1 Purpose and Background

Purpose:
The purpose of the lab is to learn about measuring the ECG and blood pressures and observing the effects of exercise on blood pressure, heart rate, and electrocardiogram (ECG).

Background

This lab will build on the class material we have covered on blood flow and pressure, cardiac contraction, regulation of heart rate and function, and the ECG.

To prepare, please review the notes and text on the ECG, the cardiac cycle, and blood pressure measurements and also read the section in your text (or any other good physiology book) on exercise.

1.1 Blood Pressure measurement

See the web site [www.ktl.fi/publications/ehrm/product2/part_iii3.htm](http://www.ktl.fi/publications/ehrm/product2/part_iii3.htm) for a description of this measurement.

Arterial blood pressure is measured by a sphygmomanometer. This consists of:

1. A rubber bag surrounded by a cuff.

2. A manometer (usually a mechanical gauge, sometime electronic, rarely a mercury column).

3. An inflating bulb to elevate the pressure.

4. A deflating valve.

Figure 1 below shows how blood pressure is measured. After the cuff is placed snugly over the arm, the radial artery is palpated while the pressure is increased until the pulse can no longer be felt, then 30 mm Hg, more. As the pressure is released the artery is palpated until the pulse is felt again. This palpatory method will detect systolic pressure only.

The auscultatory method detects diastolic as well as systolic pressure. The sound heard when a stethoscope bell (or diaphragm) is applied to the region below the cuff were described by Korotkow in 1905 and are called Korotkow’s sounds.
Figure 1: Schematic diagram of arterial blood pressure measurement by Korotkow sounds.

The artery is compressed by pressure and as the pressure is released the first sound heard is a sharp thud which becomes first softer and then louder again. It suddenly becomes muffled and later disappears. Most people register the first sound as Systolic, the muffled sound as the first diastolic and the place where it disappears as the second diastolic. It requires practice to distinguish the first diastolic, so, for our laboratory, we will record only the first sound (systolic) and the disappearance of the sound (second diastolic). These will not be difficult to elicit, and a little practice will enable you to get the same reading on a fellow student three times in succession.

1.2 Sources or error in blood pressure measurement

1.2.1 Faulty Technique

1. Improper positioning of the extremity. Whether the subject is sitting, standing, or supine, the position of the artery in which the blood pressure is measured must be at the level of the heart. However, it is not necessary that the sphygmomanometer be at the level of the heart.

2. Improper deflation of the compression cuff. The pressure in the cuff should be lowered at about 2 mm Hg per heartbeat. At rates slower than this venous congestion will develop and the diastolic reading will be erroneously high. If the cuff is deflated too quickly the manometer may fall 5 or 10 mm Hg between successive Korotkow sounds, resulting in erroneously low readings.

3. Recording the first Blood Pressure. Spasm of the artery upon initial compression and the anxiety and apprehension of the subject can cause the first blood pressure reading to be
erroneously high. After the cuff has been applied, wait a few minutes before recording the blood pressure. Make several measurements. Generally the third value recorded is the most basal.

4. Improper application of the cuff. If the rubber bladder bulges beyond its covering, the pressure will have to be excessively high to compress the arm effectively. If the cuff is applied too loosely, central ballooning of the rubber bladder will reduce the effective width, thus creating a narrow cuff. Both bulging and ballooning result in excessively high readings.

1.2.2 Defective Apparatus

A defective air release valve or porous rubber tubing connections make it difficult to control the inflation and deflation of the cuff. The aneroid manometer gauge tube should be clean.

If an aneroid manometer is used, its accuracy must be checked regularly against a standard manometer. The needle should indicate zero when the cuff is fully deflated.

2 Procedure

The lab is divided into 4 sections, the first 3 of which you should do in parallel in the usual paired groups. Half the class should start with the blood pressure measurements while the other half starts with the ECG measurements. For the final section, please form groups of 4–7 as this exercise needs more people to carry out.

