Tissue blood flow responses to external pressure using LDF and PPG
Testing a system developed for pressure ulcer research

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Att leva är inte nog;
solsken, frihet och en liten blomma måste man ha.

H. C. Andersen
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ABSTRACT

Background: Pressure ulcers are a problem for immobile individuals, and having pressure ulcers impact and restrict the daily and are often associated with pain. Pressure ulcers occur frequently and cause high costs for the health care system. The prevention of pressure ulcers by focusing on different mattresses and overlays aimed to reduce the interface pressure or the pressure exposure of the tissue. The problem is the poor evaluation of this type of equipment. There are important factors regarding pressure ulcer development, pressure, shear, temperature and humidity. People are affected by external pressure in different ways and therefore it is preferable to measure the effect of pressure as a complement to the pressure measurement and thus we consider blood flow measurements to be a suitable method.

Aims: The aim of Study I, the first part in this thesis was to investigate the existence of sacral tissue blood flow at different depths in response to external pressure in elderly individuals as a part of evaluation of a newly developed system. The aim of Study II, the second part was to evaluate a multi-parametric system combining LDF and photoplethysmography into a single probe, for the simultaneous measurement of blood flow at different depths in the sacral tissue when the tissue is exposed to external load. This new system will be used to facilitate the understanding of pressure ulcer formation.

Methods: To be able to observe tissue blood flow, the non-invasive optical methods laser Doppler flowmetry and photoplethysmography were used. In this thesis a newly developed prototype probe was used, combining the two methods. Green light and infrared light were used in the PPG instrument for penetrating the depths of approximately 2 mm, 8 mm and 20 mm depths. A HeNe laser was used to measure the superficial skin blood flow, <1 mm depth. The prototype probe, made of silicone was fixed in a stiff 10×10 cm plate. Seventeen active individuals over the age of 60 were recruited for the two studies. In Study I, the subject’s sacral blood flow and tissue thickness (using ultrasound) were measured in unloaded position and in supine position loading the area with their own body weight. In Study II, the sacral area was provoked with external load at 37.5 mmHg and 50.0 mmHg and the relative change in blood flow at different depths was observed before, during and after load.
Abstract

Results: Study I showed that the sacral tissue in elderly individuals is highly affected by load and is compressed by 60.3 ± 11.9%. The mean sacral tissue thickness was 26 ± 13 mm in unloaded tissue and 10 ± 6 mm in loaded tissue. Correlations were found between BMI and tissue thickness: both TT_{unload} r =0.68 (p=0.003) and TT_{load} r =0.68 (p=0.003). Almost all subjects had affected blood flow superficially but only occasionally deeper in the tissue and findings may indicate that the blood flow is occluded in the superficial layer before it is occluded deeper in the tissue structure. The most common response in Study II was an increase in blood flow while loading. In those occasions when the blood flow decreased, it was mostly affected at the skin surface and the reactive hyperaemia occurred more frequently in the superficial tissue structures. The blood flow responses may be different in the different tissue layers.

Conclusions: The newly developed system was found to be suitable for measuring tissue blood flow at different depths; however the prototype probe had some limitations that will be solved in the further development of the system into a thin flexible probe with ability to measure a larger area.
LIST OF PAPERS

This thesis is based upon the following papers, which will be referred to in the text by their Roman numerals (I and II):


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INTRODUCTION

Pressure ulcers are complications related to the care and treatment of primarily disabled and elderly people. Findings indicate that pressure ulcers affect a person’s life physically, socially (1-2), emotionally and mentally, (2-3) and that having pressure ulcers is often associated with pain (1-4). Young men with spinal cord injuries expressed that having a pressure ulcer had adversely affected their lives and had led to increased dependence on their partners, families and careers (5). When the impact of pressure ulcers on health-related quality of life in older patients is explored, many aspects are revealed: physical influences and limitations such as physical restrictions and lifestyle changes; social effects such as restricted social life and limitations in intimacy; psychological aspects such as self-concept changes and emotional problems; effects of pressure ulcer symptoms such as pain; and influences on general heath such as infections and restricted rehabilitation (6). Other aspects were also expressed as impact on others, financial effects, the health care provider/patient relationship and a need for knowledge.

Pressure ulcers occur frequently. In the 1980s a study found that 4% of patients in the public health services area in Sweden had pressure ulcers (7), and a study twenty years later in the same area has its findings among acute, geriatric and nursing home inpatients (8). In other parts of Swedish hospital care the prevalence is found to be higher, 23.9% (2002) and 22.9% (2006) (9). A European study in hospitals in five countries found a prevalence of 18.1% (10), and in acute health care in the US the prevalence ranged from 14 to 17% for the period 1999-2004, with 7-9% of patients developing pressure ulcers after admission (11). However, due to inconsistencies in study design, caution is needed to draw conclusions and in interpreting the results of these studies (12).

Pressure ulcers are costly to treat. A Swedish study indicates that the treatment cost for is considerable (13); the cost of nursing time spent on dressing changes is equivalent to 15m SEK annually (1999). In the Netherlands, the cost of illness from pressure ulcers has been estimated at between 362 million to 2.8 billion USD, which is a low estimate, approximately 1% of the country’s total health care budget (presented 2002) (14). The cost of treating a pressure ulcer in the UK varied from 1.064 GBP for a grade 1 ulcer to 10.551 GBP for a grade 4 ulcer, with most of the cost due to nursing time...
(15). The total cost was approximately 4% of the total National Health Service budget.

In the area of pressure ulcer prevention, there are many different kinds of mattresses and overlays available on the market to reduce the interface pressure or decrease the time the tissue is exposed to pressure. In evaluating the antidecubitus mattresses, blood flow is an important variable, among others, due to that ischemia is considered to be an important factor in pressure ulcer formation (16). The optical methods Laser Doppler flowmetry and photoplethysmography are satisfying methods for exploring blood flow as they directly reflect blood flow at different levels and are non-invasive methods (17). This thesis will investigate whether there is a possibility to combine these two techniques into a single probe and then be able to measure blood flow at four different depths simultaneously. From a future perspective, the intention is to perform measurements on patients while they are in bed.
BACKGROUND

Pressure ulcers

During the past few years, the European Pressure Ulcer Advisory Panel and the American association the National Pressure Ulcer Advisory Panel have worked together to develop common guidelines for the treatment and prevention of pressure ulcers, and have recently published their work. Their new definition of a pressure ulcer is “localized injury to the skin and/or underlying tissue usually over a bony prominence, as a result of pressure, or pressure in combination with shear” (18).

The new International NPUAP/EPUAP Pressure Ulcer Classification System classifies different pressure ulcers into four categories, with two additional categories for the US (18) (Figure 1):

- Category I: Non-blanchable erythema
  Intact skin with non-blanchable redness of a localized area, usually over a bony prominence.

- Category II: Partial Thickness
  Partial thickness loss of dermis presenting as a shallow open ulcer with a red-pink wound bed, without slough.

- Category III: Full thickness skin loss
  Full thickness skin loss. Subcutaneous fat may be visible but bone, tendon or muscle are not exposed. Slough may be present.

- Category IV: Full thickness tissue loss
  Full thickness tissue loss with exposed bone, tendon or muscle. Slough or eschar may be present.

- Unstageable/unclassified: Full thickness skin or tissue loss – depth unknown
  Full thickness tissue loss in which actual depth of the ulcer is completely obscured by slough and/or eschar in the wound bed. Until the base of the wound is exposed, the true depth cannot be determined, but it will be either a Category III or IV.
- Suspected Deep Tissue Injury – depth unknown

Purple or maroon localized area of discolored intact skin or blood-filled blister due to damage of underlying soft tissue from pressure and/or shear.

