Biophysical properties of Skin Perfusion Pressure

Study of the mechanical pressure on the skin

Clara Gregori Pla

Master Thesis
KTH Royal Institute of Technology
June 2013
This thesis summarizes the master thesis project of Clara Gregori Pla for the Master in Engineering Physics in Biomedical Physics from the Royal Institute of Technology (KTH), Stockholm, Sweden. The research was conducted in the microcirculation instruments company Perimed AB under the supervision of Kristian Svensson-Eurén. The examiner was Kjell Carlsson from KTH.

TRITA-FYS 2013:31

ISSN 0280-316X

ISRN KTH/FYS/--13:31—
“Cada dia és un nou pas, 
cada nit, un nou repòs, 
cada gota de rosada nova frescor.”

Esquirols

“Every day is a new step, 
every night, is a new rest, 
every drop of dew brings freshness.”

Esquirols
Patients with peripheral arterial disease (PAD) and critical limb ischemia (CLI) suffer from pain and discomfort in their daily life. 20% of patients with CLI will even die within the first year, mainly due to lack of proper diagnosis and treatment. For those reasons, it is absolutely vital to develop a quick and efficient way to diagnose them. One of the possible methods, and the subject of this thesis, is laser Doppler flowmetry skin perfusion pressure (LD-SPP), which is a method to measure the blood pressure of the microcirculatory flow in the skin with laser Doppler technique.

The most accurate measurement of the status of the microcirculation is radionuclide washout skin perfusion pressure, which consists in injecting a radioactive agent locally under the skin. If a pressure that stops the microcirculation is applied, the radioactive substance does not decrease through washout and the corresponding pressure has been established as the skin perfusion pressure (SPP). LD-SPP comes from the need of finding a noninvasive method to study the microcirculation in the skin.

Today there are only two easy non-invasive methods to measure microcirculation; SPP and transcutaneous oxygen (TcPO\textsubscript{2}). Both methods have advantages and disadvantages. TcPO\textsubscript{2} measures the local amount of oxygen delivered to the tissue, a functional test of the microcirculation, while SPP measures the blood pressure of the microcirculation. Both methods predict wound healing, indicate PAD, diagnose CLI and can also be used to decide amputation levels. Even though the clinical data and experience is bigger for TcPO\textsubscript{2} since the technique is widely used, TcPO\textsubscript{2} is affected by edema, anemia, callus skin and inflammation and results must be verified with an oxygen inhalation test to be reliable. SPP is not affected by the mentioned factors, the equipment can be cheaper and it is quicker; however, SPP is sometimes difficult to perform, since the wounded area must be covered with a pressure cuff. (1)(2) Taking all these facts into consideration, LD-SPP can be a good alternative tool to TcPO\textsubscript{2} to diagnose PAD and CLI.

There are three different methods of SPP measurement: radionuclide washout, photoplethysmography (PPG-SPP) and LD-SPP. Radionuclide washout SPP is an invasive measurement of SPP which consists in injecting a contrast radioactive agent into the bloodstream to observe any blockages. LD-SPP is a noninvasive method to measure SPP which consists in placing a monitor of microcirculation (in our case is a laser Doppler probe) on the skin, placing a pressure cuff on it, and inflating the pressure cuff until the microcirculation flow signal disappears. When the cuff pressure decreases, the microcirculation flow signal eventually returns; this pressure corresponds to SPP. PPG- SPP and LD-SPP are done in the same way but using different techniques to detect the microcirculatory flow. PPG-SPP is a photo sensor detecting the intensity shift of the skin due to changes in the microcirculatory flow and laser Doppler uses the Doppler shift of laser light reflected on moving red blood cells. Both techniques achieve the aimed goal but LD is considered to be more sensitive to small changes in the flow.(2) Because of the instruments available in the company, the chosen method to perform SPP in this project has been LD-SPP.

LD-SPP has been performed for more than 30 years. During this time several researchers, especially J. J. Castronuovo, have given credit to the LD-SPP as a tool to diagnose CLI and PAD (1)(2)(3)(4)(5)(6)(7)(8). However, there are different technical aspects that need to be properly understood and other well-identified issues that require solving.

The main problem to solve in LD-SPP method concerns the fact that when the air pressure in the pressure cuff is measured, such pressure has until now been assumed to correlate to the pressure applied by the probe holder to the skin. However, this is an indirect measurement that has never been properly evaluated until now. Then, the main goal is to construct a probe holder that can measure the actual mechanical pressure applied onto the skin and the microcirculation status on the skin.

Another question addressed in this project is to establish whether the temperature induces a change in SPP. Temperature brings an increment of vasodilation and reduction of basal metabolic rate. Then blood flow is increased, thus the Doppler signal rises and gives a better signal which is easier to interpret. It is unclear if these metabolic changes caused by temperature influence SPP. If the laser Doppler just increases the signal with no change of SPP, it will be an enormous help for the physicians and for this project to recognize the SPP value on the monitored data.
Moreover there is another problem on the technique: the pressure cut-off value used to decide the severity of the patient is 30 or 40 mmHg for the majority of the research groups, but they do not all agree on one number (1)(4)(8)(5). Even though this problem cannot be solved in this project, it has been discussed.

To proceed with the project, first of all, the influence of temperature on the SPP measurements is checked with the heating probe 457, the Periflux 5010 laser Doppler unit and the Periflux 5020 unit. If the temperature is not influencing the results, all the measurements can be performed with high temperature.

Secondly, a laser Doppler probe holder with a force sensor is designed in order to measure cuff pressure and mechanical pressure at the same time. To find suitable mechanical pressure measurement devices is part of the study; thus, Flexiforce force sensor is selected. Then, the measurements are compared to the pressure measurement from the air pressure device. Testing is conducted on a limb prototype. Different probe holder sizes and different probe holder and cuff placements are investigated.

Finally, measurements are performed on healthy volunteers. Since no correlation is found between the cuff pressure and the mechanical pressure, different body placements with different probe holders (without using the force sensor) are studied with LD-SPP.

Regarding the main result, no correlation is found between the cuff pressure and the mechanical pressure on the skin. This failure was mainly due to a non-homogenously loading of the probe holder by the cuff or from the non-repeatable force sensor. On a limb prototype it is found that the size and the shape of the probe holders are well correlated to the force sensed on the surface of foam. Nevertheless, when the probe holder increases on height, then non-linear results are found. This effect could arise from edge effects of the probe holder. Results show that feet SPP is lower than legs SPP. However, high standard deviations are found when measuring SPP with different probe holders and in different body positions. A reason could be that the skin is really heterogeneous, thus the probe holder occludes the blood flow depending on the thickness and characteristics of the tissue underneath. Another reason is the loading of the probe holder by the cuff; completely different results are obtained if the cuff is not covering completely the surface of the probe holder and loading it equally. The last results to comment concerns the temperature influence on SPP. These results show that temperature does not influence evidently, but SPP increases in every measurement if repeated results are performed continuously.
ACKNOWLEDGEMENTS

First of all I would like to dedicate this Master Thesis to my parents, always supporting me during all this time. They made everything for me, making it possible for me to finish my studies, first in Barcelona and now in Sweden. I want to thank very especially also to my sister Nu, the Best sister that you can imagine, always supportive, good advisor and lovely, I would not be what I am without you. And I cannot forget my Swedish family, Peter and Susanne, for all the family warmth since the first plain to Sweden.

I would like to thank also specially my supervisor, Kristian, because he gave me the great opportunity to work in a really interesting project, in a welcoming company such Perimed AB. Thanks a lot for your constant advice, for your endless help and for your answers always with a smile. I cannot forget Karin, Anders and Reyhan for the helpful discussions and ideas to build up this project. Jörgen and Håkan for the energy invested on my project. And to all Perimed.

To my friends, always there for the good and bad moments. Mattiiia, Gaby, Rosita, Xita, Ludovico, Paulins and Ther, for being here from miles away. Charles, Simon and Mathilde for making this master a great personal experience. Antoine, Sibel, Victor, Bea and Hedwig for a real friendship and love needed in a foreign country.

And of course, I cannot forge to thank Pauet, for his advice and corrections, care, attention and for standing me always.
## ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABI</td>
<td>Ankle-brachial index</td>
</tr>
<tr>
<td>CLI</td>
<td>Critical limb ischemia</td>
</tr>
<tr>
<td>CT</td>
<td>Computer tomography</td>
</tr>
<tr>
<td>LDF</td>
<td>Laser Doppler flowmetry</td>
</tr>
<tr>
<td>LDPI</td>
<td>Laser Doppler perfusion imagers</td>
</tr>
<tr>
<td>LDPM</td>
<td>Laser Doppler perfusion monitors</td>
</tr>
<tr>
<td>LD-SPP</td>
<td>Laser Doppler skin perfusion pressure</td>
</tr>
<tr>
<td>MRA</td>
<td>Magnetic resonance angiography</td>
</tr>
<tr>
<td>PAD</td>
<td>Peripheral arterial disease</td>
</tr>
<tr>
<td>PPG-SPP</td>
<td>Photoplethysmography skin perfusion pressure</td>
</tr>
<tr>
<td>PU</td>
<td>Perfusion units</td>
</tr>
<tr>
<td>SPP</td>
<td>Skin perfusion pressure</td>
</tr>
<tr>
<td>TBI</td>
<td>Toe-brachial index</td>
</tr>
<tr>
<td>TcPO$_2$</td>
<td>Transcutaneous oxygen</td>
</tr>
</tbody>
</table>
**TABLE OF CONTENTS**

LIST OF FIGURES ................................................................................................................................. 1

LIST OF TABLES ......................................................................................................................................... 3

LIST OF EQUATIONS .................................................................................................................................. 4

INTRODUCTION ........................................................................................................................................ 6

Blood flow and blood pressure ................................................................................................................ 6

Macro- and microcirculation ............................................................................................................... 6

Cardiovascular diseases ....................................................................................................................... 7

Laser Doppler flowmetry ....................................................................................................................... 10

The Doppler effect ................................................................................................................................ 10

Depth sensitivity ................................................................................................................................ 11

Calibration ......................................................................................................................................... 12

Heat stimulation ................................................................................................................................ 12

Laser Doppler flowmetry skin perfusion pressure ................................................................................. 14

SPP cut-off value ................................................................................................................................ 14

AIMS ........................................................................................................................................................ 18

MATERIAL AND METHODS ......................................................................................................................... 20

Temperature dependence of SPP .......................................................................................................... 20

Correlation between the pressure in the cuff and the pressure in the probe holder on a limb prototype ........................................................................................................................................ 23

Conditioning and calibration of the force sensor .............................................................................. 23

The first measurements ..................................................................................................................... 25

Tested parameters ................................................................................................................................ 27

Correlation between the pressure in the cuff and the pressure in the probe holder on human beings ........................................................................................................................................ 30

New probe holders ............................................................................................................................ 30

Before measuring on volunteers ....................................................................................................... 31

Measuring on volunteers .................................................................................................................. 31

Further measurements .......................................................................................................................... 33

RESULTS ...................................................................................................................................................... 36

Temperature dependence of SPP .......................................................................................................... 36

Correlation between the pressure in the cuff and the pressure in the probe holder on a limb prototype ........................................................................................................................................ 38

Tested parameters ................................................................................................................................ 38

Correlation between the pressure in the cuff and the pressure in the probe holder on human beings ... 42

Correlation between the Force and the Pressure on the skin ........................................................... 42

SPP of the different probe holders in the same placements. ............................................................ 43

SPP from different positions of the limbs .......................................................................................... 44

Further measurements .......................................................................................................................... 45

CONCLUSIONS ............................................................................................................................................ 48
LIST OF FIGURES

