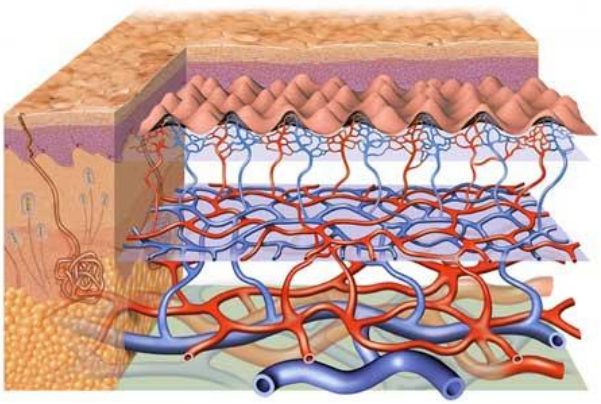
# Microcirculatory oscillations in patients during anesthesia and in intensive care patients

Svein Aslak Landsverk



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**To Nora and Selma**

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## 2. List of abbreviations

ACh = Acetylcholine

AU = Arbitrary Units

AVAs = Arteriovenous Anastomoses

CO =Cardiac Output

HR = Heart Rate

ICU = Intensive Care Unit

LDF = Laser Doppler Flowmetry

NO = Nitric Oxide

OR = Operating Room

SD = Standard Deviation

SkBF = Skin Blood Flow

SNA = Sympathetic Nervous Activity

SNP = Sodium Nitroprusside

ΔPP = Respiratory Variations in Pulse Pressure

ΔPOP = Respiratory Variations in Photoplethysmographic Waveform Amplitude

## 3. List of papers

This thesis is based on the following scientific papers, which are subsequently referred to by their Roman numbers:

**Paper I:**

Landsverk SA, Kvandal P, Kjelstrup T, Benko U, Bernjak A, Stefanovska A, Kvernmo H, Kirkeboen KA. Human Skin Microcirculation after Brachial Plexus Block Evaluated by Wavelet Transform of the Laser Doppler Flowmetry Signal.

Anesthesiology. 2006; 105(3):478-84

**Paper II:**

Landsverk SA, Kvandal P, Bernjak A, Stefanovska A, Kirkeboen KA. The Effects of General Anesthesia on Human Skin Microcirculation Evaluated by Wavelet Transform.

Anesthesia&Analgesia. 2007; 105(4):1012-9

**Paper III:**

Landsverk SA, Hoiseth LO, Kvandal P, Hisdahl J, Skare O, Kirkeboen KA. Poor Agreement Between Respiratory Variations in Pulse Oximetry Photoplethysmographic Waveform Amplitude and Pulse Pressure in Intensive Care Unit Patients.

Anesthesiology. Accepted may 2008

# 4. Introduction

The skin is the largest organ of the human body and displays several functions that are essential for preservation of life. The most obvious is that it serves as a barrier between the external environment and internal organs, both physically and as a part of the immune system. It produces hormones, like vitamin D, and is an important communicative organ. The skin is also of vital importance for regulation of temperature. About 20% of all thermal input to the central thermoregulatory system, mainly the hypothalamus, is transmitted from the skin (1). Nociceptors and mechanosensors in the skin transmit information to the central nervous system. This information can induce behavioral changes, such as avoiding painful stimulus, or regulate efferent nerve traffic direct to the skin vasculature or sweat glands. The skin vasculature also receives information from the cardiovascular and respiratory sensors, as part of the baroreflex system to regulate blood pressure (2;3). Both vasculature and sweat glands are influenced by emotions and stress. When the influence of hormones or medications is added to the list of factors that can influence skin response, the complexity of this system is obvious. These aspects have several important implications in medicine, especially related to diagnostics. There is a current trend in modern medicine to focus on non-invasive diagnostic methods of the microcirculation, both to detect early signs of diseases and to monitor treatment of pathophysiological conditions, such as sepsis and shock (4). To be able to use these methods in patients during surgery, or in the intensive care unit, knowledge about the impact of anesthetics and sedative drugs on microcirculation is of vital importance. Several in vivo studies on the effect of anesthetics on microcirculation exist in animal models (5;6). However, in order to implement non-invasive diagnostics into daily practice, clinical studies are imperative. The aim of this thesis was to explore the impact of some common anesthetics methods on human skin microcirculation. This knowledge was then applied to question a non-invasive diagnostic approach for detecting fluid responsiveness in intensive care unit ( ICU) patients.

### 4.1 Anatomy of skin circulation

The vascular system of the skin is organized in horizontal layers of plexus (7). This is illustrated figure 1. Most superficially, below the epidermis, is the capillary network that reaches into the papilla. These capillaries arise from the subepidermal plexus that consists of arterioles, metarterioles and venules. Below this, in the area of the dermis and the subcutis, the deep plexus of arteries and veins arises. In the glabrous regions, or nonhairy region of the skin (fingers, palmar surfaces of hands, soles of feet and in lips) a direct communication between arteries and veins exists, known as arteriovenous anastomoses (AVAs). These vessels are located between the two deepest layers of plexa, and are under strict nervous control, allowing large amounts of blood to be distributed to the skin, when opened.

**Figure 1**

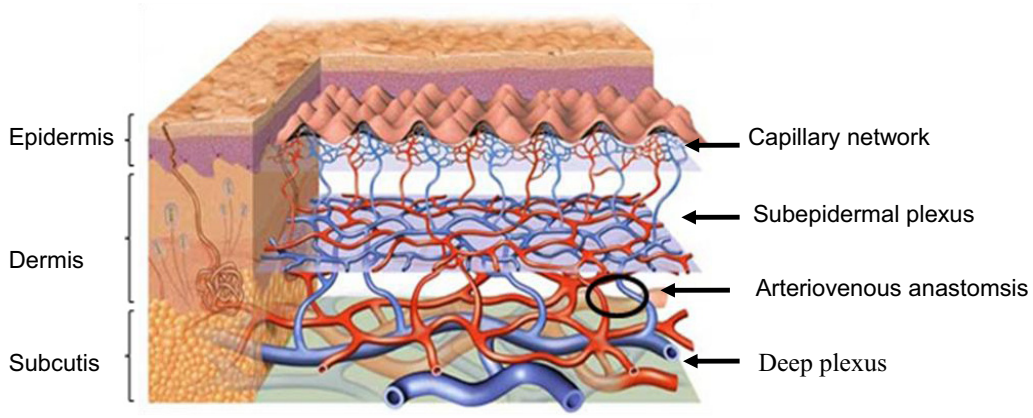


*Figure 1. Anatomy of human skin, focusing on the vascular system. Modified from Skin Care Forum Issue 40, www.scf-online.com, Cognis GmbH, with permission. This permission also applies for the figure on the front page.*

## 4.2 Regulation of skin blood flow

*Central regulation*

Under normal conditions, skin blood flow (SkBF) represents about 5% of cardiac output. SkBF can be reduced to a minimum during hypothermia or hypovolemia and increased up to a maximum of 60% of cardiac output (CO), or 6-7 liter/min, during heat stress. Such a large SkBF would require an additional increase of CO and a redistribution of blood flow from the splanclinic circulation (8). To be able to mediate these large adjustments, the vessels are innervated by two different sympathetic nerves, which is unique for the human skin. These are known as the sympathetic vasoconstrictor and vasodilator nerves. The knowledge of this dual sympathetic system has existed since the experiment by Grant and Holling in 1938 (9). They showed that sympathectomy in normothermic subjects only increased SkBF slightly, whereas it increased much more during heat stress. When performing sympathectomy during heat stress, the high SkBF dropped to levels of normothermic subjects. These findings are later confirmed by others (10;11).

The noradrenergic vasoconstrictor nerves innervate all skin vessels, both in glabrous and non-glabrous skin, using norepinephrine as a transmitter and neuropeptid Y as a cotransmitter (12;13). During normothermia this system plays the main role for regulating SkBF, as all vessels have a basal vascular tonus corresponding to basal sympathetic nervous impulse rate. The AVAs are only innervated by noradrenergic vasoconstrictor nerves. As core temperature increases and capacity of the vasoconstrictor system to induce vasodilation is exceeded, the active vasodilator system becomes activated. During heat stress this system is in fact responsible for about 80-90 % of the increase in SkBF. During resting conditions, sweating starts at the same threshold value. However, this threshold value can be different during exercise or in situations where the baroreflex system is activated (14). Mechanisms regarding the vasodilator system are not fully understood. The coupling to the cholinergic sympathetic

nerves innervating the sweat glands has long been recognized, as persons with antihidrotic ectodermal dysplasia (congenital absence of sweat glands) are not able to respond to heat stress with active cutaneous vasodilatation (15). Atropine does not block this response, indicating that the system is more complex (11). A present hypothesis is that active vasodilatation is caused by acetylcholine (ACh) together with a cotransmitter, possibly vasoactive intestinal peptide, in addition to involvement of histamine and nitric oxide (NO) (16).

