Suvi Tiinanen

METHODS FOR ASSESSMENT OF AUTONOMIC NERVOUS SYSTEM ACTIVITY FROM CARDIORESPIRATORY SIGNALS
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METHODS FOR ASSESSMENT OF AUTONOMIC NERVOUS SYSTEM ACTIVITY FROM CARDIORESPIRATORY SIGNALS

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Abstract

A cardiorespiratory system is highly regulated via the autonomic nervous system (ANS), whose function can be quantified noninvasively by analyzing electrocardiogram (ECG), blood pressure (BP) and respiration signals. Several conditions and illnesses are linked with imbalance of the ANS.

This thesis aimed to develop methods for describing the ANS regulation of a cardiovascular system from short-term cardiorespiratory measurements. More specifically, the role of breathing rate and its effects on traditional frequency domain based cardiovascular indexes describing ANS control is addressed.

The main contributions are as follows: 1) an adaptive filtering based method to remove respiratory influences from cardiovascular signals and indexes was developed. The adaptive filter reduced the bias caused by low respiration rate, enabling the usage of spontaneous respiration measurement protocol over controlled respiration. 2) Methods to quantify respiratory sinus arrhythmia (RSA) index from cardiovascular signals were developed as well: two methods utilizes adaptive filtering and either the measured respiration signal or the ECG-derived respiration signal and one method uses independent component analysis. Developed RSA index methods allow varying respiration rates making them physiologically more accurate than traditional high frequency power with fixed respiration rate, often used as RSA index. 3) Tools for studying the power and the frequency of low frequency (LF) oscillations of cardiovascular signals were developed, including a time-frequency representation for analyzing varying data. An experimental study was conducted with patients of continuum of cardiovascular risks. According to results, aging decreased the frequency of LF oscillation, whereas coronary artery disease decreased it further. 4) Two new ECG-derived respiration (EDR) methods utilizing decomposition techniques were developed. The proposed methods yielded statistically significant improvements over previously developed EDR methods. EDR method enables to get respiratory information from ECG, which in its turn reduces needed modalities in ANS quantification.

This thesis provides methods to quantify indexes describing the ANS function more accurately by acknowledging the respiration effects. The results of this thesis may be utilized in various application areas, ranging from clinical to physiology research up to commercial health, wellness and sport products.

Keywords: baroreflex sensitivity, ECG-derived respiration, heart rate variability, low frequency oscillation, respiratory sinus arrhythmia
Tiinanen, Suvi, Menetelmiä autonomisen hermoston aktiviteetin määrittämiseen käytäen kardiorespiratorisia signaleja.
Oulun yliopisto, Lääketieteellinen tiedekunta, Tieto- ja sähköteknikan tiedekunta; Center for Machine Vision and Signal Analysis
Acta Univ. Oul. D 1521, 2019
Oulun yliopisto, PL 8000, 90014 Oulun yliopisto

Tiivistelmä

Autonominen hermosto sääteli tarkasti sydän- ja verenkiertoelimistözä sekä hengitystä. Autonomisen hermoston toimintaa voidaan analysoida laskennoiden avulla mitatuista elektrokardiogrammissa (EKG, sydänsähkökäyrä), verenpaine- ja hengityssignaaleista.

Väitöskirjassa kehitettiin menetelmiä sydän- ja verisuonijärjestelmän autonomisen säätelyn kuvaamiseksi lyhentämällä kardiorespiratorisista tallenteista. Erityistä huomiota on kiinnitetty hengityksen vaikutukseen perinteisiin muutoksiin, jotka ovat autonominen hermoston toiminnan merkittävä tekijä.

Väitöskirjan päätuloksia ovat:
1) uusi adaptiivinen frequency domain (FD) analyysi hyödyntäen potilaan hengitystaajuusvaihtelun ja EKG-signaalin. Tämä menetelmä voidaan käyttää kliiniseen tiedon toiminnan mittaamiseen.
2) uusi adaptiivinen frequency domain (FD) analyysi hyödyntäen potilaan hengitystaajuusvaihtelun ja EKG-signaalin. Tämä menetelmä voidaan käyttää kliiniseen tiedon toiminnan mittaamiseen.
3) uusi adaptiivinen frequency domain (FD) analyysi hyödyntäen potilaan hengitystaajuusvaihtelun ja EKG-signaalin. Tämä menetelmä voidaan käyttää kliiniseen tiedon toiminnan mittaamiseen.

Väitöskirja tarjoaa menetelmiä autonomisen hermoston toiminnan mittaamiseksi huomioimaan erityisesti hengityksen vaikutus estimoituja parametreihin. Väitöskirjan tuloksia voidaan soveltaa kardiorespiratorisista signaleja hyödyntävissä sovelluksissa aina kliiniseen työnsä myötä fysiologian tutkimukseen ja kaupallisiin hyvinvointi-, terveys- ja urheilusovelluksiin.

Asiakirjat: barorefleksiherkkyys, EKG-johdet tuhottavat signaalit, matalataajuinen vaihtelu, respiratorinen sinä arytmiin, sykevariaatio
To Minna S.

"Life is suffering" M.K.
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Oulu August 2019

Suvi Tiinanen
### Abbreviations

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tbody>
<tr>
<td>α</td>
<td>Alpha coefficient technique of BRS estimation</td>
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<tr>
<td>ANS</td>
<td>Autonomic nervous system</td>
</tr>
<tr>
<td>AR</td>
<td>Autoregressive</td>
</tr>
<tr>
<td>BP</td>
<td>Blood pressure</td>
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<td>BPV</td>
<td>Blood pressure variability</td>
</tr>
<tr>
<td>BEI</td>
<td>Baroreflex effectiveness index</td>
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<td>BRS</td>
<td>Baroreflex sensitivity</td>
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<tr>
<td>CAD</td>
<td>Coronary artery disease</td>
</tr>
<tr>
<td>CB</td>
<td>Controlled breathing</td>
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<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>DM</td>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiography</td>
</tr>
<tr>
<td>EDR</td>
<td>ECG-derived respiration</td>
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<tr>
<td>FFT</td>
<td>Fast Fourier transform</td>
</tr>
<tr>
<td>FIR</td>
<td>Finite impulse structure</td>
</tr>
<tr>
<td>Fs</td>
<td>Sampling frequency</td>
</tr>
<tr>
<td>HF</td>
<td>High frequency band around 0.15-0.4Hz</td>
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<tr>
<td>HR</td>
<td>Heart rate</td>
</tr>
<tr>
<td>HRV</td>
<td>Heart rate variability</td>
</tr>
<tr>
<td>HT</td>
<td>Hypertension</td>
</tr>
<tr>
<td>ICA</td>
<td>Independent component analysis</td>
</tr>
<tr>
<td>LF</td>
<td>Low frequency band around 0.04-0.15Hz</td>
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<tr>
<td>LMS</td>
<td>Least-mean-square</td>
</tr>
<tr>
<td>MI</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>PNS</td>
<td>Parasympathetic nervous system</td>
</tr>
<tr>
<td>PLF</td>
<td>Prevalent low frequency</td>
</tr>
<tr>
<td>PSD</td>
<td>Power spectral density</td>
</tr>
<tr>
<td>PCA</td>
<td>Principal component analysis</td>
</tr>
<tr>
<td>RESP</td>
<td>Respiration flow signal</td>
</tr>
<tr>
<td>RRI</td>
<td>Heart beat time interval</td>
</tr>
<tr>
<td>RAA</td>
<td>Renin–angiotensin–aldosterone system</td>
</tr>
<tr>
<td>RSA</td>
<td>Respiratory sinus arrhythmia</td>
</tr>
<tr>
<td>SB</td>
<td>Spontaneous breathing</td>
</tr>
<tr>
<td>SBP</td>
<td>Systolic blood pressure</td>
</tr>
<tr>
<td>SNS</td>
<td>Sympathetic nervous system</td>
</tr>
<tr>
<td>Acronym</td>
<td>Definition</td>
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<tr>
<td>---------</td>
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<tr>
<td>STD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>TF</td>
<td>Transfer function technique of BRS estimation</td>
</tr>
<tr>
<td>VLF</td>
<td>Very low frequency band around 0.003-0.04Hz</td>
</tr>
<tr>
<td>ULF</td>
<td>Ultra low frequency band below 0.003Hz</td>
</tr>
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List of original articles

This thesis is based on the following publications, which are referred throughout the text by their Roman numerals:


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1 Introduction

1.1 Background

Already early humans were probably aware of their heartbeat and breathing, especially during situations like physical exercise and sexual arousal when these vital parameters are changing. Similarly, they might have been noticed their pulsating arteries and veins. When methods to quantify time and measurement devices developed, also these physiological phenomena were quantified for the first time as heart rate (ca. 300 BC), breathing frequency (in 1707) and blood pressure (in 1847), respectively. Measurement and sensor technology development and computerization during the 20th century have resulted in a growing amount of measurements of continuous cardiorespiratory signals and research in the field, including signal processing and analysis of the measurements (Billman, 2011).

A cardiorespiratory system is highly controlled via the autonomic nervous system (ANS) to maintain the homeostasis in the body. Dysfunction of ANS regulation, also referred to as autonomic imbalance, is mainly characterized as increased sympathetic drive and decreased parasympathetic/vagal drive. Autonomic imbalance has a massive role linking several disorders and conditions to cardiovascular diseases that holds number one position in cause of deaths -- both globally and in Finland (THL 2016, WHO 2017). Cardiovascular diseases generate considerable economical load and human suffering yearly.

Autonomic imbalance may increase heart rate and conductivity through increased sympathetic activation, which increases cardiac load and thus exposes to cardiovascular diseases. Because ANS affect hormonal secretion, autonomic imbalance may increase oxidative stress, reduce vasodilation, increase chronic inflammation, and promote atherosclerosis progression, inducing cardiovascular diseases. In addition, risks and conditions such as smoking, psychological and work stress, sleep disturbances, metabolic syndrome and loss of cardiorespiratory fitness, result in autonomic imbalance, and thus are risk factors for cardiovascular diseases (Thayer, Yamamoto, & Brosschot, 2010).

The ANS function can be quantified noninvasively by measuring electrocardiogram (ECG), blood pressure (BP) and respiration signals and performing computerized analysis. The ANS regulation of cardiorespiratory system accomplishes characteristic oscillating patterns to beat-to-beat time series that can be derived from ECG and BP. The most important ANS regulation target is heart
rate, and its beat-by-beat variation analysis is referred to as heart rate variability (HRV) analysis. Similarly, blood pressure varies constantly, while respiration affects both heart rate and blood pressure generating respiratory sinus arrhythmia (RSA) phenomenon.

Autonomic imbalance may be assessed noninvasively using cardiorespiratory signal analysis. Above mentioned risks and conditions and several pathophysiological situations involving, e.g. cardiovascular, psychiatry, and neurological illnesses, affect and/or be affected by the ANS impairment. Different patient groups and healthy controls have been a base to study the ANS control mechanisms during pathological process (Caetano & Alves, 2015; Thayer et al., 2010). Clinical applications of cardiovascular signal processing, especially HRV, includes risk marker of different illnesses and prognostic marker of endpoints. For instance, low HRV after myocardial infarction predicts a greater risk of sudden death (La Rovere, Bigger, Marcus, Mortara, & Schwartz, 1998).

Physiology research has benefitted greatly from the cardiorespiratory signal processing. Different animal models, pharmacologic manipulation and provocative measurement protocols combined with development of signals processing methods have given insights into cardiovascular control mechanisms during the last three decades (Billman, 2011). The measurement and analysis of cardiorespiratory signals have a remarkable role in cardiology, both in clinical work and heart research. The 21st century has been the golden age for health, wellness and sport applications and devices that utilize cardiorespiratory signals providing quantified health parameters for the user (Lewis & Short, 2010; McCratty & Shaffer, 2015). These categories include also mobile phone apps and sensors (Bruining et al., 2014).

Signal processing methods, algorithms and models have been applied and developed to produce indices to describe the ANS regulation of cardiorespiratory system. An all-embracing model of a cardiovascular control system cannot be easily built apart from a large number of researches. The reason for this is the complexity of the ANS control of cardiorespiratory system. The challenge is to have simplified, cost-effective yet as physiologically right as possible methods to describe the ANS regulation of the cardiovascular system. The purpose of this thesis is to provide methods to quantify ANS regulation from short-term cardiorespiratory measurements with special emphasis on respiration effects.
1.2 Main objectives

This thesis focuses on cardiorespiratory signal processing where ECG, continuous BP and respiration flow signals are used to quantify ANS regulation. The research is cross-disciplinary, combining biosignal processing and heart and physiology research. The thesis is divided into four specific objectives briefly described below.

Objective 1: Remove the effect of respiration from cardiovascular signals and indexes

Heart rate intervals (RRi) and systolic blood pressure (SBP) series, derived from ECG and BP signals, are commonly used to describe the ANS regulation of cardiovascular system. Respiration modulates both RRi and SBP and thus the indices describing the ANS function, such as low and high frequency powers, are affected as well. Usually, breathing rate of human is about 0.25Hz, which is on the high frequency (HF) band (0.15-0.4Hz). However, the breathing rate can be low for some people, being on the low frequency (LF) range as well (0.04-0.15 Hz). Hence, the frequency domain parameters describing the cardiovascular function can be biased, depending on the breathing rate. Therefore, the very first objective of the thesis work was to develop a method to remove the respiration effects from cardiovascular signals and parameters.

Objective 2: Estimate the RSA index from cardiovascular signals

An RSA phenomenon is mainly generated by the parasympathetic branch of ANS. Therefore, the RSA index serves a noninvasive index describing the parasympathetic nervous activity. It is known that increased RSA protects the heart, while the decreased RSA is a prognostic indicator for cardiovascular diseases (Bental, Shamailov, & Paton, 2013), for example, the decreased RSA is a risk of sudden cardiac death among myocardial infarction patients (Peltola et al., 2008).

Several methods to describe RSA index do exist. A commonly used parameter to describe RSA is to quantify HF power of the RRi oscillations. A varying respiration pattern may complicate the traditional RSA analysis, however. Therefore, the objective was to develop novel methods to describe RSA by utilizing decomposition techniques.

