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Background

1 | Anatomy and Physiology

The following chapter outlines the functions of the cardiovascular system and focuses on its microcirculatory part. Further the phenomena of vasomotion is illustrated.

1.1 Macrocirculatory system

The main function of the cardiovascular system is the blood supply of the whole body and the transportation of metabolites. The propulsion of this is the heart. It generates the systolic blood pressure through the strength of left ventricle. The pressure difference between the heart and the periphery emerging from there, ensures the blood flow. The blood flows from regions with high pressure, like the aorta, to regions with low pressure, like the periphery.[**martini2012**]

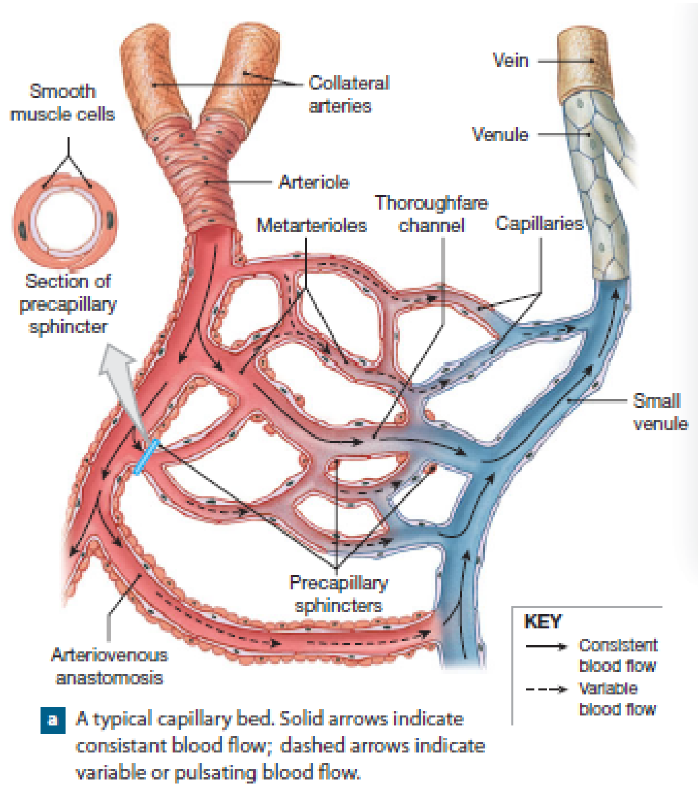
The heart supplies the body through the systemic and the pulmonary circuit with blood. Through these circuits the heart regulates the blood allocation with adjustment of stroke volume and heart frequency. The oxygen-rich blood accumulates in the left ventricle. From there the blood is pushed out through the aortic valve into the aorta and via the arteries spread into the whole body. The venous system returns the meanwhile low in oxygen blood back to the heart into the right atrium. From there the blood flows into the right ventricle and is pushed out through the pulmonary valve into the lung arteries. In the lungs gas exchange of the blood happens. Subsequent the oxygen-rich blood flows via the pulmonary veins back to the left heart to supply the body.[**martini2012**]

As mentioned, there are two types of vessels, arteries and veins. The difference between those two types of vessels is that arteries transport the blood away from the heart and veins solely transport blood to the heart. There are also some differences in the structure of arteries and veins. Arteries consist of three different layers, tunica interna, tunica media and tunica externa. The tunica interna consists of vascular endothelium, the tunica media consists of smooth muscle cells and elastic fibres, the tunica externa consists of connective tissue and also elastic fibres. Furthermore, there are two different types of arterial vessels. In arteries of the elastic type prevail the elastic fibres in the tunica media. This allows an abrupt extension of the vessel during the systole and ensuing constriction, due to this the blood is transported. This phenomena is called windkessel function. In arteries of the muscular type prevail the muscular fibres in the tunica media. This allows regulation of the lumen by constriction and dilatation, whereby the resistance and the blood flow in the organs is regulated.[**martini2012**]

Venous vessels are similarly structured like arterial vessels, however they are thinner and have also semilunar valves inside, to inhibit back flow inside the vessels. This system is supported by the skeletal muscles which help to hold up blood flow. The arterial and the venous vessel system are connected through the capillary system in the microcirculatory system.[**martini2012**]

1.2 Microcirculatory system

The heart and larger arteries and veins are associated with the cardiovascular system, but those are only used for transportation of blood. Instead it is the capillaries, that permeate most tissues, that is responsible for the perfusion of tissue. These are the only vessels which permit exchange between the vessel and the surrounding interstitial fluids. Factors that affect tissue perfusion is cardiac output, peripheral resistance and blood pressure. Capillaries are made not of single individual fluid conductors like veins and arteries, but instead formed into capillary beds. Here they work as a interconnected network of vessels. As mentioned before the arteries decrease in size the further they expand into the peripheral system. The small arteries divide into arterioles which further divide into dozen of capillaries. The capillaries merge into a venule after the blood has been deoxygenated. A capillary is divided into two segments, first the metarteriole and second the capillary. The blood flow between arterioles and venules can also be a direct connection, made by an arteriovenous anastomosis. This works as a bypass diverting blood flow around the capillary bed. An example of the structure of the capillary bed can be seen on figur 1.1.[**martini2012**]



Figur 1.1: The basic structure of a capillary bed, with arteriole on the left side of the bed and a venule on the right[**martini2012**].