Figure 1. Illustrations of the different categories in the International NPUAP/EPUAP Pressure Ulcer Classification System. Printed with permission from the NPUAP.

It is important that nursing care be able to identify patients at risk of developing pressure ulcers. Important predictors, known as intrinsic factors, are immobility (19-20) and high age (7, 20-21), poor nutritional status (22-23) and sensory impairment (24). These intrinsic factors consider the person’s state of health. There are further factors important in pressure ulcer development, so-called extrinsic factors, and these can be divided into primary and secondary factors. The first primary factor is the pressure the person is exposed to while resting; there is reason to believe that pressure exceeding the pressure in the capillaries increases the risk of pressure ulcer development (25). The second primary factor is the shear forces in the contact surface between the person and the underlay that may cause the capillaries to stretch or pleat and impair tissue perfusion (24, 26). The negative effect of impaired tissue perfusion may worsen if the temperature in the tissue increases, which is considered to be the third primary factor. The increase in temperature in the skin surface is due to an accumulation of body heat as a result of poor (or no) transport of heat in the underlay. An example of a secondary factor is an
accumulation of moisture on the skin surface, which is considered to make the skin more vulnerable to external influence (24, 26).

To prevent pressure ulcer development it is important to use materials in beds and wheelchairs that reduce extrinsic influences. A wide range of pressure-relieving equipment is available; the purpose of these antidecubitus mattresses/overlays is to facilitate tissue perfusion and reduce the accumulation of heat and moisture in the skin/mattress interface. They are constructed to either reduce the time the tissue is exposed to high pressure (27) or reduce the actual interface pressure by allowing a larger area to carry the body weight (28). The uncontrolled purchase of mattresses has been criticized; reasons for criticism include: the number and type of the different mattresses may have been insufficient to meet current needs; the mattresses may not have been effective; the mattresses may have been made available to the wrong patients; the mattresses were not used properly; or the mattresses replaced other forms of nursing care aiming to prevent pressure ulcers (29).

In the field of pressure ulcer prevention research as well as commercially, the focus has traditionally been on pressure measurements, but external pressure affects individuals very differently. Young healthy persons are less affected by loading than are patients with hemiplegia (23), and geriatric and paraplegic patients are more likely to have decreased skin blood flow than are young and healthy people (26, 30). It is therefore preferable to measure tissue blood flow in the exposed tissue instead of interface pressure, while pressure measurements can provide additional information in the evaluation of antidecubitus mattresses (17). The commercial market has long used pressure measurements; in this aspect, pressure measurements have a value.

**Microcirculation**

**The function of the capillaries**

The structure of microcirculation affected by pressure and shear includes blood vessels smaller than 100 µ. From the terminal arterioles, the blood flows into a system of capillaries (capillary bed) for assembly into the venules and the vein system. The capillaries are known as exchange vessels and their main function is the exchange of oxygen, nutrients and metabolites between the blood and the tissue cells. For this exchange to work, the capillary wall
consists of a single layer of endothelial cells and a basement membrane. The distribution of the blood in the capillary system is regulated by the precapillary sphincters, located at the entrance of the capillary bed. When a sphincter is relaxed the blood flows into the capillary bed, and when the sphincter contracts the flow decreases or even ceases. Normally, the blood flows through the capillaries due to alternating contraction and relaxation of the smooth muscle cells of the arterioles and the precapillary sphincters, a process called vasomotion. When the metabolic need is low, blood flows through a small part of the capillary network (31-32). (Figure 2)

The skin consists of the epidermis (the outermost layer), consisting mostly of keratinocytes, and the dermis, under the epidermis, consisting mainly of connective tissue. In the thicker dermal tissue, the blood vessels, nerves, glands and hair follicles are embedded. The epidermis and dermis are connected by a basement membrane, and the capillary network extends from the deeper vessels through the upper part of the dermis called the papillary dermis. The capillaries form capillary loops that supply the most superficial parts of the dermis and deeper layers of epidermis (31, 33). (Figure 3)
Regulation and control

The systems that control the smooth muscle cells in the blood vessels work centrally or locally, but their response is the same: the constriction or dilatation of blood vessels (34). The central control provides optimal circulation homeostasis such as cardiac output, blood pressure, venous flow, blood volume and thermoregulation for the individual. There are three main mechanisms that practice the central control: vasoconstrictor nerves, vasodilator nerves and humoral control.

The local control focuses on the separate tissues’ capillary exchange, as well as the regional control of blood flow and blood flow distribution to provide optimal nutritive blood flow. Other focus is the auto-regulation of blood flow. The local control is driven by three mechanisms: myogenic pacemaker activity, local myogenic control and local metabolic control.

Myogenic pacemaker activity controls the basal tonus of the vessels. The myogenic basal tonus is located primarily in the pre-capillary vessels. Local metabolite accumulation, as a result of reduced blood perfusion through the closure of pre-capillary sphincters, responds with dilatation as negative feedback and helps to stabilize the blood flow.

The myogenic control plays an important role in blood flow regulation and responds to changes in transmural pressure, the pressure difference between...
the inside and outside of the vessel (35). An increase in transmural pressure results in a constriction of the vessel, and vice versa (36). The myogenic response exists in different vascular beds and in a variety of vessels (36), and both pre-capillary resistance vessels and pre-capillary sphincters are involved.

The chemical-metabolic control responds to metabolite concentration in the tissue and is responsible for the most important vessel reaction for the local tissue, the functional hyperaemia. This results in an increase in the nutritive blood flow when the tissue’s metabolism increases. Examples mechanisms involved in metabolic vasodilatation are, transmitters from nerve endings and the endothelium (36), such as nitric oxide and especially prostaglandins (37); and decrease in PO\(_2\) (36).

Pressure-induced vasodilatation leads to an increase in blood flow and is thought to entail the protection of the tissue from ischemia during mechanical loading when the applied pressure is below the level at which blood vessels become occluded (35, 38-39). There are indications that the underlying myogenic response is most dominant between 15 and 20 mmHg of loading, but at the same time sacral skin blood flow increased when pressure exceeded 35 mmHg (35). Both healthy younger and healthy older persons exhibit an increase in blood flow at low levels of pressure (<32 mmHg) (40). These findings indicate that there are many mechanisms involved in pressure-induced vasodilatation and, despite the development of pressure-induced vasodilatation, that pressure application can impair blood flow (38).

A rapid reduction or elimination of the external pressure that has caused ischemia in the tissue results in a great increase in skin blood flow to above baseline levels (39), a process called reactive hyperaemia. The reaction is explained by the reduction in myogenic basal tone caused by the very low transmural pressure during the period of occlusion. But the accumulation of metabolites during the period of occlusion also has an impact on the reactive hyperaemic response. Little is known about intrinsic physiological responses of skin to pressure-induced ischemia or the characterization of post-loading hyperaemic response. In one study, hyperaemia was studied in healthy humans by monitoring blood perfusion using Laser Doppler imaging (LDI) in heel skin while loading. It was found that peak post-loading responses depended on applied load magnitude and duration of application. Heels loaded for 10 min showed peak hyperaemic responses that increased with increasing load magnitude up to 120 mmHg. When heels were loaded with 120 mmHg for 5-10 min, the peak hyperaemic perfusion level was 74% of maximal vasodilatory state (measured as heat response). Hyperaemic recovery
time increased with both load duration and magnitude; 4.8 min while loading for 5 min, (41).