Other central regulatory mechanisms also influence SkBF. Experiments with Lower Body Negative Pressure, a method to induce hypovolemia by pooling blood into the lower limbs (17), show the impact of the baroreflex system on SkBF (3). Vasoconstriction as result of activating the baroreflex system includes both the active vasoconstrictor and vasodilator system. This is important, because it is then possible to redistribute blood to vital organs also during heat stress. However, when measuring skin sympathetic nervous activity with microneurography in response to baroreflex tests, the results have been conflicting (18). In addition to these central regulatory mechanisms, pain, stress and emotions also affect SkBF. This can be mediated both by the active vasoconstrictor system and the vasodilator system, illustrated by a PhD student defending his thesis with cold, clammy hands and a facial flush.

*Local regulation*

Changes in local temperature can also influence SkBF. In response to local heating, or by other stimuli, afferent nerve c-fibers release neurotransmitters that induce vasodilatation. This is known as the axon reflex. This is the first part of a biphasic response, induced by local heating. If local heating continues, the secondary response seems to be mediated by NO (19). This response seems independent of the active vasodilator system (20). Local response to cooling, however, requires an intact sympathetic vasoconstrictor nervous system. A local reduction in temperature is sensed by cold-sensitive afferent nerves initiating release of norepinephrine that causes vasoconstriction (21).

An important local regulating mechanism is the metabolic need of the skin, first of all oxygen. When the demand of oxygen in the tissue exceeds the supply, vasodilatation is initiated in small arterioles, metarterioles and precapillary sphincters due to an increase of vasoactive substances such as adenosine, carbon dioxide, histamine, potassium ions and hydrogen ions. It has also been proposed that the vascular smooth muscle cells of metarterioles and precapillary sphincters dilate as a direct result of reduced oxygen tension, or a lack of other nutritive agents within the vascular wall (22).

Pressure sensitive receptors in vascular smooth muscles can also induce vasodilation when increased blood pressure is detected. This is known as the myogenic response (23). It has been shown that vascular smooth muscle cells have the ability to perform cyclic changes of diameter both independent of external influence, but also possibly modulated by the myogenic response. These rhythmical contractions are most likely caused by repetitive release and reuptake of calcium from the sarcoplasmatic reticulum, which spreads and synchronizes between vascular smooth muscle cells through gap junctions (24). Finally, the endothelium of the vessel wall has an important role in adjusting blood flow. Increased blood flow can be sensed by the endothelial wall as shear stress, which initiates production of NO, and relaxes the vascular smooth muscle cells. The endothelium also produces other vasoactive substances, such as prostanoids and endothelin.

The constant changes of the vessel diameter as a local response to pressure, metabolic need, shear stress, and the" intrinsic pacemaker" in the vascular wall, are known as vasomotion and induce continuous changes, or oscillations, in skin microcirculation.

### 4.3 Oscillations in skin microcirculation

When SkBF is measured with laser Doppler flowmetry (LDF), the oscillatory nature of the skin microcirculation, with different frequencies, is evident. The most obvious oscillations are those produced by the heartbeat and by the respiration. Even though these oscillations are small in the microcirculation, it is easy to identify their origin, regardless of the method used, due to their synchrony in different tissues and correlations to electrocardiogram and respiratory rate.

The stroke volume produced by the heartbeat creates a pulse wave that is transmitted through the whole cardiovascular system, including the microcirculation. The effect of spontaneous breathing is related to changes in the intrathoracic pressure that influences venous return. The negative intrathoracic pressure during inspiration increases venous return, pulmonary flow and CO. Due to the pulmonary transit time for blood, about 2 seconds, the increase in stroke volume is seen first during the expiration (25). During mechanical ventilation this effect is opposite and larger, as described later.

Slower and larger oscillations than those related to respiration, have been known since the intervention of the microscope. It is generally accepted that these oscillations are related to sympathetic nervous system (SNA) and to local activity of the vascular wall. However, the mechanisms that relate to the different frequency intervals have been more conflicting. Kastrup et al. (26) indentified two slow oscillations in human skin. One slow oscillation (β-wave) with an average frequency at about 1-2 each minute was related to SNA because it disappeared during nerve block. Similar findings have been confirmed by other. However, Strauss et al. (27) found that the oscillations related to SNA occurred at higher frequencies.

In the study by Kastrup et al. (26) the second (α-wave) had an average about 6-7 cycles each minute and was related to the vascular wall, because it was unaltered during nerve block. These oscillations had smaller amplitude and lacked the synchrony characterizing the oscillations related to SNA, respiration and heartbeat. These oscillations are related to myogenic activity of

the vascular smooth muscles (28-31). Our group has also demonstrated oscillations slower than those generated by the SNA. We have previously demonstrated that these oscillations are related to the vascular endothelium, as iontophoresis with ACh increased the relative amplitude to a greater extent than sodium nitroprusside (SNP) selectively in this frequency interval (32). In a later study, we showed that this effect was inhibited by $N^G$-monomethyl-L-arginine, an inhibitor of NO synthesis, and reversed by L-arginine, the substrate for NO synthesis (33).

Centrally mediated oscillations of the skin microcirculation (heartbeat, respiration and SNA) are characterized by their synchrony, in contrast to oscillations of local origin (vasomotion) that are characterized by a more chaotic pattern.

## 4.4 Impact of anesthesia on the cardiovascular system

The impact of anesthesia on the cardiovascular system is a large and complex theme. Thus, this part is mainly limited to drugs and anesthetic methods used in our studies.

*Regional anesthesia*

A peripheral regional block, such as the brachial plexus block, has the potential to induce changes in both local and central hemodynamics. The local vascular effects are mainly related to the nerve block and the sympathetic impairment, which will cause loss of the basal tone with vasodilation and increased tissue perfusion as a result. In contrast to epidural or spinal anesthesia, central hemodynamic changes are not related to the nerve block per se, but to the systemic effect of local anesthetics or the supplemental drugs.

Systemic effects of local anesthetics are seen in accidental intravenous administration, or in some cases, by absorption due to high doses in the tissue. The effect on the heart is the most pronounced. All local anesthetic agents have a dose-dependent negative inotropic effect, bupivacaine more than lidocaine. They also exert electrophysiological effects that differs both quantitatively and qualitatively between the different drugs. However, in general local anesthetic agents prolong the conducting time. High doses of local anesthetics may also have a

direct effect on the peripheral vascular smooth muscle cells. This effect is considered biphasic, with vasoconstriction at lower doses and vasodilatation at higher doses (34). Additionally, there are reports that local anesthetics impair endothelium-mediated vasodilatation (35-37). However, a study using venous occlusion plethysmography on the human forearm did not confirm this (38).

*General anesthesia*

Anesthetic and sedative drugs have a major influence on the macro- and microcirculation. Even though there are differences between them, they share some common characteristics related to hemodynamic effects. With the exception of ketamine and etomidate, they all suppress SNA dose-dependently, leading to decreased systemic vascular resistance. Changes in heart rate (HR) depend, among other factors, on the impairment of the baroreflex system. Many of the anesthetic drugs also exert a dose-dependent negative inotropic effect on the cardiac muscle cells.

Propofol are known to be a strong suppressor of the SNA, which reduces blood pressure. This reduction in blood pressure may also be connected, although controversial, to reduced cardiac contractility (39). HR often remains unchanged despite the reduced blood pressure. This effect has been related to the impairment of the baroreflex system. As to direct effects on the vascular wall, studies are more conflicting (40-43).

Fentanyl is reported to maintain hemodynamic stability. HR is usually reduced, with only slight effects on cardiac contractility. There is also little impact on baroreflex sensitivity. However, SNA is often reduced. Fentanyl, like other opioids, might have direct effects on the vascular smooth muscles that can initiate vasodilation (44).