Each capillary entrance is controlled by a precapillary sphincter, which is composed of smooth muscle cells, that are able to contract or relax and thereby limit access of blood flow to certain capillaries. The blood flows relatively slow within the capillaries giving time for the two way exchange of nutrients and wastes. [**martini2012**]

1.2.1 Vasomotion

The flow within the capillaries varies. This is among other thing due to the earlier mentioned precapillary sphincters opening and closing. The opening and closing of sphincters is part of the autoregulation process performed at a local level, to control the blood flow. The vascular system does not contain blood enough for every vessel a capillary beds to be filled with blood. Therefore only 25% of the vessels in a capillary bed contains blood, and vessels activity needs to be well coordinated. Thermoregulation and control of nutrition balance are the primary functions of the microcirculatory system. Local changes in concentration of chemicals and interstitial fluids eg. dissolved oxygen concentrations in tissue modulates the vascular smooth muscles activity. Constriction and dilation of the vessel is thereby regulated by this periodic activity, also known as vasomotion. [**martini2012**, **geyer2004**]

Under normal circumstances cardiac output remains stable and the control of local blood flow happens through local peripheral resistance within local tissues. The regulation of cardiovascular activity is controlled by local homeostatic mechanism. These make sure that demands such as oxygen and nutrients are meet and wastes are disposed.[**martini2012**]

Physiological mechanism controlling vasomotion are not yet fully understood, but vascular smooth muscle activity has been shown to be roughly proportional to the tissue's metabolic demand for oxygen.[**geyer2004**] Further have some factors that trigger homeostatic mechanism to alter the vasomotion been said to have an impact. Factors that trigger dilation is called vasodilators and can be some of the following:[**martini2012**, **geyer2004**]

- Decreased oxygen level or increased CO2 level
- Lactic acid or other acids generated from tissue cells
- Nitric oxide NO released from endothelial cells
- Rising concentrations of potassium ions or hydrogen ions in the interstitial fluid
- Chemicals released during local inflammation
- Elevated local temperature

A vasodialation will result in increased oxygen, nitrients, buffers released to recreate homeostasis. Factors that stimulate contriction is called vasoconstricters and can happen due to following:[**martini2012**]

- Damaged tissue
- Aggregating platelets

2 | Hemodynamics

Hemodynamics explains the movement or flow of blood. It is influenced by parameters like blood pressure, blood volume, cardiac output, blood composition, etc. It is possible to measure some of the hemodynamic parameters non-invasive, and also to calculate parameters.[**martini2012**, **thiriet2008**]

2.1 Physiological Base

The regulation of the blood pressure happens with baroreceptors in the walls of the big arteries in chest and neck area. These receptors register the changes of the elongation of the vessels and transmit this information to medulla oblongata. With the received pressure informations initiates the medulla oblongata, if necessary, regulatory measures. For the short-term regulation is the sympathicus responsible. Both, middle-term and long-term regulation, is made by the kidneys. For middle-term regulation messenger substance are released, which entail vasoconstriction. The long-term regulation occurs per pressure diuresis or reabsorption in the kidneys.It is possible to measure different blood pressures at different places in the cardiovascular system, for example the mean arterial pressure (MAP). The MAP increases in relation to the stroke volume and decreases when blood flows into the peripheral system. The central venous pressure (CVP) states the pressure in the venous system and complies approximately the pressure in the right ventricle. CVP depends on the filling volume of the venous system. Cardiac output, total periphery resistance and the viscosity of blood affect the blood pressure.[**martini2012**, **thiriet2008**]

The cardiac output (CO) states the blood volume, which is pumped by the heart per time unit (HR). The calculation of the CO as follows.[**martini2012**]

$$CO = HR \times strokevolume \tag{2.1}$$

The total periphery resistance (TPR) is the flow resistance of the systemic circulation and results from the sum of all vessel resistances. TPR depends on MAP , CVP and CO. [**martini2012**]

$$TPR = \frac{MAP - CVP}{CO} \tag{2.2}$$

2.2 Physical Base

To consider the hemodynamics, it is possible to draw conclusions by analogy of physical laws. Especially of Ohm's law $R = \frac{U}{I}$ or rather $I = \frac{U}{R}$. A special case of Ohm's law constitutes Hagen-Poiseuille's law in the field of fluid dynamic and rheology. Hagen-Poiseuille's law describes the laminar flow of an homogeneous Newtonian fluid through a rigid pipe depending on characteristics of the fluid and of the pipe.[**noordergraaf2011**, **thiriet2008**]