Figure 1: Blood macrocirculation and microcirculation in the human body (reproduced from Perimed AB).................................................................6
Figure 2: Auscultatory method to measure the macrocirculation blood pressure with a pressure cuff (in green), air pump pressure (represented by the pressure graph) and a stethoscope (in black).......................7
Figure 3: A: Source stationary B: source moving to the left (as indicated by the arrow).................................10
Figure 4: Detection of a red cell flux by LDF (reproduced from (12)).................................................................11
Figure 5: Calculated wavelength-dependent penetration depth of light into tissue (blood volume 5%, oxygenation 80%, water content 80%,) over a wavelength range from 500 nm to 100 nm (reproduced from (15)). ...................................................................................11
Figure 6: A: Representative tracing of the local heater set temperature and the skin temperature at the local heater-skin surface interface during a local heating protocol. B: Representative tracing of the blood flow response to the local heating protocol. Values are expressed as a percentage of maximal blood flow during infusion with 50 mM sodium nitroprusside (reproduced from (17)).................................13
Figure 7: Example set-up for SPP measurements (reproduced from Perimed AB)..............................................14
Figure 8: SPP measurement. Microcirculation flow signal (in PU) from the laser Doppler (channel one). Pressure (in mmHg) from the pressure cuff (channel three). SPP pointed out after cuff deflation. (Reproduced from Perimed AB). .................................................................14
Figure 9: Logistic regression analysis of patients (n=29) who were not thought to require vascular reconstruction to heal and were managed with local debridement, minor amputation, or both correlating a given SPP with probability of healing. (10) .............................................................................16
Figure 10: First set-up for SPP measurements. ..................................................................................................20
Figure 11: The 457 Perimed probe holder and the 457 Perimed laser Doppler probe inside A: from above. B: from below. ...................................................................................................................................................21
Figure 12: 457 Perimed laser Doppler probe A: lateral view B: front view showing the sender and receiver fibers...........................................................................................................................................21
Figure 13: A: Heating laser Doppler probe placed inside the Perimed probe holder, and both set on the calf on one volunteer. B: Cuff and probe holder placed in the calf before starting the measurements...21
Figure 14: Channel 1: blood flow signal in PU. Channel 2: temperature signal on the skin on °C. Channel 3: pressure in the pressure cuff in mmHg. ...........................................................................................................................................22
Figure 15: Recommended and initial circuit of the Flexiforce sensor. ............................................................23
Figure 16: Flexiforce sensor A-201. It has a thickness of 0.208 mm, length of 197 mm and sensing area of 9.53 mm diameter. Upper and lower pucks of 8 mm of diameter or 1 mm of height were stack on the sensing areas. ...........................................................................................................................................23
Figure 17: Vertical walls that supported a horizontal map containing boundaries to place the 110 g piece in the middle of the force sensor ...........................................................................................................................................24
Figure 18: Ring on top of the loading area to be used for loading. Perimed probe holder in contact to the other loading area. .................................................................................................................................24
Figure 19: Preliminary set-ups with different foams A: 1.3 cm foam without the cuff. B: 0.3 cm foam with the cuff set to start the measurements. ...........................................................................................................................................25
Figure 20: Second set-up A: inner set-up fixed with tape on 0.3 cm thick foam. B: inner set-up representation. ...........................................................................................................................................26
Figure 21: Piece of 1.1 N used to calibrate and check the performance of the sensor before every measurement. ...................................................................................................................................................27
Figure 22: Set-up example for the study of height. 27.6 mm height case. A: inner set-up pieces. B: inner set-up with inflated cuff and 0.3 cm foam. ...........................................................................................................................................28
Figure 23: Inner set-up examples of the study of contact area in the cuff. A: Perimed probe holder piece on top and 1.3 cm foam. B: 12.5 cm length semicylindrical piece on top and 0.3 cm foam. .........................28
Figure 24: Inner set-up example for the study of contact area in the foam. 5 cm diameter piece case. A: without the cuff and 1.3 cm foam. B: with the cuff around and 0.3 cm foam ...........................................................................29
Figure 25: Nonforcesensor probe holder with the laser Doppler probe set inside. A: from above. B: from above. C: force diagram. ...........................................................................................................................................30
Figure 26: Forcesensor1 probe holder with the laser Doppler probe and the force sensor set inside A: from below. B: from above. C: force diagram. ...........................................................................................................................................31
Figure 27: Forcesensor2 probe holder with the laser Doppler probe and the force sensor set inside A: from above. B: from below. C: force diagram. ...........................................................................................................................................31
Figure 28: A: First serie and second serie set-ups considering the respective shift of the probe holders between them. B: probe holders and cuff set-up on a volunteer lying on his stomach facing the bed. ...

Figure 29: From left to right: Low probe holder, High probe holder, Perimed probe holder, Nonforcesensor probe holder. .................................................................

Figure 30: Channel 1: blood flow signal in PU channel 2: skin surface temperature; channel 3: pressure in the pressure cuff in mmHg. A: Blood perfusion average of 12.5 ± 2.3 PU at body temperature and with no cuff occlusion, 0 mmHg. B: Blood perfusion average of 30.3 ± 4.9 PU at 40 °C and with no cuff occlusion, 0 mmHg. .................................................................

Figure 31: Linear regressions obtained when studying different inner-setup heights on different foams. ................................................................................................................

Figure 32: Linear and second-degree polynomial regressions from equal inner set-ups. ....................

Figure 33: Linear regressions using different surface contact pieces of the inner set-up on the cuff. ..... 

Figure 34: Linear regressions using pieces with two different diameters under the force sensor. .......

Figure 35: First serie set-up. ..............................................................................................................................

Figure 36: The correlation between the cuff pressure and the force in the front leg with the Forcesensor2 probe holder during the first serie of measurements. ..........................................................

Figure 37: First serie set-up of measurements. From left to right: Low probe holder and High probe holder in the left leg. Nonforcesensor probe holder and Perimed probe holder in the right leg. ....

Figure 38: Force diagram of the inner set-up on top of the protolimb surrounded by the pressure cuff. 

Figure 39: Set-up for “chamber” method.................................................................

Figure 40: Set-up for “dynamometer” method........................................................................
LIST OF TABLES
Table 1: Different Fointaine classification stages to classify PAD (adapted from (9))................................. 7
Table 2: Different Rutherford classification stages to classify PAD (adapted from (9)). ............................... 8
Table 3: Reference SPP values for healing and for predicting CLI and PAD. ........................................... 15
Table 4: Different SPP values in both healthy and ischemic volunteer limbs (reproduced from (6)). ......... 16
Table 5: Different properties of the sensor......................................................................................... 25
Table 6: Pieces designed for further tests and testing foams. ............................................................... 26
Table 7: Set-ups of the different probe holders in different body placements for the four groups of measurements.................................................................................................................. 32
Table 8: SPP and probe temperature values for a volunteer and averaged SPP for all the volunteers. ..... 36
Table 9: Study of increase of SPP. The data was averaged for each recording number and for all the volunteers........................................................................................................................................... 37
Table 10: SPP values increases between the second and the rest recordings for all the four different probe holders in the calves. These data was averaged for the eleven volunteers. ......................... 37
Table 11: R² for each group of data obtained........................................................................................... 43
Table 12: SPP sample data for one volunteer, the SPP averaged values in black are the values to be considered for further calculations. The first recording is excluded................................................. 43
Table 13: SPP difference in percentage................................................................................................ 44
Table 14: SPP difference in percentage................................................................................................. 44
Table 15: SPP sample data for one volunteer in the left calf with the High probe holder and Low probe holder. ........................................................................................................................................ 45
Table 16: SPP sample data for one volunteer. ....................................................................................... 46
Table 17: Average results from subtracting the SPP value of the first serie from the second serie for all the volunteers ........................................................................................................................................ 46
Table 18: Average results from subtracting the SPP value of the different probe holders from the SPP value of the Nonforcesensor probe holder for all the volunteers. ................................. 46
LIST OF EQUATIONS

Equation 1: $\Delta f = (\Delta v/c) f_0$ ................................................................. 10
Equation 2: $F = m \times g$ ................................................................................. 24
Equation 3: $P = F/A$ ........................................................................................... 38
INTRODUCTION

Blood flow and blood pressure
Blood pressure is the pressure exerted by circulating blood upon the walls of blood vessels and it is one of the principal vital signs. The human cells, the basic structures in the human body, obtain $O_2$ and nutrients from the blood flow in the capillaries, the smallest vessels, and discharge $CO_2$ and other metabolic waste products into it.

Macro- and microcirculation
The blood pressure in the circulation is principally due to the pumping action of the heart. Differences in the mean blood pressure are responsible for blood to flow from one location to another in the circulation. Mean blood pressure reduces as the circulating blood moves away from the heart through arteries and further on, through the arterioles and capillaries due to viscous losses of energy. The circulation in the terminal arterioles, capillaries and venules is called microcirculation; it is present in the vasculature embedded within organ tissues. This contrasts with the macrocirculation, which transports blood to and from the organs, as depicted in Figure 1.

![Figure 1: Blood macrocirculation and microcirculation in the human body (reproduced from Perimed AB).](image)

Blood pressure varies between a minimum and a maximum value, known as diastole and systole, during each heartbeat. The diastolic pressure is the minimum pressure in the arteries, which is around 80 mmHg, and occurs near the beginning of the cardiac cycle when the ventricles, the pumping chambers in the heart, are filled with blood. The systolic pressure, which is around 120 mmHg, is the peak pressure in the arteries and occurs near the end of the cardiac cycle when the ventricles are contracting. The pressure to be measured in this project is the systolic pressure on the capillaries. If the pressure is appropriate, a correct exchange of nutrients and waste products in the cells should occur, unless there are further complications to be diagnosed.

A standard and spread way of measuring the systolic pressure is blocking the blood pressure, and then, releasing the blockage slowly until the highest pressure (systolic pressure) appears. It is common to use the auscultatory method when measuring the macrocirculation. This method consists on blocking the blood pressure while pumping air to a pressure cuff around the limb. Thereafter, the air is released slowly from the pressure cuff until the first beat (the systolic pressure) is heard through a stethoscope, Figure 2. Looking at the pressure value in the pump when hearing the first beat, the systolic pressure can be obtained. If the air is released until the cuff is empty, there is one point where the beats cease; this point is the diastolic pressure. However, the microcirculation is the circulation to be measured in this project instead of the macrocirculation. Laser Doppler flowmetry is used instead of the auscultatory method. Laser Doppler flowmetry is explained in an upcoming section.
The rate of mean blood pressure depends on several aspects. It depends mainly on the resistance to flow presented by the blood vessels; gravity affects it via hydrostatic forces (e.g., during standing), valves in veins, breathing, and pumping from contraction of skeletal muscles. Thus the measurements in this project were carried out in a silent atmosphere and in an horizontal position of the volunteer’s body.

**Cardiovascular diseases**

**Peripheral Arterial Disease**

Peripheral arterial disease (PAD) is a narrowing of blood vessels that restricts blood flow. It mostly occurs in the legs, but it is sometimes seen in the arms. More restrictedly speaking, PAD includes a group of diseases in which blood vessels become restricted or blocked. Typically, the patient has peripheral arterial disease from arteriosclerosis, a formation of fat on the inner walls of the blood vessels. Blood clots are another process leading to PAD, which restrict blood flow in the blood vessels. In some cases PAD may occur suddenly, for instance when there is an embolism or when a clot clot rapidly develops in a blood vessel already restricted by an atherosclerotic plaque; consequently, the blood flow is quickly cut off.

Even though veins and arteries can be affected, the disease is usually arterial, that is where PAD name stems from.

