Assessing the sympatho-vagal balance in children and neonates
an initial exploration of methods and measurements

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Abstract

An initial exploration is made of the possibilities of researching the sympatho-vagal balance in neonates and children at the Saint Joseph hospital in Veldhoven. Based on the medical research protocol for such a study and a physiological model an assessment is made of the methods and devices to be used.
Working principle of both Periflux and Finapres, first impressions of measurements performed on them and a part of the literature about them are presented.
Finally a comparison is made between the blood pressure variability from the Finapres measurements and the variability obtained from intra-arterial measurements in neonates, both in time and frequency domain.
Conclusions from this comparison are:

a.) The standard method for evaluating the sympatho-vagal balance in the frequency domain cannot be used on Finapres measurements in neonates,
b.) in the time domain comparisons can be made using the systolic blood pressure variability, and
c.) in all analysis methods the measurement should be taken as its own base-line, because of large differences between individual measurements.

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Chapter 1 Clinical setting and research questions

As an introduction a description of the clinical setting of the research and the underlying questions will be given below.

1.1 General clinical setting

The neonatal intensive care unit (NICU) at the Saint Joseph hospital[1] is the one of two such units in the Netherlands that has no medical faculty directly associated with it. Being one of ten such NICUs in the Netherlands, it serves the medical needs of seriously ill and often prematurely born infants from the larger part of the South East of the country. At the moment 14 incubators or cribs can be placed at the NICU in Veldhoven. Within the next couple of years this will be expanded to 30 places.
While there is no affiliation with a medical faculty, a strong cooperation with Technische Universiteit Eindhoven (TU/e) exists, primarily through the Clinical Physics department. One of the results of this cooperation is the PINO-project (Physiologisch Informatievoorzieningssysteem voor Neonataal Onderzoek = physiological information-system for neonatal research), in which physicians, clinical physicists and university students collaborate in physiological research. As a part of this project, which started in 1990, the technical infrastructure was created to digitally store and analyse patient data provided by the clinical monitors.
In part because of the PINO-project a unique situation has been created in which the physicians are offered technical research possibilities beyond what is possible at other NICUs and the university students and clinical physicists have a place to develop and test clinical data acquisition and analysis systems with a continuous inflow of patient data.
In addition to the NICU, and partly as a result of its presence, more recently an obstetric high care unit (OHCU) was opened at the Saint Joseph hospital. Women who experience serious problems during pregnancy are admitted to this unit.
The combination of the NICU and OHCU at the same hospital with the addition of the technical support, creates an ideal environment for clinical research concerning perinatal problems. A small number of clinical research projects have already been started and plans for extending the cooperation with the TU/e are being developed at the moment.

1.2 Investigating the autonomic nervous system

One of the clinical studies, mentioned in 1.1, is an investigation of the mechanisms of the fetal origins hypothesis, which links growth retardation in the fetal or infant stages of life to cardiovascular diseases in adulthood. This implies the assumption that somehow these diseases are 'programmed' in early life. A more extensive description of this hypothesis' background will be given in Chapter 3.
One of the possible mechanisms by which this programming takes place, and the one that is to be investigated in the Saint Joseph hospital, is an altered functioning of the autonomic nervous system. The general idea behind it is that if programming takes place by means of an altered autonomic function, it might be possible to detect this alteration before apparent symptoms are seen. Once diagnosed, it could also be possible to start treatment early on thus lowering the risk of actually developing cardiovascular defects.
Although monitoring of signals at the NICU can, as a result of the PINO-project, be done routinely, there is no way to actually assess the working of the autonomic function yet. Exploring the question

[1] Because of a merger with another hospital (Diaconnessenhuis, Eindhoven) from January 1st, 2002 the St. Joseph hospital is known as Maxima Medical Centre, location Veldhoven. In this report the old name will still be used.

how to reliably and effectively assess the autonomic system and find differences in its working between test groups is the primary goal of this study.
A medical specialist has worked out a proposal for a clinical study. This, in combination with a global physiological model, serves as guideline for the investigation of the technical feasibility.

1.3 Considerations in clinical research

Doing research in a clinical environment comes with a specific set of problems. Basically the problems encountered can be divided into three classes.
The first group consists of the problems caused by the environment. A clinic's main goal is to provide medical care. This implies that the staff and infrastructure is dedicated to this goal, research being an additional effort, and can not interfere with medical care.
The second group contains the difficulties caused by the subjects to be investigated. The first one in this group is the impossibility to switch part of a human being off to research a part of its system, so the measurement outcome can always be influenced by effects that are not sought after. Sometimes certain illnesses and medication are known to interfere with systems to be researched, which excludes these patients from the research program. On the other hand, a patient should preferably not be discomforted and certainly not be put in the risk of being harmed for the sake of research. These conditions limit the group of research subjects to those who are willing to cooperate, receive a certain kind of treatment, so that the research does not cause any extra harm or discomfort, and do not have any illness or medication preventing them from being included. All in all this makes the pool of test persons rather small.
The third group of problems lies in the medical measurement equipment. In most cases some kind of dedicated hard- and software is used to perform measurements in a clinic. These devices are developed to perform standardised measurements and present them accordingly. In research, a large degree of freedom is demanded to adapt and experiment with the equipment in order to set it up in such a way that it measures and outputs the desired results. More often than not in medical equipment the flexibility is limited. Systems tend to be closed, output being limited to the standard results and manufacturers are rarely willing give complete insight into operations performed in data-acquisition and analysis. Adapting the system itself is hardly an option, since safety regulations would demand it to be thoroughly tested and approved after every adaptation. In some cases it is possible to obtain some kind of 'raw' signal from the device, which can then be processed using other, more flexible systems. This is what is done in the PINO-project.

1.4 Measurement infrastructure

In the past measurements at the NICU were done through an integrated data-acquisition network, which could be operated from a separate technical room. In the course of 2001 a mobile acquisition station was set up. A more detailed description of this mobile station is given in Appendix A.
The main advantage of the mobile data acquisition station is the fact that it enables measurements at every possible location within the hospital, with any kind of device with an analogue output signal. It offers flexibility in both acquisition and analysis of data. On the other hand, the need of the presence of the equipment in room interferes with daily routine of nurses and medical staff and the chance of disturbing the patient is larger. These factors have to be taken into consideration for every measurement or test done.

1.5 Set-up of this report

In this report both the global physiological model (Chapter 2) and an overview of the proposed clinical research project (Chapter 3) will be described.
Chapter 4 and Chapter 5 will give a more in depth into the non-invasive techniques and devices used to assess the autonomic nervous system.

Then in Chapter 6 and Chapter 7 a method for spectral analysis and a comparative investigation of blood pressure variability in neonates are presented. In this comparison, the Finapres blood pressure monitor is compared with standard intra-arterial measurements.
Finally, in Chapter 8, some recommendations for the assessment of the autonomic function by means of blood pressure variability and skin blood flow measurements will given.

Chapter 2 Cardiovascular regulation

As mentioned in Chapter 1, a general physiological model and a clinical research protocol serve as guideline for this study. In this chapter the physiological model will be presented. In physiological textbooks, like [1] and [2], a more extensive description can be found.

2.1 The nervous system

The nervous system is a communications network through which a creature can interact with its surroundings. Both anatomically and functionally it can be divided into its central and peripheral parts. The central nervous system consists of the brain and the spinal cord. The peripheral nervous system is a communications network, which electrochemically transfers information from the rest of the body to the central system (afferent) or from the central system back to the muscles and organs (efferent). In the central nervous system information is gathered and processed and the desired action determined.
Alternatively, the nervous system can be divided into the somatic and the autonomic nervous system[2]. The somatic system is primarily dedicated to the conscious interaction of the creature with its (external) surroundings, whereas the autonomic system's main task is the upkeep of a constant internal environment (homeostasis). As our interest lies in the functioning of the autonomic nervous system, the remainder of this chapter will be on this part. A schematic overview of the functional subdivision of the nervous system is given in Figure 1.

2.2 The autonomic nervous system

The central parts of the autonomic system are located in the hypothalamus and the higher levels of the limbic system. Its main task is the upkeep of the constant internal environment (homeostasis). Communications with distant parts of the body can take place through hormonal as well as neural systems. The peripheral part of the autonomic nervous system can be divided into an afferent and an efferent part. The efferent part of the autonomic nervous system can, in turn, be subdivided into the sympathetic and the parasympathetic systems.
Basically, the effects of the sympathetic and parasympathetic systems on the innervated organs are antagonistic: Stimulation by the parasympathetic system has exactly the opposite effect of stimulation by the sympathetic system. The parasympathetic system stimulates anabolic processes in the organ and slows catabolic processes down. The sympathetic system stimulates the catabolic processes and slows the anabolic down. Sympathetic activity is therefore linked to a heightened state of alertness or activity, while parasympathtic activity is associated with rest and digestion of food.
Not all vegetative organs are innervated by both systems. For example, the width of the pupil of the eye is regulated by two antagonistic muscles, one of which is innervated only sympathetically and the other one is innervated only parasympathetically. Thus for each organ there are three possibilities: 1. The organ is only innervated by the sympathetic system, 2. The organ is only innervated by the parasympathetic system, 3. The organ is innervated by both systems. In Table 1 an overview is given of the innervation of some of the vegetative organs.

[2] In literature both the names vegetative nervous system and autonomic nervous system are used. Vegetative nervous system, because it controls the vegetative organs in order to keep up the homeostasis. Autonomic nervous system, since information reception and processing in this part of the nervous system is not performed consciously. 'Autonomic nervous system' will be used here.

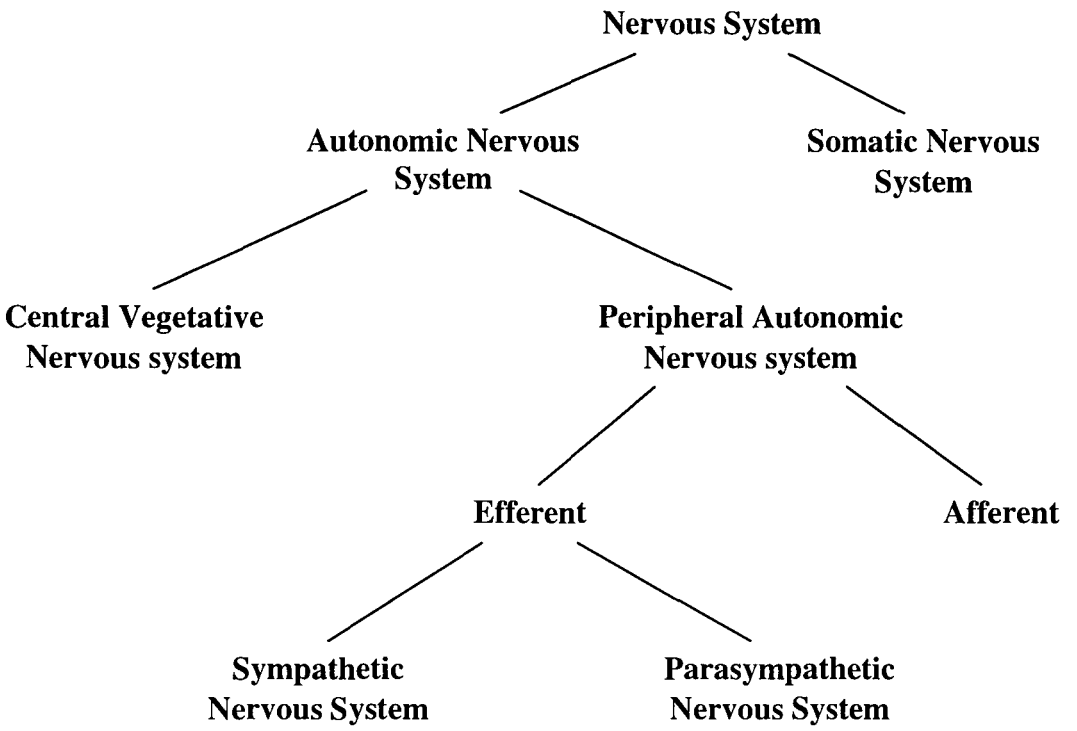

Figure 1: Organisation of the nervous system on a functional level.

Organ	*Sympathetic effect*	*Parasympathetic effect*
Heart	Acceleration	Retardation
Arterioles	Constriction	
Bronchi	Dilatation	Constriction
Iris	Pupil dilatation	Pupil narrowing
Bladder	Relaxation	Contraction

Table 1: Overview of the effects of the sympathetic and parasympathetic nervous system on various organs [4]. It should be noted that the width of the pupil (iris) is regulated by the antagonistic effect of the two systems, but each of the regulating muscles is only innervated by one of the systems.

Anatomically, the sympathetic and parasympathetic systems are remarkably similar. Counting the central autonomic nervous system as the primary vegetative centre, they both have three neurological centres in which signals are transferred between different neurons. The main difference is that the innervation by the parasympathetic system takes place through direct innervation from the central nervous system to the organs, while the sympathetic system forms more of a network, as can be seen in Figure 2.
Biochemically there is a difference in the transmission of the stimulus from the nervous system to the innervated structure. The innervating fibres (axons) of the parasympathetic system trigger the organ by secretion of acetylcholin, which is quickly disintegrated by acetylcholinesterase. The axons of the sympathetic nervous system generally trigger the innervated organ by secretion of norepinephrine, which can, in certain circumstances, be transformed into epinephrine. Unlike acetylcholin, both the norepinephrine and the epinephrine can not be disintegrated by locally present enzymes. This causes the sympathetic stimulation to last longer, and maybe even spread out over a larger area of the organ.
This subtle difference is a beautiful example of the functionality of the regulation in the human body. The parasympathetic triggers, stimulating the anabolic processes of the body, have a short duration. This enables the body to delay its anabolic functions quickly, and initiate a catabolic state (higher level of alertness, and possibility to perform physical labour) whenever danger might occur.

The opposite effect of retarding the catabolic and stimulating the anabolic functions is not as vitally important for the safety and survival of the organism and can therefore be slower.

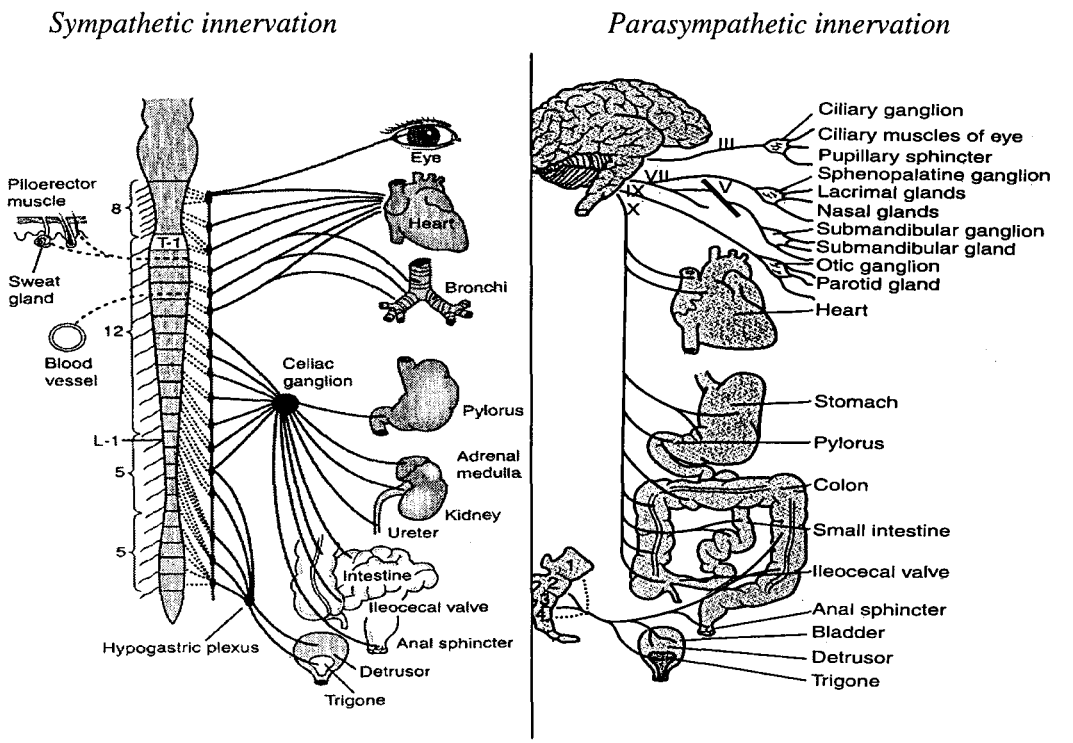

Figure 2: Innervation of viscerous structures by the sympathetic and the parasympathetic nervous system. As is apparent, the nerves in the sympathetic system are interlinked in several centres, while the parasympathetic nerves innervate the organ more directly [3].

2.3 Regulation of the arterial blood pressure

The organs, especially the brain and the kidneys, need a constant supply of blood. Since the blood flow depends directly on the mean arterial blood pressure, this pressure has to be kept at a certain level. On the other hand, blood vessels and organs can be damaged when the blood pressure is too high. Therefore the arterial blood pressure has to be kept within relatively tight limits at all times, even during a change of posture or exercise. The regulation providing a stable blood flow through the organs is called the short-term blood pressure regulation, as opposed to the long-term blood pressure regulation, which regulates the filling of blood vessels over the course of years.
Focusing on the neural regulation of the short-term blood pressure regulation, two contributions can be found: sympathetic and parasympathetic. Sympathetic activity causes the heart to accelerate and the small blood vessels (arterioles and venules) to constrict. The constriction of venous vessels causes an increased blood flow to the heart. In combination with an increased heart rate this results in a higher cardiac output. The constriction of arterial vessels causes a higher flow resistance of the blood. The combination of an increased cardiac output and an increased resistance causes the arterial blood pressure to rise.
When the sympathetic activity decreases, the heart will decelerate and vasodilatation will occur. This results in a lower resistance and a lower cardiac output, and thus in a lower blood pressure. Parasympathetic activity can only cause further deceleration of the heart, resulting in a lower cardiac output. It has no direct effect on the resistance and capacity of the blood vessels.
In Figure 3 the system for short-term blood pressure regulation is summarized.