Objective 3: Develop methods to study the LF power oscillations

LF power oscillations of RRi and SBP series describe the ANS regulation mechanisms of cardiovascular system. However, the mechanisms producing the LF
oscillation frequency are yet uncertain even though some evidence exists that increased frequency has prognostic value regarding cardiac diseases (Molgaard, Hermansen, & Bjerregaard, 1994; Veerman, Imholz, Wieling, Karemaker, & van Montfrans, 1994; Takalo, Korhonen, Majahalme, Tuomisto, & Turjanmaa, 1999). Several methods to estimate the frequency of oscillations exist and the aim was to develop tools to study LF oscillation frequency during physiological tests and among cardiovascular patients.

Objective 4: Develop novel ECG-derived respiration (EDR) methods

Breathing rate and volume are important parameters in several diseases. In laboratory conditions, breathing parameters are measured using spirometer or respiration belts, which both may affect persons’ natural respiration pattern and are sensitive to noise. In addition, these methods as such are not optimal for ambulatory monitoring. EDR methods to derive respiration pattern have been developed for some time already. The respiration pattern induces into ECG mechanically and via ANS, enabling capturing it by mathematical methods. The particular aim here was to improve and develop decomposition based EDR methods.

1.3 Original publications and authors’ contributions

In Paper I, a method that reduces the distortion effect in baroreflex sensitivity estimators caused by low respiration rate is developed. The method enables the usage of spontaneous breathing protocol in baroreflex sensitivity estimation. The author was mainly responsible for the study design and manuscript preparation, developed the algorithm and conducted the further implementation and data analysis. Dr. Mikko Tulppo presented the original problem in BRS estimation and Prof. Tapio Seppänen gave an idea to approach the problem. Dr. Tulppo and Prof. Seppänen supervised the work and proofread the manuscript. Dr. Tulppo also provided the used database.

Papers II and III present new algorithms to estimate the RSA component from cardiovascular signals. The author developed the algorithms, implemented and performed data analysis. The author was mainly responsible for manuscript preparations. Dr. Kiviniemi and Dr. Tulppo provided physiological aspects and ideas for the manuscripts and proofread the manuscripts. Prof. Seppänen supervised both papers and helped in paper finalization.

Paper IV aimed to study the low frequency oscillation of cardiovascular signals.
The author developed the software that was used for data analyses, wrote technological part of the paper and reviewed the paper. Prof. Seppänen has been supervising the computer software development. The person mainly responsible for Paper IV was Dr. Kiviniemi. Dr. Hautala, Dr. Norton, Dr. Frances, Dr. Nolan and Prof. Huikuri gave physiological and clinical insights for Paper IV and reviewed the manuscript.

Paper V aimed to study the low frequency oscillation of cardiovascular signals using spectrogram methods to demonstrate the rapid change in low frequency oscillation frequency during dynamical physiological test. The author implemented the algorithms, produced the results and prepared the manuscript. Dr. Kiviniemi and Dr. Tulppo provided data in Paper V, helped in physiological aspects and reviewed the paper. Prof. Seppänen helped in finalizing the paper and supervised the work.

New methods to obtain the respiration signal from single lead ECG were developed in Paper VI. The author developed the methods, performed data analysis and wrote the main parts of the manuscript. M.Sc. Kai Noponen developed the statistical analysis part of the study and wrote the manuscript part, respectively. In addition, M.Sc. Noponen gave ideas for algorithm development and revised the manuscript. Dr. Antti Kiviniemi and Dr. Tulppo gave original ideas for the paper and provided scenarios where developed methods are needed. Prof. Seppänen supervised the work and participated in manuscript revision.

In addition to methods developed during this thesis work, the computer software for cardiorespiratory signal processing was also implemented in Matlab environment and compiled to work as a stand-alone application in the Windows environment. Thus far, the software has been used in several studies of co-operation partners.
2 Literature review

This chapter provides current literature with relevant terms and definitions related to short-term cardio-respiratory signal processing. The literature review is divided into two sections: the first one reviewing the anatomy and physiology of cardiorespiratory system and its regulation mechanisms, and in the second part, the emphasis is on relevant signal processing methods and parameters.

Cardiovascular and respiratory system together is abbreviated as a cardiorespiratory system. The main function of the cardiorespiratory system is to maintain the homeostasis by meeting metabolic demands over a range of highly variable internal and external conditions in the body.

2.1 Autonomic nervous system regulation of cardiorespiratory system

The nervous system is divided into a central nervous systems (CNS), consisting of a brain and a spinal cord and a peripheral nervous systems (PNS). The PNS is further divided into autonomic nervous system (ANS) and somatic nervous system. The CNS gathers information outside and inside the body using the PNS, processes collected information, organizes reflexes and responses and sends output signals to the body. The most important functionalities of the brain, regarding this thesis work, are control centers of the heart, circulatory system and respiration, which are located in the medulla oblongata of the brainstem. The ANS regulates the function of organs mostly involuntarily by controlling the contraction of cardiac and smooth muscles and certain secretory and metabolic processes (Ganong, 2005). However, there is a growing body of evidence that individuals practicing yoga, meditation, and relaxation may exert at least some voluntary control over their blood pressure and heart rate that are normally regulated by the ANS (Lehrer & Gevirtz, 2014; Olex, Newberg, & Figueredo, 2013). The main tasks of ANS regulation regarding this thesis are control of the heart rate, blood pressure and respiration rate and flow (Bakewell, 1995).

The ANS is anatomically and functionally divided into parasympathetic (PSN) and sympathetic nervous systems (SNS), controlling organs, usually in an antagonistic way. The role of adrenergic SNS is accelerating and stimulating the organ function being the opposite of the cholinergic PSN control, i.e. smoothing and relaxing. The SNS enables the body to be prepared, e.g. for a fear or a fight reaction. The stimulation of PSN has an opposing effect; energy is restored and
body relaxed (Bakewell, 1995; Ganong, 2005). However, not all organs are regulated in an antagonistic way, for instance, blood vessel constriction and dilation (tonus) are regulated by the SNS only. In addition, both ANS divisions may be largely active occasionally compared to a situation where usually other ANS branch is dominant (Tulppo, Kiviniemi et al., 2005; Tulppo, Huikuri et al., 2005; Tulppo et al., 2011).

2.1.1 The heart

The heart is a hollow muscular organ with four chambers and situates in mediastinum, the space between the lungs. The heart can be regarded as two serial pumps from which the right one pumps deoxygenated blood from systemic circulation to pulmonary circulation (from body to lungs) and the left one pumps oxygenated blood from pulmonary to systemic circulation. The heart undergo alternating periods of relaxation, called diastole and contraction, referred to as systole, allowing the chambers to fill up blood and pump it out, and the pressure difference between aorta and right chamber to be accomplished which maintains the blood circulation. The heart has a valve system to maintain a unidirectional flow through the heart and the blood vessels (Ganong, 2005; Klabunde, 2004).

Cardiac excitation

Heart contraction and relaxation are accomplished using myoelectric signals spreading over the entire heart in an organized and timely way. The heart has specific conduction system through which the depolarization wave spreads towards the various parts of the heart. The conduction system consists of the sinoatrial node (SA node), internodal atrial pathways, atrioventricular node (AV node), bundle of His and its branches, and the Purkinje system.

Cell depolarization refers to a rapid change of electronic membrane potential towards positive voltage via ion channel currents. Repolarization refers to a change of electronic membrane potential towards negative voltage, respectively. The depolarization wave spreads from atria to ventricles and from conducting pathways to contractile cardiac muscle cells via gap junctions. The depolarization of the conducting pathway triggers the coordinated mechanical contraction of the heart recognised as a heartbeat. After the depolarization, the repolarization wave spreads from the ventricle to the atria and the heart relaxes. The organized cellular electrical activity of the heart generates macroscopic currents within the body, establishing
potential differences on the surface of the body that can be acquired as ECG using biomedical instrumentation amplifier (Goy, Stauffer, Schlaepfer, & Christeler, 2013).

Heart rate regulation

“A healthy heart is not a metronome”

(Shaffer, McCraty, & Zerr, 2014)

When heart rate (HR) is examined on a beat-to-beat basis, it is obvious that the HR is not a constant but instead varies all the time depending, among other things, on breathing, circadian rhythm, emotion, exercise and stress conditions. The HR variations result from complex, continuous and nonlinear interactions between a number of physiological regulation mechanisms that can be neural, hormonal, and mechanical control systems at both local and central levels (Reyes del Paso, Langewitz, Mulder, van Roon, & Duschek, 2013). The HR is determined as the amount of heartbeats per time interval (beats/min). Resting HR varies widely between different persons, from about 50 beats/min to 100 beats/min. The maximum HR is partly age-dependent so that aging gradually decreases the maximum HR (Hainsworth 2004).

The ANS regulation is the major contributor of the HR. A heart control center responds to sensory inputs from the body and higher brain center inputs from the cerebral cortex and limbic system, by adjusting the HR via sympathetic and parasympathetic (vagal) outflow (Shaffer & Venner, 2013). Generally, sympathetic activation increases the HR, conducting velocity and force of contraction, while parasympathetic stimulation of the heart has an opposite effect (Bakewell, 1995). The HR represent the net effect of parasympathetic and sympathetic activation. Under resting conditions, the parasympathetic branch is dominant. An increase of the HR can be accomplished by shifting the balance of the ANS towards the sympathetic branch. Therefore, HR reflects the relative activity of the sympathetic and parasympathetic systems (Klabunde, 2004).

Several reflex systems affect the HR, either accelerating it via sympathetic fibers or decelerating it via parasympathetic fibers, or both. Some common reflexes responsible for changing the HR are baroreflex (Eckberg & Sleight, 1992; Kirchheim, 1976), Bainbridge (atrial) reflex (Ledsome & Linden, 1964; Carswell, Hainsworth, & Ledsome, 1970), chemoreflex (Sampson & Hainsworth, 1972) to mention only a few. The baroreflex is the most dominant reflex response affecting
the HR as a response to blood pressure control (Hainsworth 2004). It is described in Section 2.1.2 in more details.

Respiratory sinus arrhythmia

The heart rate shows periodic variations synchronously with respiration. The heart rate accelerates during inspiration and decelerates during expiration. This phenomenon is called respiratory sinus arrhythmia (RSA) and it results from the complex interaction between the respiratory and cardiovascular systems (Eckberg, 1983a). The RSA seen in the HR is thought to illustrate a healthy phenomenon improving the efficiency of the gas exchange and reducing cardiac work by increasing pulmonary circulation during inspiration (Hayano, Yasuma, Okada, Mukai, & Fujinami, 1996; Ben - Tal, Shamailov, & Paton, 2012).

The central, neural, humoral and mechanical feedback mechanisms together generate the RSA (Anrep, Pascual, & Rossler, 1936; Jordan & Spyer, 1987; Grossman, Wilhelm, & Spoerle, 2004). For instance, hemodynamic reflexes and thoracic stretch receptors have an influence on the RSA (Hirsch & Bishop, 1981a; Akselrod et al., 1985). The RSA may also be generated by phasic increases in venous return, which has a direct mechanical influence on sinus node (Triedman & Saul, 1994; Sleight et al., 1995). Traditionally, the neural genesis of the RSA is thought to be mainly mediated through the changes in the cardiac vagal activity that is generated by CNS (Katona, Poitras, Barnett, & Terry, 1970; Katona & Jih, 1975). During inhalation, the vagal outflow is inhibited which accelerates the HR. Conversely, during exhalation, the vagal outflow is restored which decelerates the HR (Eckberg & Eckberg, 1982). The phenomena can be modelled as “common cardiopulmonary oscillator” having the RSA and the respiration frequency as its parallel outputs (Denver, Reed, & Porges, 2007; Eckberg, 2003).

2.1.2 Hemodynamics

Blood circulates from higher to lower pressure as the heart generates altering pressure conditions within each heartbeat. The blood is pumped from the left ventricle of the heart through a complex network of arteries, arterioles, and capillaries, and then returned through venules and veins to the right chamber of the heart. The energy stored in the elastic artery walls is released during diastole, enabling continuous blood flow. Parallel coupled arteries with adjustable diameter, enable a blood volume regulation in the different parts of the body (Ganong, 2005).
Systolic blood pressure (SBP) is measured during heart contraction as a maximum BP value. Diastolic blood pressure (DBP) is measured when the heart relaxes and BP reaches its minimum (Salvi, 2012).

**Blood pressure regulation**

The heart must create enough pressure to maintain circulation. However, BP should not be too high, either, because it creates extra load for the heart and damages smaller vessels. Therefore, the BP is constantly highly regulated. The regulation of the BP is either a short-term or a long-term regulation. Mean arterial pressure (MAP, mmHg) is defined with the equation $MAP = CO \times TPR$, where $TPR$ refers to total peripheral resistance resistance and cardiac output ($CO$, ml/min) is defined as $CO = HR \times SV$. Stroke volume ($SV$, ml/beat) is defined as the difference between the ventricular end-diastolic and end-systolic volumes. Hence, the major regulation targets regarding the BP control are $CO$, $TPR$ and $SV$. A number of factors influence the $CO$, $TPR$ and $SV$, and for this reason, the total endogenic regulation of BP is rather complex.

The cardiovascular control center regulates the BP neurally by sympathetic and parasympathetic outflow. The sympathetic outflow increases the BP via effector organs that are the heart, blood vessels, adrenal gland (medulla) and kidneys. The mechanisms that change the BP are fast direct neural cardiovascular effects and slower neuroendocrine mechanisms. The cardiovascular effects include, e.g. the change of diameter of the blood vessels (regulation of $TPR$) regulated through sympathetic outflow and the control of the heart pumping action (regulation of $CO$). Neuroendocrine mechanisms include release of catecholamines from adrenal gland and activation of renin–angiotensin–aldosterone (RAA) system in kidneys. RAA activation increases the BP via several mechanisms. In addition, $TPR$ is affected by local metabolic control in organs and by blood viscosity, which is determined by the amount of red blood cells (Ganong, 2005).

The most important mechanism for a short-term regulation of blood pressure is the baroreflex, described in the next Section. In addition, several other reflexes and responses have an effect on BP, e.g. the reflexes affecting the HR affect the BP also by changing CO. Bunches of physiological variation in BP exist, such as age that will gradually increase the BP. The BP is at a higher lever with males, and circadian rhythm decreases the nightly BP and increases the BP in daytime, reaching its top value on mornings. Cardiovascular responses associated with certain behaviours and emotions can change the BP. For instance, the “fight-or-
flight”-response activates the SNS and increases the BP. Mental stress and emotional excitement may increase the BP. In addition, exercise has many complex effects on the cardiovascular system. Generally, dynamic (muscular) exercise increases SBP. During static (isometric) exercise, both SBP and DBP increase (Hainsworth 2004). Respiration periodically influences the BP, decreasing it during inspiration and increasing it during expiration. This effect is mainly due to cyclical mechanical compression of thoracic cage.