Aetiology of pressure ulcers

The aetiology of pressure ulcers is a complex phenomenon and is not fully understood. There is no consensus on the origin of a pressure ulcer or at which depth the process of ulceration starts. The most common understanding is that the primary cause of pressure ulcers is tissue ischemia from applied pressure (42-43), but some researchers have questioned this accepted approach (44). Two theories can be distinguished based on the depth at which the ulceration begins (45). The traditional theory states that the process starts on the skin surface and if the pressure is not relieved, the ulcerations progress deeper through the epidermis and upper dermis, down to the deep tissue and muscle, i.e. top-to-bottom ulcer formation (16, 46). This theory relies on observations of the histological changes in the tissue at varying sites. The initial change occurs in the vessels of the papillary dermis, and is then followed by necrosis in the skin structures at increasing depths (46), and involves the two processes of occlusion of the blood vessels by external pressure and endothelial damage of arterioles and microcirculation due to the application of disruptive and shearing forces (16). The second theory states that ulceration occurs in a bottom-to-top fashion (47-48). This theory is based on the initial pathologic changes occurring in the muscle, since muscle is more sensitive to pressure than skin is, due to the higher metabolism in muscle which leads to a higher sensitivity to ischemia (47). This theory also relies on observations of the histological changes in the tissue, but over bony prominences (47-48). These two theories might be seen as contradictory, but both may be able to be applied, depending on where on the body the pressure ulcer appears. It is therefore necessary to focus on the development of techniques that will allow us to explore the influence of applied pressure on the tissue at different depths.

Pressure ulcer research

Previous studies have attempted to explore the relationship between applied pressure or interface pressure and pressure ulcer development. Most studies have focused on four different responses in tissues exposed to pressure:
Background

histopathological changes, circulatory changes, chemical responses and biomechanical characteristics. There is great variance of these variables between the studies as well as duration of applied pressure, which makes it difficult to compare their results and summarize the knowledge in this field. Nevertheless, there are interesting results in this area.

Many studies have evaluated histopathological changes in tissue exposed to pressure, using animal models, mostly rodents (49-53) but also pigs (47, 54-55), and engineered epidermis (56). One study compares its data with previous histopathology data from studies on rats, and states that with pressure exposure between 15 min and one hour a pressure over 32 kPa (240 mmHg) causes cell death in muscle, and with exposure for two hours or longer a pressure over 9 kPa (67 mmHg) always causes muscle damage (52). This stresses the importance of time as a factor in pressure ulcer development. This type of study has several limitations. Animal studies and artificial experiments make it difficult to transfer the results to the human condition. The methods are very experimental or invasive, far from a clinical situation, making it problematic to implement the conclusions in clinical situations involving pressure ulcer development.

Other studies have focused on the circulatory changes in tissue exposed to pressure. Blood flow measurements using photoplethysmography in seated geriatric patients show that patients can have blood flow occlusion below a pressure exposure of 20 mmHg but that healthy young men needed a pressure exposure of at least 120 mmHg to have occlusion (26). Wavelet-based spectrum analysis of sacral skin blood flow showed that there was an increase in blood flow of compressed tissue in response to alternating pressure compared to constant pressure (57) and therefore suggest alternating pressure reduce the pressure ulcer risk. By characterizing the hyperaemic response post to heel loading using LDPI, a study points out that maybe the risk for relative heel brake down could be better classified (58). These studies are easier to interpret as they were performed on humans; however, the different body sites used in the studies may have very different conditions, and applying the results found at one body site to another may produce the wrong implications and must be done with caution.

Other studies measure IL-1α release (56) and interstitial fluid pressure (51) as markers of damage due to applied pressure. The results indicate that even if there is no visible damage after exposing the epidermis to 6.7 kPa (50 mmHg) there is an IL-1α release indicating tissue damage (56). The study of interstitial fluid pressure (IFP) used invasive needles, and the measurements interacted and affected tissue damage (51). The authors found that damage at 10 kPa (75
mmHg) and 70 kPa (525 mmHg) only appeared when combined with IFP and that damage of 250 kPa (1875 mmHg) was severe with IFP. They draw parallels between their results and a study by Kosiak in 1961, the results of which showed very low pressure values for tissue damage, and state that Kosiak also used a measurement technique involving needles. This result is interesting and may indicate that one of the most referred to studies in the pressure ulcer research tradition may not be as reliable as it is thought to be.

Some studies have used mechanical testing of the tissue to explore characteristics such as stiffness, internal stress, elastics and viability (52, 56, 59). Recently, the use of computer models to simulate the tissue’s reactions to pressure has been seen as a new approach to exploring changes deeper in the tissue (52-53). The MRI technique has been developed to try to see tissue damage and to relate the interface pressure to the internal local mechanical condition of the compressed tissue. These bio-mechanical studies are based on theories that state that pressure ulcer development is caused mainly by sustained compression of the tissue (50, 60-61).

Loading time and magnitude play a major role in the development of pressure ulcers, and no consensus has been reached about the levels of tissue damage; the studies above use fixed levels. There is therefore a need for clinically relevant study situations in which the effect of the applied pressure can be evaluated. Since there is great variation in tissue viability and responses to external loading between individuals, only measuring pressure is not enough; assessing the blood flow as well is more suitable, preferably at different depths simultaneously (16).
AIMS

The development of a multi-parametric system is ongoing and a prototype of the system is evaluated in this thesis. If the system is suitable, a further object (for the dissertation) is to compare pressure and blood flow in the tissue, between healthy persons and patients in need of pressure ulcer prevention, lying on standard mattresses and pressure-relieving or pressure-reducing mattresses.

The medical problems of fundamental importance in this research are:
- How are pressure and blood flow distribution related to each other?
- Do pressure ulcers start in the epidermis and then progress deeper, or do they start in the deeper tissue and progress from the bottom to the top?
- How do the mechanisms for the development of pressure ulcers interfere with different types of mattresses for healthy persons or risk patients?
- How do pressure and temperature in different combinations affect the blood flow and the risk for pressure ulcer development?

Aims of the thesis

The aim of Study I was to investigate the existence of sacral tissue blood flow at different depths in response to external pressure in elderly individuals as a part of evaluation of a newly developed system.

The aim of Study II was to evaluate a multi-parametric system combining LDF and plethysmography into a single probe, for the simultaneous measurement of blood flow at different depths in the sacral tissue when the tissue is exposed to external load. This new system will be used to facilitate the understanding of pressure ulcer formation.
METHODS

Optical methods

To be able to observe tissue blood flow, the optical, non-invasive laser Doppler flowmetry (LDF) and photoplethysmography (PPG) techniques are satisfying methods, as the blood flow is directly reflected at different levels in the tissue (17). If these two techniques are combined into a single probe it will enable measurement of blood flow at different depths simultaneously, and improve the investigation of pressure ulcer formation. The mixture of LDF and PPG was chosen in order to be able to explore the most superficial skin blood flow using LDF as well as blood flow in deeper tissue layers using PPG. The combination of the two techniques also provides possibilities to compare different techniques; LDF was considered the reference method in this thesis.

Laser Doppler flowmetry

Laser Doppler flowmetry has been used extensively for the evaluation of tissue perfusion (23, 62-63). It is a method used in many areas like diabetes microangiopathy, peripheral vascular diseases, pharmacological applications and skin diseases (64). LDF has several advantages: It is relatively cheap, has low operator bias as it is easy to handle, and is well validated (65).

