

Text Reference

1. Arkhipov, A *et al*. *Myoglobin Case Study*. Retrieved October 20, 2006 from http://www.ks.uiuc.edu/Training/CaseStudies/pdfs/myoglobin.pdf