**Baroreflex physiology**

The main function of the baroreflex is to prevent wide BP fluctuations. Arterial baroreceptors are mechanoreceptors locating in blood vessel walls of carotid sinus and aortic arch. Baroreceptors are constantly monitoring the BP and respond to a stretching of a vessel by changing their firing rate of action potential conducted to brain. Increased BP activates the baroreceptors and increase impulse rate conducted to CNS, respectively (Casadei 2004). Decreased BP has an opposing effect. Baroreceptors can adapt to a new pressure level, which is important when hypertension is developing (La Rovere et al. 1995). CNS integrates the impulses and as a response parasympathetic and sympathetic outflow regulates the heart and peripheral circulation. In the case of increasing BP, the HR and TPR are decreased to lower the BP via parasympathetic activation and sympathetic inhibition, respectively. Therefore, baroreceptors control the HR, cardiac contractility and vasoconstriction in order to regulate the BP (Hainsworth 1995; Klabunde, 2004).

Because it is much simpler to measure HR than TPR, the dynamic estimate of the baroreflex, referred to as baroreflex sensitivity (BRS), is generally estimated from HR only. Numbers of methods to estimate the BRS from SBP and RR interval time series have been developed. BRS estimation methods are described in Section 2.2.3. The relationship between the SBP and RR interval is found to be a nonlinear sigmoidal curve with a threshold and saturation (Di Rienzo et al., 2001).

The arterial baroreflex is believed to be a major contributor in the production of BP and HR oscillations. Due to the delays in baroreflex loop, a shift in BP is not corrected immediately but after the delay. Then, the reflex response for the BP shift modulates the baroreflex again, which in its turn, after a delay, causes a new BP shift, with a lower magnitude. These kinds of processes continue until BP reaches the set point causing a faded BP oscillation at a resonance frequency that depends on the time of the delay in the system. The frequency of generated oscillation equals twice the magnitude of the delay (Grodins & James, 1963; Hammer & Saul, 2005).
Parasympathetic (vagal) response in HR, i.e. in the case of increasing BP, is faster than sympathetic response, in a steady-state condition, it is about 800ms (Borst & Karemaker, 1983). HR sympathetic response differs from the vagal response by developing more slowly. The latency is up to 5s and then the HR gradually increases to a new level in 20-30s (Hainsworth 2004). Therefore, the HR and BP oscillation occurs at a frequency of about 0.1 Hz (10 s oscillation period) (deBoer, Karemaker, & Strackee, 1987a; Saul et al., 1991; Vaschillo, Lehrer, Rishe, & Konstantinov, 2002). The delays in vasomotor regulation are longer, causing about 0.03 Hz oscillations for cardiovascular signals (Magosso, Biavati, & Ursino, 2001; Vaschillo et al., 2002). It is debated whether baroreflex could produce some longer, from seconds to minutes, oscillating components also (Barrett & Malpas, 2005; Thrasher, 2005).

Mayer waves

Mayer waves are defined as vasomotor waves seen in the BP at frequency, which is lower than respiration rate (0.25Hz) (Mayer, 1876). Mayer waves occur at 0.1Hz (10 sec wave) in humans and are seen also in the RR interval time series. However, the genesis of Mayer waves is still under debate because several theories have been conducted producing a sort of “enigma” of Mayer waves (Julien, 2006). Two most common theories that explain the underlying mechanisms behind Mayer waves are central oscillations of sympathetic activity (Kaminski, Meyer, & Winter, 1970; Pagani et al., 1986; Preiss & Polosa, 1974) and resonance frequency of dynamic baroreflex function presented in the previous section (Bertram, Barres, Cuisinaud, & Julien, 1998; deBoer, Karemaker, & Strackee, 1987b; Julien, Chapuis, Cheng, & Barres, 2003). The evidence for central oscillation theory arises from animal studies where baroreflex influences have been eliminated, e.g. by chirurgical denervation. However, the data is not consistent and the frequency after baroreflex loop opening differs from a usual Mayer wave. Thus, it is assumed that the central nervous system may generate some endogenic oscillation pattern, but in physiological circumstances, Mayer waves are generated mostly by baroreflex (Julien, 2006). Therefore, manipulations and vasoactive drugs may change the LF power due to modulation of autonomic outflow by baroreflex.

Mayer waves are generally quantified, as low frequency (LF) oscillation of cardiovascular system and several widely applied methods to estimate the LF oscillation exist (see, Section 2.2.2 below). Mayer waves are enhanced during sympathetic activation such as physical activity, emotional reactions, standing or
head up tilt test (Cooke et al., 1999; Furlan et al., 2000) and are strongly attenuated by pharmacological blockade of alpha-adrenoreceptors, which decreases BP (van de Borne et al., 2001). Therefore, several studies have proposed that by measuring the amplitude of these oscillations provides an indirect measure of sympathetic nervous activity. However, contradictory studies have also been made that resulted in decreased LF oscillation in situations like aging and in some cardiovascular diseases where sympathetic activation is thought to be present (Ng, Callister, Johnson, & Seals, 1993; Veerman et al., 1994; van de Borne et al., 2001; Mussalo, Vanninen, Ikaheimo, Laitinen, & Hartikainen, 2003), indicating the complexity of the regulation mechanisms. It has been suggested that one task for Mayer waves is to liberate nitric oxide (vasodilator) from endothelium by cyclic changes of vascular shear stress, which could benefit the end organs (Julien, 2006).

### 2.1.3 Respiration

“Alive or just breathing?”

*(Killswitch Engage: 2nd Album 2002)*

Respiration refers to an intake of oxygen from air and removal of carbon dioxide from the body. Respiration includes inspiration and expiration phases. The synonym for respiration is ventilation. During inspiration, inspiratory muscles (diaphragm, scalene muscles and external intercostal muscles) contract, which expands the chest and air pass to lungs. During expiration, however, air moves outwards passive, inspiration muscles relaxes and the chest and lung volume reduces. During increased ventilation, abdominal wall muscles may also be recruited to help the expiration. The respiratory passages include the upper tract that is composed of the nasal and oral cavities, the pharynx and the larynx. The lower respiratory tract is composed of the tracheobronchial tree and the lung parenchyma with alveoli. The gas is changed between alveoli and lung capillaries by passive diffusion (Peracchia, 2014).

**Respiration regulation**

A characteristic respiratory pattern is generated in respiratory center in medulla oblongata of the CNS, which controls the respiratory muscles. Final respiratory output is influenced by a complex interaction between reticular formation of the brainstem and higher centers, including the limbic system and cortical structures.
Controlled respiratory parameters include respiration rate, which is normally about 12-20 breaths/min and depth of breathing, i.e. tidal volume being normally around 500ml in adults.

Respiration is primarily regulated for metabolic and homeostatic purposes. The control variables for respiration are partial pressure of carbon dioxide (pCO₂), partial pressure of oxygen (pO₂) and pH of the artery blood, which are constantly monitored by central and periphery chemoreceptors. Similarly, mechanoreceptors in the chest wall response to stretch of intercostal muscles, pulmonary stretch sensors in the tracheal and bronchial walls respond to increasing lung volume and muscle spindles in the respiratory muscles respond to changes in airway resistance in the lung and chest wall. Furthermore, irritant sensors in the bronchial mucosa, J-sensors in the alveolar walls, heat and cold sensors affect the respiration. Cardiovascular sensors affect also the respiratory center. Besides respiration flow and tidal volume, the ANS controls dilation of the bronchial branches via smooth muscle contractions such that sympathetic stimulus dilates the bronchial branches allowing more air to pass through the lungs and parasympathetic vice versa.

It is noteworthy that respiration is under voluntary control while speaking, singing, diving, etc. that allows all of the feedback sensor mechanisms to be overridden by cortical inputs. If homeostasis of internal environment deteriorates too much by voluntary control, autonomic control of respiration takes over, e.g. during syncope caused by breath holding.

Emotion expressions such as happy, fear, pain and joy and reflexes from sneezing, coughing, yawning and swallowing affect also the respiration (Ganong, 2005; Homma & Masaoka, 2008). As a feedback response, either respiration rate and/or tidal volume are changed in an appropriate way that causes variation in respiration rate and tidal volume during spontaneous breathing. The respiration variation is similar to beat-to-beat variation that is present in HR due to cardiovascular control mechanisms, but has received much less attention (Bruce, 1996; M. A. Cohen & Taylor, 2002).

While the RSA is determined by the modulation of heart rate by respiratory activity, cardioventilatory coupling is a triggering of respiration by cardiac activity. The relationship between HR and respiration is thought to be bi-directional and complex, creating synergies that promote healthy physiology. Cardiac and respiratory activities are linked both functionally and anatomically through highly overlapping brainstem networks (Baumert, Javorka, & Kabir, 2015; Garcia, Koschnitzky, Dashevskiy, & Ramirez, 2013). The clinical relevance of cardiovascular coupling arises from the fact that altered cardiovascular function
and imbalance of ANS control is commonly associated with respiratory diseases and dysautonomias (Trang, Boureghda, Denjoy, Alia, & Kabaker, 2003; Weese-Mayer et al., 2006; Cazzola et al., 2012). Respiratory dysfunction can negatively impact cardiovascular health and vice versa (Garcia et al., 2013).

2.2 Short-term cardiovascular signal processing

“Variability is the law of life…”

(William Osler, physician and educator, 1849–1919)

Ancient Greek physician Herophilus was the first to describe the heart rate and measure it with a water clock ca. 300 BC. It was 1707 when English physician John Floyer invented “The Physician Pulse Watch” that he used to tabulate the heart rate and respiration rate under several physiological conditions. In 1733, the Rev. Stephen Hales reported variation in blood pressure level and beat-to-beat interval in a glass pipe connected to carotid artery of a horse. In 1847, Carl Ludwig used a smoked drum kymograph to record the amplitude and timing of the arterial blood pressure wave of a dog. Ludwig was the first who also properly documented an increasing heart rate during inspiration and decreasing heart rate during expiration, a phenomenon which became to known as respiratory sinus arrhythmia (RSA) (Billman, 2011).

British physiologist Augustus Waller measured the first human electrocardiogram in 1887. Few years’ later, Dutch physiology William Einthoven invented galvanometer based device, more sensitive compared to Waller’s devise. In addition, Einthoven distinguishes the ECG deflections, and described the ECG features of several diseases (Billman, 2011).

Development of digital signal processing (Cooley & Tukey, 1965) and computer technology in the 1960s enabled analysis of beat-to-beat variations in the time domain. During the 1970s-1980s, power spectral analysis has been applied to study the oscillating components of the heart rate and the blood pressure. Several research groups performed pioneering work creating the physiological basis of cardiovascular variability analysis (Akselrod et al., 1981; B. Hyndman & Gregory, 1975; B. W. Hyndman, Kitney, & Sayers, 1971; Kay & Marple Jr, 1981; Malliani, Pagani, Lombardi, & Cerutti, 1991; Myers et al., 1986; Pagani et al., 1986; Penaz, Honzikova, & Fiser, 1978; Pomeranz et al., 1985; Sayers 1973). Since the 1990s, the field of cardiovascular variability analysis has been rapidly expanded with several application areas including clinical utility. Lately, other methods for
quantifying cardiovascular signal variability such as geometric and nonlinear methods, baroreflex sensitivity (BRS) estimation and heart rate turbulence have also been developed (Acharya, Joseph, Kannathal, Lim, & Suri, 2006; Kleiger, Stein, & Bigger, 2005). In addition to spectral analysis, methods such as multiparameter modelling (Baselli et al., 1994; Faes et al., 2004; Nollo et al., 2001; Porta, Baselli, Rimoldi, Malliani, & Pagani, 2000), higher-order spectrum estimation (Chua, Chandran, Acharya, & Lim, 2008), time-frequency and time-varying methods (Pola, Macerata, Emdin, & Marchesi, 1996; Mainardi, 2009) have been developed for cardiovascular signal analysis.

2.2.1 Cardiorespiratory signals

Cardiovascular variability signals regarding this thesis are ECG, continuous BP and respiration flow/volume signal. In addition, many other modalities where beat-to-beat variation occur exist, for instance, muscle sympathetic nervous activity (MSNA) from peroneus nerve and total peripheral resistance using ultrasound measurements. They all can serve a way to explore ANS regulation of cardiorespiratory system and are used, e.g. in more complex modelling, but not reviewed here because they are not in the scope of this thesis.

Measurement protocols

Noninvasive cardiorespiratory signals are acquired to investigate the role of ANS control of cardiovascular system both in clinical studies and in research purposes. The ANS control is complex while multiple active regulation mechanisms with several responses and reflexes influence the cardiorespiratory signals. Things like body position, emotional state, ingested food, medication and other substances may have an impact on ANS regulation (Freeman, 2006; Freeman & Chapleau, 2013). Therefore, special attention should be paid while performing the measurements to obtain comparable, reproducible and reliable results. Caffeine and nicotine are usually prohibited for at least 3-4 h before the measurements and alcohol for 8 h. If possible, sympathomimetic and anticholinergics drugs should be stopped for 24-48h. Directly before testing, the patient should be laid down or seated for about 30 min in a quiet room with neutral temperature and humidity (Jaradeh & Prieto, 2003).

This thesis work operates with short-term cardiovascular signals. Short-term refers to signals that are measured usually during 2-15 minutes. Most often, the used signal length in the laboratory measurements is 5 minutes, which is adequate
to distinguish the major oscillations of the ANS regulation (Heart rate variability: Standards of measurement, physiological interpretation and clinical use, 1996). Short-term measurements are performed in laboratory conditions with supine, sitting or standing positions and with spontaneous breathing (SB) or controlled breathing (CB). Maneuvers elucidating physiologic, pharmacologic, or pathologic changes in ANS function may be used in the measurements (Kleiger et al., 2005). Long-term cardiovascular signal analysis usually implies to 24-hour recordings and it should always distinguished from short-term analysis (Heart rate variability: Standards of measurement, physiological interpretation and clinical use, 1996). Long-term recordings can be used to assess ANS responses during normal daily activities in health, disease, and in response to therapeutic interventions, e.g. exercise or drugs, and are particularly useful for risk stratification (Kleiger et al., 2005).