**Symptoms**

The main symptom is pain in the affected area. Since this disease is seen mainly in the legs, the pain and other symptoms usually occur when walking. The symptoms may disappear when resting. As the disease becomes worse, symptoms occur all the time, even at rest. At the most severe stage of the disease, when the blood flow is greatly restricted, gangrene can develop in the areas lacking blood supply. There are different stages according to the severity of PAD. These stages were classified by Fontaine and Rutherford as shown on the following Table 1 and Table 2.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Asymptomatic</td>
</tr>
<tr>
<td>II</td>
<td>Intermittent claudication. This stage takes into account the fact that patients usually have a very constant distance at which they have pain</td>
</tr>
<tr>
<td>IIa</td>
<td>Intermittent claudication after more than 200 meters of pain-free walking</td>
</tr>
<tr>
<td>IIb</td>
<td>Intermittent claudication after less than 200 meters of walking</td>
</tr>
<tr>
<td>III</td>
<td>Rest pain. Rest pain is especially troubling for patients during the night</td>
</tr>
<tr>
<td>IV</td>
<td>Ischemic ulcers or gangrene (which may be dry or humid)</td>
</tr>
</tbody>
</table>

Table 1: Different Fointaine classification stages to classify PAD (adapted from (9)).
**Stage** | **Symptoms**
--- | ---
I | Asymptomatic
II | Mild claudication
III | Moderate claudication – The distance that establishes mild, moderate and severe claudication is not specified in the Rutherford classification, but it is mentioned in the Fontaine classification as 200 meters
IV | Severe claudication
V | Rest pain
VI | Ischemic ulceration not exceeding ulcer of the digits of the foot
VII | Severe ischemic ulcers or frank gangrene

Table 2: Different Rutherford classification stages to classify PAD (adapted from (9)).

**Risk factors**
There are several factors that may increase the probability of PAD, for instance: smoking, diabetes, obesity (a body mass index over 30), high blood pressure (140/90 mmHg or higher), high cholesterol (total blood cholesterol over 240 mg/dl, or 6.2 mmol/l), increasing age (especially after reaching 50), high levels of homocysteine (a protein component that helps to maintain the tissue), a family history of PAD, heart disease, and/or stroke. (9)

**Diagnosis**
PAD can be diagnosed by comparing the blood pressures taken above and below the point of pain. The area below the pain (downstream from the obstruction) will have a much lower or undetectable blood pressure reading. There are several techniques to diagnose PAD; the most commonly used in the hospitals are angiography, ankle-brachial index (ABI), toe-brachial pressure (TBP), computed tomographic angiography (CT), magnetic resonance angiography (MRA), Doppler and ultrasound (Duplex) imaging and skin perfusion pressure (SPP). (9) Some of them are briefly defined later on.

If the patient smokes, it is highly advised to stop smoking immediately. Exercising is basic to treat PAD. Infections in the affected area should be treated promptly. Surgery may be required to attempt treatment of clogged blood vessels. Considering the last stages, limbs with gangrene must be amputated to prevent the patient from dying.

**Critical Limb Ischemia**
Critical limb ischemia (CLI) is defined as limb pain occurring at rest, or impending limb loss caused by severe compromise of blood flow to the affected extremity. Although the hallmark of PAD is an inadequate blood flow to supply vital oxygen demanded by the limb, CLI occurs right after chronic lack of blood supply, setting off several pathophysiologic (the functional changes associated with or resulting from disease or injury) events that lead to atrophic lesions, rest pain of the legs, or both.(10)

The international consensus regarding CLI is defined as follows: any patient with chronic ischemic rest pain, ulcers, or gangrene attributable to objectively proved arterial occlusive disease.(9) It is important to note that CLI is not to be confused with acute occlusion of the distal arterial tree; instead, it is a process that occurs in a range frame of months to years and, if left untreated, it leads to a limb loss because of lack of adequate blood flow and oxygenation through the distal extremities. (10)

CLI is a severe manifestation of PAD; then, the patients would be placed in the more severe ends of the Fontaine (stage III-IV) or Rutherford classification (grades V-VII), see Table 1 and Table 2 respectively.

SPP can diagnose both CLI and PAD, which is of high relevance since 20% of CLI patients die within the first year.(11)

**Methods of diagnosis**
In order to diagnose both PAD and CLI, several methods can be used.
Microcirculation methods:

**Laser Doppler Flowmetry Skin Perfusion Pressure (LD-SPP):** is a noninvasive method to measure the blood pressure of the microcirculatory flow in the skin at 1-2 mm skin depth, by means of a laser Doppler using the Doppler shift of laser light reflected on the moving red blood cells. SPP measures in millimeters of mercury (mmHg) the pressure at which blood flow first returns to the capillaries.

**Radionuclide washout SPP:** an invasive measurement of SPP which consists in injecting a contrast radioactive agent subcutaneous and study the washout time. The radio nucleotide radiation will decline due to washout by the microcirculatory flow. If the microcirculation is blocked by a pressure, the decline of radiation will stop and the pressure of the microcirculation can be correlated with a pressure cuff.

**Photoplethysmography SPP (PPG SPP):** a non-invasive measurement of SPP by means of a photo sensor detecting the intensity shift of the skin due to changes in the microcirculatory flow.

**Transcutaneous oxygen \((\text{TCPO}_2)\):** non-invasive measurement reflecting the amount of \(\text{O}_2\) that has diffused from the capillaries, through the epidermis, to an electrode.

Macrocirculation methods:

**Ankle-brachial index (ABI):** a non-invasive method that compares the blood pressure in the feet to the blood pressure in the arms in order to determine how well the blood is flowing. Normally the ankle pressure is at least 90 percent of the index of the arm pressure; with severe narrowing it may be less than 50 percent. If an ABI reveals an abnormal ratio between the blood pressure of the ankle and arm, more testing is needed before making a diagnose.

**Toe-brachial index (TBI):** a non-invasive method that compares the blood pressure in the toe with the blood pressure in the arms in order to determine how well the blood is flowing. TBI is performed when the ABI is abnormally high due to plaque and calcification of the arteries in the leg. TBI is unaffected by calcified vessels.
Laser Doppler flowmetry

Laser Doppler flowmetry (LDF) is a non-invasive diagnostic method of measuring blood flow in tissue. This technique is based on measuring the Doppler shift induced by moving red blood cells.

The Doppler effect

The Doppler effect (Doppler shift) is the change in frequency of a wave, or other periodic element, for an observer moving relative to its source. If the source and the observer are still, an observer sees the light wave with the same wavelength and frequency as it was emitted, see Figure 3 A. When the source of the waves is moving towards the observer, each successive wave crest is emitted from a position closer to the observer than the previous wave. As the arrival time between successive waves is decreased, the distance between successive wave fronts reduces. This leads to an increase of the frequency. As depicted in the Figure 3 B, the distance between successive wave fronts is reduced for the observer on the left side of the figure. When the source of waves is moving away from the observer, each wave is emitted from a position farther from the observer than the last wave. Thus each wave takes slightly more time to reach the observer than the previous one, then the distance between successive wave fronts increases. This leads to a reduction of the frequency for the observer on the right side of the figure.

![Figure 3: A: Source stationary B: Source moving to the left (as indicated by the arrow).](image)

The difference between the observed and emitted frequencies is directly proportional to the speed of the source towards or away from the observer, given the laser Doppler light equation:

\[
\Delta f = \left( \frac{\Delta v}{c} \right) f_0
\]

\(\Delta f = f - f_0\) is the difference between the emitted and observed frequencies.

\(\Delta v = v_r - v_s\) is the velocity of the receiver relative to the source: it is positive when the source and the receiver are moving towards each other, and negative when they are moving apart.

\(c\) is the speed of light.

Looking at Figure 4 it can be seen that the laser light is conducted to the skin via fiber optics. In the skin, a small fraction of the light is reflected by moving red cells with a shifting frequency (Doppler effect), whereas the rest is reflected by the same frequency. Both reflected beams are transmitted to the receiving optical fiber. Velocity and concentration of the blood cells in movement can be measured from the output of the LDF instrument. (12)
There are two types of laser Doppler instruments: laser Doppler perfusion imagers (LDPI), which enables to creation of images of the blood flow, and laser Doppler perfusion monitors (LDPM), which is the observation of blood perfusion in a single measurement point (13)(14). In this project LDPM is used.

The major advantage of the laser Doppler techniques in general is their non-invasiveness and their ability to measure the microcirculatory flow of the tissue and fast changes of perfusion during provocations. The technique can measure perfusion quantitatively (although relative) in real time.(13)

However, the technique has some limitations: the influence of optical properties of the tissues on the perfusion signal, motion artifact noise, biological zero problem (signal when there is no flow), unawareness of the depth of measurement, absence of absolute units for the perfusion signal and low perfusion signal (3)(4)(13). These three last limitations are of special interest to this project; therefore, they are more thoroughly explained in this section.

**Depth sensitivity**

The measuring depth depends mainly on both biological and optical aspects. On one side, it depends on tissue properties such as the structure and density of the capillary beds, temperature, pigmentation and oxygenation. On the other side, it depends on the wavelength of the laser light and on the distance between the sending and receiving fibers in the laser Doppler probe.

Since the optical absorption by blood and, to a smaller extent, the scattering level of the tissue differ significantly for green, red and infrared light (see Figure 5) this may be utilized to measure the blood flow in tissue volumes of different size and depth. (13)

On Figure 5 the wavelength dependence of the penetration depth of light into tissue can be seen. Green light (543 nm) has a smaller penetration depth, 0.33 mm, into tissue than both red light (633 nm) and infrared light (800 nm), 3.14 mm and 4.3 mm respectively. (15)
Another aspect to be mentioned is the changing of the source-to-detector separation. Measurements with a flow model show that a larger separation between source and detector increases sensitivity to deeper flows, whereas a smaller separation between source and detector measures more superficially. (16)

Human skin is the largest organ of the body and has an average thickness of 1-2 mm. LDF measuring depth should be then of the order of 0.5-1 mm. Considering penetration depth and source-to-detector separation and, in order to reach this depth, a probe with a fiber separation of 0.25 mm and a 780 nm wavelength laser are used in this project. These characteristics correspond to the probe holder 457 from Perimed.

Calibration
Standardization is required to compare the level of perfusion in different measurements and from different instruments owing to the fact that the laser Doppler perfusion signal is a relative measure of flux. Hence the stability of the instrument can be checked, as well as the linearity of the instrument’s response to blood flow. The relationships between different instruments can be established, and the reading of the instrument to real perfusion can be related (if it is possible).

So far, there is no gold standard available for the calibration of the laser Doppler instrument for perfusion measurements. The problem is that the distribution of blood vessels in tissue and optical properties is heterogeneous, thus it is difficult to calibrate an instrument to measure absolute blood flow per unit volume of tissue. (13)

Even though is not the aimed gold standard, a simple method is used for frequent and easy calibration of laser Doppler instrumentation. It uses an aqueous suspension of polystyrene microspheres in a fixed concentration, called a motility standard. The Doppler shift generated by the Brownian motion of the particles in the suspension is used to calibrate the system’s overall integrity for a comparison of measurements at different time intervals. (13) Since the measured volume is unknown, absolute perfusion values cannot be determined, and measurements are expressed in an arbitrary unit called perfusion units (PU). In this project the probes are calibrated considering that the Brownian motion of our particles equals to 250 PU. Calibration in this project is performed with motility standard Periflux 1000 developed by Perimed AB.

Heat stimulation
Another issue is the action of heat. In human beings, local heat below pain sensation evokes vasodilation, so increase of blood flow; this is mediated by both neurogenic reflexes and locally released substances. (17)

Many factors can have an influence on the response, but in general, local heating evokes an initial dilation response that peaks in a few minutes, followed by a brief nadir, and then a secondary dilation to a plateau that can be sustained. Each dilation is thought to be innervated by two different parts of the nervous system: adrenergic vasoconstrictor system (stimulation by adrenaline hormone) and cholinergic vasodilator system (stimulation by choline hormone). (17)(18)

As it can be observed in Figure 6, there are distinct responses to the local heating. In human beings the skin temperature is around 30 °C, but if the skin is heated until 40°C and kept at constant temperature as it is shown in Figure 6 B, two responses are clearly seen. The regular flow shape before heating is called baseline flow; after heating, a rapid increase in blood flow is found; thereafter, a transient drop follows and, finally, there is a secondary progressive rise to a plateau. After prolonged heating (50 min), and despite maintaining a high skin temperature, blood flow begins to decline in some subjects. (17)

Even though it has been studied that the blood flow increases, it is not clear whether this increase affects the SPP values. This question is one of the goals of this project.
Figure 6: A: Representative tracing of the local heater set temperature and the skin temperature at the local heater-skin surface interface during a local heating protocol. B: Representative tracing of the blood flow response to the local heating protocol. Values are expressed as a percentage of maximal blood flow during infusion with 50 mM sodium nitroprusside (reproduced from [17]).
Laser Doppler flowmetry skin perfusion pressure

Laser Doppler flowmetry skin perfusion pressure (LD-SPP) is a noninvasive method to measure the blood pressure of the microcirculatory flow in the skin at 1-2 mm skin depth. SPP measures in millimeters of mercury (mmHg) the pressure at which the blood flow first returns to the capillaries.