2.1 Arterial blood pressure measurement

The subject lies down with both arms resting comfortably at his sides or sits quietly with arm hanging down, elbow slightly bent—the goal is to have the arm at the same level as the heart. Wrap the sphygmomanometer cuff about the arm so that it is at heart level. The air bag inside the cuff should overlay the anterior portion of the arm about an inch above the antecubital fossa—the depression on the inside of the elbow joint. Note the “Artery” label and the arrow that should sit over the center of the inside of the elbow. The cuff should be wrapped snugly about the arm.

**Palpatory method:** Palpate the radial pulse with the index and middle fingers near the base of the thumb on the anterior surface of the wrist. While palpating the radial pulse, rapidly inflate the cuff until the blood pressure manometer reads 200 mm Hg pressure. Set the valve on the rubber bulb so that the pressure leaks out slowly (about 5 mm per second). Continue palpating the radial pulse, and watch the manometer while air leaks out of the cuff. Note the pressure at which the pulse reappears.

Record the pressure: ______ mm Hg.

This is Systolic pressure as detected by palpation. Allow the pressure to continue to decrease, noticing the changes in the strength of the radial pulse.

**Auscultatory method:** Elevate the pressure in the cuff 20 mm Hg higher than the pressure at which the radial pulse reappeared in A. Apply the stethoscope bell lightly against the skin in the antecubital fossa over the brachial artery. There will be no sounds heard if the cuff pressure is higher than the systolic blood pressure because no blood will flow through the artery beyond
the cuff. As the cuff is slowly deflated, blood flow is turbulent beneath the stethoscope. It is this turbulent flow that produces Korotkow’s sounds. Laminar flow is silent. Thus when the cuff is deflated completely, no sounds are heard at the antecubital fossa. Deflate the cuff completely and allow the subject to rest for a few minutes. **DO NOT REMOVE THE CUFF**

**Palpatory and Auscultatory methods simultaneously:** Palpate the radial artery, and elevate the pressure in the cuff to 20 mm Hg. Higher than that at which the radial pulse reappeared. Apply the stethoscope to the skin over the brachial artery, and allow pressure to leak slowly from the cuff. Note the pressures:

1. At which the radial pulse is first felt: __________ mm Hg.
2. At which the sound is first heard with the stethoscope: __________ mm Hg.

The pressure at which the sound was first heard is recorded as systolic blood pressure. Allow the pressure to continue to fall. The Korotkow’s sounds grow more and more intense as the pressure is reduced. Then they suddenly acquire a muffled tone and finally disappear.

The pressure observed at the first muffled tone is the first Diastolic pressure.

The pressure observed when the sound disappears is the second diastolic pressure for this laboratory. (Note: In practice you should record both diastolic pressures.)

Repeat the blood pressure determination at least three times, or until sufficient proficiency is acquired that agreement is obtained between consecutive readings. Blood pressure is recorded with the systolic pressure reading first,

* e.g., 120/80 means Systolic 120 mm Hg; Diastolic 80 mm Hg.

Pulse Pressure is the difference between Systolic and Diastolic blood pressure.

**How do the measurements from these two methods compare? Which do you think was more accurate and why? Try and explain any differences in results.**

### 2.2 Venous blood pressure

#### 2.2.1 Estimation of Venous Pressure

One can measure the approximate venous pressure by noting how much above the level of the heart an extremity must be so that hydrostatic and venous pressures are equal. At that point, there is barely enough venous pressure to lift blood against the hydrostatic pressure of the elevate limb.

With the subject sitting quietly next to a bench, with one arm lying on the bench-top, observe the veins on the back of the relaxed hand. While the subject is reclining, passively raise and lower the subject’s arm and observe for filling and collapsing of the veins of the back of the hand. Measure the distance in millimeters from the position where the veins are just barely collapsed to the level of the heart (in the sitting subject approximately at mid-thorax. This will give the venous pressure in mm of blood.

Venous pressure in mm of blood ________________ =

The specific gravity of blood is 1.056.

The specific gravity of mercury is 13.6.