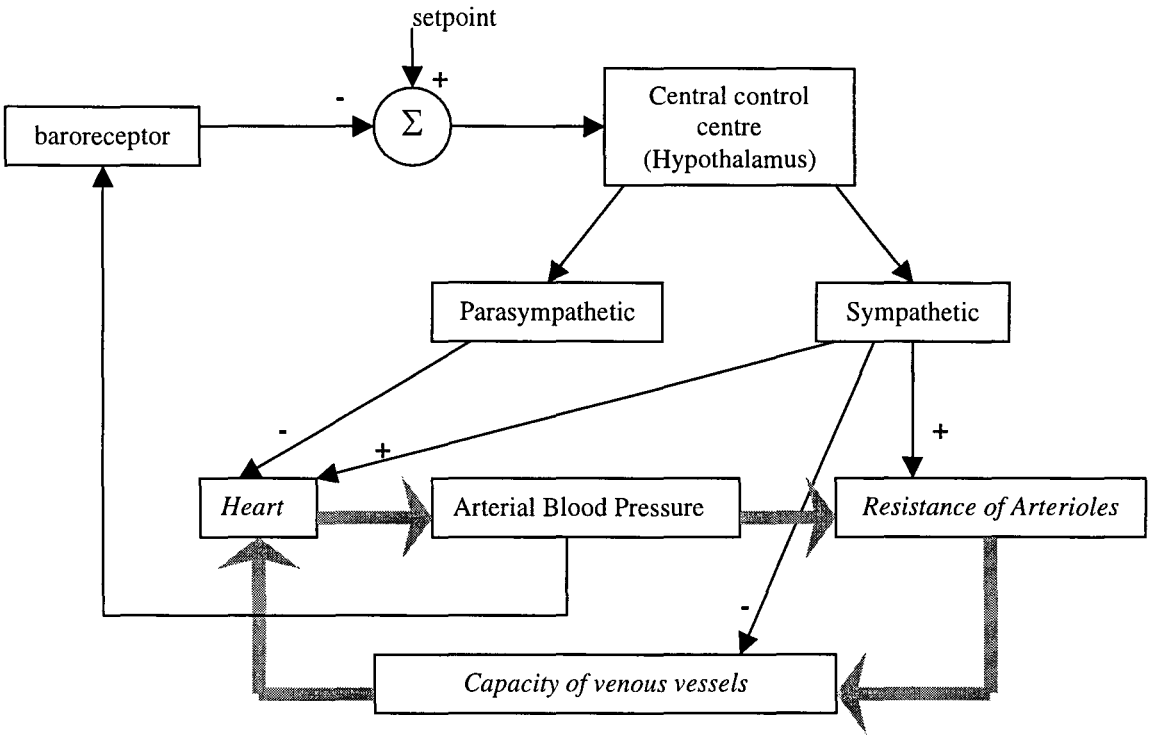

Figure 3: Schematic overview of the neural, short–term regulation of the arterial blood pressure. Terms in italics are under direct influence of the autonomic nervous system. The nature of the influence of sympathetic or parasympathetic activity is indicated next to the arrow.(Adapted from [2])

2.4 Fluctuations of the arterial blood pressure

In 2.3 the driving force of the blood pressure regulation was already indicated to be the need of a constant blood flow through the vital organs. There is however no need for a constant blood pressure in the arterial part of the circulation. Since it would be extremely complex to keep a constant arterial pressure with the heart pumping an amount of blood into the arteries periodically, evolution has allowed a fluctuating arterial blood pressure. As a matter of fact three types of fluctuations can be recognised.

The first type of fluctuation is associated directly with the pumping action of the heart. The pressure is highest directly after the heart pumped the blood into the arteries (systolic pressure) and lowest just before the next heart beat occurs (diastolic pressure). A typical example of a blood pressure wave can be seen in Figure 4.

Secondly, fluctuations associated with respiration can be seen. These so called Traube-Hering waves consist of a drop off in blood pressure during the beginning of the inspiration. This is caused by a lowered venous return to the left half of the heart, resulting from the sudden capacity growth of the lungs. Even during inspiration this is followed by a rise in blood pressure, due to an increased output of the left half of the heart resulting from the larger venous return from the lungs. During expiration a general decrease in venous return occurs. The right half of the heart suffers this effect instantaneously, while the decrease of the capacity in the lungs keeps the return to the left side up for a short time.

Thirdly, low frequency fluctuations (0.03–0.05 Hz for adults), called Mayer waves, are found. They are assumed to be associated with some kind of low frequency oscillations of the output of the vasomotor centre. What exactly causes these fluctuations is not known.

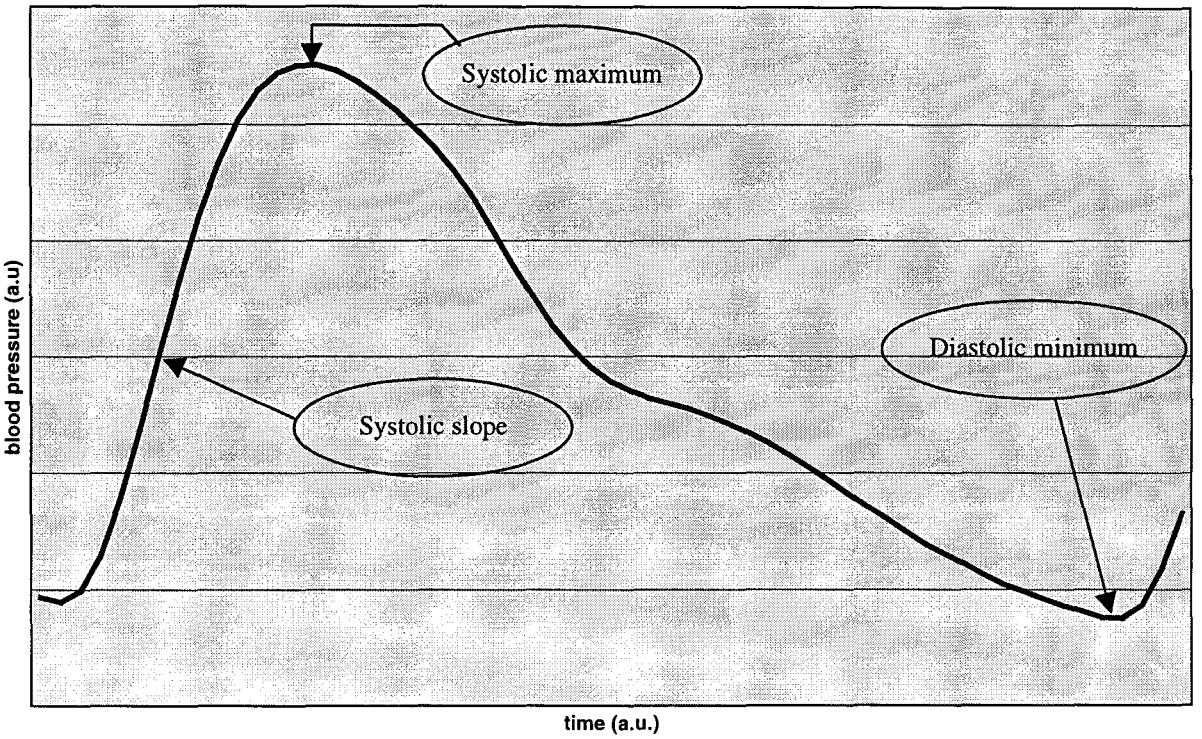

Figure 4: A typical blood pressure wave. The fluctuation is caused by the pumping action of the heart. The picture represents slightly more than one heart beat. This wave is taken from an intra-arterial measurement done at the NICU.

2.5 Heart rate and blood pressure variability

In 2.3, the regulation of the arterial blood pressure has already been discussed. Both the heart rate and the blood pressure are under the influence of the sympathetic and the parasympathetic system (cf. Figure 3). In maintaining the required blood flow to the vital organs, both the sympathetic and the parasympathetic system will be active to some extend at all times. This activity will cause both blood pressure and heart rate to show fluctuations, the so-called blood pressure variability and heart rate variability. Since, because of the differences in transmitter substance described in 2.2, the parasympathetic system can affect the innervated organs quicker than the sympathetic system; parasympathetic regulation can take place at a higher frequency than the sympathetic regulation. The sympathetic system can affect the innervated organs within several seconds, limiting the frequency to several tenths of a Hertz, whereas the parasympathetic system affects the innervated organs within several tenths of a second accounting for frequencies up to a couple of Hz [4].
This distinction in response time creates an opportunity to measure the sympathetic and parasympathetic activity, by recording the fluctuations in heart rate and blood pressure: high frequency fluctuations are caused by the parasympathetic system, and low frequency fluctuations can be caused by both the sympathetic and the parasympathetic system.
Analysis of the ECG signal provides information about the regulation of the heart rate. Some of the clinical studies analyse the heart rate variability to find information about the parasympathetic and sympathetic activity (e.g. [11]). The primary reason for doing so is that ECG measurement can be performed easily without harming the patient. Continuous blood pressure measurements are more complex. Blood flow to the vital organs is however, as mentioned before, the driving force behind the regulation. Blood pressure is related more directly to the flow than the heart rate is. Therefore, blood pressure variability should provide more basic information.

2.6 Skin blood flow

As described in 2.5, information about the sympathetic and parasympathetic activity can be gathered from the heart rate and blood pressure variability. As can be seen in Figure 3, information about the sympathetic nervous system can also be found in the regulation of the width of the arterioles.
The width of the arterioles is directly associated with the perfusion of the tissue surrounding them. Therefore, when sympathetic activity increases the perfusion of this tissue should decrease. The most accessible tissue in the human body is, of course, the skin. From measuring the (relative) perfusion of the skin one should be able to say something about the sympathetic activity.
Using this parameter to assess the autonomic nervous system has its pros and cons. The main 'con' is that, as in measuring heart rate variability, the measured quantity is not the quantity that is most strictly regulated. The skin is not a vital organ, arterioles providing the skin blood flow are constricted sympathetically to decrease the flow and increase the blood flow to the vital organs. The most important 'pro' lies in the fact that the arterioles are only innervated sympathetically and the regulation of their width more directly says something about the sympathetic activity.

Chapter 3 BIRTH-study

As mentioned in the first chapter, the aim of this study is to explore the ways by which to assess the autonomic nervous system. A protocol for a clinical research project on this subject was written by a medical specialist [5]. In this chapter an overview of this 'Birth weight In Relationship To Hypertension'-study will be given as a back-ground.

3.1 Fetals Origins Hypothesis

There appears to be an epidemiological relationship between intra and extra uterine growth retardation and cardiovascular diseases including diabetes mellitus, hypertension and coronary heart failure.
In the late 1980s and early 1990s a number of surveys was done on this relationship. Two of the most extensive ones were performed by Barker. Both of them were performed retrospectively using birth data from maternity hospitals in Sheffield and Hertfordshire from between 1905 and 1930. In the first of the surveys Barker showed that death rates from coronary heart disease were almost 3 times as high among those weighing 8.2kg or less than those weighing 12.3kg or more at age 1 year. The second one shows a negative trend between death rates from cardiovascular disease and birth weight, head circumference and ponderal index[3].
Also several researchers in Britain associated low growth rates up until the age of one year with increased prevalence of risk factors for cardiovascular disease. These relations parallel those with death rates from cardiovascular disease. Typically, the association is seen in children born small for gestational age (SGA) rather than those born prematurely. A relationship between the duration and the timing of the undernutrition and the specific risks later in life was found from surveys in humans and animal experiments. The most common illness found proved to be hypertension (elevated blood pressure).
The outcome of these studies led to the formulation of the fetal origins- or Barker-hypothesis stating that undernutrition in early life permanently affects a range of organs and tissues, among which liver and blood vessels.
Looking more specifically into the risk of elevated blood pressure, a survey was done by Law in 1996 who found that blood pressure decreased with increasing birth-weight. Increased risk of hypertension was found in cases in which the placenta was large in relationship to the birth weight. Also an elevated maternal systolic blood pressure was associated with higher blood pressure in childhood.
In an effort to link the undernutrition early in life to hypertension later on, several biological mechanisms have been proposed. These are hormonal effects, changes in the vascular structure (e.g. loss of elasticity in the vessel walls), disfunctioning of the endothelial cells in blood vessels, abnormal renal development, and an altered autonomic cardiovascular function. For all of these mechanisms indications have been found in clinical experiments.

3.2 Analysis of the signals

At the Saint Joseph hospital, a choice was made to look into the last of the mechanisms mentioned in 3.1: Undernutrition in the early stages of child development causes an altered autonomic function.

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[3] *Ponderal index is defined as:*

$$Ponderal\ \ index = \frac{BW}{L^3}$$ *, with BW = Birth Weigth, L = Length, the ponderal index depends on the chosen units*

In the previous chapter the occurrence of variability in heart rate and blood pressure as a result of the cardiovascular regulation, was already mentioned. Analysis of the variability of heart rate and blood pressure can be performed in both the time and frequency domain to assess the sympathetic and parasympathetic activity.
Analysis in the frequency domain allows the possibility to calculate the energy in predefined frequency intervals. Interpreting the high frequency energy as a result of the parasympathetic activity and the low frequency energy as a combined result of the sympathetic and parasympathetic activity, the energy ratio of the two intervals provides a measure of the balance between the types of autonomic nervous stimulation: the sympatho-vagal balance. This ratio of the low frequency and high frequency energy (LF/HF-ratio) is highly dependent on the definition of the frequency bands. Whereas the limits for the LF interval and the lower limit for the HF interval is more or less predetermined by the physiological process of the sympathetic and parasympathetic stimulation, the upper limit for the HF interval should be high enough to include the Traube-Hering waves, which are directly related to the respiratory frequency. For adults and children this has led to the following consensus on the definition of the frequency interval:

Very Low frequency	up to 0.04Hz
Low Frequency	0.04Hz – 0.15Hz
High Frequency	0.15Hz – 0.4Hz

This allows a respiratory rate of 24/min. For neonates no such consensus has been established. The respiratory frequency exceeds that of adults and can be as high as 90/min. Therefore the upper limit of the HF interval should be 1.5Hz. Taking examples from earlier studies [5] the HF interval is defined to be 0.4 to 1.5Hz in neonates.
Using these definitions the sympatho-vagal balance can be assessed from either ECG signal or the blood pressure variations. The specifics of the techniques used in frequency analysis will be described in Chapter 6.

For the analysis of the blood perfusion measurements no analysis method is preferred. Based on the nature of the signal a method will have to be designed. Some proposals will be offered later.

3.3 Research groups

In paragraph 3.1 it has already been stated that the risk factors as a result of growth retardation were found in children who were small for gestational age, rather than in those who were born prematurely. In search of differences in the autonomic function the BIRTH-study focuses on two age groups to be investigated. The first group consists of neonates, the second of children at the age of 8-10 years. The neonates are recruited from the NICU, the school children from a NICU follow-up program.
To find proof of a defect in autonomic control the neonatal group is subdivided into four. The groups to be considered are full-term infants who are appropriate for gestational age (AGA), full-term infants who are small for gestational age (SGA), pre-term infants who are AGA and pre-term infants who are SGA. In the population of school children (8-10years) only pre-term borns are included because they can be more easily recruited from the follow-up program. This group is divided into SGA and AGA.

3.4 Provocations

In the populations described in 3.3 a difference in regulation could be found in a constant environment. However, if there is a difference to be found between regulatory systems, it should be most obvious when the demands on the systems are highest A difference in the autonomic regulation between the test-groups is therefore most likely to occur when the change in situation is highest. The state of the test person should therefore change from as anabolic (parasympathetic) as possible to as catabolic as possible (sympathetic) over a short period of time.

This would mean provoking the regulatory systems to a large extend. In clinical research provocations are regularly found, for example in provoking syncope (cf. Chapter 4).
The provocations proposed to be used in the BIRTH-study are a passive head-up tilt and cold provocation. A passive head-up tilt requires the test person to lie comfortably down in a controlled and save environment, thus entering an anabolic state. After acclimatisation to this environment the subject is moved into an up-right position, without requiring any muscle action from the person. This change of posture has a double effect. First there is a heightened state of alertness causing more sympathetic activity. Secondly, the change in posture requires the complete cardiovascular system to be regulated again, to ensure the blood flow to the vital organs. Through the heart rate and blood pressure variability the sympatho-vagal balance can be assessed before and after the tilt.
Cold provocation again requires the test-person to be comfortable and in a controlled environment. A cold pad is applied locally to one of the extremities. The application of the cold pad should cause the arterioles in the skin to constrict in protection of the vital organs, thus causing a decreased blood perfusion.

3.5 Measurement devices

In order to register the changes in sympatho-vagal balance and skin perfusion, measurements have to be done during the provocations described above. As described in Chapter 1, it is demanded that the patient be harmed as little as possible in clinical research. When there is no medical indication to perform measurements, the patient should not be harmed at all. This demand puts restrictions on the techniques and devices to be used. It is impossible however to do any kind of research using provocations, without disturbing the test-person. The goal is therefore to limit this disturbance to the absolute minimum.
To prevent any risk of infection the measurements should preferably be performed non-invasively. For determining the sympatho-vagal balance from the heart rate this can be done by standard ECG measurements. The variability in the blood pressure can however not be determined by a standard clinical, non-invasive type of measurement. The oscillometric[4] blood pressure measurements, which are performed routinely, do not provide a continuous signal of the blood pressure wave and can therefore not be used for beat-to-beat analysis in the time or frequency domain. The clinical way to get a continuous blood pressure measurement is invasive and can therefore not be used for research purposes only. Relying on this method would make it difficult to get a sufficient neonatal test-population, and would make the blood pressure measurement impossible in school children. The Finapres however is capable of producing a continuous blood pressure wave non-invasively, by using a finger cuff. This device is not routinely used for monitoring blood pressure, and there are still a lot of questions about it. More information about working principle and possibilities of this device can be found in Chapter 4.
For measuring skin perfusion, laser doppler techniques are available. These devices are used for research purposes and measuring perfusion after for example skin transplants. A description of this technique is given in Chapter 5.

Summary

A short description of the aims and techniques of the BIRTH-study can be given as follows: The BIRTH study aims to find proof of an altered autonomic function in children with a growth retardation early in life, thus providing proof of a proposed mechanism for the Barker hypothesis. The autonomic function will be investigated by means of heart rate and blood pressure variability and skin blood perfusion in neonates and schoolchildren, both in resting condition and during suited provocations. The measurement techniques will need to be non-invasive.

[4] this method is also called the sphygmomanometric method. A description of it can be found in [1].

Chapter 4 Variability measurements

With the aim to find differences in sympatho-vagal balance between subjects, regulation of both the heart rate and the blood pressure may give adequate access to the autonomic function. In 4.1 an impression of the literature on variability measurements during tilt testing will be given, explaining both the expected effects and the requirement for the provocation. Also, it will lead to the preference of actually measuring the blood pressure variability, in addition to the heart rate variability, as this may provide information lost otherwise. Therefore in 4.2 a closer look is taken at the Finapres and the possibility to record continuous blood pressure waves non-invasively.

4.1 Tilt-testing

In order to assess the sympatho-vagal balance, tilt-testing is a common procedure. The procedure itself is described in Chapter 3. The focus here will be on the effect of tilt-testing on the sympatho-vagal balance, and the possibility to assess it from the analysis of blood pressure variability.

4.1.1 Expected physiological effect

As mentioned before the passive head-up tilt requires the subject to be in a state that is as anabolic as possible. In this state a maximum in parasympathetic activity and a minimum in sympathetic is reached. Bringing the subject into this state is usually done by laying him down in a comfortably warmed room for some time.
When the patient is subsequently tilted upright (without any somatic action), the blood flow to the brain would show a considerable drop-off as a result of hydrostatic effects. The properties of the cardiovascular system have to change instantly to keep the blood flow to the brain at a sufficient level and prevent syncope.
The sudden change of posture puts the subject in danger (of oxygen shortage in the brain), and therefore in a maximum state of alertness. This should be accompanied by a maximum in sympathetic activity and a minimum in parasympathetic activity.
During a tilt-test the subject undergoes a fast transition from maximum anabolic situation to a highly catabolic situation. If any changes in the autonomic system are to be found, the chance of finding them will be highest during such a transition.

4.1.2 Spectral analysis in combination with tilt-testing

As mentioned earlier the energy in different intervals in the frequency domain, should be indicative of the parasympathetic action (HF band) and the sympathetic plus parasympathetic activity (LF band). The LF/HF ratio is therefore found to be a good indication of the sympatho-vagal balance in adults [6].
Clinical confirmation of this statement is widely available from research after syncope. Syncope constitutes an inability of the cardiovascular system to keep the blood flow to the brain sufficiently high, and is thus an indication of a failing autonomic regulation. In tilt-tests performed on syncope patients, usually no difference was found in LF/HF ratio before tilting. However after tilting the subjects, those who developed syncope, the heart rate variability showed a significantly different response [7][8][9][10][11].
In healthy subjects generally an increase in energy content of the LF band is seen upon tilting, combined with a decrease in HF energy. Meanwhile in the syncope patients the LF and HF energy stays constant or shows a slight decrease [8][9][11]. Indicating a lack of sympathetic activity in syncope patients. These effects can also be found in children suffering from syncope [12].
Based on heart rate variability, there seems to be a good correlation between deviating energies in the defined frequency bands and a defect in sympathetic activity, leading to syncope. In blood pressure variability studies [10][13] a drop-off in LF energy was found just before fainting in syncope patients.