Midazolam, have only a modest impact on the cardiovascular system. Blood pressure may be slightly reduced, whereas HR and CO are usually unaltered. However, in combination with

other anesthetic drugs, like opioids, there is a synergistic impact on blood pressure that exceeds the effect of the drugs alone (45).

## 4.5 Impact of mechanical ventilation on cardiovascular signals

Mechanical ventilation induces cyclic changes in cardiac filling, CO, arterial blood pressure and in the peripheral circulation, illustrated in figure 2. This is explained by: 1) Reduction of right ventricular preload because vena cava and the right atrium are compressed during increased inspiratory pressure. 2) Right ventricular afterload increases due to the increase alveolar pressure. Because of the pulmonary transit time of blood (approximately 2 s), the decreased right ventricular output during inspiration, causes a decrease in left ventricular filling and stroke volume, only a few heartbeats later, usually during the expiratory period. The initial increase of stroke volume and pulse pressure during inspiration relate to: 3) Left ventricular preload increases because the positive alveolar pressure squeezes blood into the left side. 4) The positive intrathoracic pressure during inspiration assists left ventricular ejection due to a relative decrease in left ventricular afterload (25).

**Figure 2.**

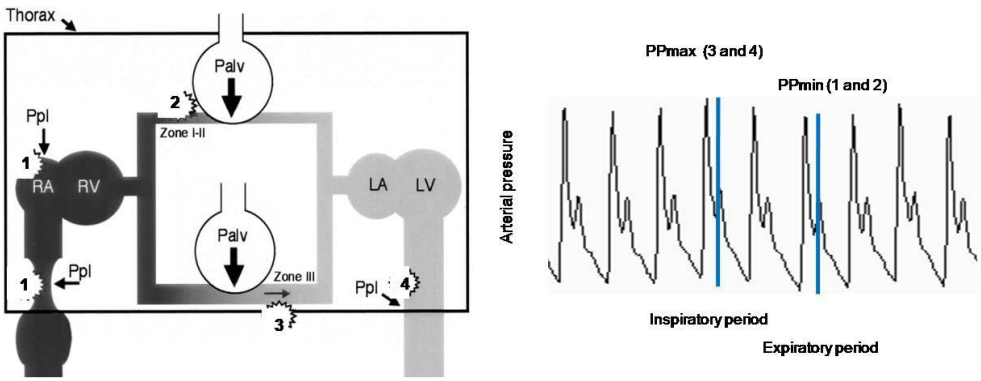


*Figure 2 . Impact of mechanical ventilation on arterial blood pressure due to following mechanisms: Reduction of right ventricular preload (1), increased right ventricular afterload (2), positive alvolar pressure squeezes blood to the left side (3), increased intrathoracic pressure assists left ventricular ejection (4). Modified from Michard F. Anesthesiology.2005;103(2):419-28, with permission.*

These effects (mainly mechanism #1) are increased during hypovolemia and causes changes in stroke volume and pulse pressure during a respiratory cycle.

### 4.6 Fluid responsiveness

When patients have symptoms of cardiovascular failure, such as a low blood pressure, the first questions related to treatment would be: “Should I give fluid?” Usually this question has been answered by trying to evaluate preload. However, the key is whether the patient responds to fluid by increasing CO. If not, fluid could potentially be harmful, like worsening of pulmonary oedema in heart failure. Fluid responsiveness is defined as an increase in CO, or stroke volume by 10-15 % in response to fluid challenge (46). As a dynamic parameter, respiratory variations in blood pressure (ΔPP) have been shown to predict fluid responsiveness better than traditional static parameters, such as central venous pressure or pulmonary artery wedge pressure, in mechanical ventilated patients (47). Respiratory variations in pulse oximetry photoplethysmographic waveform amplitude (ΔPOP) have been proposed as a non-invasive alternative to ΔPP. Several studies have demonstrated correlations between ΔPP and ΔPOP (48-50). However, due to the complexity of the photoplethysmographic signal, many investigators have questioned whether the pulse oximetry and the method of calculating ΔPOP, can be used to identify fluid responsiveness.

## 5. Aims of the thesis

The aim of this thesis was to improve knowledge about oscillations in human skin microcirculation and the impact of anesthesia. This knowledge was then applied to question a photoplethysmographic method for detecting fluid responsiveness in ICU patients. The following hypotheses were tested:

1. We hypothesized that changes related to brachial plexus block, primarily the sympathetic block can be detected by LDF, iontophoresis, and wavelet analysis of the human skin microcirculation.

2. We hypothesized that wavelet transform of laser Doppler flowmetry signals can detect changes in the microcirculation induced by general anesthesia.

3. We hypothesized that the variability of ΔPOP would be larger than that of ΔPP, when calculations were performed continuously in 10-15 minutes for ICU patients, making the agreement between the two methods poor.

4. We hypothesized that LDF in fingertips could improve the detection of slow oscillations and highlight the relationship between acral skin microcirculation in fingertips and the variability in ΔPOP.

# 6. Summary of the papers

## 6.1 Paper I

**Introduction:** Human skin microcirculation shows rhythmical variations, or oscillations, related to the heartbeat, respiration, SNA, and to local mechanisms generated from the microvascular wall. By performing wavelet transform of the LDF signal, five characteristic frequencies, in the interval 0.095-1.6 Hz, can be demonstrated in human skin microcirculation. The endothelial function can be further evaluated with iontophoresis with ACh and SNP. In Paper I, we wanted to investigate alterations in these periodic oscillations, induced by brachial plexus block.

**Methods:** 13 healthy, hand surgery patients, were anesthetized with brachial plexus block, using bupivacaine, lidocaine and epinephrine. Measurements with LDF (one LDF probe on each arm) and iontophoresis with ACh and SNP were obtained before and after brachial plexus block. Wavelet transform of the LDF signal was performed.

**Results:** In the anesthetized arm LDF after brachial plexus block increased from 19 (12-30) to 24 (14-39) arbitrary units (AU), ($P < 0.01$). A significant increase was also seen in the contra-lateral arm from 17 (14-32) to 20 (14-42) AU, ($P < 0.01$). After brachial plexus block, spectral analysis revealed a significant reduction in relative amplitude of the oscillatory components within the 0.0095-0.021 ($P < 0.001$) and 0.021-0.052 Hz ($P < 0.001$) intervals, in the anesthetized arm.

**Conclusion:** Alterations in skin microcirculation induced by brachial plexus block can be evaluated by wavelet transform of the LDF signal. Brachial plexus block reduced the oscillatory components within the 0.0095-0.021 and 0.021-0.052 Hz intervals of the LDF signal. These alterations were related to inhibition of SNA and possibly to an impairment of endothelial function.

## 6.2 Paper II

**Introduction:** The skin microcirculation can be evaluated non-invasively by LDF and iontophoresis with both an endothelium-dependent (ACh) and -independent agent (SNP). Spectral analysis of the LDF signal, using wavelet transform, shows periodic oscillations of five characteristic frequencies in the range of 0.0095-2 Hz. In paper II we wanted to investigate alterations in skin microcirculation induced by general anesthesia, with emphasis on these periodic oscillations.

**Methods:** 11 healthy patients undergoing jaw surgery were included. Skin microcirculation was measured with LDF (two LDF probes located on the same arm) and iontophoresis with ACh and SNP before and during general anesthesia using propofol, fentanyl and midazolam. The LDF signals were analyzed using wavelet transform.

**Results:** Skin perfusion increased from 18 ± 8 to 25 ± 10 AU ($P = 0.039$) in probe 1 and from 18 ± 7 to 24 ± 8 AU ($P = 0.026$) in probe 2, during general anesthesia. There were highly significant reductions in spectral amplitudes in the 0.0095-0.021, the 0.021-0.052, and the 0.052-0.15 Hz frequency interval and a significant increase in the 0.15-0.6 Hz frequency interval, in both probes. General anesthesia had no effect on the difference between ACh and SNP on relative amplitudes in the 0.0095– 0.021 Hz frequency interval ($P < 0.001$).

**Conclusion:** Alterations in skin microcirculation induced by general anesthesia can be evaluated by wavelet transform of the LDF signal. General anesthesia reduced the oscillatory components of the perfusion signal related to SNA, myogenic activity and the component modulated by the endothelium. However, the iontophoretic data did not reveal a specific effect on the endothelium. The increase in the 0.15-0.6 Hz interval is related to the effect of mechanical ventilation.