Skin Perfusion pressure is performed by placing a monitor of microcirculation (in our case is a laser Doppler probe) on the skin (see Figure 7), placing a pressure cuff on it, and inflating the pressure cuff until the microcirculation flow signal disappears. After a few seconds without flow signal, the pressure in the cuff is decreased, letting the air out slowly (see Figure 8). While the cuff pressure decreases the microcirculation flow signal eventually returns, this pressure corresponds to SPP (see Figure 8).

The main requirement for body position for recording the measurements is that the height level of the measured parts coincides with the level of the heart (7), as it is shown in Figure 7. The measurements in the present project are taken at supine position.

**SPP cut-off value**

SPP value is a reference value that measures the probability of healing of injuries and ulcers, concerning the pressure measured on the skin. This reference value also helps diagnose lethal diseases such as critical limb ischemia (CLI) and peripheral arterial disease (PAD), previously described. There have been several statistical studies about the significance of the SPP cut-off value as it is shown in Table 3. Summarizing, according to the bibliography, it can be said that the interval between 30-40 mmHg is the critical range. For instance, as a consequence, any ulcers and injuries below this will not heal since there is not enough blood supply that reaches the tissues.
Table 3: Reference SPP values for healing and for predicting CLI and PAD.

<table>
<thead>
<tr>
<th>REPORT</th>
<th>CRITERIA</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castronuovo, Adera, Smiell and Price, 1997(5)(3)</td>
<td>&lt; 30 mmHg</td>
<td>CLI</td>
</tr>
<tr>
<td>Lo, Sample, Moore and Gold, 2009(1)</td>
<td>&lt; 30 mmHg</td>
<td>Wound unlikely to heal</td>
</tr>
<tr>
<td></td>
<td>≥ 30 mmHg</td>
<td>Wound likely to heal</td>
</tr>
<tr>
<td>Yamada, Ohta, Ishibashi, Sugimoto, Iwata, Takahashi and Kawanishi, 2007(8)</td>
<td>&lt; 40 mmHg</td>
<td>wound unlikely to heal and severe PAD</td>
</tr>
<tr>
<td></td>
<td>&gt; 40 mmHg</td>
<td>Wound likely to heal</td>
</tr>
<tr>
<td>Adera, James, Castronuovo, Byrne, Deshmukh and Lohr, 1995(4)</td>
<td>&lt; 30 mmHg</td>
<td>Wound unlikely to heal</td>
</tr>
<tr>
<td></td>
<td>≥ 30 mmHg</td>
<td>Wound likely to heal</td>
</tr>
</tbody>
</table>

In case of gangrene, amputation is the only possible solution and SPP is a tool to decide on the level were the amputation-wound will heal. Figure 9 shows experimental results considering SPP cut-off value when deciding on amputation when suffering from ulcers. All foot lesions and amputation wounds in group I healed, but not all of them in group II, specially below 30 mmHg. Vascular reconstruction or major amputation may have been required instead just local debridement or minor amputation, owing to the low SPP values.

Figure 9: SPP values for all limbs. Group I patients (n = 32) required vascular reconstruction or major amputation in the opinion of vascular attending surgeon. Group II patients (n = 29) were not thought to require vascular reconstruction to heal and were managed with local debridement, minor amputation, or both (reproduced from (10)).

Another study (Figure 9) shows that SPP values between 20 and 30 mmHg do not predict healing with great accuracy. In contrast, an SPP value less than 20 mmHg and a SPP value greater than 30 mmHg predict the outcome of local therapy quite accurately. (10)
Figure 9: Logistic regression analysis of patients (n=29) who were not thought to require vascular reconstruction to heal and were managed with local debridement, minor amputation, or both correlating a given SPP with probability of healing. (10)

The majority of the publications about SPP set a general cut-off value of 30 or 40 mmHg. However, a research group found different SPP values in different parts of the body, Table 4. It is suggested that the results of SPP are getting lower when the measurement is done far from the heart. Therefore, even lower values, stemming from less body supply, are obtained when patients suffer from CLI and PAD.

<table>
<thead>
<tr>
<th>LEVEL</th>
<th>Normal Mean SPP</th>
<th>Ischemic mean SPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brachial</td>
<td>52 ± 3</td>
<td>55 ± 8</td>
</tr>
<tr>
<td>AboveKnee</td>
<td>50 ± 5</td>
<td>46 ± 4</td>
</tr>
<tr>
<td>BelowKnee</td>
<td>42 ± 4</td>
<td>22 ± 4</td>
</tr>
<tr>
<td>Dorsal foot</td>
<td>43 ± 4</td>
<td>10 ± 2</td>
</tr>
<tr>
<td>Dorsal toe</td>
<td>55 ± 5</td>
<td>16 ± 4</td>
</tr>
<tr>
<td>Plantar toe</td>
<td>73 ± 5</td>
<td>17 ± 3</td>
</tr>
</tbody>
</table>

Table 4: Different SPP values in both healthy and ischemic volunteer limbs (reproduced from (6)).

The checking of different SPP values in different parts of the limbs was studied in this project.
AIMS

The first question to address is whether the temperature induces a change in SPP. Temperature brings an increment of vasodilatation and reduction of basal metabolic rate. Then blood flow is increased, thus the Doppler signal rises and gives a better signal which is easier to interpret. It is unclear if these metabolic changes caused by temperature influence SPP. If the laser Doppler just increases the signal with no change of SPP, it will be an enormous help for the physicians and for this project to recognize the SPP value on the monitored data.

The second question to address, and the main problem, in LD-SPP method concerns the fact that when the air pressure in the pressure cuff is measured, this pressure has been assumed to correlate to the pressure applied by the probe holder to the skin. However, this is an indirect measurement that has never been properly evaluated. To give an example of how uncertain is the assumption of correlation between the pressure in the cuff and the pressure applied by the probe holder to the skin: if the pressure cuff is attached very tight on top of the probe holder, the cuff will definitively cause a pressure onto the probe holder and consequently, onto the skin; nevertheless, the air pressure will still show 0 mmHg.

Several concepts were to be studied additionally along the project: the optimal methodology to get results, the optimal size of the probe holder, and the optimal placement of the cuff and the optimal probe holder.

Summing up all the stated problems to be addressed, the main goal a priori was to construct a probe holder that could measure the actual mechanical pressure applied onto the skin. This new probe provided another tool, the mechanical pressure, to evaluate SPP and to correlate the indirect measurement of pressure in the pressure cuff with the new mechanical pressure applied onto the skin.
MATERIAL AND METHODS

Temperature dependence of SPP

The first question to address is whether temperature influences SPP. If the laser Doppler increased only the blood flow signal with no change of SPP, it would be helpful to recognize the SPP value in the clinic.

The volunteers were a group of 25 people (17 men and 8 women) with a mean age of 48 years (from 28 to 75 years), none of them with diagnosed circulation problems. Three out of 25 volunteers repeated the measurements, therefore 28 packs of measurements were obtained. Two out of 28 packs of measurements were rejected due to several inconsistencies. Each pack of measurements consisted of three recordings at body temperature plus three recordings at T=40°C and, in each recording blood perfusion, probe temperature, and cuff pressure were obtained.

Figure 10 shows the first set-up used in this project. Pressure cuffs, 10 and 12 cm wide (Hokansson, USA) were used to measure cuff pressure. The width of the cuff required depends on the width of the limb to be measured. The cuff should be 20% wider than the diameter of the limb on which it is to be used (19). A laser Doppler heating probe was located underneath the cuff. The target place was the middle calf.

![Figure 10: First set-up for SPP measurements.](image)

A Periflux 5000 (Perimed, Sweden) monitored the temperature, the pressure and the blood perfusion with four different monitor units, from left to right in Figure 10: two Periflux 5010 laser Doppler perfusion monitoring units to measure blood perfusion, a Periflux 5020 temperature unit used to perform local heat provocation with two connectors for thermostatic laser Doppler probes and temperature measurement, and a Periflux 5050 pressure unit used to control cuff pressure deflation. A Periflux 472 digital/analog converter was used to send digitalized signals to the Periflux 2.5 software, since in all the other parts of the project the output signal was partly analog.

Before starting, the calibration of the different monitor units was performed. The laser Doppler unit was calibrated with a Periflux 1000 Calibration Probe (Perimed, Sweden). Temperature and pressure were calibrated considering initial known values.

The 457 Perimed probe holder (see Figure 11) holds the heating laser Doppler probe (see Figure 12). The probe holders were designed to avoid tilting of the probe holder when inflating the cuff, to ensure
contact between the probe holder and the skin and to study how the shape of the support influenced the SPP values. The 457 Perimed probe has 10 mm of diameter and 8 mm height.

![Figure 11: The 457 Perimed probe holder and the 457 Perimed laser Doppler probe inside A: from above. B: from below.](image)

![Figure 12: 457 Perimed laser Doppler probe A: lateral view B: front view showing the sender and receiver fibers.](image)

The pressure cuff was placed in a way that the Perimed probe holder was exactly in the middle of the surrounding cuff, as it can be seen in Figure 13.

![Figure 13: A: Heating laser Doppler probe placed inside the Perimed probe holder, and both set on the calf on one volunteer. B: Cuff and probe holder placed in the calf before starting the measurements.](image)

The next step was to obtain a stable baseline from the laser Doppler probe, this took around 1 minute after the placing the cuff and the probe holder. The baseline is the received signal from the laser Doppler probe showing the blood perfusion (see Figure 14).
As it can be observed in Figure 14, the recording part was to be started as soon as a stable baseline was obtained. The pressure cuff was inflated (see channel 3 in minute 10) until the microcirculation disappeared (depicted in channel 1). The cuff was maintained inflated at 150 mmHg during 30 seconds, as it can be seen in channel 2. This was the time needed in order to stabilize the blood flow, obtaining a nearly flat and low intensity signal shown in channel 1.

After this waiting time the cuff was deflated (channel 3 in Figure 14). The deflation was linear at a speed of 3.4 mmHg/sec, controlled by the PF 5050 pressure unit. Channel 1 shows the change of the blood flow from a low-flow signal to a normal flow signal when deflating the cuff. The cuff pressure at the time the microcirculatory flow returns is defined as the SPP. This routine was repeated twice more at body temperature.

After the three first recordings, the laser Doppler probe was heated until a temperature that should bring a vasodilatation response without feeling pain, around 40 °C (17). According to the bibliography, 30 minutes are needed to achieve full blood flow increase (17); however, only two minutes were considered since the duration of the test in the hospitals plays an important role when choosing the medical method to be used. Moreover, after two minutes the flow had already increased significantly. Thereafter, three more measurements with the same previous routine were performed at 40 °C.

Finally, the systolic brachial pressure (arm blood pressure) was measured in all volunteers.
Correlation between the pressure in the cuff and the pressure in the probe holder on a limb prototype

When measuring SPP, the air pressure in the pressure cuff has been assumed to correlate to the pressure applied by the probe holder on the skin. However, this is an indirect measurement that has never been properly evaluated. This correlation was tested on a limb prototype with a selected force sensor and with different probe holder sizes on top of different hardness of foams.

Conditioning and calibration of the force sensor

The selected and purchased sensor Flexiforce (Tekscan, USA) is composed by two layers of substrate made of polyester film. On each layer, a conductive material (silver) is applied, followed by a pressure-sensitive ink layer. When force is applied, the conductance increases and the force value can be obtained since the conductance is proportional to the force.