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1 If it is difficult to see the veins on the back of the subject’s hand, have the subject hang the hand loosely down, with muscles relaxed. If veins do not become visible, slap the back of the hand gently few times to stimulate blood flow.
Compute the venous pressure in mm Hg using the equation

\[ \text{mm of blood} \times \text{Sp.Gr. of blood} = \text{mm of mercury} \times \text{Sp.Gr. of mercury} \]

Venous pressure in mm Hg. =

2.3 ECG Measurement

![Heart Lead Field](image)

Figure 2: Electrocardiographic lead field.

The “limb leads” are part of the legacy of Wilhems Einthoven, developer of the string galvanometer and winner of the 1926 Nobel Prize for his advances in electrocardiography. The idea was to capture the projection of the cardiac dipole in the frontal plane based on an equilateral triangle coordinate system. The underlying formalism of the limb lead (and the Frank lead) system is the lead field, a function that projects a current dipole source to any point on the body surface as shown in Figure 2. The lead field vector \( \mathbf{H} \) is specific to a set of electrode locations and when multiplied by the current dipole vector \( \mathbf{P} \), the result is a scalar value equal to the potential difference between the electrodes of the lead.

The goal of this part of the lab is just to learn the basics of measuring an ECG. Figure 3 illustrates the limb lead ECG, with three electrodes forming three difference measurements or “leads”. We will use the remaining electrode as the reference in each case and record the lead from the other two electrodes. Note the polarity of each of the limb leads and try to mimic them in your measurements.

The steps in setting up this basic ECG measurement are as follows:

1. Identify the following four sites on the torso of the subject and use an alcohol swab to **thoroughly** clean the skin beforehand:
   - Right anterior shoulder, just below the clavicle
   - Left anterior shoulder, just below the clavicle
   - Left lower ribs, near the mid-axillary line (equivalent to left leg) Note: the mid-axillary line runs along the side of the thorax mid way between front and back of the chest; the axilla are the armpits.
   - Right lower ribs, near the mid-axillary line (equivalent to right leg)
2. At each site, apply one of the disposable, pre-gelled ECG electrodes. Find locations as free of subcutaneous fat and muscle as possible.

3. Using the bundled ECG connector wires and short splitter cables, connect the lead wires in set of three to into the connectors that run to the inputs of the 4-channel bioamplifier so that you can record all three leads at once. **Take careful note of the polarity of the leads and make connections accordingly.** For example, to record Lead I, this would require:

   - Right anterior shoulder: -, G2 input, blue dot on the connector
   - Left anterior shoulder: +, G1 input, yellow dot
   - Right leg (or lower torso): reference, COM, green dot

   You can check that you have proper polarity by comparing the measured signals to the sample in Figure 5 below.

4. For the bioamplifier start with the following settings:

   - Switch calibrator switch to “USE”
   - Set the LO FREQ. setting to 0.1.
   - Set Amplification to 5 (and turn the “ADJ. CAL” screw all the way to the left; the resulting gain is approximately 1700.
   - Set HI FREQ. to 1 kHz
   - Make sure to use the same settings for all channels.

5. Put a T-connector on any two of the outputs of the bioamplifier, with two BNC cables, one to a channel on the oscilloscope (both ends of the cable should be BNC) and the other to the A/D input box connected to the computer. Connect the A/D ground input (“Ain Grnd”) to the ground of the oscilloscope (near the power switch).

6. On the oscilloscope:
Figure 4: Circuit diagram for the limb lead measurements

- On the oscilloscope, push the Menu button and for each channel, first set the tracing position with the small knob and then select DC coupling with one of the screen buttons.
- Vertical scaling control knob so that it is the same on both channels and start with a setting of 2 or 5 V/division.
- Adjust the horizontal control knob to .2 SEC/DIV.
- Trigger should be set to AUTO.