### 6.3 Paper III

**Introduction:** ΔPP have been shown to identify fluid responsiveness in mechanically ventilated ICU patients. A correlation between ΔPP and respiratory variations in ΔPOP has previously been demonstrated. Knowledge about the repeatability of the methods is important when agreements between the two methods are to be evaluated. However, no such data existed. In paper III, based on knowledge of how SkBF continuously changes or oscillates, we hypothesized that the variability of ΔPOP would be larger than that of ΔPP when calculations were performed continuously over a long recording period.

**Methods:** Respiration, continuous invasive blood pressure, pulse oximetry, and LDF were recorded in 14 mechanically ventilated ICU patients. 70 comparisons between ΔPP and ΔPOP were calculated in a custom maid program, for each of the 14 patients.

**Results:** For all patients, ΔPP was 5.8 ± 2.6% and ΔPOP was 13.7 ± 5.8% ($P < 0.001$). There was a larger intraindividual- (8.94 vs. 1.29, $P < 0.001$) and interindividual (26.01 vs. 5.57, $P < 0.001$) variance of ΔPOP than of ΔPP. In six patients there was no significant correlation between ΔPP and ΔPOP. ΔPP values were above the 13% threshold value in 0.7% of the cases. In contrast, 39% of the ΔPOP values were above the 15% threshold.

**Conclusion:** A large variability of ΔPOP and a poor agreement between ΔPP and ΔPOP, limit ΔPOP as a tool for evaluation of fluid responsiveness in ICU patients. This is in contrast to ΔPP, which shows small variability.

# 7. Methodological considerations

There are several ways to investigate skin microcirculation. The microscope is still one of the most common. Through the years, new technologies have been added and improved the microscope, making it possible to visualize different tissue elements and different tissue structures, using fluorescence or light of different wavelength. Video or high resolutions images from living tissue, called intravital microscopy, give important insight into microvascular dynamics. However, this method has usually been restricted to studies in animals. A further development to improve the image quality of this microscope is the orthogonal polarization spectroscopy. This exists in a hand held version that can be used on humans. Perfusion of the skin can also be evaluated by thermograhy, transcutan oximetry, photoplethysmography and LDF/laser Doppler imaging. These perfusion monitoring techniques can be combined with iontophoresis or microdialysis, to test microvascular function in response to drugs or to external stimuli, like local heating.

In this thesis, LDF, iontophoresis and photoplethysmography were used. The signals were decomposed by wavelet transform to gain further information on microvascular dynamics. These measurements were performed in human skin in different clinical settings.

## 7.1 Study design

Two of the studies (I and II) in this thesis are experimental, clinical studies on previously healthy, non-smoking patients and volunteers. Paper III is a clinical, observational study, in ICU patients. In study I and II, patients undergoing hand or jaw surgery were included, respectively. As a control group, healthy volunteers were used in both studies. Study I was performed at Oslo Orthopedic Center, in the same room throughout the whole study. Study II was performed in the same operating room at Ulleval University Hospital. In both studies, experimental conditions, such as temperature and light were aimed to be constant during the measurements. In study III,

patients admitted to the intensive care unit at Ulleval University Hospital were included. These patients were all deeply sedated and mechanically ventilated. This group was heterogeneous regarding cause of admittance to the ICU, treatment and medication. However, they were regarded as hemodynamically stable. No interventions, such as fluid challenges, additional medication or procedures were performed during the measurements. In this study, no efforts were maid to control the surrounding experimental conditions, such as temperature.

## 7.2 Laser Doppler flowmetry

LDF is a continuous, non invasive, method to detect real-time changes in microvascular blood flow. The method has been used for almost 30 years (51). Laser light, with wavelength of approximately 780 nm, is submitted from an optical fiber into the tissue. In skin, it penetrates about 1 mm down into the epidermis. Some of the light is reflected back to collecting optical fibers and transferred to the perfusion monitor for processing (figure 3). Reflected light from static structures, such as connective tissue, differs from moving structures like red blood cells, because reflections from moving object will create a frequency shift in accordance to the Doppler effect. By analyzing the spectrum of the reflected light it is then possible to calculate the concentration of moving blood cells and their average velocity. The product of these parameters is known as Flux. Since the volume of the tissue, measured with LDF is not accurately defined and the red blood cells move in all direction under the probe, Flux represents a semi-quantitative measurement of SkBF, expressed in AU. LDF measurements from the skin reflect perfusion in capillaries, arterioles, venules and dermal vascular plexa, and a major part of the signal reflects thermoregulatory perfusion (52). In this thesis the Flux signal is referred to as LDF signal.

**Figure 3**

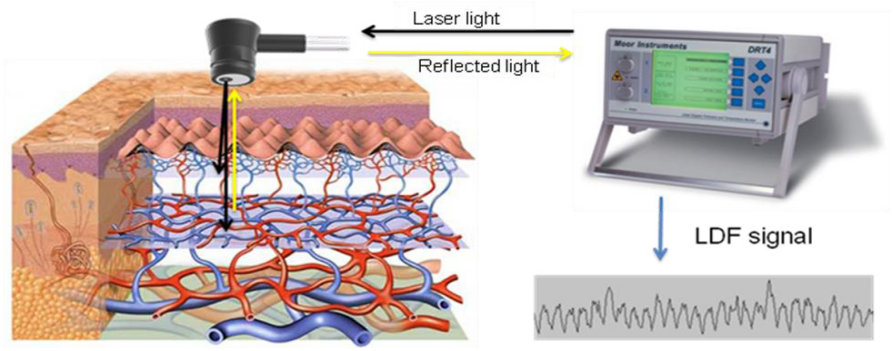


*Figure 3. Illustration of the LDF set up. The probe measures concentrations of red blood cells and their average velocity. The product of these parameters is knows as Flux. Modified from Skin Care Forum Issue 40, www.scf-online.com, Cognis GmbH, with permission.*

In study I and II, LDF measurements were performed on the lower forearm. In study III, the LDF probe was attached on the fingers bilaterally. The LDF measurements in all three studies were obtained using a two-channel flowmeter (MoorLAB server/satellite, Moor Instruments, Axminster, Devon UK) for basal, unstimulated recordings. A sampling frequency of 40 Hz and a time constant of 0.1 s were selected.

### 7.3 Iontophoresis

Iontophoresis is a non-invasive method for transdermal delivery of polar drugs by means of a small electrical current. It is then possible to test microvascular response to a variety of drugs without any systemic effects (53). The response is measured continuously by a LDF probe, which is placed above the iontophoretic chamber where the test substance is deposited (Fig. 4A). A constant current stimulator is used to provide a direct current for the drug iontophoresis. To test endothelium-dependent vasodilatation and endothelium-independent vasodilatation in skin microcirculation, ACh and SNP can be used, respectively. This generates a characteristic LDF response as illustrated in figure 5. This method to examine endothelial function in skin

microcirculation has been used for about 20 years (54-56). While ACh infusion in larger vessels induces vasodilatation mediated by NO, from the endothelium (57), the effect

**Figure 4**

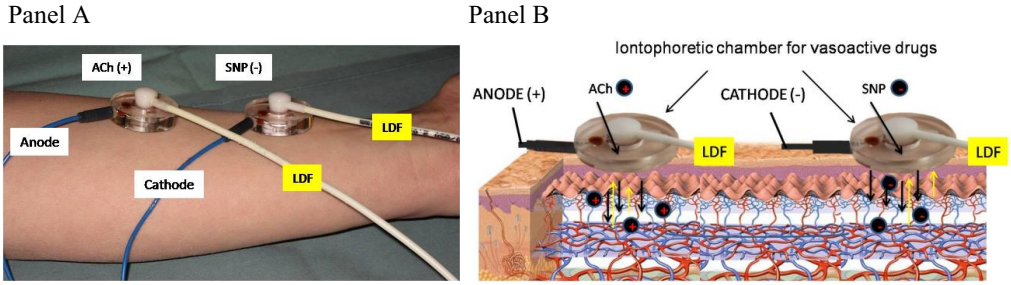


*Figure 4. Experimental set up of iontophoresis on lower forarm (Panel A). The iontophoretic drugs, such as ACh or SNP, are delivered under the LDF probe in the iontophoretic chamber. At the anode the positive charged ACh-ion will be transferred into the skin. At the cathode, the negative charged SNP-ion will be transferred into the skin. (Panel B) The local effects on the skin microcirculation can then be monitored by LDF. Figure in Panel B is modified from Skin Care Forum Issue 40, www.scf-online.com, Cognis GmbH, with permission.*

of ACh on skin microcirculation is more complex (53;58). Data suggest that the receptor-mediated response also involves prostaglandins and endothelium-derived hyperpolarizing factor, as well as NO. Thus, microvascular dilation in response to ACh is not solely dependent on NO. Additionally, iontophoresis has the potential to induce an axon reflex that contributes to the increase in SkBF. This effect can be reduced by pre-treatment of local anesthetics (59) or by using low current or low total charge during iontophoresis (60).

