

Lab #2: Blood pressure and peripheral circulation

Vertebrates have a **closed circulatory system** where the blood is always enclosed within blood vessels or the heart. Blood is pumped from the **heart** (the central pump) to the **vasculature**: the **arteries**, **capillary beds** (sites of delivery to tissues), the **veins**, and back to heart. Two important implications are that: (1) **blood pressure varies along the circuit** and (2) **blood pressure can be regulated** at points along the circuit.

For very active animals (e.g., mammals and birds) or very large animals (especially very tall animals that have more gravity to resist), the ability to regulate blood pressure is critical — active animals will need more oxygen delivered at a faster rate, especially to the most metabolically active tissues, and larger animals will require much more pressure to reach all of their tissues.

Blood pressure in the arteries leaving the heart are always at very high pressure as compared to the low pressure in the veins in the legs or the even lower pressure in capillary beds at the tissues. Blood pressure drops as the blood vessels branch again and again, increasing the cross-sectional area of the circuit, until it reaches the capillaries where the tissues experience **relatively constant, low pressure** to facilitate **diffusion**. The slow flow at the capillaries facilitates diffusion of oxygen, nutrients, and carbon dioxide and other wastes between the blood and the tissues that are bathed by the capillaries. Therefore, pressure varies depending on how **far the vessels are from the heart**, the **cross-sectional area** of the blood vessels, as well as **gravity**. However, at any given point along the circuit, blood pressure remains fairly constant.

At most times, blood pressure is regulated to maintain a relatively constant pressure, however, there are times when circulation needs to be adjusted locally. A well-known example is the **Fight-or-Flight response**, which occurs, for example, when an animal sees a predator or anticipates a fight. The sympathetic nervous system dominates and causes a ramp-up of circulation to deliver more energy to the skeletal muscles: increased **cardiac output** (= **heart rate** x **stroke volume**) and **blood pressure**, and increased blood flow to the lungs and skeletal muscles. Adjustments to blood flow are not simply an adjustment of heart function, but also constriction or relaxation of the **vasculature** (blood vessels: arteries, veins, capillaries). Constricting blood vessels will reduce their cross-sectional area and increase blood pressure and flow.

Local changes in circulation are under **nervous** and **hormonal** control. Regulation of blood flow in the vertebrate circulatory system occurs by three primary mechanisms: 1) **local receptors** (nervous system) to detect levels of metabolic activity (e.g., carbon dioxide receptors), 2) **sympathetic** and **parasympathetic** (autonomic nervous system) control of the vasculature including capillary beds at the tissues, and 3) **endocrine** (hormonal) control of the vasculature.

In this lab, we will measure blood pressure of volunteers using a finger pulse transducer, a stethoscope, a blood pressure cuff (sphygmomanometer), and changes in peripheral circulation by measuring the volume of the extremities using a belt with a force transducer. We will do a series of learning exercises and then conduct an experiment on changes in peripheral circulation during simulated dives (the dive response).

Equipment

PowerLab
Finger pulse transducer
Stethoscope
Blood pressure cuff
Blood pressure gauge (sphygmomanometer) with pod port

Exercise 1: Measurement of blood pressure

The pressure in the arteries varies during the cardiac cycle. The largest muscles of the heart are in the **ventricles**. Blood pressure is at its highest immediately after the **ventricles** contract to push blood into the arterial system (**systolic pressure**) and declines as the ventricles relax to fill with blood before pumping again. Just before the ventricles contract, blood pressure is at its lowest (**diastolic pressure**).

Traditionally, systemic arterial blood pressure is measured using a **stethoscope** and a blood pressure cuff connected to a blood pressure gauge called a **sphygmomanometer** (sss-fig-no-ma-nom-eter). The sphygmomanometer is calibrated in pressure units of mmHg (millimeters of mercury). Modern instruments use compressed air as a hydraulic fluid to transmit the force of your pulsing blood.

1. Measure blood pressure on a human volunteer using **auscultation** (listening through a stethoscope) and a sphygmomanometer (protocol 4.1). Repeat allowing each group member to take the measurement.
2. Measure blood pressure using the PowerLab system and LabChart (protocol 4.2). Measure each group member several times, making sure to comment your data trace.

Questions for thought...

1. Does the time of the first Korotkoff sound (systolic pressure heard through the stethoscope), correspond with the first appearance of blood flow (as measured by the finger pulse)? Why or why not?
2. Would slowing the rate of pressure release from the cuff make your readings of the first appearance of blood flow more accurate? What problems might be caused by slowing pressure release?
3. Does the time that diastolic pressure is heard through the stethoscope correspond with anything particular in the blood flow signal? Can you, therefore, use pulse measurement to replace the stethoscope?
4. How much variation in measurement of diastolic and systolic pressures was observed within and between individuals? What are potential sources of variation in these estimates?