Blood is an inhomogeneous suspension of liquid and corpuscular components, whose viscosity η depends on more factors than the temperature, and is consequently no Newtonian fluid. Nevertheless it is possible to draw conclusions by analogy out of Hagen-Poiseuille's law for the computation of the hemodynamics.[**noordergraaf2011**, **thiriet2008**]

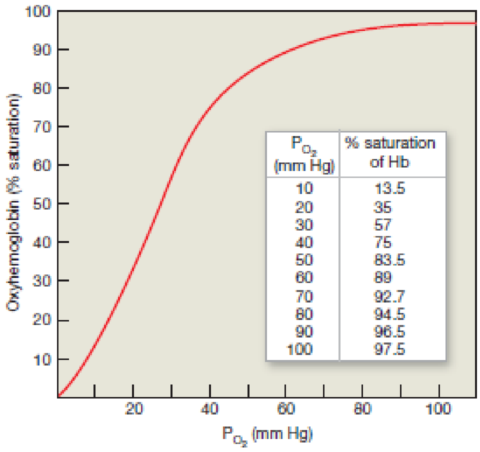
$$\frac{V}{t} = \frac{r^4 \times \pi \times \Delta P}{8 \times \eta \times I} \tag{2.3}$$

Here is the volume flow equivalent to the electrical current I and the pressure difference ΔP to the electric voltage U. Thus, the calculation of the resistance as follows.[**noordergraaf2011**, **thiriet2008**]

$$R = \frac{8 \times I \times \eta}{r^4} \tag{2.4}$$

Thereby volume flow increases 16 times and the resistance decreases 16 times for double radius r.[**noordergraaf2011**, **thiriet2008**]

In the blood, oxygen is tied to a large extent by hemoglobin. The oxygen saturation (sO_2) describes the percentage of the oxygenated hemoglobin. With rising oxygen partial pressure (pO_2) increases the oxygen saturation. This relation between sO_2 and pO_2 is showed by the oxygen binding curve.[**martini2012**, **hasan2013**]



Figur 2.1: The oxygen binding curve shows the relation between sO_2 and pO_2 [**martini2012**].

The oxygen content (cO_2) of the blood depends on both, the oxygen saturation and the oxygen partial pressure. cO_2 is calculated by the sum of the hemoglobin bounded and the physical dissolved oxygen.[**martini2012**, **hasan2013**]

$$cO_2 = Hb \times 1,34 \frac{ml}{g} + pO_2 \times 0,003 \frac{ml \times dl}{mmHg} \tag{2.5}$$

The available oxygen (DO_2) is calculated by the oxygen content of the blood and the cardiac output.[**martini2012**, **hasan2013**]

$$DO_2 = cO_2 \times CO \tag{2.6}$$

The consumption of oxygen (VO_2) is calculated by the difference between the available oxygen in the arterial and the venous blood and the cardiac output.[**martini2012**, **hasan2013**]

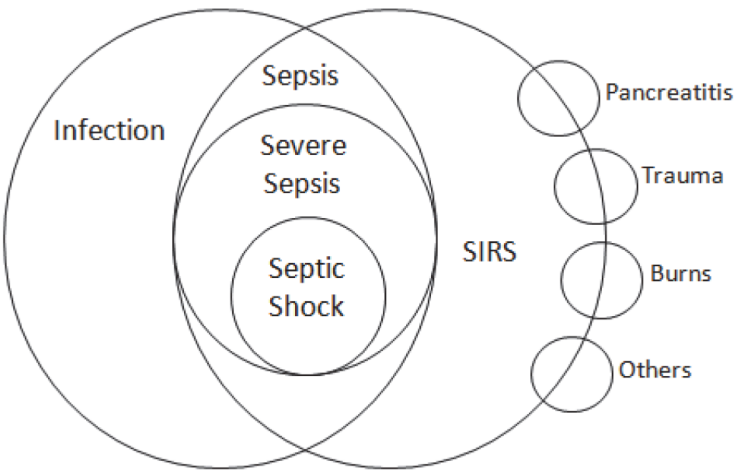
$$VO_2 = (c_aO_2 - c_vO_2) \times CO \tag{2.7}$$

Therefore one can draw a conclusion from the available and the consumption of oxygen to the cardiac output.

3 | Vasomotion in disease

This chapter describes pathologic incidents in the cardiovascular system and organs during sepsis. Sepsis is used as an example of a disease where vasomotion plays a role.