LDF uses laser (monochromatic) light that penetrates the skin. Moving objects in the tissue, e.g. red blood cells (RBC), reflect the light, which is Doppler broadened as a frequency shift. The technique detects the shift as an estimate of the perfusion and is presented in arbitrary units (Volts). There is a linear relationship between perfusion and the two factors of velocity (vRBC) and the concentration of moving red blood cells (cRBC) (62, 66-67):

\[
\text{perfusion} = <vRBC> \times cRBC
\]
Methods

Generally, there are two types of Laser Doppler modalities used: Laser Doppler perfusion monitoring (LDPM) and Laser Doppler perfusion imaging (LDPI). In this thesis only the LDPM technique is regarded.

Photoplethysmography

Photoplethysmography (PPG) has been used in many different clinical applications, for example monitoring physiological responses in the clinic such as heart rate, blood pressure, cardiac output, respiration and blood oxygen saturation (68). Vascular assessment is another area in which the PPG technique has been used, for examining things such as arterial disease, arterial compliance and ageing, endothelial function, venous insufficiency, microvascular blood flow and tissue viability. Another area is autonomic function measurements, like vasomotor function and thermoregulation, variability of heart rate and blood pressure, orthostatis and neurological assessment. PPG is used in many commercially available medical devices due to its low price and small components (68). The PPG signal can be divided into two parts, an AC signal and a DC signal. The AC signal is a pulsatile signal, synchronous with the heart rate. It correlates directly with the blood flow and reflects the arterial blood flow in the tissue (69) and orientation of the red blood cells (RBC) (70-71). The DC signal is a slowly varying signal reflecting total blood volume (72), and reflects vasomotor activity, respiration and thermoregulation (68).

A light source emits light of a certain wavelength towards the area of investigation. PPG is based on light that penetrates the tissue being absorbed, scattered and reflected; the reflected light can be detected by a photo detector. Blood absorbs mostly more light than the surrounding tissue does, and therefore a reduction of the amount of blood gives an increase in the intensity of the detected light. The wavelength and distance between light source and photo detector also determines the depth of penetration (69). Green light is suitable for measurement of superficial skin blood flow, and infrared (IR) or near IR is better for measurements of the deep tissue (muscle) blood flow (73).
The optical probe

In this thesis, a three channel PPG instrument (Department of Biomedical Engineering, Linköping University, Linköping, Sweden) was used together with a Laser Doppler flowmeter with a HeNe laser, wavelength 632.8 nm (PeriFlux Pf2b, Perimed, Järfälla, Sweden). This system was developed by the research team and created by the engineers at the department of Biomedical Engineering at Linköping University, Linköping, Sweden. The system consists of both hardware and software to allow for the combination of the two methods.

Green light (560 nm) and near infrared light (810 nm) were used in the PPG instrument for penetrating the tissue in different depths. The probe consisted of three pairs of light-emitting diodes (LED) placed symmetrically around a photo detector (Figure 4). The distance from the photo detector was 5 mm for the green LEDs, and 10 mm and 25 mm for the IR LEDs. This combination of wavelengths and distances gave the penetration depths of approximately 2 mm, 8 mm and 20 mm. One Laser Doppler fibre optic probe was also integrated and inserted between the IR LEDs, with a measurement depth of less than 1 mm.

All the components of the prototype probe were integrated onto a silicone plate, fixed on a stiff plate measuring 10*10 cm. The probe was integrated into the test bench even with the surface of the bench, to avoid any influence from the probe itself during the test situation.

Figure 4. Schematic view of the optical probe with all the components: the LEDs symmetrically placed around the photo detector and a Laser Doppler fibre inserted between the IR LEDs.
Methods

Other methods

The sacral tissue was measured by using a digital ultrasound system (HDI 5000, Philips Medical Systems, ATL Ultrasound, Bothell, WA, USA) with a linear transducer (L7-4). The sacral crest was located visually and the tissue thickness between the skin surface and the bone was calculated directly in the system (Figure 5).

![Figure 5. Ultrasound measurements of tissue thickness in unloaded and loaded sacral tissue in one individual.](unloaded-loaded-sacral-tissue.png)

A pressure mapping system (Xensor Pressure Mapping System X236, Anatomic Sitt, Norrköping, Sweden) was used to measure pressure distribution in the sacral area and contact area in that area. It consisted of an underlay (45 cm × 45 cm) with integrated pressure sensors (4 sensor/square inch) and with the ability to measure pressure ranging between 10-220 mmHg. The system also provided the total area with an active pressure sensor, i.e. contact area (Figure 6).
Figure 6. Example of data from the pressure mapping system, showing the pressure distribution and contact area in the sacral area in one subject.
SUBJECTS AND PROCEDURES

Subjects

Seventeen individuals (six men and eleven women) were recruited to participate in the study situations. Most of the subjects were recruited through a non-profit organization in the local area. The inclusion criteria were that they were over 60 years old, considered themselves healthy, lived an active life and managed daily life without assistance.

Procedures

The subjects participated in two different test situations. In the first test situation, presented in Study I, ultrasound measurements of the tissue thickness in the sacral area were performed in both loaded and unloaded tissue. The measurements were performed by a highly experienced technician.

The participant’s body height and weight were registered, and a point 2-3 cm from the subject’s medial sacral crest was marked as the point of measurement. The participant lay in a supine position on a test bench of wooden plate with a 10 × 10 cm hole in it, covered with a blanket. The transducer was led through the hole, and measurements of the unloaded tissue were performed. A Plexiglas plate was then fitted in the hole in line with the surface of the bench, and measurements of the subject’s tissue thickness, loaded with their own body weight, were performed.

In the second test situation, blood flow measurements were performed using the newly developed probe, described above. Blood flow was recorded with a sampling frequency of 75 Hz (Labview 6.1, National Instruments, Kista, Sweden) and analysed by an in-house-developed program (IMT, Linköping University, Linköping, Sweden). Both the AC signal and the DC signal were recorded and analysed, but only the data from the AC signal are used in this thesis. The blood flow was assessed as occluded when there were no pulsations at the AC signal at PPG and the flux value at the LDF was close to zero and without pulsations. All measurements were performed at the same
time of day and in the same room, and the room temperature was noted (digital thermometer, type 565, Schwille elektronik, Kircheim, Germany).

At first, the subject rested in a supine position for 15 min on the test bench and then heart rate, body temperature (ThermoScan 6022, Braun, Kronberg, Germany), skin temperature pre-and post-measurement (IR thermometer, Raytek Raynger ST, Santa Cruz, CA, USA), and blood pressure (Speidel & Keller, Jungingen, Germany) were noted. Then, the subject was placed in a prone position and the probe was fixed by double-fastened, non-allergenic tape at the sacral area to be measured (Figure 7a).

After another ten minutes of rest, the session of blood flow measurement in prone position began. The baseline data was used in both Studies I and II. It started with a five-minute period of measurement while the tissue was unloaded. Then, a period (presented in Study II) of five minutes started, loading with 37.5 mmHg weight at the sacral tissue. Then, the tissue was unloaded for five minutes, followed by five minutes of loading with 50.0 mmHg, followed then by five minutes of unloaded tissue. The weight was laterally fixed but horizontal movement was possible, so the weight was not affected by breathing movement.

After the loading session, the probe was fixed in the test bench even with the surface and the subject was turned to a supine position to rest for ten minutes (Figure 7b). Five minutes of loading with the subject’s own body weight followed, and blood flow and interface pressure were recorded. These measurements are presented in Study I.