Aono [13] studied a group of 11 hypertensive and 12 normotensive elderly subjects. He found, before and after tilting, no differences in heart rate variability and no significant response to tilting in LF and HF powers. No perinatal data of their subjects is known, but this group may be closest to the projected test population in the BIRTH study, be it not in age.
In blood pressure they found a higher LF variability in hypertensive patients in resting conditions, but the relative change in power by tilting was significantly lower than in their normotensive counterparts. In the heart rate variability no significant differences were found.

Apparently, after tilt testing syncope patients can be separated from their healthy test subjects, based solely on energy in the LF an HF energy bands of the heart rate variability. Hypertensive elderly subjects have a different response in blood pressure variability compared to normotensive counterparts. In heart rate variability this difference is not found.
The largest difference in response in LF power found in the heart rate variability of syncope patients was 17% with respect to the pre-tilt values. In the BIRTH-study, the aim is to look at patients that have not developed any symptoms yet, so the differences found must be assumed to be smaller. Aono's [13] study gives an indication that the blood pressure variability may show differences earlier than the heart rate variability does.

4.2 Finapres

4.2.1 General information about the apparatus

Theoretically blood pressure variations give more fundamental information than heart rate variability. This is supported by the study in hypertensive patients [13]. As mentioned in Chapter 3 the short-term variability of the arterial blood pressure can not be obtained from the standard oscillometric measurements. This method is however sufficient to perform most of the clinical measurements with.
For determining the blood pressure variability a measurement value is needed every heart beat. Continuously tracking the arterial blood pressure can provide the required information. About 25 years ago, such a tracking could only be achieved by inserting a catheter into an artery. This fluid filled catheter is then connected to a pressure transducer, producing a pressure dependent electrical signal.
Because of risk of infection, a catheter is only used when there is a clinical indication to do so; that is, when blood samples have to be taken often, fluid has to be brought into the blood circulation, or oscillometric measurements do not provide enough information for the medical staff.
With the introduction of the Finapres[5] in the early 1980s, measurement of a continuous arterial blood pressure wave without the need of a catheter became available. The Finapres was originally designed as an alternative to invasive monitors in IC units. Because of its cost and its limited added value in daily clinical care, its main use has always been in research. The principle and working of the Finapres will be described below.
The original developer of the Finapres, TNO, as well as commercial providers of the apparatus, do not produce or sell it anymore. A successor is currently available from TNO, called Portapres.
For the feasibility study presented here, a device has been generously on loan from the Electrical Engineering department of the Technische Universiteit Eindhoven.

4.2.2 Plethysmomanometry

The Finapres measures arterial blood pressure indirectly by plethysmomanometry. This technique was introduced in 1973 by the Czech physiologist Peñáz [14]. He used a plethysmogram and the principle of the 'unloaded vascular wall'.
A plethysmogram is measured using infrared light and a photo-cell. Placing the light source at one side of an extremity, and the photo-cell at the opposite, the amount of infrared light received by the

[5] Finapres = FInger Arterial PRESure

photo-cell is a function of the blood perfusion. This method was already used on for example the foot, hand and finger.
If we assume just one artery to be present in between the light source and the photo-cell, the plethysmogram can be described as:

$$\varphi(t) = \varphi(r(t), \alpha(t)) \qquad \textit{Equation 1}$$

(with φ = the amount of light received by photo-cell, t = time, r = radius of the artery, α = all influences other than the radius of the artery)
Factors contributing to α are for example the bone structure and muscular build-up of the extremity. Changes in these influences can be found on a longer time-scale than the fluctuations in the arterial radius [15]. They are therefore assumed to be constant over the measurement time. Thus:
$\alpha(t) = \alpha$
We then can set φ to be:

$$\varphi(t) = f(r(t)) + g(\alpha) \qquad \textit{Equation 2}$$

(with f and g arbitrary functions of their arguments)
Next we express f as an infinite power series in $r(t)$, resulting for the plethysmogram in:

$$\varphi(t) = \sum a_n r^n(t) + g(\alpha) \qquad \textit{Equation 3}$$

If more than one artery contributes to the plethysmogram, $r(t)$ might be calculated as effective radius for the combined arteries.
In Equation 3, it has also been assumed that function f does not change over the course of the measurement. This assumption implies that the properties of the artery do not change during the measurement.

In a normal state the arterial wall is stretched, because of the blood pressure inside. After applying the cuff the air pressure is raised until the arterial volume is about one third of its normal value, in that way unloading the wall [14]. For unloading the vascular wall the pressure in the cuff must be as high as the blood pressure inside the artery. In Figure 5 the situation after cuff application is schematically depicted.

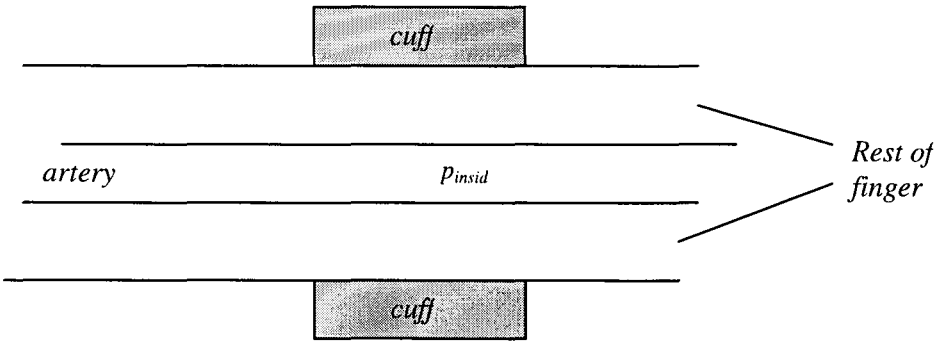

Figure 5: Schematic cross section of finger with one artery and applied finger cuff.

When recording a plethysmogram $\varphi(t)$ can be seen to fluctuate, indicating that r fluctuates and that therefore the force F on the arterial wall is fluctuating. The force F on the arterial wall can be calculated as:

$$F = (p_{inside}(t) - p_{cuff}(t)) \cdot A \qquad \textit{Equation 4}$$

(with A = the surface of the vessel wall, F = Force on the wall (positive when directed to the outside), p_{cuff} = the pressure in the cuff)

Peñáz's method for blood pressure measurement revolves around keeping the vascular wall without load at all times; therefore keeping F on the wall 0, resulting in a constant radius $r(t)$ of the vessel. The condition that F is zero can only be met if $p_{inside}(t) = p_{cuff}(t)$ at any given time t (Equation 4).

The derivative of $\varphi(t)$ can be calculated from Equation 3:

$$\frac{d\varphi(t)}{dt} = \frac{d}{dt}\left[\sum a_n \cdot r^n(t) + g(\alpha)\right] = \sum a_n \cdot r^{n-1}(t)\frac{dr}{dt} \qquad \textit{Equation 5}$$

From which can concluded that the plethysmogram should be constant if the radius of the artery does not change. Leading to the following mathematical definition of the basic principle of the Peñáz method:

$$\frac{d\varphi(t)}{dt} = 0 \Leftrightarrow \frac{dr(t)}{dt} = 0 \Leftrightarrow p_{inside}(t) = p_{cuff}(t) \qquad \textit{Equation 6}$$

In the figure below, a schematic overview is given how this principle was put to practice. The measured quantity is the air pressure in the cuff, which is equal to the pressure in the artery as long as the plethysmogram is kept constant.

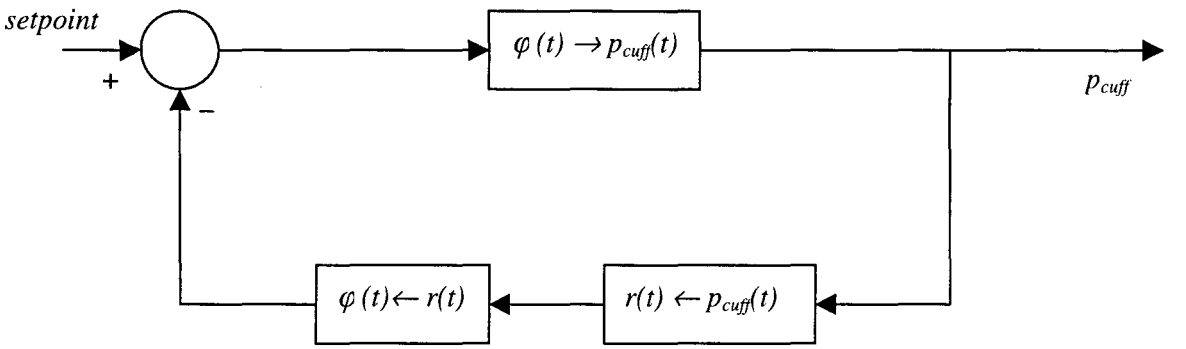

Figure 6: Schematic overview of the plethysmomanometric measurement set-up.

4.2.3 Description and specifications

After the principle was presented by Peñáz, it was implemented in the Finapres, which became available in the late 1970s. A schematic drawing of this apparatus is given in Figure 7.
The cuff is a flexible, transparent air chamber, containing the infrared light and photo-cell. It is shaped slightly conical to fit the middle phalanx of the middle finger. A two-sided velcro strip is attached to the outside of the air chamber for application of the cuff.
The front-end unit is a small 'box'. The connection tube between front-end unit and cuff is just about 10cm long, minimizing the impedance effects in the air tube. Into this system the plethysmogram readings are fed. It controls and measures the air pressure in the cuff and sends the measurement values to the main unit.
The main unit has a number of inputs and outputs. The main outputs are pressurized air and control signals for the front-end unit, and the measurement signal. In the Finapres device we had at our disposal (Finapres 5, TNO) the measurement signal was accessible through a built in plotter, a LCD display or as an analogue signal on a BNC connector.

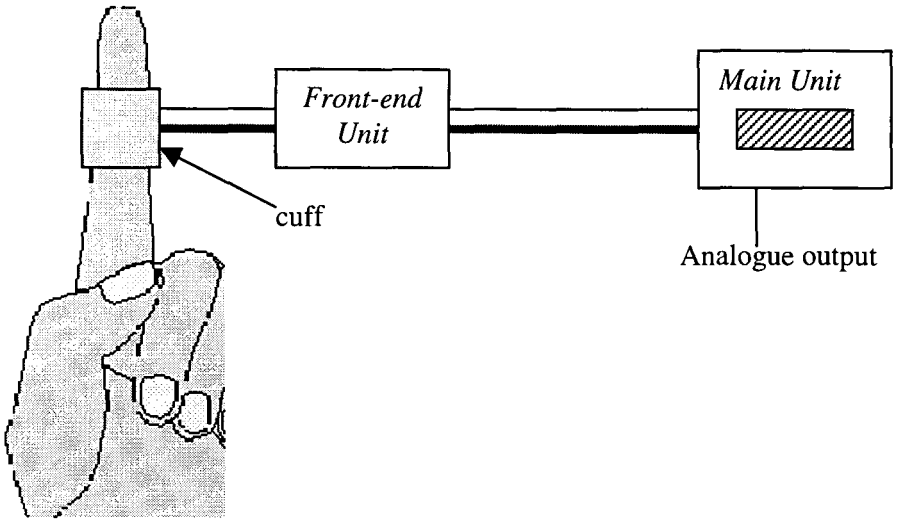

Figure 7: Schematic set-up of the Finapres. The thick dark grey connections represent air tubes, the thin black lines are electronic connections.

The analogue signal on the BNC-connector is a 200Hz-sampled wave-form at 100mmHg/V, with linear interpolation between measurement points. The resolution on this output is 5mV, corresponding with 0.5mmHg. According to the manufacturer's specifications the Finapres has an internal accuracy of 4.5mmHg. The measurement range is 10 to 300mmHg.
The LCD display simply gives mean, systolic and diastolic pressures and the heart rate during the measurement. Also errors messages are displayed here, as well as some parameters during start-up and calibration procedures.

On the input side the main unit has a number of keys for controlling the plotter, starting/stopping the measurement and calibrating the outputs. One calibration function that needs to be treated in a little more detail is the calibration called Physiocal.
In the previous paragraph the plethysmogram was discussed. Under the assumption that all other influences other than the fluctuation of the blood pressure take place on a longer time scale, the influences on the plethysmogram were taken to be constant. However, the influence of the smooth muscle in the arterial wall changes within minutes. The contraction and relaxation of these muscles can cause the unloaded arteries to expand or contract. This leads to measurement errors in the Finapres signals since it keeps the arterial size constant.
In its standard operating mode the Finapres corrects for this effect by recalibrating after a number of heart beats. During the Physiocal procedure, no output signal is available. It can be found as a staircase form in the pressure graph.
The number of heart beats between Physiocal calibrations can be as high as 70. For obtaining continuous records for, for example, Fourier analysis, the recalibration can be switched off.

4.2.4 Measurements using the Finapres

When measuring blood pressure, it always has to be kept in mind that there is no such thing as 'the blood pressure'. The arterial blood pressure measured by the oscillometric method, an intra-arterial catheter or plethysmomanometry is only a local value. In the vascular system distortion of the blood pressure wave occurs. In the arm an increasing systolic and a decreasing diastolic pressure can be seen from shoulder to hand [1].
Also hydrostatic differences can strongly influence the outcome of blood pressure measurements. These hydrostatic differences can be prevented by measuring with the probe at heart level. For the Finapres a systematic underestimation of blood pressure, compared to the value measured at the

upper arm, of (7±10)mmHg (μ±2σ) was reported in the manual. It is therefore advised to keep the cuff at 10cm below heart level during the measurements, to correct for this difference [15].
Studies comparing Finapres measurements with established methods give a more differentiated picture of the accuracy of the Finapres. Based on comparison with either oscillatory measurements [16][17][18] or intra-arterial measurements [17][19][20], different values for bias and accuracy where found. The values for four of these studies are summarized in Table 2. In general, the bias found is within an acceptable range for clinical purposes. The problem is the standard deviation of the difference, which indicates a large inaccuracy in individual measurements.

	Oscillatory			*Intra-arterial*	
Author	Musso	Tanaka	Tanaka	Jones	Epstein
Number of test persons	308	217	38	15	10
Age group	17-83y	4-16y	18-45y	'young'	Adult
Systolic pressure difference (mmHg)	-3±36	-1.9±22.2	-0.8±19.0	-1.81±27.12	+5.8±23.8
Diastolic pressure difference(mmHg)	-15±25	-5.1±18.2	-6.2±20.0	-7.81±25.78	+8.2±18.6

Table 2: Summary of 4 comparative studies [16][17][18][20]. Values are given in μ±2σ, difference of Finapres with respect to the established method. (Jones compared 2 versions of Finapres with the intra-arterial line. Because of the age of the device we used, the 'old' values are taken for comparison here. The updated version from 1992, used by Jones gives (−0.57±16.70)mmHg for systolic and (-8.77±17.50)mmHg for diastolic pressures).

In two cases this leads to the conclusion that the Finapres is not sufficiently accurate for clinical monitoring [17][20]. Musso [16] turns things around and states that the oscillatory measurements are inaccurate, assuming the Finapres is giving a correct outcome. Tanaka [18] simply states that the Finapres used on children is as accurate as it is on adults. In general, the average bias in the systolic pressure seems to be less than the bias in diastolic pressure. The uncertainty in the bias is slightly less for diastolic pressure.
In repetitive measurements the Finapres has a larger tendency to show over- or underestimation [18]. This phenomenon is ascribed to the action of the smooth muscle in the arterial wall. Also vasoconstriction or vasodilatation caused by for example cold, exercise, illness or drug use, may influence the outcome of the Finapres measurements [15].
The largest possible cause for errors is however operator related. The fitting and application of the cuff can influence the stability and outcome of Finapres measurements [15]. Correct application of the cuff is reported to be awkward, especially in non-cooperative patients [20].

Variability analysis

The biases reported above limit the possibility of using the Finapres for clinical blood pressure measurements. However, since we aim to assess the sympatho-vagal balance, systolic or diastolic values of the measurements are not of as much interest as the variations in them. A study comparing the Fourier spectrum of the blood pressure variability of the Finapres data with that of intra-arterial measurements was done by Omboni [19]. A good correlation between the spectra was found between 0.025 and 0.35Hz. Furthermore, overestimation of power in frequency bands up to 0.14Hz were found for systolic values. Diastolic and mean values produced similar estimations in the three frequency bands considered (0.025-0.07Hz, 0.07-0.14Hz and 0.14-0.35Hz).
Although some other researchers indicate that the Finapres is usable for variability analysis [18][21], in their articles no such analysis is presented.

Measurements in children and neonates

Although the Finapres was originally designed for use in adults, it has been successfully used on children (some as young as 4 years) in a number of cases [18][22] - [27]. Measuring with a finger cuff should therefore be fairly standard in the proposed test group of 10-12 year old school children. Since the smallest available cuff is still too large for the neonatal finger, the Finapres can not be used in the standard way on this test group. Drouin [21] put an adult finger cuff around the wrist of

the neonate and compared the outcomes with data obtained from an intra-arterial measurement in the umbilical artery. Reportedly, the recorded curves were 'similar' for the measurement range of 25mmHg to 50mmHg. For systolic and diastolic blood pressure values between method differences of (+1.8±6.6)mmHg (μ±2σ) and (+0.11±3.8)mmHg, respectively, were found. It should be mentioned that selected 5s intervals were used for analysis, and no long-term continuous measurements were presented. No reports of frequency domain analysis of Finapres measurements on neonates are available.

First hands-on impressions

After connecting the Finapres to the MIDAS data acquisition station (cf. Appendix A) an extensive series of measurements were run to 'get a feel' for the working of and the external influences on the Finapres. Measurements were performed on members of the clinical physics department, medical staff and neonates admitted to the NICU. A short list of impressions about the use of the Finapres:

- The general shape of the Finapres signal looks similar to blood pressure waves. In neonates its shape is more or less comparable to invasively measured blood pressure waves. The Finapres signal tends to be more 'noisy' and unstable than the invasive signal. In adults a strong tendency for double systolic peaks is found.
- Application of the cuff is as reported awkward. In cooperative adult test subjects the stability and shape of the obtained signal seem to depend on the application of the cuff. Instructions in the manual are interpreted differently by individual operators. In neonates the application of the cuff is in itself is a tedious task. Often leading to a need of re-application, in order to receive a signal from the Finapres.
- Movement artefacts are easily caused and occur often in neonates. Since we did not fixate the neonates in any way, continuous artefact free measurement series longer than 2 minutes are rare.
- The adult test subjects voiced some discomfort caused by the pulsation of the cuff. Also a initial response of arm movement could often be seen in neonates on switching the machine on

4.3 Discussion

Based on the reported measurements in syncope patients, no difference in either the heart rate or blood pressure variability should be expected between the proposed test groups in resting conditions, since the BIRTH-study aims to research differences in autonomic regulation in subjects that have not developed any symptoms yet.
Tilt-testing seems to be a good method to provoke a transition from a anabolic to a catabolic state of the subject and should therefore certainly be used.
Also, blood pressure variability seems to indicate differences in autonomic nervous function in hypertensive elderly patients, where heart rate variability does not. Therefore, the possibility of finding a difference in sympatho-vagal balance should be higher when assessing the blood pressure variability, rather than only the heart rate variability.