Sepsis is a condition, that develops on behalf of systemic inflammatory response syndrome (SIRS) with pressence of an infection or bacteria in the tissue, within the body which triggers an immune response. This response often overburdens the immune system, to fight the inflammation or bacteria. The infection or bacteria can be anywhere in the body's tissue. Some of the normal macrohaemodynamics of sepsis are abnormal body temperature, abnormal heart rate, oxygen extraction and abnormal blood pressure.[**plunta2010**, **kanta2014**] Sepsis is increasing condition in the population. Different studies suggest estimates of the incidence of sepsis, but the identification of diagnosis of sepsis can vary, why the numbers may also vary from place to place.[**baudouin2008**, **kanta2014**] The Dr. Foster Organisation found an increase by 53% from 1996 to 2002 in the hospitals in the United Kingdom. Causes for the increase in incidence can be due to the increasing elderly population, with cronic conditions undergoing invasive procedures [**baudouin2008**]. Sepsis is often associated with three stages, sepsis, severe sepsis and septic shock. This is illustrated in figure 3.1.



Figur 3.1: Relation between SIRS and infection. Showing the stages in sepsis and some of the causes of SIRS which include pancreatitis, trauma, burns etc.[**kanta2014**]

3.1 Sepsis

Under the stage of sepsis several things happen, mainly to the micro circulatory system at the capillary level, that leads to impaired homeostasis in the body. Infection or other bacteria that is responsible for causing some irregularity is present in the blood and in the tissues around the vessels. Among the first things that happens when the body encounters an infection, white blood cells are recruited to release molecules that will fight the infection. Molecules that interact with the endothelial in the blood vessels, like nitric oxide (NO), are released. The interaction causes the vessels to dilate to increase the blood vessel diameter and permeability. The increased diameter slows down the blood flow, which causes a drop in blood pressure. Also the vessels permeability is increased. This reaction happens multiple places in the body where there is infected tissue present and will cause systemically vessel dilation. The characterization of sepsis is SIRS as a result of infection.[**baudouin2008**, **kanta2014**]

3.2 Severe sepsis

When the permeability of the vessels is increased there will be more fluid in the tissue and the cells will get less oxygen because the oxygen has a harder time to get to the demanding tissue. Also the endothelial of the vessels will get damaged when the white blood cells try to destroy the pathogens. This triggers coagulation and clotting is formed in the damaged areas in the blood vessels. These clots can break off into the blood and cause further harm eg. stroke. At a point the damaged vessels leads to more leakage, because there will be a point where the coagulation cannot keep up. Organs will start to dysfunction at this stage. The characterization of severe sepsis is presence of sepsis with organ dysfunction and hypoperfusion is often included in this state. The mortality rate for patients with severe sepsis are about 25 to 30% [**baudouin2008**, **kanta2014**].

3.3 Septic shock

Septic shock occurs when the body has undergone sepsis for a greater duration of time. This stage is characterized by a condition with hypotension even after adequate fluid resuscitation is given. Because of lactic acidosis the cells are not getting a sufficient supply of oxygen and therefore the cells will begin to die. This can lead to a very dangerous state, where organs begin to fail because they get to damaged to function. When multiple organs get damaged the state in septic shock reaches multiple organ failure also called multiple organ dysfunction syndrome (MODS)[**baudouin2008**, **kanta2014**]. The mortality for patients with septic shock are in the region of 40 to 70% [**kanta2014**].

4 | Infrared Thermal Imaging

The following chapter will include an introduction to infrared imaging, where some general concepts and physical principals will be explained. Furthermore it will be elaborated how a device measures infrared radiation.

Infrared imaging is an technique that utilizes infrared radiation emitted from nearly any objects. The existence of infrared radiation was first discovered in 1800 by Sir Frederick William Herschel. His experiments lead to the knowledge that there were a light spectrum beyond the visual spectrum humans are able to perceive.[**ignacio2017**, **optris2009**]

Infrared thermography is commonly used to calculate surface temperatures and important concepts in the understanding of this are heat and temperature. Temperature is a measure for the internal energy within an object and can be defined as the average kinetic energy of the object. Heat is the energy that passes from a warm object to another colder object. A warm object will decrease in internal energy and a cold object will increase due to the temperature difference and therefore the heat transfer. In the human body, a constant temperature is keep, due to several factors and therefore the temperature will not decrease even though a heat transfer to the surrounding environment occurs. The environmental temperature do have an impact on how large the heat transfer gradient is. If a body is in a cold environment, the emitted heat will be greater than the absorbed. In the same way, if the environment is much warmer than 37°C, a greater absorption than emission will occur and the body will increase in temperature.[**ignacio2017**]