Now ensure that you have a good signal on the oscilloscope, make adjustments as necessary, and then:

1. Note the sensitivity of the ECG to motion of the subject and experiment to create the best conditions.
2. Save an image of the ECG on the oscilloscope using the memory function—you will have to use the memory function once for each channel and save each one in a different reference location on the oscilloscope.
3. From the saved signals, compute the period and heart rate for your subject.
4. Using the acquisition program, record a baseline ECG; perform the same measurements of heart rate.
5. Try swapping electrode connections around so that you measure and record all three limb leads. Also try using another lead, e.g., the right leg, as the reference and repeat the measurements. **Do the signals change noticeably when the reference changes?**
2.4 Response to exercise

The goal of this part of the lab is to record the response of a test subject to moderate exercise. For this, each team needs 4–6 people organized as follows (See Figure 6):

1) Subject: in comfortable clothes with ECG electrodes applied and blood pressure cuff applied loosely around an upper arm.

2) Blood pressure monitor: stationed at the side of the subject with stethoscope and blood pressure manometer and bulb in hand. This person will carry out the BP measurements during the breaks in the exercise.

3) Pulse monitor: stationed on the other side of the subject, this person’s job is to measure heart rate during the breaks in the exercise.

4) ECG/Computer operator: sitting at the bench, this person’s task is to make ECG measurements and record all other measurements from the blood pressure and pulse monitors. This person is also responsible for tracking the time and setting the pedal frequency. Use the ECG lead combination that produced the largest amplitude signals of the three possibilities.

There are two protocols for these experiments, but before beginning, let the subject warm up and make sure he/she is comfortable on the bike and has selected a comfortable gear and resistance setting to be able to complete 8–10 minutes of pedaling with moderate exertion.
2.4.1 ECG measurement

For this part of the protocol, use the same ECG setup as above, recording all 3 limb leads simultaneously on the computer and monitoring at least 1 of them on the oscilloscope.

2.4.2 Setting exercise workload with the metronome

To ensure a constant or controlled load, we will use a metronome to determine the pedaling cadence (rate) of the subject. Make the own metronome from a signal generator, as follows:

1. Set the Agilent (for Hewlett-Packard) 33120A function generator to the following settings:
   - Function: square wave
   - Amplitude: about 1 VPP (volts peak-to-peak)
   - Duty cycle: 20%
   - DC offset: 0.0

2. Connect a T-connector from the output of the function generator and connect one side of it to the Channel 2 input of the oscilloscope. Use the oscilloscope to monitor the output of the function generator, especially its frequency.

3. Connect a cable from the other end of the T-connector to the BNC/Banana converted and then to the adapter cable to a 1/8" female plug for the headphones. Adjust volume with the headphone controls and the amplitude control of the function generator.
4. Adjust the frequency of the signal generator to a level that the subjects find comfortable. Sample the signal with the oscilloscope and note the period and associated frequency.

2.5 Tips

Some additional technical aspects to note:

- Carry out measurements during the breaks at the end of each 2-minute interval as quickly as possible! Figure 7 shows such a measurement taking place. The subject will recover during these breaks and this will reduce the accuracy of the study; the breaks should be no longer than 30 seconds.

- Measure pulse rate using the count during 15 s as soon as the subject stops pedaling.

- Subjects should try and be as still as possible during the breaks and the ECG operator is responsible for measuring during an interval when the signals are as quiet and stable as possible.

- Adjust the A/D converter range for the acquisition program so as to capture the signal with the best possible resolution. Try and reduce baseline drift as much as possible; if the problem persists, try turning the Bioamplifier to AC coupling. Note that turning the oscilloscope to AC will appear to improve the baseline instability but that this effect does not pass to the A/D converter, which measures DC amplitudes.

Figure 7: Simultaneous measurements of heart rate and blood pressure during a break in the exercise.
2.5.1 Constant load protocol

1. Give the subject a 5-minute recovery period after the warmup and take resting measurements of BP, pulse, and ECG. Set the metronome to the cadence you worked out beforehand with the subject.

2. **Exercise 2 minutes:** Let the subject pedal at the set rate for 2 minutes and then stop and as quickly as possible, measure blood pressure and pulse, and take a sample of the ECG on the computer. Keep the breaks below 30 s.