Exercise 2: Measurements of peripheral circulation

Objectives

To demonstrate basic principles of peripheral circulation using blood pressure data from the extremities. What you would expect based on relative distance from the heart and gravity (and whether the location

is above or below the heart)?

Procedure

1. Brainstorm with your lab group to develop some simple experiments to demonstrate principles of peripheral circulation. What are some good hypotheses for peripheral blood pressure?
2. What are some good locations to measure (or other simple manipulations) for comparison? Make sure you place the stethoscope on a major artery or vein such as the medial vein on the forearm, or the small saphenous vein on the calf. Ask for help if you don't know where they are. Be specific when you write up your methods or we will not understand what you did.
3. For each experiment, **determine both systolic and diastolic blood pressure.**

Notes:

1. *You will need to recalibrate the blood pressure force transducer after each time you move the cuff*
2. Place the instruments directly on the skin (not through clothes).
3. When measuring from foot, please wash toe before attaching pulse transducer to prevent any fungal contamination.
4. *****Always Release the cuff pressure as soon as you are done taking data*****

Analysis

Compare systolic and diastolic pressure for each of your treatments versus an appropriate control. Choose well to best test your hypotheses.

Questions for thought...

1. Were there any significant differences in the blood pressure obtained from your treatments?
2. How much does blood pressure change for each treatment? What could explain it? Does it seem reasonable?
3. How much variation is there among members of your group? What are sources of variation in these estimates?

Exercise 3: The Dive Response

When an air-breathing animal dives, it voluntarily holds its breath while the tissues continue to use oxygen. The **dive response** is a reflexive response that reorganizes circulation to maintain blood flow to the most essential organs -- the brain, eyes, and myocardium (heart muscle), while reducing blood flow to the peripheral tissues including musculature of the limbs and thorax, lungs, and renal system. Remarkably, all vertebrates have a dive response. The responses vary greatly between taxa, with some of the most pronounced being in whales and diving seals.

A primary feature of the dive response is a **diving bradycardia** (slowing of the heart rate), which results in a dramatic drop in **cardiac output** (cardiac output = **heart rate** x **stroke volume**). You might think that this would result in a dramatic drop in blood pressure as well, but in addition to reduced cardiac output, another component of the dive response is **peripheral vasoconstriction**, where the blood vessels of the peripheral tissues are constricted or even closed. As a whole, the dive response preserves circulation around the vital organs while cutting it off to the peripheral tissues. Oxygen becomes depleted and carbon dioxide and lactate build up in the tissues during a dive. When the animal resurfaces, there is a recovery period characterized by more rapid heart rate and ventilation to absorb

more oxygen and flush out lactate and carbon dioxide.

Objectives

You will investigate the effects of the diving response on heart rate and peripheral circulation in humans during simulated dives. First, you will examine the effect of holding your breath, then you will examine the effects of simulated dives. Finally, you will do a series of experiments to determine which stimuli contribute to triggering the dive response.

Additional Required Equipment

Respiratory Belt Transducer

Wash basin, Ice, Thermometer

Paper strips, Duct tape

Use the Dive settings file

Procedures

One person will serve as the experimental subject. If time permits, each person in the group should take turns being the experimental subject in order to have sufficient data.



Figure 6. Attachment of the respiratory belt transducer to the calf for leg volume measurement.

A. Set up and calibration

1. Use the Exercise 2 setup with the addition of the Respiratory Belt Transducer to input 3 to measure leg volume. Ask your TA for the proper settings.
2. Attach the respiratory belt snugly to the calf (Fig 6). Place the sphygmomanometer cuff around the subject's thigh. Duct tape may be required to secure the cuff in place.
3. Record for 10 seconds and stop. Scale the Pulse channel and the Leg Volume channel (channel 3) to fully display the data.
4. Open the digital stopwatch (WatchIt) from the computer's software icon set (bottom of screen).

Measuring peripheral circulation using leg volume changes (the idea)

You will measure peripheral circulation by assessing venous pooling in the leg for a standard time interval. By cutting off circulation in the leg for 30 sec. using the sphygmomanometer cuff on the upper thigh, venous return of blood will be prevented, causing the blood to pool. The resulting leg volume difference (maximum-minimum: T1-T2; Figure 7) is a measure of peripheral circulation. The respiratory belt transducer senses stretch and can be used to measure relative calf volume (Figure 6). Compare resting, experimental, and recovery periods.

