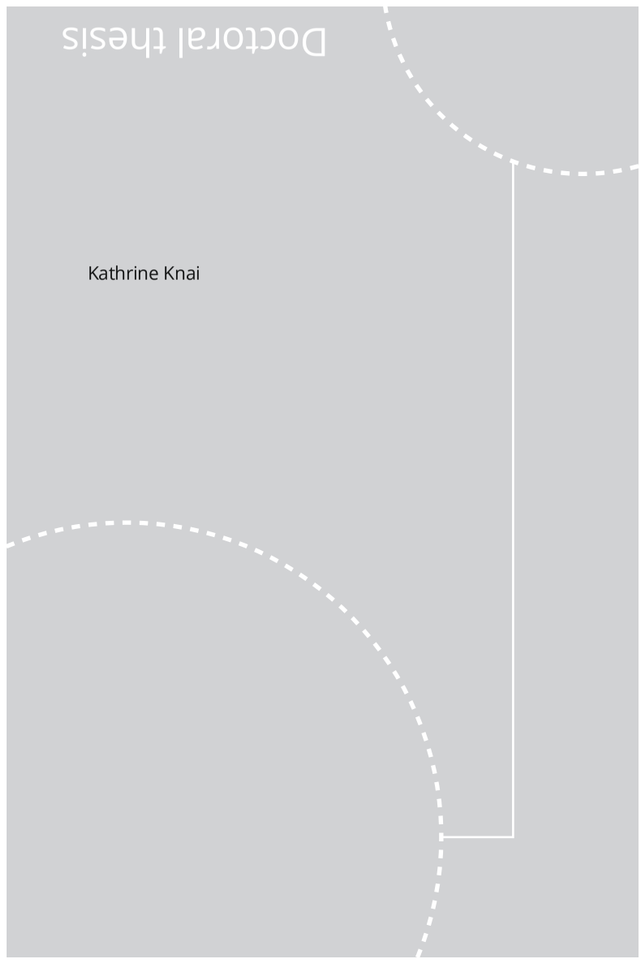
Doctoral theses at NTNU, 2020:302

Kathrine Knai

Oscillations in biological signals



ISBN 978-82-326-4952-5 (printed ver.)
ISBN 978-82-326-4953-2 (electronic ver.)
ISSN 1503-8181

NTNU
Norwegian University of
Science and Technology

NTNU
Norwegian University of Science and Technology
Thesis for the Degree of
Philosophiae Doctor
Faculty of Medicine and Health Sciences
Department of Circulation and Medical Imaging

Doctoral theses at NTNU, 2020:302

NTNU
Norwegian University of
Science and Technology

Kathrine Knai

Oscillations in biological signals

Thesis for the Degree of Philosophiae Doctor

Trondheim, October 2020

Norwegian University of Science and Technology
Faculty of Medicine and Health Sciences
Department of Circulation and Medical Imaging

NTNU
Norwegian University of
Science and Technology

NTNU
Norwegian University of Science and Technology

Thesis for the Degree of Philosophiae Doctor

Faculty of Medicine and Health Sciences
Department of Circulation and Medical Imaging

ISBN 978-82-326-4952-5 (printed ver.)
ISBN 978-82-326-4953-2 (electronic ver.)
ISSN 1503-8181

Doctoral theses at NTNU, 2020:302

Printed by NTNU Grafisk senter

Oscillasjoner i biologiske signaler

Denne avhandlingen presenterer fire studier hvor EKG-, blodtrykks- og glukosesignaler analyseres med mål om å identifisere oscillatoriske fysiologiske prosesser som er viktig for organismens motstandsdyktighet og utvikling av sykdom.

En oscillasjon er definert som en gjentakende svingning rundt en sentralverdi. Menneskets fysiologi inneholder oscillatoriske prosesser med stor variasjon i varighet og virkeområde – fra raske variasjoner i proteiner og elektrolytter inne i den enkelte celle, til kvinnens hormonsyklus som går over flere uker og påvirker hele organismen. De oscillatoriske prosessene henger tett sammen med reguleringsmekanismer, og mange oscillasjoner som er avdekket i biologiske signaler er vist å være direkte resultater av autonom regulering. Tap av biologiske oscillasjoner reduserer kompleksiteten i biologiske systemer, noe som ses ved aldring og sykdom. Det er postulert at biologiske oscillasjoner inneholder klinisk relevant informasjon som kan implementeres i intelligente alarmsystemer og verktøy for diagnostikk og prognostikk. For at denne informasjonen skal kunne implementeres i slike verktøy, må den være spesifikk for ulike tilstander og generaliserbar mellom individer med samme tilstand. Vi har utforsket biologiske signaler i jakt på slik informasjon.

I første artikkel undersøker vi tre ulike analysers evne til å avdekke oscillasjoner i et blodtrykkssignal. Vi fokuserer på tidsfrekvensanalyser som fremstiller oscillasjonenes variasjon over tid og illustrerer hvordan de ulike analysene har ulik tidsoppløsning ved lave frekvenser. I andre artikkel analyserer vi glukosesignaler fra gris. Vi finner en oscillasjon med frekvens rundt 0,01 til 0,02 Hz, som ikke tidligere er beskrevet. I dette arbeidet ser vi at oscillasjonene ikke er konstant tilstede, men kommer og går. I tredje og fjerde artikkel analyserer vi tidsserier av hjertefrekvens, systolisk blodtrykk og amplituden av EKG-ets R-

bølge fra henholdsvis friske og hjertekirurgiske pasienter. Vi finner oscillasjoner i alle tidsserier, men ser store variasjoner mellom ulike individer. I artikkel tre finner vi at R-bølgens amplitude inneholder langsomme oscillasjoner, og viser eksempler hvor de er synkronisert med oscillasjoner i systolisk blodtrykk og hjertefrekvens. I fjerde artikkel viser vi at sammensetningen av oscillasjoner i de analyserte tidsseriene ikke viser noen systematisk endring etter hjertekirurgi.

Den overordnede konklusjonen av avhandlingen er at oscillasjoner i EKG- og blodtrykkssignaler fra ulike individer viser store variasjoner. Vi har ikke funnet klare gruppespesifikke fellestrekk, og har dermed ikke klart å avdekke informasjon som egner seg for implementering i verktøy for klinisk beslutningsstøtte. Store metodologiske utfordringer må løses før slik teknologi kan tas i bruk i klinikken.

Kandidat: Kathrine Knai
Institutt: Institutt for sirkulasjon og bildediagnostikk
Hovedveileder: Nils Kristian Skjærvold
Biveileder: Petter Aadahl
Finansieringskilde: Norges teknisk-naturvitenskapelige universitet (NTNU), Helse Midt-Norge

Ovennevnte avhandling er funnet verdig til å forsvares offentlig for graden PhD i medisin og helsevitenskap. Digital disputas finner sted fredag 16. oktober 2020, kl. 12.15.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

First, I want to thank my main supervisor **Nils Kristian Skjærvold** for your guidance and support through the last seven years. I first met Nils Kristian when I was thinking of applying to the Medical Student Research Programme, and immediately fell for his fascination with, and curiosity for, physiology. Our journey has not been straightforward, and we have several times had to reset our minds. Through this process, Nils Kristian has always had faith in me and my ideas. Second, I want to thank my co-supervisor **Petter Aadahl**. With his ability to see the bigger picture, he has reminded me to focus on the *why* in research – "Why have we done this? Why is it important?". This focus has been invaluable, especially in the work of finishing this thesis.

Further, I want to thank the **Medical Student Research Programme** and the **Faculty of Medicine and Health Sciences, NTNU**. Thanks to the programme, I got the opportunity to start my research during my studies, and the faculty made it possible for me to finish my work after graduating.

I want to thank the research group **Human Monitoring and Modelling** for providing invaluable contributions to the methodology of this thesis, and **Klinisk sirkulasjonsfysiology** for contributing with clinical and physiological insight.

Thank you **Oddveig Lyng** for your guidance in and devotion to large animal experiments. The animal lab is where it all started, and even if the work is included only as a small part of this thesis, this period was very important for my introduction to research. I want to thank **Bjørn Gardsjord Lio** and **Fredrik Einar Tobias Axelsson** for their assistance in collecting data from cardiac surgery patients. **Geir Kulia, Harald Sperre** and **Marta Molinas,** thank you for your contributions to the signal processing in Paper I.

All my **friends**, thank you for helping me maintain a balance between work and leisure. Finally, a special thanks to my **family** for your love, encouragement and support. Most of all, thank you **Magnus**, for your love, patience and for never losing faith in what I can achieve if I try my hardest.

LIST OF PAPERS

This thesis is based on the following four papers:

Paper I: Knai, K., Kulia, G., Molinas, M. & Skjaervold, NK. Instantaneous Frequencies of Continuous Blood Pressure. A Comparison of the Power Spectrum, the Continuous Wavelet Transform and the Hilbert–Huang Transform. *Adv. Data Sci. Adapt. Anal.* **09**, 1750009 (2017)

Paper II: Skjaervold NK, Knai K, Elvemo N. Some oscillatory phenomena of blood glucose regulation: An exploratory pilot study in pigs. *PLOS One.* **13**, e0194826 (2018)

Paper III: Knai, K. & Skjaervold, NK. R-wave amplitude exhibits slow oscillations. An exploratory pilot study in healthy individuals. *Manuscript submitted.*

Paper IV: Knai, K, Aadahl, P, Skjaervold, NK. Cardiac surgery does not lead to loss of oscillatory components in circulatory signals. *Physiol. Rep.* **8**, e14423 (2020)

ABBREVIATIONS

BP	Blood pressure
CABG	Coronary artery bypass grafting
CWT	Continuous wavelet transform
ECG	Electrocardiography; electrocardiogram
HR	Heart rate
HHT	Hilbert-Huang transform
HRV	Heart rate variability
iAmp	Interpolated R-wave amplitude
iHR	Interpolated heart rate
IMF	Intrinsic mode function
iSBP	Interpolated systolic blood pressure
LDF	Laser Doppler flowmetry
Loess	Locally estimated scatterplot smoothing
NIBP	Noninvasive blood pressure
PPG	Photoplethysmography
SBP	Systolic blood pressure

SUMMARY

This thesis is a study of electrocardiography, blood pressure and glucose signals in the search for oscillatory physiological processes that are of importance for resilience and the development of disease.

An oscillation is defined as a repetitive variation about a central value. Physiology shows oscillating behaviour over large spatial and temporal scales – from rapid variations of proteins and electrolytes on a cellular level to the female hormone cycle ranging over weeks and affecting the whole organism. The oscillatory processes are tightly linked to regulatory mechanisms, and several oscillations identified in biological signals are shown to be direct results of autonomic regulation. The loss of biological oscillations reduces the complexity of biological systems, which is seen with ageing and disease. It is believed that information about biological oscillations, if correctly extracted from biological signals, can be used in patient monitoring, diagnostics and prognostics.

In the first paper, we explore three different analyses' capabilities for identifying oscillatory components in a blood pressure signal. We focus on time-frequency analyses, which capture the time-variability of the oscillations, and we illustrate how such analyses have different temporal resolution among low frequencies. In the second paper, we analyse glucose recordings from pigs, identifying a previously not reported oscillation with frequency 0.01-0.02 Hz. Further, we observe that the oscillations are not constantly present, but rather come and go. In the third and fourth papers, we analyse time series of heart rate, systolic blood pressure and R-wave amplitude in healthy and cardiac surgery patients, respectively. We identify oscillatory components in all variables and subjects, showing large interindividual variations. In paper three, we identify slow oscillations in R-wave amplitude and illustrate cases where they are synchronized with oscillations in systolic blood pressure and heart rate. In paper four, we do not see distinct changes in the oscillatory distributions after cardiac surgery.

The overall conclusion of this thesis is that the oscillatory distributions of electrocardiography and blood pressure signals of healthy and cardiac surgery patients are highly heterogenous and do not hold features that are either group-specific or

common for both groups. Hence, we have not been able to identify information suitable for implementation in clinical decision tools. We are doubtful regarding biological oscillations' capability of solely providing such valuable information. Consequently, there are major technological challenges that need to be overcome before automated tools are ready for clinical use.

1 INTRODUCTION

Every day, at hospitals worldwide, doctors perform day-to-day evaluations of their patients' clinical state by using anamnesis, clinical examination, blood tests, medical imaging and other technical equipment. The sickest patients are continuously monitored with equipment such as electrocardiography (ECG), invasive blood pressure (BP) and pulse oximetry so that a deterioration can be discovered at an early stage. The information that is extracted from these signals often consists of absolute values, such as heart rate (HR), respiratory rate, systolic and diastolic BP and oxygen saturation. Together, these measurements are called vital parameters, and their values at different time points are used to evaluate the patients' development over time.

There is a current opinion that the mentioned signals contain information that can be linked to physiological processes and used for clinical purposes. This information is not visible to the naked eye but should be possible to identify by using the correct analyses. One prominent characteristic of these signals is that they oscillate. An oscillation is defined as a repetitive variation about a central value. The signals' oscillating behaviour seems quite organized, as all mentioned signals are dominated by the heart's pulsation and the respiration. However, when delving deeper into the material, several slower oscillatory components are discovered, revealing irregular and complex signals.

Heart rate variability (HRV) analyses, the study of the naturally occurring variation in the time interval between heart beats, have shown reduced variability with disease and ageing. Conventional frequency analyses have identified several characteristic frequency peaks in ECG and BP signals, and they have been associated with autonomic regulation. Thus, the identified frequency components are linked to the system's overall ability to compensate for internal and external perturbations, and the integrity of biological oscillations may serve as a hallmark of healthy systems.

Nevertheless, several questions remain unanswered. Researchers have tried to link complexity to prognosis and to identify underlying physiological mechanisms of the observed oscillations. However, studies show contradictory findings. For a long time,

researchers looked at different oscillating systems separately. Current efforts have been focused on the interaction of coupled oscillating systems and the external environment.

This thesis is a study of oscillations in ECG, BP and blood glucose recordings, with the overall aim to assess whether healthy and diseased individuals hold specific or common features that help us distinguish between them. The postulated benefit is that such information can be implemented in future clinical decision tools.

2 BACKGROUND

2.1 Biological oscillations

2.1.1 Oscillations in normal physiology

Biological oscillations are found in all living creatures, from simple cells to complex multicellular organisms. Examples of biological oscillations are circadian rhythm, menstrual cycle, pulsatile release of hormones, neurone activity, heart beats and respiration. The origin of these oscillations can be traced back to intracellular pulsatile processes, such as cyclic variations of electrolytes and regulatory proteins (1,2).

The physiology of advanced organisms exhibits a complex regulation over large spatial and temporal scales. The spatial scale is constituted by anatomical and physiological structures, ranging from cell-to-cell interactions in micrometres, to heart-to-main vessels interactions in metres. The temporal scale ranges from nerve potentials in microseconds to the female hormone cycle over several weeks. Altogether, internal oscillatory processes interact with each other and with the external environment under the control of feedback systems (3). The observed result is a complex oscillatory profile exhibiting irregular nonlinear dynamics. It is not known if the complex dynamics are an essential feature of biological systems and regulatory mechanisms, or if they are secondary to internal and external perturbations (3).

Homeostasis is known to describe biological systems in physical and chemical balance. This term implies that the system is static. Some authors have suggested that homeokinesis is a better term, which is defined by Que et al as *"the ability of an organism functioning in a variable external environment to maintain a highly organized internal environment fluctuating within acceptable limits by dissipating energy in a far-from-equilibrium state"* (4). This way, homeokinesis reflects the situation where variables are moving within an interval and can always be returned to their imagined set point. This set point is termed an attractor, and the interval an attractor basin (5). Whenever dominant physiological variables are within the limits of the attractor basin, they will be dragged back towards the attractor. When the variables cross out of the

basin's limits, they move towards a new attractor or a random fluctuation, depending on the system's underlying dynamics. Healthy systems with large attractor basins have greater ability for dealing with external exposure, and thus greater ability for compensation. This resilience is connected to the underlying regulatory mechanisms of the system and is therefore linked to biological oscillations.

Circulatory oscillations

Circulatory signals have been extensively investigated with traditional frequency analyses, extracting frequency components from the signals. Most prominent are the oscillations constituted by the heart and the respiration, giving frequency components at approximately 1 Hz and 0.2-0.3 Hz. Slower frequency components have been identified and attributed to different parts of autonomic regulation. HRV analyses of interbeat time series extracted from ECG signals have traditionally defined four main frequency components: *high frequencies* at 0.15-0.4 Hz; *low frequencies* at 0.04-0.15 Hz; *very low frequencies* at 0.003-0.04 Hz; and *ultralow frequencies* below 0.003 Hz (6). It has been suggested that the frequency components are linked to the following regulatory mechanisms: *high frequencies* representing respiration-dependent parasympathetic activity, giving HR variations termed respiratory sinus arrhythmia (7); *low frequencies* representing baroreflex activity, probably being mediated by both the sympathetic and parasympathetic systems (8); *very low frequencies* and *ultralow frequencies* requiring further elucidation, but suggested as being generated by thermoregulation and the renin-angiotensin system, and the circadian oscillation in HR, respectively (6,9). Corresponding frequency peaks have been identified by analysing human blood flow signals with the continuous wavelet transform (CWT) (10). The *low frequency:high frequency ratio* is suggested to represent sympathovagal balance (11). In BP signals, a 10-sec oscillation termed *Mayer waves* is identified and said to result from an oscillatory sympathetic vasomotor tone (12). *Mayer waves* have been seen to occur in synchrony with HRV *low frequency* components (13). However, we cannot avoid mentioning that findings are ambiguous, possibly being caused by factors such as study population, pharmacological profile, experimental protocol and others (14).

Blood glucose oscillations

The pulsatile release of insulin from the beta-cells of the pancreas has been known for several decades and has been examined in both *in vitro* and *in vivo* studies. Periodicity was early discovered in Langerhans β-cells in rats (15). Insulin is released in synchronized bursts with a periodicity of approximately five minutes. The amount of insulin released with each burst is constantly changing depending on the current blood glucose level. A study of plasma glucose and insulin levels in humans discovered oscillations with a mean period of 13 minutes, the plasma glucose cycle being 2 minutes in advance of the plasma insulin (16).

2.1.2 Oscillations with ageing and disease

Ageing and disease are associated with change of the oscillatory profile and loss of complexity of biological signals. Lipsitz and Goldberger first proposed that ageing is characterized by a progressive loss of physiologic complexity (17), which is supported by several other studies of cardiovascular dynamics (18–20). In 1995, Lipsitz suggested that the complexity of biological signals is a potential marker of vulnerability to disease (21), as it represents a reduction of the system's overall resilience. In addition to making the subject vulnerable to disease, reduced complexity is simply a feature of the diseased (22). As far back as the 1960s, it was found that foetal distress resulted in alterations in HRV before any change in HR occurred (23). A general decomplexification of ECG power spectra has been found in paediatric intensive care unit patients (24). In patients with spinal cord injury, cardiovascular autonomic dysfunction leads to reduced power in the high- and low-frequency range of HR and BP time series (25). In trauma patients, reduced complexity of HR, measured by multiscale entropy, is associated with higher mortality (26). This association is found over a diversity of injuries and is suggested to represent some common underlying pathophysiological mechanism. Analyses of blood glucose oscillations in intensive care patients find the same association between reduced complexity and increased mortality (27,28). HRV indices have also shown to have prognostic relevance in heart failure patients (29).

Looking closer at patients with coronary heart disease, we see that reduced HRV and increased postinfarction mortality were described by Wolf et al. in 1978 (30). This was supported by a work by Kleiger et al., which stated that HRV could be used to identify patients with a history of myocardial infarction and increased risk of sudden death (31). Further, HRV is known to decrease after cardiac surgery and to remain reduced for up to six months (32–34). Contrary to findings in patients with myocardial infarction, such a reduction's relevance in predicting mortality is debated, as explained in more detail by Lakusic et al. (35).

2.2 Measuring biological oscillations

Biological signals are continuous recordings of measurable biological variables. In this thesis, focus is directed towards ECG, BP and glucose signals. In Paper II, we measure blood glucose with a continuous intravascular glucose sensor, which detects relative glucose changes through volume changes in a hydrogel matrix. BP can be measured both invasively and noninvasively. Continuous BP measurements are traditionally obtained through an intraarterial cannula. From the cannula, the BP is directly transmitted through a fluid-filled system and measured by a bedside manometer. Papers I and IV include invasive BP signals. Researchers have made large efforts on providing a reliable method for noninvasive continuous BP measurements. Through advanced mathematical algorithms, we are now able to estimate the BP from noninvasive signals, such as the photoplethysmogram (PPG). Paper III includes noninvasive BP (NIBP) signals recorded with the volume clamp method. Volume clamp involves wearing a small finger-cuff that is cyclically inflated and deflated to keep the blood volume in the finger constant, measured with PPG. The PPG signal is normally pulsatile, but when blood flow is kept constant, the signal is flat. The counter pressure generated by the finger cuff directly corresponds to the arterial pressure and is measured with a manometer (36).

2.2.1 Characteristics of biological signals

A signal is a representation of how one variable varies with another variable. Biological signals often describe how parameters such as electrical voltage and BP vary with time. A system is something that produces an output signal in response to an input signal. The

circulatory system produces the output signal BP, bp(t), in response to the heart pulsation. A time series is a series of data points and is, unlike a signal, always indexed by time. Heart depolarisation and BP variations are continuous processes. When collecting ECG and BP recordings, we are not able to record continuously. Instead, we reduce the time interval between two successive data points by collecting a high number of data points per time. Thus, you have a time series with a sampling rate so high that the output looks similar to a continuous signal. Therefore, in this thesis, the terms signal and time series overlap. However, I will use the term signal when talking about raw data, such as ECG and BP recordings, and restrict the use of time series to HR, systolic BP (SBP), and other variables that are extracted from the examined signals.

Many signal processing methods are based on mathematically or electronically generated signals, which are linear and stationary. When applying these analyses to biological signals, which neither are linear nor stationary, and often contain considerable amounts of noise, you meet challenges.

Linearity

In linear systems, the output of the system varies directly with respect to the input to the system. In signal processing, linear signals have the advantage that the method of superposition can be used. Superposition is the process where the signal is broken into simple components for analysis before the results are reunited. This way one complicated problem is split into several easy problems.

Most biological signals are not linear, and signal processing algorithms that require linearity may give poor results.

Stationarity

A signal is stationary when the underlying statistical properties of the system producing it do not change over time. This does not mean that the output signal is static, just that the way it changes does not itself change. Hence, the mean, variance and autocorrelation structure remain unchanged over time.

Biological signals are mostly nonstationary. Sometimes an observed trend (change in mean) can be caused by a short observation time, as the mean would have returned to the baseline value if the recording were maintained. Nevertheless, biological signals seldom fulfil the requirements of stationarity.

Noise

Noise is unwanted disturbance in a signal. The signal is produced by the system being explored, whereas noise is produced by various other factors. Electrical systems produce noise that is linked to the underlying physics of the system, such as thermal motion and fluctuations of the electric current. This noise is termed internal noise. Additionally, noise can occur from external factors, such as movements, temperature fluctuations and body posture. The signal-to-noise ratio describes the amount of noise in the signal, relative to the signal itself.

Biological signals often contain high amounts of noise, as they are recorded from humans. Naturally, the movement-induced noise will be higher in awake patients, and especially patients walking around in wards, compared to bedbound intensive care patients. Signals such as the ECG and laser Doppler flowmetry (LDF) often contain large amounts of noise, as they are recorded through sensors that are attached to the skin. The LDF will also contain noise if there are movements in the fibre optic cable. Invasive BP recordings are often more robust to noise, as they are obtained intravascularly. However, the fluid-filled systems through which the pressure is transmitted are susceptible to movements and other disturbances.

2.2.2 Analysing biological signals

Spectral analyses

Scientists distinguish between time-domain analyses and frequency-domain analyses based on whether the signal is analysed with respect to time or frequency. Time-domain analyses explore how the signal changes over time, and frequency-domain analyses show how the signal consists of different frequencies. As the frequency-domain methods' results are illustrated through frequency spectra, they are often termed *spectral*

analyses. Traditional HRV analyses are performed in the time-domain, evaluating the variation of the HR time series in total or over shorter segments (6). The most widely used frequency-domain method is the Fourier transform, introduced by Joseph Fourier in the 19th century (37). It decomposes the original signal to a sum of simple harmonics, illustrating the averaged frequency distribution of the total signal length in a power spectrum. Sometimes one wishes to combine time and frequency analyses, illustrating the time-variability of the frequency content of a signal. Such analyses are called time-frequency analyses and are an extension of the original frequency analyses. Many of these analyses are based on the original Fourier transform, including the short-time Fourier transform and the CWT, to mention some. The results of time-frequency analyses are presented in time-frequency-power distributions: three-dimensional plots with time on the x-axis, frequency on the y-axis and power illustrated by colour (Figure 1). Sometimes frequency is substituted by period, representing the duration of one cycle of the oscillation. Increasing period corresponds to decreasing frequency.