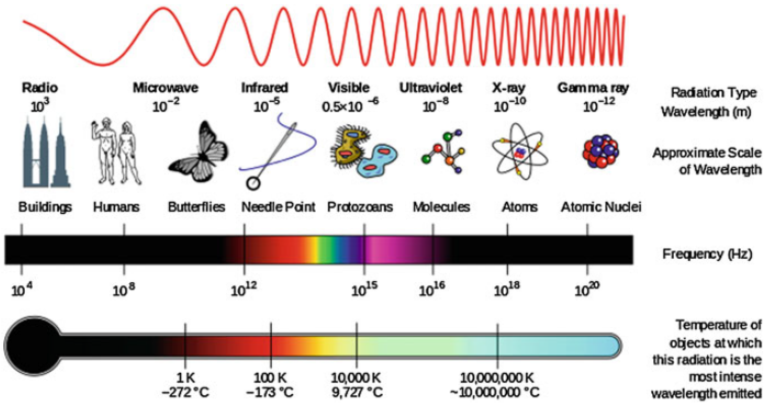
4.1 Physical principals

Any object above absolute zero emits energy-electromagnetic radiation depending on its temperature. Absolute zero is $0K$ or -273.16°C. To put that into perspective the human body has a temperature around 37°C.[**ignacio2017**, **optris2009**]

Electromagnetic radiation is a propagation of energy trough a medium without the transportation of mass. An electromagnetic wave is made of the relationship between frequency f, wavelength λ and the speed of light c. This is stated in the equation of wave motion.[**ignacio2017**]

$$\lambda = \frac{c}{f} \tag{4.1}$$

Depending on the frequency and wavelength certain characteristics arise from what is called the electromagnetic spectrum. The electromagnetic spectrum is the electromagnetic energy that is emitted. This extends from radiation of low energy such as radio waves and infrared, to waves of higher energy in form of eg. X-rays. A graphical representation of the electromagnetic spectrum can be seen on figur 4.3.[**ignacio2017**]

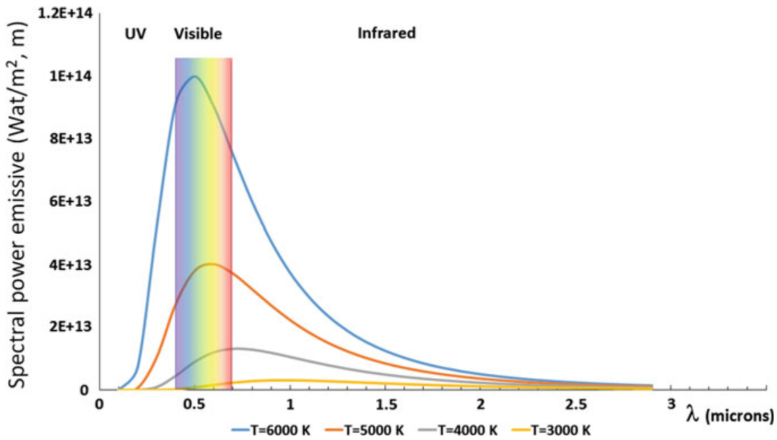


Figur 4.1: The electromagnetic spectrum with wavelength, emitters, frequency and temperature.[**ignacio2017**]

Infrared radiation is also known as thermal radiation because of the relationship between temperature and infrared radiation. Temperature of the human body permits radiation in the infrared spectrum, but objects of much higher temperature are capable of emitting radiation in the visible and UV spectrum. This has to do with the difference between object and environmental temperature. If the temperature of these are relatively close to each other, the radiation emitted will be within infrared wavelengths. Infrared radiation has a wavelength from 769 nm to 1 mm. Objects emit more radiation in some region regions compared to others. Because of this is the infrared spectrum classified in the three regions, near, middle and far infrared. Near is between 769 nm and 2.5 μm, middle 2.5 μm to 50 μm and far 50 μm to 1 mm. The human body emits most radiation in the far infrared part, and most thermal cameras are build with this in mind. Near and middle cameras are used to measure gases.[**ignacio2017**]

4.2 Measuring thermal energy

The theory of the black body is important to understand the absorption and emission of light relative to temperature, because the theory of the black body is used to describe the laws of infrared radiation and its relationship to temperature. The black body is an ideal perfect emitter of infrared radiation because it absorbs all electromagnetic radiation permitted to it, and it emits the same amount of radiation as it absorbs, the absorption and emission are both equal to one. Spectral emissive power, also denoted E_λ is the energy emitted by a surface in relation to time and range of wavelength. Figure 4.2 shows an graphical illustration of spectral emissive power of the black body for specific wavelengths when the temperature changes. [**ignacio2017**]



Figur 4.2: Spectral power emissive as a function of wavelength for different temperatures [**ignacio2017**]

The knowledge of this principle helps in the understanding of how infrared radiation behaves, and how temperature affects the wavelength of the signal. The radiation from the human body which has a temperature at 37°C emits the maximum energy of 9.3μm, which means that most of the radiation is in the far infrared spectrum.[**ignacio2017**]

Physical laws including Wien's displacement law and Stefan-Boltzmann's law are important for explaining how the infrared radiation behaves at different temperatures. [**ignacio2017**]