The measurements were validated by comparing the heart pulsations on the computer screen with palpation of the radial artery pulse to control the agreement between the two.
Subjects and procedures

Figure 7. Figure a shows a subject in prone position and Figure b shows a subject in supine position when performing measurements.

Ethical considerations

The studies were performed according to the World Medical Association Declaration of Helsinki of 1989, and the Research ethical committee in Linköping, Sweden, has approved the studies in this thesis (Dnr M166-06). Participation in this study was grounded on informed consent from the participants, both oral and written, and they were told that they could stop their participation at any time without giving a reason for doing so. The registration of background variables was performed manually according to coded protocols. The measurement data of blood flow and pressure was done electronically. When the ultrasound measurement was performed, the thickness measurement registration was done according to a protocol and the digital images were saved to the research data base of the physiological clinic at the hospital. These protocol and images were not unidentified; unidentification was performed while processing the data. The coded list and protocol were kept separate and safe.

The methods used in the two studies were non-invasive, and were not associated with discomfort or pain. The participants needed to lie with their
sacral area exposed, which may have entailed a risk of needing to defend their integrity. This was prevented by covering their back with blankets and asking about individual needs or wishes. The written information to the participants also included specific descriptions of the procedures.

During the blood flow measurements, the participants rested on a wooden bench, which may feel hard and uncomfortable. The measurements were thus performed as quickly as possible and the time spent in the same position did not exceed 20 minutes, which can be considered acceptable. This very limited bed rest will not be a problem for healthy individuals who are normally ambulatory.

During the ultrasound measurement, a gel was used to achieve good contact with the surface. The gel may feel cold, but the discomfort was minimized by forewarning the participants before placing the probe on their body. The gel contains nitric chloride and will not cause dermal reaction.

The risk to the participants through taking part in the studies was considered non-existent; nor are there actual benefits for the participants, but in the future there will be benefits for those in need of pressure ulcer prevention, who can be offered validated and effective antidecubitus mattresses.

**Statistical methods and calculations**

The background data was distributed normally and was therefore presented in terms of mean ± standard deviation. Differences in age and body mass index (BMI) between genders were compared using an independent sample $t$-test. Sub-analysis of the background data was performed because five of the participants had a cardiovascular diagnosis. No significant differences were found between the participants without diagnosis and those with diagnosis, and the material in the thesis is therefore presented in the studies as one group.

In Study I, differences in skin temperature pre- and post-measurement were compared using a paired sample $t$-test, as were differences in unloaded and loaded tissue thickness.

Blood flow response presented in Study I was used as dependent variable, and the two endpoints of non-existing and existing blood flow were presented. For comparing blood flow in unloaded and loaded tissue, respectively, the McNemar Chi Squared Test was used. Pearson Correlations were performed
Subjects and procedures

in Study I between BMI, tissue thickness, tissue thickness while loading, and contact area.

The mean arterial pressure presented in Study I, MAP, was calculated as a function of diastolic pressure, DP, and systolic pressure, SP: MAP = DP + (SP – DP)/3.

The body mass index was calculated as BMI = weight in kilograms / (height in meters) x (height in meters). The WHO BMI classification of underweight is BMI < 18.5, normal range of BMI is between 18.5-24.99 and overweight is BMI ≥ 25.0 (74).

The tissue thickness presented in Study I was calculated directly in the ultrasound system and a mean value from three different pictures in each situation was registered.

The relative changes in blood flow in per cent, presented in the published version of Study II, were presented as a single box plot for each variable on the category axis. Box plots showed the median, quartiles and extreme values for the variable. In these, the relative changes in percent are shown on the y-axis and the points in time on the x-axis for each depth on the basis of blood flow at baseline.

In Study II blood flow was calculated by computing the mean amplitudes for the AC signal at the PPG and the mean values for the LDF signal. The computed time periods were between 15 and 20 s and were chosen based on the quality of the signal.

A significance level of p < 0.05 was considered to be significant. All statistical analyses were performing using SPSS® for Windows, version 13.0-15.0 (Statistical Package of Social Sciences, SPSS Inc., Chicago, IL).
RESULTS

Background characteristics

Six males and 11 females were recruited to the studies. Mean age was 68.5 ± 7.1 years and mean body mass index was 24.3 ± 2.4. All background measurements such as heart rate, blood pressure, skin temperature, body temperature were within the reference intervals and are presented in Table 1. There were no significant differences in regard to gender.

Table 1. Mean and Standard Deviation in the individuals’ conditions relevant to microcirculation observed before blood flow measurements were performed.

<table>
<thead>
<tr>
<th>Condition</th>
<th>All (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>68.5 ± 7.1</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.3 ± 2.4</td>
</tr>
<tr>
<td>Heart rate (b/min)</td>
<td>66 ± 7</td>
</tr>
<tr>
<td>Systolic pressure (mmHg)</td>
<td>129.1 ± 13.6</td>
</tr>
<tr>
<td>Diastolic pressure (mmHg)</td>
<td>74.4 ± 8.8</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>92.6 ± 9.3</td>
</tr>
<tr>
<td>Ambient temperature (°C)</td>
<td>23.1 ± 0.6</td>
</tr>
<tr>
<td>Body temperature (°C)</td>
<td>36.4 ± 0.4</td>
</tr>
<tr>
<td>Skin temperature prior to measurement (°C)</td>
<td>32.3 ± 1.8</td>
</tr>
<tr>
<td>Skin temperature after measurement (°C)</td>
<td>32.7 ± 0.8</td>
</tr>
<tr>
<td>Differences in skin temperature (°C)</td>
<td>0.5 ± 1.9</td>
</tr>
</tbody>
</table>

Five participants had a medical history of cardiovascular problems (Table 2). One participant used tobacco and one had an irregular pulse. The participants were calm and relaxed during the test situations and no one expressed any discomfort or pain.
Results

Table 2. Overview of the individuals’ vascular diseases and medications. * Irregular pulse.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age</th>
<th>Pulse</th>
<th>Systolic blood pressure (mmHg)</th>
<th>Diastolic blood pressure (mmHg)</th>
<th>Vascular diseases</th>
<th>Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Woman</td>
<td>65</td>
<td>62</td>
<td>135</td>
<td>80</td>
<td>Hypertension</td>
<td>1, 2, 3, 4</td>
</tr>
<tr>
<td>8</td>
<td>Man</td>
<td>79</td>
<td>48*</td>
<td>140</td>
<td>70</td>
<td>atrial fibrillation, hypertension Paroxysmal atrial fibrillation</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>Woman</td>
<td>62</td>
<td>66</td>
<td>120</td>
<td>80</td>
<td>Hypertension</td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>Woman</td>
<td>65</td>
<td>64</td>
<td>120</td>
<td>70</td>
<td>Hypertension, cardiac infarction, heart valve insuff.</td>
<td>3, 5, 6</td>
</tr>
<tr>
<td>16</td>
<td>Woman</td>
<td>87</td>
<td>66</td>
<td>135</td>
<td>65</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1=β1 receptor-selective antagonist, 2=ACE inhibitor, 3=calcium antagonist, 4=anticoagulant (warfarin), 5=ASA, 6=loop diuretic

Physiological responses

Tissue conditions

Sacral tissue conditions were presented in Study I. Mean sacral tissue thickness (TTunload, unloaded tissue) was $26 \pm 13$ mm, and while loading with the subject’s own body weight, tissue thickness (TTload) was $10 \pm 6$ mm and the reduction was significant ($p<0.0005$). The compression of the tissue while loading was $60.3 \pm 11.9\%$. There were no differences between men and woman regarding tissue thickness (TTunload and TTload). When lying on the test bench, the subject’s mean contact area on the back was $188.0 \pm 72.0$ cm².