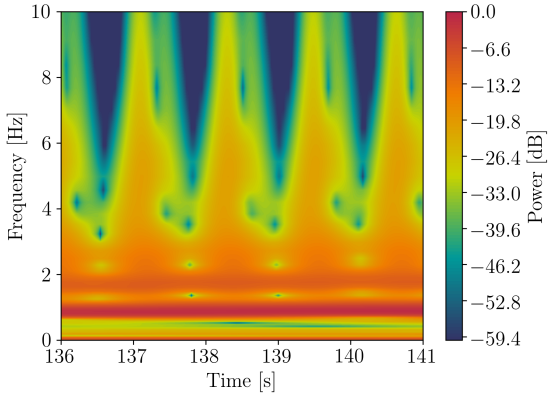


Figure 1. CWT spectrum

A time-frequency-power distribution illustrated by Figure 2 of Paper I. This CWT spectrum shows a 5-second extraction of the CWT applied to a 5-minute continuous BP signal. Reprinted from Adv. Data Sci. Adapt. Anal., 2017, Vol 9, Knai et al, *Instantaneous Frequencies of Continuous Blood Pressure. A Comparison of the Power Spectrum, the Continuous Wavelet Transform and the Hilbert-Huang Transform* (38), page 1750009-4, Figure 2. Paper distributed under a Creative Commons license, and can be shared without obtaining additional permissions from World Scientific.

The Hilbert-Huang transform (HHT) was introduced in 1998 by Norden Huang (39). It was originally developed for analysing nonstationary ocean waves for NASA and is unlike Fourier-based spectral analyses not based on the use of predefined simple harmonics. Thus, it is a data-driven approach adaptive to the data being analysed. It decomposes the original signal to the fewest monocomponents possible to describe the signal, and the results are illustrated in time-frequency-power distributions, called Hilbert spectra (Figure 2).

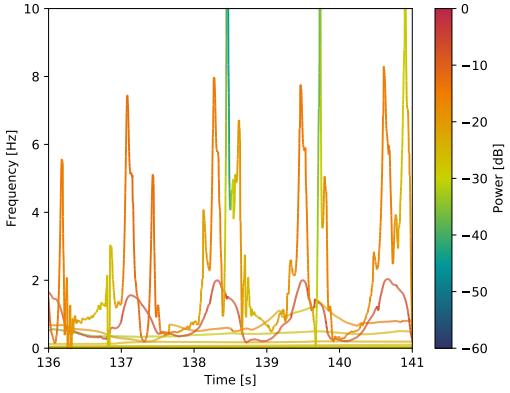


Figure 2. Hilbert spectrum

A time-frequency-power distribution illustrated by Figure 5 of Paper I. This is the same 5-second extraction as in Figure 1, illustrated with a Hilbert spectrum. Reprinted from Adv. Data Sci. Adapt. Anal., 2017, Vol 9, Knai et al, *Instantaneous Frequencies of Continuous Blood Pressure. A Comparison of the Power Spectrum, the Continuous Wavelet Transform and the Hilbert-Huang Transform* (38), page 1750009-6, Figure 5. Paper distributed under a Creative Commons license, and can be shared without obtaining additional permissions from World Scientific.

Curve fitting

Curve fitting is the process of creating a curve that fits a series of data points. It involves both interpolation, where you get an exact fit to the data, and smoothing, where a smooth line that approximately fits the data is created. In this thesis, we use a spline interpolation in the preprocessing algorithm and local regression to fit a smoothed curve to the data. The latter was originally developed for scatterplot smoothing and is therefore called locally estimated scatterplot smoothing (Loess).

Correlation analyses

Correlation is any association between two variables – to which degree two variables move in relation to each other. Causal variables always correlate, but not all correlated variables are causally dependent. In signal processing, correlation analysis is used to look for correlation between different signals or to look for serial dependence within one signal. Cross-correlation measures the correlation of two signals as a function of the displacement of one relative to the other. Thus, features that are present for shorter segments of the signal or that are present in both signals but shifted in time are identified. Autocorrelation is the correlation of a signal with a copy of itself that is shifted in time. The induced delay makes it possible to look for repeating patterns within the signal, such as oscillations.

Quantification of complexity

There is no clear definition of complexity. However, complex systems are built up by components that interact in multiple ways and with the external environment, resulting in organized and disorganized behaviour that cannot be predicted from the components alone (40). Hence, the complexity of the system is greater than the sum of its parts. Linking this to biological signals, complexity is related to the degree of information in

the signal, the predictability of the signal and the ability to describe the signal in a simple manner (Figure 3) (41).

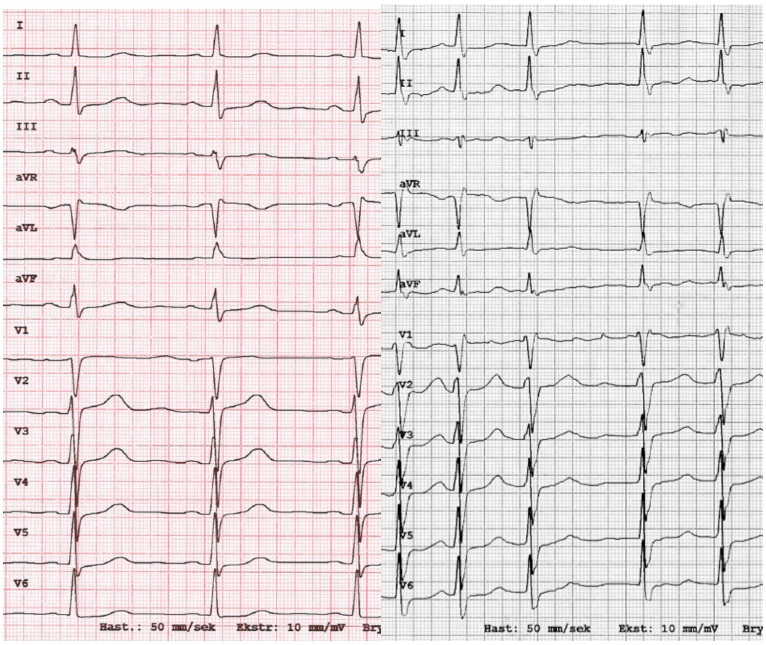


Figure 3. Complexity of biological signals

Illustrated by ECGs showing sinus rhythm (left) and atrial fibrillation (right). From the definition of complexity, atrial fibrillation is more complex, as it is less predictable and more challenging to describe. Reprinted by permission from Assoc. Prof. Jan Pål Loennechen's lectures for medical students at NTNU.

The definition of complexity is too diffuse to provide a quantitative measure that applies universally. Several complexity analyses are based on entropy, which classifies disorder in physical systems and information content of signals. Fractal analyses, such as detrended fluctuation analysis, illustrate the fractal behaviour of signals (42).

2.2.3 Why study biological oscillations? Motivation for the thesis

There are two statements forming the base of this thesis:

1) Specific oscillations of biological signals are linked to known physiological mechanisms, and they tend to disappear with disease.

2) The overall complexity of biological systems is linked to the systems' resilience and is reduced with age and disease.

Since the early development of the field, researchers have postulated that the discovery of biological oscillations would lead to medical advances (22). As oscillatory processes are linked to the subject's resilience and might show specific changes with disease, this information could be used in monitoring, diagnostics and prognostics. For this purpose, one needs to identify features of the signals that are specific for given diseases, but generalizable across patients. Considering the technological development during the past decades, we are surprised that such tools are not yet implemented in the clinic. When developed, the technology would be easy to implement, as it is based on signals that are already widely obtained.

Biological signals are nonlinear, nonstationary and contain considerable amounts of noise. Thus, the methodology is equally important as the specific research question being asked. We need to establish robust methods for both preprocessing and analysis of biological signals. If meant for clinical use, the methods should be quick, robust and easily adaptive to different biological signals.

Altogether, we want to make contributions to both the knowledge base of the field and its technological development.

3 AIMS

The overall aim of this thesis is to study the oscillatory distributions of biological signals in both healthy and cardiac surgery patients and to assess whether they hold specific or common features that can be implemented to clinical decision tools.

The specific aims of each paper are as follows:

Aim I: Investigate the performance of three different spectral analyses – the Fourier transform, the CWT and the HHT – on an invasive BP signal. How well do they identify the signal's oscillatory components and their time-variability?

Aim II: Investigate oscillatory components of 24-hour blood glucose recordings of pigs with the aim of deciphering slow blood glucose oscillations in an intact porcine model with the CWT. Is the CWT able to identify slow oscillations in blood glucose signals?

Aim III: Investigate the information content in ECG and BP recordings of healthy subjects by extracting three time series: SBP, HR and the amplitude of the ECG's R-wave. Does R-wave amplitude exhibit slow oscillations, and do these correspond with known slow oscillations in SBP and HR?

Aim IV: Investigate the development of the frequency distributions of SBP, HR and the amplitude of the ECG's R-wave of patients undergoing coronary artery bypass grafting (CABG). Do circulatory frequency distributions show loss of oscillatory components with cardiac surgery, and does this loss represent a reduction in complexity?

4 MATERIALS AND METHODS

4.1 Data

The work presented in this thesis is based on three study populations. Papers I and IV are based on data collected from patients scheduled for CABG. Paper I includes a continuous BP recording of one patient, whereas Paper IV includes continuous BP and ECG recordings of the total population of eight patients. Paper II is based on 24-hour continuous blood glucose recordings collected from four pigs. Paper III is based on the *Fantasia database* (43,44), which includes continuous NIBP, ECG and respiration recordings from 20 healthy individuals.

4.1.1 Cardiac surgery patients

From March to May 2016, patients scheduled for standard CABG at Trondheim University Hospital, Norway, was invited to participate in the study, recruiting a total of 10 patients. Two patients (patient 6 and patient 9) were excluded due to nonsinus rhythm at one or several time points of the recording. Other exclusion criteria were left ventricular ejection fraction below 0.5, severe valve disease, right ventricular failure, pulmonary hypertension and severe postoperative haemorrhage. Written consent was collected prior to data collection. The study protocol was approved by the Regional Committee for Medical and Health Research Ethics (reference: 2015/2019/REK midt). Confidentiality was strictly maintained throughout the study.

Data collection was performed in two sessions: before and after surgery. The data were collected with hardware and software provided by *ADInstruments* (Oxford, UK): PowerLab 16/35 and LabChart 8.1.3. The study equipment included a 3-electrode ECG, an LDF sensor and an arterial cannula inserted into the left radial artery. Additionally, subjects 1, 2 and 3 had an infrared PPG finger sensor attached. The preoperative recordings were collected with the patients resting in bed in a quiet room without disturbances in the thoracic surgery ward. The patients did not receive premedication prior to surgery, and surgery was performed under general balanced anaesthesia (thiopental, fentanyl, isoflurane and propofol). During surgery, the study equipment was

removed before it was reattached using new ECG patches and a new arterial cannula inserted into the right radial artery. The postoperative recording was collected from the patients arrived in the thoracic intensive care unit until the next morning.

For Paper I, a 5-min extraction of the preoperative BP recording of patient 1 was selected and exported to the software s2s (Signal Analysis Lab, Grimstad, Norway) for analysis.

For Paper IV, 30-min selections of the BP and ECG recordings of all eight subjects were exported as mat.files and analysed in R, version 3.5.1 (45). The PPG and LDF recordings of subject 1 were included for subanalysis. We subdivided the data into four situations: preoperatively (A); postoperatively, on respirator (B); postoperatively, after extubation (C); and postoperatively, the next morning (D).

Figure 4 shows an extraction of the raw PPG, ECG, BP and LDF signals of patient 1.

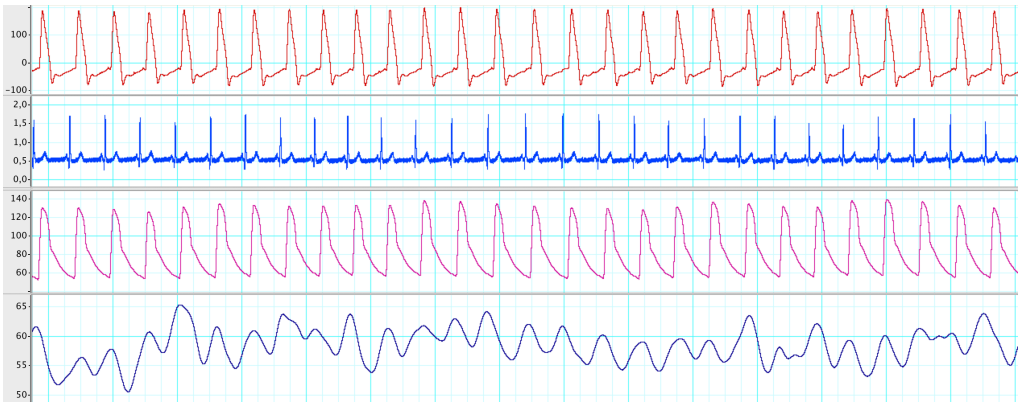


Figure 4. Raw signals of the cardiac surgery patients
Raw signals of the cardiac surgery patients, illustrated by the PPG (red), ECG (blue), BP (pink) and LDF (purple) signals of patient 1.

4.1.2 Pig blood glucose data

The experiments were originally performed in 2014 to validate intravascular glucose sensors over 24 hours. Four outbred pigs (25% Duroc, 25% Yorkshire, 50% Norwegian Landrace, 22–28 kg) were included in the study after approval from the Norwegian State Commission for Animal Experimentation. All the animals received humane care in accordance with the European Convention for the Protection of Vertebrate Animals used

for Experimental and Other Scientific Purposes. Details regarding premedication, anaesthesia, respirator adjustments and fluid therapy are explained in Paper II, and details regarding glucose sensor specifications and calibration procedures in (46). Two glucose sensors (GlucoSet, Trondheim, Norway) were inserted in each superficial femoral artery and connected to the in-house glucose monitor. The animals were kept sedated on a respirator for 24 hours before they were euthanized. The recordings were exported as csv.files (Figure 5) and analysed in R, version 3.1.1 (45).

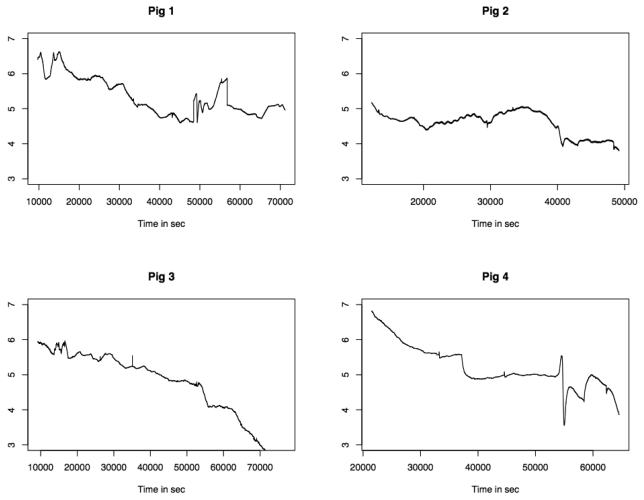


Figure 5. Raw blood glucose signals

Raw blood glucose signals of pigs 1-4. The durations of the recordings vary between approximately 50 000 and 70 000 seconds. Reprinted from PLOS One, Vol 13, Skjaervold et al, *Some oscillatory phenomena of blood glucose regulation: An exploratory pilot study in pigs* (47), page 3, Figure 1. Paper distributed under a Creative Commons license, and can be shared without obtaining additional permissions from PLOS.

4.1.3 The Fantasia database

We used prerecorded data from the *Fantasia Database* (43). ECG, respiration and NIBP recordings from twenty healthy subjects, ten young and ten elderly, were exported from *PhysioNet* (44) as mat.files and analysed in R, version 3.5.1 (45).

Figure 6 shows the raw ECG, NIBP and respiration signals of subject *f2y01*.

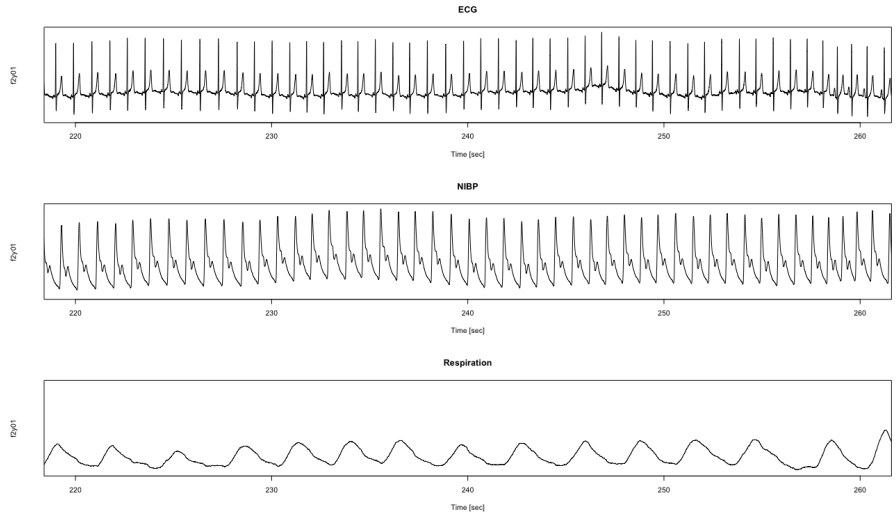


Figure 6. Raw signals from the Fantasia database
Raw signals from the Fantasia database (43) illustrated for subject *f2y01*. ECG in the first row, NIBP in the second row and respiration in the third row.

4.2 Preprocessing

In Papers III and IV, the BP and ECG signals are preprocessed to three time series: SBP, HR and R-wave amplitude. Baseline wander is removed from the ECG signals by applying a Savitzky-Golay smoothing filter before further analysis (48). We define the SBP and R-wave amplitude as the maxima of the BP and ECG, respectively. The HR is defined as: $HR = 60/RR_i$, where RR_i is the time interval in seconds between R-peak i and $i+1$ of the ECG. Some episodes of noise are misclassified as heartbeats; thus, we remove values outside the interquartile range from the SBP, RR-intervals and R-wave amplitude before further calculation. To provide evenly sampled time series, we perform a cubic spline interpolation to a sampling frequency of 10 Hz. The final variables are called interpolated SBP (iSBP), interpolated R-wave amplitude (iAmp) and interpolated HR (iHR). An overview of the preprocessing is shown in Figure 7.

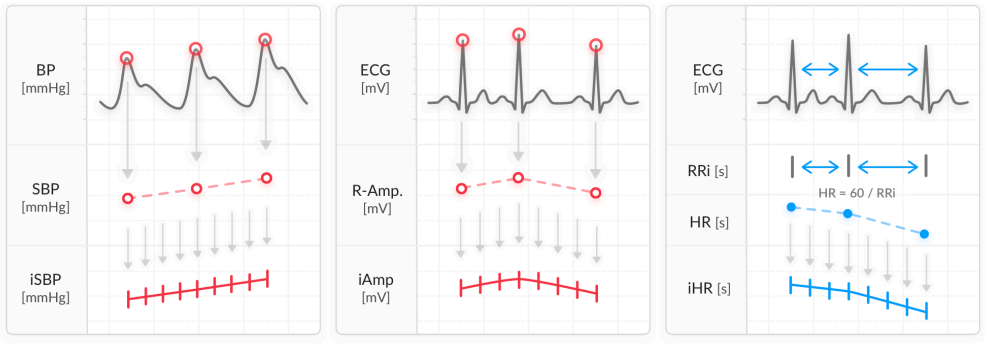


Figure 7. The preprocessing of BP and ECG signals

Preprocessing of BP and ECG signals generated the variables iSBP, iAmp and iHR. The SBP and amplitude of the R-peak are defined as the maxima of the BP and ECG, respectively. The HR is calculated from the time interval between two subsequent R-peaks of the ECG (RRi [s]). The time series are interpolated to a sampling rate of 10 Hz. Illustration: T. Aasnes. Reprinted from Physiol. Rep., Vol 8, Knai et al, *Cardiac surgery does not lead to loss of oscillatory components in circulatory signals* (49), page 3, Figure 1. Paper distributed under a Creative Commons license, and can be shared without obtaining additional permissions from John Wiley & Sons, Inc.

4.3 Analyses

The computation for Paper I is performed in cooperation with a third-party technical consultant firm named *Signal Analysis Lab AS,* using the software *s2s* (Signal Analysis Lab, Grimstad, Norway). Paper I involves comparing the performance of the power spectrum, the CWT and the HHT on a 5-min continuous BP signal.

In Paper II, 24-hour recordings of blood glucose in pigs are analysed with the CWT to examine the oscillatory profile of the pigs' glucose levels. The first 200 minutes of all glucose recordings are discarded prior to analysis due to extensive instability and sensor calibration.

In Paper III, we analyse NIBP- and ECG-signals from young and elderly healthy subjects. In Paper IV, we analyse selections, representing key events of the perioperative course, of the BP- and ECG-signals recorded on cardiac surgery patients. We use the CWT to identify oscillatory components present in iSBP, iAmp and iHR. In a subset of

cases, we examine the oscillations' phase differences by using the CWT for bivariate time series and Loess. Further, we perform a cross-correlation analysis on the Loess-extracted oscillatory components, identifying at which time lag the correlation is highest, and thus at which relative displacement the studied variables oscillate.

4.3.1 Fourier analyses

Fourier analyses encompass a vast spectrum of analyses decomposing signals to a sum of simple harmonics (Fourier transformation) and rebuilding the signals from the identified components (Fourier synthesis) (37,50). Fourier analyses include both simple frequency analyses, illustrating the frequency distribution of the complete signal, and time-frequency analyses, illustrating the frequency distribution as a function of time. As there are a variety of different Fourier analyses, none will be explained in detail.

4.3.2 Continuous wavelet transform

The CWT is a convolution of a signal with functions generated by the mother wavelet (51). The mother wavelet is a waveform of limited duration and an average value of zero. We use the Morlet wavelet, which by mathematical definition is a Gaussian enveloped cosine wave. It has been widely used for investigations of biological signals, especially the ECG (52). In the convolution process, it is shifted in time and stretched and shrunk, quantifying different frequency components' presence in the signal at different time points. The results are presented in time-frequency-power distributions, called CWT spectra. The results can also be displayed in average wavelet power spectra, illustrating the averaged frequency distribution of the total signal length. The CWT for bivariate time series identifies frequency components that are present in two time series with a significance level of 0.05. The results are presented in cross-wavelet spectra where oscillations that are significantly present in both time series are marked by white lines and their phase difference by arrows.

4.3.3 Hilbert-Huang transform

The HHT is a data-driven approach adaptive to the data being analysed (39). It decomposes the signal with the empirical mode decomposition, which defines the upper

and lower envelopes of the signal by identifying and drawing a line through the extrema. Further, the mean envelope is defined and subtracted from the original signal. When the mean envelope fills the requirements for an intrinsic mode function (IMF), it is defined as the first IMF. The process of defining envelopes and subtracting them from the signal is repeated until one has a monotonic line, which is considered the residual. Therefore, the empirical mode decomposition defines the fewest monocomponents possible to describe the signal, illustrated by IMFs with decreasing frequency. From the IMFs, the instantaneous frequency is defined as the time-derivative of the phase (53). The results are presented in time-frequency-power distributions called Hilbert spectra. To avoid blockage of the frequency components of interest, IMFs with high time-variations are excluded from the final plots.

4.3.4 Loess regression

Loess is a nonparametric regression that combines different regression models with a k-nearest-neighbour-based model (54). It applies classical regression models, such as least squares regression, to short segments of the signal. Further, it uses a weight function so that data points close to the point of estimation are weighted higher than data points further away. When performing the analysis, one specifies how long the segments of estimation should be, and thus how much of the signal that should be used to fit each local polynomial. Further, one specifies the degree of the local polynomials being fitted to the data.

In Papers III and IV, we decompose the time series with Loess to visually examine the individual time-series' oscillations and their phase differences. We apply the regression several times, each time subtracting the smoothed curve from the signal, providing a set of frequency components of increasing frequency. We name the extracted components Loess #1, Loess #2 and Loess #3. By generating plots of the components of the different variables, we examine their phase differences, illustrated in Figure 8.