The force sensor was integrated with the electronic box with help from the electronic department of Perimed AB, see Figure 15. After the first group of measurements, the V_T power has been changed from -1V to -0.165 V to avoid saturation.

Before starting the measurements, the sensor had to be conditioned and calibrated. The User Manual was followed thoroughly and the next steps were required to condition the sensor:

In order to get an even distribution of the force on the sensor area, “pucks” were placed on the sensing area. Two pucks, shown in Figure 16, were to be designed. One puck was set on each side of the sensing area. It was needed, since the contact area of the load was larger than the sensing area. Double-side stickers were used to fix the sensing area with the pucks.

110 % of the maximum test load was placed onto the sensor for approximately three seconds, repeating the procedure five times. However, since the highest test load was unknown at the beginning, the first five sensors were not conditioned properly and thus the results were disregarded. The initial load test to condition was 50 N. The rest of the sensors have been conditioned at 70 N.

Once the sensor was conditioned, a two-step calibration was required.
Four different weights of 50, 110, 300 and 700 g were used in order to obtain the linear relation between the input value and the output value. The timeframe between the measurements was considered to be 30 s.

Once the linear regression was found, the values were calibrated in the program considering the weight of the objects, so its actual theoretical force in Newton units since:

Equation 2: \( F = m \times g \)

\( m \) is the mass and \( g \) is gravity.

Initial problems of the sensor

The sensor was found to be very sensitive, placing the weight slightly different brought very different results. Then double-side stickers were set between the pucks and the upper and lower contact pieces, in order to improve the repeatability. Moreover, two vertical walls on top of an horizontal card were build, where the card had the exact proper loading placement drawn on the upper side (Figure 17). The measurements of the force exerted by the 110 g calibration piece were repeated twenty times and averaged. Once repeatable values were obtained with the 110 g piece, other weights were tested.

![Figure 17: Vertical walls that supported a horizontal map containing boundaries to place the 110 g piece in the middle of the force sensor.](image)

Another experiment in order to study the sensor’s behavior, was to remove the upper puck that was on top of the loading area. Then the puck was replaced by a ring in order not to cover all the sensing area (see Figure 18). Completely different results were found. That means that the pucks should be set carefully on the loading area so as to cover the same surface above and below.

![Figure 18: Ring on top of the loading area to be used for loading. Perimed probe holder in contact to the other loading area.](image)

As it can be seen in the Table 5, the force sensor had several properties that could lead to non-valid results. These properties were studied.
<table>
<thead>
<tr>
<th>Property</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity (error)</td>
<td>± 3%</td>
</tr>
<tr>
<td>Repeatability</td>
<td>± 2.5% of full scale (conditioned sensor, 80% force applied)</td>
</tr>
<tr>
<td>Hysteresis</td>
<td>&lt;4.5% of full scale (conditioned sensor, 80% force applied)</td>
</tr>
<tr>
<td>Drift</td>
<td>&lt;5% per logarithmic time scale (constant load of 90% sensor rating)</td>
</tr>
<tr>
<td>Response Time</td>
<td>&lt;5 microseconds</td>
</tr>
<tr>
<td>Output Change/Degree F</td>
<td>Up to 0.2% (~0.36% / °C). Loads &lt;10 lbs, operating temperature can be increased to 165°F (74°C).</td>
</tr>
</tbody>
</table>

Table 5: Different properties of the sensor.

The linearity error was overcome by averaging several measures. The hysteresis was checked measuring the 100 g piece with and without the 300 g piece several times. The hysteresis effect was studied, it was considered insignificant from the results obtained. No further studies were performed about drift. Response time was measured with a pressure cuff and was established to be around 1 sec. The room was kept all the time between 23°C and 25 °C. Summing all the effects, the output value had a margin of error of 5-10 %.

The first measurements

Cuff pressure versus mechanical pressure (from the force sensor) correlation was obtained first on a limb prototype. A plastic cylinder of 10 cm of diameter and 60 cm long was used instead of a human limb. Two foams of different thickness were set around the cylinder; these were used to simulate different thickness of skin: 0.3 and 1.3 cm thickness. Twenty followed recordings at five different pressures were initially performed using a hand pump to fill the cuffs. By using a compressor, a more consequent filling of the cuffs was achieved and only four recordings of each pressure were needed. Initially, the cuff pressures 50 mmHg, 75 mmHg and 100 mmHg were used. At least ten recordings for each pack of measurements were needed in order to find repeatable values.

The first measurements were performed with the sensor on contact with the surface of the foam. The force sensor was placed under the Perimed probe holder. The cuff surrounded the limb prototype in order to exert pressure on the sensor when being inflated and deflated. See Figure 19 B. It is to be stated that the heating probe holder was not measuring in this part of the project. It was only used to check how its placement in the set-up influenced the mechanical pressure results.

However, the pucks moved from the loading area and the Perimed probe holder moved from its initial place due to torsion and other non-vertical forces from the cuff. The results were not repeatable.
Then, the sensor was situated on top of the probe holder in order to obtain more repeatable results (see Figure 20); consequently, a larger surface area was in contact with the protolimb to bring more stability to the inner set-up. The inner set-ups were the probe holder prototypes in this part of the project. A Stabilizing piece was designed and placed on top of the sensor. This semicylindrical piece, shown in Figure 20, was designed to measure, on the force sensor, only radial forces from the cuff pressure cuff. Furthermore, tape was carefully set to fix the inner set-up on the protolimb as shown in Figure 20 A. Consequently, the repeatability increased considerably.

**Figure 20:** Second set-up A: inner set-up fixed with tape on 0.3 cm thick foam. B: inner set-up representation.

**Inner set-up**

Several pieces were designed for further tests. These pieces, together with the force sensor, conformed the called inner set-up. The inner set-up was set between the cuff and the foam. All the different designed pieces are listed in Table 6.

<table>
<thead>
<tr>
<th>Cylinders to be situated under the force sensor</th>
<th>Semicylinders to be situated on top of the force sensor</th>
<th>Foams</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 cm diameter × 1.5 cm height</td>
<td>10.3 cm radius × 1 cm height × 11.3 cm length</td>
<td>1.3 cm thickness</td>
</tr>
<tr>
<td>4 cm diameter × 0.85 cm height</td>
<td>4 cm radius × 1 cm height × 15 cm length</td>
<td>0.3 cm thickness</td>
</tr>
<tr>
<td>5 cm diameter × 1.5 cm height</td>
<td>4 cm radius × 1 cm height × 3.2 cm length (called Stabilizing piece)</td>
<td></td>
</tr>
<tr>
<td>5 cm diameter × 0.85 cm height</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5 cm diameter × 1.5 cm height</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5 cm diameter × 0.85 cm height</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6: Pieces designed for further tests and testing foams.

During the rest of the project, the 110 g piece (1.1 N) was used to check whether the force sensor was properly calibrated and undamaged before each measurement, see Figure 21.
An issue to take into account was the saturation. During the first measurements, the values got saturated at 20 mmHg. Therefore, the $V_{\text{total output}}$ was changed from -1 V to -0.156 V to get lower resistivity by the sensor, in other words, to get higher values of force. Now the saturation level was situated above the 50% of the load to be used of the sensor, which was the proper interval range to be used.

**Non-linearity**

Non-linear results were found during the measurements, even though the sensor had already been conditioned and calibrated. The possible origins could be the electronic box, the sensor or/and the cuff.

The electronic box was checked with the help of several resistances and the output values of the electronic box were compared. Regarding the sensor, different weights were used to check the linearity. Finally, about the cuff, different pressure cuff values of 125, 100, 75, 50 and 25 mmHg were exerted on the different pieces on top of the sensor; these pieces had different surface area values and heights. The electronic box and the sensor were working in a linear way, but not the cuff when using inner set-up pieces of different height. These results are to be commented in an upcoming section.

**Methodology in each measurement:**

First the force sensor was checked to work with the 1.1 N piece. Calibration was performed if needed. Then, the three parts of the inner set-up (a cylindrical piece on top of the foam, the force sensor in between, and a semicylindrical piece on top) were glued with double-sided adhesive strips. Tape was set to fix the inner set-up on the foam (see Figure 20 A). Next, the room temperature was noted followed by the starting the measurements, twenty measurements were performed for each pressure value with the manual pump and four measurements for the automatic pump. Once the measurements were completed, the position of the different pieces of the inner set-up and the pucks of the force sensor were checked from possible shifts. To ensure that the results were reliable, the whole set-up was demounted and mounted again and the process was fully repeated until repeatable results were found, around ten times.

**Tested parameters**

The cuff was exercising mechanical force on the inner set-up, thus the inner set-up would exert the same force on the foam (the patients’ skin). However, different characteristics of the inner set-up were thought to bring different force values on the foam. Four values were studied: the height of the inner set-up, the contact area of the inner set-up in the cuff, the contact area of the inner set-up in the foam, and foam thickness on top of the protolimb. The data obtained was the correlation of the mechanical pressure, pressure from the force sensor, with the cuff pressure.

**Height**

In order to check the effect of the height of the inner set-up, four different inner set-ups were used on top of two different foams. The cylindrical pieces that were lying under the force sensor were: 8.5, 15, 23.5 and 30 mm high, see Table 6. The piece on top of the force sensor was the Stabilizing piece. The total heights of study were consequently 21.1, 27.6, 36.1, 42.6 mm. The foams were 0.3 and 1.3 cm thick. See Figure 22 with an example of set up for this section.
Contact area in the cuff

In order to check the effect of the surface contact area of the top part of the inner set-up in the cuff, four different inner set-ups have been used. The pieces on top of the force sensor were a semicylinder with radius of 10.5 cm and a semicylinder with radius of 4 cm (both described in Table 6), the Stabilizing piece and the Perimed probe holder. The piece under the force sensor was a 2 × 2 cm hard plastic square of 1.5 mm of thickness. The total height was 11.7 mm. The foam used for this study was 0.3 cm thick. See Figure 23 with two examples of set-ups of this section.

Contact area in the foam

In the case of really small probe holders a little contact area in the skin could cause the probe holders to lean and thus, to obtain misleading results. The importance of the contact area in the foam is to be studied.

In order to check the effect of the contact area of the inner set-up in the foam, two different inner set-ups were used. The pieces under the force sensor were two cylinders with different widths, 2.5 and 5 cm, from Table 6. The piece on top of the force sensor was the Stabilizing piece; it was chosen since the results were more repeatable with this piece when studying the other characteristics. The total height was 11.7 mm. The foams were 0.3 and 1.3 cm thick. See example of the inner set-up in this study in Figure 24.
Figure 24: Inner set-up example for the study of contact area in the foam. 5 cm diameter piece case. A: without the cuff and 1.3 cm foam. B: with the cuff around and 0.3 cm foam.

**Foam thickness**

For almost every different set-up, measurements with both thin and thick foam were obtained. In the case of just one foam, the thin foam was chosen since the pressure values obtained were higher, hence the force changes were easier to differentiate.
Correlation between the pressure in the cuff and the pressure in the probe holder on human beings

The limb target is changed to real human limbs of volunteers after the previous studies. The main question is finally to be studied: how the air pressure in the pressure cuff correlates to the pressure applied by the probe holder to the skin.

New probe holders

From the initial conclusions of the second part of this project, it was decided that the new probe holder should be minimized, cylinder shaped like the cuff around it and without sharp edges. Then, the following designed prove should have a high repeatability.

Consequently three different designs were firstly obtained. The material was chosen to be hard plastic in order to both avoid conductive problems with the sensor in the case of metals, and to avoid the probe holder to bend and, thus, transmit the proper force to the force sensor. The height in the three probe holders was minimized taking into account the height of the laser Doppler probe, pucks and force sensor and mathematically considering that the minimum thickness of the material to be used was 0.5 mm. The probe holders were semicylindrically shaped and the edges were rounded. These probe holders were lined-up with the laser Doppler on the skin contact area. The first tests were performed on the protolimb of the previous part. The probe holders were the following:

Nonforcesensor probe holder: In Figure 25 it can be seen the first probe holder with no force sensor. In A, the space in the middle of the probe holder contains the laser Doppler probe.