The interface pressure attained pressure values of at least 220 mmHg in the sacral area. However, in two individuals (one woman and one man) the pressure values were much lower, showing that their sacral tissue was not exposed to loading as high as that of the other 15 participants.

The correlation between TTunload and TTload was $r=0.88$ ($p<0.0005$). The correlations between BMI and tissue thickness, was presented in Study I: both TTunload $r=0.68$ ($p=0.003$) and TTload $r=0.68$ ($p=0.003$). Furthermore, there were correlations between sacral contact area and tissue thickness, both TTunload $r=0.50$ ($p=0.043$) and TTload $r=0.58$ ($p=0.014$) and BMI $r=0.60$ ($p=0.011$). No
correlations were found between compression and any of the variables age, BMI, TT_{unload} and TT_{load}.

**Blood flow responses**

While loading with a participant’s own body weight lying in a supine position, blood flow (BF_{load}) was more affected in the superficial parts of the tissue (presented in Study I) (Table 3).

<table>
<thead>
<tr>
<th>Subject</th>
<th>Unloaded tissue thickness (mm)</th>
<th>Tissue thickness while loading (mm)</th>
<th>Laser Doppler (depth of measurement 1 mm)</th>
<th>Green Doppler (depth of measurement 2 mm)</th>
<th>Superficial IR Doppler (depth of measurement 8 mm)</th>
<th>Vascular Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Man</td>
<td>11</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Man</td>
<td>15</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Woman</td>
<td>7</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>Man</td>
<td>12</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Woman</td>
<td>21</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>Woman</td>
<td>22</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>Woman</td>
<td>28</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>Man</td>
<td>18</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>Woman</td>
<td>39</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>Woman</td>
<td>28</td>
<td>10</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>Woman</td>
<td>27</td>
<td>11</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>Man</td>
<td>24</td>
<td>13</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>13</td>
<td>Woman</td>
<td>27</td>
<td>13</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>14</td>
<td>Man</td>
<td>32</td>
<td>13</td>
<td>-</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>Woman</td>
<td>39</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>16</td>
<td>Woman</td>
<td>41</td>
<td>19</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>17</td>
<td>Woman</td>
<td>57</td>
<td>22</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Only two of 16 participants had existing BF_{load} measured with Laser Doppler, compared with 11 of 17 participants measured with superficial IR light at PPG (Table 4).

<table>
<thead>
<tr>
<th>Participants with existing BF_{load}</th>
<th>Laser Doppler &lt;1 mm</th>
<th>Green light (PPG) 2 mm</th>
<th>Superficial IR light (PPG) 8 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>16</td>
<td>16</td>
<td>17</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.0005</td>
<td>0.001</td>
<td>0.031</td>
</tr>
</tbody>
</table>
Results

In Study I, at each depth of measurement, the participants were divided into two groups: those who had non-existing BF_{load} and those who had existing BF_{load} in the sacral area while loading with their own body weight. The two groups were compared with regard to age, BMI, MAP, TT_{unload}, TT_{load}, compression and sacral contact area. In two variables, TT_{load} and contact area, there were differences between the groups. Those who had non-existing BF_{load} measured with green light PPG (2 mm depth) had less TT_{load} (p = 0.002) than those who had existing BF_{load}. Those who had non-existing BF_{load} had less contact area than those who had existing BF_{load}, measured with both green light PPG (p = 0.009) and IR light PPG (8 mm) (p = 0.028). No other differences were found between the two groups.

In Study II, the pattern of blood flow responses that occurred most frequently was described as “the standard appearance” (Figure 8). This pattern was seen when measuring with PPG in ten participants. Measuring with LDF, the blood flow showed more variance and no pattern was discerned. The standard response was found as an increase in blood flow while loading, higher at 50.0 mmHg than at 37.5 mmHg (seen in seven participants). During the periods of unloading, the blood flow rapidly decreased to a level near baseline.

![The standard appearance](image)

Figure 8. Example of the standard appearance of the blood flow responses in one participant described in relative change in per cent. t1 is baseline, t2 is the point in time during 37.5 mmHg load, t3 is directly after removing the load, t4 is just before 50.0 mmHg load, t5 is during 50.0 mmHg load, t6 is directly after removing the second load, and t7 is just before ending the session.
Relative change measured with PPG (Study II) showed an increase in blood flow while loading; the change was greater using superficial IR light than green light. The deep IR light showed the greater variances of the three PPG channels, and the changes were similar between the two sessions of load (Table 5).

Table 5. Relative change in per cent of the blood flow with median, quartiles and extreme values at the seven points in time. \( t_1 \) is baseline, \( t_2 \) is the point in time during 37.5 mmHg load, \( t_3 \) is directly after removing the load, \( t_4 \) is just before 50.0 mmHg load, \( t_5 \) is during 50.0 mmHg load, \( t_6 \) is directly after removing the second load, and \( t_7 \) is just before ending the session.

<table>
<thead>
<tr>
<th></th>
<th>Laser Doppler</th>
<th>Green light (PPG)</th>
<th>Superficial IR light (PPG)</th>
<th>Deep IR light (PPG)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Relative change, %</td>
<td>Relative change, %</td>
<td>Relative change, %</td>
<td>Relative change, %</td>
</tr>
<tr>
<td>( t_2 )</td>
<td>median</td>
<td>14.0</td>
<td>31.1</td>
<td>43.6</td>
</tr>
<tr>
<td></td>
<td>( q_1 )</td>
<td>-28.2</td>
<td>9.5</td>
<td>8.8</td>
</tr>
<tr>
<td></td>
<td>( q_3 )</td>
<td>106.9</td>
<td>37.1</td>
<td>54.9</td>
</tr>
<tr>
<td>( t_3 )</td>
<td>median</td>
<td>13.8</td>
<td>2.3</td>
<td>10.1</td>
</tr>
<tr>
<td></td>
<td>( q_1 )</td>
<td>-0.5</td>
<td>-1.3</td>
<td>-1.7</td>
</tr>
<tr>
<td></td>
<td>( q_3 )</td>
<td>88.0</td>
<td>16.8</td>
<td>23.5</td>
</tr>
<tr>
<td>( t_4 )</td>
<td>median</td>
<td>11.3</td>
<td>2.6</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td>( q_1 )</td>
<td>-9.4</td>
<td>-5.4</td>
<td>-3.4</td>
</tr>
<tr>
<td></td>
<td>( q_3 )</td>
<td>35.7</td>
<td>7.8</td>
<td>10.6</td>
</tr>
<tr>
<td>( t_5 )</td>
<td>median</td>
<td>-6.9</td>
<td>29.2</td>
<td>59.9</td>
</tr>
<tr>
<td></td>
<td>( q_1 )</td>
<td>-54.2</td>
<td>14.1</td>
<td>21.2</td>
</tr>
<tr>
<td></td>
<td>( q_3 )</td>
<td>26.4</td>
<td>46.8</td>
<td>99.6</td>
</tr>
<tr>
<td>( t_6 )</td>
<td>median</td>
<td>57.4</td>
<td>9.0</td>
<td>15.4</td>
</tr>
<tr>
<td></td>
<td>( q_1 )</td>
<td>26.0</td>
<td>-0.1</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td>( q_3 )</td>
<td>110.6</td>
<td>24.3</td>
<td>84.1</td>
</tr>
<tr>
<td>( t_7 )</td>
<td>median</td>
<td>18.9</td>
<td>5.5</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>( q_1 )</td>
<td>-5.0</td>
<td>-4.1</td>
<td>-9.7</td>
</tr>
<tr>
<td></td>
<td>( q_3 )</td>
<td>47.6</td>
<td>13.1</td>
<td>24.8</td>
</tr>
</tbody>
</table>

Three participants showed decreases in blood flow while loading, measured with PPG using superficial IR light and deep IR light. In measurements in two participants, there was loss of data from the green PPG channel during load, but the measurements at the periods of unloading do not contradict the possibility that there could be decreases in blood flow even in this depth.
Thirteen participants showed a decrease in blood flow while loading measured with LDF, and three had non-existing blood flow.