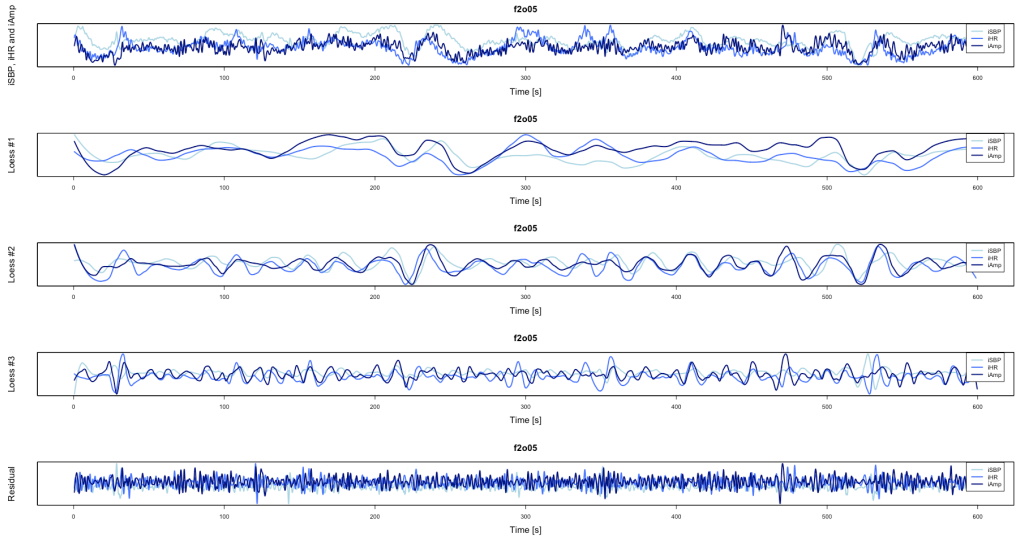


Figure 8. Decomposing the time series with Loess

Loess regression illustrated by Figure 5 from Paper III. The first row shows the time series of iSBP, iHR and iAmp. Loess #1-3 are plotted in rows two to four, and the residuals are shown in row five.

4.3.5 Cross-correlation analysis

The cross-correlation analysis calculates the correlation of two time series as a function of the displacement of one relative to the other – the cross-correlation function. The cross-correlation function illustrates the correlation of two time series at different time lags, the time lag representing the relative displacement. By defining the maximum, we identify at which time lag the correlation is highest, and thus at which relative displacement the studied variables oscillate.

5 RESULTS

5.1 Paper I

The Fourier-based power spectrum, the CWT and the HHT were applied to a 5-min continuous BP signal. The power spectrum illustrates the frequency distribution of the signal without capturing its time-varying nonstationary properties. It identifies the HR at 0.8 Hz. Considering slow frequencies, it shows increasing power below 0.1 Hz, without unambiguously identifying any specific frequency peaks. The CWT and the HHT identify corresponding slow frequency components and their time-variability. More specifically, they identify oscillations at approximately 0.04 Hz and just below 0.01 Hz. It is arguable if the CWT also identifies the 0.02 Hz oscillation, which is seen in the Hilbert spectrum. The CWT has considerably lower temporal resolution at low frequencies than the HHT.

5.2 Paper II

The CWT was applied to 24-hour continuous blood glucose recordings of four pigs in general anaesthesia. We illustrate the presence of a previously not reported oscillation with frequency 0.01-0.02 Hz. Further, we illustrate several frequency components, most prominent the known 0.001-0.002 Hz oscillation. We observe that the oscillations are not constantly present, but rather phenomena that come and go.

5.3 Paper III

NIBP and ECG recordings from twenty healthy subjects were decomposed to time series of SBP, HR and R-wave amplitude and analysed with the CWT. We illustrate the presence of slow oscillations in R-wave amplitude and some cases with corresponding slow oscillations in SBP and HR. Slow oscillations in R-wave amplitude are mainly found in the old subgroup, as the young subgroup shows a domination of the respiration. The R-wave amplitude is the single variable with the best identification of the respiration. The variables oscillate with time lags of a few seconds for both respiratory and slow oscillations.

5.4 Paper IV

BP and ECG recordings of eight cardiac surgery patients were decomposed to time series of SBP, HR and R-wave amplitude and analysed with the CWT. Four situations were used to illustrate the development through the perioperative course. We identify oscillatory components in all variables, patients and situations. We illustrate frequency distributions that change through the perioperative course, but the observed changes do not display any trend or system. We present one case with loss of a distinct 25-second oscillation after surgery and another where noise is misclassified as an oscillation.

6 DISCUSSION

6.1 Methodological considerations

6.1.1 Data

Cardiac surgery patients

Our study population is small, consisting of only eight patients. We recruited patients scheduled for CABG over a period of three months in 2016. The recruited patients are heterogeneous individuals featuring different medical backgrounds, pharmacological profiles and general health. However, altogether, the group holds common features such as high age and coronary heart disease, which, to some extent, correspond with the overall population of CABG patients. One could raise the question of selection bias, as we recruited patients over a short time period and excluded patients with serious illnesses, such as heart failure, valve disease and perioperative complications. However, we investigate universal physiological features without performing statistical hypothesis testing or other comparisons on the group level. The comparisons we performed are only between situations of the perioperative course, and in such cases, the patients serve as their own controls. Interpretation of the results must be done with these aspects in mind, and the results' generalizability should be investigated in larger study groups.

To minimize autonomic activation and artefacts caused by postural changes, the patients remained lying down during data collection. For Paper I, we used the last 5 minutes of the preoperative recording of patient 1 to maximize the time for stabilization. The data were collected with research hardware and software to secure complete control of filtering and preprocessing algorithms applied to the data. To avoid putting the patients through unnecessary stress by inserting two arterial cannulas prior to surgery, we used different arterial cannulas pre- and postoperatively. A consequence of this could be different absolute values of the BP recordings before and after surgery. However, we believe that the frequency distributions of the signals are unchanged. Vasoactive and analgesic medications and fluids were administered postoperatively according to the

individual patients' clinical state. Thus, the patients may have received different amounts of medications, with varying contributions to their oscillatory distributions.

One could ask if the studied population already has compromised circulatory frequency distributions prior to surgery. This could be a result of coronary heart disease, comorbidities that are not included to the exclusion criteria, medications and other factors. We know that reduced HRV after myocardial infarction increases the risk of death (30,31). We explore a group with stable coronary heart disease, and we do not know if reduced HRV or loss of oscillatory components are features of their baseline oscillatory distributions. If so, it could explain why further loss of oscillations is not seen after cardiac surgery. For future studies, one could consider looking at valve surgery patients without coronary heart disease.

Pig blood glucose data

In large animal studies, the number of animals is kept low due to ethics considerations, high cost and complex methodology. In Paper II, data from already performed experiments were used to describe general qualitative phenomena of blood glucose regulation. To study the generalizability of the findings, one must perform new experiments with a larger study group. For exploration of any underlying physiological cause of the observed oscillations, future experiments should include some kind of intervention.

The animals were kept under general anaesthesia for 24 hours, where the primary aim was to validate the glucose sensors. The pigs received a bolus of insulin at the beginning of the experiments. When performing analyses for Paper II, this period was not included. General anaesthesia, prone position and artificial ventilation may have affected the glucose regulation. However, this is difficult to quantify, and must, as with all large animal experiments, be taken into consideration when interpreting the results.

In all animal experiments, "the three R's" must be considered (55,56):

Replacement: This study requires recordings from intact animals, as we are studying complex physiological mechanisms originating from an interplay of regulatory mechanisms.

Reduction: We use glucose recordings from four animals that were already collected. As such, the animal sacrifices were already made, and our study contributed to increasing the amount of information provided by the experiments.

Refinement: Our group has extensive experience in planning and performing experiments involving large animals. The project manager has long experience with human anaesthesia and was present during all experiments. The animals were euthanized while still sedated, referred to as so-called "acute experiments". Altogether, the suffering of the animals was kept to an absolute minimum.

The Fantasia database

The database consists of 40 subjects, 20 of them including all three recordings of interest: continuous NIBP, ECG and respiration signals. The NIBP signals are uncalibrated and noninvasive, recorded with the volume clamp method. Working with uncalibrated signals is advantageous, as you do not have to deal with short, flat segments caused by the calibration (Figure 9). However, the absolute values of the signals are not necessarily correct. We believe the oscillatory content, and thus, the frequency distributions of the signals are unchanged. To avoid problems when comparing signals with different absolute values, we perform analyses that are independent of scale.

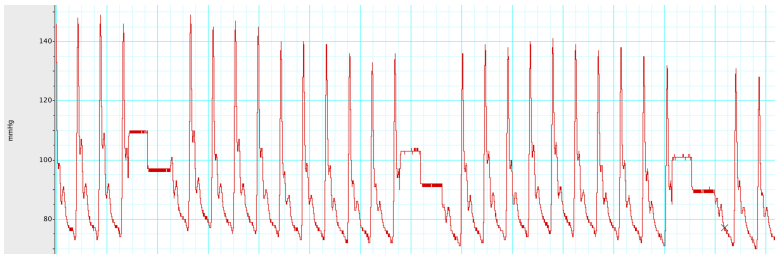


Figure 9. Calibrated NIBP signal

A calibrated NIBP signal showing the characteristic flat segments. This is a test recording performed with our research equipment, PowerLab 16/35 (ADInstruments, Oxford, UK).

When working with the uncalibrated NIBP signals, we discovered that the absolute values had a tendency of increasing over short segments and stabilizing on a new level (Figure 10). According to the volume clamp method (described in *Background: Biological signals*), the finger cuff is cyclically inflated and deflated to keep the PPG-signal flat. It is assumed that with a flat PPG-signal, the pressure inside the cuff equals the pressure inside the artery (36). We can only speculate on the underlying cause of the abrupt increase of pressure values. It is likely to assume that it is caused by some methodological factor, such as movement or changed placement of the cuff, and not a true increase in BP. The challenge with such steps is that they might mimic an oscillation, affecting the results. When choosing 10-min periods for analysis, we tried to avoid these segments. As many of the ECG signals also contain some segments of noise, and we had to select the same period for both signals, we had to accept some noise in the final periods. By removing outliers in the preprocessing, we removed short events of noise yielding extreme absolute values. However, the stepwise increases of the NIBP signals' absolute values were not considered as outliers, as the signals stabilized at the new level.

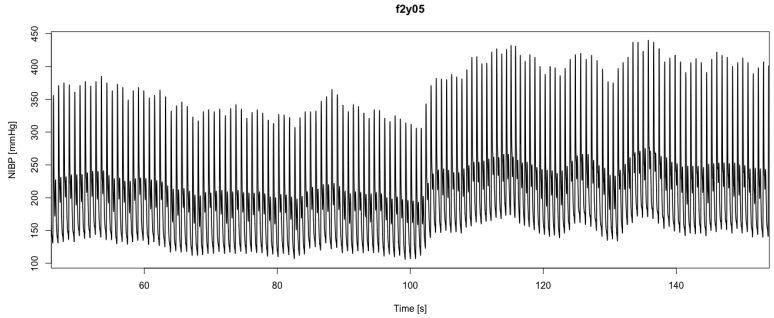


Figure 10. Variations in absolute values of uncalibrated NIBP signals

A steep increase in absolute values of the NIBP signal is seen just after the 100-second mark.

6.1.2 Noise handling and preprocessing

There are several things that can be done to reduce noise, both in the experimental setting and through preprocessing. All electrodes should be tightly attached, cords should be left untouched and the subject should be resting. In clinical settings or if the study protocol involves some kind of activity, these requirements can only partly be met.

Preprocessing involves all actions or changes that are made to the data prior to analyses. It might involve screening data for out-of-range values, missing values and impossible data combinations (Sex: Female, Prostatic cancer: Yes). In the field of signal processing, preprocessing involves data preparation through selection of time period, filtering and others.

In this thesis, we determined that it is impossible to provide noise-free recordings, even under controlled, experimental settings. After having selected nearly noise-free time periods, we saw that the remaining noise mainly included high-frequency irregularities in the baseline of the raw signals. As our main interest is slow oscillations, we are not interested in frequencies above the HR. Therefore, we developed a preprocessing algorithm, generating new time series based on the maxima of the raw signals. The variables SBP, HR and R-wave amplitude were established, as we wanted to look at the same aspects of the two signals: the maxima of the BP signal (SBP), the maxima of the ECG signal (R-wave amplitude) and the HR, which could have been extracted from either signal. We chose to extract the HR from the ECG, as this is in accordance with HRV analyses. With these variables, we have the opportunity to distinguish between oscillations in the oscillatory frequency from oscillations in the amplitude (Figure 11).

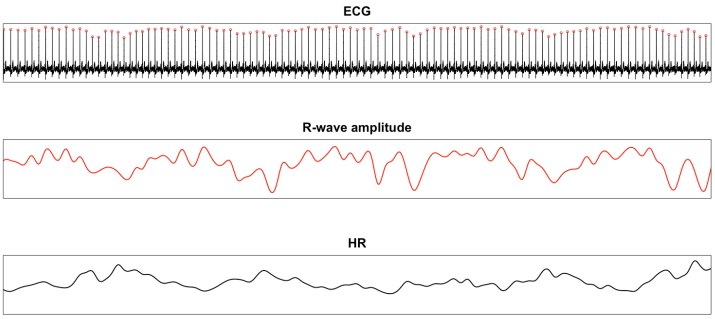


Figure 11. Amplitude vs frequency modulations of biological signals

An illustration of how an ECG signal can show oscillatory behaviour through both amplitude and frequency variations. The R-wave amplitude shows cyclic variations, even after baseline removal. Oscillations in HR time series have been extensively investigated with HRV and spectral analyses.

To check if our algorithms were applied correctly to circulatory signals, we used the *Fantasia database* (43,44). The recordings in the *Fantasia database* contain less noise than the ones we collected from cardiac surgery patients and are therefore easier to work with. The main aim of this work was to explore the oscillatory phenomena of the different variables and how they interact. Having worked with ECG signals for years, we had a suspicion that the R-wave amplitude contains oscillations slower than the respiration. We started exploring the literature and discovered the work of Brody from 1956, describing the theoretical model of ventricular preload's influence on R-wave amplitude (57). We found several works corroborating this theory through animal experiments (58–61). In humans, we found studies exploring R-wave amplitude changes in relation to coronary heart disease, intravascular volume status and mechanical ventilation (62–65). We found only one study plotting and inspecting the oscillatory behaviour of R-wave amplitude and relating this to the HR (66). One common feature of the cited articles is that they quantify amplitude variations by some measure of dispersion (e.g., variance) or only look at short-time variations, with emphasis on the respiration. We did not manage to find any publications describing slow oscillations in R-wave amplitude. We wanted to explore if R-wave amplitude also contains slow oscillations and their relation to slow oscillations in SBP and HR.

Amplitude variations can result in the whole signal drifting, called baseline wander. For BP recordings, one does not specifically talk about baseline wander, as amplitude variations are actual variations of systolic and diastolic BP. In regard to the ECG, baseline wander is considered to originate from various and to some extent uncontrollable causes, such as respiration, body movements and variations in electrode impedance (67–69). Therefore, baseline wander of ECG signals is commonly removed during preprocessing. One of the investigated subjects in Paper III showed a clearly sinusoidal baseline variation with phase approximately 20 seconds. Figure 12 shows a 10 min-selection of the ECG, without prior baseline-removal, and corresponding CWT of the iAmp.

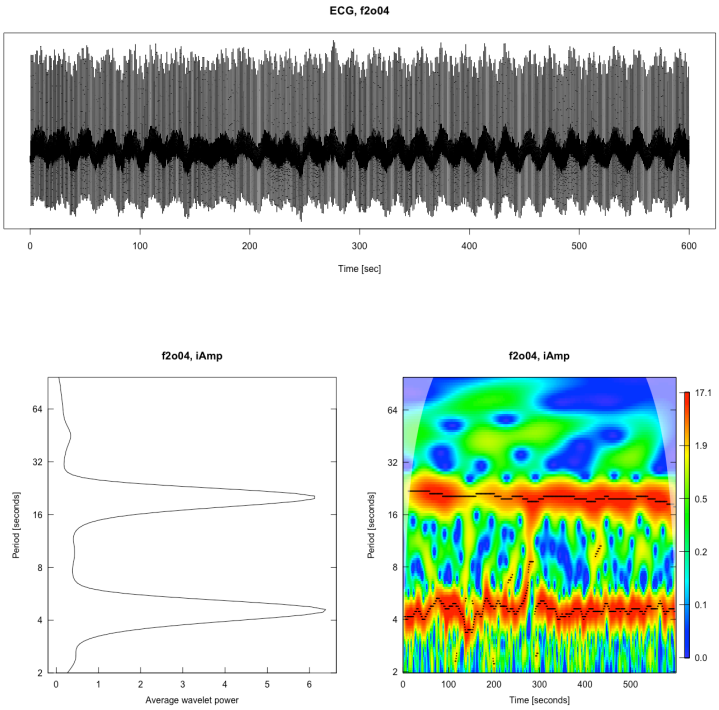


Figure 12. Removal of baseline wander in ECG recordings

The ECG and CWT of iAmp of subject *f2o04*, without prior baseline-removal. We see a clear sinusoidal oscillation with phase approximately 20 seconds in the ECG baseline, confirmed in the CWT of the iAmp. The CWT also illustrates the respiration with period just above 4 seconds.

From our point of view, it is unlikely that this oscillation is caused by noise, as noise is characterized by high-frequency and/or irregular fluctuations. Similar baseline variations are not observed in any of the other subjects in the study, reducing the probability of a methodological cause. We were not able to conclude an underlying cause, having observed the phenomenon in only one subject. Nevertheless, we believe this raises questions to the current view of baseline variations not providing valuable information and the widespread practice of removing them prior to analyses.

One can raise the question of bias in the process of selecting noise-free segments for analysis. When we first started our work with the cardiac surgery patients, we wanted to analyse the complete recordings using time-frequency analyses. Thus, the development through the perioperative course could be directly illustrated with time-frequency

spectra. As the preoperative recordings are much shorter, this would have made our results noncomparable, as the lowest possible identifiable frequency is related to signal length. Therefore, we decided to illustrate the time dimension through defining four situations and extracting 30-min sections representing each situation. The time points of important events were written down during data collection and used when selecting periods. The ECG and LDF signals showed large amounts of noise, especially during extubation and after waking up. This noise was most likely caused by movements and could not be avoided without impairing the quality of the patient care. Therefore, when choosing periods for analysis, we had to compromise between the quality of the signals and the time point of the perioperative course. Regarding the LDF signals, noise-free segments could only be provided in a subset of the patients, and they were therefore excluded from the main analyses. In one patient, the LDF signal was included in a sub-analysis to examine one specific identified oscillation.

6.1.3 Analyses

Quantification of complexity

As described in *Background*, the complexity of a system is caused by multiple interacting components, which further contributes to the information content of signals obtained from the system, the predictability of the signals and the ability to describe the signals in a simple manner (40,41). Oscillatory components of biological systems represent underlying components that interact and produce the behaviour of the system as a whole. They increase the information content and may reduce the predictability of biological signals. We used this as a basis for relating the number and distribution of oscillatory components to the overall complexity of the system. Altogether, we believed that frequency and time-frequency analyses could be used as surrogates for complexity analyses, as they decompose the signal and depict the number of components that are needed to describe the original signal. By this, we postulated that highly complex systems contain many oscillating components showing time-varying properties, possible to decipher with frequency- and time-frequency analyses. Having completed the work, we see that this approach involves challenges. The generated spectra showed a high number of oscillatory components and large interindividual variations. Altogether, a

quantitative comparison of complexity was difficult to provide. Instead, we looked for situations with loss of distinct oscillatory components, representing a reduction in the overall complexity (Paper IV). This is an insufficient approach if the aim is to develop a tool for defining different subjects' resilience through the quantification of complexity.

Spectral analyses

All Fourier-based spectral analyses are hampered with deficiencies in regard to analysing biological signals, as the signals are seldom linear or stationary. Nonlinearity and nonstationarity can lead to low precision in the final spectra, as high numbers of waves or wavelets are required to describe the original signal. Another limitation considering Fourier and wavelet analyses is that they are bound by a time-frequency resolution based on the Heisenberg-Gabor limit. This is the origin of windows in such spectra, and a trade-off between spectral and temporal resolution is unavoidable (70). The mathematical basis of this is known as the uncertainty principle of signal processing, which is given by $\Delta t \cdot \Delta f \leq \frac{1}{4\pi}$, where t represents the time and f the frequency. With decreasing frequency, f, the time-resolution decreases, as the time-interval, Δt, increases. As slowly oscillating processes are of high interest in biological research, this represents a challenge when using such analyses.

Originally, we planned to analyse all signals with the CWT. We planned to perform the analysis on the total signal length and were therefore interested in illustrating the time-variability. We discovered the HHT, which promises higher time-resolution. As the HHT is computationally challenging we engaged *Signal Analysis Lab* to help us with the analyses. We decided to start our work with a methodology article, both to check that it was possible to use the HHT on biological signals and to compare the performance of different spectral analyses (Paper I). In the work of Paper I, we experienced that the high time-resolution provided by the HHT made it difficult to quantify and separate the different oscillatory components, both within and between subjects. The oscillatory components tended to overlap, making it difficult to quantify their frequency. The most prominent high-frequency components showed extremely large time-variations,

blocking the view of slower oscillatory components (Figure 13). To be able to see the oscillations of interest, these oscillations had to be removed from the final spectra.

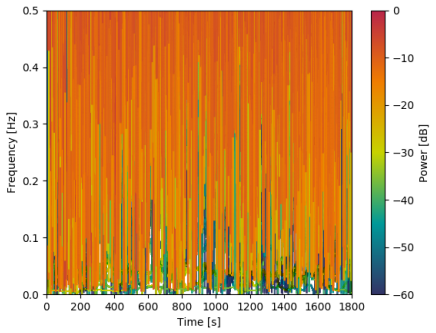


Figure 13. Oscillations with large time-variations

A Hilbert spectrum with an oscillatory component showing large time-variations. In this case, it is both difficult to quantify the frequency of the oscillation, and the oscillation blocks the view of other, slower, oscillations.

With this knowledge, we hesitated to continuate the use of the HHT, as we were planning to analyse larger datasets and perform comparisons on the group level. Additionally, we had become aware of the extent of the computational skills required to perform the HHT correctly. To avoid being dependent on help from others, we directed our focus back towards the CWT. To investigate its performance on slow oscillations, we used glucose signals obtained from pigs in 2014. As the signals were 24 hours long, they were ideal for the identification of slow oscillatory phenomena. In this work, we experienced that the CWT identified, with acceptable time-resolution, oscillations as slow as 0.0002-0.0001 Hz. However, we discovered that it was challenging to identify localized, slow oscillations in long signals by subjecting the complete signal to the CWT. In those cases, we had to identify the oscillations by visual inspection and confirm their presence by applying the CWT to shorter segments of the signal.

Analysis of phase differences

In the literature, when describing phase differences, it is said that two signals are in phase when there is no time lag between the two. Thus, in all situations where there is a time

lag between two signals, they are said to be off phase. Two signals oscillate in antiphase when the time lag is close to half a period. Phase shift is presented in angles, calculated as a fraction of one whole turn.

In this thesis, we do not compare an oscillation with a shifted version of itself, but rather compare oscillations that are identified in different time series. We do not calculate phase shift in angles but specify the time lag in seconds. Therefore, we restrict the use of the term *off phase* to examples where the oscillations do not follow each other. More specifically, if an oscillation has a period of 100 seconds, and the lag between the different time series is 2-3 seconds (Figure 5, Paper III), we say that the variables oscillate *in phase*. Additionally, we specify which time series or variable is leading. If an oscillation with a period of 4-5 seconds shows a similar displacement between variables (Figure 3, Paper III), we would say that the variables oscillate *off phase*, as the time lag is approximately half a period. This is in accordance with Figure 14.

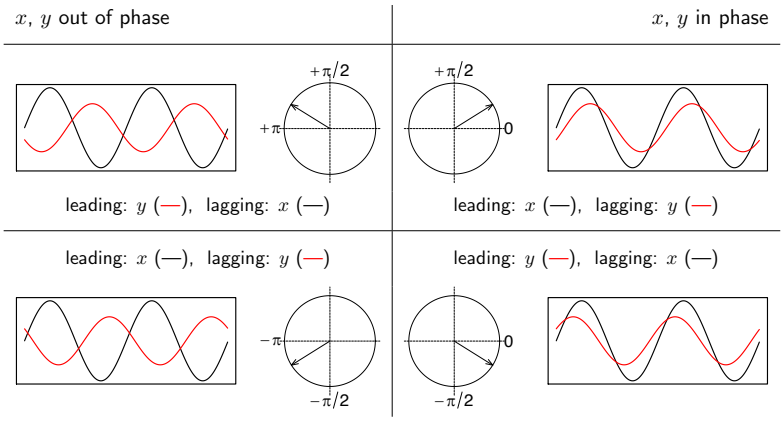


Figure 14. Phase differences and their interpretation

An illustration of phase angles at different phase differences of signals x and y. Reprinted from *Wavelet Comp 1.1, A guided tour through the R package* (71) with permission from the authors.