3. **Exercise 4 minutes:** Let the subject pedal another 2 minutes and repeat measurements.

4. **Exercise 6 minutes:** Let the subject pedal another 2 minutes and repeat measurements.

5. **Exercise 8 minutes:** Exercise for another two minutes and then measure again. Stop the exercise at this point but keep subject sitting on bicycle.

6. **Recover 2 minutes:** no pedaling, repeat measurements.

7. **Recover 4 minutes:** no pedaling, repeat measurements.

8. **Recover 6 minutes:** no pedaling, repeat measurements.

9. Let subject relax and cool down.

2.5.2 Graded load protocol

The goal for this protocol is to apply a graded stress to the subject and observe the response. For this, have the subject select a gear that he/she can maintain over a cadence range of about 60–90 rpm. The subject will spend 2 minutes at each cadence, then stop for measurements, then continue at an increased cadence for 2 minutes, and so on.

Work out beforehand a sequence of cadences and associated periods that will span at least 60–90 rpm in 4 steps.

1. Give the subject a 5-minute recovery period and take resting measurements of BP, pulse, and ECG. Set the metronome to produce a cadence rate of 60 bpm.

2. **Exercise 2 minutes:** Let the subject pedal at the set rate for 2 minutes and then stop and as quickly as possible, measure blood pressure and pulse, and take a sample of the ECG on the computer. During the measurement break, set the new cadence on the metronome.

3. **Exercise 4 minutes:** Let the subject pedal another 2 minutes at the new cadence and repeat measurements. Increase the cadence again.

4. **Exercise 6 minutes:** Let the subject pedal another 2 minutes at the new cadence and repeat measurements. Increase the cadence again.

5. **Exercise 8 minutes:** Let the subject pedal another 2 minutes at the new cadence and repeat measurements. Stop the exercise at this point but keep subject on bicycle.

6. **Recover 2 minutes:** no pedaling, repeat measurements.

7. **Recover 4 minutes:** no pedaling, repeat measurements.
8. **Recover 6 minutes:** no pedaling, repeat measurements.

9. Let subject relax and cool down; feed water but hold off on the cake until the end of the next protocol.

## 3 Lab Report

As with the previous report, concentrate on presenting the results and discussion of them rather than the methods and background sections. You may choose to include the discussion with the results or have separate results and discussion sessions. It is up to you.

Include results and discussion for the following parts of the lab:

**Blood pressure:** include all the pressure measurements and answer all the questions in the lab description. Be sure to include all three results of the measurements and explain reasons for the variation you might see.

**ECG:** include tracing of 1-3 beats from each of the electrode arrangements you measured; describe any differences in signal morphology you see. For at least one tracing, add arrows to mark each of the major features of the ECG: P, Q, R, S, and T waves, ST segment, PQ segment, and TQ segment.

**Exercise:** create a table and graphs for both systolic and diastolic blood pressures, heart rate using palpation, and heart using the measured ECGs for each of the exercise protocols; these should all be graphs of the measured value, *e.g.*, heart rate, as a function of time through your the protocol. Discuss the results of these graphs—did they go as you expected? What do they suggest about the body’s response to exercise? Did you see any changes in ECG shape with exercise? Specifically, what changes did you see in blood pressure with exercise—provide a model of what happens during exercise and discuss how the data you measured support that model.

**Mechanisms:** Describe briefly the physiological mechanisms of as many as possible of the responses to exercise that you observed. Specifically, make sure to explain the different factors that will alter blood pressure and decide which ones might be dominant from your data.

**Experimental problems:** Describe any experimental challenges you had to face in the lab and how you dealt with them or how you would plan to deal with them were you to repeat these experiments in the future.

**Miscellaneous:**

- When displaying the results of the ECG recordings, try and use the same scaling on the axes so that it is possible to compare results between different recordings.

- You can, in principle, do all the graphs with Excel but please try and use MATLAB if possible. This suggestions is especially important if you wish to do any signal processing such as filtering of the ECG signals. If you have questions, simply ask the TA or me for explanations or suggestions.