Reactive hyperaemia was seen in four participants using PPG. Measuring with LDF, reactive hyperaemia was seen in 14 participants. In four participants, a different response occurred. A rapid increase was observed directly after removing the load, but instead of the gradual decrease described in the literature (Figure 9a), the blood flow continued to increase during the whole period, i.e. five minutes (Figure 9b).

One participant showed no response in blood flow during load using PPG, but the blood flow decreased far below baseline at all three depths after the two periods of unloading the tissue.
DISCUSSION

Methodological considerations

Laser Doppler flowmetry is an optical non-invasive method for measuring blood flow perfusion in the tissue, in terms of a flux value corresponding to the number of cells times the average velocity of moving red blood cells. The advantages of Laser Doppler techniques are their non-invasive quality, their ability to measure the microcirculatory aspects of the tissue, and their ability to detect rapid changes due to provocation of the perfusion. There are limitations as well: there is no exact depth of measurement, the perfusion signal is influenced by the optical properties of the tissue, there are problems with motion artefacts, and there is a perfusion signal at no flow condition (biological zero signal). There are no quantitative units for perfusion; the perfusion is given as a flux value (75).

In this thesis, the limitations are handled in several ways. The depth of measurement depends on the wavelength and fibre separation used. Utilising a wavelength of 780 nm wavelength and using a standard fibre, the depth is 0.5-1 mm (75). In this thesis, a He-Ne laser was used utilising a wavelength of 632.8 nm, and Monte Carlo simulations using He-Ne laser show a measurement depth of 0.20-0.24 mm depending on the blood content (76). This result indicates that the depth of measurement is very superficial and probably just a few hundred µm in the present studies. Regarding the influence of the optical properties of the tissue, as well as the flux value, the design of the present studies only focuses on individual measurements, with the individual serving as his/her own reference during an intervention. There was no intention to compare actual blood flow on a group level or over time, as the blood flow shows great variation between individuals and from day to day, due to the present tissue circulatory condition. It is therefore not clinically relevant to perform time to time blood flow measurements, but LDF will be suitable for this study design. One theoretical limitation is that the technique is based on the assumption that there is only a single Doppler shift in the tissue. In reality, there is multiple scattering in highly perfused tissue (75). This will not be a problem in the present study design, as the sacral area has low basal
perfusion levels and the prototype system that is used handles only relatively small perfusion levels. The motion artefacts are mainly a problem for the Laser Doppler perfusion imager system, as the laser beam is placed at a distance from the tissue. In Laser Doppler perfusion monitoring, the probe is attached directly to the skin surface and the movements are then minimised. The fact that the light delivery and detection are on the same probe also minimises the motion artefacts (77). Handling the issue of biological zero is of crucial importance. In the sacral area, it is not possible to achieve totally occluded flow, and therefore no reference flux value.

In Study I, while lying in a supine position the flux value was very close to zero, a value received when the instrument was calibrated according to the manual with a solid surface, and no pulsations were seen either. These findings offer convincing evidence that the blood flow was occluded. This was not an issue in Study II, as the relative change in blood flow was presented and was not a flux value.

The PPG technique has for many years been used extensively to measure blood perfusion of the skin. However, the exact origin of the PPG signal is not fully understood. The pulsatile component (AC) of the signal is dependent on the pumping action of the heart (78) and reflects the peripheral pulse synchronous with the heart beat. Historically the AC component has been assumed to reflect pulsatile volume changes due to varying lumen of the vessel with each heart beat. However, in vitro studies utilising rigid tubes has demonstrated a pulsatile (AC) PPG signal synchronous with pulsatile pressure (and pulsatile blood flow) (79). The implication is that the reflected/scattered light and the AC signal vary both with volume changes and with orientation of red blood cells. Despite different explanations of the origin of the AC component, there is strong evidence that the AC signal is related to blood flow. An absent AC signal indicates no pulsatile flow. The limitations regarding LDF described above are similar for the PPG technique (even the techniques are based on different physical processes)(68) and are handled in the same way in this thesis.

During the last few years efforts have been made to monitor physiological parameters from deeper vascular compartments in humans. Based on suitable combinations of optical wavelengths and distances between light source(s) and photo detector(s) it has been possible to e.g. monitor fetal oxygen saturation using pulse oximetry and PPG (80) and fetal heart rate using NIR PPG (81). In the latter case a wavelength of 900nm was used with a source-to-detector separation of 40mm and the distances between the maternal skin and the fetal skin was 40-50 mm.
Other examples of applications using similar modality of the PPG technique are studying blood flow in the patellar bone (79) and monitoring blood flow variations in the tibial anterior and trapezius muscles (73, 82). The latter cases as well as unpublished studies show that different optical PPG probes may discriminate between superficial skin blood flow using 560nm and a source-to-detector separation of 3.5mm and muscle blood flow using a wavelength of 806 or 880nm with a source-to-detector distance of 10, 20 or 25mm. As a consensus from experimental assessments in these studies one may assume that pulsatile blood flow variations may be monitored from approximate depths of 2mm using 560nm, 8-10mm using 810nm (source-to-detector separation = 10mm) and a depth of 20mm using 806 or 880nm (source-to-detector separation = 10mm).

Evaluation of the probe

The two studies discussed in this thesis presented the first opportunity to test the newly developed prototype probe. The purpose of the test situations, which were fairly different from realistic clinical situations, was mainly to evaluate the prototype probe as part of the procedure leading to a probe designed for human clinical studies. The evaluation of the probe consisted of two parts: a physiological part focusing on the ability to detect relevant blood flow responses, and part focusing on the technical aspects of the prototype.

In Study I, the ultrasound measurements were preformed in order to characterise the area where the blood flow measurements were performed and relate the results to the theoretical depths of measurement using PPG and the level of compression of the loaded tissue.

The prototype probe was a solid stiff plate, and to be able to measure when the participants were lying in a supine position (like in a bed) it needed to be integrated into the test bench even with the surface. This was to avoid the probe itself influencing the test situation. This resulted in a hard test bench, and the interface pressure between the participant and the bench was very high. These pressure values were unexpectedly high, and the pressure map system usually used for clinical measurements (like in bed) reached its maximum value of 220 mmHg. Therefore, the pressure values are not presented in Study I; instead, it is noted that the participants were exposed to very high pressure values (least 220 mmHg). This turned out to be an extreme test situation, and we will not see this kind of interface pressure in the patient/bed surface. This problem indicates the importance of further development into a thin, flexible probe that will provide measurements in bed,
which is a clinically relevant test situation. However, here the blood flow responses in one of the most extreme situations were observed, and this can be seen as an investigation of the endpoint of the interface pressure from lying on a totally stiff wooden board.