Several test protocols to explore the ANS function of cardiovascular system are also developed (Freeman, 2006; Hilz & Dütsch, 2006). These tests are provocative to autonomic cardiovascular reflexes and their usage gives important insights into ANS function. Due to the complexity of the ANS, tests are not necessarily inducing either purely sympathetic or parasympathetic outflow. ANS function tests include, among others Valsalva maneuver, which is used for evaluating sympathetic functions with BP responses and parasympathetic functions using the HR responses (Sarnoff, Hardenbergh, & Whittenberger, 1948; Smith et al., 1996), deep breathing used to explore parasympathetic function via RSA modulation (Jaradeh & Prieto, 2003), orthostatic test that is parasympathetic stimulus (Furlan et al., 2000). Mainly sympathetic stimulus is achieved via BP raise which is achieved via isometric handgrip exercise test (Ewing, Martyn, Young, & Clarke, 1985; Seals, Chase, & Taylor, 1988a), cold pressor test (Lafleche, Pannier, Laloux, & Safar, 1998), passive head-up tilt test and mental arithmetic tests (Freeman & Chapleau, 2013; Jaradeh & Prieto, 2003). Pharmacological or nonpharmacological interventions are a widely used tool to assess human autonomic function (Tulppo et al., 2001; J. J. Goldberger, Challapalli, Tung, Parker, & Kadish, 2001; Tulppo et al., 2005). In addition to cardiorespiratory signal analysis, other modalities may be used to explore ANS function. These include measurements of neurotransmitter levels, microneurography, thermoregulatory sweat test, galvanic skin response (GSR) to mention only a few (Freeman & Chapleau, 2013).
**Signal acquisition**

In addition to measurement protocols, special emphasis needs to be put on proper signal acquisition to obtain good quality signals for the analysis. Cardiorespiratory signals, i.e. ECG, continuous BP and respiration flow/volume regarding this thesis, are acquired simultaneously or with known time delays using attached sensors and a suitable recording device.

The ECG is acquired by recording the electrical activity of the heart with electrodes placed on a person’s body surface. Commonly, Ag/AgCl electrodes are used in ECG measurements and placed on the body to standardized locations, e.g. according to 12-lead ECG. Single-channel ECG with lead II is often used in cardiovascular signal analysis. The ECG consists of its characteristic waves P, Q, R, S and T generated by the depolarization and repolarization of the atria and ventricles (Clifford, Azuaje, & McSharry, 2006a; Goy et al., 2013).

The gold standard method of measuring BP is invasive intra-arterial catheter which, however, is not a very convenient method outside the operating rooms (Langton & Stoker, 2001; Ward & Langton, 2007). Therefore, continuous arterial BP signal is usually acquired noninvasively using finger arterial pressure monitoring such as Finapres™ (Imholz, Wieling, van Montfrans, & Wesseling, 1998). Continuous noninvasive methods utilize Penaz principle which states that a generated force by a body can be determined by measuring the opposing force that prevents the disruption (Penaz, 1973). In finger arterial pressure monitoring, a pressure of a cuff around a finger is continuously adjusted to maintain the vascular volume constant value. A light emitting diode within the cuff shines light through the finger, which is proportional to the volume of the blood and detected on the other side. The amount of light absorbed is kept constant via cuff pressure that equals to the arterial blood pressure. The cuff pressure is measured, and absolute BP values are obtained using calibration with an arm cuff (Ward & Langton, 2007). The performance of noninvasive methods against the invasive method by intra-arterial catheter has been validated (Imholz et al., 1998; Omboni et al., 1993). Methodical issues with calibration may have an impact on spectral estimation (Kiviniemi et al., 2014). In addition, the method may be less accurate in hypotensive patients or those with vascular insufficiency. Positioning and tightness of a cuff may lead to variation in measurements (Langton & Stoker, 2001).

Lung function can be measured with several different modalities, depending on the viewpoint, but in the case of cardiovascular signal processing, it is most useful to have continuous respiration flow signal or volume signal. Some of the
cardiovascular parameters and indices are highly correlating with respiration flow estimate and the respiratory information may provide reliability of the analysis. Respiratory volume is a derivative for flow so it can be derived from flow or vice versa. The gold standard for respiration flow is spirometry (Miller et al., 2005), which can be undertaken typically using a mouthpiece or a mask, including a separate nose blocker that ensures the airflow goes through the measurement sensor only. The spirometer equipment itself may cause unpleasant user experience. Spirometry measures either continuous flow or volume signal as a function of time. Another commonly used measurement devise for respiration flow/volume measurement is respiratory effort belts that are elastic belts placed around the rib cage and abdomen of the subject to measure breathing. The respiratory belts are more convenient to use but they have some disadvantages such as the subject’s body position and breathing style may distort the calibration and signal modelling (Seppanen, Alho, & Seppanen, 2013; Tobin, 1992). Finally, thermistor based temperature sensors are also used for respiration flow estimation. Usually, temperature sensors are not very sensitive and circumstances such as room temperature may distort the measurements.

Acquired physiological signals are AD-transformed and recorded with suitable sampling frequency larger than 250Hz to guarantee the robust R peak detection and resolution of ≤ 4 ms (Heart rate variability: Standards of measurement, physiological interpretation and clinical use, 1996; Kuusela, 2013). Nowadays, in laboratory environments, computer storage properties and recording devises are improved, enabling good quality signals to be easily recorded. In the cases such as health applications and mobile data, recording function may set some limits to signal quality. Figure 1 below presents the simultaneously acquired ECG, BP and respiration signals.

**Pre-processing and beat-to-beat parametrization**

Continuous ECG, BP and respiration signals are first parametrized and preprocessed using standardized methods (Heart rate variability: Standards of measurement, physiological interpretation and clinical use, 1996). Beat-to-beat variation in ECG and BP signals reflect the regulation mechanism of the ANS that have oscillating nature. The parameters of interest regarding this thesis work are heart rate intervals (RRI) and systolic blood pressure (SBP) derived from the ECG and continuous measurement of blood pressure.
Robust algorithms and good quality of the signals ensure the accuracy of fiducial point estimation of a signal that is often R peak in ECG and maximum value in BP signal (Kamath MV & Fallen EL 1995). RRi or heart rate variability (HRV) signal is obtained by aligning consecutive RR time intervals (msec or sec). The time series are called as tachogram for the series of RRi and systogram for the series of SBP values. Examples of the tachogram and systogram are presented in Figure 1. It should be noted that other beat-to-beat parameters such as diastolic blood pressure (DBP) and mean arterial pressure (MAP) could also be extracted depending on the control system that is under examination.

Because series of RRi and SBP are collected using irregular time spans due to variable duration of adjacent heartbeats, special attention should be paid when spectrum analysis is performed. There are two options to overcome this problem: interpolating the data evenly or perform the spectrum analysis in the cycles/beat – plane instead of Hz-plane (Deboer, Karemaker, & Strackee, 1984; Malliani et al., 1991). Spectrum analysis in the cycles/beat –plane may affect the morphology, the measurement units of the spectra, and the relevant spectral parameters, and thus the interpolation is a method that is more favorable. Various interpolation methods such as linear (Lippman, Stein, & Lerman, 1994) and spline interpolation methods (Press, Teukolsky, Vetterling, & Flannery, 1996) with a sampling rate 2-4 Hz are used (Heart rate variability: Standards of measurement, physiological interpretation and clinical use, 1996). Special attention should also be paid for the phase relationships and the synchronization while deriving the RRi and SBP series, especially if intersignal properties are studied.

Visual inspection and validation of the data is advisable to perform to observe, e.g. ectopic beats, arrhythmic events, missing data or noise in a data. These kinds of errors may be decreased using proper data interpolation methods. Noise reduction should apply according to noise source induced to signals, e.g. notch filtering for 50Hz electric interference removal (Heart rate variability: Standards of measurement, physiological interpretation and clinical use, 1996; Kuusela, 2013). Often, baseline is removed using detrending methods such as Savitski-golay
(Orfanidis S.J, 1996). In short-term analysis, trend removal should only be used for frequencies lower than oscillating component of interest.

Fig. 1. Short segments of simultaneous ECG, continuous blood pressure and respiration signals and their derivative beat-to-beat signals.

2.2.2 Cardiovascular variability analysis

Heart rate and blood pressure variability

Heart rate variability (HRV) is defined as the variation over time of the period between consecutive heartbeats and depends on the current regulation of the heart rate (HR). The normal HR variation is due to autonomic neural regulation, i.e. ANS control, of the cardiorespiratory system. The HRV analysis refers to computer based analytical tools that are used to assess overall cardiac health and ANS regulation. HRV analysis is usually performed both in constant steady-state
circumstances and during modulation or stimulation, e.g. during standard provocative tests (Freeman, 2006; Heathers, 2014; Thayer et al., 2010).

Methods for quantifying HRV may be categorized as time domain, spectral or frequency domain, geometric, and nonlinear methods (Kleiger et al., 2005). Short-term recordings, which are utilized in this thesis, are mostly analyzed with frequency domain methods, for which reason these methods are described in more detail in the next Section. The rest of the HRV methods are mainly used to analyze the long-term data such as 24h Holter recordings, which, however, are not in the scope of this thesis, and thus are not reviewed here. A comprehensive review of these methods can be found, e.g. from (Cygankiewicz & Zareba, 2013; Shaffer et al., 2014; Xhyheri, Manfrini, Mazzolini, Pizzi, & Bugiardini, 2012).

A first report towards standardization of HRV analysis methods was published 1996 (Heart rate variability: Standards of measurement, physiological interpretation and clinical use, 1996). Later, a huge number of publications where HRV methods are used have been published and criticism towards HRV methods has been presented (Heathers, 2014; Sassi et al., 2015). Furthermore, HRV methods are still not widely used in clinical settings. The reasons for this include, among others, the fact that the selection of analysis method may produce a different scale of results, the length of electrocardiogram recordings may vary affecting the results, and that HRV is highly sensitive to measurement protocols, emotions, etc. Additionally, there is lack of agreed normative values for HRV, without which it is clinically difficult to classify HRV.

Blood pressure variability (BPV) may be quantified using partly similar methods than are used in HRV analysis. Especially frequency domain methods are adopted for BPV analysis. From BPV, contrary to HRV, it is known that an increased variability represents a major risk factor for the occurrence of fatal events (Floras, 2013; Mancia & Parati, 2003; Parati et al., 2003; Tozawa, Iseki, Yoshi, & Fukiyama, 1999).

**Power spectral analysis of cardiovascular signals**

Power spectral density (PSD) analysis of the beat-to-beat variations of RR intervals and BP series has become a widely used method to quantify cardiac autonomic regulation (Akselrod et al., 1981; Akselrod et al., 1985; M. A. Cohen & Taylor, 2002; Heathers, 2014; Taylor & Eckberg, 1996). The PSD of the signal is a linear transformation from time domain to frequency domain. Several methods have been developed for PSD estimation in the literature (Bendat J. & Piersol A., 1980;
Haykin S., 1996; Ifeachor E. & Jervis B., 1993; Proakis J. & Manolakis D., 1996) and two methods are commonly applied with the beat-to-beat data. These include nonparametric Fast Fourier Transform (FFT) based methods (Akselrod et al., 1981; Sayers 1973) and parametric autoregressive (AR) modelling (Baselli & Cerutti, 1985) based spectrum estimation. The limitation of FFT and AR based algorithms is that the signal to be analyzed should be at least weakly stationary. The weak stationarity means that the joint probability distributions of a stochastic signal do not change over time, indicating that the mean and the variance should not change. Conventional stationarity testing may be applied to prior analysis (Heart rate variability: Standards of measurement, physiological interpretation and clinical use, 1996; Bendat J. & Piersol A., 1980).

FFT based algorithms are nonparametric methods that include, e.g. periodogram, Bartlett’s method and the Welch’s method (Proakis J. & Manolakis D., 1996). Two latter methods divide the analyzed data into segments, which reduces the variance of the mean spectrum estimate. Welch's method is an improvement of the periodogram spectrum estimation and Bartlett's methods, which use overlapping (typically 50%) windows. Hamming, Hann or Gaussian windows are typically used for segments to reduce spectral leakage that a rectangular window may cause.

The AR model is the most popular parametric method. In parametric methods, the observed cardiovascular signal is first modelled as an output of a linear filter with a white noise as an input. Then, the PSD is obtained from the estimated filter coefficients. The assumption is that each value of the process depends only on a weighted sum of the previous values from the same process plus "noise". There are several criteria to evaluate an optimal model order such as final prediction error, Akaike’s information criterion (AIC) and minimum description length (MDL) (Proakis J. & Manolakis D., 1996). Model order criteria work if the signal is a pure parametric process, which is rarely the case, and thus these criteria can only be used as guidelines. A general guideline is to use model order 8 to 20 (Heart rate variability: Standards of measurement, physiological interpretation and clinical use, 1996).

The main advantage of FFT based methods is their computational efficiency compared to an AR method (Heart rate variability: Standards of measurement, physiological interpretation and clinical use, 1996). The AR method, on the other hand, produces smoother spectra, which are more easily interpreted. In the case of short data length, spectral resolution in the AR method is better. Generally, both methods have been evaluated to give reasonable results (Heart rate variability:

The PSD described above may be calculated for analyzing the frequency content of beat-to-beat cardiovascular signals that are RRi and SBP series regarding to this thesis work. Cross spectrum analysis is used to analyze the correlation between these signals at different frequencies. A cross-spectral density is described as a Fourier Transform of the cross-covariance function of the signal. The squared coherence function reflects linear correlation between signals (Bendat J. & Piersol A., 1980).

Spectral components and indices

Cardiovascular oscillations have been traditionally divided into four primary frequency bands or components that are the high frequency (HF, 0.15-0.4Hz), low frequency (LF, 0.04-0.15Hz), very-low frequency (VLF, 0.0033-0.04Hz), and ultra-low frequency (ULF, < 0.003 Hz) bands (Heart rate variability: Standards of measurement, physiological interpretation and clinical use, 1996). The VLF, LF and HF powers are determined from both short-term and long-term recordings when ULF band can only be determined from long-term recordings. The ULF band may reflect circadian oscillation, temperature regulation, metabolism and the function of renin-angiotensin (Heart rate variability: Standards of measurement, physiological interpretation and clinical use, 1996; Bonaduce et al., 1994). The data obtained from long-term recording is usually analyzed in 5 min segments and overall powers are calculated averaging the 5 min segments (Heart rate variability: Standards of measurement, physiological interpretation and clinical use, 1996).