**Figure 5**

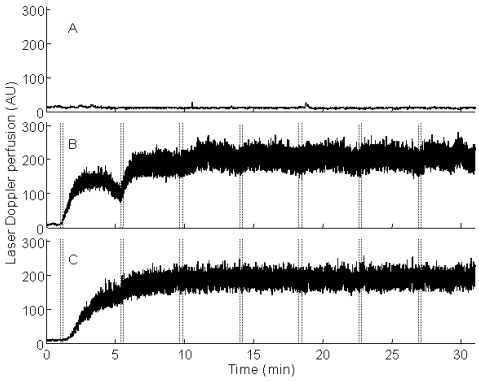


*Figure 5. Unstimulated (A) and iontophoretic stimulated SkBF with ACh (B) and SNP (C) measured with LDF. From Landsverk et al. Anesthesiology 2006;105:478–84, with permission.*

In study I, two combined probe holders for iontophoresis and perfusion measurement (MIC1-E ION, Moor Instruments, Axminster, Devon, UK) of opposite polarity, were attached to the volar side of the forearm. A charge of 2 mC (100 μA for 20 s) followed by a 240-s response measurement period after each iontophoresis was used. The iontophoresis was repeated seven times. This protocol was the same that was used in two previous studies performed by our group (33;61). In study II, we used a protocol with charges of 0.75 mC (75 μA for 10 s), 1.5 mC (150 μA for 10 s), 3.0 mC (150 μA for 20 s) and 6.0 mC (200 μA for 30 s) with a response measuring period of 300 s after each charge of the iontophoresis. This protocol has also previously been used (32). They are also in accordance with the lower total charge, but over the limit for current (60). Despite the limitations regarding this method, it is still used when studying endothelial function in human skin microcirculation (62).

## 7.4 Pulse oximetry photoplethysmographic waveform

The pulse oximetry photoplethysmographic waveform is a non-invasive signal related to the pulsatile volume of blood in tissue. From the beginning it was displayed on the monitor as additional information to the arterial oxygen saturation, so that the clinician could evaluate the reliability of the arterial oxygen saturation values by relating it to the quality of the waveform. Due to its similarity to the arterial blood pressure curve, it has later been subjected to great attention as a possible tool for evaluating hemodynamics. The photoplethysmographic waveform is generated by two light-emitting diodes, red and infrared light, sent through a fingertip or an earlobe, and collected by a photodetector on the other side (transmission mode). The light absorbed is then dependent on the amount of blood and the hemoglobin they carry, present in the tissue, which changes with pulsation. An increase of erythrocytes and the light absorbing hemoglobin, following a pulsation of blood, increase both the optical density and the distance of light through the tissue. This decreases the light that can be collected by the

photodetector. Thus, the original photoplethysmographic waveform is opposite compared to the arterial blood pressure curve. To be able to relate the signal better to the increase in volume and pressure in the tissue, most manufactures of pulse oximeter "flip" the signal giving it the form usually seen on the monitor. Additionally, the signal is also pre-processed to optimize the measurement of arterial oxygen saturation, not focusing on the wave form. Thus, even if several authors report that they use a "raw" signal from the analogue output of the monitor, this is misleading as long as a commercial pulse oximetry is the source of the signal. In study III the photoplethysmographic waveform data were obtained from a Nellcor pulse oximeter (Nellcor, OxiMax 451N5, CA, USA). As seen from the figure 6, the photoplethysmographic waveform is affected by several factors, such as changes in vascular tone from all tissue compartments present in the fingertip. The amplitude of the pulse oximetry photoplethysmographic waveform is narrowed during vasoconstriction.

**Figure 6**

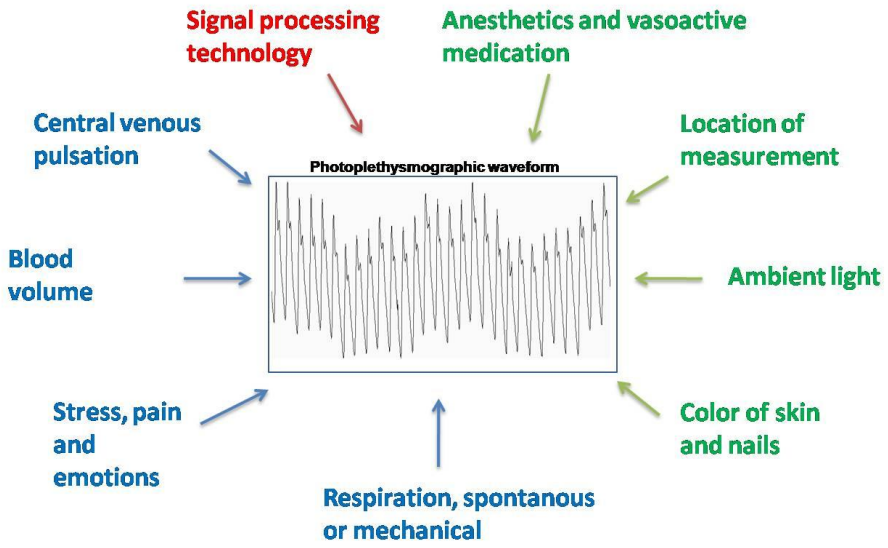


*Figure 6. Illustration of the photoplethysmographic waveform signal and different technical, physiological and external factors, which can influence this signal.*

## 7.5 Signal analysis and wavelet transform

When looking at a hemodynamic signal on the monitor screen, such as ECG, the window is usually narrow to be able to look at details of the QRS complex or other parameters that can be related to heart disease. However, if the interest is HR variability, a wider window should be used. Thus, the information available from the signal sometimes depends on the window length. From our studies, this can be illustrated by looking at the different frequencies present in the LDF signal. In figure 7C (the narrowest window) it is possible to identify oscillations related the heartbeat and respiration, but in A (the most wide window), these are not seen; only the large and slow oscillations are available.

**Figure 7**

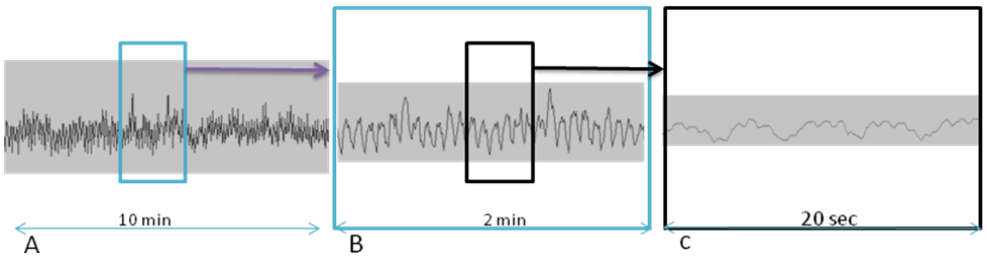


*Figure 7. Illustration of the same LDF signal using three different time windows. A 10 minute recording (A), a 2 minute recording (B) and a 20 second recording (C) are shown.*

Another, and often better way of obtaining information about the frequency component of a signal, include mathematical transformation, or a spectral analysis, such as Fourier or wavelet transform. The Fourier transform, perhaps the most common used, is suitable when the signal is stationary. This means that the different frequencies are present at all time. Thus, they do not change. LDF signals from human skin are, like other biological signal, non-stationary by nature. Like HR variability, the characteristic frequencies of the LDF signal continuously change with time. This implies the necessity for good low frequency resolution and good time resolution for higher frequency components. This can be achieved by wavelet transform.