6.2 Discussion of the main results

The main motivation for this thesis was to establish robust methods for analysing biological signals and to use these to extract information from biological signals suitable for implementation to clinical decision tools. Through Papers I, II and III, we have developed a method for preprocessing and analysing biological signals. Summarized, we preprocess the raw signals by defining the maxima and creating new time series based on their absolute values (SBP and R-wave amplitude) or the oscillatory rate (HR). We found that the CWT identifies the same frequency components as the HHT and that high temporal resolution can be challenging in situations where you are comparing subjects and time points. We have explored the frequency distributions of glucose in pigs and circulatory signals in healthy and cardiac surgery patients. Looking at our findings altogether, there are some aspects we want to highlight:

Oscillations in amplitude and frequency. We find oscillations in different aspects of biological signals – both frequency and amplitude. This might be explained by a direct link between the two – frequency ↔ amplitude. However, it could reflect different, synchronized physiological processes that leave different traces in biological signals.

Heterogeneity. In Papers III and IV, we have explored frequency distributions of circulatory signals from cardiac surgery patients and healthy individuals. The healthy study population of Paper III was further subdivided into a young and an old group. We have performed comparisons between individuals, groups and over time. Altogether, our findings display huge intra- and interindividual variations, making it impossible to define features specific for healthy, diseased, ageing or cardiac surgery. Further, our findings do not directly translate to the specific frequency bands (high, low, very low and ultra-low frequencies) that are described in the literature (6). One could speculate how the power would distribute between the different bands if we operated with frequency intervals instead of presenting the original frequency spectra. However, these bands have been established as researchers have identified peaks at the given frequencies (7,8,10,11), to which our findings do not correspond. We see the use of frequency bands as a simplification of a highly complex field.

Come and go. In Paper II, we describe oscillations that are not present all the time, but rather come and go. This might contribute to the described large intra- and interindividual variations. It also represents a risk of overseeing localized oscillations when analysing long signals, as described in *Methodological considerations*.

Complexity. There are some studies stating that they quantify complexity from frequency spectra (24,72). However, they present spectra that are much less variable than our results, and simply quantify the degree of power among different frequency bands. Our idea was to do this with a much higher precision. As described in *Methodological considerations*, the large heterogeneity made quantifying this complexity challenging, not to mention comparing subjects and/or situations.

We have sketched three factors that might explain the mentioned features of our findings and the discrepancies with earlier findings in the field:

1) Reality is not as simple as earlier described. Reality is actually as heterogenous and time-varying as described in this thesis. Thus, earlier findings are either erroneous or representing only small aspects of reality. If so, the development of clinical decision tools would meet large challenges regarding generalizability.

2) The measured signals only partly reflect the underlying physiology. Science is largely based on a reductionistic approach, where complex problems are divided into smaller and simpler units that are investigated separately (73). Some problems are solvable this way, but sometimes you lose the perspective of the system as a whole. We explore BP, ECG and glucose recordings with the aim of gaining knowledge about physiology. The signals are believed to represent a sum of all physiological mechanisms that affect the variable being measured. As a consequence, we cannot know to which extent the signals hold features that are important to physiology and if important features are not visible in the signals. To increase the possibility of identifying oscillatory processes, we chose to extract three variables representing different aspects of the signals and analysed them separately. Of the extracted variables, the R-wave amplitude is probably the least robust, as skin contact, electrode placement and body movements might disturb the findings. In addition, the physiological correlate of variations in R-wave amplitude is less explored.

3) There are methodological issues. The deviation from earlier findings might be caused by methodological issues, such as sample size, noise and/or choice of analysis. Sample size is the major limitation of this work. Heterogeneous results are the main reason for us not being able to conclude in accordance with the motivation and aims sketched when starting the project. We do not know if heterogeneity is a feature of the general population, or only of the explored samples. However, heterogeneity is seen in all papers of this thesis, giving us reason to believe that this is also the case for larger samples. Regarding the analyses, we know that all Fourier-based analyses are hampered with limitations in regard to biological signals. We have illustrated low temporal resolution among low frequencies, but still the CWT identified the same frequencies as the HHT (Paper I). Thus, we can expect our results to be correct when the analyses are applied to noise-free signals and when the frequency range is reasonable in relation to signal length. In Paper IV, we present a case where noise is misclassified as a true oscillation (Supplementary Figure 2, Paper IV). To avoid misinterpreting our results, we have inspected all raw signals and time series and related them to the frequency spectra. Nevertheless, we are left wondering if some of the identified oscillations represent noise and not true physiological oscillators. As we have collected data in controlled, experimental settings, this emphasizes the challenges one will meet when developing tools that are meant to be automated and used in everyday clinical practice.

Altogether, our findings imply that biological oscillations cannot be used in clinical decision tools. The major reason for this, we believe, is caused by human physiology being much more heterogenous and complex than earlier believed. However, we cannot be completely sure that methodological challenges are not the actual problem. If so, technology is the limiting factor for future achievements.

6.2.1 Future perspectives

Going back to the motivation for this thesis, we used two statements as the basis: *1) Specific oscillations of biological signals are linked to known physiological mechanisms, and they tend to disappear with disease*, and 2*) the overall complexity of biological systems is linked to the systems' resilience and is reduced with age and disease*. For this to be possible to implement in clinical decision tools, we need to *identify features of biological signals that are specific for given diseases, but generalizable across patients*. A complex physiology with large interindividual variations makes the statements invalid, and the following requirement impossible. This is supported by an early work by Ary Goldberger (22) that is pioneering in the field of complexity and one of the first studies describing loss of complexity with disease. The fact that the technology is still not implemented to clinical practice can indicate that we have met the same problems as many before us. In 2001, Leon Glass stated that *"the field of biological rhythms have not yet led to medical advances, although several directions are under active consideration"* (3). This statement came 20 years after the first description of oscillatory components of interbeat time series (7). Today, after another period of 20 years, increasing numbers of advanced mathematical algorithms are being developed (74,75), but the technology is still not seen in everyday clinical practice. Large efforts are made to develop wireless and more precise sensors, alongside with implementing a vast number of variables for automatic monitoring systems (76). However, the focus on systems based on biological oscillations alone seems to have slowed down. The observed failure to show definite progress makes us highly hesitant to expect future breakthroughs in the field. If possible, we believe further development is dependent on less focus on specific oscillations and their underlying physiological mechanisms. Only with a broader perspective, perhaps with use of systems biology, can the whole system be taken into consideration, and the observed heterogeneity might be explained (77). For implementation in clinical practice, the methods must be based on equipment that is affordable and widely available, in addition to involving analyses that are applicable across subjects and not dependent on manual validation. Among challenges that need to be overcome, we will highlight noise-handling and the development of an algorithm for quantification of complexity.

The field of biological oscillations is built upon the view that oscillations are a vital feature of all living organisms, and thus important in themselves. According to Cheong and Levchenko, this might not always be true: *"the ease with which oscillations can emerge also should increase our caution about the natural tendency to ascribe functional significance to all observed oscillatory processes"* (78).

7 CONCLUSIONS

Compared to conventional frequency analyses, time-frequency analyses provide higher precision when analysing nonstationary signals and have the advantage of illustrating the signals' time-variability. The CWT and the HHT, both time-frequency analyses, have similar capabilities for identifying oscillatory components of biological signals, although the CWT has considerably lower temporal resolution at low frequencies than the HHT.

Biological oscillations show time-variability through period, amplitude and presence. We illustrate synchronization of respiratory oscillations in SBP, HR and R-wave amplitude. R-wave amplitude contains slow oscillations, and some cases show synchronized slow oscillations in SBP and HR. Slow oscillations in R-wave amplitude might be a result of preload variations in accordance with the Brody effect, but we cannot conclude on their underlying physiological mechanisms based on this work.

The frequency distributions of SBP, HR and R-wave amplitude of cardiac surgery patients show only one case with loss of a distinct oscillatory component after surgery. At the group level, the surgery does not induce systematic changes in the frequency distributions, and the observed high variety seems to represent interindividual variations more than factors of the performed surgery.

The overall conclusion of this thesis is that the oscillatory distributions of ECG and BP signals of healthy and cardiac surgery patients are highly heterogenous and do not hold features that are either group-specific or common to both groups. Hence, they do not hold information suitable for implementation in clinical decision tools.

8 ERRATA

In *Discussion, Methodological considerations,* (page 46, line 10) the following sentence was corrected with the correct analysis (the HHT, not the CWT): Additionally, we had become aware of the extent of the computational skills required to perform the HHT correctly.

Location was included for Signal Analysis Lab (Grimstad, Norway) on page 28 and 31, and for ADInstruments (Oxford, England) on page 39.

References for Figure 1 (page 19), Figure 2 (page 20), Figure 5 (page 29), Figure 7 (page 31) and Figure 14 (page 47) is included in the figure legends and in the reference list. Additionally, reference for Figure 1, paper III, is included.

In the legend of Figure 6 (page 30), the correct reference (Iyengar et al, 1996) for the Fantasia database is included.

9 REFERENCES

1. Hess B, Boiteux A. Oscillatory Phenomena in Biochemistry. Annu Rev Biochem. 1971;40(1):237–58.

2. Dupont G, Combettes L, Bird GS, Putney JW. Calcium Oscillations. Cold Spring Harb Perspect Biol. 2011 Mar;3(3).

3. Glass L. Synchronization and rhythmic processes in physiology. Nature. 2001 Mar 8;410(6825):277–84.

4. Que C-L, Kenyon CM, Olivenstein R, Macklem PT, Maksym GN. Homeokinesis and short-term variability of human airway caliber. J Appl Physiol. 2001 Sep 1;91(3):1131–41.

5. Hunt BR, Kennedy JA, Li T-Y, Nusse HE. The Theory of Chaotic Attractors. Springer Science & Business Media; 2013. 522 p.

6. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart Rate Variability Standards of Measurement, Physiological Interpretation, and Clinical Use. Circulation. 1996 Jan 3;93(5):1043–65.

7. Akselrod S, Gordon D, Ubel FA, Shannon DC, Berger AC, Cohen RJ. Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. Science. 1981 Jul 10;213(4504):220–2.

8. Pomeranz B, Macaulay RJ, Caudill MA, Kutz I, Adam D, Gordon D, et al. Assessment of autonomic function in humans by heart rate spectral analysis. Am J Physiol-Heart Circ Physiol. 1985 Jan 1;248(1):H151–3.

9. Shaffer F, McCraty R, Zerr CL. A healthy heart is not a metronome: an integrative review of the heart's anatomy and heart rate variability. Front Psychol. 2014 Sep 30;5(1040).

10. Bracic M, Stefanovska A. Wavelet-based analysis of human blood-flow dynamics. Bull Math Biol. 1998 Sep;60(5):919–35.

11. Pagani M, Lombardi F, Guzzetti S, Rimoldi O, Furlan R, Pizzinelli P, et al. Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympatho-vagal interaction in man and conscious dog. Circ Res. 1986 Aug;59(2):178–93.

12. Julien C. The enigma of Mayer waves: Facts and models. Cardiovasc Res. 2006 Jan 4;70(1):12–21.

13. Madwed JB, Albrecht P, Mark RG, Cohen RJ. Low-frequency oscillations in

arterial pressure and heart rate: a simple computer model. Am J Physiol-Heart Circ Physiol. 1989 Jun 1;256(6):H1573–9.

14. Pagani M, Lucini D, Porta A. Sympathovagal balance from heart rate variability: time for a second round? Exp Physiol. 2012;97(10):1141–2.

15. Chou HF, Berman N, Ipp E. Oscillations of lactate released from islets of Langerhans: evidence for oscillatory glycolysis in beta-cells. Am J Physiol-Endocrinol Metab. 1992 Jun 1;262(6):E800–5.

16. Lang DA, Matthews DR, Peto J, Turner RC. Cyclic Oscillations of Basal Plasma Glucose and Insulin Concentrations in Human Beings. N Engl J Med. 1979 Nov 8;301(19):1023–7.

17. Lipsitz LA, Goldberger AL. Loss of "Complexity" and Aging: Potential Applications of Fractals and Chaos Theory to Senescence. JAMA. 1992 Apr 1;267(13):1806–9.

18. Kaplan DT, Furman MI, Pincus SM, Ryan SM, Lipsitz LA, Goldberger AL. Aging and the complexity of cardiovascular dynamics. Biophys J. 1991 Apr 1;59(4):945–9.

19. Umetani K, Singer DH, McCraty R, Atkinson M. Twenty-Four Hour Time Domain Heart Rate Variability and Heart Rate: Relations to Age and Gender Over Nine Decades. J Am Coll Cardiol. 1998 Mar 1;31(3):593–601.

20. Takahashi ACM, Porta A, Melo RC, Quitério RJ, da Silva E, Borghi-Silva A, et al. Aging reduces complexity of heart rate variability assessed by conditional entropy and symbolic analysis. Intern Emerg Med. 2012 Jun 1;7(3):229–35.

21. Lipsitz LA. Age-related changes in the '"complexity"' of cardiovascular dynamics: A potential marker of vulnerability to disease. Chaos Interdiscip J Nonlinear Sci. 1995 Mar 1;5(1):102–9.

22. Goldberger AL. Non-linear dynamics for clinicians: chaos theory, fractals, and complexity at the bedside. The Lancet. 1996 May 11;347(9011):1312–4.

23. Hon EH, Lee ST. Electronic evaluation of the fetal heart rate. VIII. Patterns preceding fetal death, further observations. Am J Obstet Gynecol. 1963 Nov 15;87:814–26.

24. Goldstein B, Fiser DH, Kelly MM, Mickelsen D, Ruttimann U, Pollack MM. Decomplexification in critical illness and injury: relationship between heart rate variability, severity of illness, and outcome. Crit Care Med. 1998 Feb;26(2):352–7.

25. Claydon VE, Krassioukov AV. Clinical correlates of frequency analyses of cardiovascular control after spinal cord injury. Am J Physiol-Heart Circ Physiol. 2008 Feb 1;294(2):H668–78.

26. Riordan WP, Norris PR, Jenkins JM, Morris JA. Early Loss of Heart Rate Complexity Predicts Mortality Regardless of Mechanism, Anatomic Location, or Severity of Injury in 2178 Trauma Patients1. J Surg Res. 2009 Oct 1;156(2):283–9.

27. Brunner R, Adelsmayr G, Herkner H, Madl C, Holzinger U. Glycemic variability and glucose complexity in critically ill patients: a retrospective analysis of continuous glucose monitoring data. Crit Care. 2012 Oct 2;16(5):R175.

28. Lundelin K, Vigil L, Bua S, Gomez-Mestre I, Honrubia T, Varela M. Differences in complexity of glycemic profile in survivors and nonsurvivors in an intensive care unit: A pilot study. Crit Care Med. 2010 Mar;38(3):849–54.

29. Ho KKL, Moody GB, Peng C-K, Mietus JE, Larson MG, Levy D, et al. Predicting Survival in Heart Failure Case and Control Subjects by Use of Fully Automated Methods for Deriving Nonlinear and Conventional Indices of Heart Rate Dynamics. Circulation. 1997 Aug 5;96(3):842–8.

30. Wolf MM, Varigos GA, Hunt D, Sloman JG. Sinus arrhythmia in acute myocardial infarction. Med J Aust. 1978 Jul 15;2(2):52–3.

31. Kleiger RE, Miller JP, Bigger JT, Moss AJ. Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. Am J Cardiol. 1987 Feb 1;59(4):256–62.

32. Kuo C-D, Chen G-Y, Lai S-T, Wang Y-Y, Shih C-C, Wang J-H. Sequential changes in heart rate variability after coronary artery bypass grafting. Am J Cardiol. 1999 Mar 1;83(5):776–9.

33. Demirel Ş, Akkaya V, Oflaz H, Tükek T, Erk O. Heart Rate Variability After Coronary Artery Bypass Graft Surgery: A Prospective 3-Year Follow-Up Study. Ann Noninvasive Electrocardiol. 2002;7(3):247–50.

34. Lakusic N, Mahovic D, Sonicki Z, Slivnjak V, Baborski F. Outcome of patients with normal and decreased heart rate variability after coronary artery bypass grafting surgery. Int J Cardiol. 2013 Jun 20;166(2):516–8.

35. Lakusic N, Mahovic D, Kruzliak P, Cerkez Habek J, Novak M, Cerovec D. Changes in Heart Rate Variability after Coronary Artery Bypass Grafting and Clinical Importance of These Findings. BioMed Res Int. 2015;2015.

36. Penáz J. Criteria for set point estimation in the volume clamp method of blood pressure measurement. Physiol Res. 1992;41(1):5–10.

37. Kreyszig E. Advanced Engineering Mathematics. 9th ed. Wiley; 2006. 1245 p.

38. Knai K, Kulia G, Molinas M, Skjaervold NK. Instantaneous Frequencies of Continuous Blood Pressure a Comparison of the Power Spectrum, the Continuous Wavelet Transform and the Hilbert–Huang Transform. Adv Data Sci Adapt Anal. 2017

Oct 1;09(04):1750009.

39. Huang NE, Shen Z, Long SR, Wu MC, Shih HH, Zheng Q, et al. The empirical mode decomposition and the Hilbert spectrum for nonlinear and non-stationary time series analysis. Proc R Soc Lond Math Phys Eng Sci. 1998 Mar 8;454(1971):903–95.

40. Johnson N. Simply Complexity: A Clear Guide to Complexity Theory. Oxford, England: Oneworld Publications; 2009. 202 p.

41. Goldberger AL, Moody GB, Costa MD. Variability vs. Complexity. Physionet.org. 2012. Available from: https://archive.physionet.org/tutorials/cv/

42. Goldberger AL, Amaral LAN, Hausdorff JM, Ivanov PCh, Peng C-K, Stanley HE. Fractal dynamics in physiology: Alterations with disease and aging. Proc Natl Acad Sci U S A. 2002 Feb 19;99(Suppl 1):2466–72.

43. Iyengar N, Peng CK, Morin R, Goldberger AL, Lipsitz LA. Age-related alterations in the fractal scaling of cardiac interbeat interval dynamics. Am J Physiol-Regul Integr Comp Physiol. 1996 Oct 1;271(4):R1078–84.

44. Goldberger AL, Amaral LAN, Glass L, Hausdorff JM, Ivanov PC, Mark RG, et al. PhysioBank, PhysioToolkit, and PhysioNet: components of a new research resource for complex physiologic signals. Circulation. 2000 Jun 13;101(23):E215-220.

45. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2018. Available from: https://www.R-project.org

46. Skjaervold NK, Solligård E, Hjelme DR, Aadahl P. Continuous Measurement of Blood Glucose: Validation of a New Intravascular Sensor. Anesthesiology. 2011 Jan;114(1):120–5.

47. Skjaervold NK, Knai K, Elvemo N. Some oscillatory phenomena of blood glucose regulation: An exploratory pilot study in pigs. PLOS ONE. 2018 Apr 2;13(4):e0194826.

48. Nahiyan KMT, Amin AA. Removal of ECG Baseline Wander using Savitzky-Golay Filter Based Method. Bangladesh J Med Phys. 2017 Mar 9;8(0).

49. Knai K, Aadahl P, Skjaervold NK. Cardiac surgery does not lead to loss of oscillatory components in circulatory signals. Physiol Rep. 2020;8(9):e14423.

50. Proakis JG, Manolakis DG. Digital Signal Processing. Fourth. New Jersey: Pearson; 2003.

51. Fugal DL. Conceptual Wavelets in Digital Signal Processing: An In-depth, Practical Approach for the Non-mathematician. United States: Space & Signals Technical Pub.; 2009. 382 p.

52. Addison PS. Wavelet transforms and the ECG: a review. Physiol Meas. 2005 Oct 1;26(5):R155–99.

53. Boashash B. Estimating and interpreting the instantaneous frequency of a signal. I. Fundamentals. Proc IEEE. 1992 Apr;80(4):520–38.

54. Cleveland WS, Devlin SJ. Locally Weighted Regression: An Approach to Regression Analysis by Local Fitting. J Am Stat Assoc. 1988;83(403):596–610.

55. Russell W, Burch R. The Principles of Humane Experimental Technique. Wheathampstead: Universities Federation for Animal Welfare; 1959.

56. Tannenbaum J, Bennett BT. Russell and Burch's 3Rs Then and Now: The Need for Clarity in Definition and Purpose. J Am Assoc Lab Anim Sci. 2015 Mar 1;54(2):120–32.

57. Brody DA. A Theoretical Analysis of Intracavitary Blood Mass Influence on the Heart-Lead Relationship. Circ Res. 1956 Nov;4(6):731–8.

58. Nelson CV, Chatterjee M, Angelakos ET, Hecht HH. Model studies on the effect of the intracardiac blood on the electrocardiogram. Am Heart J. 1961 Jul 1;62(1):83–92.

59. Horan LG, Andreae RL, Yoffee HF. The effect of intracavitary carbon dioxide on surface potentials in the intact canine chest. Am Heart J. 1961 Apr 1;61(4):504–14.

60. Angelakos ET, Gokhan N. Influence of venous inflow volume on the magnitude of the QRS Potentials in vivo. Cardiologia. 1963;42:337–48.

61. Nelson Clifford V., Rand Peter W., Angelakos Evangelakos T., Hugenholtz Paul G. Effect of Intracardiac Blood on the Spatial Vectorcardiogram. Circ Res. 1972 Jul 1;31(1):95–104.

62. David D, Naito M, Chen CC, Michelson EL, Morganroth J, Schaffenburg M. R-wave amplitude variations during acute experimental myocardial ischemia: an inadequate index for changes in intracardiac volume. Circulation. 1981 Jun;63(6):1364–71.

63. Degre S, Longo B, Thirion M, Stoupel E, Sobolski J, Berkenboom G, et al. Analysis of exercise-induced R-wave-amplitude changes in detection of coronary artery disease in patients with typical or atypical chest pain under digitalis treatment. Cardiology. 1981;68 Suppl 2:178–85.

64. Cannesson M, Keller G, Desebbe O, Lehot J-J. Relations Between Respiratory Changes in R-Wave Amplitude and Arterial Pulse Pressure in Mechanically Ventilated Patients. J Clin Monit Comput. 2010 Jun 1;24(3):203–7.

65. Lee CK, Rinehart J, Canales C, Cannesson M. Comparison of automated vs. manual determination of the respiratory variations in the EKG R wave amplitude for the

prediction of fluid responsiveness during surgery. J Comput Surg. 2014 Jan 10;1(1):5.

66. Amoore JN. Amplitude variations in electrocardiographic S and R waves during sleep. South Afr Med J Suid-Afr Tydskr Vir Geneeskd. 1981 Aug 8;60(6):232–6.

67. Sörnmo L. Time-varying digital filtering of ECG baseline wander. Med Biol Eng Comput. 1993 Sep 1;31(5):503.

68. Blanco-Velasco M, Weng B, Barner KE. ECG signal denoising and baseline wander correction based on the empirical mode decomposition. Comput Biol Med. 2008 Jan 1;38(1):1–13.

69. Luo Y, Hargraves RH, Belle A, Bai O, Qi X, Ward KR, et al. A Hierarchical Method for Removal of Baseline Drift from Biomedical Signals: Application in ECG Analysis. Sci World J. 2013.

70. Burrus CS, Gopinath RA, Haitao G. Introduction to Wavelets and Wavelet Transforms. New Jersey: Prentice Hall; 1998.

71. Roesch A, Schmidbauer H. WaveletComp: Computational Wavelet Analysis. 2018. Available from: https://CRAN.R-project.org/package=WaveletComp

72. Lipsitz L A, Mietus J, Moody G B, Goldberger A L. Spectral characteristics of heart rate variability before and during postural tilt. Relations to aging and risk of syncope. Circulation. 1990 Jun 1;81(6):1803–10.

73. Ahn AC, Tewari M, Poon C-S, Phillips RS. The Limits of Reductionism in Medicine: Could Systems Biology Offer an Alternative? PLOS Med. 2006 May 23;3(6):e208.

74. Kralemann B, Frühwirth M, Pikovsky A, Rosenblum M, Kenner T, Schaefer J, et al. In vivo cardiac phase response curve elucidates human respiratory heart rate variability. Nat Commun. 2013;4:2418.

75. Shekatkar SM, Kotriwar Y, Harikrishnan KP, Ambika G. Detecting abnormality in heart dynamics from multifractal analysis of ECG signals. Sci Rep. 2017 Nov 9;7(1):15127.

76. Davoudi A, Malhotra KR, Shickel B, Siegel S, Williams S, Ruppert M, et al. Intelligent ICU for Autonomous Patient Monitoring Using Pervasive Sensing and Deep Learning. Sci Rep. 2019 May 29;9(1):1–13.

77. Ahn AC, Tewari M, Poon C-S, Phillips RS. The Clinical Applications of a Systems Approach. PLOS Med. 2006 May 23;3(7):e209.

78. Cheong R, Levchenko A. Oscillatory signaling processes: the how, the why and the where. Curr Opin Genet Dev. 2010 Dec 1;20(6):665–9.