The required non-invasive measurement of the blood pressure variability is more complex than the measurement of the heart rate variability. The Finapres does provide continuous blood pressure measurement by plethysmomanometry, and obtaining these measurements from school children should not be too troublesome.
As the sympatho-vagal balance is associated with the energy contends of defined frequency bands of the variability signal, analysis in the frequency domain seems the best method. Reportedly Finapres signals represent the intra-arterial measurements adequately enough to perform frequency analysis of the blood pressure variability on them.
On the other hand, blood pressure measurements obtained with the Finapres should be treated with extreme care. No importance can be assigned to the absolute outcomes as the inaccuracy with respect to established methods of blood pressure measurements is too high.

Furthermore, cuff application is awkward and error prone. Wrong application of the cuff may influence the outcome of the measurement, and the effect of wrong cuff application on the variability of the blood pressure signal is not known.

In neonates the Finapres can be used, with the same considerations as in school children, by putting the cuff on a wrist instead of a finger. However, a study comparing variability analysis performed on such a Finapres signal with the same analysis on intra-arterial measurements has yet to be published. In Chapter 7 such a comparison will be made for patients in resting conditions.

During the course of this research, no tilt-testing was performed, and therefore no experimental data linking Finapres and tilt-testing obtained. From the first impressions with the Finapres problems might be caused by movement. There will probably be a need to strap the measurement wrist or finger into a fixed position. This may cause anxiety and a disturbance of the anabolic state the patient is supposed to be in before tilting. Testing in this area will be needed, if the use of the Finapres is decided on.

Chapter 5 Skin blood perfusion

As the arterioles contract under influence of the sympathetic nervous system, measuring the blood perfusion of the skin could give access to information about the regulation of the width of the arterioles. In this chapter insight is given into the proposed provocation method and the known regulation of skin blood flow in neonates (5.1). A method and device to measure the skin blood flow non-invasively is discussed in 5.2.

5.1 Cold provocation

Several provocation methods can be used to influence the skin perfusion. In the BIRTH-study cold provocation is proposed to be used. In 5.1.1 the description of the procedure and the expected effects are given. The known phenomena seen in the skin perfusion of neonates are shortly discussed in 5.1.2.

5.1.1 Expected physiological effect

Cold provocation, like tilt-testing, requires the patient to be comfortable, preferably having minimum sympathetic activity before the provocation. As arterioles constrict under sympathetic activity, in an anabolic state maximum vasodilatation is expected to occur. Applying a cold pad, or ice to the skin of e.g. the forearm or lower leg provokes a sympathetic reaction.
In order to protect the vital organs from cooling down, as cold blood flows from the provocation site to through the body, the blood vessels providing nutrition and oxygen to the skin are constricted. Thus the flow is reduced.
Prolonged cooling, or a progressively lower temperature may cause a sudden increase in blood flow through the provocation area; a reflex to prevent loosing body parts. However this may cause damage to the vital organs as the blood temperature drops.
A defect of the sympathetic nervous system may cause a changed process of contraction in the arterioles.

5.1.2 Skin perfusion differences in neonates

Skin perfusion measurements are often used in research to give an indication of the vasomotor tone. The vasomotor tone concerns the regulatory systems determining the contraction or dilatation of the blood vessels.
Experimental data show the skin perfusion in the finger to be independent of the local systolic blood pressure even after provocation [28]. Two of the methods that can be used to provoke a reaction in skin blood flow are pin pricking and cold provocation. When using local cold provocation the skin blood flow decreases, while pin pricking has an opposite effect in healthy untreated subjects [29]. Cold provocation should be easier to perform in a controlled manner, and is preferred in the BIRTH-study.
Measurements performed on infants have shown that both full-term and pre-term infants are able to control their skin blood perfusion during provocations. Also a development in the control of the arterioles can be found over the first 3 weeks of life [30]. Pre-term infants seem to have an attenuated response to cold provocation compared to full-term infants. In pre-term infants the response developed with post-natal age [31], indicating an immature vasomotor control at birth.
A development in the oscillations of skin blood perfusion is also found in full-term infants. However, this group reaches oscillation patterns similar to adults at the end of their first week of life, whereas pre-term infants have not reached the stage of development of a full term baby at birth after their first week [32].

In the regulation of the skin blood flow a distinction can be made between full-term and pre-term neonates. The question is whether this regulation is related to the birth weight as well and whether the pre-term infants ever make up for the difference at birth. From the literature mentioned above it

seems that both skin blood perfusion and the fluctuations in the perfusion may give access to the regulation of the skin blood flow, and should be considered.
In the BIRTH-study the aim of the cold provocation tests is to provide insight into the regulation of arteriole width, subject to the sympathetic system. Cold provocation should provide the necessary transition from anabolic to catabolic state.

5.2 Periflux

5.2.1 General description of the apparatus

As described in 5.1.2, the regulation of the skin blood flow may be influenced by provocations, proving that it is under the influence of the autonomic nervous system. Measurement of the skin blood flow can be performed by commercially available laser Doppler flowmeters, like the Periflux. A description of the working principle of laser Doppler flow meters can be found in 5.2.2.
The use of laser Doppler flowmeters is not daily routine. It is used for research purposes and in specific situations, for example for measuring the perfusion after a skin transplant. Only during the last couple of months of this study a Periflux device (Periflux PF2) was available at the Saint Joseph hospital. The apparatus was provided by the laboratory for vascular research at the St. Radboud Hospital in Nijmegen for the duration of this study.

5.2.2 Laser Doppler Flowmetry

The Periflux's working principle is called Laser Doppler Velocimetry (LDV). The Doppler effect underlying this principle can be formulated as:

$$\frac{\Delta\lambda}{\lambda} = \frac{v}{c} \qquad \textit{Equation 7}$$

(with $\Delta\lambda$ = the change in wave length upon reflection, λ = the wavelength of the incident light, v = velocity of the reflecting object, c = the velocity of light)
When using a LDV on skin tissue, a laser beam is aimed at the skin. The light will enter into the skin and undergo multiple scatterings, thus resulting in an almost isotropically illuminated measurement volume [33].
Cells traversing the measurement volume will reflect the light incident on them, causing it to undergo a Doppler shift. The light in the measurement volume will then contain a mix of unshifted wavelength λ_{laser}, and shifted wavelengths $\lambda_{laser}+\delta\lambda$.
Magnitude of the shifted signal is dependent on the number of moving cells in the measurement volume. The frequency depends on their velocity. However, because of the frequent reflection and scattering in the measurement volume, the direction of movements of the cells is not of direct interest to the mixture.
Considering multiple reflections on the moving cells with constant velocity v, the Doppler shift is:

$$\delta\lambda = \sum a_n \cdot \left(\frac{v}{c}\right)^n \cdot \lambda_{laser} \qquad \textit{with integer } n \geq 1 \qquad \textit{Equation 8}$$

(with $\delta\lambda$ = the Doppler shift in wavelength, v = velocity of the moving cells, c = velocity of light, λ_{laser} = wavelength of the incident laser light)
The coefficients a_n depend on the number and nature of the reflections.
The light in the measurement volume is then made up of wavelengths λ_α:

$$\lambda_\alpha = \lambda_{laser} + \delta\lambda_\alpha \qquad \textit{Equation 9}$$

As the velocity of light is much higher than the cell velocity in any tissue it is save to assume $v/c << 1$. From elementary scattering the maximum absolute value of a_n can be shown to be of the order n, therefore $|\delta\lambda_\alpha| << \lambda_{laser}$.

The relationship between wavelength and frequency is known to be:

$$c = f \cdot \lambda$$

(with c = the velocity of light, f = the frequency of the light, λ = the wavelength of the light)
Therefore as λ undergoes a shift $\delta\lambda$, also the frequency f undergoes a shift δf. Translating Equation 9 into frequencies gives:

$$f_\alpha = f_{laser} + \delta f_\alpha \qquad \textit{Equation 10}$$

(with f_α =frequency of the light, f_{laser} = the original frequency of the laser light, δf_α = the change in frequency due to Doppler shift(s))
Combining different frequency modes f_α gives rise to frequencies:

$$f_- = \left| f_\gamma - f_\kappa \right|$$
$$f_+ = f_\gamma + f_\kappa \qquad \textit{Equation 11}$$

(with f_γ and f_κ resulting light frequencies from scattering and Doppler shifts, defined like f_α in Equation 10)
From the measurement volume a light sample is taken by a photo-detector, and transferred to an electrical signal. As the band-width of the electrical circuit is not adequate for processing the high frequency components with f_{laser}, f_+, or f_α (each ~10^{15}Hz), these are filtered out automatically. The remaining components have wavelength f_-:

$$f_- = \left| (f_{laser} + \delta f_\gamma) - (f_{laser} + \delta f_\kappa) \right| = \left| \delta f_\gamma - \delta f_\kappa \right| \qquad \textit{Equation 12}$$

The total signal after the effective filtering is made up of f_- components calculated for all wavelength components f_α. Calling the resulting signal *A(f):*

$$A(f) = A\left(\left| \delta f_\gamma - \delta f_\kappa \right| \right) \qquad \textit{Equation 13}$$

For use on skin tissue, this signal appears to have a frequency range of 0 to 15kHz [33]. The signal A(f) is a stochastic function of the number of blood cells in the measurement volume and their velocity, and thus a measure of the perfusion of the measurement volume.
From this function a well-defined flow related parameter can be calculated:

$$F = K \int_{f_{low}}^{f_{high}} f \cdot A(f)\, df \qquad \textit{Equation 14}$$

(with f_{low} = the low cut-off frequency, f_{high} = the high cut-off frequency, f = the frequency, $A(f)$ = the stochastic function of the Doppler shifts, and K = a constant of proportionality)

5.2.3 Description and specifications

A schematic view of the Periflux device is given in Figure 8.
The probe consists of 1 optical output for the laser light and 2 optical inputs for the measurement signal. It is therefore coupled to the main unit with an optic fibre cable containing three optic fibres. A probe holder is generally attached to the skin using a two-sided adhesive ring. For local heating of the skin a special heated probe holder can be attached to the main unit separately.

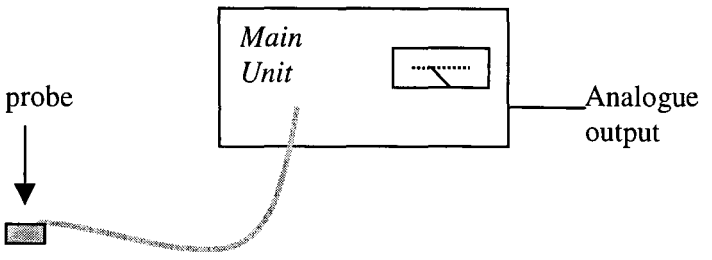

Figure 8: A schematic view of the Periflux, The thick grey connection represents the optic fibre cable, the black line an electric connection.

The most important outputs of the main unit are the laser light from a 2mW He-Ne laser, coupled into the optic fibre, a power out for the heated probe holder and the signal outputs: an analogue signal on a BNC connector, an analogue display and an audio signal. The output signal is also available on a J300 connector. No recording options are available on the main unit. In our tests we used the analogue BNC output.

The laser output connected to the skin surface illuminates a hemisphere of between 1mm and 1.5mm radius. All cells moving through this hemisphere contribute to the Doppler signal at the output side. By occluding the flow to a particular system, it can be shown that in a 'stationary' situation the measured flow is not entirely zero. The contributions still present during such an occlusion are caused by the vibrations of the skin cells and random movement of stationary plasma cells. In a normal situation these contributions a negligible [33].
The algorithm by which the Periflux calculates the flow percentage is based on the relationship given in Equation 14. The lower frequency limit is 20Hz, the upper limit can be chosen to be either 4kHz or 12kHz. A change between these upper limits of course influences the outcome of the measurement, as it limits the maximum cell velocity to be taken into account.
Movement of the probe with respect to the measurement volume or movement of the optic fibre cable can cause artefacts in the signal. A movement artefact filter can be used to filter the artefacts out. The presence of a detected movement artefact is indicated by an LED on the main unit, regardless whether the artefact filter is on or off. When the artefact filter is on, the signal is locked at a constant value when, thus providing no new information during this period.

Measurements using the Periflux

The output signal of the Periflux does not have any physical significance. It is given in percentages of full range deflection rather than e.g. average velocity of the cells or the volume of plasma flowing through the measurement volume per second. With the gain selector switch the range of the device can be adapted to the flow range to be measured. When comparing the flow range these values should of course be taken into account.
The problem to assign physical significance can be led back to fact that the measurement volume is both depending on the dimensions of the laser output and the signal input fibres [34] and the local properties of the skin. Therefore an exact volume of the illuminated skin is unknown.
The fact that the signal depends on both application, specifications of the individual probe and the patient, leads to the conclusion that only measurements using the patient as its own base-line can be used for the perfusion values.
When using laser Doppler flowmetry during provocations, the movement artefacts should be expected to occur frequently [35], making correct measurements difficult to obtain. As the movement artefact filter actually disturbs the data-acquisition the artefacts should be prevented by fixating both the probe and the optic fibres. This implies fixating the patient as well, which may interfere with the desired anabolic state.

First hands-on impressions

As we had the Periflux available for only a limited amount of time and a damaged optic fibre cable delayed the use of it, experiences with the device are limited. After setting up the data-acquisition,

some initial measurements were performed on a healthy adult test person. A short list of impressions:

- Operation of the apparatus and set-up are fairly simple. The need for the laser to warm-up for about 60 minutes could be a problem in using the device as part of a mobile data-acquisition set-up.
- Care has to be taken in handling the probe. The probe holder on the main unit (used when the device is switched on, but no measurement is performed) does not hold the probe adequately, resulting in the probe head falling out frequently. The risk of damaging the probe or optic fibres is present. Especially in working with conscious children, the laser beam may cause additional risks.
- Detected movement artefacts are easily caused.
- The signal obtained proved to depend strongly on the pressure applied to it. A probe-holder should be used to get a standard location of the probe relative to the skin surface.

The measurements were performed in two situations: at rest after acclimatisation, and during cold provocation with melting ice.
The baseline measurement was performed with the test person sitting up-right, after having acclimatised for about an hour at room temperature. No probe heating was used. The probe was placed in a probe-holder applied to the inside of the left forearm, which was held at a constant level. The signal obtained should fluctuations, as expected. The measurement was run for about 5 minutes. The average flow found was 47% with a standard deviation of 7%, at gain 10.
After this measurement a pack of melting ice-cubes was pressed to the forearm distal to the probe. All other conditions were kept the same. The measurement was run for about 3 minutes. The average flow found was 34%, the standard deviation was 10% (gain was 10).
After removing the ice the subject blood flow was not back at the base-line level after 5 minutes. Registrations during re-application of the ice show a drop-off in average flow, although no clear slope can be identified in the signal.

5.3 Discussion

In investigation of the Barker hypothesis, base-line differences in skin perfusion between the AGA and SGA test group may exist. However, non-invasive skin blood perfusion measurements do not provide an objective physical measure for the perfusion. Therefore other means of investigating the regulation should be sought after.
Some parameters that may show a difference in autonomic regulation are relative change upon cold provocation, time domain changes upon cold provocation or frequency domain analysis with or without cold provocation.
The first of these proposed techniques has already been used in finding distinctions between neonates in several stages of development [30][31].
Additionally, the time scale on which the perfusion changes upon provocation may be indicative for the response of the autonomic nervous system. To get a clear picture of the development of the perfusion in time the high frequency oscillations, associated with the pumping of the heart, should be filtered out before analysis. The slope in the perfusion graph, or the time difference between base-line and minimum flow during provocation could be good parameters.
In the frequency domain the frequencies of the oscillations in skin blood perfusion may provide an indication. More likely however, a change of the energy contend in the defined low frequency band may be witnessed upon provocation as a result of the increased sympathetic activity.

From the first impressions the Periflux seems to provide a stable signal, when movement of the probe an optic fibres are prevented. Preventing these artefacts requires fixating both optic fibres and the subject, which may be undesirable when getting him/her to feel comfortable.

Cold provocation may be assumed to be a good provocation method for the skin blood perfusion. There is however no conclusion yet about the method of data analysis. Base-line perfusion values

are probably not suited, because they depend on the individual patient. In the frequency domain, the oscillation frequencies in the skin perfusion may provide an objective measure. However, analysis of the changes in the skin blood perfusion during provocation, will probably provide a better opportunity to find any existing differences between the testgroups. Both time and frequency domain methods should be used.

Chapter 6 Analysis of blood pressure waves

In Chapter 7 a comparison of Finapres measurements in neonates with intra-arterial blood pressure measurements is presented. This comparison will be performed in both the time and the frequency domain. During the course of the PINO-project a lot of effort has been put into the analysis of blood pressure and heart rate variability in the frequency domain. In this chapter an overview is given of the consecutive steps in the frequency analysis of the variability of the blood pressure waves. This method is largely based on the existing method.

6.1 Preparation of the signal

As mentioned in 2.5, in the blood pressure signal three types of fluctuations can be recognized. Since the fluctuations associated with the pumping of the heart have little to do with the neural regulation of the blood pressure, they have to be filtered out. Research done in the past [36] showed that systolic and diastolic blood pressure values can be viewed as independent signals. It is therefore a logical step to take the consecutive systolic or diastolic values as the signal to be analysed. In doing so, the continuous nature of the measurement signal is discarded, since there is only one measurement value in every heart beat. The signal analysed is therefore the beat-to-beat systolic or diastolic blood pressure variation.
The detection of the systolic/diastolic blood pressure values creates a set of not equidistantly sampled data: a value is measured every heart beat, and the duration of a heart beat is subject to variability of its own. In order to reliably perform frequency analysis later on, times of occurrence of the diastolic or systolic pressures should be linked to their values. Therefore the detected extreme values are passed through a 'sample and hold' system, keeping the last detected value until the next

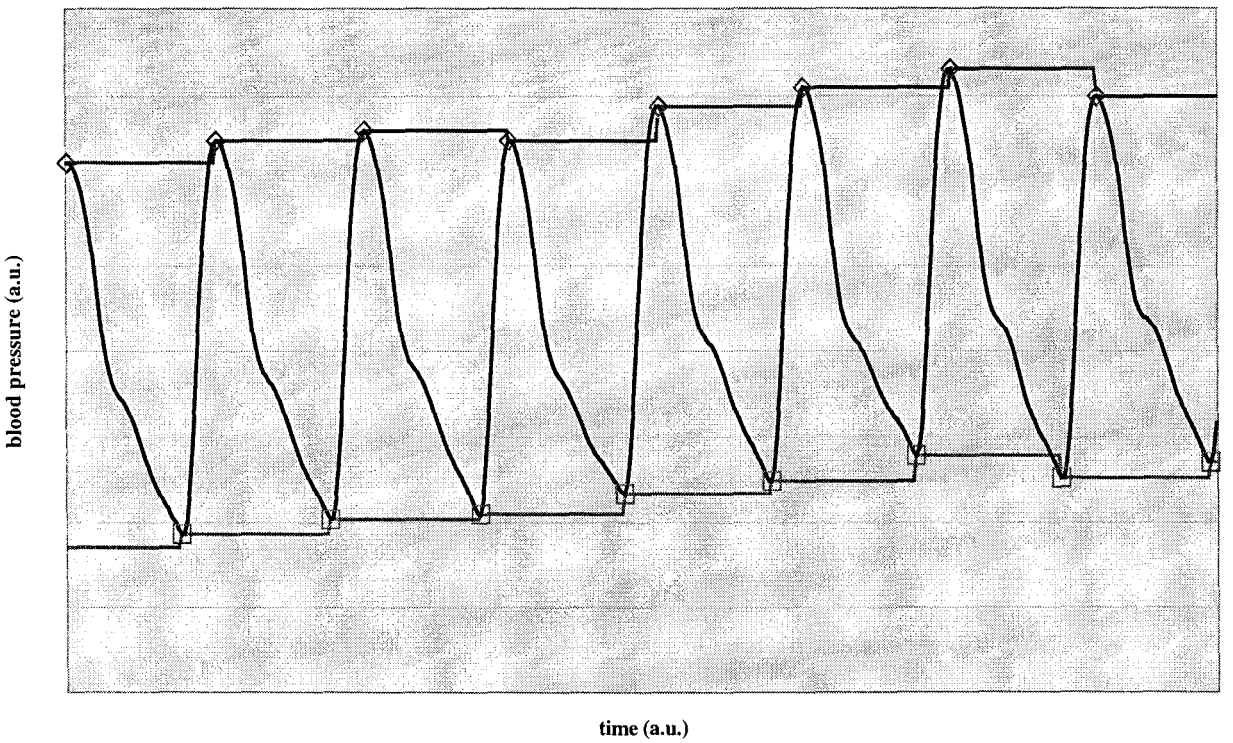

Figure 9: Graphic of the creation of a systogram/diastogram from a continuous blood pressure wave. The fluctuating black line is the blood pressure, the squares indicate the systolic/diastolic values, the top red continuous line (stepped) is the systogram, the bottom blue continues line (stepped) is the diastogram.

one is measured. In this way the systogram or diastogram is created (cf. Figure 9). In this systogram or diastogram the first order blood pressure fluctuations have been filtered out and the time information has been saved to allow accurate analysis.