*Wavelet analysis*

The wavelet transform is performed with an adjustable window length to obtain an optimal frequency resolution for the low frequency content and optimal time localization for the high frequency content (11, 12). Slow events are analyzed with a long window, while faster events are analyzed with a shorter window. In addition, the continuous wavelet transform enables logarithmic frequency resolution. The continuous wavelet transform, based on the Morlet mother wavelet, was calculated within the frequency interval between 0.0095 Hz and 1.6 Hz in paper I and 0.0095 and 2 Hz in paper II. The increase up to 2 Hz was to include higher HR than approximately 100 beats per minute. Later in this thesis we will not distinguish between this two intervals and denote it as 0.0095 and 2 Hz. Slower oscillations than 0.0095 have been described but will not be commented further in this thesis (61).

**Figure 8**

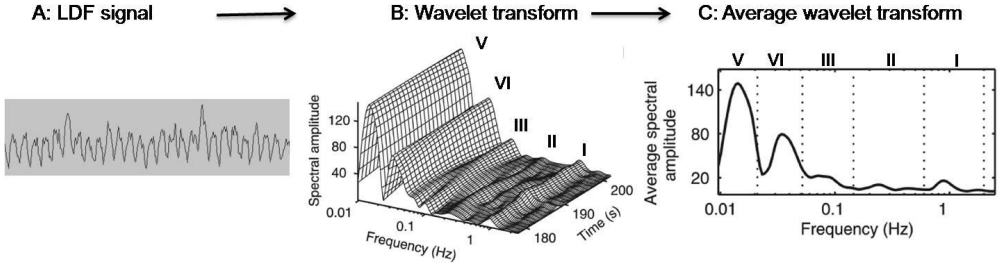


*Figure 8. Illustration of a LDF signal (A). This signal is spectral analysed with wavelet transform (B) and then to an average wavelet transform (C). Five frequencies can be observed. These frequencies relate to: Beat of the heart (I), Respiration (II), Myogenic activity (III), SNA (IV), Endothelium (V). Modified from Landsverk et al. Anesth Analg. 2007;105(4):1012-9, with permission.*

Programs for the calculation of wavelet transform and graphical presentations were written in MatLab (MatWorks). As Illustrated in figure 8, a LDF signal (A) generates a 3-dimentional wavelet transfom (B) and then by averaging in time, a 2-dimentional average wavelet transform is created (C). In the interval 0.0096-2 Hz, periodic oscillations with five different characteristic frequencies are observed. The interval 0.6-2 Hz relates to the heartbeat, 0.145-0.6 Hz to

respiration, 0.052-0.145 Hz to myogenic activity, 0.021-0.052 Hz to SNA and 0.0096-2 to the endothelium. The mean amplitude of the whole interval (0.0096-2 Hz) *(*denoted as *mean amplitude)* is first determined, and then the amplitude of each particular frequency interval *(*denoted as *absolute amplitude*) is calculated. The *relative amplitude* is then defined as the ratio between the *absolute amplitude* at a particular frequency interval and the *mean amplitude* of the entire spectrum (11). Spectral power analyses are not presented in any of the papers. In paper I, only relative amplitude is presented. Both absolute and relative amplitude were given in the first submission of this paper, but due to recommendations of the reviewers, only relative amplitude was kept. In paper II, both values are given.

## 7.6 Data acquisitions and analyses

In study I and II, HR, intermittent non-invasive blood pressure and respiratory rate were manually recorded in an Excel sheet. LDF data were transferred from the DRT4 and the moorLAB server/satellite to a Laptop PC, using a company made software (moorsoft v1.2). This software was used to calculate values of LDF and generating text files that were analyzed using wavelet transform. Programs for the calculation of wavelet transform and graphical presentations were written in MatLab (The MatWorks, MA, USA). In study III, HR, invasive blood pressure, respiration rate and the photoplethysmographic data were transferred from the analogue output of the monitor (Marquette Solar 8000i, GE Healthcare) and the Moorlab server to an analogue-to- digital converter (NIDAQPad-6015, National Instruments, TX, USA) and then to a laptop PC (Dell Latitude D620) using a data acquisition software (VI logger, National Instruments, TX, USA).

Calculations of ΔPP and ΔPOP were performed in a custom made program in LAB VIEW v8.2 (National Instruments, TX, USA). A respiratory cycle was chosen manually and the program would then display the corresponding blood pressure, pulse oximetry plethysmographic waveform and LDF curve. ΔPP and ΔPOP were calculated automatically. ΔPP, the percentage change in PP, was calculated as described by Michard and coworkers: $\Delta PP\ (\%) = 100 \times ([PP_{max} - PP_{min}]/[(PP_{max} + PP_{min})/2])$. Pulse pressure (PP) was calculated as the difference between systolic and diastolic arterial pressures. Maximal PP ($PP_{max}$) and minimal PP ($PP_{min}$) values were determined over the same respiratory cycle.

POP waveform amplitude, expressed in millimetres, was measured from beat-to-beat as the vertical distance between peak and preceding valley through the waveform. Maximal POP ($POP_{max}$) and minimal POP ($POP_{min}$) were determined over the same respiratory cycle. ΔPOP was calculated using a formula similar to that for ΔPP: $\Delta POP\ (\%) = 100 \times ([POP_{max} - POP_{min}]/$

[($POP_{max}$ + $POP_{min}$)/2]). Calculations of ΔPP and ΔPOP were repeated over three consecutive respiratory cycles and averaged.

### 7.7 Statistics

In all three papers anthropometric and hemodynamic data are presented as mean±standard deviation (SD). In paper I, flow and wavelet data are presented as median and total range or box plot, because a substantial part of the data was not normally distributed. This had been the usual way of presenting data in previous articles related to wavelet transform of LDF data. Thus, the Wilcoxon signed rank test for comparison of dependent samples was used to evaluate differences in skin perfusion and wavelet data in basal probes. Differences for the wavelet data between ACh and SNP probes were evaluated by the Mann–Whitney rank sum test. In paper II, these data were presented as mean and SD and therefore evaluated with the t-test to compare dependent samples for the basal probes while differences between ACh and SNP probes were evaluated by a paired t-test. In this study, some of the flow and waved data were still not normally distributed. However, on the recommendation from one of the reviewers and our local statistician we chose to evaluate the distribution based on histogram, making the flow and wavelet data normally distributed.

Based on pilot studies we performed sample size calculations before study I. We found that with $\alpha = 0.05$ and a desired power of 80%, we would need a minimum number of 10 patients to show a 50% decrease of the amplitude in the 0.021-0.052 Hz frequency interval. In paper I we included 13 patients and in paper II, 11 patients were included. However, due to large variations in some of our results, and the number of variables calculated, we can not exclude the possibility of having type 2 errors in our statistical calculations.

In paper III, ΔPP and ΔPOP were compared using a Pearson correlation coefficient, linear regressions analysis and a Bland-Altman plot (63). Intra- and interdividual variance were

calculated by a linear mixed model for repeated measures, using the lme function in R (64). Mixed models take into account the dependency in repeated measurements by adding variance components (random effects). The overall variance was then divided in two terms, intraindividual and interindividual (65). In the Bland-Altman plot, two sets of lines, representing limits of agreement, were presented. One standard, independent of the increase in average values and the other regression based, representing situations where the difference between the methods changes with increasing average values (66). In both cases, the limits of agreement were adjusted for the mixture of between and within-subjects values according to Bland-Altman (66). Instead of ANOVA, we chose a linear mixed model for repeated measures (65).

In paper I and II, data were analyzed in Sigmastat (Systat Software Inc, Richmond, California, USA). In paper III, statistical calculations were performed by SPSS 14.0 (SPSS Inc., Chicago, IL, USA), and the R version 2.5.1 (www. r-project.org). A *P* value of 0.05 was considered statistically significant.

# 8. General discussion

The main results will be discussed related to the aims of each study.

Paper I: *We hypothesized that changes related to brachial plexus block, primarily the sympathetic block could be detected by LDF, iontophoresis, and wavelet analysis of the human skin microcirculation.*

There was a significant increase in skin perfusion and skin temperature both in the anesthetised arm and the control arm after the anesthetic procedure, with no difference between them. However, when performing spectral analyses of the perfusion signal we were able to detect reduction of the two slowest oscillatory components in the anesthetized arm. In accordance to previous studies we relate the frequency interval of 0.021-0.052 Hz to sympathetic nervous activity (26;67) and the reduction of this frequency interval to the sympathetic block. Others have related the sympathetic oscillations to around 0.1 Hz (27). In study I, this frequency interval was completely unaltered.