10 APPENDIX – PAPERS I TO IV

Paper I

Advances in Data Science and Adaptive Analysis
Vol. 9, No. 4 (2017) 1750009 (9 pages)

DOI: 10.1142/S2424922X17500097

Instantaneous Frequencies of Continuous Blood Pressure a Comparison of the Power Spectrum, the Continuous Wavelet Transform and the Hilbert–Huang Transform

Kathrine Knai
Department of Circulation and Medical Imaging
Norwegian University of Science and Technology
Trondheim, Norway
kathrikn@ntnu.no

Geir Kulia
Department of Electronics and Telecommunications
Norwegian University of Science and Technology
Trondheim, Norway

Marta Molinas
Department of Engineering Cybernetics
Norwegian University of Science and Technology
Trondheim, Norway

Nils Kristian Skjaervold
Department of Circulation and Medical Imaging
Norwegian University of Science and Technology
Trondheim, Norway

Received 7 July 2017
Accepted 15 September 2017
Published 3 November 2017

Continuous biological signals, like blood pressure recordings, exhibit nonlinear and non-stationary properties which must be considered during their analysis. Heart rate variability analyses have identified several frequency components and their autonomic origin. There is need for more knowledge on the time-changing properties of these frequencies. The power spectrum, continuous wavelet transform and Hilbert–Huang transform are applied on a continuous blood pressure signal to investigate how the different methods compare to each other. The Hilbert–Huang transform shows high ability to analyze such data, and can, by identifying instantaneous frequency shifts, provide new insights into the nature of these kinds of data.

Keywords: Instantaneous frequency; biological signals; power spectrum; continuous wavelet transform; Hilbert–Huang transform.

1. Background

Continuous biological signals, like blood pressure recordings, exhibit both nonlinear and nonstationary properties which must be considered during their analysis [Usui and Toda (1991)]. The fractal nature of biological signals is already thoroughly studied by methods as detrended fluctuation, multiscale entropy and Poincaré analyses, where the complexity of the overall signal is indexed [Seely and Macklem (2004)]. In order to decipher the oscillatory components of the signal, frequency analyses are required. The Fourier transform was introduced in the 19th century by Joseph Fourier, a method for decomposing a signal to a sum of simple harmonics [Kreyszig (2006)]. The Discrete Fourier transform is the foundation for several spectral analysis methods, such as the power spectrum [Proakis and Manolakis (2003)] and the continuous wavelet transform (CWT) [Burrus *et al.* (1998)]. The CWT is a time-frequency analysis method for identifying the nonstationary behavior of the signal and has been used to identify several dominant frequency bands in physiological time series [Bracic and Stefanovska (1998)]. The Hilbert–Huang Transform (HHT) was introduced in 1998 by Norden Huang [Huang *et al.* (1998)]. It was originally developed for analyzing nonstationary ocean waves and is, unlike the aforementioned methods, not based on the Fourier Transform. When studying oscillations in the circulatory system, one will easily discover the frequencies constituted by the heart beats and the respiration (1 Hz and 0,2–0,3 Hz, respectively). In heart rate variability (HRV) analyses of human ECG-signals, one has defined three main frequency components: high frequencies (HF) at 0.15–0.4 Hz; low frequencies (LF) at 0.04–0.15 Hz; very low frequencies (VLF) at 0.003–0.04 Hz; ultralow frequencies (ULF) below 0.003 Hz [Task Force (1996)]. The regulatory origin of these components have been studied to a great extent [Shaffer *et al.* (2014); Li *et al.* (2011)], but there is a need for more knowledge on the time-changing properties of these frequencies and the transferability to other biological signals, such as blood pressure. Motivated by the interest in exploring these frequency components, three different techniques are investigated in this paper to evaluate their capabilities in detecting instantaneous frequencies. The power spectrum, CWT, and HHT are applied on a real-life continuous blood pressure signal to investigate how the different methods compare to each other in terms of instantaneous frequency capture, focusing on the LF and VLF-range. The ULF are only identifiable in recordings longer than 5 min and thus beyond the scope of this paper.

2. Study Material

The signal examined in this paper is a 5 min' invasive blood pressure recording from one patient scheduled for coronary heart surgery at Trondheim University Hospital, Norway. Written consent was collected prior to data collection. The study protocol is approved by the Regional Committee for Medical and Health Research Ethics (REC). The signal is recorded with the patient resting in bed during a stable coronary condition, the morning before surgery. An arterial cannula was placed

in the patient's left radial artery using the standard technique; the patient did not receive any premedication prior to data collection. The data are sampled by PowerLab (ADInstruments) with a sampling rate of 400 Hz, and exported to s2s, a software provided by Signal Analysis Lab, for analysis.

3. Results

3.1. *Fourier-based spectral analysis*

Figure 1 shows the Fourier-based power spectrum [Proakis and Manolakis (2003)] of frequencies below 1 Hz of the continuous blood pressure signal. From this, a frequency peak around 0.8 Hz is identified. We can also see increasing power in the frequency components below 0.1 Hz, but it is not possible to unambiguously identify any specific frequency peaks in this range.

The power spectrum in Fig. 1 cannot capture the time-varying nonstationary properties of the frequency. The time-varying blood pressure was therefore analyzed using CWT as shown in Fig. 2.

A frequency centered just below 1 Hz, shown in red, corresponds to the heart rate and the frequency peak at 0.8 Hz in the power spectrum (Fig. 1). There also seems to be high power in the LF- and VLF-range, better visualized in Fig. 3.

There seems to be higher power around 0.04 Hz and just below 0.01 Hz, but as the CWT is limited by a physical time-frequency resolution, it is challenging to determine any nonstationary properties of these lower bands. The CWT optimizes the resolution by applying time-frequency scaling, giving high frequency resolution and lower temporal resolution in the lower band and higher temporal resolution with lower frequency resolution in the upper bands [Burrus *et al.* (1998)].

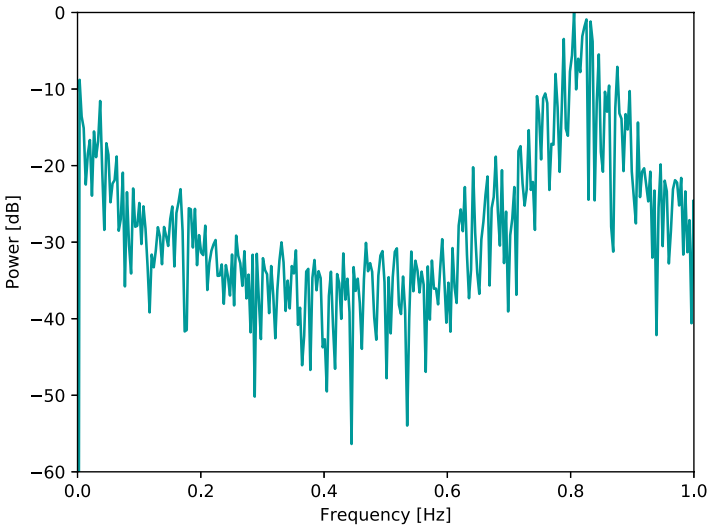


Fig. 1. Fourier-based power spectrum of the continuous blood pressure.

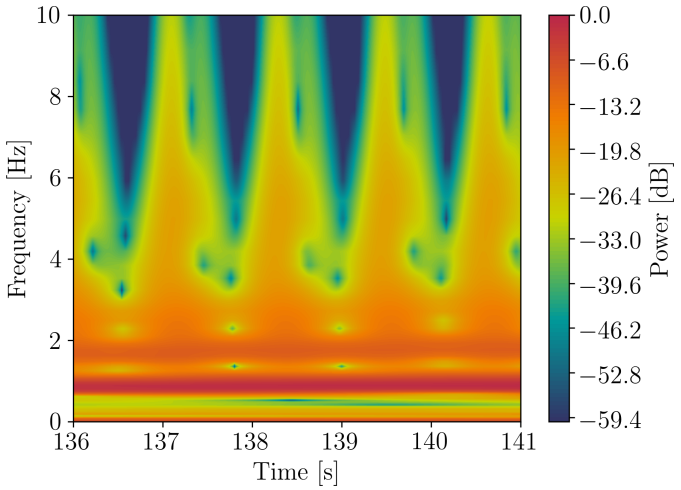


Fig. 2. CWT of the continuous blood pressure. The spectrum shows a 5 s extraction of the 5 min analyzed and frequencies up to 10 Hz.

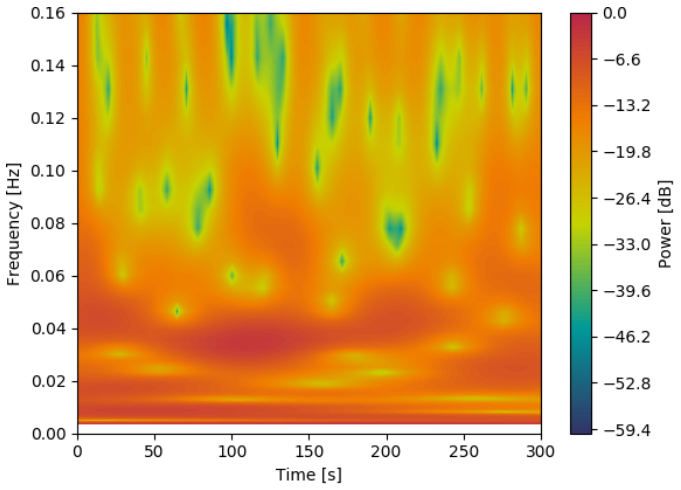


Fig. 3. CWT of the invasive blood pressure signal, showing frequencies below 0.16 Hz.

3.2. *Hilbert–Huang transform*

The HHT is, unlike Fourier-based spectral analysis methods, not based on the use of simple harmonics or predefined mother wavelets and is therefore not bound by the time-frequency resolution [Burrus *et al.* (1998)]. Instead, it uses the Empirical Mode Decomposition (EMD) to decompose the raw blood pressure signal BP(t) into moncomponents with varying amplitudes and frequencies called Intrinsic Mode Functions (IMFs) so that

$$x(t) = r(t) + \sum_{i=1}^{n} c_i(t), \tag{1}$$

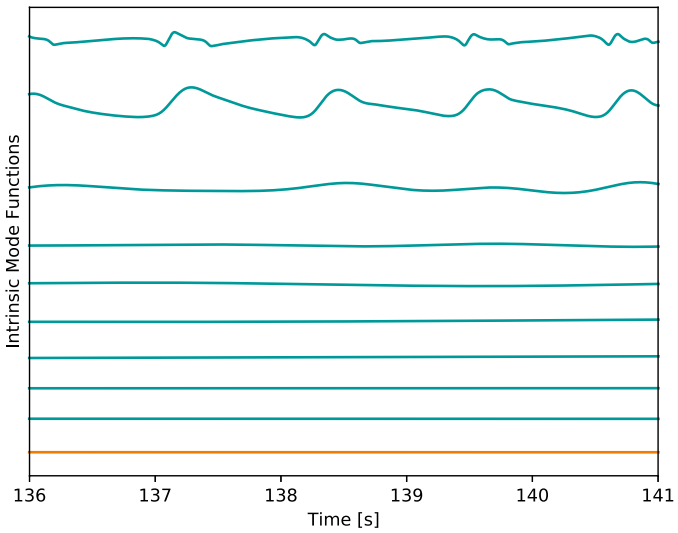


Fig. 4. The IMFs of $x(t)$, visualized by the same 5 s period as in Fig. 2. The residual is shown in orange.

where $x(t) = \mathrm{BP}(t)$ is the time-varying blood pressure, $c_i(t)$ is the intrinsic mode function number i that $x(t)$ consists of, and n is the total number of IMFs. The raw signal $x(t)$ were decomposed using EMD, and a 5 s sequence of the IMFs is shown in Fig. 4.

Each of the IMFs $c_i(t)$ that $x(t)$ consists of, can be written in the form

$$c_i(t) = a_i(t)\cos\theta_i(t), \tag{2}$$

where $c_i(t)$ is bound to only have one local extrema for each zero-crossing, and $a_i(t)$ and $\theta_i(t)$ are unambiguously defined [Huang *et al.* (1998); Hahn (2003)]. From this, the instantaneous frequency is defined as the time-derivative [Boashash (1992)] of the phase so that

$$f(t) = \frac{1}{2\pi}\frac{d\theta(t)}{dt}. \tag{3}$$

We can then define a Hilbert spectrum as

$$H(f,t) = \sum_{i=1}^{n} \begin{cases} a_i, & f = f_i(t) \\ 0, & \text{otherwise} \end{cases}. \tag{4}$$

Figure 5 shows the Hilbert spectrum of $x(t)$. The spectrum shows frequencies corresponding to the frequencies in the CWT; the heart frequency just below 1 Hz and several low frequencies. Frequencies lying above the heart rate are mainly caused by reflections of the closing of the aortic valve and are beyond the scope of this paper. However, they are useful for validating the CWT and HHT as both specters should constitute approximately the same time-varying high frequency-components. Figure 6 shows the CWT (Fig. 2) and the HHT (Fig. 5) overlapped,

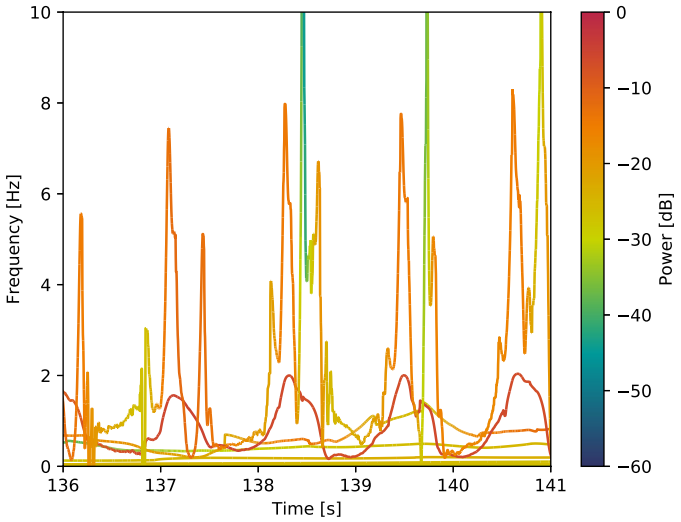


Fig. 5. Hilbert spectrum of $x(t)$, showing frequencies up to 10 Hz and the same 5 s period as in Figs. 2 and 4.

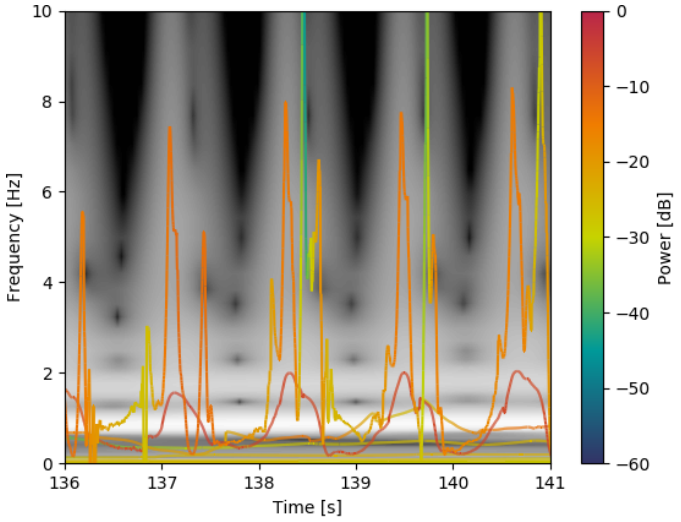


Fig. 6. The continuous wavelet transform in Fig. 2 shown in grayscale, overlapped with the Hilbert spectrum in Fig. 5.

with the CWT in grayscale, white illustrating high power and black illustrating low power. We clearly see that the shape of the highest frequency components resembles each other.

Figure 7 shows a Hilbert spectrum of frequencies below 0.16 Hz. The high frequency IMFs are discarded, and only the four lowest frequency components are displayed. One IMF constitutes large time variations and is therefore difficult to evaluate. The three other IMFs are varying around 0.04 Hz, 0.02 Hz and 0.01 Hz.

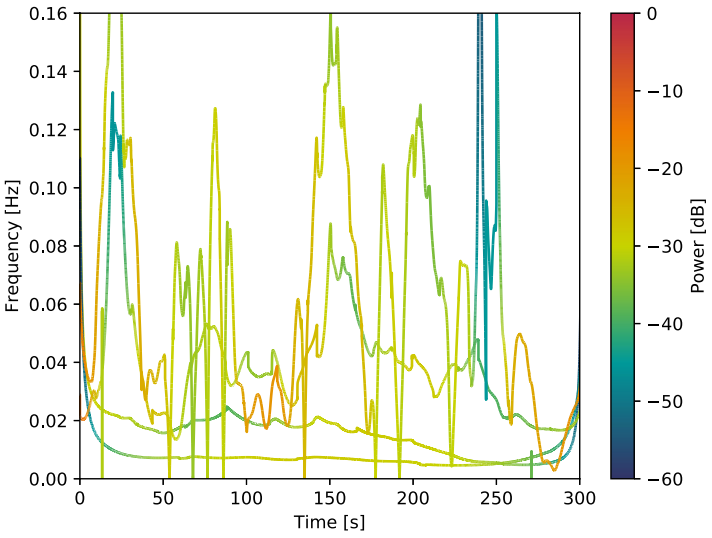


Fig. 7. Hilbert spectrum of the four lowest IMFs of $x(t)$.

For better visualization of the performance of the CWT compared to the HHT, Fig. 8 shows an overlapping of the specters in Figs. 3 and 7. The CWT is shown in grayscale. We see that areas with high power in the CWT (white and light gray) only partially correspond to areas with high power in the Hilbert spectrum. In the discussion, we suggest that this is caused by poor resolution of the CWT and removal of IMFs that would have blocked the view of the CWT. There is better correspondence between the areas with low power, seen as black spots in the CWT.

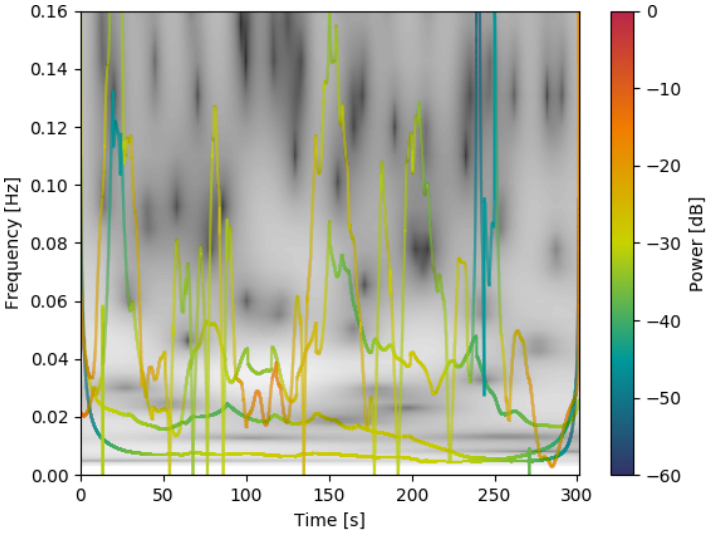


Fig. 8. The continuous wavelet transform in Fig. 3 shown in grayscale, overlapped with the Hilbert spectrum in Fig. 7.

4. Discussion

4.1. *Comparison of the methods*

The Power spectrum differs from CWT and HHT as it shows the frequency distribution in the signal under the assumption of time invariant amplitude and frequency values. By this assumption, the power spectrum cannot show any variation over time. The power spectrum identifies a frequency peak around 0.8 Hz corresponding to the heart rate, which is also seen in the CWT and HHT spectra. Considering frequencies below 0.1 Hz, the power spectrum does not unambiguously identify any specific frequency peaks, mainly caused by the spectral leakage in the Fourier Transform.

Both the CWT and HHT identify time variations in the high frequency range, but in the low frequency range, the CWT has considerable lower temporal resolution than the HHT. The CWT identifies two bands with high power around 0.04 Hz and just below 0.01 Hz. The HHT identifies the same frequencies and additionally a frequency around 0.02 Hz. It also illustrates the time-varying properties, especially seen in the 0.04 Hz-component. When comparing CWT and HHT in the low-frequency range in Fig. 8, one sees that the CWT has large areas with medium to high power (light gray and white) and smaller areas with low power (black). Thus, it could seem that the CWT performs better when it comes to identifying where there are no frequencies. On the other hand, the explanation could be that the areas with frequencies are smaller than illustrated and areas without frequencies are bigger than illustrated. Thus, the areas without frequencies are hidden and shown as small spots in the final specter. Some of the areas in Fig. 8 shows high power in the CWT but no power in the Hilbert spectrum. This would probably be avoided by showing more IMFs in the Hilbert spectrum, but we chose not to include them as they blocked the view of the CWT.

As mentioned in the introduction, biological data often exhibit both nonlinear and nonstationary properties which must be considered during their analysis. This is arguably the major reason for spectral leakage and low accuracy when analyzing such data with Fourier-based spectral analyses. HHT, being a data-driven approach designed to capture instantaneous frequency, is well suited for analyzing such data, and can, by identifying instantaneous frequency shifts, provide new insights into the nature of these kinds of data.

4.2. *Methodological considerations*

When analyzing biological data, one must always consider what may have influenced the data other than the physiological phenomenon one wants to investigate. To minimize autonomic activation and artifacts caused by postural changes, the patient was kept lying during data collection, and the data used for analysis was recorded after 55 min of rest. The patient did not receive any premedication prior to data collection. For correct pressure recordings, the transducer was kept in the same

height as the arterial cannula. The data were collected with research hardware and software, not patient monitors used in the clinic. Such patient monitors filter the raw signal in high extent and has several algorithms for identifying abnormalities such as fall in blood pressure, arrhythmias, etc. Using research equipment gives the researcher complete control of how the raw signal is processed before analysis. This is an advantage as it can be difficult to know exactly which pre-processing is used in clinical equipment. In later studies on these data, we are primarily interested in looking at slow oscillations, and therefore the maximum frequency of interest is the heart rate ($\sim$1 Hz). The blood pressure signal $x(t)$ was oversampled with a sampling rate of 400 Hz to avoid aliasing [Proakis and Manolakis (2003)].

Acknowledgments

This work was supported by research grants from the Faculty of Medicine, Norwegian University of Science and Technology and Department of Anaeshesia and Intensive Care Medicine, Trondheim University Hospital. We would like to thank Harald Elias Bjerke Sperre for contributing to the HHT analyzes. We would also like to thank Bjorn Gardsjord Lio and Fredrik Einar Tobias Axelsson for assistance in data collection.

References

Boashash, B. (1992). Estimating and interpreting the instantaneous frequency of a signal. I. Fundamentals. *Proc. IEEE*, **80**: 520–538.

Bracic, M. and Stefanovska, A. (1998). Wavelet-based analysis of human blood-flow dynamics. *Bull. Math. Biol.*, **60**: 919–935.

Burrus, C. *et al.* (1998). *Introduction to Wavelets and Wavelet Transforms.*

Hahn, S. L. (2003). On the uniqueness of the definition of the amplitude and phase of the analytic signal. *Signal Process.*, **83**: 1815–1820.

Huang, N. E. *et al.* (1998). The empirical mode decomposition and the Hilbert spectrum for nonlinear and non-stationary time series analysis. *Proc. R. Soc. Lond. A*, *Math. Phys. Eng. Sci.*, **454**: 903–995.

Kreyszig, E. (2006). *Advanced Engineering Mathematics.* 9th edn. Wiley, NY.

Li, H. *et al.* (2011). Hilbert–Huang transform for analysis of heart rate variability in cardiac health. *IEEE/ACM Trans. Comput. Biol. Bioinform.*, **8**: 1557–1567.

Proakis, J. G. and Manolakis D. G. (2003). *Digital Signal Processing.* 4th edn. Pearson, UK.

Seely, A. J. E. and Macklem, P. T. (2004). Complex systems and the technology of variability analysis. *Crit. Care*, **8**: 367–384.

Shaffer, F., McCraty, R. and Zerr, C. L. (2014). A healthy heart is not a metronome: An integrative review of the heart's anatomy and heart rate variability. *Front. Psychol.*, **5**.

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (1996). Heart Rate Variability Standards of Measurement, Physiological Interpretation, and Clinical Use. *Circulation*, **93**: 1043–1065.

Usui, S. and Toda, N. (1991). An overview of biological signal processing: Non-linear and non-stationary aspects. *Front. Med. Biol. Eng. Int. J. Jpn. Soc. Med. Electron. Biol. Eng.*, **3**: 125–129.

Paper II

PLOS ONE

RESEARCH ARTICLE

Some oscillatory phenomena of blood glucose regulation: An exploratory pilot study in pigs

Nils Kristian Skjaervold[1,2]*, Kathrine Knai[1], Nicolas Elvemo[3]

1 Department of Circulation and Medical Imaging, Norwegian University of Science and Technology, Trondheim, Norway, **2** Department of Cardiothoracic Anaesthesia and Intensive Care Medicine, Trondheim University Hospital, Trondheim, Norway, **3** Glucoset AS, Trondheim, Norway

* nils.k.skjaervold@ntnu.no

Citation: Skjaervold NK, Knai K, Elvemo N (2018) Some oscillatory phenomena of blood glucose regulation: An exploratory pilot study in pigs. PLoS ONE 13(4): e0194826. https://doi.org/10.1371/journal.pone.0194826

Editor: Antonio Gonzalez-Bulnes, INIA, SPAIN

Received: November 3, 2017

Accepted: March 9, 2018

Published: April 2, 2018

Data Availability Statement: Original data is to be found as supporting information.