6.2 Frequency domain analysis

The variability signal to be analysed is described in 6.1. The sample and hold signal created from the blood pressure recordings can in principle be transferred to the frequency domain for further analysis. The most common way to do this is by Fourier transformation [37]. In order to limit the required calculation capacity the number of data points should be kept to a minimum and the algorithms as efficient as possible.

6.2.1 Fast Fourier Transform

To reduce the calculation capacity needed to perform the analysis, the Fast Fourier Transform (FFT) is preferred over the more general Digital Fourier Transform (DFT). For the FFT-algorithm the number of data points must be an integer power of 2. This condition can be tampered with by zero padding or interpolation, but just as easily be taken into account by chosing signal lengths and sample frequencies appropriately. If both record length (in s) and sample frequency (in Hz) are powers of 2 the resulting number of data points is such a power as well.
The algorithm used calculates a one-sided power spectrum based on the FFT algorithm [36]. Because the signal is real, the two-sided power spectrum, or the amplitude spectrum for that matter, would be symmetric, and no information is lost in calculating the one-sided power spectrum. Besides, eventually the aim is to compare different patients or measurement methods, which means that any consequent choice of a correct method of frequency analysis would suffice. For comparison with results at other research sites, a standard method is preferred, though.

6.2.2 Resampling

In an attempt to determine the locations of the systolic or diastolic blood pressures in the signal accurately, the blood pressure waves are sampled at 128Hz in the data acquisition. Since the maximum heart rate expected to be measured is about 3.3Hz (200/min), and there is only one measurement value every heart beat, the sample and hold signal is oversampled. To reduce the number of measurement points resampling is performed on this signal.
In chosing the resampling frequency, the Nyquist sampling theorem has to be taken into consideration. The Nyquist (critical) frequency is calculated as:

$$f_N = \tfrac{1}{2} f_S \qquad \textit{Equation 15}$$

(with f_N = Nyquist critical frequency, f_S = sample frequency)
The sampling theorem states that a signal band-width limited to the frequencies smaller than the Nyquist frequency, is completely determined by its samples [38]. This is means that a continuous signal with a limited frequency band-width can be digitally sampled without losing any information. On the other hand, when the signal is not band-width limited, frequencies higher than the Nyquist frequency will be displayed incorrectly in the spectrum. This phenomenon is called aliasing. It can only be prevented, not corrected for after sampling.
After peak/minimum detection the signal has a variable natural sampling frequency equal to the heart frequency. This implies that there is also a variable natural 'Nyquist frequency' of half the heart frequency. As the maximum heart frequencies to be considered are about 3.3Hz, at best frequencies up to about 1.6Hz are correctly recorded. There is, therefore, no reason to choose a higher resampling frequency than 4Hz.
The resampling is executed by applying a boxcar interpolation on the signal. The boxcar is a square wave of 0.5s width and amplitude 1. The interpolation at every resampled point is calculated by the convolution of the sample and hold signal and the boxcar centred on the resample location. This method has the additional advantage of serving as a low pass filter with a cut-off frequency of 2Hz [36].

The resampling by this boxcar method constitutes a convolution operation in the time domain. After calculation of the power spectrum, the effect of this convolution on the outcome can be corrected for by dividing through the power spectrum of the boxcar [37].

Cardiac aliasing

Special care has to be taken with respect to the natural sampling frequency f_H (heart frequency) of the blood pressure signal. This means that, by the Nyquist theorem, only frequencies between $-½f_H$ and $+½f_H$ can be correctly displayed. By resampling at a higher frequency no information can be added to the signal.
In adults, information with a frequency outside the $[-½f_H,+½f_H]$ is only present in exceptional situations. However, in neonates respiratory rates higher than half the heart rate are common, as the heart rates range generally between 120 and 160/min and the respiratory rates from 40 to 70/min. Therefore aliasing of the Traube-Hering waves may occur, seriously influencing the energy content of the defined frequency bands. The effect of aliasing under influence of the heart frequency is called cardiac aliasing.

6.2.3 Record length

Through the sampling theorem, the minimum sampling frequency is determined by the maximum frequency present in the signal. The length of the signal to be analysed determines the frequency resolution of the spectrum. Using the FFT, the number of data points in the time domain is equal to the number of data points in the frequency domain:

$$N = f_S \cdot T \qquad \textit{Equation 16}$$

(with N = number of data points, f_S = sampling frequency (Hz), T = length of the signal (s))
There are N frequency intervals or bins defined in the spectrum, ranging from $-½f_S$ to $+½f_S$, which means each bin has width:

$$\frac{\frac{1}{2}f_S - (-\frac{1}{2}f_S)}{N} = \frac{f_S}{f_S \cdot T} = \frac{1}{T} \qquad \textit{Equation 17}$$

Because a Fourier transform can theoretically only be calculated from an infinitely long signal, the finite record is repeated in time. After repetition the reciprocal value of the finite signal length is displayed in the first non-zero frequency bin. All lower frequency- and DC components are displayed in the zero frequency bin.
As is described in Chapter 3, the lowest frequency band to be analysed starts at 0.04Hz. With the first non-zero frequency bin located from 1/(2T) up to 3/(2T) the record length should at least be 37.5s, to prevent overestimating frequency components in the LF band caused by the artificially introduced repetition in the signal. For this reason the record length is chosen to be at least 64s, the lowest power of 2 above 37.5.

6.2.4 Spectral leakage

Taking a limited section of a signal is effectively the same as multiplying an infinitely long signal with a square wave in the time domain. This may cause spectral leakage of power into the neighbouring frequency bins of the spectrum [39].
The multiplication with a square wave in the time domain causes infinitely long power spectrum to be convoluted with the FFT of the square wave. This does not cause any distortion, if the signal is periodic with the length of the square wave being an integer number of periods. In any other case however, discontinuities in the signal, as the finite record is repeated in time for purpose of the Fourier transform, cause spectral leakage to occur [36].
Two obvious measures can be taken to reduce the effects of spectral leakage on the spectrum. The first measure is removing any DC components from the variability signal, thus reducing the power at zero frequency and therefore also reducing spectral leakage.

The other one is multiplying the record with a tapered window in the time domain. Tapered windows can be designed in many forms. The choice of the specific form is of little importance compared to the condition that window is zero at the edges [40]. Because of the shape of the FFT of a tapered window, it causes spectral leakage to occur with every signal, even when it would not have occurred if a square wave had been used. However, the effects of the spectral leakage on the shape of the spectrum are a lot smaller if the signal does not answer the requirements for periodicity as described above [36]. Since in general a physiological signal does not have the required periodicity, a Parzen window is used to counter the effects of spectral leakage.
After calculation of the power spectrum, it should be corrected for the multiplication with the Parzen window by dividing it through the squared average value of the window [37].

6.2.5 *Accuracy of the FFT algorithm*

From the operations above, the resulting signal is a 64s, 4Hz resampled record of the systogram/diastogram, which is multiplied by a Parzen window. To assess the autonomic function in the frequency domain the one-sided power spectrum is calculated [36]. From this spectrum the energy content of the LF and HF frequency bands can be calculated for interpretation.
However, for the physiological signals at hand the standard deviation in the estimation of the power is equal to its value. To improve on this accuracy, a stationary signal longer than the needed 64s is obtained. After resampling the signal is split into n_s independent 64s records. Calculating the power spectrum of each section and averaging over the n_s records causes a reduction in the normalised random error ε by a factor $1/(\sqrt{n_s})$ [36]. (The normalised random error is defined as

$$\varepsilon = \frac{\sigma(P(f))}{P(f)}$$

(with $P(f)$ = the one sided power value, and $\sigma(P(f))$ = its standard deviation; If $\sigma(P(f)) = P(f)$, $\varepsilon = 1$)
Optimal reduction of the error is achieved if half-overlapping intervals are used. Since the intervals are not statistically independent in that case, and because a Parzen window is used, ε is calculated to be [36]:

$$\varepsilon = \sqrt{\frac{9n_s - 1}{8n_s^2}} \qquad \textit{Equation 18}$$

Having established that a 64s record length is the minimum that can be used in 6.2.3, the aim is to obtain a stable signal which is as long as possible. In the PINO-project 192s (5 half overlapping intervals) have been used, reducing ε to 0.47.

6.2.6 *Summary and interpretation*

In the table below the consecutive operations performed on the systogram/diastogram are given, along with the reason to preform them and the paragraph in which it is discussed.
After having calculated the power spectrum of the signal as described above, parameters can be calculated from this spectrum to judge the working of the autonomic nervous system. In the previous chapters, this is already described.
Linking the HF band to parasympathetic activity, while linking the LF band to sympathetic and parasympathetic activity, a measure of the autonomic activity can be defined [6]. From observing the development in the spectrum under different circumstances enables researches to follow the reaction of the autonomic nervous system under these circumstances.

When measuring in neonates cardiac aliasing may occur quite frequently. In fact, in order to correctly display spectrum at the upper limit of the defined HF band (1.5Hz), the heart rate should be 180/min. This is not within the common range of heart rates for neonates.

Action	*Reason*	*Par.*
Resampling	Low pass filtering and data reduction	6.2.2
Splitting up in 64s half overlapping records	64s: resolution in the frequency domain Half overlapping intervals: increase of accuracy	6.2.3 6.2.5
DC removal of the records	Avoiding spectral leakage	6.2.4
Multiplication with 64s Parzen window	Avoiding spectral leakage	6.2.4
FFT power spectrum	Tranfer to frequency domain	6.2.1
Averaging FFTs from records	Increase of accuracy	6.2.5
Dividing through squared average value of the Parzen window	Correction of effect Parzen window on energy content	6.2.4
Dividing through power spectrum of the boxcar	Correction for convolution in time domain	6.2.2

Table 3: Operations performed in calculating the Fourier transform.

When the heart rate is lower, the effect of the cardiac aliasing can be assumed to be small only when the respiratory rate is lower than half the heart rate, at all times. When this is not the case, aliasing of the Traube-Hering waves will occur and the precise effect on the spectrum and the energy contends of the LF and HF band will depend on the exact values of both the heart rate and the respiratory rate.
In the comparison made in the next chapter, no attention will be paid to this, as the effects of the aliasing should be the same in both measurement methods. In Chapter 8 it will be briefly discussed.

Chapter 7 Comparison of Finapres with intra-arterial measurements

In this chapter a comparison will be described between measurements of the blood pressure variability performed with Finapres with measurements by means of an intra-arterial catheter. Reason to do so is twofold: it gave the opportunity to work out the particulars of the Finapres in a systematical way, and such a comparison had to be made before being able to use the Finapres for spectral analysis in neonatal blood pressure signal, as mentioned in Chapter 4.

7.1 Introduction

In this chapter a comparison of Finapres measurements with intra-arterial blood pressure measurements will be given. As mentioned in Chapter 3 it would facilitate inclusion of patients in the BIRTH-study, if the blood pressure variability can be measured non-invasively. The choice to work with neonates was made partly because of the availability of the patients in the NICU, whereas school children with an intra-arterial catheter are usually not nursed in Veldhoven.
The aim is judging whether the frequency analysis as described in Chapter 6, gives the same results if performed on simultaneous Finapres and invasive measurements. A number of studies have been done in adults (cf. Chapter 4), but no study using Finapres signals for frequency analysis with neonates was found.
Drouin [21] did use the Finapres on neonates and concluded the measurements to be in agreement with those performed invasively. However this test did not include comparison of the blood pressure variability in the time or frequency domain. We will first attempt to repeat the result of the study performed by Drouin and then take into account the variability as well. Analysis of the blood pressure variability will be compared in both time and frequency domain.
In comparing the Finapres outcomes with those of the intra-arterial measurements, it should always be kept in mind that differences between the two result from both differences due to the measurement systems and differences due to a difference in physiology between the measurement locations.

7.2 Research population and methods of measurement

The 6 neonates included in this comparison are recruited from the patients on the NICU. They were required to have an intra-arterial blood pressure measurement, put in place on medical indication. No other criteria for inclusion were used.
In cooperation with a neonatologist, both the ECG and blood pressure measurements were disconnected from the monitoring system and connected to the monitor on MIDAS (cf. Appendix A). Also the Finapres was connected to MIDAS.
The available Finapres finger cuffs are too large to fit the neonatal finger. Drouin [21] applied an adult size cuff to the forearm of the neonate. In our measurements we used this method as much as possible, if both wrists were in use for catheters, or we were not able to obtain a signal from the forearm, other parts of extremities were used. We were also able to obtain data from the upper arm and the lower leg. In all cases we used the same cuff, size small. The neonates were all lying belly down in an incubator, their arms and legs approximately at heart level. No correction for hydrostatic effects was performed either during measurements or data processing.
Of the patients included in this comparison 4 had the intra-arterial catheter applied to the umbilical artery (cf. Table 4) and the Finapres to the left wrist. Of the remaining two patients, one had the Finapres connected to the left lower leg and the invasive catheter to the right wrist. The connections were exactly reversed in the last included test patient. As distortion of the blood pressure wave-form is known to occur in the extremities because of changing properties of the arteries, the data from the last two patients should be viewed separately from the other four.

After application of the cuff, the Finapres was switched on and allowed to calibrate until the intervals between calibrations (Physiocal) reached 60 heart beats. Then calibration was switched off and data-acquisition started.
Simultaneously ECG, the intra-arterial blood pressure and blood pressure from the Finapres were sampled at 128Hz and stored on the MIDAS PC. Measurements ran for 5 to 10 minutes at a time[6].
After the first measurement, the Finapres was switched off and the cuff released for a short period to allow full perfusion of the hand and forearm again. Then the after re-applying the cuff, another measurement series was obtained in most cases. The measured blood pressures ranged from 30mmHg to 65mmHg, heart rates were roughly between 120/min and 160/min
It appeared rather hard to get a stable signal from the Finapres for periods longer than about 2 to 3 minutes because of movement artefacts.
For six included patients we were able to obtain sections of 128s of stable signal, which was manually chosen from the measurement series. The start of these sections was chosen to be as shortly after the calibration as possible.

Systolic and diastolic value detection

After chosing the 128s sections of signal, both the Finapres signal and the invasively measured pressure were passed through a detection procedure for systolic peaks and diastolic minima. This procedure is an adapted version of the detection procedure developed earlier in the PINO-project. A full description of the procedure and the adaptations made can be found in Appendix B.
After detection had taken place, the outcome was checked manually. If any errors in the detection did occur, they could be corrected; either by interpolating a missing peak/minimum, or deleting a falsely detected value. The result was a 2D array of locations and systolic/diastolic values for both the Finapres and the intra-arterial catheter.
For the neonates included in this comparison, the systolic blood pressure values range 40 to 60mmHg. The diastolic values vary from 20 to 40mmHg.

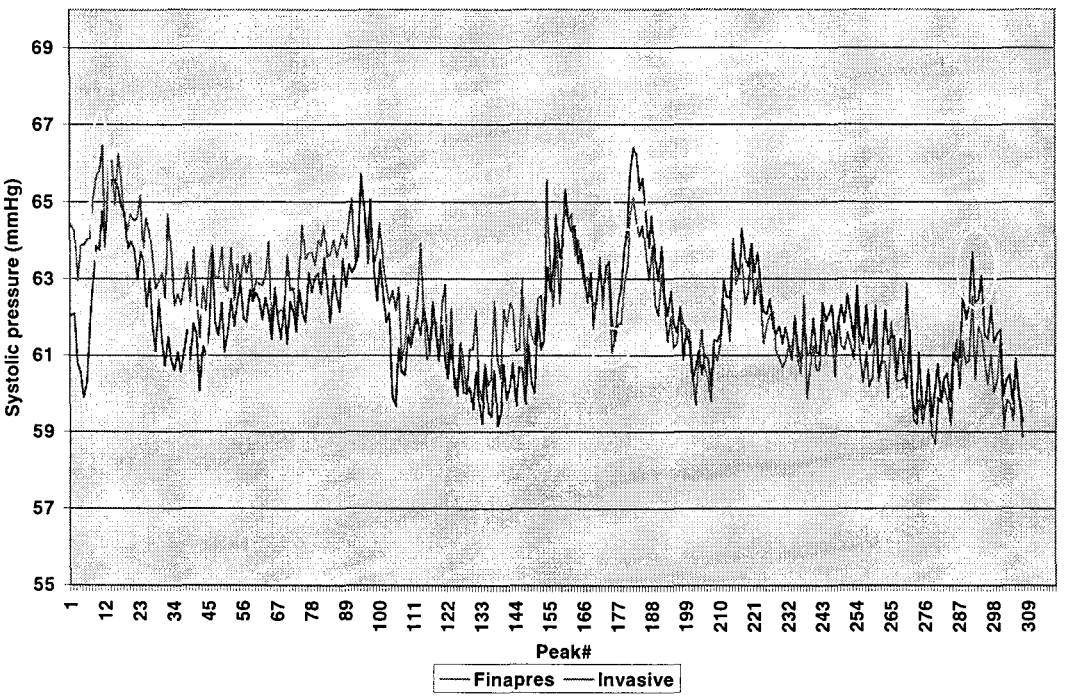

Figure 10: Graph of detected systolic values in Finapres and invasive measurements. The ellipses give examples of characteristic peaks that are matched.

[6] Some of the early measurement series were allowed to run for 20 minutes. This was corrected abandonned later, only parts of these series shorter than 10 minutes after calibration were used for further analysis.