**Figure 9**

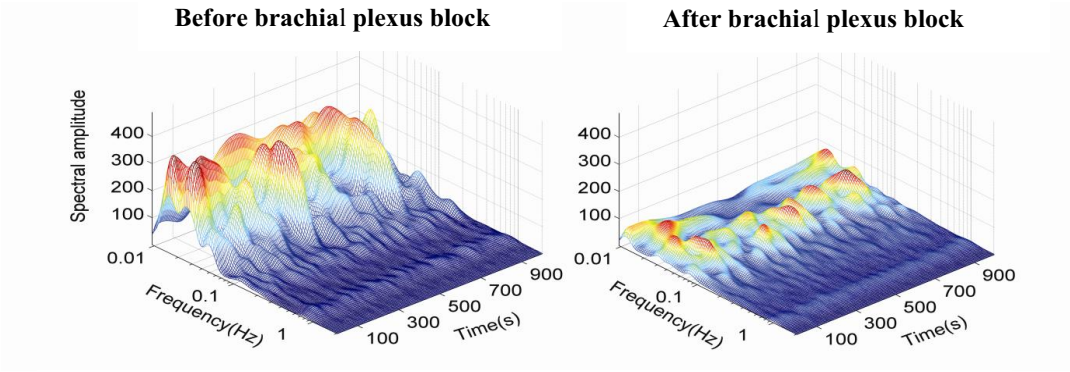


*Figure 9. A three-dimensional wavelet transform of a LDF signal from a patient before and after brachial plexus block. From Landsverk et al. Anesthesiology 2006;105:478–84, with permission.*

The slowest oscillation was also reduced in the anesthetized arm. Previously, this oscillation (around 0.01 Hz) has been related to the vascular endothelium, because iontophoresis with ACh increased selectively the normalized amplitude to a greater extent than SNP in this frequency interval. In accordance with this interpretation, the difference between ACh and SNP in this frequency interval were abolished after brachial plexus block. We therefore interpreted the reduced amplitude of this oscillation as an effect on the vascular endothelium activity induced by the brachial plexus block. This is in contrast to a study performed with venous occlusion plethysmography on the lower arm (38), which found no influence on the endothelium due to brachial plexus block. The different interpretation made in study I and II regarding this frequency interval is commented in the discussion of paper II.

In study I, we presented the wavelet data using only relative amplitude. This is in contrast to in study II, were absolute and relative amplitude were presented. Based on Figure 4 (Panel A and B) in paper I, one could get the impression that "nothing happened" in the control arm after brachial plexus block. This was not the case. When looking at the absolute amplitude from the wavelet analysis in the control arm, all frequency intervals increased, explaining why the relative amplitude was unaltered. We believe that epinephrine used as a supplement to the local anesthetics could cause these changes. It has previously been shown large spatial variability of LDF recording in human forearm, and it is likely that these vessels both reflect a passive perfusion depending on blood pressure and various degrees of sympathetic innervations. This has been demonstrated by Lossius et al. (28) were LDF in forearm and finger pulp was correlated to blood pressure. In a study by Silverman et al. (68) it was found different response to phenylephrine in acral skin (palm of the hand) and forearm when measuring SkBF with LDF. Thus, the forearm skin microcirculation is less sympathetically innervated than in the palms and finger pulp, and is more influenced by blood pressure.

We believe that additional information could have been obtained by changing the protocol. By measuring acral skin blood flow, using a LDF probe on the finger pulp in study I (and II), we could have made the comparisons between our three studies better. However, we only had four probes and choose iontophoretic measurements. An alternative would have been to perform measurements on several locations before and after the procedure.

Paper II: *We hypothesized that wavelet transform of laser Doppler flowmetry signals can detect changes in the microcirculation induced by general anesthesia.*

The effects of general anesthesia, using propofol, fentanyl and midazolam on the central hemodynamic were as expected. Blood pressure was substantial reduced and HR was unaltered. SkBF increased, however, the temperature remained unchanged. The main finding in this study was that general anesthesia induced major changes in the oscillations in human skin microcirculation.

**Figure 10**

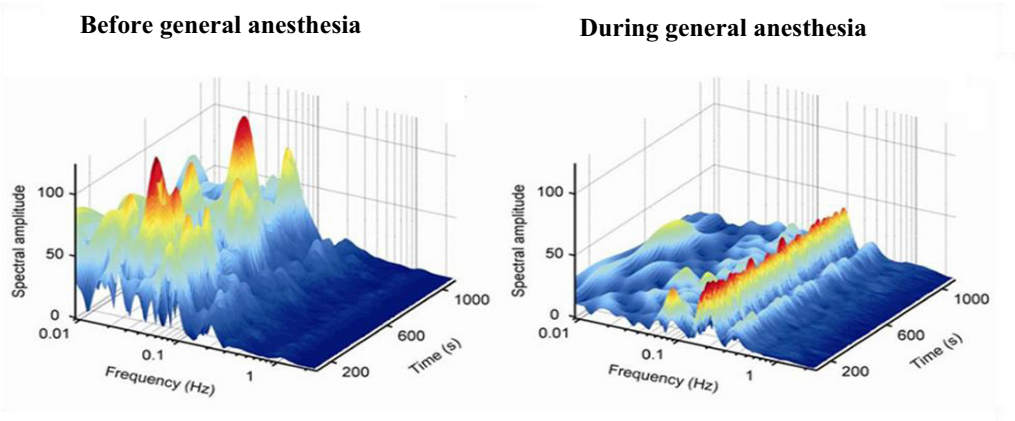


*Figure 10. A three-dimensional wavelet transform of a LDF signal from a patient before and during general anesthesia. From Landsverk et al. Anesth Analg 2007;105(4):1012-9, with permission.*

The amplitude of the oscillations related to respiration was clearly increased. This is an important observation because it shows that the respiratory variations due to mechanical ventilation seen in blood pressure, and in the pulse photoplethysmographic waveform, can be demonstrated by LDF. It is likely to believe that these respiratory variations would be larger if the patients had been hypovolemic. However this is based on assumptions and not on data from study II. Another important finding in study II is the reduction of the amplitude related to myogenic activity and vasomotion. This is in contrast to the finding in study I, were this interval was unaltered. As it has been proposed that the physiological role of vasomotion is to improve perfusion, it should be important that common anesthetics and sedative drugs impair this effect. These effects of general anesthesia on vasomotion, have been demonstrated in vivo, animal models (5;6), but as far as we are aware of, not in human skin microcirculation. The amplitude of the frequency interval related to SNA was also reduced, as anticipated. This is in accordance to the suppressive effect of general anesthesia on SNA that mediates to the cardiovascular system.

The amplitude of the slowest interval, which can be modulated by ACh, was clearly reduced, but this finding was not supported by the iontophoretic data. In study I, we concluded that endothelial function was impaired due to a reduction of the same amplitude in the slowest oscillation, supported by the iontophoretic data. There are important differences between study I and II, regarding methods used. In the iontophoretic protocol in study I, a charge of 2 mC was used seven times, with a response time of 240 second between each iontophoretic period. This was the same protocol used in a previous study (33). However, in study II we changed the protocol by gradually increasing the current to charges of 0.75 mC, 1.5 mC, 3.0 mC, and 6.0 mC with 300 seconds between each iontophoretic period. It was believed that a protocol with increasing doses of ACh and SNP, compared to a static protocol, would give additional information. Thus, we cannot exclude the possibility that the different iontophoretic protocols

could have influenced the conclusions. This issue was not highlighted in the discussion in paper II. Additionally, large variability of the iontophoretic data is a limitation of the study and we choose to use the word "possible" regarding the effect of brachial plexus block on endothelial function.

Paper III: *We hypothesized that the variability of ΔPOP would be larger than that of ΔPP, when calculations were performed continuously in 10-15 minutes in ICU patients, making the agreement between the two methods poor.*

In study III, we found that the within- subject or intraindividual variability of ΔPOP was much larger than ΔPP. In fact the variability of ΔPOP was so large that more than every third of the calculation of ΔPOP were above the threshold value that would indicate fluid responsiveness when the value of ΔPP would not. Thus, it would not be possible to obtain a representative value of ΔPOP in a time frame of 10-15 minutes in ICU patients in our study. This large variability made the agreement between the two parameters poor.