Wien's displacement law tells that the wavelength of the peak of the black body radiation curve decreases as the body temperature increases. This law can be used to describe different wavelengths according to the temperature of the black body which emits the radiation. Wien's law has the following equation:

$$\lambda_{max} = \frac{a}{T} \tag{4.2}$$

In Wiens displacement law 'a' denotes the Wien's displacement constant, this constant has the value $2.897 * 10^{-3} mK$. 'T' denotes the absolute temperature in kelvin. 'λ_{max}' denotes the wavelength of emission peak with unit in meters.[**ignacio2017**]

Stefan-Boltzmann's law tells that small changes in temperature will lead to big changes in emissive power. This is seen in Stefan-Boltzmann's equation because it states that the total emissive power is proportional to the fourth power of the absolute temperature. [**ignacio2017**]

$$E = \varepsilon * \sigma * T^4 \tag{4.3}$$

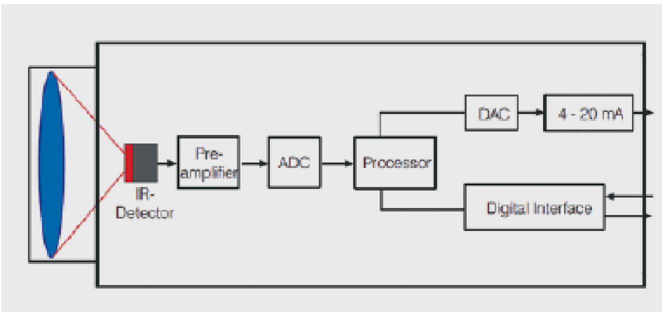
In Stefan-Boltzmann's equation 'E' denotes the total emissive power with unit W/m^2. 'σ' denotes the Stefan-Boltzmann's constant, this has a value of $5.67 * 10^{-8} W/m^{-2}K^{-4}$ and 'T' is the temperature in kelvin. 'ε' denotes the emissivity and is normally not a part of the Stefan-Boltzmann's law, but part of the modified Stefan-Boltzmann's equation, because it is used for calculation of temperature in most thermal cameras. Emissivity is different for all materials. Skin have an emissivity between 0.95 to 9.99, why these values typically are used when assessing the temperature of the skin of the human body with thermography. This law is important when considering infrared thermography because the sensitivity when calculating the temperature from the emissive power is big. [**ignacio2017**]

Region of interest

Region of interest (RIO) is an important consideration when doing measurements with thermal imaging. One of the reasons why this is an important aspect is because it is the RIO that lays the foundation of the data that goes into the statistical analysis. A minimum of 25 pixels is recommended for the RIO to reduce error in the data. To get the best measurement the RIO should be filling most of the image to get the best thermal data and better resolution in the area you want to measure. [**ignacio2017**]

Thermal cameras

The radiation emitted from an object is focusing the RIO onto a detector via a lens in the thermal camera. The radiation emitted to the detector generates an electric output proportional to the radiation. The output is then undergoing amplification before further signal processing that digitalizes the signal into pixels. This allows the final output signal to be viewed as a temperature for the object on an monitor, this is also illustrated on figure 4.3. [**optris2009**]



Figur 4.3: Simplified block diagram of an standard infrared camera.[**optris2009**]

The infrared radiation is made into an electrical signal in the camera, this data can be used in calculating the temperature for an object by knowing certain variables and putting these into Stefan Boltzmann's equation 4.3. The emissive power denoted E, is the radiation that the detector in the camera is getting. The variables σ are known and ε is specified for the object that is being measured, eg. the human skin with emissivity between 0.95 to 0.99. The temperature are then the only variable to calculate and this is done for each pixel in the image to make up the complete thermal image of the object. Each pixel will be representing one thermal data. [**ignacio2017**]

5 | The use of infrared imaging in vasomotion

In the following chapter an introduction to different techniques of measuring vasomotion will be giving. Here their methods and applicability for measuring vasomotion will be presented, with main focus directed towards thermal imaging as applied technique.

5.1 Techniques of measuring vasomotion

For some time it has been the interest of scientists and health care professionals to get a better understanding of the mechanisms that control and regulate local blood flow in the microcirculatory system[**sagaidachnyi2014**, **sagaidachnyi2017**, **Geyer2004**, **liu2012**]. Visualization of the vessels in the skin and the way these behave can be important for assessment of stages of sepsis as mentioned before in, but also in peripheral vascular disease, the results of skin reconstructive surgery, wound and ulcer management.[**liu2012**, **kanta2014**]

For measuring regulation in the peripheral blood flow, it is assumed that these oscillating changes are the source of thermal waves propagating from microvessels toward the skin's surface. Especially thermal imaging uses this concept.[**sagaidachnyi2017**] Furthermore a correlation between skin temperature in fingertips and blood flow oscillations has been found[**sagaidachnyi2014**]. There are multiple different techniques of measuring blood flow in the peripheral circulatory system. Some of these are: capillaroscopy, laser Doppler, laser speckle contrast and orthogonal polarisation spectral imaging. These have been used differently trying to quantify functional aspects of skin vasculature.[**liu2012**]