The HF component is around 0.15-0.4Hz and reflects parasympathetic (vagal) activity related to the respiratory cycle (B. W. Hyndman et al., 1971). The LF power band is around 0.04-0.15 Hz and modulated mainly with baroreflex control (Akselrod et al., 1985) reflecting both the sympathetic and parasympathetic activity. The mechanisms how respiration induces changes in HR via the RSA phenomena and further the BP (Eckberg, 1983a; Eckberg, 2003) and the genesis of LF band oscillations (Saul et al., 1991; Taylor & Eckberg, 1996) are described in Sections 2.1.1 and 2.1.2, respectively.

The regulatory mechanisms that are generating the VLF component have not been as well defined as the LF and HF components. The VLF is assumed to reflect thermoregulation, vasomotor activity and renin-aldosterone system (Shaffer 2007, Cyganievic 2013). However, recent experimental evidence suggests that the heart
intrinsically generates the VLF oscillation and that these oscillations are modulated by sympathetic activity (Armour 2003, Shaffer 2007). Increases in resting VLF power may reflect increased sympathetic activity and factors that increase sympathetic activation can cause it to cross over into the lower region of the LF band (Shaffer et al., 2014).

The absolute powers, i.e. as ms² for RRi and as mmHg² for SBP series, in the above-mentioned spectral bands are calculated from the PSD estimates. The power components are often presented as normalized units (nu), which represent the relative value of LF and HF power component in proportion to the total power from which the VLF component is first subtracted. The normalization of the components minimizes the effect of large intersubject variation that may occur in total powers (Heart rate variability: Standards of measurement, physiological interpretation and clinical use, 1996).

In addition, LF/HF ratio is usually determined (Heart rate variability: Standards of measurement, physiological interpretation and clinical use, 1996; Pagani et al., 1986), which is characterized originally as “sympatho-vagal balance”. Nowadays, its usage as a pure index of that has been challenged, as the regulatory mechanisms of the LF oscillations reflects the baroreflex function and constitutes vagal activity as well (Heathers, 2014; Porges, 2007; Rahman, Pechnik, Gross, Sewell, & Goldstein, 2011; Tiller, McCraty, & Atkinson, 1996). Peak or center frequencies in the bands may also be described as a characteristic frequency of oscillating component. The characteristic frequency of a signal is usually described as a frequency of a highest spectral peak, i.e. the mode frequency, mean frequency or median frequency (Stulen & De Luca, 1981).

**Time-frequency representation and time-varying methods**

Traditional power spectral analysis of cardiovascular signals presumes the signal to be stationary. Therefore, methods to overcome this limitation have been developed to better explore the ANS function in various situations, e.g. in provocative stress testing, sleep or daily-life activities and to describe and quantify the changing spectra parameters and indices of transient data (Mainardi, 2009). Time-frequency representation (TFR) of a signal provides both time and spectral information simultaneously compared to as PSD, which can only serve the frequency content of a signal with good spectrum resolution. Time-varying methods implies to algorithms, which have time-dependent parameters.
The quantification of the power and frequencies of the LF and HF components or a tracking of a certain physiological phenomenon such as respiration frequency may be approached using various time–frequency methods. These algorithms include, e.g. short-term Fourier transform (STFT) (Vila et al., 1997), time-varying autoregressive (TVAR) analysis (Bianchi et al., 1994; Bianchi, Mainardi, Meloni, Chierchiu, & Cerutti, 1997), time–frequency representations (TFR) (Novak & Novak, 1993) and wavelet decomposition (Toledo, Gurevitz, Hod, Eldar, & Akselrod, 2003). In addition, empirical mode decomposition (EMD) and complex demodulation (CD) techniques have been applied to track time-varying components of cardiovascular data (Mainardi, 2009). The drawback of TFR and time-varying methods is their complexity and computational load, which have been limiting factors, especially in ambulatory applications.

In STFT, data is first divided into shorter segments of equal length, and then the Fourier transform is computed separately on each segment. Therefore, the Fourier spectrum is obtained as a function of time and is usually plotted as a spectrogram, which is squared magnitude of the STFT. There is always a trade-off between the time and frequency spectrum resolution when selecting the segment size (Vila et al., 1997).

In the time-varying AR model (TVAR), the cardiovascular variability signal (e.g. HRV signal) is first modeled as an output of the model by estimating model parameter vector at each time instant. When the current estimate of the AR parameter vector is available, the time–frequency information is obtained by computing a time-varying spectrum from the parameter vector. AR model parameter estimation may be accomplished with several algorithms such as smoothed Kalman filter (Tarvainen, Georgiadis, Ranta-aho, & Karjalainen, 2006a) and recursive least-squares (RLS) estimation (Bianchi et al., 1997; Cerutti, Mainardi, & Bianchi, 2002).

Wigner–Ville distribution (WVD) is defined as the Fourier transform of the instantaneous autocorrelation function of a signal. The WVD is quadratic form of TFR from which we can reason that there are cross-terms (interference terms) present making the interpretation of TFR more difficult. The cross-terms can be attenuated, however, using suitable transformation of Cohen’s class (L. Cohen, 1995). The smoothed pseudo-Wigner–Ville (SPWV) has Cohen’s class of frequency and time smoothing windows making the method preferable for the analysis of cardiovascular signals (Novak & Novak, 1993; Pola et al., 1996).

In wavelet analysis, the signal is transformed using wavelets that are corresponding to the cosine and sine unit vectors. Wavelet analysis is based on
recursive sums and differences of the vector components that correspond to different frequency bands. Wavelet analysis allows a multi-resolution analysis of data with different scales and is a popular TFR method (Mainardi, 2009; Toledo et al., 2003).

Applications and clinical utility

Since the very first step of HRV analysis, the clinical emphasis has been on exploring ANS control in cardiac diseases (Bigger et al., 1992; Wolf, Varigos, Hunt, & Sloman, 1978). The clinical relevance of the HRV was first noticed with fetuses whose beat-to-beat variability correlated with fetal viability (HON & LEE, 1963). HRV analysis has been applied in various pathophysiological conditions and it has shown its ability as an independent predictor of deteriorated prognosis in several illnesses by impaired autonomic activity. These illnesses include, among others, myocardial infarction, congestive heart failure, coronary artery disease and essential hypertension. In addition the ANS function has been found impaired using HRV analysis in several non-cardiac diseases such as diabetes mellitus and diabetic neuropathy, end-stage renal disease, multiple sclerosis and several psychiatric disorders such as depression and schizophrenia (Cygankiewicz & Zareba, 2013; Kleiger et al., 2005; Thayer et al., 2010; Xhyheri et al., 2012). The most important clinical HRV application is that impaired autonomic activity, recognized as elevated sympathetic and lowered vagal activities, has been shown to be an independent predictor of mortality after myocardial infarction (Bigger et al., 1992; Huikuri et al., 2000; Kleiger, Miller, Bigger, & Moss, 1987; Stein, Domitrovich, Huikuri, & Kleiger, 2005; Wolf et al., 1978).

Besides clinical applications, cardiovascular signal analysis has several other applications. One major field of HRV applications includes sport, exercise and rehabilitation applications (C. C. da Silva, Pereira, Cardoso, Moore, & Nakamura, 2014; V. P. da Silva, de Oliveira, Silveira, Mello, & Deslandes, 2015; Lewis & Short, 2010; Plews, Laursen, Stanley, Kilding, & Buchheit, 2013; Prinsloo, Rauch, & Derman, 2014). A lately emerging area where cardiovascular variability is used to treat a bunch of disorders is biofeedback interventions. HRV and other ANS measures are used in an intervention session where a person is aware of practicing brain-heart connection to improve, e.g. HRV impairment or other illnesses (Baldwin & Hammerschlag, 2014; Conder & Conder, 2014; Lehrer & Gevirtz, 2014; Manea, Comsa, Minca, Dragos, & Popa, 2015; Wheat & Larkin, 2010). Affective computing and emotion recognition have been applied to cardiovascular.
signal variability analysis and is also an emerging application area (Honna & Masaoka, 2008; Koelsch & Jancke, 2015; Kortelainen et al., 2012; G. Park & Thayer, 2014; Valderas, Bolea, Laguna, Vallverdu, & Bailon, 2015).

2.2.3 Baroreflex sensitivity estimation

Baroreflex sensitivity (BRS) is described as the amount of response in the HR due to a change in BP expressed in units of ms/mmHg. Methods for BRS estimation can be categorized as “classic” and “modern” techniques that are utilising spontaneous RRI and SBP variability signals (Di Rienzo, Castiglioni, Mancia, Pedotti, & Parati, 2001). In “classic” techniques, an external stimulus is used to evoke a change in BP or to trigger the baroreceptors. BP change is evoked e.g. using vasoactive drugs (La Rovere, Pinna, & Raczak, 2008), Valsalva manoeuvre (Sarnoff et al., 1948; Smith et al., 1996) or a neck chamber (Eckberg, Cavanaugh, Mark, & Abboud, 1975; Eckberg, 1977). Linear association between SBP and RR interval is assessed by Pearson’s correlation coefficient as a quantitative index of BRS (La Rovere et al., 2011).

In “modern” techniques, spontaneous fluctuations of BP and HR are used making the measurements more comfortable for subjects and providing new ways to perform measurements in various situations, also outside the laboratory environment. Several studies have showed a correlation between drug-induced and modern methods and accepted modern techniques as an alternative for invasive “classic” BRS techniques (Parlow, Viale, Annat, Hughson, & Quentin, 1995; Pellizzer, Kamen, Jackman, Brazzale, & Krum, 1996; Watkins, Grossman, & Sherwood, 1996).

“Modern” techniques to assess BRS include a sequence method in the time domain (Bertinieri et al., 1985; Bertinieri et al., 1988; Di Rienzo, Bertinieri, Mancia, & Pedotti, 1985) two spectral methods, alpha coefficient (Pagani et al., 1988) and transfer function techniques (Robbe et al., 1987). In the sequence method, first, the sequences where both SBP and subsequent RR interval change in the same direction, are identified. Next, the regression slopes are calculated for each identified sequence. Finally, the estimate of BRS is obtained by calculating the average value of all slopes.

The alpha coefficient (α) method is based on the assumption that the SBP and RRI are highly linearly correlated at HF and LF bands, and on the hypothesis that the correlation at these two frequency bands is due to the baroreflex coupling. First, the PSD of RRI and SBP series, and their coherence function are computed.
Absolute powers in the LF and HF band are calculated from the frequencies where coherence criterion > 0.5 holds. Then, the square-root ratio of RR and SBP powers for both LF and HF bands are computed as BRS(LF) and BRS(HF) estimates. Depending on the application, a mean value of BRS(LF) and BRS(HF) may be calculated as BRS(mean).

The classical open loop model for baroreflex control can be described as a single input single output system between SBP (input) and RR intervals (output). Transfer function of the open loop model is described as:

\[ H(f) = \frac{S_{SBP,RR}(f)}{S_{SBP}(f)} \]  (1)

where \( S_{SBP}(f) \) is PSD of SBP and \( S_{SBP,RR}(f) \) is the cross-spectrum of the SBP and RRI signals. The gain of \( H(f) \) determines the BRS (Robbe et al., 1987). The BRS is quantified as an average transfer function gain in LF range. Previously, a coherence > 0.5 has been applied but lately it is advised that the whole LF band, regardless of coherence values, could be used (La Rovere et al., 2008; La Rovere et al., 2011).

The main limitation of “modern” BRS techniques is the simplification made that the baroreflex control system is thought to be open, i.e. all the changes in RR intervals is baroreflex feedback origin. In reality, the feedforward effect does exist, i.e. HR and thus RR intervals affect the BP, as mentioned in Section 2.1.2. Therefore, open loop models lump together both feedforward and feedback effects (Bertinieri et al., 1988; Porta et al., 2000).

While the “traditional” and “modern” BRS estimation methods are the most commonly applied BRS methods, in the last few decades, several more complex mathematical models have also been proposed to better clarify the functioning of the baroreflex control mechanisms (Di Rienzo, Parati, Radaelli, & Castiglioni, 2009). These models may approach the baroreflex modelling either by black box or by the physiological descriptions, considering the causality issues between RR intervals and BP. Models are, however, more complicated. In addition, e.g. the modelled nonlinearities in the system, the type of used variables, the baroreflex resetting phenomenon, and the capability to include some specific real cardiovascular dynamics, such as respiratory or Mayer wave differ between models.

Overall, the open loop models are easier to apply, and they are used more often than complex models, which, however, mimic the physiology better. It has been observed that the BRS gain is continuously modulated over time because of central influences aimed at optimizing the response of the cardiovascular system to the
daily life demands (Smyth, Sleight, & Pickering, 1969; Eckberg, 1977; Pagani et al., 1988). Partly from this reason, the reproducibility of BRS measurement is not that good in both open loop and closed loop models. In addition, respiration conditions affect the estimation, which is why controlled respiration is usually used. In the studies with the sequence method, it has been noticed that not all increasing or decreasing SBP ramps affect the baroreflex-driven changes in RRI ramps. This phenomenon is quantified as the ratio between the number of spontaneous SBP ramps followed by a reflex RRI change and the total number of SBP ramps, named as baroreflex effectiveness index (BEI). With healthy subjects, the BEI is about 0.20 indicating that non-baroreflex mechanisms such as central inhibitory influences or interferences exist as well (Di Rienzo et al., 2001).

The BRS is assessed both in cardiovascular research to explore an autonomic control of cardiovascular system and in a clinical setting. Several studies have shown that the BRS have a prognostic value in congestive heart failure, myocardial infarction, diabetes mellitus and hypertension (Parati, Di Rienzo, & Mancia, 2000). An impairment of the baroreflex may cause dysregulation of BP, leading to an increased BP variability with sudden pressure drops on shifting from supine to standing position as well as pressure rises with an increased risk for fatal events such as myocardial infarction and stroke (Di Rienzo et al., 2009).

2.2.4 Respiratory sinus arrhythmia quantification

The physiological background of RSA phenomenon was described in Section 2.1.1. As it is stated, the RSA is thought to describe mostly vagal activity of the ANS function (Eckberg, 1983a; Fouad, Tarazi, Ferrario, Fighaly, & Alicandri, 1984). The understandings of the physiological mechanisms mediating the respiratory induced cardiovascular fluctuations remain still incomplete, however. Various methods to quantify the RSA index from the HR oscillations have been developed during the last few decades.