This is in contrast to findings in three previous studies (49;69;70) where there was a significant correlation between ΔPP and ΔPOP in ICU patients. The heterogeneity of our patients regarding sedative and vasoactive drugs and cause of admission to ICU, could explain some of the difference between their and our findings. However, we believe that the main cause of difference relates to our investigation of the reproducibility in each patient. As far as we were able to read the previous three papers, this was not performed in these studies. In the study by Cannesson et al. (69) and Wyffels et al. (70), there are no detailed information on how the values of ΔPP and ΔPOP were selected from the recordings. In the study by Feissel et al. (49) calculation of ΔPOP was, as far as we were able to understand, based on a few randomly selected respiratory cycles. The correlation between ΔPP and ΔPOP has primarily been

investigated in the perioperative setting, where the potential for this non-invasive method is greater than in the ICU, since most ICU patients have an arterial line. Several studies have also demonstrated that fluid responsiveness can be predicted using ΔPOP (48;50). During general anesthesia, the intraindividual variability in ΔPOP could be less than seen in our study, due to a more suppressed SNA and less use of vasoactive drugs. This effect of general anesthesia on SNA oscillations in SkBF was demonstrated in study II, although measurements were performed on the lower forearm. Finally, the calculation of ΔPP demonstrated a good repeatability, and is therefore robust in different clinical situations.

Paper III: *We hypothesized that laser Doppler flowmetry in fingertips could improve the detection of slow oscillations and highlight the relationship between acral skin microcirculation in fingertips and variability in ΔPOP.*

LDF were measured on the pulp of the thumb bilaterally to detect oscillations in skin microcirculation that could interfere with ΔPOP, especially the synchronous oscillations related to SNA. When performing the analysis, the calculations of ΔPOP were influenced by changes in perfusion, clearly demonstrated by LDF, but also visible in the photoplethysmographic waveform as seen in figure 11. This figure illustrate that there is a major change in ΔPOP, compared to ΔPP during sudden changes in perfusion. Although the variability would be less when averaging data over 3 respiratory cycles, as performed in our study, the effect of one or two high, or low values would still have the potential to alter the final ΔPOP value used to evaluate fluid responsiveness. Interestingly, large, slow oscillations were seen in 6 of our 14 patient, even though these patients were deeply sedated (MAAS 0-1).

**Figure 11**

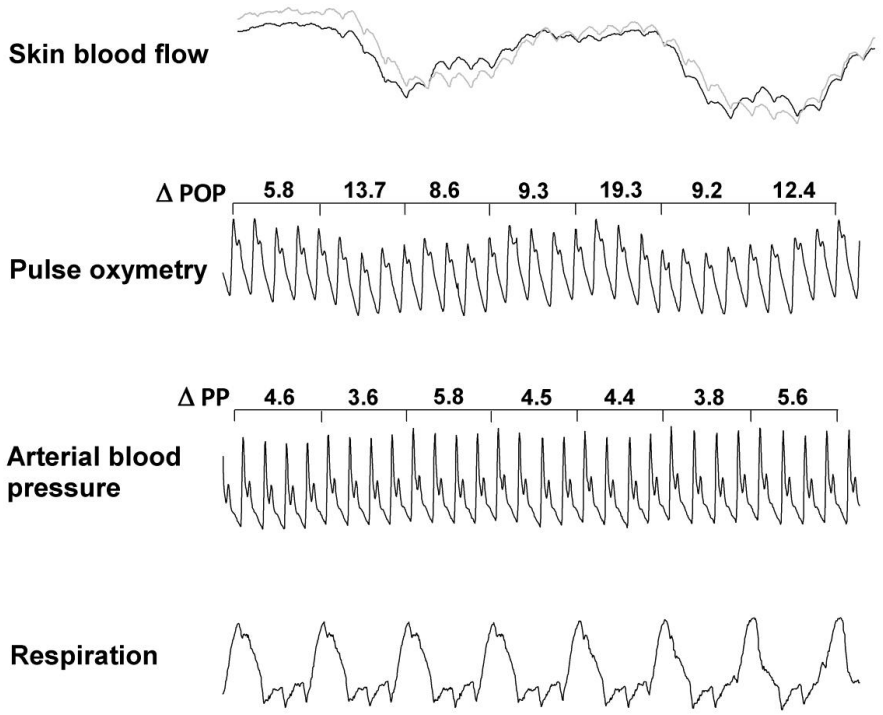


*Figure 11. Illustration of the effect of mechanical ventilation on arterial blood pressure, pulse oximetry and SkBF from an intensive care patient. From Landsverk et al. Anesthesiology, accepted May, 2008, with permission.*

Other mechanisms than slow oscillation, might also contribute to the variability and lack of agreement. By using average values of LDF, we related different vascular perfusion levels in the skin microcirculation to ΔPOP. However, we found no correlation between the average values of LDF and ΔPOP. We also compared the patient group that demonstrated a significant positive correlation between ΔPP and ΔPOP, and ΔPOP values below the accepted threshold limits, with the rest of the patients. However, there was no significant difference between the two groups, in parameters most likely to reflect vascular tone (LDF, temperature, doses of

norepinephrine and central venous oxygen saturation). It must be noted that the numbers of patients in these statistic calculations are few and that spatial variability is large regarding measurements LDF. The vascular tone, and not only the change in it, is probably important for the values of ΔPOP. However, by using LDF in this study we were not able to show this.

# 9. Conclusions

1. Hypothesis 1 (Paper I):

Decomposing the LDF signal by wavelet transform on a logarithmic scale and the use of iontophoresis can detect and identify microcirculatory changes, such as inhibition of SNA and endothelial activity, in human skin after brachial plexus block. To our knowledge, this has not previously been shown.

2. Hypothesis 2 (Paper II):

Decomposing LDF signal by wavelet transform on a logarithmic scale can detect and identify microcirculatory changes, such as inhibition of SNA and vasomotion, in human skin during general anesthesia. To our knowledge, this has not previously been shown.

3. Hypothesis 3 (Paper III):

A large variability of ΔPOP and a poor agreement between ΔPP and ΔPOP, limit ΔPOP as a tool for evaluating fluid responsiveness in ICU patients. This is in contrast to ΔPP where the reproducibility is good. To our knowledge this has not been previously shown.

4. Hypothesis 4 ( Paper III):

Large, slow oscillations, clearly demonstrated by LDF, are one of the mechanisms for the large variability in ΔPOP. Average value of LDF did not highlight other factors explaining the large variability in ΔPOP.

# 10. Clinical implications and future perspectives

1. Vasomotion is a fundamental property of the microcirculation and may improve tissue oxygenation, especially in conditions with limited perfusion. If a common combination of anesthetics, as used in our study II, impairs vasomotion, this could have relevance for a large group of patients. We believe that the methods used in studies I and II, in combination with methods to measure tissue oxygenation, should highlight this issue further.
2. In study III we showed that, due to the large variability of ΔPOP, there is no "representative" value of ΔPOP from a photoplethysmographic waveform recording in ICU patients, to demonstrate fluid responsiveness. Future studies investigating ΔPOP and fluid responsiveness must document the reproducibility of this method and previous enthusiastic reports on the ability of ΔPOP to predict fluid responsiveness should be interpreted in this context.
3. Study III could have relevance for the implementation of ΔPOP perioperatively. A method for detection of fluid responsiveness perioperatively should be independent of the different clinical situations in the operating room, such as surgical stress reactions due to different levels of anesthesia, vasoactive drugs or other medical interventions. Comparing the two methods on a heterogeneous group of ICU patients is therefore useful. Presently, we are performing a new study aiming to investigate intraindividual variability of ΔPOP in patients undergoing major abdominal surgery in general anesthesia.
4. We believe that the pulse oximetry photoplethysmographic waveform display clinical useful information regarding hemodynamics. The advantage is that it is non-invasive and the pulse oximeter is always "around". Based on data from study III and the ongoing study described above, we are presently working on a method, based on wavelet transform, to improve the photoplethysmographic waveform as a tool to evaluate fluid responsiveness.

# 11. Reference List


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