Funding: The cost of the animal experiments and the salaries of NKS and KK were provided by Norwegian University of Science and Technology. The glucose sensors used were fabricated and provided by GlucoSet AS who provided support in the form of salaries for author NE who, as CEO and shareholder of GlucoSet AS, was involved in study design, analysis and interpretation of data and writing of the paper. GlucoSet AS did not have any additional role in the data collection or decision to

Abstract

It is well-known that blood glucose oscillates with a period of approximately 15 min (900 s) and exhibits an overall complex behaviour in intact organisms. This complexity is not thoroughly studied, and thus, we aimed to decipher the frequency bands entailed in blood glucose regulation. We explored high-resolution blood glucose time-series sampled using a novel continuous intravascular sensor in four pigs under general anaesthesia for almost 24 hours. In all time series, we found several interesting oscillatory components, especially in the 5000–10000 s, 500–1000 s, and 50–100 s regions (0.0002–0.0001 Hz, 0.002–0.001 Hz, and 0.02–0.01 Hz). The presence of these oscillations is not permanent, as they come and go. This is the first report of glucose oscillations in the 50–100 s range. The origin of these oscillations and their role in overall blood glucose regulation is unknown. Although the sample size is small, we believe this finding is important for our understanding of glucose regulation and perhaps for our understanding of general homeostatic regulation in intact organisms.

Introduction

A key feature of physiological regulation is the oscillations and pulsations that are apparent in all advanced organisms. These are believed to be of importance for several regulatory processes, and are seen in different organ systems such as the endocrine system, the respiratory system, the circulatory system, the nervous system, and others. The underlying physiological bases for the oscillatory patterns observed in global variables, is believed to be pulsatile and synchronization mechanisms at lower spatial and temporal levels. [1–3]

The pulsatile release of insulin from the beta-cells of the pancreas has been known for several decades and has been examined in both *in vitro* and *in vivo* studies [4,5]. Insulin is released in synchronized bursts with a periodicity of approximately five minutes. The amount of insulin released with each burst is constantly changing depending on the current blood glucose level (BGL). Even in periods of stable BGL, the consecutive bursts are varying, possibly due to the system perturbing itself to fine-tune its regulation. When studying the BGL in intact organisms

publish. The specific role of NE is articulated in the 'author contributions' section.

Competing interests: Nicolas Elvemo is CEO and shareholder of GlucoSet AS who was involved in study design, analysis and interpretation of data and writing of the paper. The glucose sensors used were fabricated and provided by GlucoSet AS. There are no further patents, products in development or marketed products to declare. This does not alter our adherence to all the PLOS ONE policies on sharing data and materials, as detailed online in the guide for authors.

with massive repetitive measurements, oscillations with a periodicity of approximately fifteen minutes have been found [6]. However, studies of continuous BGL measurements from subcutaneous sensors indicate that a normal BGL entails a complex regulatory pattern [7][8]. Furthermore, this pattern seems to "decomplexify" both as patients develop diabetes mellitus and as a consequence of critical disease [9–12]. This indicates that there could be several distinct oscillatory components in the native BGL regulation that are yet to be discovered.

We have the developed a method to study BGL changes over time in animals with a highly accurate and quickly responding continuous intravascular sensor [13]. In previous studies, we found that this sensor was able to detect these small oscillations in the BGL [14]. Therefore, in this study, we aimed to decipher BGL oscillations in longer time series in intact pigs.

Materials and methods

Animals, anaesthesia and study protocol

The study was approved by the Norwegian State Commission for Animal Experimentation (Oslo, Norway). A total of four domestic pigs were used in the studies (22–28 kg), and they were acclimatized and treated in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. The animals were premedicated with intramuscular diazepam 10 mg and azaperone 400 mg. Anaesthesia was induced through an intravenous access on the external surface of theear with atropine 1.0 mg, fentanyl 8.0 μg/kg, thiopenthal sodium 4.0 mg/kg and ketamine hydrochloride 8.0 mg/kg. Before intubation, 5 ml of 40 mg/ml lidocaine was applied to the larynx. The animals were ventilated in pressure control mode on a ventilator (Dameca, Copenhagen, Denmark) with initial values of FiO_2 at 0.30, a tidal volume of 10 ml/kg, PEEP at 6 cmH_2O and respiratory frequency of 18/min adjusted as needed in order to maintain $PaCO_2$ at 4.5–5.5 kPa. Anaesthesia was maintained by isoflurane 0.5–1.0%. Based on clinical response this was supplemented with boluses of fentanyl 50 μg/ml as needed. Intravascular volume was maintained by a bolus of acetated Ringer's solution 10 ml/kg, followed by a continuous infusion of 10 ml/kg/h throughout the experiment. 5000 IU heparin was administered i.v. to prevent clot formation. The animals were kept on the ventilator for almost 24 hours before euthanasia with pentobarbital 100 mg/kg.

After surgical cut-down, the animals were fitted with a central venous line for fluid and medicine administration in their right internal jugular vein and an arterial line in their left carotid artery. Two intravascular glucose sensors (GlucoSet, Trondheim, Norway) were inserted in each superficial femoral artery after surgical cut-down, and connected to the glucose monitor. Details of the glucose sensor with pre-insertion two-point calibration as well as repetitive post-insertion one-point calibrations are described in [13].

Data handling and analyses

The calibrated glucose signal was exported for analysis with the statistical software "R" version 3.3.0 with the "WaveletComp" package [15,16]. We removed the first 200 min of the sampled data in each series since these were periods of large instability and calibration of the sensors. The rest of the data from the entire study time until sacrifice of the animals are included in the study and are presented in Fig 1. The rest of the data were imported into the statistical software, and a combination of visual inspections and quantitative time-frequency analysis with continuous wavelet analysis was applied, as described in the Results & Discussion section.

The continuous wavelet transform (CWT) is a convolution of the original signal with a function generated from the so-called "mother wavelet" [17]. The mother wavelet is a waveform of limited duration with an average value of zero. In the convolution process, it is shifted

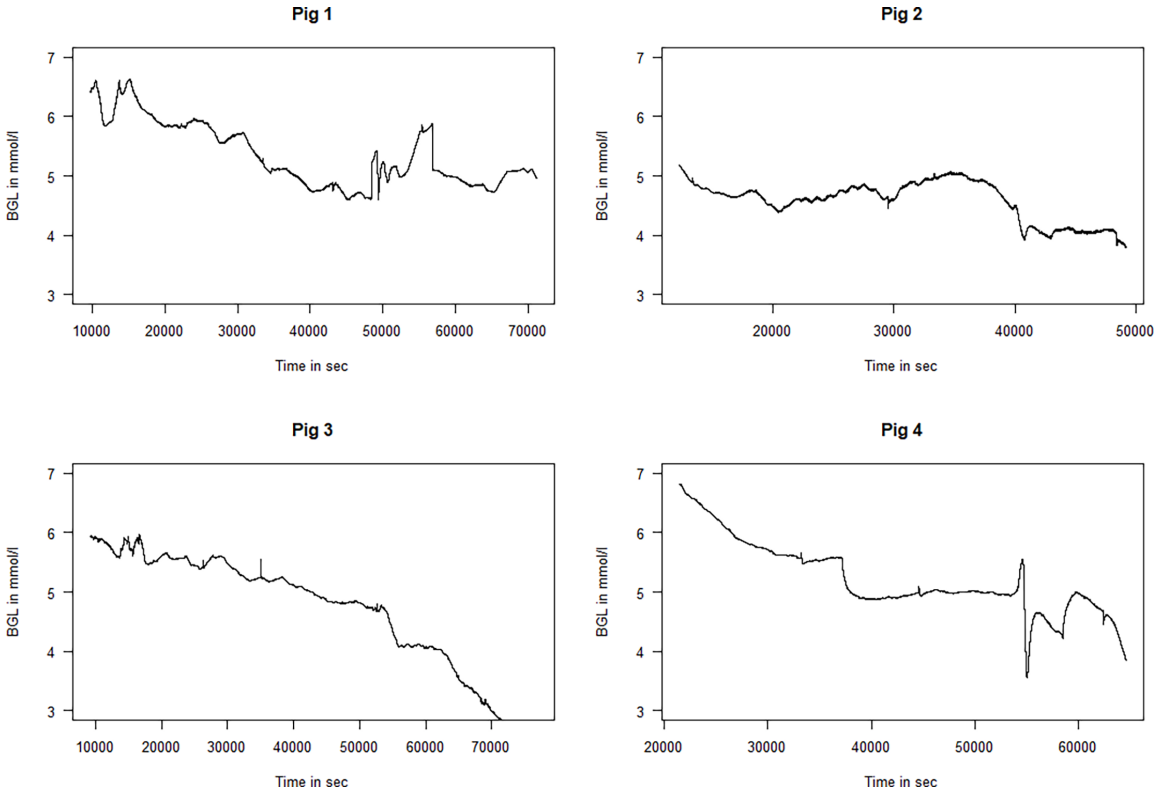


Fig 1. Time series of the glucose values for the four animals. The length of the time series and thus the scaling on the x-axes different between individual animals (BGL = Blood glucose level).

https://doi.org/10.1371/journal.pone.0194826.g001

in time and stretched and shrunk through the use of a scaling function. By stretching and shifting the mother wavelet in time, the CWT identifies the correlation of different frequencies at different time points. The final wavelet power spectrum is made by making a 3D-display of the correlation values (degree of match = power) and indicates the power by colour.

The most frequently used mother wavelet and the one used in this paper, the Morlet wave, is by mathematical definition a Gaussian enveloped cosine wave [17,18]. To illustrate the oscillatory phenomena, the presence of which varied, we performed the CWT on selections of the time series. Instead of illustrating frequency in Hz (number of cycles per second), we use period (the duration of time of one cycle), specified in seconds.

Results & discussion

All recordings were mainly performed with BGLs in the range of 4 to 6.5 mmol/l; however, as seen from Fig 1, the BGLs of all animals slowly declined throughout the studies. Some of the BGLs of the animals were very low at the end of the experiment, and the data for these periods were discarded before analysis. When qualitatively studying the BGLs of the four animals (Fig 1) a few oscillatory periods can clearly be seen. In Pig 1 and to some extent in Pig 3, one can

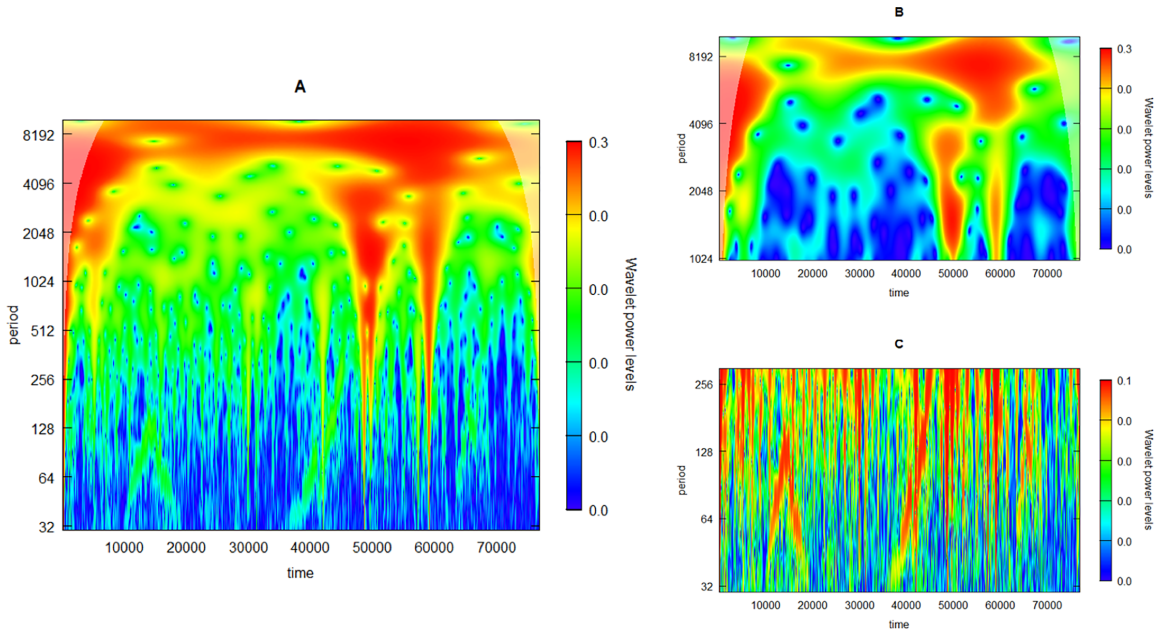


Fig 2. Wavelet power spectrum from the continuous wavelet transform (CWT) of the entire time series from Pig 1. The plot depicts the presence of distinct periods throughout the time series, with the time in the experiment in seconds at the abscissa, the time of the respective periods on a logarithmic scale at the ordinate, and the "power" of distinct periods as a function of the time-series shown in colours according to the scale next to the plots. The CWT covering the whole range of periods from 30–10000 sec only reveals the slow oscillation at approximately 8000 sec (A), which is highlighted when focusing in on the slow periods (B). The CWT of the whole time-series focusing on the high-frequency oscillations at 30–300 sec does not yield any meaningful result (C).

https://doi.org/10.1371/journal.pone.0194826.g002

see a very slow wave with a period of somewhere between 5000 and 10000 sec (0.0001–0.0002 Hz ≈ 1 ½ h period). In particular, in some parts of Pigs 2, very distinct oscillations with a periodicity of approximately 1000 sec (0.01 Hz ≈ 15 min) can be observed.

When examining the data in details, as we will below, there are oscillatory components to be found with periodicity ranging from 50 to 5000 sec. These oscillations constitute time-changing properties and should therefore be analysed by a time-frequency method such as the CWT. However, since the oscillatory components are not present throughout the recording and the power of the oscillations compared to the overall signal is so low, it is difficult to merely analyse the signal in its entirety. We subjected the entire time series of Pig 1 to a CWT, looking for periodicity from 30 to 10000 sec, but we had difficulty in deciphering any meaningful information apart from very slow oscillations at a periodicity of approximately 8000 sec (Fig 2A). When repeating the analysis only for the slowest period, we obtained a clearer visualization of this 8000-sec oscillation (Fig 2B). We then repeated the procedure for the shortest periods we studied, 50–100 sec. For these periods, the analysis did not perform well, only indicating some interesting periods in the high frequency range, especially between 10000 and 20000 sec and at 40000 sec in the time series (Fig 2C).

Before going deeper into the faster frequencies, we created a complete time series CWT of the slowest frequencies and performed these analyses for Pigs 2, 3, and 4. Pig 2 also had a low-frequency component at 5000–10000 sec throughout the series, although with larger time-variations than Pig 1. Pig 3 had a very complex oscillatory pattern with an increasing decay in BGL

throughout the time-series, that seems to disturb the analysis; however, the 5000–10000-sec oscillatory component can to some extent be seen at the beginning and end of the series. In Pig 4, this component is also present and is most visible in the first and last part of the CWT analysis but less evident in the middle of the analysis period. The lack of such a component in the middle could be caused by several large abrupt changes, artefacts, and the presence of other, more powerful higher-frequency oscillations (Fig 3).

As seen from Fig 1, Pig 2 had very distinct oscillations with a periodicity of some 1000 sec throughout the second quarter of the time series. We therefore specifically searched for this

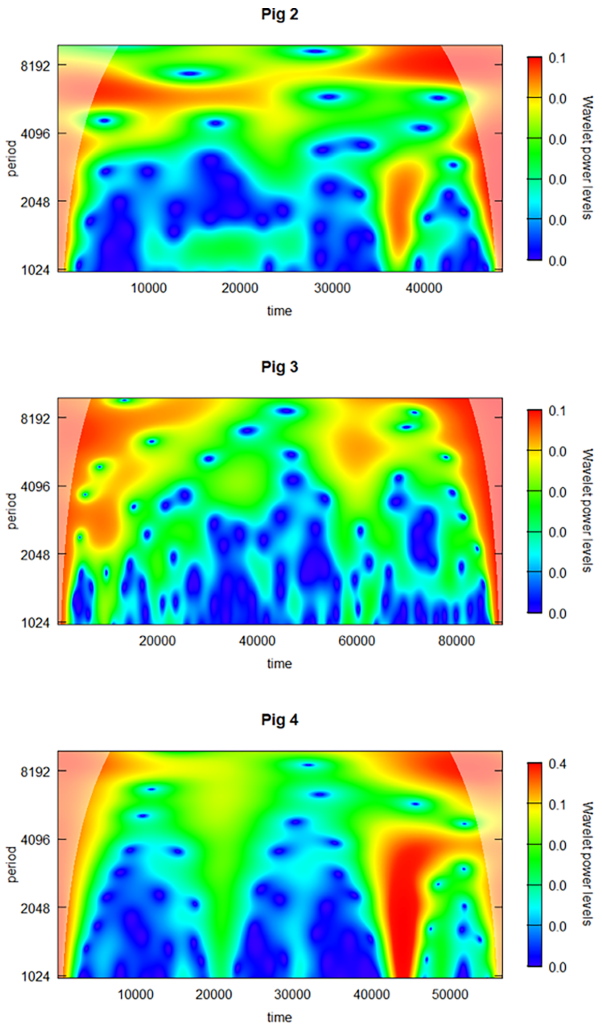


Fig 3. Wavelet power spectrum of the continuous wavelet transform of slow oscillations (1000–10000 seconds) of Pig 2, Pig 3 and Pig 4.

https://doi.org/10.1371/journal.pone.0194826.g003

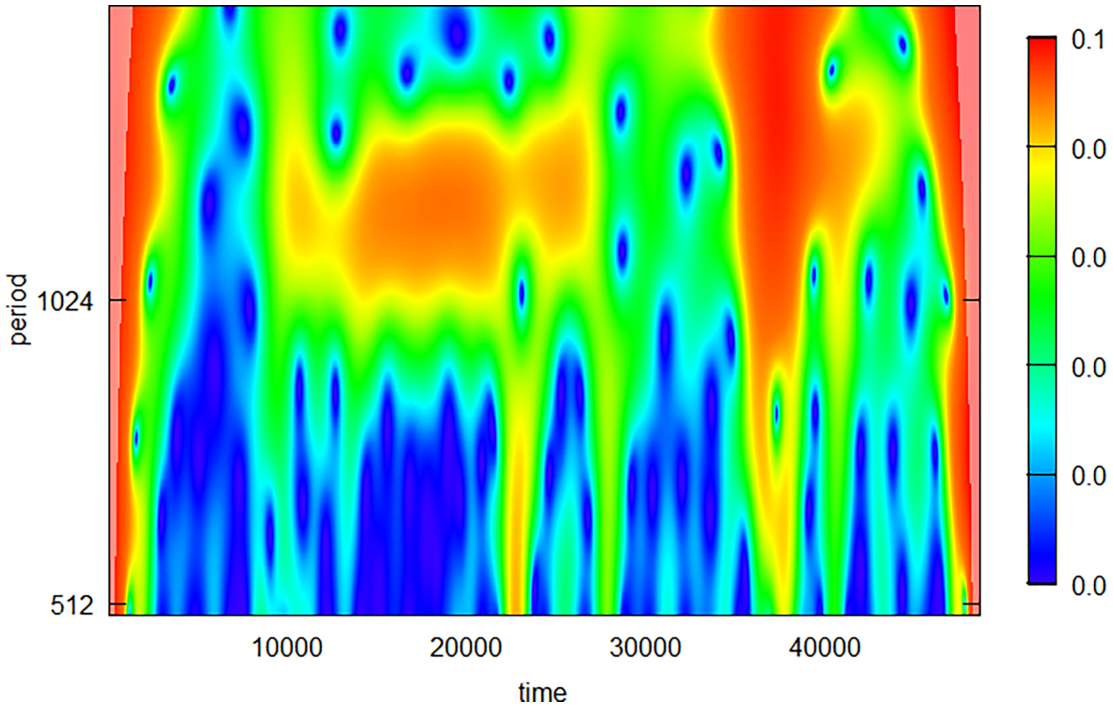


Fig 4. Wavelet power spectrum of the continuous wavelet transform of Pig 2 with a periodicity set to 500–2000 sec to identify the 1000-sec oscillatory component in the second quarter of the time series. This period is somewhat visualized in orange colour in the plot. The high power seen at 35000–40000 sec in the time series is caused by the steep decay in BGL at this time period.

https://doi.org/10.1371/journal.pone.0194826.g004

component in this one animal to quantify this component, looking in the range from 500 to 2000 sec. The CWT result is somewhat interesting as it shows both some of the strengths and the weaknesses of the method. One can see the approximately 1000-sec oscillation between 1000 and 3000 sec in the time series, but it does not stand out as very powerful and clear (Fig 4). This is probably caused by a combination of edge effects, artefacts, and other oscillatory components somewhat overshadowing the period we are examining.

When looking into the individual time series in more detail, especially searching for faster oscillatory periods, some interesting features appear. Pig 1 has quite a few periods with distinct oscillations in the 50–100 sec range, and Fig 5 depicts some of the most impressive periods. As shown, the exact periodicity varies some, and sometimes it changes in a linear fashion within small time periods; for example, in Fig 5 panel B, there is a quickly oscillating component that seems to have a linear increase in periodicity. Fig 6 depicts a CWT of this individual time period. Here, we have both an average power spectrum that does not consider the time dimension and therefore does not yield much information (left panel), and a time-frequency plot where the oscillatory component is very visible. The latter plot clearly shows how the oscillatory component linearly increases its period from 50 to 300 sec.