![Nonforcesensor probe holder with the laser Doppler probe set inside. A: from below. B: from above.](image)

Forcesensor1 probe holder: Figure 26 shows the third probe holder with force sensor. The goal in this case was to transmit only part of the force from the cuff. In this case, the cuff exerted a force directly to all the skin in contact, and only the laser Doppler probe exerted a certain force measured by the force sensor (see Figure 26 C).
As in the first probe holder, the height was minimized and it had a similar shape.

**Forcesensor2 probe holder**: Figure 27 shows one of the force sensor probe holders. The goal in this case was to transmit all the radial force from the cuff to the force sensor, and the same force from the force sensor to the skin (see Figure 27 C).

As in the first probe holder, the height was minimized and it had a similar shape. In order to improve stability, one groove was carved in the upper piece of the probe holder as it can be seen on the right bottom part in Figure 27 B.

**Before measuring on volunteers**

Before being able to continue with the measurements on people, another electronic box was needed to be able to use the two force sensors at the same time. Help from one expert in electronics from Perimed AB was required.

In order to obtain the same output voltage value from the two electronic boxes, the adjustable resistance of the new electronic box was tuned with the help of a voltmeter; the output voltage of the both electronic boxes was adjusted to 0.5580 V.

Checking the performance of the new electronic box with the force sensor, non-expected results were obtained. The output value was non-zero. However, when using a resistance instead of the force sensor, the value obtained was correct. The ground of the circuit was not properly set. The sensors were calibrated separately as performed in the first part of the project: four different weights of 50, 110, 300 and 700 g were used in order to calibrate the force sensor and its mechanical pressure in the software. Further measurements were performed to check the air tubes and connections.

**Measuring on volunteers**

Once the different set-ups were ready to be used and checked, the measurements were carried on 17 volunteers (11 men and 6 woman) with a mean age of 45.3 years (range 28 to 56 years) in this first part of the force SPP measurements on volunteers.
**Methodology**

First the temperature of the heating probe holders was adjusted to 40 °C. Again, the two force sensors were checked to work with the 1.1 N piece and calibration was performed if needed. The legs of the volunteers were left without occlusion and the volunteers at supine position. It was important that the feet and the legs were at the same height as the heart. Moreover, the atmosphere was quite to keep the blood pressure constant. Thus, the measurements could proceed: the three support probe holders were set in the three different sides as stated Table 7. A double sticker between the skin and the probe holders as well as tape around the probe holders kept the set-up attached to the skin. Then the pressure cuffs were set around the limbs with the probe holder on top, leaving two fingers of free space between the probe holder and the cuff. It is to be noted that, during the first recording of every group of measurements, it was checked that the cuffs were equally tight and that the probe holders had not tilted. Thus the data of the first recording was not used. If the setup was not properly set, the probe holders and the cuffs were set again. The three following measurements were the data used in further calculations.

The second, third and fourth series of measurements were performed with the same routine as explained in the previous paragraph, placing the different support probe holders according to Table 7.

<table>
<thead>
<tr>
<th></th>
<th>Foot dorsum</th>
<th>Foot planum</th>
<th>Front leg</th>
<th>Calf</th>
</tr>
</thead>
<tbody>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; serie</td>
<td>Forcesensor1 probe holder</td>
<td>Nonforcesensor probe holder</td>
<td>Forcesensor2 probe holder</td>
<td>none</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; serie</td>
<td>Forcesensor2 probe holder</td>
<td>Forcesensor1 probe holder</td>
<td>none</td>
<td>Nonforcesensor probe holder</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt; serie</td>
<td>none</td>
<td>Forcesensor2 probe holder</td>
<td>Nonforcesensor probe holder</td>
<td>Forcesensor1 probe holder</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt; serie</td>
<td>Nonforcesensor probe holder</td>
<td>none</td>
<td>Forcesensor1 probe holder</td>
<td>Forcesensor2 probe holder</td>
</tr>
</tbody>
</table>

Table 7: Set-ups of the different probe holders in different body placements for the four groups of measurements.

**Tested parameters**

Three different aspects were studied: how the air pressure in the pressure cuff correlates to the pressure applied by the probe holder to the skin, which is the main goal; the SPP values obtained with the three new probe holders; and the SPP values of four different positions in the limbs.
Further measurements

It was decided to check other variables since the main goal of the first part was not accomplished. The **height** and the **contact surface on the cuff** of different probe holders were now checked. These probe holders had already been studied previously on the protolimb in the previous part of this project. The following measurements were taken in 11 volunteers (7 men and 4 women) with a mean range of 40 (range 28 to 50 years).

Four different probe holders were situated in pairs on the calves, in a way that the center of the cuff in each leg was situated in the middle of every pair of probe holders (see Figure 28 A). The volunteers were laying on their stomach to measure easily in the calves as it can be observed in Figure 28 B.

There were two series of measurements in order to check the repeatability when removing the set up and setting it again. First the temperature of the heating probe holders was adjusted to 40 °C. Six measurements were made for each pair at the same time in each leg, then, the cuffs and the probe holders were removed. The second serie stared after moving 4 cm all the probe holders in one side to avoid measuring at the same skin spot (see Figure 28 A) and adjusting the set-up again. Hence six further measurements were recorded. Like before, the first recording was just to check that the set-up was working properly.

![Figure 28: A: First serie and second serie set-ups considering the respective shift of the probe holders between them. B: probe holders and cuff set-up on a volunteer lying on his stomach facing the bed.](image)

The results obtained were not real SPP values since the probe holders were not lying in the middle of the cuffs. However, the correlations between the probe holders placed on the same limb were still of scientific interest.

Characteristics of the four probe holders, showed in Figure 29:

- **Low probe holder**: 25 mm of radius and 18 mm of height. Checking the height effect and repeatability.
- **High probe holder**: 25 mm of radius and 25 mm of height. Checking the height effect and repeatability.
- **Perimed probe holder**: 25 mm of radius, 9 mm of height, 0.0005 m$^2$ of area. No sharp edges. Checking the area effect and repeatability.
- **Nonforcesensor probe holder**: 10 mm of height, semicylindrical shape, 0.002 m$^2$ of area. Checking the area effect and repeatability.
Figure 29: From left to right A: Low probe holder and High probe holder B: Perimed probe holder and Nonforcesensor probe holder.
RESULTS

Temperature dependence of SPP
The main goal in this part of the project is to determine if the temperature affects the SPP value. The increase of temperature brings an increase of blood flow which entails a more intense laser Doppler signal, as it can be observed in Figure 30.

A

Figure 30: Channel 1: blood flow signal in PU channel 2: skin surface temperature; channel 3: pressure in the pressure cuff in mmHg. A: Blood perfusion average of 12.5 ± 2.3 PU at body temperature and with no cuff occlusion, 0 mmHg. B: Blood perfusion average of 30.3 ± 4.9 PU at 40°C and with no cuff occlusion, 0 mmHg.

Six SPP measurements have been obtained from twenty-six volunteers (see Table 8). The first three measurements were at body temperature and the last three were at 40°C.

<table>
<thead>
<tr>
<th>Recording number</th>
<th>SPP (mmHg)</th>
<th>T (°C)</th>
<th>Averaged SPP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>49.2</td>
<td>31.1</td>
<td>58.1 ± 15.5</td>
</tr>
<tr>
<td>2</td>
<td>52.7</td>
<td>32</td>
<td>59.2 ± 14.6</td>
</tr>
<tr>
<td>3</td>
<td>56.9</td>
<td>32.9</td>
<td>61.4 ± 16.2</td>
</tr>
<tr>
<td>4</td>
<td>58.1</td>
<td>39.8</td>
<td>63.7 ± 14.8</td>
</tr>
<tr>
<td>5</td>
<td>59.9</td>
<td>39.8</td>
<td>63.3 ± 15.1</td>
</tr>
<tr>
<td>6</td>
<td>63.8</td>
<td>39.8</td>
<td>63.4 ± 14.8</td>
</tr>
</tbody>
</table>

Table 8: SPP and probe temperature values for a volunteer and averaged SPP for all the volunteers.

When performing the measurements, it was noticed that the SPP slightly increased from the first to the sixth recording in each volunteer (see Table 8). The difference between the first recorded value and the following values (until six recordings) was calculated in order to study this effect. Moreover, the SPP expected values, and its spread depending on the volunteer, can be observed in the last column.

The increase between recordings was calculated in Table 9. Healthy volunteers were hypotensive and hypertensive (see the standard deviation of the last column of Table 8); thus pressure changes were lower or higher respectively. Relations between two measured parameters were calculated in percentage change in order to overcome this fact in the rest of the project.
Table 9: Study of increase of SPP. The data was averaged for each recording number and for all the volunteers.

Table 9 shows that the biggest SPP increase is 7.1% from the first to the second recording. SPP increases 1.4% between the body temperature last measurement and the first measurement at 40°C (between the third and the fourth recordings).

A second temperature study was performed using the data of the last part of this project to clarify if this SPP increase was due to temperature or there was another drift. Six measurements at 40°C were recorded when the height and the contact surface effects of the probe holders where studied on volunteers (see Table 10). In this study the first recording was excluded, since it was observed that the inner set-up could move to an equilibrium position when the cuff was inflated on it for the first time.

Table 10: SPP values increases between the second and the rest recordings for all the four different probe holders in the calves. These data was averaged for the eleven volunteers.

From Table 10 it can be observed that SPP tended to increase on time. SPP increases 6.6% in total.

The main result from this study is that there is no evidently SPP change due to temperature. SPP increased 8.3% during the five last measurements (neglecting the first one), and SPP increased 6.6% during the five measurements at 40°C in the last study (the data of the first measurement was not obtained).
Correlation between the pressure in the cuff and the pressure in the probe holder on a limb prototype

The correlation between the cuff pressure and the real mechanical pressure exerted on the probe holder was first tested on a limb prototype with different inner set-ups on top of two foams of different thickness. The inner set-ups were different prototypes of possible probe holders to measure SPP and the foams represented different kinds of skin tissue. Each inner set-up consisted of a piece on top of the force sensor, a force sensor, and another piece underneath. See Figure 20 B with an example of inner-set up for this section. The mechanical pressure was calculated with a force sensor considering the contact surface area of the inner set-up with the cuff. See Equation 3, were $F$ is force, $P$ is pressure and $A$ is area:

$$P = \frac{F}{A}$$

Height, contact area in the cuff and contact area in the foam of the inner set-up were studied as well as the foam thickness. The values of the pressure of the cuff and the pressure from the force sensor were the data obtained for each study.

Tested parameters

**Height**

In order to check the effect of the height of the inner set-up, four different inner set-ups have been used. The cylindrical pieces that were lying under the force sensor were: 8.5, 15, 23.5 and 30 mm high, (see Table 6). The piece on top of the force sensor was the Stabilizing piece. The total heights of study were consequently 21.1, 27.6, 36.1, 42.6 mm. Figure 31 shows the linear regressions of the force responses when inflating the cuff at 50, 75 and 100 mmHg. Thin lines represent 0.3 cm thick foam and thick lines represent 1.3 cm thick foam.

![Study of height in different foams](image)

In Figure 31 two main groups of measurements can be observed. The mechanical pressure increases, respect to the cuff pressure, with the height of the inner set-up for all the foams. However, the mechanical pressure increased faster for the thinner foam measurements. It is to be noted that the linear regressions from 50, 75 and 100 mmHg values do not reach the mechanical pressure of 0 mmHg.
in the thin foam case, as it is expected practically when the cuff pressure is 0 mmHg. Thus the heights of the inner set-up, the sensor, the electronic box and the cuff were checked as possible origins of this unexpected behavior. After several tests, the sensor, the electronic box and the cuff were disregarded, leaving only the height of the inner set-up as the possible problem.