5.2 Laser Doppler flowmetry

In a study from Geyer et al. vasomotion is investigated trough the use laser Doppler flowmetry (LDF) as recording technique. In the study vasoregulation variables are sought quantified. LDF is a non invasive approach to measuring changes in vasomotion. The technique register changes in the depth of 1 mm, and works like Doppler ultrasound, utilizing the shift in frequency, but instead of ultrasound it uses light reflected from red blood cells.[**Geyer2004**]

5.3 Thermal imaging

In other studies made by a Russian group lead by Sagaidachnyi et al. the use thermal imaging has been used to study vasomotion. In their studies they seek to get better understanding of the relationship between blood flow oscillations and temperature oscillations, and see if they are able to recreate the flow oscillation from temperature recording. Recordings of flow where done by Photoplethysmography and temperature of the skin by thermal imaging. The recordings were made on a small point of the fingertip. Trough their work, five frequency bands were identified as vasomotion activity, and are following: endothelial (0.005–0.02 Hz), neurogenic (0.02-0.05 Hz), myogenic (0.05-0.15 Hz), respiratory origin (0.15-0.4 Hz) and cardiac origin (0.4-2.0 Hz).[**sagaidachnyi2017**, **sagaidachnyi2014**]

some more about why they used it and how

5.4 Summation

For instance, the depression in the amplitude of endothelial blood flow oscillations is considered to be an indicator of endothelial dysfunction and a precursor of many kinds of cardiovascular disorders such as arterial hypertension, cardiac ischemia, and atherosclerosis. The increasing amplitude of blood flow oscillation within the neurogenic frequency band is characterized by a decrease of vascular resistance and an increase of volume blood flow through the arteriovenous shunt.

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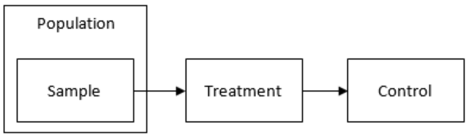
Experimental method

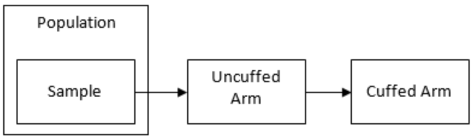
6 | paired t-test

The used method of the study design is called in series design. This enable to compare the situation before and after treatment within each subject.



Figur 6.1: The procedure of studies with in series design.

Within this study the arms of the subjects will be cuffed. To avoid any carry-over effect, the measurements on the normal arm will be done first. That means beginning with the control and then the "treatment".



Figur 6.2: The procedure of this study with in series design.

The paired t-test is used to check the difference of means of two conditional samples. This test is usually used to compare "before treatment"and "after treatment". The tested hypotheses are (where $\delta = \mu_1 - \mu 2$)[**dodge2008**]:

- $H_0 : \delta = 0$ no difference between uncuffed and cuffed arm
- $H_1 : \delta \neq 0$ a difference between uncuffed and cuffed arm

In this case, there is a relation between the microcirculatory and the macrocirculatory system shown, if the null hypothesis is rejected.

It is requisite that the sample size of the of both samples is identical.

Following some useful formulas[**dodge2008**]:

- difference within the subjects

$$d_i = x_{i2} - x_{i1} \tag{6.1}$$

with $i = 1, 2, ..., n$

- mean of the difference

$$\bar{d} = \frac{1}{n}\Sigma d_i \tag{6.2}$$

with $i = 1, 2, ..., n$

- standard deviation

$$s_d = \sqrt{\frac{\Sigma(d_i - \bar{d})^2}{n-1}} \tag{6.3}$$

- test variable

$$T = \frac{\bar{d}}{\frac{1}{\sqrt{n}} s_d} \tag{6.4}$$

- degrees of freedom

$$n - 1 \tag{6.5}$$

- decision rule for rejecting the null hypothesis

$$|T| > t_{n-1,\frac{\alpha}{2}} \tag{6.6}$$

6.1 Subjects

Eight healthy subjects within the age of .. - 52 where taking part in the experiment as volunteers, three men and one female. The research is done as a pilot study, why it is presumed that the sample size of eight is sufficient. The research focuses on assessing the microvasular system with the hand as a window on healthy subjects, why the following inclusion and exclusion criteria have been formed:

Inclusion criteria

- Subjects should have at least one hand to perform the measure on
- The cuff should be able to fit the arm circumference
- The subject should be able to sit still for a greater extend of time

Eksclusion criteria

- Health conditions that sets the subject in risk of injury when conducting the experiment like high blood pressure.

  - Systolic blood pressure over 140
  - Diastolic blood pressure over 90
- Age under - years old
- Age over - years old
- Obesity to a greater extend
- Parkinson disease

The experiment was performed in a small laboratory office at Hjørring hospital in of Northern Denmark.