Getting the leg volume measurement:

1. Set the Marker to a region just prior to cuff deflation in the leg volume channel (Figure 7).
2. Use the Waveform Cursor to determine the relative change in leg volume for the data trace when the cuff was deflated.

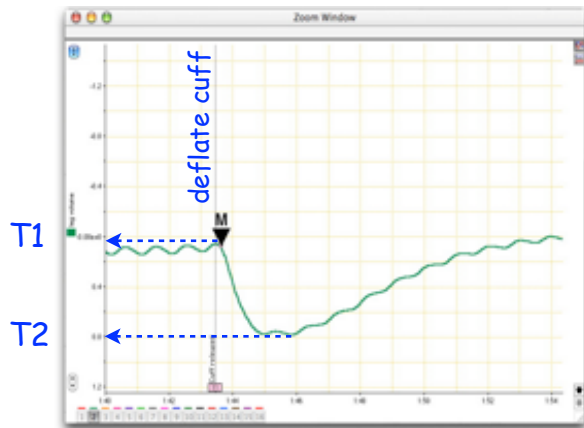


Figure 7. Zoom Window view of Marker and Waveform Cursor tools for analyzing leg volume change during a simulated dive.

B. Experimental Procedure: Breath holding

1. Place the respiratory belt on the calf (Figure 6) and the sphygmomanometer cuff on the upper thigh. You may need to use duct tape to close it.
2. Record the subject's resting pulse for 15 seconds.
3. **Measure leg volume measurement at rest:** Inflate to 60 mmHg, hold it at that pressure for exactly 30 seconds, then quickly release the pressure (Figure 7). ****Deflate cuff after taking data****
4. **Measure during breath hold (~1min):** Have the subject take one or two deep breaths, exhale partially, and then hold their breath while they place their head down on the lab bench. Comment to mark the start of breath hold. Use the timer DVM window to help keep track of time. Measure leg volume 30 seconds into the breath-hold. ****Deflate cuff****
5. **Measure during recovery:** at 30 sec into the recovery. Comment. ****Deflate cuff****

C. Experimental Procedure: Dive response

1. Fill your bucket with icewater deep enough to submerge your face up to your temples. Use a thermometer to monitor temperature. *Note: The receptors that trigger the dive response are in the temples, so it is critical that the temples be submerged in order to see the dive response.*
2. **Resting:** Have the subject stand or sit in front of the cooler, position their face just above the water's surface, and remain motionless. In this position, record the subject's resting pulse for 15 sec. Perform a leg volume measurement, and continue to record the subject's resting pulse for an additional 15 seconds.
3. **Simulated Dive:** (Practice simulated dive and recovery with a dry run before conducting the experiment.) While recording, have the subject take a deep breath, exhale partially, and then hold their breath while immersing their face up to their temples in the pan of water for 30 sec. Perform a leg volume measurement. One member of the group should tap the subject on the back at 10-second intervals to help them keep track of the time and prevent anxiety. Comment start and end of dive. **NOTE: Minimize movement in the Finger Pulse Transducer. NOTE: Do not force the subject to remain submerged.**
4. **Recovery:** Tap the subject on the back to tell them to surface. Comment. Make sure the subject remains still. After 30 seconds of recovery, perform a leg volume measurement. Continue to record for another 15 seconds.

Analysis

1. Compare the heart rate and leg volumes before, during and after a breath-hold.
2. Compare the heart rate and leg volume before, during and after simulated dives.

Questions for thought...

1. Compare your results of heart rate during breath holding with those from simulated dives. Are they the same?
2. What factors could explain differences between breath holding and a “dive”? Have you eliminated any hypotheses?
3. Compare the percent change in heart rate during dives among the members of your group. Is the relative bradycardia similar between individuals? Or is it the absolute difference that is similar?
4. Do your results for leg volume suggest that peripheral circulation changes during a breath-hold?
5. Did your peripheral circulation increase or decrease during a “dive”?
6. Why do you think the diving response is considered advantageous?

Additional Experiment

The experiments above provide a basic demonstration of the dive response. However, the experiment involved multiple stimuli simultaneously. Brainstorm how you might identify the components which are actually “triggering” the dive response by isolating stimuli. Are these components all necessary? Are they additive?

Each group should design an experiment to isolate one potential stimulus responsible for triggering the dive response and perform it. Get your idea approved by your TA. Share your results with the other groups. Make sure you explain your methods carefully (including your logic) in your lab report.