The HF power of RRI series is most often used as an RSA index (Hayano et al., 1996; Pomeranz et al., 1985). The methods to quantify the RSA index can be roughly divided into time domain, frequency domain and time-varying methods. In addition, SD1 of the Poincare plot, which is classified as a geometric HRV method, has been used as a measure for RSA (Brennan, Palaniswami, & Kamen, 2001). Next, the selected key methods to describe the RSA index are presented.

In addition to FFT and AR based PSD methods used in spectrum analysis to obtain HF power as the RSA index, wavelet analysis has been applied as well.
The advantage in wavelet analysis is that weak stationary assumption is not needed (Houtveen & Molenaar, 2001). Time domain method, RMSSD, is determined as the root mean square of differences between successive RR intervals and used as RSA index (Penttilä et al., 2001; Stein, Bosner, Kleiger, & Conger, 1994). The RSA index has been also determined using peak–valley estimation (pvRSA); (Eckberg, 1983b; Grossman et al., 2004; Katona & Jih, 1975) by subtracting the shortest RRi during inspiration from the longest RRi during the expiration (respiration signal needed). Compared to frequency domain analyses, the advantage of time domain methods is that the analysis can be performed for shorter time segments.

Time-varying methods such as a point process framework for heart beat dynamics has been proposed to assess RSA index within an adaptive point process filtering algorithm. The extension of a point process framework to a bivariate model with respiration as a covariate has also been proposed (Barbieri, Matten, Alabi, & Brown, 2005; Chen, Brown, & Barbieri, 2009). Multivariate autoregressive (MAR) modelling, whose parameters are estimated dynamically at each time instant with different algorithms such as Kalman smoothing algorithm, are proposed to quantify RSA index (Kuoppa, Lipponen, & Tarvainen, 2013; Tarvainen, Georgiadis, Ranta-aho, & Karjalainen, 2006b). Time-varying methods can produce indices describing ANS function dynamically at each time-instant. The drawback is however the amount of computation capacity.

Some evidence exist that respiration rate and tidal volume have an influence on RSA, which may be independent from cardiac vagal outflow (Ritz & Dahme, 2006). The RSA oscillation increases according to decreased respiration rate and maximal RSA occurs at the respiration rate of 0.1 Hz (Hirsch & Bishop, 1981a; Mehl sen, Pagh, Nielsen, Sestoft, & Nielsen, 1987).

### 2.2.5 ECG derived respiration

Methods to extract a respiratory signal from the ECG signal, i.e. ECG derived respiration (EDR), have been developed for the first time several decades ago (Moody, Mark, Zoccola, & Mantero, 1985; Pinciroli et al., 1985; Wang & Calvert, 1974). The EDR signal is defined as a surrogate respiration that should provide at least respiratory rate and the temporal pattern of the respiration, which correlate to respiration flow. Respiration is one of the main contributors in the variation of the HR and BP inducing them via RSA mechanism described in Section 2.1.1. There is a clear need for respiratory information in cardiovascular signal analysis like in
HRV, BPV and BRS analysis. At least respiratory rate would be beneficial when, e.g. evaluating the reliability of the RSA index, BRS, and vagal control via HF power. The analysis of previously recorded data without simultaneously acquired respiration signal, e.g. the analysis of Holter data, would benefit the existence of reliable EDR signals.

Several techniques to measure direct respiration signal exist, which are presented in Section 2.2.1, but their usage may be limited due to the interference with the natural breathing, limited measurement capacity, and motion artefacts among others. For instance, in applications outside laboratory such as ambulatory monitoring, stress testing, and sleep studies they are not convenient to use. In laboratory environment, controlled respiration rate via, e.g. metronome, visual paced stimulus, etc. is often used. However, it is not fully known how controlled breathing affects the ANS function and hence the ANS parameters and some evidence exists that controlled breathing affect the cardiovascular system itself (Larsen, Tzeng, Sin, & Galletly, 2010).

Respiration modulates several bioelectric and -mechanical signals such as ECG (Einthoven 1950), impedance plethysmography (L. Mason, 2002) and thorax acceleration signals (Drummond, Bates, Mann, & Arvind, 2011), which makes the indirect measurement of respiration very attractive. Particularly ECG is nowadays easily obtained with well-developed measurement devices and is cost-effective with disposable electrodes. A thorax movement along with the respiration causes rotation of the electrical axis of the heart and thus affects the ECG morphology beat-by-beat. During each respiration cycle, the heart is stressed and compressed within the inspiration and expiration. In addition, thorax impedance varies due to filling and emptying of the lungs. R-wave modulation is induced by changes in relative position of electrodes during respiration cycle (Malmivuo & Plonsey, 1995; Moody et al., 1985). As already stated in Section 2.1.1, heart rate varies according to respiration via RSA phenomena, which is induced neurally and mechanically (Hirsch & Bishop, 1981b; Larsen et al., 2010; Wang & Calvert, 1974).

Methods to obtain the EDR signal may be divided into methods that utilize beat morphology (Wang & Calvert, 1974), methods that are based on HRV/RR intervals series (Womack, 1971; Correa, Laciar, Torres, & Jané, 2008) and methods combining beat morphology and HRV (Boyle, Bidargaddi, Sarela, & Karunanithi, 2009; Orphanidou et al., 2009) based methods. Single lead methods usually exploit R-wave amplitude (Khaled & Farges, 1992; C. L. Mason & Tarassenko, 2001), QRS area (De Chazal et al., 2003; S. Park, Noh, Park, & Yoon, 2008; Sobron, Romero, & Lopetegi, 2010), Wavelet transform (Boyle et al., 2009; Yi & Park, 2002)
or some other beat feature (Langley, Bowers, & Murray, 2010; Widjaja, Varon, Dorado, Suykens, & Van Huffel, 2012). Multiple lead methods utilize either variations in angle of mean electrical Axis (AMEA) (Caggiano & Reisman, 1996; Moody et al., 1985) or vector cardiograph (VCG) (Bailón, Sörnmo, & Laguna, 2006). Generally, EDR algorithms based on beat morphology are more accurate than EDR algorithms based on RR interval series due to noise and baseline variation issues. Practical applications are simpler to implement using single lead methods (Clifford, Azuaje, & McSharry, 2006b).
3 Research contributions

This chapter summarizes the research contributions of the original Papers I–VI organized in sections according to specific practical objectives described in more detail in Section 1.2. Adaptive filtering is utilized in Papers I, II and IV to remove the respiratory effects from cardiovascular signals. Papers II-IV provide methods for RSA index estimation from RRi and SBP series. Tools for analyzing dynamics and frequency of LF oscillations of RRi and SBP series are described in Papers IV and V. Finally, Paper VI proposes two novel ECG derived respiration (EDR) methods.

3.1 Removing the effect of respiration from cardiovascular signals

Respiration affects the RRi and BP series via several mechanisms (Badra et al., 2001; Eckberg, 1983a; Grossman et al., 2004). If breathing rate is low, it can distort the traditional PSD based analysis and indexes in the frequency domain. Metronome-guided CB protocol (0.25Hz) is usually applied to standardize respiration during the measurements (Heart rate variability: Standards of measurement, physiological interpretation and clinical use, 1996). Yet, the CB may be uncomfortable, difficult to perform and even have an influence on cardiovascular control system itself. The least-mean-square (LMS) adaptive filtering based method was developed to cancel the respiration effects. Paper I demonstrate how respiration induced effects can be reduced in BRS estimation (Section 3.1.1). Adaptive filtering was also utilized in RSA index estimation in Papers II and IV (Section 3.2) and in Paper IV to study the dynamics and frequency of LF oscillations of RRi and SBP series (Section 3.3.1).

3.1.1 Enhancement of baroreflex sensitivity estimation

As presented in Section 2.2.3, widely used spectral open loop methods to estimate BRS from short-term cardiovascular signals may be biased, if breathing rate is lower than 0.25Hz. A small preliminary study was conducted to demonstrate the overestimation of BRS values during SB compared to corresponding BRS values during CB at frequency of 0.25Hz (Tiinanen, Seppänen, & Tulppo, 2005). Paper I aimed to provide a computational method to reduce the bias in the BRS estimation allowing the usage of SB rate lower than 0.25Hz in measurements.
**Data**

Papers I, II and III utilize the same data described next. The study group consisted of 24 healthy male subjects (mean age = 34 years). ECG, BP and respiration signals were acquired during 5 min of SB and 5 min of metronome-guided CB at 0.25Hz in supine position (fs = 200 Hz). Series of RRi and SBP values were derived as stated in Section 2.2.1. The subjects were divided into two groups according to subject’s SB rate. Group 1 (n = 12) consists of subjects whose SB rate is within the LF band (0.04-0.15Hz) and Group 2 (n = 12) consists of the subjects whose SB is within the HF band (0.15-0.4 Hz).

**Implementation and results**

From the signal processing point of view, the challenge was the spectrally overlapping signals in Group 1. When respiration rate is low, it induces power to regions in series of RRi and SBP values from which the BRS is calculated distorting the BRS analysis. As a solution, the respiration-induced component was estimated and extracted from the series of RRi and SBP values by the LMS adaptive filter (Widrow et al., 1975). The LMS filter was selected because of its good stability, efficiency and simple structure. Furthermore, the effect of sampling rate and making the signals time-synchronous before adaptive filtering were addressed as important preprocessing steps in Paper I. The series of RRi and SBP values are characteristically irregularly sampled and reference respiration was evenly sampled at the original sampling frequency (200Hz). Therefore, the signals were resampled, and Fig. 2 presents the block diagram of signal pre-processing steps and LMS adaptive filter structure used in Paper I. The BRS estimation was conducted in LF range using both alpha and transfer function methods explained in Section 2.2.3.

The data analysis was conducted such that first, the BRS analysis was conducted without adaptive filtering in LF range. The results revealed that a slow SB rate distorts the BRS(LF) values by producing highly overestimated BRS values. In addition, persons that breathed spontaneously at a higher rate seemed to get higher BRS(LF) values compared to breathing at controlled 0.25Hz rate.

The simulation study was conducted to verify the filter function. Subjects’ own respiration component was first removed with adaptive filter, then, a known respiration component was added to RRi and SBP series. At the last stage of simulation, the adaptive filter was applied to remove the added component. Simulation results revealed that the adaptive filter was able to remove the added
component without affecting the signal spectral characteristics. Absolute spectral powers decreased during simulation 6.9-15% depending on the signal and spectral range. The change in BRS values however was minimal indicating that the filter works correctly.

**Fig. 2.** Block diagram of pre-processing steps and LMS adaptive filter structure of the developed algorithm to remove respiration effect from cardiovascular signals (Paper I).

The results of Paper I showed that the two resampling methods (irregular/regular) and the adaptive filtering technique used do not have an effect on the BRS(LF) estimates when breathing rate is controlled at 0.25Hz (p < 0.05) or SB rate was on HF range (Group 2, p < 0.05). However, when spontaneous breathing rate was low (Group 1), the obtained BRS(LF) values with adaptive filtering are significantly (p < 0.05) smaller compared to the values obtained without filtering.

The conclusive statement in Paper I was that, when SB rate was used in measurements, the BRS(LF) estimates differed according to subjects SB frequency. The developed adaptive filter is able to remove the effects of respiration on BRS(LF) values and thus reduce the bias that slows respiration pattern causes in BRS estimation.
3.2 Respiratory sinus arrhythmia quantification from cardiovascular signals

RSA is commonly quantified using HF power at 0.15-0.4Hz (Section 2.2.4). Breathing rate may, however, change depending on the used breathing protocol and other methods describing RSA are then more favourable. RSA is an important index describing the ANS function.

Paper II and III present two new algorithms to quantify RSA. In Paper II, which is an extension for the technology proposed in Paper I, the respiration pattern is obtained using ECG-derived respiration (EDR) method and thus measured respiration, is not needed. The algorithm presented in Paper II widens the application area of RSA index for setups where only one modality, the ECG, is needed. Paper III utilizes independent component analysis (ICA) showing its potential in cardiovascular signal processing. The method proposed in Paper I was applied in Paper IV for RSA index estimation with a bigger study group across the continuum of risk of cardiovascular diseases.

3.2.1 RSA component extraction by combining adaptive filtering and PCA-derived respiration

Data

The same database used in Paper I, which is described in the previous section, was adopted in Paper II.
4 Implementation and results

Paper I presents a method to reduce the respiratory effect in BRS estimation using adaptive filtering solution. The method removes the estimated respiration component, i.e. RSA index, from RR interval and SBP series. The drawback in Paper I was that the measured respiration signal is needed, which made it appealing to develop a method that utilizes ECG-derived respiration (EDR), described in more detail in Section 2.2.5. In Paper II, the method, namely EDR-LMS, that combines LMS adaptive filtering and EDR was developed to extract the RSA component from the RRi and SBP time series. A novel top performing principal component analysis (PCA) based method to produce EDR was adopted from the literature (Langley et al., 2010) and its ability to act as a reference respiration was tested in Paper II.

The performance of EDR-LMS algorithm was evaluated comparing obtained remainder LF and HF powers and peak frequencies against LMS filter with reference respiration. The peak frequencies of the bands were slightly decreased (p < 0.05) in Group 1 (SB rate < 0.15Hz) after removing the RSA component with both LMS and EDR-LMS, indicating that a low respiration rate induces the most dominant peak in the LF range. There were no significant differences in obtained peak frequencies between compared methods. The powers in the bands after filtering yielded quite similar results with both methods (p < 0.05) except a slight difference in LF powers in Group 1 (filtering with reference respiration: 858 ± 461 ms² and filtering with EDR-LMS: 996 ± 434 ms², without filtering: 2412 ± 1446 ms²). What is more, the filtering did not change the peak characteristics or the spectral power of the respiratory-free band.

According to results, both respiration signals (real or EDR) were able to extract the RSA component from RRi and SBP series with quite similar performances without significantly distorting remaining spectral characteristics. The result enables the usage of EDR-LMS method in RSA component extraction.
4.1.1 RSA component extraction using independent component analysis

Data

The database used in Paper I and II (described in Section 3.1.1) was used in Paper III as well.