Different inner set-ups with different heights were placed under the force sensor. The pressures to measure were also 10 and 25 mmHg in order to check what was happening near the mechanical pressure of 0 mmHg. Second-degree polynomial regressions of the mechanical pressure of the force response are represented by thick lines in Figure 32, the cuff pressure was inflated at 10, 25, 50, 75 and 100 mmHg for the different height pieces. The linear regressions of the force response are represented by thin lines. The cuff pressure was again inflated at only 50, 75 and 100 mmHg. The foam was 0.3 cm thick.

![Figure 32: Linear and second-degree polynomial regressions from equal inner set-ups.](image)

Linear and second-degree polynomial regressions are plotted and compared in Figure 32. It can be observed that the mechanical pressure is smaller in the polynomial fit than in the linear regression at 0 mmHg of cuff pressure. At the measurements’ serie at 10, 25, 50, 75 and 100 mmHg cuff pressures, the correlation coefficient ($R^2$) for all the three second-degree polynomial regressions is $R^2=0.998$ and a maximum of $R^2=0.971$ if the fits are linear instead; where $R^2$ shows how accurately the equations describe the data, being $R^2 = 1$ the best fit. However, at the measurements’ serie of 50, 75 and 100 mmHg cuff pressures, the $R^2$ of the linear regressions are between 0.998 and 0.995.

The second-grade polynomial regression is the best fit. The differences between the mechanical pressure and the 0 mmHg pressure cuff were small: 12 mmHg for the 21.1 mm height inner set-up, 18 mmHg for the 27.6 mm height inner set-up, and 15 mmHg for the 36.1 mm height inner set-up.

**Contact area in the cuff**

Four different inner set-ups have been used in order to check the effect of the surface contact area (area of the top part of the inner set-up) with the cuff. The pieces on top of the force sensor were a semicylinder of 10.5 cm radius, a semicylinder with radius of 4 cm, the Stabilizing piece and the Perimed probe holder (see Table 6). The piece under the force sensor was a $2 \times 2$ cm hard plastic square of 1.5 mm of thickness. The total height was 11.7 mm. The Figure 33 shows the linear regression of the force response when inflating the cuff pressure at 10, 25, 50, 75 and 100 mmHg. The foam was 0.3 cm thick.
Figure 33: Linear regressions using different surface contact pieces of the inner set-up on the cuff.

The results obtained were similar for the two semicylindrical pieces used, and cuff and mechanical pressure had similar values in both cases.

Considering the Stabilizing piece and the Perimed support, the values obtained followed the tendency of the other studies: the mechanical pressures were higher than the cuff pressures. It is to be noted that the mechanical pressure almost corresponded to cuff pressure with the 10.3 cm radius and 4 cm radius pieces on the top of the force sensor.

Contact area in the foam

In order to check the effect of the contact area of the inner set-up on the cuff, two different inner set-ups were used. The pieces under the force sensor were two cylinders with different widths, 2.5 and 5 cm (see Table 6). The piece on top the force sensor was the Stabilizing piece (see Table 6). The total height was 11.7 mm. Figure 34 shows the linear regression of the force response when inflating the cuff pressure at 10, 25, 50, 75 and 100 mmHg. The linear regressions are represented by thin lines for 0.3 cm thick foam and by thick lines for 1.3 cm thick foam.
Figure 34: Linear regressions using pieces with two different diameters under the force sensor.

The mechanical pressure values for the two different pieces in contact with the 0.3 cm foam showed a small pressure difference of about 20 mmHg along the linear regression. The slope of the two linear regressions was the same but not the origin of coordinates. Similar linear regressions between these two inner set-ups were found when setting the thicker foam.

**Foam thickness**

Figure 31 and Figure 34 show that the foams did not bring the same results in any inner set-up case. A minimum difference of 20 mmHg and a maximum difference of 500 mmHg, of mechanical pressure at the same cuff pressure, were measured between the 0.3 cm thick and 1.3 cm thick foams.
Correlation between the pressure in the cuff and the pressure in the probe holder on human beings

After studying height, contact area in the cuff, contact area in the foam and foam thickness, three different laser Doppler probe holders were designed: Forcesensor2, Forcesensor1 and the Nonforcesensor, introduced before in the Method, see Figure 27, Figure 26 and Figure 25 respectively. Then, three different aspects were studied: the main goal, the correlation of the mechanical force with the cuff pressure in every designed probe holder on the skin; the SPP values obtained with the three new probe holders in the same body positions; and the SPP values from four different positions of the limbs. Four series of measurements were performed in different positions of the limbs as shown in Table 7 in Methods. The first serie is exemplified in Figure 35.

Correlation between the Force and the Pressure on the skin

The correlation between the force obtained from the force sensor and the pressure obtained from the cuffs was studied in every probe holder and adjusted into a linear regression. A linear regression example is plotted in Figure 36. $R^2$ for all the linear regressions is listed in Table 11.

Figure 35: First serie set-up.

Figure 36: The correlation between the cuff pressure and the force in the front leg with the Forcesensor2 probe holder during the first serie of measurements.
Table 11: $R^2$ for each group of data obtained.

In Table 11 it can be observed that none of the $R^2$ values approaches 1, the best fit. The data obtained from the force sensor, the mechanical pressure, was not considered in further studies because of the low precision.

**SPP of the different probe holders in the same placements.**

After disregarding the first measurement of each group of the pressure cuff, the following three measurements were averaged for all the volunteers, obtaining one averaged SPP value for each probe holder and for each site (see Table 12). These pressure cuff values were the data to be used for further calculations.

<table>
<thead>
<tr>
<th>PROBE HOLDER TYPE</th>
<th>Forcesensor2 probe holder (mmHg)</th>
<th>Forcesensor1 probe holder (mmHg)</th>
<th>Nonforcesensor probe holder (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1st SERIE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PROBE HOLDER TYPE</td>
<td>Front leg</td>
<td>Dorsal foot</td>
<td>Planum foot</td>
</tr>
<tr>
<td></td>
<td>27.4</td>
<td>87.9</td>
<td>123.8</td>
</tr>
<tr>
<td></td>
<td>24.8</td>
<td>86.6</td>
<td>116.2</td>
</tr>
<tr>
<td></td>
<td>26.1</td>
<td>87.6</td>
<td>117</td>
</tr>
<tr>
<td>SPP average</td>
<td>26.1 ± 1.3</td>
<td>87.6 ± 0.7</td>
<td>117 ± 4.2</td>
</tr>
<tr>
<td><strong>2nd SERIE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BODY SIDE</td>
<td>Dorsal foot</td>
<td>Plantar foot</td>
<td>Calf</td>
</tr>
<tr>
<td></td>
<td>73.5</td>
<td>97.6</td>
<td>68.2</td>
</tr>
<tr>
<td></td>
<td>76.5</td>
<td>96.1</td>
<td>80.1</td>
</tr>
<tr>
<td></td>
<td>76.3</td>
<td>95.6</td>
<td>73.5</td>
</tr>
<tr>
<td>SPP average</td>
<td>76.3 ± 1.7</td>
<td>96.1 ± 1.1</td>
<td>73.5 ± 6</td>
</tr>
<tr>
<td><strong>3rd SERIE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BODY SIDE</td>
<td>Plantar foot</td>
<td>Calf</td>
<td>Front leg</td>
</tr>
<tr>
<td></td>
<td>81.6</td>
<td>66.2</td>
<td>59.5</td>
</tr>
<tr>
<td></td>
<td>87.9</td>
<td>63.2</td>
<td>59.6</td>
</tr>
<tr>
<td></td>
<td>78.5</td>
<td>68.9</td>
<td>62.8</td>
</tr>
<tr>
<td>SPP average</td>
<td>81.6 ± 4.8</td>
<td>66.2 ± 2.9</td>
<td>59.6 ± 1.9</td>
</tr>
<tr>
<td><strong>4th SERIE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BODY SIDE</td>
<td>Calf</td>
<td>Front leg</td>
<td>Dorsal foot</td>
</tr>
<tr>
<td></td>
<td>40.8</td>
<td>43.9</td>
<td>88.3</td>
</tr>
<tr>
<td></td>
<td>51.8</td>
<td>35.4</td>
<td>94.6</td>
</tr>
<tr>
<td></td>
<td>50.5</td>
<td>37.6</td>
<td>83.2</td>
</tr>
<tr>
<td>SPP average</td>
<td>50.5 ± 6</td>
<td>37.6 ± 4.4</td>
<td>88.3 ± 5.7</td>
</tr>
</tbody>
</table>

Table 12: SPP sample data for one volunteer, the SPP averaged values in black are the values to be considered for further calculations. The first recording is excluded.
The standard deviation was calculated for every group of four measurements, for each serie and body position in all the volunteers (twelve for each volunteer), to understand its repeatability. The averaged standard deviation was $3.5 \pm 0.9$ mmHg.

Considering the Nonforcesensor probe holder as a reference, the difference between the SPP value from the two probe holders with force sensor and between the value from the Nonforcesensor probe holder were calculated. Then, these differences were averaged for all the volunteers (see Table 13):

<table>
<thead>
<tr>
<th></th>
<th>Front leg</th>
<th>Dorsal foot</th>
<th>Plantar foot</th>
<th>Calf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonforcesensor probe holder – Forcesensor2 probe holder (%)</td>
<td>$31.5 \pm 21.8$</td>
<td>$5.6 \pm 31.3$</td>
<td>$7.9 \pm 21.6$</td>
<td>$3.9 \pm 29.6$</td>
</tr>
<tr>
<td>Nonforcesensor probe holder – Forcesensor1 probe holder (%)</td>
<td>$7.2 \pm 35.1$</td>
<td>$29.7 \pm 15.8$</td>
<td>$3.9 \pm 26.1$</td>
<td>$1.1 \pm 30.2$</td>
</tr>
</tbody>
</table>

Table 13: SPP difference in percentage.

When calculating the standard deviation average for each difference, high numbers were obtained (see Table 13). The values obtained between the probe holders were similar in six out of the eight relations calculated previously.

**SPP from different positions of the limbs**

The SPP value can be studied in different parts of the body from the sample data in Table 12. The parts of interest were the upper and lower feet, the front part of the leg and the calf.

Again, our results are compared to a reference, in this case the reference was the front leg position. All the different considered positions were compared to the front leg for the different probe holders for all the volunteers (see Table 14).

<table>
<thead>
<tr>
<th></th>
<th>Dorsum foot – front leg</th>
<th>Planum foot – front leg</th>
<th>Calf – front leg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forcesensor2 probe holder (%)</td>
<td>$90.9 \pm 69.3$</td>
<td>$81 \pm 68.7$</td>
<td>$56.1 \pm 64.3$</td>
</tr>
<tr>
<td>Forcesensor1 probe holder (%)</td>
<td>$61.6 \pm 72.7$</td>
<td>$60.9 \pm 53.9$</td>
<td>$40.8 \pm 62.1$</td>
</tr>
<tr>
<td>Nonforcesensor probe holder (%)</td>
<td>$54.1 \pm 61.3$</td>
<td>$57.1 \pm 64.2$</td>
<td>$28.2 \pm 44.9$</td>
</tr>
</tbody>
</table>

Table 14: SPP difference in percentage.

Again, a high standard deviation was found for all the relations. The lowest SPP values were found in the front legs, followed by the calves, and the highest values were found in the feet.
Further measurements
Further studies were performed since the main goal was not achieved and there were volunteers available to continue the project.

Then, different parameters studied in the limb prototype were considered on human beings. The height and the contact area on the cuff were studied with two pairs of probe holders. Each calf was considered as a single study.

The different probe holders were set as depicted in Figure 37 in the first serie of measurements. In the second serie all the probe holders were removed and placed again 4 cm away from its initial place. Two series of six measurements were performed in the calves of each volunteer, see Table 15 for sample data. The average of the last five measurements was the data used for the study.