Another consequence of lying of the wooden board (Study I) was that two of the participants’ sacral tissue was not exposed to high pressure due to their body constitution. Their buttocks were able to resist the load so that the sacral area was not loaded in the same extent as the other participants. This was revealed by the pressure map, and these two individuals were the only ones with existing blood flow measured with LDF and presumably were not exposed to the same study conditions as the other participants were (Figure 10). However, these two participants were not excluded from the study since this was a consequence of their body constitution and not the intervention. Lying on a mattress, the effect of different body constitutions will disappear as they are supportive of the whole body surface.

![Figure 10. Schematic view of the pressure map when the problems with low pressure occurred in two individuals. The buttocks are relieving the sacral area, which leads to low pressure values in this area due to the subject’s body constitution.](image)

There were problems due to the stiffness of the prototype probe in relation to body constitution in Study II as well. In some cases, reactive hyperaemia was detected despite no previous decrease in blood flow being seen. Likewise, in some cases there was a decrease or occlusion of blood flow but no reactive hyperaemia was detected. Reactive hyperaemia is a strong indicator of previous ischemia (32). There are therefore grounds to assume that if reactive hyperaemia is detected, the tissue has been exposed to ischemia despite a lack of previous decrease in blood flow. If the participants’ body constitution provides a small gradient of the skin surface in the sacral area, the skin is likely to slide in relation to underlying tissue layers while loading (Figure 6a).
Since the probe has only one detector and therefore measures at a single point, there is a risk that measurements were not performed at the exact same point when loading and unloading the sacral area. Similarly, ischemia may still be present in the tissue even if no reactive hyperaemia was detected or only a single occasion of reactive hyperaemia was detected.

This problem involving movement of the probe was also due to the test situations with the participants lying in a prone position. In further studies in supine positions with a flexible probe, this will not be a problem. Development of the probe with more than one detector will provide a matrix of measurement points resulting in a larger measurement volume. This will increase the possibility to detect possible tissue ischemia despite movements of the different tissue layers.

Finally, there were occasions in both Studies I and II where the light intensity of the reflected light in the PPG instrument was too high (the instruments indicated overload). This phenomenon is due to the facts that more light is reflected in compressed tissue than in tissue under unloaded conditions, and that different body sites/levels have different blood perfusion. Therefore it is important to evaluate the probe on the actual body site and using PPG instrumentation with extended measurement facilities.

In conclusion, the problems that occurred are due to the design of the prototype probe. The problems of extreme pressure values, contact problems due to body constitution, failure to detect all the occurring occasions of decrease in blood flow and reactive hyperaemia and signal disturbances will be solved as the probe is developed further. It will be a thin, flexible probe with the ability to measure tissue blood flow in a larger vascular volume in patients lying in bed.

**Discussion of the physiological results**

Two types of loading were performed in the two studies. In Study I, the participants loaded the sacral tissue with their own body weight, a situation that occurs while lying in a bed, for example. In Study II the load was controlled, with weights of 37.5 mmHg and 50.0 mmHg. In Study I, the LDF (measurement depth <1 mm) and PPG measurements with green light (measurement depth 2 mm) and superficial IR light (measurement depth 8 mm) were used. In Study II, deep IR light (measurement depth 20 mm) was added. The two studies presented in this thesis focus on detecting relevant physiological tissue responses to external load. The traditional way to study
tissue condition is to measure the actual pressure the tissue is exposed to, often presented as interface pressure. In Study I, the sacral tissue was exposed to very high pressure (at least 220 mmHg), which is an extreme situation. In a supine position lying on a standard mattress, mean pressure values in the sacral area were previously found to be $30.7 \pm 9.0$ mmHg in healthy individuals and $38.2 \pm 13.3$ mmHg in patients (23). The tissue was highly affected in this situation with compression values of about 60%, which is in accordance with other findings in sitting patients (83). The loading values in Study II of 37.5 mmHg and 50.0 mmHg may be more clinically relevant ones that can be compared with resting in bed in a supine position.

The most common blood flow response in the two studies was an increase in blood flow while loading. This increase can be explained by a number of responses. First is the fact that all of the participants were active and relatively healthy. The increase is a compensatory response to protect the tissue, and is seen in healthy individuals exposed to external pressure (38-40). Other studies state that individual conditions are more important than age (20). There is a methodological explanation as well: The tissue being compressed while loading affects the strength of the signal (68). Compared to unloaded tissue, the light from the PPG penetrates more deeply into the compressed tissue to a certain depth. This results in more compact tissue with a larger blood volume, which leads to an increase in the PPG signal.

The results in Studies I and II indicate that all occasions of decrease in or occlusion of blood flow as well as reactive hyperaemia were not detected by the prototype probe due to its limitations, described above. This makes it hard to make characterizations and draw comparisons between the subjects who had affected blood flow and those who did not. Nonetheless, the responses in blood flow that have been detected are reliable and truthful. The reactive hyperaemia that was detected supports the findings of a decrease in or occlusion of blood flow. This is also supported in the literature, when PPG and LDF are used in a similar way to detect blood flow responses in human muscle (73). In Study II, the results indicate that the blood flow was highly affected at the skin surface, shown by LDF. Reactive hyperaemia was present in 14 individuals post-loading, indicating that ischemia was present during load. This was surprising, as the tissue was exposed to relatively low pressure (maximum 50.0 mmHg). Previous findings have shown that pressure values of at least 120 mmHg were required to occlude the blood flow in sitting young individuals (26). On the other hand, the hospitalized geriatric group showed occluded blood flow below pressure values of 40 mmHg. This large variance in pressure values that cause ischemia indeed point out the need for
evaluation of the effect of pressure in groups prone to pressure ulcer development.

In conclusion, the physiological responses observed are important in pressure ulcer prevention. The responses support the statement that this newly developed technique with combined PPG and LDF is suitable for detecting tissue blood flow responses to external pressure. When the prototype probe is further developed, it will offer an improved possibility to characterize humans with impaired blood flow while loading and to compare different groups with regard to tissue blood flow responses.
CONCLUSION

Sacral tissue is highly compressed by external load of at least 220 mmHg. In all three (approximately <1, 2 and 8 mm) examined tissue layers, blood flow was severely affected on several occasions during load. There seems to be a difference in sensitivity to external pressure in the different tissue layers, as the blood flow in the superficial tissue layers is occluded before the blood flow deeper in the tissue.

The most common blood flow response in the sacral tissue while loading at 37.5 mmHg and 50.0 mmHg was an increase in blood flow, but when the blood flow decreased during load it was mostly affected at the skin surface.

The possibility to measure blood flow at different depths provided interesting findings and indicates that the blood flow responses may be different due to the depths of measurements. Reactive hyperaemia may occur more frequently in the superficial layers of the tissue post loading with 37.5 mmHg and 50.0 mmHg. The study showed that LDF complements the PPG.

The new system is satisfactory for tissue blood flow measurements at different depths. The physiological blood flow responses detected by the system are well documented in previous literature. The studies showed that the LDF complements the PPG.

There are limitations with the prototype probe that has been used in this thesis. The design of the probe lead to an experimental test situation where problems occurred that will be solved in a clinical situation with subjects in bed. Further, the design also failed to detect all the blood flow responses that were probably present during external load of the sacral tissue. Further development into a thin flexible probe with the ability to measure a larger area will solve the problems present in this thesis.
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