Implementation and results

Paper III utilizes independent component analysis (ICA) which is a computational technique for separating linearly mixed unknown source signals of measurements (Hyvärinen & Oja, 2000). ICA is widely applied in different data sets and it has been gained acceptance in blind source separation. The sources are assumed to be non-Gaussian and mutually independent. Several ICA algorithms exist and FastICA software package was chosen to use in Paper III. FastICA is developed by the inventor group of ICA, and it can be downloaded freely from http://www.cis.hut.fi/projects/ica/fastica/.

Flowchart in Fig. 3 describes the developed algorithm to separate the RSA component from the series of the RR intervals. The hypothesis was that the series of RR intervals are a linear mixture of RSA component and series of RRI. Since the variance of each independent component is unit variance, the linear regression modelling is used to scale the components in order to calculate the absolute power spectrum estimates for the remainder and extracted RSA component.

\[
RR = \alpha RR_{EM}^N + \beta RR_{RSA}^N = [RR_{EM}^N \ RR_{RSA}^N] \theta = \bar{RR} \theta
\]  

(2)

where \( \theta = \begin{bmatrix} \alpha \\ \beta \end{bmatrix} \).

From equation (2) it can be estimated that: 
\[
\hat{\theta} = \bar{RR}^+RR ,
\]
where \( \bar{RR}^+ \) is pseudoinverse.

The performance of the developed algorithm was tested with a specific simulation study. The simulation was performed such that first the known RSA component was added to the original tachogram signal and then the ICA algorithm presented above was applied to extract the RSA. A residual analysis was performed for the extracted RSA component by ICA and original added RSA component.
Before simulation, a person’s own RSA component was removed with the adaptive filtering method described in Paper I to reduce the confusing effect a second RSA component in a signal may affect. Two RSA components that had different frequencies (low and high frequency range) were arbitrary obtained from Group 1 and 2 and used in the simulation study.

After RSA extraction by the ICA method, residual analysis was performed in the time and frequency domain. The extracted RSA follows the shape of simulated RSA (3.3 – 5.4 % RMS error of total variability), and thus it could be concluded that ICA was able to remove RSA without changing the power content of heart rate intervals (p < 0.05).

Fig. 3. Flowchart describing the ICA based algorithm to obtain the RSA component from the series of RRi.
4.1.2 RSA component estimation using adaptive filtering

Data

The healthy non-smoking participants were divided into young (YOUNG, 25–40 years, n = 39) and older (OLDER, 50–75 years, n = 40) groups. The elderly patients with diagnosis (n = 106) formed three subgroups as follows: patients with essential hypertension formed a hypertensive (HT, n = 36) group, the patients with coronary artery disease without (CAD, n = 28) and with type 2 diabetes (CAD + DM, n = 42) formed their own subgroups. Standard lead-II ECG, continuous finger BP and breathing by plethysmography were collected for 5 min at supine position while participants breathed spontaneously. Sampling frequency was 1000 Hz.

Implementation and results

The method proposed to remove respiratory induced effects from cardiovascular signals in Paper I was applied in Paper IV in a way that the extracted RSA component was further utilized as an index describing the ANS function. The performance of RSA index was tested with a larger study group across the continuum of risk of cardiovascular diseases. As it was expected, RSA index was higher among subjects with low SB rate compared with those with higher SB rate in YOUNG (2314 ± 3055 vs. 713 ± 669 ms², p = 0.017), OLDER (517 ± 333 vs. 292 ±549 ms², p = 0.008) and HT groups (399 ± 347 vs. 158 ±197 ms², p = 0.039). However, among CAD and CAD + DM groups, the RSA index was not different between subjects with lower and higher SB rates (in CAD: 331 ± 308 vs. 234 ±389 ms², p = 0.118, in CAD + DM group: 413 ± 619 vs. 118 ± 139 ms², p = 0.073). The OLDER group had lower RSA index compared to the YOUNG group, while RSA index did not differ between OLD, HT, CAD and CAD + DM (p < 0.05).

In the CAD group, patients with a prior myocardial infarction (MI) had lower RSA index than those without (132 ± 159 vs. 515 ± 435 ms², p = 0.006), whereas all the other spectral indexes of BP and RRi variability were similar, including HF power. In CAD + DM group, i.e. clinically more severe stage, no differences were found between patients with and without prior MI (171 ± 167 and 270 ± 474 ms², p = 0.520).

The RSA index derived via adaptive filtering was decreased in OLDER compared to YOUNG group. Moreover, in CAD group, patients with prior MI had significantly decreased RSA index.
4.2 Tools for analysing low-frequency oscillations of cardiovascular signals

As described in the literature review, LF oscillations of RRi and SBP series reflect mainly the activity of the sympathetic and parasympathetic branches of the ANS via baroreflex, the regulation mechanisms determining the LF peak frequency and its shift, e.g. during provocative tests and among different patient groups, have been unclear (Julien, 2006; Wichterle et al., 2004).

The aim here was to develop and apply algorithms for exploring the ANS regulation by LF oscillations with experimental studies using real patient groups (Paper IV and V). It was hypothesized that the frequency of the LF oscillations in RRi and SBP might shift among different patient groups and due to used ANS test protocol. Furthermore, the frequency of LF oscillations could provide a cardiovascular risk marker.

4.2.1 Power and frequency of LF oscillations using adaptive filtering

The Paper IV was an experimental study to assess the power and the frequency of LF oscillations in SBP and RRi across the continuum of cardiovascular risk including age. Low breathing rate may distort the analysis of LF oscillations. Therefore, the adaptive filtering technique proposed in Paper I was adopted to study the LF power and frequency in Paper IV. It was hypothesized that adaptive filtering decreases the power and peak frequency of LF oscillations in SBP and RRi among the subjects with low SB rate to the levels observed in those with higher breathing frequency. Furthermore, RSA index was extracted using adaptive filtering (results reported in the previous Section 3.2).

Data

The used data is described above in Section 3.2.3.

Implementation and results

Paper IV presents a study were the power and frequency of LF oscillations in SBP and RRi series with and without adaptive filtering are examined in subgroups described in the Data section above. The objective was to assess the performance of the adaptive filter separately for subjects with lower and higher SB rate. In
addition, it was hypothesised that severity of cardiovascular risk decreases both the power and frequency of LF oscillations. Median frequency was selected to characterize the LF oscillation because spectral analysis may reveal more than one power peak at the LF band. Median frequency is defined as a frequency dividing LF band into the two segments having equal power (Stulen & De Luca, 1981). The usability of the median frequency power over the frequency of the maximum power was determined in one of our group’s previously published paper about LF oscillations (Kiviniemi et al., 2010).

According to results, the LF power and the median frequency of SBP and RRi series decreased largely after adaptive filtering compared with values without filtering in all sub groups with low breathing rate. When adaptive filtering was applied among the sub groups with higher breathing rate, any of these LF spectral variables were not altered after filtering, indicating that the filter removes only the respiratory component and the rest of the spectrum remains undisturbed. Mostly, no differences were observed with these variables after adaptive filtering between subjects with lower and higher spontaneous breathing rate.

The main results of the Paper IV regarding the ANS regulation of cardiovascular LF control revealed that aging decreased the LF power of RRi (p < 0.05) and increased the LF power of the SBP (p < 0.05) as compared to younger subjects. Furthermore, aging decreased the median frequency of the LF oscillation in the BP and RRi. As a novel finding, a further decrease in the LF frequency with utilizing adaptive filter was observed in coronary artery disease (both CAD and CAD+DM groups).

4.2.2 LF peak frequency estimation using time-frequency representation of cardiovascular signals

A tool to study altering cardiovascular oscillations and track the frequency of LF peak was developed utilizing TFR method in Paper V.

Data

The transient data for Paper V was obtained by a static handgrip exercise (HG) test. HG exercise involves an increased BP and HR due to increased sympathetic activity and a vagal withdrawal (Iellamo et al., 1999; Seals, Chase, & Taylor, 1988b). The study population consisted of 11 healthy male volunteers (n = 11, mean age = 35 years). During the measurements, subjects lay in a supine position and were
instructed to breathe at a constant metronome-guided frequency of 0.25Hz. Standard lead-II ECG, BP from a finger of the non-exercising arm and respiration signals were collected ($fs = 1000$ Hz) for 5 min without HG (baseline) and for 5 min performing the handgrip exercise (HG, 20% of maximal voluntary contraction).

**Implementation and results**

Due to the HG test protocol, the data is nonstationary, whereas traditional power spectrum analysis assumes the stationarity of the data. The traditional power spectrum analysis suffers also from poor time resolution while fast changes might remain unobserved. Time-frequency representation (TFR) methods provide one solution to analyse nonstationary signals. Smoothed pseudo Wigner-Ville distribution (SPWVD) described in Section 2.2.2 was utilized when analysing the data of Paper V.

Visual inspection of the SPWVDs revealed that 2-dimensional image plot with data scaled as RGB colours and/or 3-dimensional surface plot provided informative illustrations of the measurement protocol steps (see Fig. 4.). An algorithm for LF peak frequency estimation from the SPWVD was developed. Inspection of SPWVD revealed lots of activity in LF range which may be due to a complex regulating mechanism behind the HR and BP. Therefore, only the peaks exceeding the threshold value in the SPWVD were selected and the mean value of local maxima was calculated in the time window where LF frequency was analysed. LF peak frequency is interpreted in Paper V as a prevalent low frequency (PLF) according to (Wichterle et al., 2004). Visualization revealed also that PLF was increasing during the HG test and reaches the maximum during the last two minutes of HG exercise for both RRi and SBP. In addition, the first two minutes of the handgrip were analysed and two minutes from baseline data were selected for analysis to have the same segment length of data. Mean LF and HF powers and PLF were estimated for the defined two minutes data segments.

PLF increased during the HG test and reached the statistical significance ($p<0.05$) during the end of the HG test for both RRi and SBP. The LF power of RRi and SBP oscillations increased during the HG test. RRi oscillation in HF range decreased, referring to the withdrawal of the parasympathetic activity even though the controlled breathing rate 0.25Hz continued throughout the measurements. LF/HF ratios increased in both RRi and SBP signals during the HG test, indicating the shift to sympathetic activation.
4.3 New ECG derived respiration methods

As section 2.2.5 presents, the EDR methods have been developed for some time already. Recently, decomposition based techniques like principal component analysis (PCA) (Langley et al., 2010), and its nonlinear version, kernel PCA (KPCA) (Widjaja et al., 2012), have been applied to derive a surrogate respiration signal from single-channel ECG signal. Their performance was shown superior towards previously developed EDR methods using single channel ECG. The RSA component extraction method proposed in Paper II (Section 3.2.1) utilizes the PCA based EDR method from the literature (Langley et al., 2010) where it was assumed that the first principal component (PC1) would produce the best EDR, i.e. surrogate
respiration signal. During the analyses for Paper II, it was noticed that the PCA based EDR method needs a proper component selection algorithm to produce the best EDR signal from decomposed PCs. In addition, the good performance of PCA in EDR production encouraged us to test the well-known and widely used independent component analysis (ICA) in EDR derivation. Therefore, Paper VI proposes two new EDR methods: Adapted ICA (AICA) method and Adapted PCA (APCA), which utilizes the linear PCA method with the extension of the component selection algorithm.

4.3.1 Adapted ICA and PCA methods

Data

A freely available Fantasia database (A. L. Goldberger et al., 2000) was used in Paper VI. Twenty recordings from both young (21-34 years, n = 20) and old (68-85 years, n = 20) healthy subjects were included. Measurements were performed at supine position, subjects breathing spontaneously, and watching the movie called Fantasia (Disney, 1940). Simultaneous respiration was measured with a belt attached around the thorax and the lead II ECG was captured (fs = 250 Hz). A five min data sequence that contained no perceived movement artefacts was selected for the analysis from each subject.

Implementation and results

The data matrix for deriving the EDR was constructed by aligning consecutive QRS segments of the ECG. Because the number of ICs is the same as that of sensors/measurements, which is an ICA characteristic, and the fact that QRS complexes are highly correlated due to the redundancy in the data, the dimension of the original QRS data matrix was first reduced using the PCA. ICs were calculated using a nonlinear ICA algorithm by FastICA toolbox (http://www.cis.hut.fi/projects/ica/fastica/). ICs are also produced in random order in ICA. Therefore, it is critical to select the most suitable component from decomposed ICs as to EDR signal. For the algorithm, it was assumed that with the current data, the subject’s respiration frequency has limited variability and a peaked spectrum. First, the PSD of the decomposed ICs were estimated. Next, the global maximum of the spectrum is located and a fixed size (0.08 Hz) window is placed
around it. Finally, the spectral energy ratio between the spectrum outside the window and the spectrum within the window is calculated and the component that produces the lowest energy ratio is selected to be EDR signals in the AICA method. The algorithm allows small variability of the respiration rate; however, with a more varying respiration pattern and longer data, the proposed component selection algorithm may be applied with shorter segments.

The original linear PCA method (Langley et al., 2010) was extended in Paper VI as a APCA method such that a similar component selection algorithm that was used in AICA was applied for decomposed PCs to select the best PC to be an EDR signal. Fig. 5 illustrates the critical choice of the correct component from the decomposition of ICA or PCA. The performance of new AICA and APCA was obtained with an experimental comparison made with the original PCA algorithm that utilizes the first principal component, named PCA1 here (Langley et al., 2010), the traditional R-peak amplitude method (AMP) (C. L. Mason & Tarassenko, 2001), and the Kernel PCA method (KPCA) (Widjaja et al., 2012). Paper VI also demonstrates that the usage of smoothing spline resampling and bandpass-filtering improves the performance of all of the EDR methods.

The comparison of EDR methods was conducted with a correlation and coherence analysis between estimated EDR signals and the measured reference respiration signal. The developed AICA and APCA yielded a significant improvement with correlations: 0.84, 0.82, 0.76, 0.63, 0.37 and coherences: 0.90, 0.91, 0.85, 0.74, 0.52 between reference respiration and AICA, APCA, KPCA, AMP, PCA1, respectively. AICA and APCA were the best performing EDR methods in Paper VI regarding correlation and coherence. However, it was noticed that decomposition based EDR methods, all but AMP in this paper, failed occasionally to produce decent EDR signal. AICA had the lowest failure rate when using correlation/coherence of at least 0.6 as a threshold for acceptable performance making it a top performing EDR method.
Fig. 5. Eigenvectors (EV) for the first five PCs and their PSDs on the right and left side, respectively. The eigenvector from PC3 resembles most the respiration reference signal (bottommost plots) and is selected as a surrogate respiration signal by the developed APCA method (Paper VI).
5 Discussion

5.1 Reducing the respiratory effects from cardiovascular indexes with adaptive filtering

Breathing rate may distort the spectral cardiovascular indexes such as HF and LF powers, RSA index and BRS estimates. This is because the bands that are generally estimated from beat-to-beat oscillating time series of HR and SBP are fixed, and in the case of lower than 0.25Hz respiration rates, the spectral parameters are largely affected by respiration. The problem is often bypassed by using CB measurement protocol. However, it may be difficult and uncomfortable, and even have an impact on ANS regulation (Larsen et al., 2010).