The following step was to match the Finapres measurements to the corresponding intra-arterial values, necessary for the Bland-Altman comparison and the correlation calculations described later. The matching was done by plotting the arrays of peaks/minima in one graph and matching up characteristic features. In Figure 10 an example of such a graph is given for systolic peaks. By deleting one point from either the Finapres or the invasive signal at the beginning or the end of the signal, the characteristic peaks/minima can be matched to occur at the same index in the array.

After matching the arrays as described above, the time difference between the registration of the Finapres signal and the intra-arterial signal is calculated for the 6 patients. Results can be found in Table 4 and Table 5.
Remarkably, the Finapres signal is recorded before the intra-arterial signal. This can only mean that the processing time of the HP monitor is longer than the processing time of the Finapres measurement. The variation in the delay between patients is probably due to the fact that the systolic pressure is taken to be the maximum value of the blood pressure in an interval after the systolic slope. The exact location of the maximum is varies from patient to patient. Also the diastolic value is taken to be the minimum value in an interval before the systolic slope, causing a similar variation.

7.3 Methods for comparison

Three separate analysis methods will be followed in comparing the Finapres measurements with the intra-arterial measurements: the Bland-Altman comparison, linear correlation and frequency analysis. A description and motivation for the separate techniques will be given here.

7.3.1 Bland-Altman comparison

In a comparison of clinical measurement methods, the correlation between the measurement outcomes is not necessarily a good method. Since the exact value of the measurement is not as important as the state of the patient it represents, it is sufficient that one method matches the other method within certain limits. Bland and Altman developed a mathematical method to compare an established clinical type of measurement with an alternative one [41].
At every measurement time q two measurement values are available: $s_{est}(q)$ from the established measurement method, and $s_{alt}(q)$ from the alternative measurement. From these measurement values 2 parameters are calculated:

Average: $$A(q) = \frac{s_{est}(q) + s_{alt}(q)}{2}$$ *Equation 19*

Difference: $$D(q) = s_{alt}(q) - s_{est}(q)$$ *Equation 20*

For D(q) the mean value μ and standard deviation σ is calculated. Limits of agreement are calculated from μ and σ. For example: assuming a normal distribution of the differences, 95% of the measurement values will lie between $\mu+2\sigma$ and $\mu-2\sigma$. Looking at the upper limit and the lower. limit, a conclusion can be reached about the agreement between the two methods. In Figure 11 a Bland-Altman comparison of the blood pressure measurements at hand is given as an illustration.
For several reasons Bland-Altman comparisons can dismiss alternative techniques. First, if σ is too high, the uncertainty in measurement outcome with the alternative method is too high. Secondly, if the μ value is not close to or equal to zero, results obtained by the two methods can not directly be compared, which makes the alternative method unfit for clinical use.
From displaying the data in graphs like Figure 11 systematical errors between the two methods may be found as trends. Separate groups of measurement points in the graph may indicate inconsistencies during the measurement period.
The Bland-Altman method will be used on the systolic and diastolic pressures in order repeat the results of Drouin. Since we want to compare the variability of the blood pressure signals, the Bland-

Altman method is used also on the beat-to-beat systolic and diastolic blood pressure variations. The beat-to-beat variation is defined, for $q = 1$ to $(n-1)$, as:

$$\Delta p(q) = p(q+1) - p(q) \qquad \textit{Equation 21}$$

(with p = systolic/diastolic blood pressure (mmHg), q = index of the systolic peak/diastolic minimum, n = the total number of detected peaks/minima in the signal)
The aim is to compare the different measurement techniques in the frequency domain. A good agreement in a Bland-Altman comparison does not guarantee this to be possible, but from the comparing the beat-to-beat variations, some insight might be gained about the origins of eventual differences.

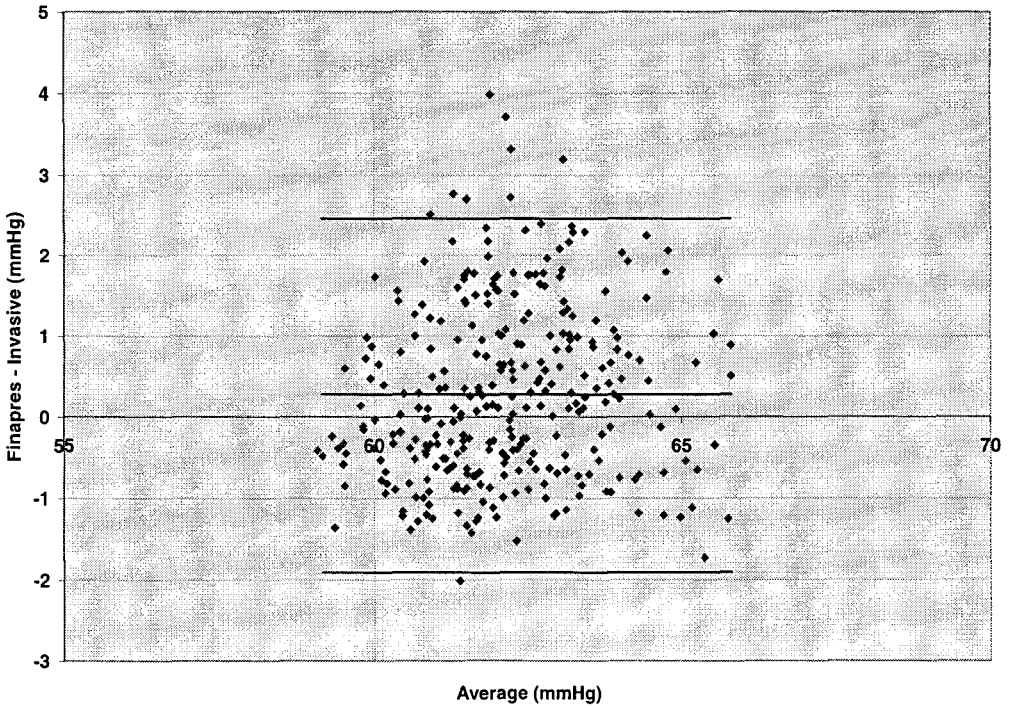

Figure 11: Bland-Altman comparison of blood pressure measurements. Established method is the invasively measured value, the alternative method the Finapres measurement. The displayed values are systolic blood pressure values, obtained from a patient measurement. The black horizontal lines represent upper limit of agreement, lower limit of agreement and the mean. The limits of agreement are $\mu+2\sigma$ and $\mu-2\sigma$. The measurement shown is selected to depict a good agreement between the methods.

7.3.2 Linear correlation

Having performed the Bland-Altman comparison, the relationships between the beat-to-beat systolic and diastolic variations of the two methods is evaluated. To guarantee a correct frequency domain analysis the relationships meet two requirements. First of all, the relationships should be linear:

$$\Delta p_{finapres} = A \cdot \Delta p_{invasive} + c \qquad \textit{Equation 22}$$

(with $\Delta p_{Finapres}$ = beat-to-beat variation of systolic/diastolic pressure measured with Finapres (mmHg), $\Delta p_{finvasive}$ = beat-to-beat variation of systolic/diastolic pressure measured with invasive catheter (mmHg), A = linear coefficient, c = off-set (mmHg))
And secondly, the off-set c should be 0mmHg and the linear coefficient A should be 1. Linearity is evaluated by the linear correlation coefficient R.
If any other relationships are (linear or not) found Finapres values can, of course, be corrected during signal processing.

7.3.3 *Frequency domain analysis*

After the analysis of the beat-to-beat variation in the time-domain, a frequency spectrum in calculated of the 128s-signals. The method for doing this is described in 6.2. The signals are divided into 3 half overlapping records.
These spectra will be evaluated on their coherence and the outcome of the LF/HF ratio calculation over the intervals defined in Chapter 3. For this purpose the power spectra will be integrated over the frequency bands, LF/HF energy ratio calculated and compared.

Coherence

Coherence provides a measure of linear correspondence between signals in the frequency domain. For real valued power spectra mathematically defined as:

$$coherence = \frac{[P_{XY}(f)]^2}{[P_{XX}(f)][P_{YY}(f)]} \qquad \textit{Equation 23}$$

(with P_{XY} the cross spectral density of X and Y, P_{XX} and P_{YY} spectral densities for X and Y, respectively)
The coherence is 1 if the spectra are perfectly linearly related and 0 if they are entirely linearly unrelated.
If the coherence between the two spectra is good, we may be able to draw conclusions, of a relative nature based on Finapres measurements, even if the values of the power calculations do not correspond.

7.4 Results

7.4.1 *Bland-Altman comparisons*

Systolic and diastolic blood pressure measurements
As can be read from Table 4, in patients 1 through 4 (all measured with umbilical intra-arterial line) there is some variation in measurement bias in the systolic pressures. Patients 1 through 3 produce consistent results with biases of the Finapres with respect to the invasive measurement ranging from –6.5mmHg to – 4.5mmHg (2σ from 1.4mmHg to 1.8mmHg). The data for patient 4 however show an average overestimation by 4.4mmHg (2σ = 5.75mmHg).
In the diastolic measurements (Table 5) an underestimation is only seen in patient 1 ((-3.45± 2.11)mmHg). Patients 2 and 3 produce an slight overestimation of the diastolic pressure ((1.70± 0.79)mmHg and (2.06±1.90)mmHg, respectively. The diastolic values for patient 4 show a an overestimation of (17.9±3.3)mmHg by the Finapres.
These data suggest some kind of measurement abnormality in patient 4. Combining the data for patients 1 through 3 (872 systolic and diastolic values) Finapres measurements differ by (–5.59±2.32)mmHg for systolic values (cf. Figure 12a).Doing the same for the diastolic values, a difference of (+0.07±5.36)mmHg is found. When looking at Figure 12b, the inter patient differences results in two separate groups of measurements in the diagram. Measurements for diastolic pressures with the Finapres in patients 1 through 3 do not correspond with each other. The systolic pressures simply have a bias with respect to the intra-arterial measurements.
The data for patient 5 show a large overestimation of the systolic pressures by the Finapres ((+17.6±4.0)mmHg) and an underestimation of the diastolic pressures ((-5.57±3.81)mmHg), indicataing a bad correspondence between the intra-arterial measurement at the ankle and the Finapres at the wrist. Surprisingly, though, patient 6 shows almost no difference in systolic bloodpressure ((+0.28±2.18)mmHg) and an underestimation in the diastolic pressure ((-4.49±1.12)mmHg). Inaccuracy is typically small in patient 6.

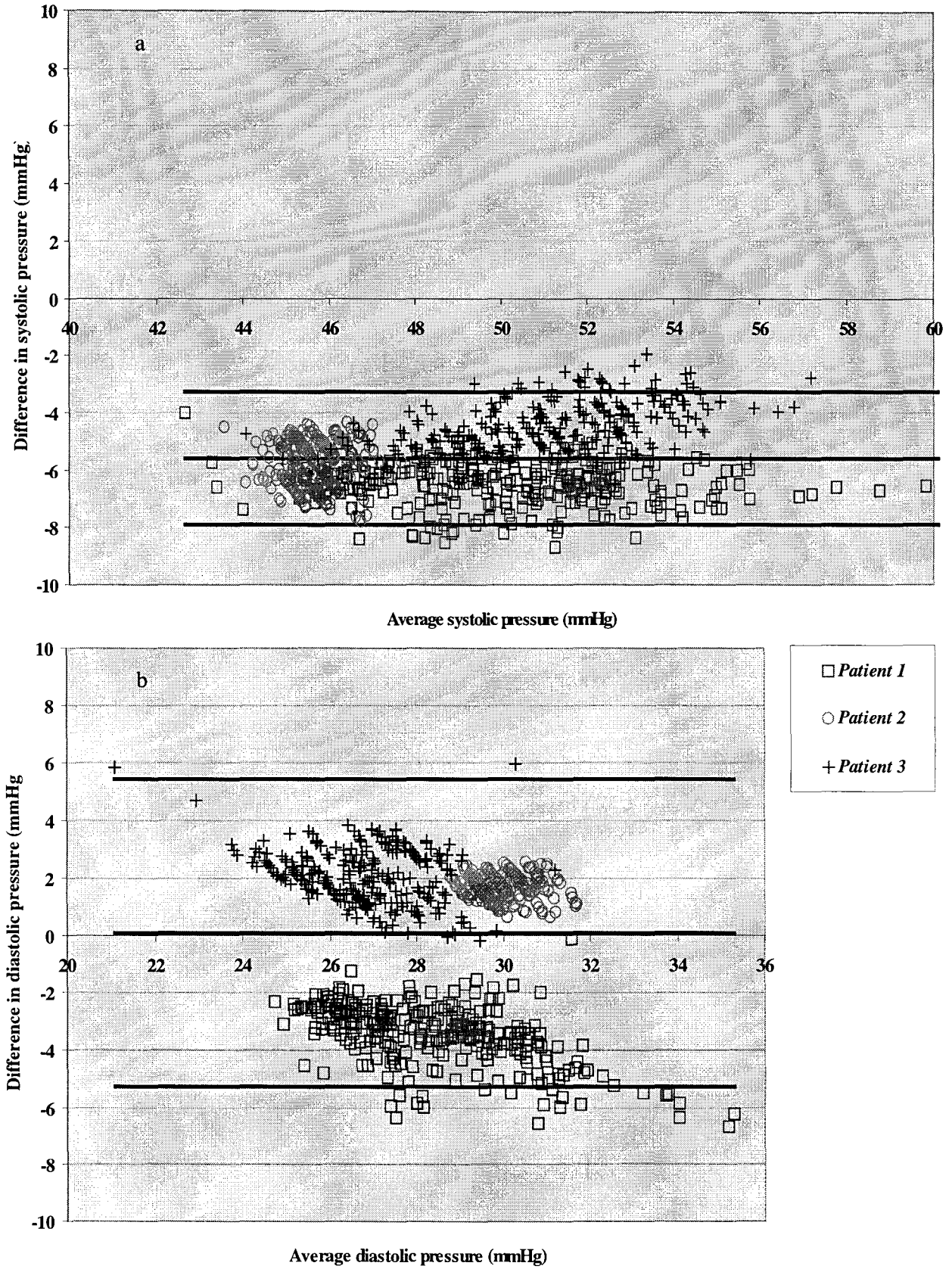

Figure 12: Bland-Altman comparison of the systolic (a) and diastolic (b) blood pressures from patients 1 through 3. At the horizontal axis the average of the Finapres and the intra-arterial measurement is displayed. At the vertical axis the difference ($p_{Finapres}$-$p_{invasive}$) is given.

The inconsistencies in patients 5 and 6 are probably due to operator error (cuff application) or inter patient differences, otherwise the opposite effect should have been found when the two are compared.

Beat-to-beat variation in systolic and diastolic pressure

When looking at the beat-to-beat variations of the pressures, Finapres seems to provide accurate tracking in all 6 patients (average biases range from -0.03 to 0.00mmHg). The average differences are within the resolution of the Finapres (0.5mmHg). Inaccuracy ranges from 0.78mmHg to 3.81mmHg.
Combining data for patients 1 through 4 (n=1191) the agreement in systolic pressure variations is good (difference = (-0.01±2.02)mmHg).
Also the agreement in diastolic beat-to-beat variations is found to be good (difference = (-0.01±0.79)mmHg). In Figure 13b a slight negative trend can be found, indicating that the Finapres systematically underestimates fluctuations in diastolic pressures.
Reviewing the individual patient data, this negative trend can be found in all of patients 1 through 4; most pronounced in patient 3. In patient 6 no such trend is found and patient 5 shows a positive trend in diastolic variations, indicating a systematic overestimation of diastolic fluctuations.

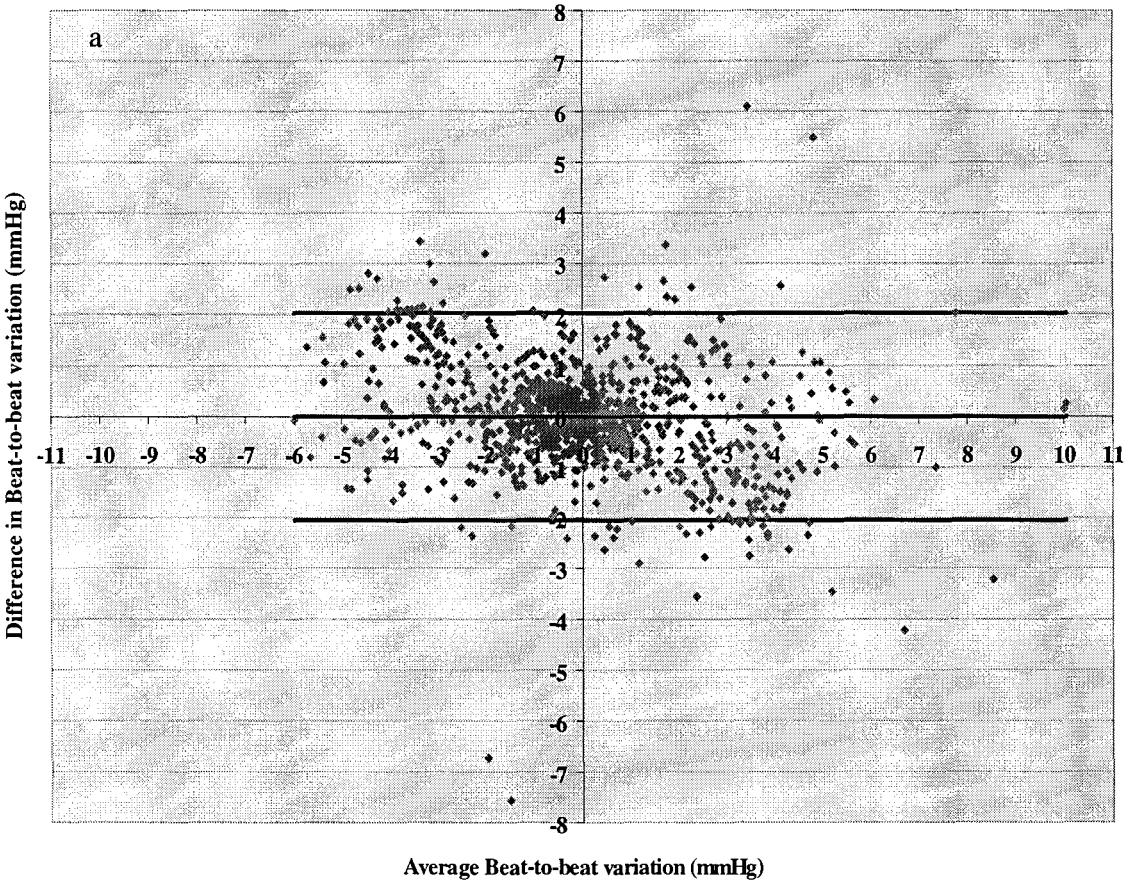

Figure 13a: for caption see below figure 13b on the next page.