7 | test setting

In this study the peripheral circulation is observed to investigate if there are changes in microcirculation during partial occlusion of blood supply. Infrared imaging is used to measure the temperature changes in the skin of the hand, which is used as an indicator for peripheral circulation. To see if there are changes in the microcirculatory system depending on flow levels in the macrocirculatory system, the test is set in two conditions. The first measurement of the hand, which is done under normal conditions, is used as a control measurement. The second measurement of the hand is done during a partial occlusion of the blood supply. The partial occlusion of the arm leads to an ischemia, which leads to a lower oxygen supply and is used as a way to mimic sepsis, which also leads to lower oxygen supply.

By first taking the control measurement under normal conditions, the carry-over effect of the occlusion is avoided. It also enables to take both measurements of each subject straight successively, what reduces inaccuracies within the setup of both experiments for each subject. Therefore a special setting, which is shown in figur 7.1 is assembled in the Regionshospital Nordjylland in Hjørring.

The subject will be placed in a upholstered chair with adjustable backrest, footrest and also armrests, which allows a good positioning of the measured hand. The measured hand is the dominant hand and must be fixed during the whole test to minimize movement bias. Hence the hand is stabled with a vacuum pillow which is covered by a micro fiber tissue to get a better background for the images. To provide a more comfortable position of the arm during the experiment the armrest of the adjustable chair is padded with some sheets under the vacuum pillow. A comfortable position in the chair is important, because the subject has to sit still and is not allowed to move during the test for at least 45 min, because the test setting just prevents very small unintentional movements. $37,5 \pm 1,0cm$ over the hand the Xenics Gobi 64017μ m GigE infrared camera is positioned with a tripod. The focus is adjusted on the hand until the wrist. The camera is via a Ethernet cable connected with a laptop, which is used to record the measurements with Xenics software with a frame rate of $\frac{50}{8}\frac{1}{s}$ and to save the files. It should be respected, that the file has to be named or rather renamed before each recording, otherwise the software will overwrite the previous file.

Figur 7.1: The test setting in the Regionshospital Nordjylland.

The subject will be placed in a upholstered chair with adjustable backrest, footrest and also armrests, which allows a comfortable positioning of the subject during the experiment. Measurements will be done on the dominant hand, which must be stabilized during the whole test to minimize movement bias. Hence the hand is stabled with a vacuum pillow which is covered by a micro fiber tissue to get a better background for the images, because microfiber . To provide a more comfortable position of the arm during the experiment the armrest of the adjustable chair is padded with some sheets under the vacuum pillow. A comfortable position in the chair is important, because the subject has to sit still and is not allowed to move during the experiment for at least 45 min, because the test setting just prevents very small unintentional movements. The Xenics Gobi 640 17μ m GigE infrared camera is positioned with a tripod over the hand. Focus is adjusted on the hand until the wrist . The camera is via a Ethernet cable connected with a laptop, which is used to record the measurements with Xeneth software with a frame rate of $\frac{50}{8}\frac{1}{s}$ and to save the XVI-files. Before each measurement is started, a unique file name has to be chosen and typed in, otherwise the software will overwrite the previous file.

First the cable connections between the camera, the laptop and the power supply have to be set. Afterwards the camera is turned on and has to warm up for about 15 min. During this the laptop should be started and the software for taking the measurements is set in operational readiness.

When the preparation of the test setting is done, the preparation for the subject can begin. At first the blood pressure of the subject is measured on the dominant arm. The blood pressure is measured three times while the subject is sitting relaxed on a chair. Out of the three measured systolic blood pressures the mean is calculated. To get the total occlusion pressure (TOP) the mean has to be multiplied by 1,3. To reduce the blood flow in the arm to 50% during the measurement within the second condition, the arm is cuffed with 30% of the TOP [**mouser2017**]. The occlusion pressure that is applied in the cuff has also to be calculated before the experiment can start. Then the cuff is affixed at the subjects dominant arm without tighten it. After that the subject can take place in the chair and the hand can be stabled with the vacuum pillow. The vacuum generator is attached to the pillow for giving the hand more stability. Next the camera needs to be positioned $37,5 \pm 1,0cm$ over the hand. The focus has to be adjusted.

If the camera is stable and the filename is modified according to the subject, the first measurement can be started for 20 min. The time needs to be measured by a stopwatch. During the whole experiment the subject is not allowed to move or speak to minimize movement bias. Directly after the first measurement the cuff on the arm of the subject is tightened with the calculated value $P_{cuff} = 0,3 \times TOP$ and the second measurement can be started for 20 min.The pressure of the cuff should be observed during the whole measurement and if necessary adjusted.

After the measurement of a subject, the next subject can be measured without preparing the test setting. It can be started with the preparation for the subject. It will take approximately one hour for each subject. For a guide during the experiment is an experimental protocol used.

XXX maybe an other picture

Del III

Data analysis