The first objective of this thesis work was to develop a method to remove the distorting effect of respiration from cardiovascular signals. The BRS estimation was selected to be the application for which the method was developed in Paper I. The preliminary work revealed the fact that the BRS estimates differed significantly depending on the respiration rate such that BRS(LF) estimates are overestimated in subjects whose SB rate is low (<0.15Hz) (Tiinanen et al., 2005) which was later reasserted in Paper I. In addition, the respiration protocol, i.e. CB at 0.25Hz vs. SB in HF range, affects the BRS(LF) estimates such that CB seemed to decrease the BRS(LF) values. This can be taken as an evidence of the statements presented in the literature that CB, which is achieved by overridden the respiration regulation via cortical inputs, may have an effect on cardiovascular control via cardiorespiratory coupling (Garcia et al., 2013; Larsen et al., 2010).

The developed adaptive filtering technique manages to remove the distortion effect from BRS(LF) estimates that a low respiration rate may induce in BRS estimation. The function of the filter was verified with a simulation study, which indicated that the non-respiratory spectral characteristics remain unaffected. The developed technique enables the usage of SB protocol replacing the CB in the measurements. The technique developed in this thesis opens up new opportunities for studying the cardiovascular system.

The LMS adaptive filter was selected due to its simplicity and computational cost-efficiency. In addition, LMS is known to be superior against faster techniques like RLS when a signal is nonstationary (Haykin S., 1996). Spectrally based open-loop BRS models are easy to apply despite of their simplifying assumptions and
are in widespread usage. Thus, it is justified to develop technical improvements that make them more usable for practical work.

Another solution to address the respiratory effect in BRS analysis would be multiparametric closed-loop models (Baselli et al., 1994; Korhonen, 1997; Takalo, Saul, & Korhonen, 2004) that have a better fit to the actual physiological system considering the bidirectional interaction and delays between the variables. The utility is decreased, however, by their parametric and complicated nature. Nevertheless, it would be interesting to compare the results obtained here using adaptive filtering vs. BRS gains by multivariate closed loop models. This is left for future work.

5.2 RSA index quantification

As second objective of this thesis, two methods to extract the RSA component from RRi and SBP series, were developed in Papers II and III to overcome the distortion problem that a SB rate lower than 0.25Hz may cause. In addition, RSA index was extracted in Paper IV using the technology presented in Paper I with real patients across the continuum of cardiovascular risk. The RSA index is generally thought to measure the cardiac vagal outflow.

In Paper II, the adaptive filter was combined with EDR signal named as LMS-EDR technique. The method enables the analysis of RRi and SBP series without a need for measured respiration signal. The RSA component was removed with LMS-EDR technique without significant distortion in remaining spectral characteristics compared as to adaptive filtering with real respiration signal as a reference.

In Paper III, an ICA-based method was developed to extract the RSA component from cardiovascular signals. The limitation of the developed method is that a reference respiration is needed in the developed algorithm. ICA may be applied as a “self-referencing” version but was left for future work. However, ICA showed great potential to discriminate different oscillating components of cardiovascular signals.

According to results of Paper IV, the RSA index derived via adaptive filtering was decreased in OLDER, HT, CAD and CAD + DM groups compared to YOUNG group. The patients with prior MI in the CAD group had a significantly decreased RSA index as compared to those without MI, which indicates further ANS dysregulation after MI.
An issue regarding the RSA index is the fact that both respiration rate and tidal volume have substantial influence on the magnitude of the RSA index, which is independent of the cardiac vagal outflow (Grossman & Taylor, 2007). This issue has often been ignored while RSA algorithms have been developed and applied. It has been hypothesized that the influence of the respiration rate on RSA magnitude is about linear and some tools for correcting the time domain based RSA index according to respiration rate have been developed (Schulz, Ayala, Dahme, & Ritz, 2009). The purpose in this thesis was to develop new methods to extract the RSA component, but the interpretation and usage with corrections according to respiration rates were not in the scope of the current work, which as such, however, is an important aspect in RSA estimation. The results of Paper IV revealed that RSA index via adaptive filtering depends on respiration rate, which is in accordance with the literature. However, among CAD and CAD + DM groups, the RSA index was not different between low and high respiration rate. This may be interpreted as dysregulation of the ANS function and thus deceased vagal outflow among CAD patients. It is known that a greater vagal outflow is recognized as cardio-protective role (Eckberg, Drabinsky, & Braunwald, 1971; Ellenbogen, Smith, & Eckberg, 1990; Kolman, Verrier, & Lown, 1976).

It would also be beneficial to compare the RSA index algorithms developed in this thesis against HF power, which is the most common method to estimate the RSA index, and the bunch of algorithms proposed in the literature (Peltola et al., 2008; Kodituwakku, Lazar, Indic, Brown, & Barbieri, 2010; Nemati, Malhotra, & Clifford, 2010; Orini, Bailón, Laguna, Mainardi, & Barbieri, 2012). In addition, RSA index obtained via adaptive filtering (Section 3.2.3) could also be combined with the new EDR algorithms developed in this thesis (Section 3.4). The performance of the RSA indexes could also be tested in time-varying conditions. These analyses are left for future work, however.

5.3 Tools to study LF physiology

While there is consensus about the HF component representing mostly the respiratory influences regulated by parasympathetic branch of the ANS, the regulatory mechanisms generating the LF oscillation is more complex (Julien, 2006; Reyes del Paso et al., 2013; Shaffer et al., 2014). Especially the frequency of LF oscillations is less studied and the mechanisms shifting the LF peak frequency are unclear. The third objective of this thesis was to serve tools to examine the ANS regulation of LF oscillations in RRi and SBP series. Firstly, different methods to
estimate the frequency of the LF power were implemented. Next, the bias in the LF oscillation estimation that a low breathing rate may induce was recognized and as a solution, adaptive filtering was applied prior to spectral analysis. Finally, a TFR based tool was developed for varying conditions such as ANS function tests.

Paper IV provided an experimental study across the continuum of cardiovascular risks. LF oscillations were examined utilizing an adaptive filter to remove the distortion effect that a low breathing rate may cause on characteristic peak frequency and LF powers. The results indicated that the adaptive filter manages to remove the respiratory effect from the power spectrum of SBP and RRi series among healthy subjects and patients with cardiovascular disorders and decreases the bias in power and frequency estimation.

The main results of the Paper IV regarding the ANS regulation of cardiovascular LF control were that aging decreased the LF power of RRi and increased the LF power of the SBP as compared to younger subjects. Furthermore, aging decreased the median frequency of the LF oscillation in the BP and RRi. A novel finding was that a LF frequency decreased further in coronary artery disease (both CAD and CAD + DM groups).

From a methodological point of view, in addition to altering respiration rate, a challenge while detecting the frequency of the LF oscillation is the PSD estimation and its parameter selection. Different spectrum estimation methods and parameters may produce a different amount of peaks in the spectrum, which challenges the interpretation of the spectrum. Furthermore, several measures for detecting the LF oscillation frequency exist and clear consensus what to use has not been drawn (Heart rate variability: Standards of measurement, physiological interpretation and clinical use, 1996). In Paper IV, the median frequency was selected to overcome the problems with several peaks in the spectrum. In addition, the median frequency is least sensitive to noise in the spectrum (Stulen & De Luca, 1981). However, median frequency is prone to detrending, especially if strong VLF power leaks into LF band.

In Paper V, smoothed pseudo Wigner-Ville representation (SPWVR), which presents the signal power with time information, was applied to analyze the HG data of healthy men. An algorithm for detecting the LF peak frequency was developed. In addition, LF and HF powers were estimated from TFRs. Developed methods managed to quantify the frequency of the LF oscillations according to visual inspection of the TFR. The obtained results of the Paper V indicated that during sympathetic activation, mainly caused by metaboreflex, frequency of LF
oscillation of SBP and RRi increases during HG exercise. LF/HF increased due to withdrawal of vagally mediated HF power of RRi series.

It is known that static exercise involves an increased BP and HR due to the increased sympathetic activity and the vagal withdrawal (Iellamo et al., 1999; Seals et al., 1988a). In addition, muscle metaboreflex is an important mechanism increasing sympathetic activity during static exercise (Sheriff, O’Leary, Scher, & Rowell, 1990). Thus, the obtained result gives important information about the frequency shift during static HG exercise. The preliminary results of Paper V were later verified with a more comprehensive study and published as a novel physiologic finding during static muscle metaboreflex exercise (Kiviniemi et al., 2010).

During the measurements, the CB rate at 0.25Hz was in use, which may have had an impact on results (Larsen et al., 2010). Further, it was noticed that tidal volume of respiration tended to increase during the HG exercise. The increased tidal volume apparently increased the HF power of SBP series through increased intrathoracic pressure changes. Apparently, HF variation in SBP is mostly from pressure changes, while HF in RR intervals reflects more vagal outflow.

The peak frequency was detected using the maximum peak in the spectrum (Wichterle et al., 2004). However, according to later experimental studies (Kiviniemi et al., 2010), the median frequency would be better in case there are several peaks in the spectrum. Future studies include the comparative studies of the developed SPWVR method with other time-varying methods.

5.4 ECG derived respiration

The final objective of this thesis was to improve the single-channel ECG derived respiration methods. Respiratory rate and volume are important parameters in the quantification of the ANS regulation. Furthermore, present methods for capturing respiratory information especially outside laboratories have limitations due to their usability and noise sensitivity. Therefore, EDR methods produce attractive and cost-effective modality to indirectly measure respiration.

Recently developed decomposition based techniques, principal component analysis (PCA), and its nonlinear version, kernel PCA (KPCA) have been shown to be top-performing methods in single-channel EDR analysis (Langley et al., 2010; Widjaja et al., 2012). Two new methods, adapted independent component analysis (AICA) and adapted principal component analysis (APCA) algorithms, were developed in Paper VI. The APCA method is an extension for linear PCA technique.
presented in the literature (Langley et al., 2010). In addition, it was shown that the usage of smoothing spline resampling and bandpass-filtering improves the performance of all EDR methods. Coherence and correlation with real respiration signals were used to evaluate the performance of developed methods. According to results, the performance of the developed AICA and APCA was the best compared to methods in the literature.

The developed component selection algorithm for AICA and APCA was quite straightforward but nevertheless showed great ability to capture the respiratory pattern. The challenge to select a correct component for EDR signal was noticed first while preparing the Paper II where EDR signal was utilized in adaptive filtering. In that work, a more complex and cost-expensive least mean square filtering based algorithm to select the correct component from linear PCA decomposition was developed. Nevertheless, varying respiration patterns may limit the utility of the proposed component selection algorithm, which assumes the respiration rate is rather stable. A solution could be the segmentation of ECG data into shorter time windows, but the more complex algorithm proposed in Paper II could be applied instead. These approaches are left for future work.

Occasionally, the correlation dropped significantly with all decomposition based methods, something that was already acknowledged in a paper by (Widjaja et al., 2012). Visual inspection of the components revealed that in those situations the decomposition (PCA, ICA or KPCA) itself failed to generate the decent respiration-like signal. However, the failure rate was smallest in AICA, while KPCA failed most. The failures were more apparent, the more variation in the respiration signal had. However, the AICA was best performing, as there were subjects with more varying respiration where all the other methods failed except AICA.

Another EDR algorithm based on statistical mean shape was also developed during the thesis work, not included this thesis (Noponen, Tiinanen et al. 2012). The developed decomposition based EDR methods in Paper VI were compared with that slightly outperforming it (data not published). The future plans regarding EDR involves the testing the performance and computational cost of the developed algorithms with long-term measurements. It is known that the complexity of the method increases the computational cost. Thus, a simple amplitude method would be more cost-effective. Long-term measurements probably would need preprocessing steps like segmenting to overcome problems due to computational cost. It would be also interesting to combine the methods developed in this thesis
such that the RSA index could be estimated using adaptive filtering and the AICA/APCA method in clinical studies.
6 Summary

This thesis proposes new methods to describe the ANS regulation of cardiovascular system from short-term measurements of single channel ECG, continuous blood pressure and respiration signals. Special emphasis is put on a low breathing rate and its effects on traditional frequency domain based cardiovascular indexes describing ANS regulation.

The main contributions of this thesis are the following: Firstly, a method to remove respiratory influences from cardiovascular time series and indexes was developed. The distortion effect of low respiration rate was demonstrated in baroreflex sensitivity estimation. Further, the developed LMS adaptive filter reduced the bias of low respiration rate, which enables the usage of a spontaneous respiration measurement protocol over controlled respiration.

Secondly, three methods to extract RSA component from cardiovascular time series were developed: two methods exploiting adaptive filtering and either the measured respiration signal or the ECG-derived respiration signal and one utilises independent component analysis showing its potential in cardiovascular signal analysis. All developed RSA methods quantifies it within the respiration rate, which makes then physiologically more accurate RSA indexes than traditional HF power with a fixed respiration rate.

Thirdly, tools for studying the power and the frequency of LF oscillations in cardiovascular signals were developed. Special emphasis was put on the distortion effect of respiration in both the frequency and the power of LF oscillation. An experimental study was conducted applying an adaptive filter with patients of continuum of cardiovascular risks. Aging decreased the frequency of LF oscillation, whereas coronary artery disease decreased it further. A time-frequency representation with an algorithm to track the LF oscillation was develop for analysing nonstationary data, e.g. during ANS provocative tests.

Fourthly, two new ECG-derived respiration (EDR) methods were developed: adapted independent component analysis (AICA) algorithm and an extend of the normal linear PCA technique with the best principal component selection (APCA, adapted PCA). The proposed AICA and APCA yielded statistically significant improvements over previously developed single channel EDR methods.

This thesis provides methods to quantify ANS function more accurately by acknowledging the respiration effects. The results of this thesis may be utilized in various applications, ranging from clinical to physiology research up to commercial health, wellness and sport products.
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