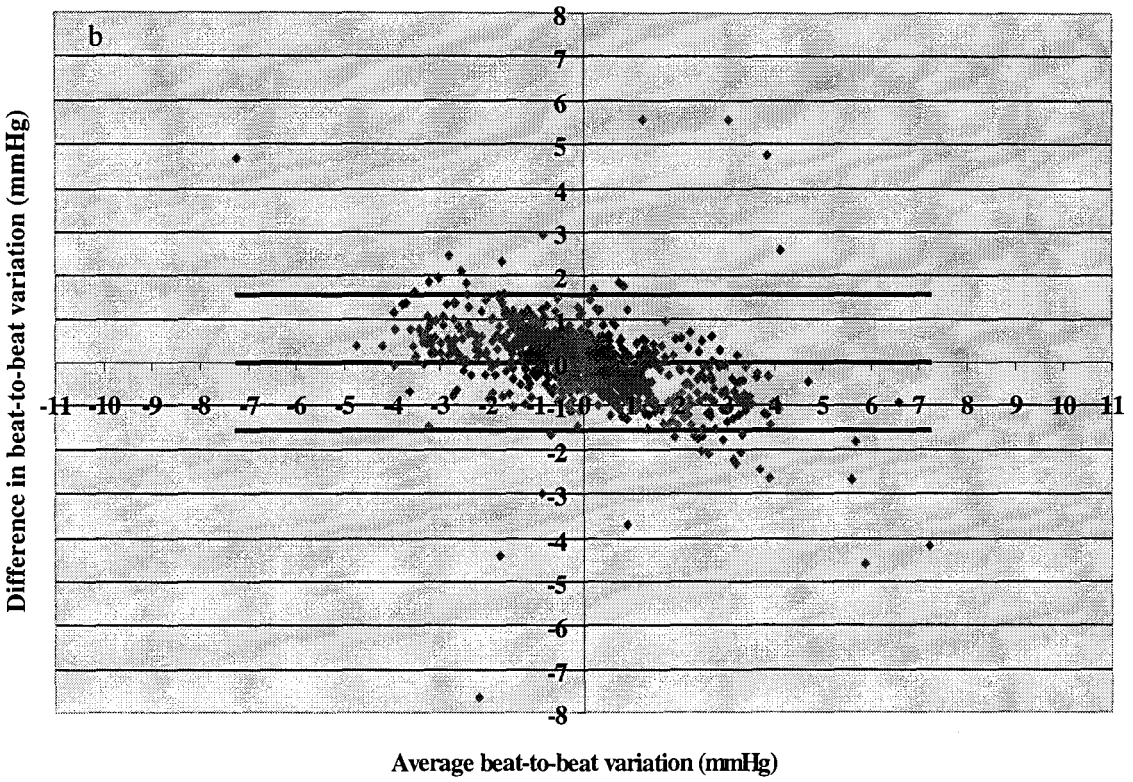

Figure 13: Bland-Altman comparison of beat-to-beat variations in systolic blood pressure from patients 1 through : systolic pressure in a, diastolic in b. The horizontal axis displays the average variation of the two methods, the beat-to-beat variation being defined by Equation 21 ($A(q) = \frac{1}{2}(\Delta p_{Finapres}(q)+\Delta p_{invasive}(q))$). The vertical axis shows the corresponding differences ($D(q) = \Delta p_{Finapress}(q)-\Delta p_{invasive}(q)$).

For the systolic variations, the individual patient data show negative trends in both patients 2 and 4; a pronounced positive trend is found in patient 6. Examples of the trends in individual patients are given in Figure 14 (taken from the systolic variation values). A possible explanation for these trends may be found in the physiology of the patient. Responses in the arm may be less strong compared to those in the torso and variations in the leg could be less strong compared to those in the arm. Assuming this is true, there is no explanation for finding a positive trend in the diastolic variations of patient 5, while the data for patient 6 shows the same in systolic variations.
There is a systematic difference in Finapres measurements that is apparently not occurring in every patient, or every measurement, correspondingly. Caution should be taken when comparing variabilities, but in general, the Finapres appears to follow the variations in intra-arterial measurements well.

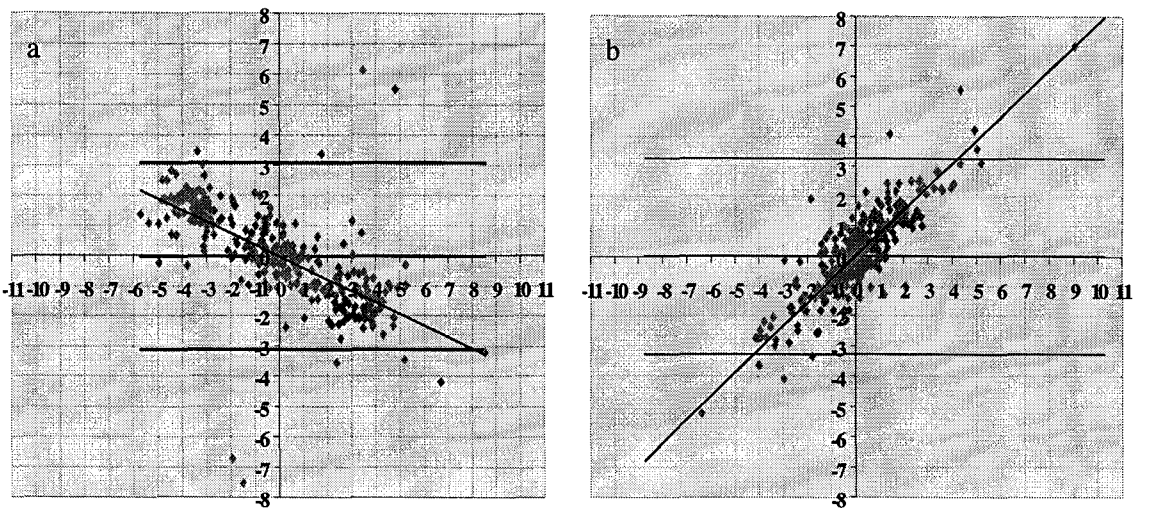

Figure 14: Bland-Altman comparison of beat-to-beat variations in systolic blood pressure for patients 4 (a) and 5 (b), The horizontal axis displays the average variation of the two methods (mmHg), the beat-to-beat variation being defined by Equation 21 ($A(q) = \frac{1}{2}(\Delta p_{Finapres}(q)+\Delta p_{invasive}(q))$). The vertical axis shows the corresponding differences (mmHg) ($D(q) = \Delta p_{Finapres}(q)-\Delta p_{invasive}(q)$).

7.4.2 Linear correlation

The relationship between the intra-arterial variations and the Finapres variations appear to be linear in all included patients. Individual patient data can be found in Table 4 and Table 5. Looking at patients 1 through 4 again, the linear correlation coefficient is higher than 0.9 for all in the systolic variations. For the diastolic variations, linear correlation is worse in patients 1 and 2 (R = 0.77 and 0.73 respectively) . This may be due to fluctuations that sometimes appear near the diastolic point in the blood pressure signal, influencing the exact value of the minima.

For patients 5 the correlation coefficient R is 0.89 for systolic and 0.71 for diastolic, indicating a better linear relationship in the systolic variations as well. The same can be said for patient 6, but the linear correlation (systolic R = 0.61, diastolic R = 0.33) is far worse than it is in patients 1 through 5, and a linear relationship is arguable.

As should have been expected from the trends in the Bland-Altman comparison, the linear coefficients *A* (cf. Equation 22) differ from one in a lot of cases. In the diastolic variations *A* ranges from 0.42 to 0.81 for patients 1 through 4. In the systolic variations it ranges from 0.62 to 1.02.

A linear fit on the combined systolic variations for patients 1 through 4 result in the following relationship:

$$\Delta p_{finapres} = 1.02 \cdot \Delta p_{invasive} + 0.01$$

Which translates to $\Delta p_{finapres} = \Delta p_{invasive}$ just considering the resolution of the Finapres.

The same fit applied on the diastolic variations produces the following relationship:

$$\Delta p_{finapres} = 0.70 \cdot \Delta p_{invasive} - 0.01$$

Which gives $\Delta p_{finapres} = 0.70 \Delta p_{invasive}$ within the resolution of the Finapres.

In general the requirement of linearity between the two measurement methods is fulfilled. However, there are considerable differences in correlation and linear coefficient between individual patients or measurements.

In patients 1 through 4 the average correlation coefficient for systolic beat-to-beat variations is 0.92 (σ =0.01), the diastolic variations it is 0.84 (σ =0.09). This proves that the systolic variations are better followed by the Finapres than their diastolic counterparts.

7.4.3 Summary of results

In the tables below the results of the Bland-Altman comparisons and correlation analysis are summarized. The values presented are outcomes of calculations on the measurement data, no correction for the resolution or inaccuracy of the Finapres has been made.

	Patient	1	2	3	4	5	6
	Birth weigth (g)	1260	2215	1440	1806	946	772
	Gestational age (w+d)	33+3	33+1	30+4	30+5	26+1	26+1
	Classification (SGA/AGA)	SGA	AGA	AGA	AGA	AGA	SGA
Number of detected peaks in signal		297	275	300	320	325	307
Measurement Location	Finapres	Left Wrist	Left wrist	Left Wrist	Left wrist	Right wrist	Left leg
	Intra-arterial	Umb	Umb	Umb	Umb	Left leg	Right wrist
Delay between	Average ($t_{invasive}$-$t_{Finapres}$) (s)	0.24	0.30	0.22	0.23	0.31	0.28
Signals	2σ (s)	0.02	0.02	0.03	0.02	0.01	0.01
Differences in Systolic blood Pressure	Average ($p_{Finapres}$-$p_{invasive}$) (mmHg)	-6.53	-5.74	-4.52	4.40	17.6	0.28
	2σ (mmHg)	1.48	1.57	1.80	5.75	4.0	2.18
Differences in beat-to-beat Variation	Average ($\Delta p_{Finapres}$-$\Delta p_{invasive}$) (mmHg)	0.00	-0.01	0.00	-0.03	0.00	-0.01
	2σ(mmHg)	1.16	0.79	2.07	3.10	3.27	1.69
Relationship of beat-to-beat Variations	A	0.97	0.62	1.02	0.63	1.94	0.73
	R	0.94	0.91	0.92	0.91	0.89	0.61
	c	0.00	-0.01	0.00	-0.03	-0.01	-0.01

Table 4: Results of the calculations of delay time, Bland-Altman comparisons and correlation of the systolic blood pressure signals for the 6 included patients.(Gestational age is given in weeks+days, umb = umbilical artery, which means that the catheter is inserted through the remnants of the umbilical cord, blood pressure is measured in the lower torso.)

	Patient	1	2	3	4	5	6
	Birth weigth (g)	1260	2215	1440	1806	946	772
	Gestational age (w+d)	33+3	33+1	30+4	30+5	26+1	26+1
	Classification (SGA/AGA)	SGA	AGA	AGA	AGA	AGA	SGA
Number of detected minima in signal		297	275	300	320	325	307
Measurement Location	Finapres	Left Wrist	Left wrist	Left Wrist	Left wrist	Right wrist	Left leg
	Intra-arterial	Umb	Umb	Umb	Umb	Left leg	Right wrist
Delay between	Average ($t_{invasive}$-$t_{Finapres}$) (s)	0.25	0.27	0.25	0.26	0.28	0.24
Signals	2σ (s)	0.02	0.02	0.01	0.01	0.01	0.02
Differences in Diastolic blood	Average ($p_{Finapres}$-$p_{invasive}$) (mmHg)	-3.45	1.70	2.06	17.9	-5.57	-4.49
Pressure	2σ (mmHg)	2.11	0.79	1.90	3.3	3.81	1.12
Differences in beat-to-beat	Average ($\Delta p_{Finapres}$-$\Delta p_{invasive}$) (mmHg)	0.00	-0.01	-0.01	-0.02	0.00	-0.03
Variation	2σ(mmHg)	1.30	0.79	1.98	1.89	2.21	1.41
Relationship of	A	0.60	0.42	0.51	0.81	0.95	0.71
beat-to-beat	R	0.77	0.73	0.91	0.93	0.71	0.33
Variations	c	0.01	0.01	0.00	-0.03	-0.03	0.01

Table 5: Results of the calculations of delay time, Bland-Altman comparisons and correlation of the diastolic blood pressure signals for the 6 included patients.(Gestational age is given in weeks+days, umb = umbilical artery, meaning that the catheter is inserted through the remnants of the umbilical cord, blood pressure is measured in the lower torso.)

The beat-to-beat blood pressure variations as measured with the Finapres seem to correspond linearly with the variations of the intra-arterial measurements in the time domain. The correlation is generally better in the systolic values, than it is in diastolic values. The linear coefficient *A*, in the relationship between the variabilities, is highly dependent on the individual patient or measurement. Comparing different patients or measurements should only be done, using parameter relative to the same measurement.

7.4.4 Frequency Domain Analysis

In Figure 15 the spectrum for patient 2 is displayed. As in all of included patients the general shape of the spectra seems to correspond. For the spectra of both the systolic and the diastolic beat-to-beat variations the coherence of the spectra is calculated and averaged over the LF and HF frequency band. Results can be found in Table 6.
All of the averaged coherence values are high enough to say that the spectra are linearly related. However, since coherence is probably high when the signal is zero, the standard deviation of the coherence should also be taken into account. With 95% of the coherence values between the (average±2 standard deviations) limits, a average of coherence of 0.83 with a SD of 0.16 (patient 6, diastolic, HF) would simply mean that 95% of the coherence values in the band is between 0.51 and 1.00, the others being under 0.51. Keeping this in mind the systolic spectrum is clearly better linearly related than the diastolic spectrum.

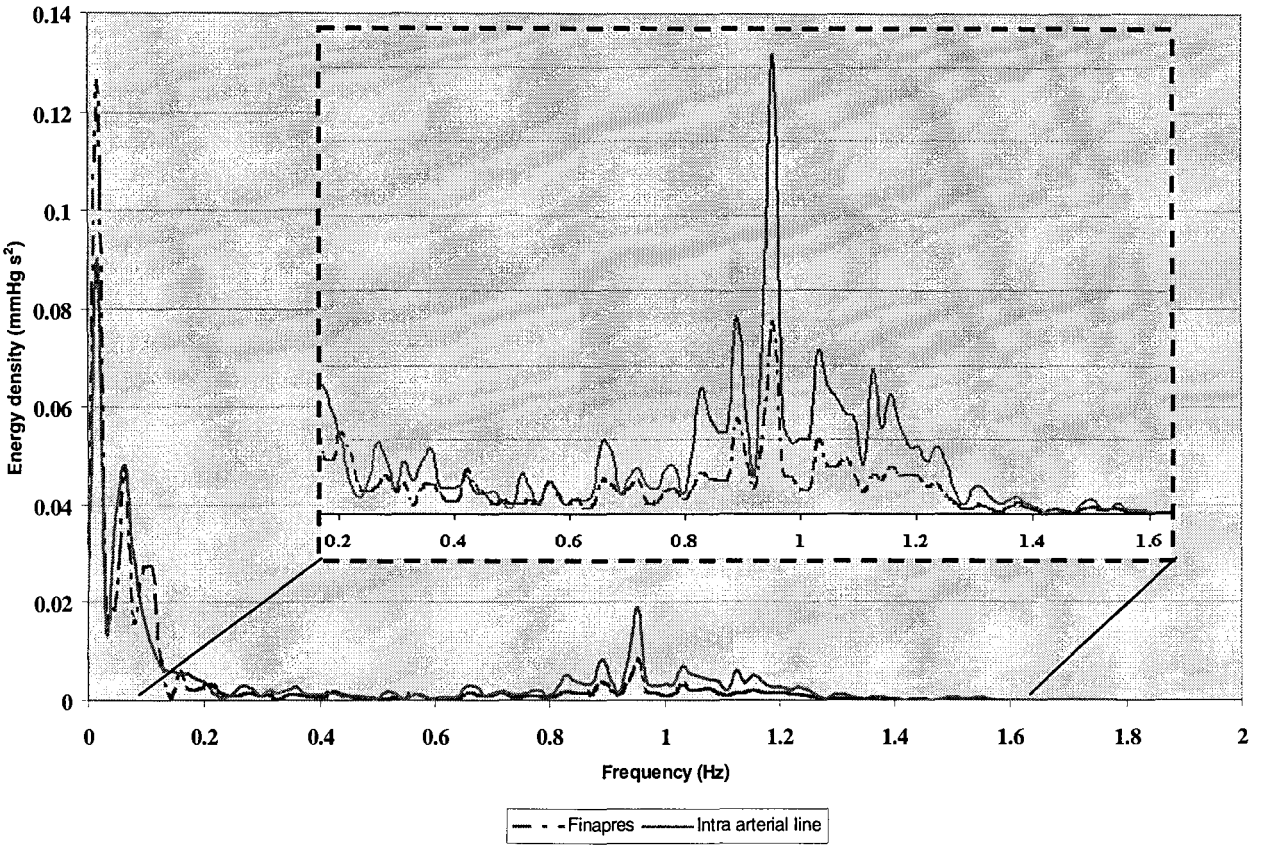

Figure 15: Spectrum of the beat-to-beat systolic variation of patient 2. Part of the spectrum is displayed on an enlarged vertical scale.

	Systolic variation spectrum				*Diastolic variation spectrum*			
	LF band (0.04-0.15Hz)		*HF band (0.4-1.5Hz)*		*LF band (0.04-0.15Hz)*		*HF band (0.4-1.5Hz)*	
	Average	*SD*	*Average*	*SD*	*Average*	*SD*	*Average*	*SD*
Patient 1	0.97	0.03	0.94	0.06	0.89	0.16	0.85	0.13
Patient 2	0.95	0.03	0.92	0.08	0.93	0.05	0.80	0.17
Patient 3	0.99	0.01	0.95	0.05	0.94	0.04	0.93	0.12
Patient 4	0.93	0.05	0.93	0.08	0.96	0.04	0.87	0.14
Patient 5	0.85	0.11	0.94	0.07	0.90	0.08	0.74	0.14
Patient 6	0.90	0.08	0.78	0.15	0.83	0.08	0.83	0.16

Table 6: Average coherence values and their standard deviation (SD) over the defined frequency bands for patients 1 through 6, as calculated from the spectra for systolic and diastolic beat-to-beat variation.

For patients 5 and 6 the coherence is worse than the coherence of patients 1 through 4 in either the LF or the HF band of the spectrum. This is consistent with the lower correlation coefficients found for them in 7.4.3.

The power spectra are integrated over the LF frequency band (0.04-0.15Hz) and the HF frequency band (0.4-1.5Hz), giving the energies in $mmHg^2s$. For both the spectrum of the systolic variations

and the spectrum of the diastolic variations the energies in the Finapres signal and the intra-arterially measured signal are compared. Results are found in Table 7.

Patient		*1*	*2*	*3*	*4*	*5*	*6*
Diastolic variations spectrum							
LF energy	Finapres	1.44E-02	1.99E-03	5.41E-03	6.78E-02	3.30E-03	2.39E-02
($mmHg^2$ s)	Invasive	2.89E-02	2.91E-03	1.37E-02	5.07E-02	5.72E-03	9.37E-03
	Difference (%)	-50	-31	-61	34	-42	155
HF energy	Finapres	3.06E-03	3.46E-04	5.44E-03	4.47E-02	2.68E-03	9.91E-03
($mmHg^2$ s)	Invasive	4.32E-03	8.62E-04	1.67E-02	2.56E-02	3.92E-04	7.62E-03
	Difference (%)	-29	-60	-67	75	583	30
LF/HF ratio	Finapres	4.71	5.76	0.99	1.52	1.23	2.41
	Invasive	6.69	3.37	0.82	1.98	14.61	1.23
	Deviation (%)	30	71	21	23	92	96
Systolic variations spectrum							
LF energy	Finapres	3.83E-02	2.28E-03	1.84E-02	6.54E-02	8.42E-03	4.14E-02
($mmHg^2$ s)	Invasive	1.29E-02	1.24E-03	3.19E-02	2.35E-02	4.60E-03	3.63E-02
	Difference (%)	196	84	-42	178	83	14
HF energy	Finapres	3.87E-02	2.40E-03	1.39E-02	6.37E-02	1.58E-02	9.38E-03
($mmHg^2$ s)	Invasive	1.26E-02	2.62E-03	2.52E-02	4.75E-02	2.74E-03	8.40E-03
	Difference (%)	207	-8	-45	34	476	12
LF/HF ratio	Finapres	0.99	0.95	1.32	1.03	0.53	4.41
	Invasive	1.03	0.47	1.27	0.49	1.68	4.32
	Deviation (%)	4	101	5	107	68	2

Table 7: Comparison of the resulting energies in the spectra. Differences are expressed in percentages of the corresponding intra-arterial energy, and can be both positive and negative. Deviations are expressed as the absolute value of the difference (%) of the intra-arterially determined LF/HF ratios.