![Figure 37: First serie set-up of measurements. From left to right: Low probe holder and High probe holder in the left leg. Nonforcesensor probe holder and Perimed probe holder in the right leg.](image)

<table>
<thead>
<tr>
<th></th>
<th>High probe holder</th>
<th>Low probe holder</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1st serie</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>55.7</td>
<td>33.9</td>
</tr>
<tr>
<td></td>
<td>54.2</td>
<td>36.2</td>
</tr>
<tr>
<td></td>
<td>57.6</td>
<td>40.4</td>
</tr>
<tr>
<td></td>
<td>66.4</td>
<td>47.3</td>
</tr>
<tr>
<td></td>
<td>59.3</td>
<td>41.5</td>
</tr>
<tr>
<td><strong>SPP average</strong></td>
<td><strong>58.6 ± 5.7</strong></td>
<td><strong>39.9 ± 3</strong></td>
</tr>
<tr>
<td><strong>2nd serie</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>49.9</td>
<td>33.9</td>
</tr>
<tr>
<td></td>
<td>55.7</td>
<td>36.3</td>
</tr>
<tr>
<td></td>
<td>57.2</td>
<td>34.7</td>
</tr>
<tr>
<td></td>
<td>57.2</td>
<td>37.1</td>
</tr>
<tr>
<td></td>
<td>56.9</td>
<td>36.5</td>
</tr>
<tr>
<td><strong>SPP average</strong></td>
<td><strong>55.4 ± 2.4</strong></td>
<td><strong>35.7 ± 4</strong></td>
</tr>
</tbody>
</table>

Table 15: SPP sample data for one volunteer in the left calf with the High probe holder and Low probe holder.
Table 16: SPP sample data for one volunteer.

The general repeatability of the different probe holders was studied: in Table 17 it is calculated how the SPP of the probe holders changes when setting the set-up again and performing the same measurements.

Table 17: Average results from subtracting the SPP value of the first serie from the second serie for all the volunteers

Table 17 shows that the probe holder with more repeatability and less standard deviation was the Low probe holder. The High probe holder has the biggest standard deviation. The Nonforcesensor probe holder has the biggest difference between series but a small standard deviation compared to the other probe holders.

The general difference obtaining SPP values between the different probe holders situated in the same limb was studied; calculations are shown in Table 18.

Table 18: Average results from subtracting the SPP value of the different probe holders from the SPP value of the Nonforcesensor probe holder for all the volunteers.

Table 18 shows that, on average, there is not a big difference of SPP between the two pairs of probe holders, with a maximum of 15.1% between the Perimed and the Nonforcesensor probe holder. However, the standard deviations are big.
CONCLUSIONS

The main goal of this work was to construct a laser Doppler probe holder with a mechanical pressure monitor in order to measure microcirculation and mechanical pressure at the same time. This probe holder would be used to understand the SPP method better; SPP is a method to study the status of the microcirculation on the skin. Hence SPP would be a good alternative tool to TcPO$_2$ to diagnose PAD and CLI. Additionally studies were performed to study whether the temperature induces a change in SPP.

The correlation between the cuff pressure and the real mechanical pressure exerted by the probe holder on the skin was first tested on a limb prototype with different inner set-ups (probe holder prototypes). Height, contact area in the cuff, contact area in the foam of the inner set-up, and the foam thickness (representing the skin) were studied to design a proper probe holder with a force sensor. Thereafter, the designed probe holders of similar size and different force characteristics were tested on volunteers. Then, the SPP value from the force sensor and from the pressure cuff was obtained at the same time with these new probe holders. Further SPP measurements were performed to check the probe holder prototypes studied on the protolimb on human beings. Furthermore, temperature studies were performed to measure SPP at body temperature versus at 40 °C.

The probe holder designed was not useful in the measurements on human beings, aimed at finding a correlation between the SPP value from the purchased force sensor versus the SPP value from the cuff pressure. The maximum correlation coefficient found was 0.523 (being 1 the best fit), meaning that it was insufficient to determine with precision the state of the microcirculation on the skin. Most probably one handicap in this project was the lack of repeatability of the force sensor, which had from 5 to 10 % of error. The results had to be performed around ten times in order to be repeatable in the protolimb study. Therefore, it was expected that it would not be easy to find repeatable results on volunteers. Measurements have to be time efficient in the clinic, so four measurements were carried out on volunteers instead of the ten measurements needed with the protolimb.

Another drawback of the force sensor was its sensitivity; for instance, it gave high output values when being slightly bended even though no force was applied. Different forces were applied to the sensor for equal cuff pressures if the cuff was not set equally loose in all the measurements. It was troublesome to set the same looseness in some body positions, specially on the feet, given its irregular shape.

Regarding the measurements on the limb prototype, it was proved that the studied parameters of the probe prototypes (height, contact surface on the cuff, contact surface on the foam and foam thickness) exerted different forces on the force sensor, where these forces were proportional to the cuff pressure. Thus, the mechanical pressure values on the surface of the foam were different depending on these characteristics, specially on thin foam. Moreover, satisfactory linear relations were found between the pressure in the cuff and the force exerted to the sensor when studying the different probe holder characteristics. Height was the only parameter with non-linear results when the height of the probe holder increased. This effect could be due to big edge effects. If the pressure cuff is at a constant pressure, then a force is exerted radially to the probe holder. However, if all the cuff on top of the probe holder is not in completely contact with the probe holder, then this force is “divided” between the contact sites of the probe holder (see wider white arrows in Figure 38). Then, if the probe holder is higher, more force will be “divided” and a higher force will be exerted radially on the top contact of the probe holder (see the red arrow in Figure 38). Moreover, these big edge effects could be the reason why the mechanical pressures found by the force sensor were normally higher than the pressure cuff.

Figure 38: Force diagram of the inner set-up on top of the protolimb surrounded by the pressure cuff.
Concerning the contact surface with the cuff, another important effect to comment is the area of the probe holder. The more area at a constant pressure, the bigger the force (see Equation 3). The same results between cuff pressure and mechanical pressure were found only in the case of inner set-ups covering all the cuff surface perpendicularly (see Figure 23 B) with two semicylindrical pieces of 11.3 cm length and 15 cm length. This equality of results could arise from a decrease of mechanical pressure due to a lack of edge effects. Concerning the contact surface with the foam, no significant differences were obtained between the different inner set-ups. Different results were obtained from the different foams. The main conclusions are obtained from the thinner foam since it was thought that the mechanics were easier to understand. Lower mechanical results were found with the thinner foam; moreover, the inner set-up travelled downwards when force was applied on it; then, probably the radial force from the cuff was exerted in different directions apart from the radial one, exerting a lower force onto the force sensor. Summing up, in this part of the project it is shown that the SPP value depends on the probe holder size and shape and on the type of surface applied.

The force measurements on humans were disregarded because of the bad correlation. However, the data obtained in this part was used to study the SPP value of the different designed probe holders and in different body positions. It was found that the SPP values on the feet were higher than on the legs. The different designed probe holders obtained similar SPP values in general, as could be expected from their similar shape. However, really high standard deviations were found for all the volunteers. The main reason for the discrepancy of the results could be the skin structure. Clear correlations were found between applied force and cuff pressure for different probe holders in the limb prototype; however, skin is not a simple foam on a plastic tube; it is a complex tissue covering a mix of muscles, bones and tendons. Moreover, skin composition varies in a few millimeters along the surface in each individual, bringing different SPP values.

Further SPP measurements were performed to check, on human beings, the probe holder prototypes studied on the protolimb. The probe holders had a high repeatability but again, the standard deviation was notorious. As in the protolimb study, it was found that the higher probe holder brought higher SPP results. These results could be explained from the edge effects.

Considering the temperature study, on one hand it was shown that temperature does not have an evident influence on the SPP measurements. SPP increased in every measurement if repeated results were performed in a raw; this increase was 8.3% if increasing the temperature to 40 °C after half of the measurements, and 6.1 % at constant 40 °C for the same number of measurements. However, in order to improve the reliability of these results, it would be appropriate to measure more than six times in a raw in each side and to repeat the measurements on more volunteers.

Regarding SPP, probably the main hassle of this technique is the loading of the probe holder by the cuff. Completely different results were obtained if the cuff was not covering completely the surface of the probe holder and loading it equally. This problem has a physical explanation (see Equation 3). On one hand, when the whole surface of the probe holder was not fully loaded, the sensed force was lower than the expected. However, when calculating the mechanical pressure, the area to consider was the area of the probe holder, not the real loading area, thus the pressure obtained should be smaller than expected. This problem was difficult to overcome. Moreover, although the probe holders were especially semicylindrical shaped to fit better under the cuff, it could be that they were not properly placed on the limbs. Consequently, torsion forces could be applied on the force sensor added to the expected radial forces, and thus, these could cause the inner set-up to move.

Maybe results on patients could show more information than on healthy volunteers; circulation problems could limit the high tissue response and hence, the blood flow may show a more regular and standardized behavior.

SPP can be a great tool to measure microcirculation but it is probably not ready to be used on patients. There is no established protocol to make the measurements; I have created my protocol; nevertheless, it seems that every researcher finds a different one. Moreover, there is no established probe to measure; this is an important point since in this project it has been shown that the probe holder size and shape affects the SPP measurements. Consequently, I believe that the considered cut-off values, 30 or 40 mmHg, are obsolete. Once the protocol and instruments are standardized, a new cut-off value should
be found on patients. However, to measure with a probe holder containing a laser Doppler probe may be useful, since the measurements in a raw (without removing the set-up, exactly at the same place in the same volunteers) have been clearly repeatable: the standard deviation for every group of four measurements in a raw was $3.5 \pm 0.9$ mmHg.

The future work of this thesis can be divided into two parts: improving the actual method or finding a new method to measure SPP.

The actual force sensor could be changed to another less sensitive sensor and with less error. Regarding the loading of the cuff, there should be a way to ensure that the cuff does not fold when loading the probe holder. Maybe the probe holder should be glued to the cuff. Then measurements should be performed carefully to avoid tilting the probe holder when pumping the cuff.

Other methods to measure SPP could bring more reliability to the method. In my opinion, that the main problem is the cuff. From Equation 3 it can be observed that the loading area influences a lot in the results. My suggestions would be intended to avoid the cuff to bend. I would keep the laser Doppler probe as a sensor for the return of blood flow.

My first suggestion is a method that measures SPP in a “chamber” situated between the target skin surface to measure and a thin square non-elastic but moldable plastic. This plastic would cover a certain area, with its centered in the point of microcirculation interest, see Figure 39. The laser Doppler probe should be placed in this point of interest under the plastic piece. Strong tape should glue the edges of the plastic on the skin without pressing the laser Doppler probe underneath. The plastic should have an air entrance to be pumped. Thus the air pressure could be increased in this “chamber”. Then the probe holder would exert a certain force into the skin to occlude the blood flow. The blood flow would come back, releasing slowly the air of the chamber; thus, the SPP value could be found. The main problem in this method would be the fact that a strong tape should be set on the skin to isolate the “chamber”; hence, it would be troublesome for the patients.

![Figure 39: Set-up for “chamber” method.](image)

My second suggestion is a method that does measure SPP, but correlates the coming back of the blood flow to a force instead of the cuff. As it can be seen in Figure 40, two dynamometers should be placed in each end of a non elastic rope. The non elastic rope would be surrounding the limb, however it should not occlude the flow. This could be checked with the laser Doppler probe. The two ends would be pulled in order to press the laser Doppler probe and thus to occlude the microcirculation blood flow. The force of this occlusion would be measured by the dynamometer. Then, the rope around the limb should be released slowly. The increase of rope distance and thus the force difference would be found again with the dynamometers. The drawback of this method is mainly that there is no cut-off force value, as there is in SPP. This reference value should be found on patients in the clinic.
With these methods, a reliable parameter could be obtained to check the microcirculation status. However, in any case the cut-off values to obtain a diagnose should be obtained in the clinic, tested from a large amount of patients and following a standard protocol.
REFERENCES