Often, one oscillatory component precedes another, or different oscillatory components are present at the same time in a fractal-like pattern. For example, in Pig 2, there is a distinct period of 50–100-sec oscillations followed by the very characteristic 1000 sec oscillation (Fig 7)

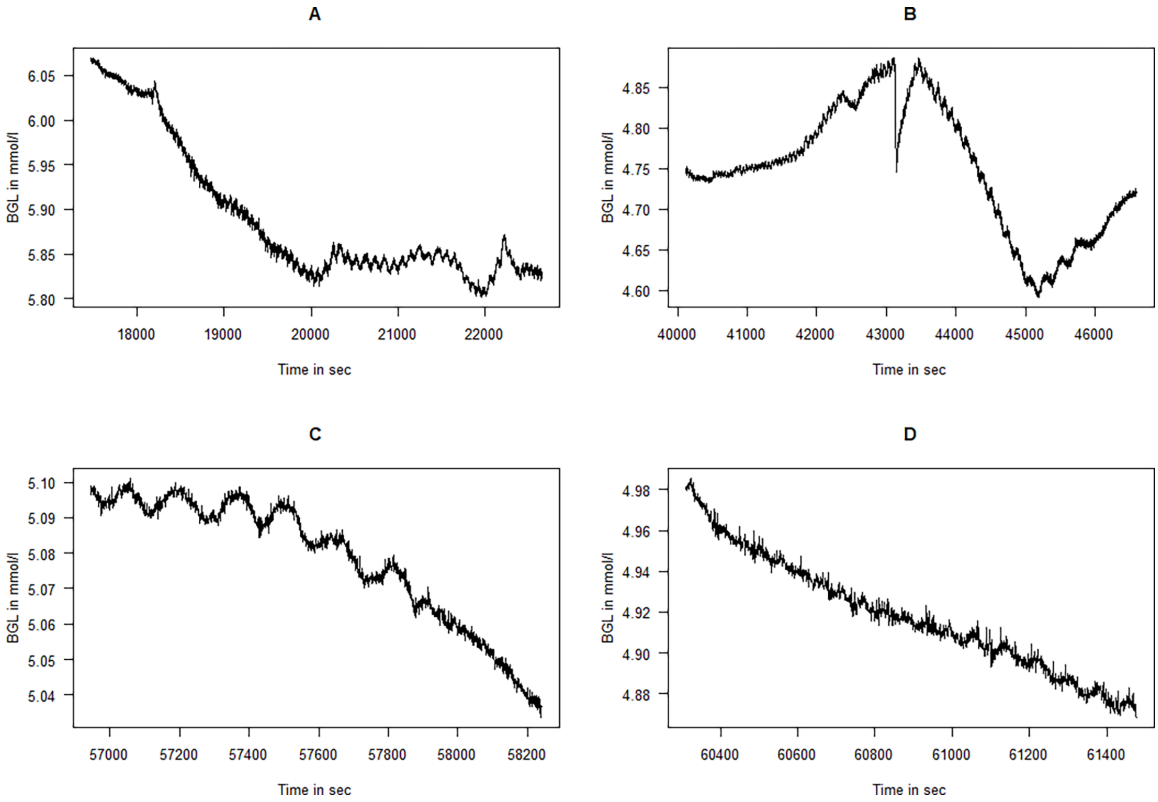


Fig 5. Some interesting periods from Pig 1 with oscillations in the 50–100 sec period range.

https://doi.org/10.1371/journal.pone.0194826.g005

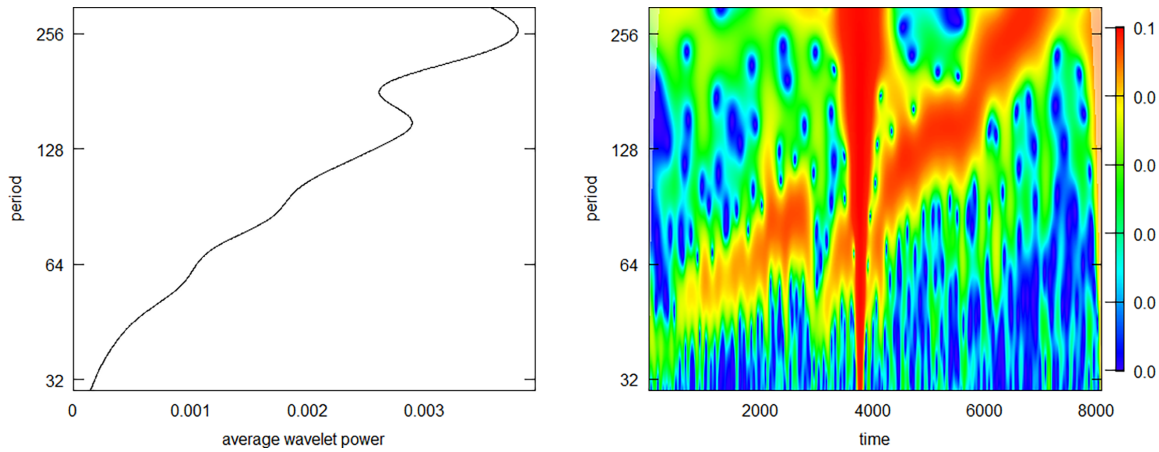


Fig 6. Continuous wavelet transform of the time series from Fig 5B where the periodicity appears to be constantly changing. The average power plot to the left only indicates that there are some periodicities in the 100–300 sec range while the wavelet power spectrum plot to the right clearly shows how the main oscillatory component has a linear rising periodicity from 50 to 300 sec throughout the time series. The abrupt drop in the middle of the series, seen in Fig 5B, yields the large artefact in the middle of the wavelet power spectrum plot.

https://doi.org/10.1371/journal.pone.0194826.g006

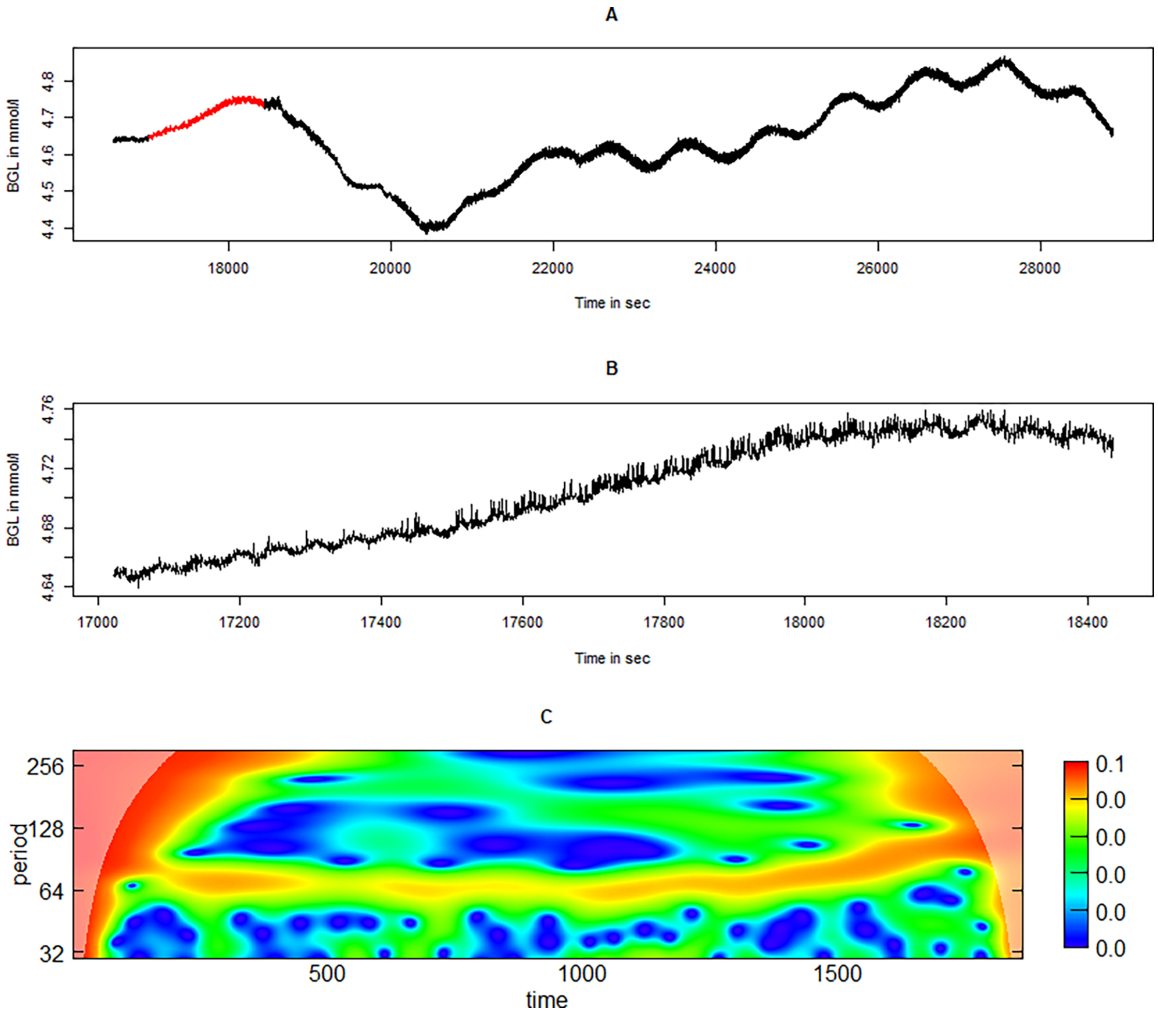


Fig 7. A period from Pig 2 in which two different oscillatory components follows each other. In Fig 7A, the 500–1000-sec slow oscillating component in the second half of the time series is shown. A period (marked in red, enlarged in panel B) seems to have a fast oscillating component at 50–100 sec. The wavelet power spectrum from the continuous wavelet transform of the time series in 7 B is displayed in 7 C, and this clearly shows the 50–100 sec oscillatory component.

https://doi.org/10.1371/journal.pone.0194826.g007

However, the fractal nature is most clearly seen in Pig 3, where Fig 8 depicts two illustrative situations in which both the 100-sec and the-1000 sec oscillations are present at the same time.

In Pig 4, we found several oscillatory components, especially in the 50–100-sec range and the 1000-sec region, as shown in Fig 9.

The current study was exploratory in its nature, and should be interpreted as hypothesis generating. We also kept in mind that we studied pigs, not humans, and the very limited number of animals observed. The results of this pilot animal study need to be confirmed in larger future studies, preferably in humans, to draw clearer conclusions. Nevertheless, these examples

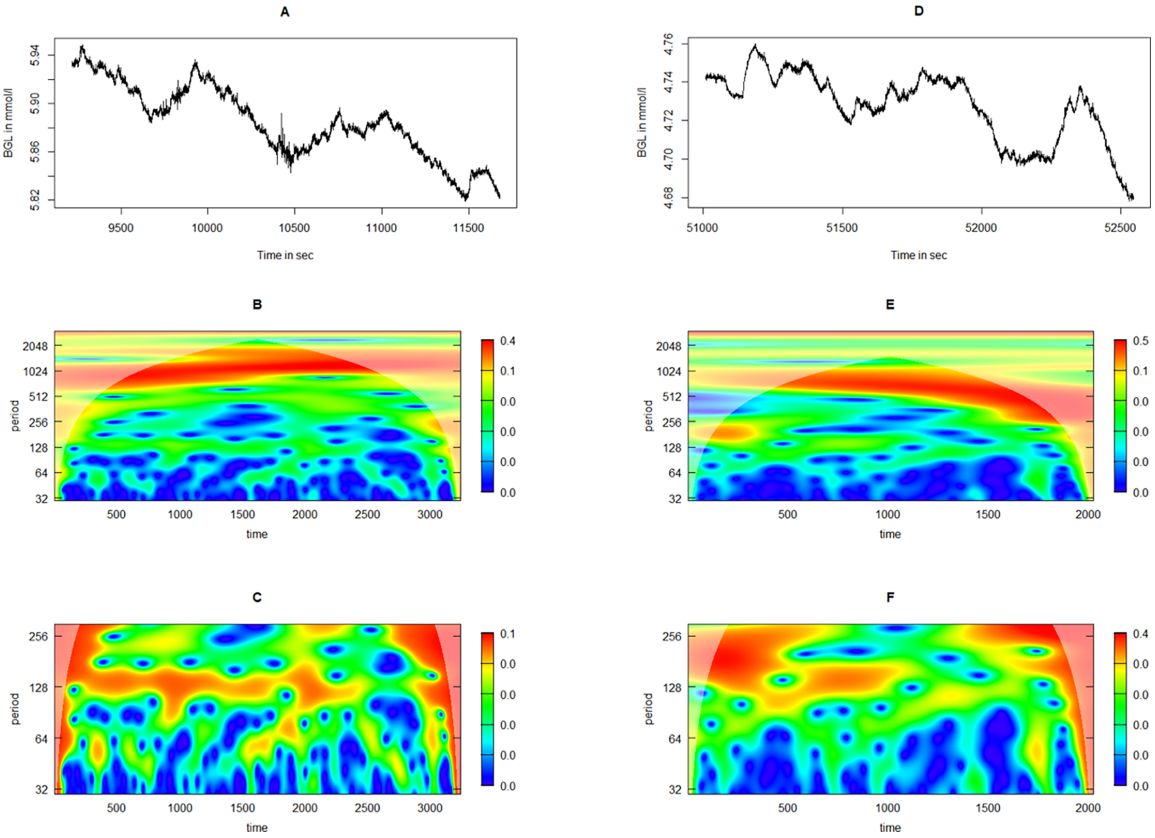


Fig 8. The fractal nature of blood glucose oscillations illustrated with two examples from Pig 3. Panels A and D depicts the time series from two situations in which the 1000-sec oscillation is clearly seen. Panels B and E depicts the wavelet power spectrum from the continuous wavelet transform from A and D, respectively, clearly showing the 1000-sec oscillatory component. However, the 50–100-sec component is poorly depicted in these figures due to the low power in the high-frequency oscillations compared with the low-frequency oscillations. Thus, panels C and F depicts the 50–100-sec components from A and D, respectively. (BGL = blood glucose level).

https://doi.org/10.1371/journal.pone.0194826.g008

show several oscillatory components in the time series of all four pigs. In our study, we find clear oscillations in the 5000–10000-sec, 500–1000-sec, and 50–100-sec regions (0.0002–0.0001 Hz, 0.002–0.001 Hz, and 0.02–0.01 Hz). This could indicate that there are three distinct oscillators within the organism that regulate the BGL. It is beyond the scope of this exploratory study to speculate regarding the physiological origin of these oscillations. However, based on our previous studies on the effect of intravenous insulin boluses on BGL changes in pigs, where the effect of each bolus has an approximately 15-min BGL-lowering effect [19], the fastest oscillations are unlikely to be caused by the pulsatile oscillation release by the beta-cells. The two slowest effects, in contrast, could be caused by such pulsatility.

The current study confirms observations of previously described oscillations using a novel sensing system, and describes a previously undescribed high-frequency oscillatiory phenomenon. There have been some concerns that the observed differences in complexity in continuous glucose measurements in different clinical situations could be caused by limitations in the

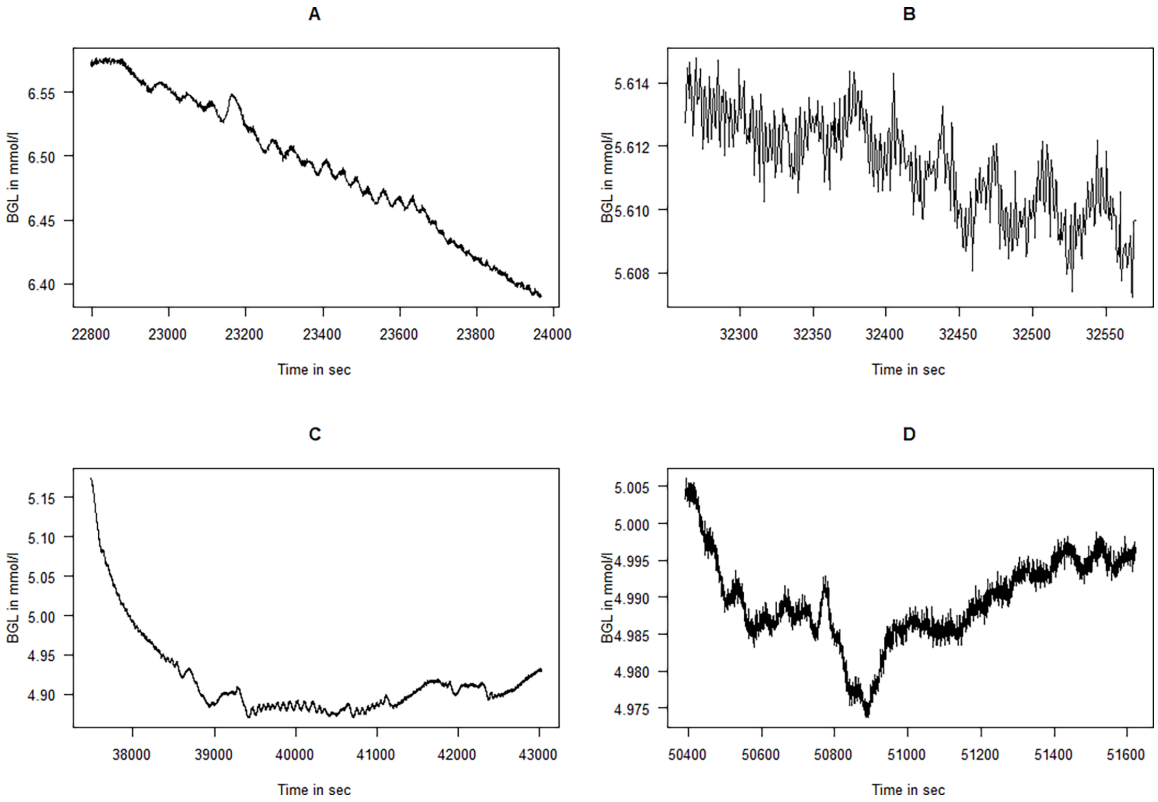


Fig 9. Some interesting periods from Pig 4 with oscillations in the 50–100-sec range and the 1000-sec range.

https://doi.org/10.1371/journal.pone.0194826.g009

sensors [20]. However, the novel sensing system used in this study has a very low signal-to-noise ratio, as seen in the figures containing unprocessed raw data (Figs 1, 5, 7 and 8). The high-frequency phenomenon is unlikely to have been picked up with slower glucose sensors. Nevertheless, the sensors used in this study have a time constant to stepwise change of some two minutes. The amplitudes of the measured oscillations are therefore likely larger than those that we measure in these experiments due to damping caused by this time-delay.

A limitation of using a novel sensing system is that it is not as well-described as a more mature system and that the results could be caused by an unknown interference or other phenomenon in the sensing system. However, the non-stationarity and physiological appearance of the oscillations make them unlikely to be caused from some unknown properties of the optical signal processing. Prior to this study, the sensors were tested in *in vitro* studies to examine interference by other physiological and pharmacological chemical factorss, and they were found to be sensitive to interferents, mainly temperature and pH. However, while these measures can change quickly in experimental conditions [21], no changes in the experimental protocol varied with the same frequency as the oscillations. Alternatively, could the observed BGL oscillations be caused by some other natural oscillatory phenomena or iatrogenic interference from medications or other interventions? All medication given

throughout a study can theoretically have some unappreciated effect on the physiological outcome studied [22], for example, isoflurane is known to interfere with the insulin/glucose system, albeit not in an oscillatory manner [23]. It is also unlikely that the oscillatory nature of the respirator with a periodicity of three to four seconds or occasional changes in anaesthesia could cause the rhythmicity observed in the BGL. Nevertheless, the current study cannot rule out that the observed oscillations were in fact caused by some previously undescribed non-glucose, high-frequency phenomenon.

Conclusion

In this exploratory study of continuous intraarterial BGL measurements in four domestic pigs under general anaesthesia, we found several interesting oscillatory components, especially in the 5000–10000-sec, 500–1000-sec, and 50–100-sec regions (0.0002–0.0001 Hz, 0.002–0.001 Hz, and 0.02–0.01 Hz). The origin of these oscillations is unknown. Further studies are needed to confirm the novel findings described in this study and to elucidate any underlying physiological mechanism of the phenomena.

Supporting information

S1 Text. A table of time (sec) in column 1 and blood glucose values (mmol/l) in column 2 from the entire recording in Pig 1.
(TXT)

S2 Text. A table of time (sec) in column 1 and blood glucose values (mmol/l) in column 2 from the entire recording in Pig 2.
(TXT)

S3 Text. A table of time (sec) in column 1 and blood glucose values (mmol/l) in column 2 from the entire recording in Pig 3.
(TXT)

S4 Text. A table of time (sec) in column 1 and blood glucose values (mmol/l) in column 2 from the entire recording in Pig 4.
(TXT)

Acknowledgments

We wish to thank Tine Hunt (GlucoSet) and Oddveig Lyng (NTNU) for invaluable help with the animal studies and Dag Roar Hjelme (GlucoSet & NTNU) for the pre-processing of signal recordings.

Author Contributions

Conceptualization: Nils Kristian Skjaervold, Kathrine Knai, Nicolas Elvemo.

Data curation: Nils Kristian Skjaervold.

Formal analysis: Nils Kristian Skjaervold.

Investigation: Nils Kristian Skjaervold, Kathrine Knai.

Methodology: Nils Kristian Skjaervold.

Writing – original draft: Nils Kristian Skjaervold.

Writing – review & editing: Kathrine Knai, Nicolas Elvemo.

References

1. Goldbeter A. Biochemical Oscillations and Cellular Rhythms: The Molecular Bases of Periodic and Chaotic Behaviour. Cambridge University Press; 1997.
2. Kaneko K. Life: An Introduction to Complex Systems Biology. Springer; 2006.
3. Varela M, Ruiz-Esteban R, Mestre de Juan MJ. Chaos, fractals, and our concept of disease. Perspect Biol Med. 2010; 53(4):584–95. https://doi.org/10.1353/pbm.2010.0003 PMID: 21037411
4. Hellman B. Pulsatility of insulin release—a clinically important phenomenon. Ups J Med Sci. 2009; 114 (4):193–205. https://doi.org/10.3109/03009730903366075 PMID: 19961265
5. Matveyenko AV, Veldhuis JD, Butler PC. Measurement of pulsatile insulin secretion in the rat: direct sampling from the hepatic portal vein. Am J Physiol Endocrinol Metab. 2008 Sep; 295(3):E569–574. https://doi.org/10.1152/ajpendo.90335.2008 PMID: 18577690
6. Lang DA, Matthews DR, Peto J, Turner RC. Cyclic oscillations of basal plasma glucose and insulin concentrations in human beings. N Engl J Med. 1979 Nov 8; 301(19):1023–7. https://doi.org/10.1056/NEJM197911083011903 PMID: 386121
7. Kroll MH. Biological variation of glucose and insulin includes a deterministic chaotic component. Biosystems. 1999 Jun; 50(3):189–201. PMID: 10400269
8. Holt TA. A chaotic model for tight diabetes control. Diabet Med J Br Diabet Assoc. 2002 Apr; 19(4):274–8.
9. Pørksen N, Hollingdal M, Juhl C, Butler P, Veldhuis JD, Schmitz O. Pulsatile insulin secretion: detection, regulation, and role in diabetes. Diabetes. 2002 Feb; 51 Suppl 1:S245–254.
10. Churruca J, Vigil L, Luna E, Ruiz-Galiana J, Varela M. The route to diabetes: Loss of complexity in the glycemic profile from health through the metabolic syndrome to type 2 diabetes. Diabetes Metab Syndr Obes Targets Ther. 2008 Aug 11; 1:3–11.
11. Lundelin K, Vigil L, Bua S, Gomez-Mestre I, Honrubia T, Varela M. Differences in complexity of glycemic profile in survivors and nonsurvivors in an intensive care unit: A pilot study. Crit Care Med. 2010 Mar; 38 (3):849–54. https://doi.org/10.1097/CCM.0b013e3181ce49cf PMID: 20068460
12. Brunner R, Adelsmayr G, Herkner H, Madl C, Holzinger U. Glycemic variability and glucose complexity in critically ill patients: a retrospective analysis of continuous glucose monitoring data. Crit Care Lond Engl. 2012 Oct 2; 16(5):R175.
13. Skjaervold NK, Solligård E, Hjelme DR, Aadahl P. Continuous measurement of blood glucose: validation of a new intravascular sensor. Anesthesiology. 2011 Jan; 114(1):120–5. https://doi.org/10.1097/ALN.0b013e3181ff4187 PMID: 21169804
14. Skjaervold NK, Ostling D, Hjelme DR, Spigset O, Lyng O, Aadahl P. Blood glucose control using a novel continuous blood glucose monitor and repetitive intravenous insulin boluses: exploiting natural insulin pulsatility as a principle for a future artificial pancreas. Int J Endocrinol. 2013; 2013:245152. https://doi.org/10.1155/2013/245152 PMID: 24369461
15. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2016. Available from: https://www.R-project.org/
16. Roesch A and Schmidbauer H. WaveletComp: Computational Wavelet Analysis. R package version 1.0. 2014. Available from: https://CRAN.R-project.org/package=WaveletComp
17. Fugal DL. Conceptual Wavelets in Digital Signal Processing: An In-depth, Practical Approach for the Non-mathematician. Space & Signals Technical Pub.; 2009.
18. Huang NE, Shen Z, Long SR, Wu MC, Shih HH, Zheng Q, et al. The empirical mode decomposition and the Hilbert spectrum for nonlinear and non-stationary time series analysis. Proc R Soc Lond Math Phys Eng Sci. 1998 Mar 8; 454(1971):903–95.
19. Skjaervold NK, Lyng O, Spigset O, Aadahl P. Pharmacology of intravenous insulin administration: implications for future closed-loop glycemic control by the intravenous/intravenous route. Diabetes Technol Ther. 2012 Jan; 14(1):23–9. https://doi.org/10.1089/dia.2011.0118 PMID: 21751892
20. Signal M, Thomas F, Shaw GM, Chase JG. Complexity of Continuous Glucose Monitoring Data in Critically Ill Patients: Continuous Glucose Monitoring Devices, Sensor Locations, and Detrended Fluctuation Analysis Methods. J Diabetes Sci Technol. 2013 Nov 1; 7(6):1492–506. https://doi.org/10.1177/193229681300700609 PMID: 24351175
21. Andrews RJ, Bringas JR, Alonzo G. Cerebrospinal fluid pH and PCO2 rapidly follow arterial blood pH and PCO2 with changes in ventilation. Neurosurgery. 1994 Mar; 34(3):466–470. PMID: 8190222
22. Langeland H, Lyng O, Aadahl P, Skjærvold N-K. The coherence of macrocirculation, microcirculation, and tissue metabolic response during nontraumatic hemorrhagic shock in swine. Physiol Rep. 2017 Apr 1; 5(7):e13216. https://doi.org/10.14814/phy2.13216 PMID: 28400499

23. Tanaka T, Nabatame H, Tanifuji Y. Insulin secretion and glucose utilization are impaired under general anesthesia with sevoflurane as well as isoflurane in a concentration-independent manner. J Anesth. 2005; 19(4):277–81. https://doi.org/10.1007/s00540-005-0341-1 PMID: 16261463

Paper III

This paper is awaiting publication and is not included due to copyright

Paper IV

DOI: 10.14814/phy2.14423

ORIGINAL RESEARCH

Physiological Reports

Cardiac surgery does not lead to loss of oscillatory components in circulatory signals

Kathrine Knai[1] | **Petter Aadahl**[1,2] | **Nils K. Skjaervold**[1,2]

[1]Department of Circulation and Medical Imaging, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, Trondheim, Norway

[2]Department of Cardiothoracic Anaesthesia and Intensive Care, Clinic of Anaesthesia and Intensive Care, Trondheim University Hospital, Trondheim, Norway

Correspondence
Kathrine Knai, NTNU, Faculty of Medicine and Health Sciences, Department of Circulation and Medical Imaging, Postboks 8905, 7491 Trondheim, Norway.
Email: kathrine.knai@ntnu.no

Funding information
Norwegian University of Science and Technology

Abstract

The circulatory system is oscillatory in its nature. Oscillatory components linked to physiological processes and underlying regulatory mechanisms are identifiable in circulatory signals. Autonomic regulation is essential for the system's ability to deal with external exposure, and the integrity of oscillations may be considered a hallmark of a healthy system. Loss of complexity is seen as a consequence of several diseases and aging. Heart rate variability is known to decrease after cardiac surgery and remain reduced for up to 6 months. Oscillatory components of circulatory signals are linked to the system's overall complexity. We therefore hypothesize that the frequency distributions of circulatory signals show loss of oscillatory components after cardiac surgery and that the observed changes persist. We investigated the development of the circulatory frequency distributions of eight patients undergoing cardiac surgery by extracting three time series from conventional blood pressure and electrocardiography recordings: systolic blood pressure, heart rate, and amplitude of the electrocardiogram's R-wave. Four 30-min selections, representing key events of the perioperative course, were analyzed with the continuous wavelet transform, and average wavelet power spectra illustrated the circulatory frequency distributions. We identified oscillatory components in all patients and variables. Contrary to our hypothesis, they were randomly distributed through frequencies, patients, and situations, thus, not representing any reduction in the overall complexity. One patient showed loss of a 25-s oscillation after surgery. We present a case where noise is misclassified as an oscillation, raising questions about the robustness of such analyses.