In the spectra of the diastolic fluctuations of patients 1 through 3, energies are underestimated by 29 to 67% with respect to the invasive measurements, leading to an underestimation of the LF/HF ratio of 30 to 71%. In patient 4 both the LF energy and the HF energy are overestimated, though not by the same percentage. The LF/HF ratio is therefore underestimated by 23%. Based on the linear coefficient an underestimation of the energies should have been found in patient 4 as well. No direct relationship between the linear coefficient calculated in 7.4.3 and the underestimation of the energies can be found.
For the spectra of the systolic variations energies are overestimated in patient 1, but by the same amount in LF and HF band. The LF/HF ratio from Finapres measurements only differs 4% from the LF/HF ratio from invasive measurements. A similar result is reached for patient 3, though here powers are consequently underestimated. A difference of 5% is found in the LF/HF ratio.
The LF/HF ratios in patients 2 and 4 differ by 101 and 107% respectively.
A linear coefficient of 1 seems to give a good agreement in LF/HF ratio. However, no corresponding agreement is found in LF and HF energies (which are over estimated by about 200% in patient 1 and underestimated by about 45% in patient 2).
For patients 2 and 4 an overestimation of the LF energy can be found, which together with a smaller overestimation (patient 4) or a underestimation (patient 2) of HF power, leads to a large overestimation of the LF/HF ratios (>100%).
In patients 5 and 6 the deviations in LF/HF ratios are large in three out of 4 cases. Only, in patient 6, using systolic variations the LF/HF ratio has a deviation of only 2%. Since this is the signal with the lowest correlation between Finapres and intra-arterial variations, this is probably a coincidence. The LF/HF ratios for patients 1 through 4 are displayed in Figure 16.

A remarkable observation is the general absence of correspondence of the LF and HF powers calculated from the systolic variations with those calculated from the diastolic correspondence.

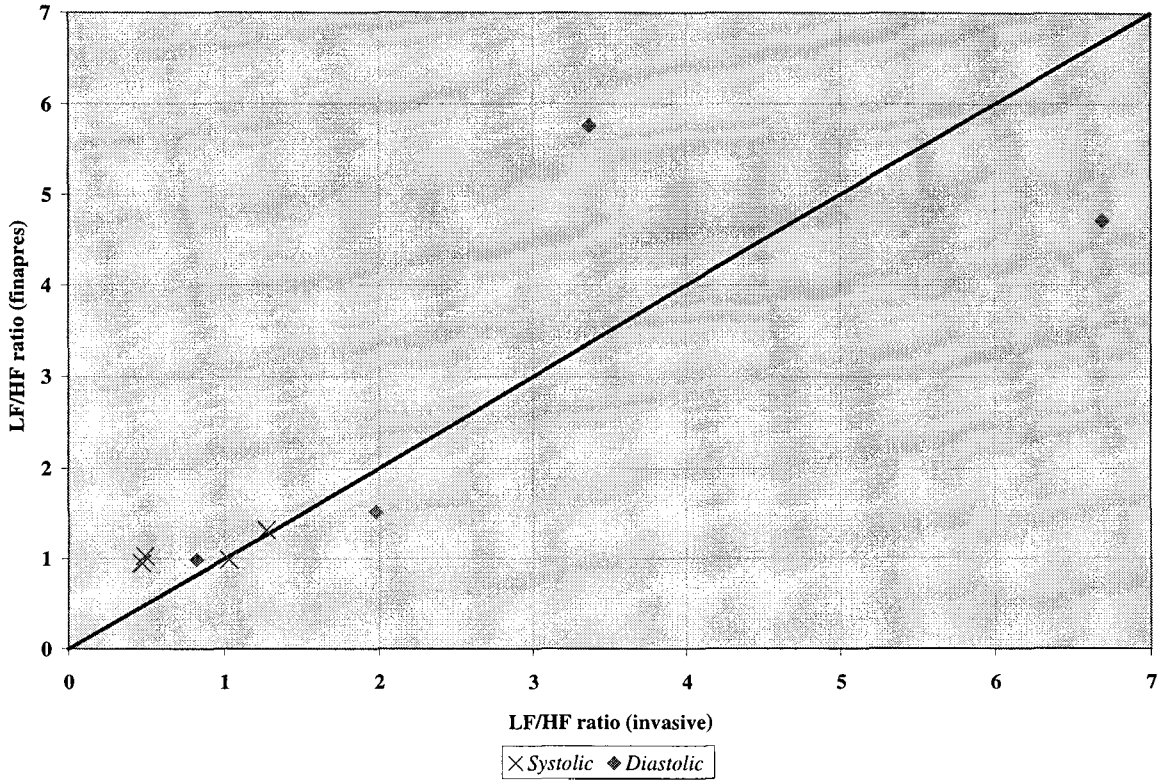

Figure 16: LF/HF ratios for patients 1 through 4, for both the systolic and diastolic pressures.

7.5 Discussion

From the Bland-Altman comparisons between the Finapres and the intra-arterial measurements it becomes apparent that the outcome of the Finapres is highly dependent on the individual measurement. This difference could be due to inter patient differences or inconsistencies in cuff application.
The agreement between the beat-to-beat blood pressure variations measured by the two systems is better, but systematic errors occur is some patients. The question whether and how a systematic difference occurs depends again on the individual measurement or patient.

Linearity between the two measurement methods is best in beat-to-beat systolic variations for patients with an umbilical intra-arterial line. The linear coefficient depends on the individual measurement or patient, making comparisons between patients awkward.
Also coherence between the spectra of the systolic variations of these patients is sufficiently high in the required frequency bands to indicate a linear relationship between the two spectra. This however does not result in corresponding energies in the LF and HF frequency bands, and thus not in corresponding LF/HF ratios.
In two cases a good correspondence between the LF/HF ratios from the systolic variations was found. Typically, this was in the patients in which the linear coefficient in the relationship between the Finapres systolic variations and the intra-arterial systolic variations is approximately 1. Since the

correspondence in calculated LF and HF energies is not visible and we have only two patients exhibiting the correspondence, no direct relationship can be assumed.
The data of patients with the intra-arterial line connected to one of the extremities produce worse linear correlation and coherence results in the variability analysis. The population is however to small to draw any conclusions.

The general conclusion should be that in use on neonates the Finapres measurement correspond best with the intra-arterial measurements when assessing the systolic blood pressure variations in the time domain. The linear relationship between finapres and intra-arterial measurements does have variable linear coefficient, and for inter patient comparisons parameters relative to the same measurement should be used.
In the frequency domain a high degree of coherence is found for the systolic variations in the LF and HF band as well. There is a complete lack of correspondence, however, when parameters like LF energy, HF energy, and LF/HF ratio are calculated from the spectra.
Therefore in neonates, the Finapres should not be used for frequency domain analysis by calculating LF and/or HF energies.
Possibly, the relative change in LF energy, HF energy or LF/HF ratio during provocation is consistent between the two methods. No experimental evidence hereof has been found though.

Chapter 8 Recommendations and conclusions

Blood pressure variability

No differences in heart rate and blood pressure variability in resting conditions are found between healthy test subjects and patients with an obvious disfunctioning in the autonomic nervous system (syncope). A pronounced difference can be found in the variability during provocation.
Therefore no difference is expected to be found between the SGA and the AGA test groups in the BIRTH study in the same conditions, since they have not developed any obvious symptoms yet. Provocation will be necessary to find any differences in the heart rate or blood pressure variability between the test groups. The proposed passive head-up tilt is adequate in provoking a clear response of the autonomic nervous system, which is visible in the heart rate and/or blood pressure variability.

Based on the literature and the first impressions with the Finapres, blood pressure variability analysis using the Finapres should be possible in school children in both the time and the frequency domain. Our first impressions and the more extensive investigation on neonates however, lead to the suspicion that inter patient differences and cuff application differences between individual measurements may cause problems in children and adults as well. Any differences between the SGA and the AGA test groups could be lost in the inaccuracy of the method.

Skin blood perfusion

At cold provocation the Periflux registers a significant drop in skin blood flow in a healthy test person. Any existing differences between the SGA and AGA test groups may be found in either skin blood flow at rest, the reaction of the skin blood flow to provocation, the time needed to complete the drop in skin blood flow on provocation, the oscillation frequencies in the skin blood flow, or the response of these oscillations to a cold provocation. Analysis in both time and frequency domain should be attempted to get a complete picture.

There are known differences between the development of the skin blood flow between pre-term and full-term neonates, and neonates are able to control the skin blood flow.

Because of the relative nature of the Periflux measurements and the inaccuracy in the measurement volume, only relative changes in the measurement values should be used for inter patient and inter measurement comparisons. (This requirement effectively makes the search for difference in rest conditions impossible).

In conclusion, there is no doubt that a response to cold provocation can be measured in the skin blood flow. The question remains whether there are any differences to be found in this response between the SGA and the AGA test groups.

Finapres and neonates

It is possible to obtain continuous blood pressure measurements from neonates by applying a Finapres cuff to an arm or a leg. In a number of cases several attempts of cuff-application failed, though.
Biases between Finapres measurements and intra-arterial measurement depend on either individual patients or individual measurements. The unpredictability of the bias makes the Finapres unsuited for blood pressure monitoring in neonates.

Beat-to-beat variations of the systolic and diastolic pressures between the Finapres measurements and the invasive measurements in neonates generally correspond. In some cases a systematic difference can be found from the Blant Altman comparisons, especially in diastolic blood pressure variations. The fact that these systematic differences do not occur in every measurement, makes the system unpredictable.

Linear correlation between the systolic blood pressure variations of the Finapres and the intra-arterial systolic pressure variations is better than the linear correlation between the diastolic blood pressure variations. For patients with an umbilical arterial line an average correlation of 0.92 was found for systolic blood pressure variations, while in the same patients, the average was 0.84 for diastolic blood pressure variations. This makes the systolic blood pressure a better signal to analyse in the time domain.
The linear coefficient in the relationship between the Finapres measurement variations and the intra-arterial measurement variations depends on the individual patient or measurement. Therefore, analysis should be carried out with the measurement as its own base-line; only relative parameters calculated from one measurement should be compared with other measurements.

The spectra of the blood pressure variability obtained from the Finapres measurements show a good coherence in the LF and HF band with those obtained from intra-arterial measurements, when the systolic blood pressures are analysed. The coherence is generally worse for the diastolic blood pressure variability.
This coherence does not result in corresponding (or even linearly related) energy contends for the LF and HF bands. There does not appear to be a relationship between the LF and HF energies calculated from the Finapres measurements and the same values calculated from the intra-arterial measurements. As a result, also the LF/HF ratios, the indication for the sympatho-vagal balance, differ between the measurement methods.
In two cases the difference in LF/HF ratios of the systolic blood pressure variability between the Finapres and the intra-arterial measurements is less than 10%, while the individual LF energies and HF energies do not correspond. In these cases the linear coefficient in the relationship between the Finapres and the intra-arterial measurement is approximately one. However, an explanation for this connection has not been found, and the number of occurrences is too small to draw any conclusions on statistical grounds.

In conclusion, the LF and HF energies of the blood pressure variability calculated in the frequency domain from the Finapres measurements on neonates do not correspond with the energies calculated from the intra-arterial measurements. In neonates, the Finapres should not be used to obtain LF energy, HF energy or LF/HF energy ratios of the blood pressure variability for inter patient comparisons.
In the time domain, the systolic values of the Finapres measurement should be used for analysis rather than the diastolic values. As mentioned inter patient comparisons should only be performed using relative parameters.
We did not have a chance to perform tilt-tests. There is a possibility that evaluation of the relative development of the energy contend of the LF and HF bands or the LF/HF ratio induced by a provocation is a reliable way to characterize the autonomic response.

HF band energy and LF/HF energy ratio in neonates

In the analysis of the blood pressure variability generally only one measurement value is taken every heart beat, usually either the diastolic or the systolic value. This results in cardiac aliasing. In adults and children the effects on the spectrum will be small, but in neonates they can be substantial. The effect is twofold: the heart rate is often not high enough to reach a 'natural Nyquist frequency' higher than 1.5Hz (the upper limit of the HF band in neonates), and the respiratory rate is higher than or just a little below to half the heart rate.

Because of the unknown effects on the spectrum, depending on both heart and respiratory rate and the ratio between them, the HF band in neonates should be treated with the utmost care.
From the literature on tilt-testing, most of the changes in the variability should occur in LF band as a result of higher sympathetic activity. The option of discarding the HF energy value and the LF/HF ratio from the analysis of neonatal data should be considered. The relative change of the energy in the LF band during provocation is probably a more reliable parameter for the functioning of the autonomic nervous system in neonates.

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The references are listed in the order they are referred to in the main text.

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Appendix A: MIDAS – Mobile data acquisition station

Recently, in 2001, a mobile data-acquisition station was introduced into the perinatal research at the Saint Joseph Hospital. The station, affectionately known as MIDAS (Mobiel Inzetbaar Data-Acquisitie Station) or 'the cart', provides flexibility in measurement location and equipment, while avoiding the costs of a fixed infrastructure.
The advantage in flexibility and cost, comes at a price as the measurement equipment has to be present in the same room as the patient. This enhances the chance on anxiety or disturbance of the patient.
In Figure A.1 a schematic overview of the key-components of the MIDAS set-up is given.

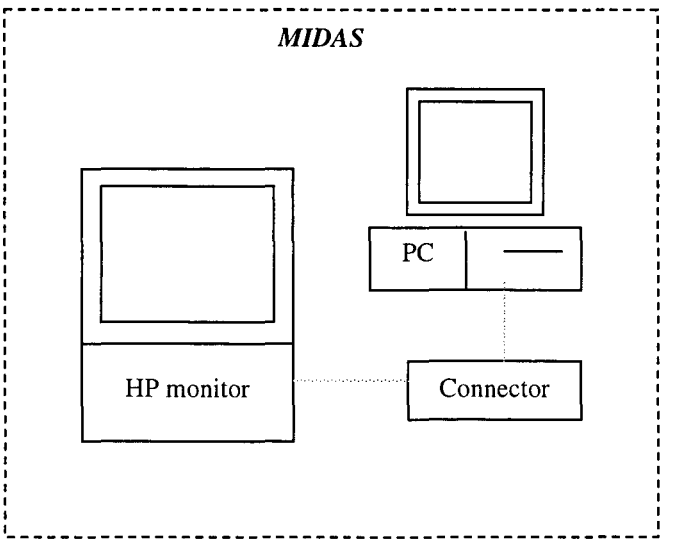

Figure A.1: Schematic set-up of the mobile data acquisition station.

The cart, on which the devices are mounted, is facilitated with a transformer, which separates the power supply for the devices from the general 230V power supply in the hospital, according to medical safety regulations.
The MIDAS set-up's most important components are the Compaq PC, with a National Instruments analogue to digital converter card (NI PCI6034E) and the external National Instuments connector-board (NI SCB68). This combination allows 8 bipolar or 16 monopolar analogue signals to be sampled and recorded simultaneously.
Also a IC monitor (HP 56S) of type in use at the NICU is installed. As it has an analogue output, signals recorded on the monitor can be sampled using the ADC. Also it provides initial processing and amplification of measured signals as ECG, temperature, intra-arterial blood pressure, and allows disposables used daily in the hospital to be used in research as well. An additional advantage is the fact that, when measuring at the NICU, the regular alarm functions can be used during measurements.
When not in use MIDAS is connected to the local area network of the clinical physics department, allowing data to be accessed from other workstations.

Data storage, processing and analysis is programmed using LabVIEW (Version 6.01i, National Instruments), either on the MIDAS PC or the workstations in the hospital.

Appendix B: Adaptation of the peak detection procedure

Systolic maxima

For the peak detection in the Finapres signal an existing procedure [40] for peak detection in blood pressure signals was adapted to the signals at hand. The procedure basically exists of three steps. The 'old' procedure can be described as:

1. Detect the local maxima in the entire signal.
2. Check for every maximum found in step 1, wether there is a systolic slope within A[7] s before it. A systolic slope is defined as an rise in pressure of at least B mmHg/s for at least C s. If the maximum doesn't meet this requirement it is discarded.
3. Check for every pair of maxima meeting the requirement in step 2, whether they are far enough apart in time. If they are not only the higher one is preserved. The time threshold is initially a fraction D of a user-defined average heart beat duration. With every new maximum found this is adapted to be:

$$Threshold\,(new) = D[\tfrac{2}{3} \cdot Threshold\,(old) + \tfrac{1}{3} \cdot Interval\ found]$$

The first adaptation is reversing the order of steps 1 and 2. This has nothing to do with the nature of the signal. Since every systolic slope has a peak associated with it, and not every peak has an associated systolic slope, it seems only logical and more time efficient to first determine the locations of the systolic slopes and then locate the peaks in the interval after them.
Then in step 3 the initial value of the threshold is no longer user-determined, but calculated to be a fraction E of the average interval between the peaks resulting from step 2. Also there is an extra built-in condition that the threshold cannot change more than F % at once. This condition was added after tests proved that missing one peak in the detection could seriously effect the possibility of detecting the following maxima.

In order to develop and test the adapted peak detection procedure 18 2-minute sections were selected from a larger set of data. The data we chosen to be relatively stable and artefact free, although some movement artefacts were allowed. This set of in total 36 minutes of Finapres measurements on neonates contained data from 8 different children. No selection was made based on the position of either the arterial line or the Finapres cuff.
From the 18 sections 10 randomly chosen ones were used to design the changes in the detection procedure (development set) and determine the best settings for the parameters in this procedure. The remaining 8 sections (test set) were kept aside and used to test the result afterwards. In Table B.1 the new and the existing parameters are given. Using these values the test set was run through the procedure.
In the test set there are 2339 peaks to be detected. Two peaks which were supposed to be found were missed (0.09%), whereas one maximum was found when it was not supposed to be detected (0.04%).

Diastolic minima

For detection of diastolic minima the procedure is generally the same as for detecting systolic peaks. The only difference is that when detecting minima, the intervals *before* the systolic slope are searched for their *minimum* value.
The existing procedure for detection of diastolic minima was adapted in the same way as described above.

[7] The capitals represent parameters used to fine tune the procedure. An overview of the parameters is given in Table B.1.

Parameter	Unity	range (estimation)	Variable	old	new
A	s	0.000 -- 0.600	Allowed time interval after slope	0.125	0.120
B	mmHg/s	?	minimal derivative on systolic slope	40.0	40.0
C	s	0.000 -- 0.100	minimal duration of systolic slope	0.040	0.035
D	1	0 – 1	fraction for recalculating threshold	1	0.7
E	1	0.5 -- 1.0	fraction for initial threshold	xx	0.8
F	1	0 – 50	maximum increase in threshold (%)	xx	20

Table B.1: Parameter values for the peak/minimum detection procedure for the Finapres signal.(xx in the table means that there is no old value available).