KEYWORDS

cardiac surgery patients, circulatory oscillations, continuous blood pressure, electrocardiogram, loss of complexity

1 | INTRODUCTION

The circulatory system is oscillatory in its nature. Oscillations can be traced back to physiological processes, such as heart contraction, respiration, and rhythmic contraction of the vasculature, and these oscillatory processes are controlled by regulatory mechanisms. The result is a highly irregular and complex oscillatory profile. Autonomic regulation is essential

for the system's ability to deal with external exposure, and the integrity of circulatory oscillations can be considered a hallmark of a healthy system. Circulatory signals, such as continuous blood pressure (BP) and electrocardiography (ECG) signals, show traces of these underlying oscillations. Traditional heart rate variability and frequency analyses have identified specific oscillations and they have been attributed to different parts of autonomic regulation (Akselrod et al., 1981; Bracic & Stefanovska, 1998; Pomeranz et al., 1985). Loss of complexity has been reported in several cardiac and non-cardiac diseases (Claydon & Krassioukov, 2008; Goldstein et al., 1998; Kleiger, Miller, Bigger, & Moss, 1987; Riordan, Norris, Jenkins, & Morris, 2009; Wolf, Varigos, Hunt, & Sloman, 1978) and simply as a feature of aging (Kaplan et al., 1991; Lipsitz & Goldberger, 1992; Umetani, Singer, McCraty, & Atkinson, 1998; Takahashi et al., 2012). Heart rate variability is known to decrease after cardiac surgery, and remain reduced for up to 6 months (Hogue, Stein, Apostolidou, Lappas, & Kleiger, 1994; Kuo et al., 1999).

There is no clear definition of *complexity*. However, complex systems are built up by components that interact in multiple ways and with the external environment, resulting in organized and disorganized behavior that cannot be predicted from the components alone (Johnson, 2009). Linking this to biological signals, complexity is related to the degree of information in the signal, the predictability of the signal, and the ability to describe the signal in a simple manner (Goldberger, Moody, & Costa, 2012). The definition is too diffuse to provide a quantitative measure of complexity that applies universally. Oscillatory components of biological systems represent underlying components that interact and produce the behavior of the system as a whole. Altogether, they both reflect the system's overall complexity (Goldberger, 1996) and are linked to underlying regulation. On this basis, the exploration of oscillatory distributions of biological signals provides information about the overall state of biological systems, which could be altered by disease or invasive procedures. If the observed changes are generalizable between patients, such information can be implemented to future monitoring tools. This is beneficial as changes in patients' clinical state could be identified and clinicians notified before overall variables such as heart rate (HR) or BP are changed.

We explore frequency and amplitude modulations of BP and ECG signals by extracting three time series: systolic BP (SBP), HR, and amplitude of the ECG's R-wave. The Brody effect states that variations in R-wave amplitude are related to ventricular preload (Brody, 1956). R-wave amplitude can thus be seen in relation with SBP and HR. By combining the frequency distributions of the three mentioned variables, we illustrate unique circulatory frequency distributions. In this work, we investigated the development of the circulatory frequency distributions of eight patients undergoing cardiac surgery. Four 30-min selections, representing key events of the perioperative course, were analyzed with the continuous wavelet transform (CWT), and average wavelet power spectra illustrated the circulatory frequency distributions. We hypothesize that the circulatory frequency distributions show loss of oscillatory components with surgery and that the observed changes persist, measured until the morning after surgery.

2 | MATERIAL AND METHODS

2.1 | Study population, ethics, and confidentiality

From March to May 2016, patients scheduled for coronary artery bypass grafting were invited to participate in the study, recruiting a total of 10 patients. Two patients were excluded due to non-sinus rhythm at one or several time points of the recording. Other exclusion criteria are left ventricular ejection fraction below 0.5, severe valve disease, right ventricular failure, pulmonary hypertension, and severe postoperative hemorrhage. Finally, we had a study group consisting of six men and two women, age ranging from 47 to 88. The patients were enumerated 1–10, with patient 6 and 9 excluded.

The surgery was performed at Trondheim University Hospital, Norway. Written consent was collected prior to data collection. The study protocol was approved by the Regional Committee for Medical and Health Research Ethics (reference: 2015/2019/REK midt). Confidentiality was strictly maintained throughout the study.

2.2 | Equipment and study protocol

Data collection was performed in two sessions: before and after surgery. The patients were lying in bed during both periods. The study equipment includes a 3-electrode ECG, a laser Doppler flowmeter (LDF) attached to the calf, and an arterial cannula inserted to the left radial artery. Additionally, patients 1–3 had a photoplethysmograph (PPG) finger sensor attached. The study equipment was provided by ADInstruments (Oxford, UK), as well as hardware and software (PowerLab 16/35 and LabChart 8.1.3). The signals were recorded with a sampling rate of 400 Hz.

The preoperative recordings were collected with the patients resting in bed in a quiet room without disturbances at the thoracic surgery ward. The duration of the recordings ranged from 47 to 86 min. The patients did not receive premedication prior to surgery, and surgery was performed under general balanced anesthesia (thiopental, fentanyl, isoflurane, and propofol). During surgery, the study equipment was removed. After surgery, the study equipment was reattached using new ECG patches and a new arterial cannula inserted to the right radial artery. The

postoperative recording was collected from the patients arrived at the thoracic intensive care unit, until the next morning. The duration of the recordings ranged from 14 to 18.5 hr.

2.3 | Data handling and preprocessing

The BP and ECG recordings were exported from LabChart as mat.files and analyzed in R, version 3.5.1, with the packages *R.matlab, signal, robustHD,* and *WaveletComp* (Alfons, 2016; Bengtsson, 2016; R Foundation for Statistical Computing, 2018; Roesch & Schmidbauer, 2018; Signal developers, 2013). We subdivided the data into four situations: preoperatively (A); postoperatively, on respirator (B); postoperatively, after extubation (C); and postoperatively, the next morning (D).

We extracted 30-min selections representing each situation and preprocessed the BP and ECG signals into three time series: SBP, HR, and R-wave amplitude (Figure 1). Baseline wander was removed from the ECG signals by applying a Savitzky–Golay smoothing filter before further analyses (Nahiyan & Amin, 2017). We defined the SBP and the R-wave amplitude as the maxima of the BP and ECG, respectively. The heart rate was defined as $HR = 60/RR_i$, where RR_i is the time interval in seconds between R-peak i and $i + 1$ of the ECG. Some episodes of noise were misclassified as heartbeats; thus, we removed outliers from SBP, RR-intervals, and R-amplitude before further calculation. To provide evenly sampled time series, we performed a cubic spline interpolation to a sampling frequency of 10 Hz. The final variables were called interpolated SBP (iSBP), interpolated HR (iHR), and interpolated R-wave amplitude (iAmp).

To examine one specific identified oscillation, the PPG and LDF signal of patient 1 were included in a subanalysis. To provide comparable results, the PPG was preprocessed with the same algorithm as iSBP and iAmp, creating a new time series of the amplitude of the signal, interpolated to a sampling rate of 10 Hz. The final variable was called PPG-iAmp. The LDF was downsampled to 10 Hz.

2.4 | Analysis

We performed the CWT to identify frequency components present in iSBP, iHR, and iAmp. The CWT is a convolution of the signal with a function generated from the *mother wavelet* (Fugal, 2009). We used the Morlet wavelet, which by mathematical definition is a Gaussian enveloped cosine wave, and has been widely used for investigation of biological signals, especially the ECG (Addison, 2005). In the convolution process, it is shifted in time and stretched and shrunk, quantifying different frequency components' presence in the signal at different time points. We presented the results in average wavelet power spectra, illustrating the averaged frequency distributions of the signals. Furthermore, we performed the CWT for bivariate time series identifying frequency components that are present in two time series with a significance level of 0.05. The results are presented in cross-wavelet spectra, with significant frequencies marked by white lines and phase differences by arrows. The CWT for bivariate time series was performed on the variable pairs, iAmp-iSBP and iSBP-iHR.

In order to visually examine the individual time-series' oscillations, we decomposed the time series with locally weighted estimated scatterplot smoothing (Loess) (Cleveland & Devlin, 1988). We applied the regression three times, each time subtracting the smoothed curve from the signal, providing a set of oscillating components of increasing frequency. The extracted components were called Loess #1, Loess #2, and Loess #3. By plotting the components of all variables together, we visually inspected their oscillating behavior and phase differences. From the CWT and Loess, we identified the components that are highly present in all variables and performed a cross-correlation analysis, which calculates the correlation of two time series as a function of the displacement of one relative to the other—the cross-correlation function (CCF). By defining CCF_{max}, we identified at which time lag the correlation is highest, and thus at which relative displacement the studied variables oscillate.

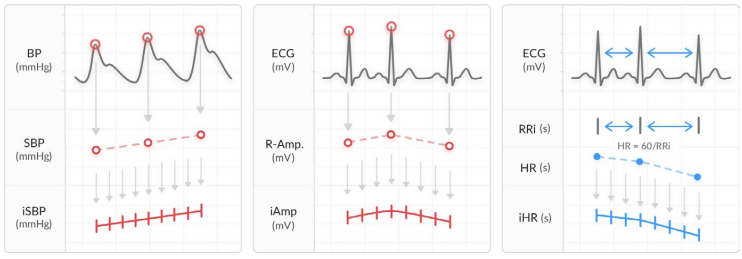


FIGURE 1 Preprocessing of the BP and ECG signals generating the variables iSBP, iHR, and iAmp. The SBP and the R-wave amplitude were defined as the maxima of the BP and ECG, respectively. The HR was calculated from the time interval between two subsequent R-peaks of the ECG (RR_i [s]). The time series were interpolated to a sampling rate of 10 Hz

3 | RESULTS

By performing the CWT on iSBP, iHR, and iAmp, we identified each patient's circulatory frequency distribution throughout the perioperative course, illustrated by situation A–D. We hypothesize that we will see loss of oscillatory components from situation A to B, and that the changes persist through situations C and D. Figure 2 shows the average wavelet power spectra of iSBP, iHR, and iAmp of all patients and situations. The spectra include periods between 10 and 1,000 s. Lower periods are not included, as the respiration is a powerful oscillatory component, overshadowing the presence of slower, less dominating oscillations.

We identified oscillatory components in all patients and situations. Patient 1 shows a distinct peak around 25 s in situation A. This oscillation is present in all three variables, and not visible in any of the situations B, C, or D. A less prominent oscillation around 800 s in 3A is observed, which arguably disappears after surgery. Patient 8 showed loss of oscillations in the range between 20 and 50 s in iHR, and in situation D, an oscillation just above 100 s is observed in all variables. We see other examples of loss of power in the mid-range after surgery, but no cases with loss of distinct oscillatory components. Altogether, we illustrate frequency distributions that change through the perioperative course, but the observed changes do not display any trend or system. Overall, the number of oscillatory components and their power are more or less randomly distributed through patients and situations. Linking this to the signals' overall complexity, we did not identify any clear reduction of such after surgery. iAmp does not show any distinct oscillatory peaks in any patients in situation B. This is due to a domination of the respiration during mechanical ventilation.

The 25-s oscillation in 1A stands out as the only oscillation that is clearly present in all variables before surgery and gone after. To examine the specific variables' oscillatory behavior, we performed a Loess regression, illustrated by Loess #2 in Figure 3.

Figure 3 shows that iSBP and iHR oscillate in phase, iHR leading. iAmp oscillates off phase with respect to the other two. By performing a cross-correlation analysis on the variable pairs, iAmp-iSBP and iSBP-iHR, we found that CCFmax of iAmp and iSBP is 0.75, with a lag of 8.2 s. The corresponding values for iSBP and iHR are 0.80 and 3.5 s. Altogether, this tells us that iHR is leading, with a time lag of 3.5 s to iSBP and 11.7 s to iAmp. Maximum correlation values of 0.75 and 0.80 are high when it comes to biological time series. The preoperative recording of patient 1 included both a PPG and a LDF signal. The signals were preprocessed as described in Methods and analyzed with the CWT. Figure 4 shows the average wavelet spectrum of all variables, and we see that the 25-s oscillation is present in the amplitude of the PPG signal but not in LDF.

Figure 2 shows an 800-s oscillation in patient 3, situation A. It is present in all three variables and is partly gone postoperatively. Looking at the raw signals and performing a Loess regression, we find that the extracted component is caused by short events of noise, thus not representing a true physiological oscillator.

Patient 8 showed loss of oscillations in the range between 20 and 50 s in iHR, and an oscillation just above 100 s in situation D that is present in all variables. Figure 5 shows the cross-wavelet spectra of iAmp-iSBP and iSBP-iHR of situation D.

Both variable pairs show high power just above 128 s, confirming that the oscillation is present in all three time series. Both variable pairs show variations in phase differences through the time course, but mostly iAmp-iSBP show arrows pointing to the lower right, meaning that they oscillate in phase, iSBP leading. iSBP-iHR show arrows pointing to the lower left, meaning that they oscillate off phase, iSBP leading. Thus, iHR and iAmp oscillate in phase, and iSBP out of phase with respect to the other two.

4 | DISCUSSION

We illustrate the frequency distributions of variables extracted from BP and ECG signals of eight patients undergoing coronary artery bypass grafting. The circulatory frequency distributions illustrate the presence of oscillatory components in all variables, patients and situations, and the oscillations are randomly distributed over the examined frequency range. The high variety seems to represent interindividual variations, more than factors of the performed surgery. Considering the heterogeneity of our findings, we have not presented information that is suitable for use in any monitoring device or other clinical decision tools.

The identified oscillations do not correspond to the distinct pattern of frequency bands that are described in the literature (Akselrod et al., 1981; Bracic & Stefanovska, 1998; Pomeranz et al., 1985). Linking the circulatory frequency distributions to the overall complexity of circulatory signals, no reduction of such is identified. Either the complexity is not reduced with cardiac surgery, or our method is not able to identify it. One case showed a 25-s oscillation that is present preoperatively (1A) and not postoperatively (1B, 1C, 1D). The oscillation is found in all three variables, and additionally in the amplitude of the PPG (PPG-iAmp). It has a frequency of 0.04 Hz, corresponding to the limit between low frequencies and very low frequencies (Task Force of the European Society of Cardiology & the North American Society of Pacing & Electrophysiology, 1996). According to the literature, low frequencies reflect baroreceptor activity, but findings are inconsistent regarding whether this activity is mediated by the sympathetic or the parasympathetic

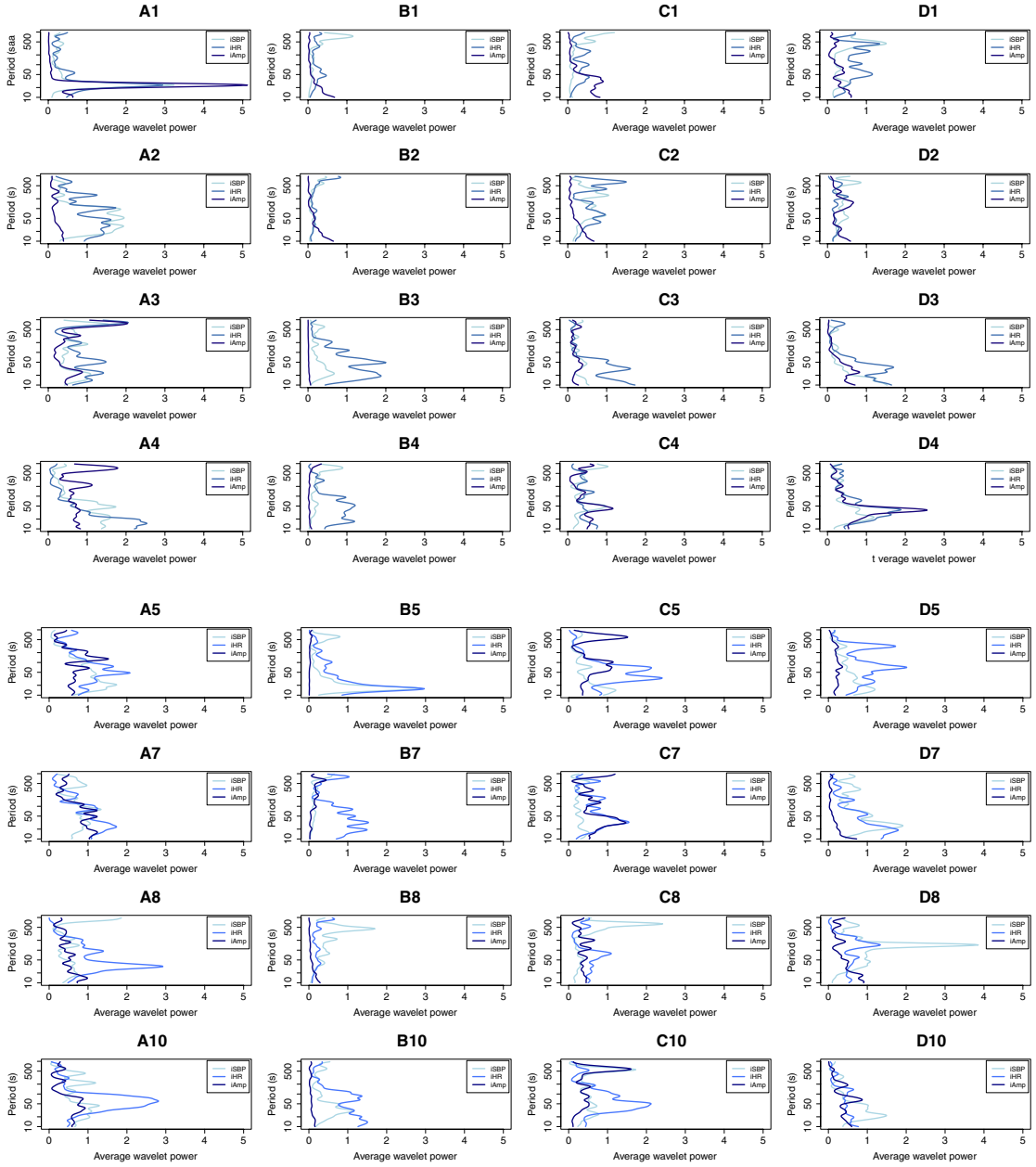


FIGURE 2 The average wavelet power spectra of iSBP, iHR, and iAmp of all patients through situation A to D. Each spectrum represents one patient in one situation, named with a number and a letter. Patients are separated by rows, and situations by columns. The situations represent key events of the perioperative course: preoperatively (A); postoperatively, on respirator (B); postoperatively, after extubation (C); postoperatively, the next morning (D). Average wavelet power is shown on the *x*-axis and period (in seconds) on a logarithmic scale on the *y*-axis. The variables are distinguished by color. Patient 1 shows loss of a 25-s oscillation between situation A and B. Patient 3 shows loss of an 800-s oscillation, and patient 8 shows loss of oscillations in the range between 50 and 100 s in iHR

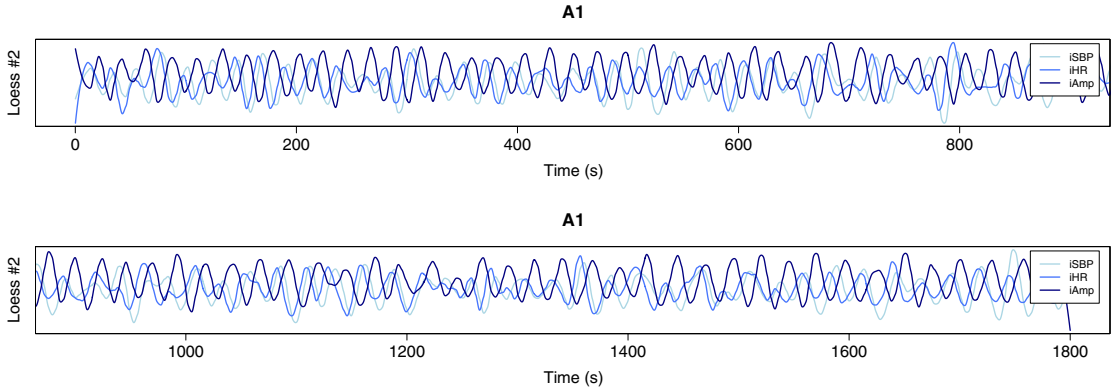


FIGURE 3 Loess regression of iSBP, iHR, and iAmp of 1A, illustrated by Loess #2 which is the second extracted component. We see that iSBP and iHR oscillates in phase, and iAmp off phase

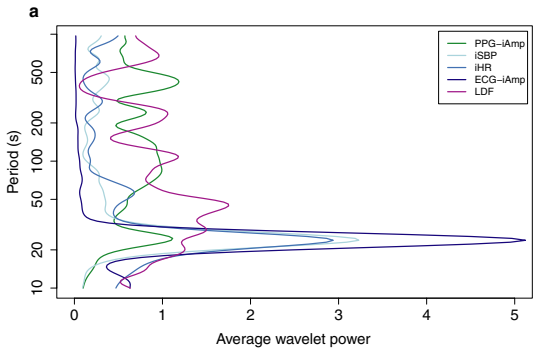


FIGURE 4 Average wavelet power spectra of 1A including PPG and LDF signals. The PPG is preprocessed with the same algorithm as iSBP and iAmp, giving a time series of the maxima of the PPG signal, called PPG-iAmp. The LDF is downsampled to a sampling frequency of 10 Hz, as the other time series. The 25-s oscillation is present in PPG-iAmp, iSBP, iHR, and ECG-iAmp, but not in the LDF

nervous system (Shaffer, McCraty, & Zerr, 2014; Task Force of the European Society of Cardiology & the North American Society of Pacing & Electrophysiology, 1996). Patient 8 showed loss of oscillations in iHR with surgery and return of an oscillation in situation D that is present in all variables. The oscillation in 8D has a frequency of 0.007 Hz, corresponding to the ultra-low frequency band. The physiological correlate to ultra-low frequencies is insecure. This study was not designed to explore underlying physiological mechanisms of identified oscillations.

We are developing methods for analyzing biological signals, both focusing on preprocessing and choice of analyses. We find that there are several challenges related to analyzing biological signals, mainly related to noise-handling. We have identified a case where noise is misinterpreted as an oscillation. This leaves us wondering which of the identified oscillations are true physiological oscillators, and which represent methodological errors. As we are not able to provide completely noise-free recordings in controlled settings of bedbound patients, we believe that tools meant for clinical use must be robust for noise. Thus, the algorithms must either remove all noise, or the results not being affected by the presence of it. We did not identify the distinct frequency bands that are reported in the literature (Shaffer et al., 2014; Task Force of the European Society of Cardiology & the North American Society of Pacing & Electrophysiology, 1996). Noise could be the problem here as well. However, the signals are mostly noise-free, so we would expect to identify the oscillations if they were present. This raises the question if the use of strict frequency intervals is a simplification of a highly complex and variable field. Features that are incorporated in biological signals, such as nonlinearity and nonstationarity, are challenging when analyzing them. We have earlier addressed the challenge by applying Fourier-based analyses to such signals, suggesting the data-driven Hilbert–Huang Transform as a better approach (Knai, Kulia, Molinas, & Skjaervold, 2017). However, the Hilbert–Huang transform is hampered by being computationally challenging and requiring thorough validation. If the developed methods at some time point are meant to be used real-time, for instance in intelligent alarm systems, the chosen analyses should be quick and easily adaptive to different biological signals. In this work, visual inspection was required to secure that the algorithms applied correctly and to validate the results, identifying the case where noise was misinterpreted as an oscillation.

4.1 | Methodological considerations

Our study population is small. We recruited 10 patients scheduled for coronary artery bypass grafting over a period

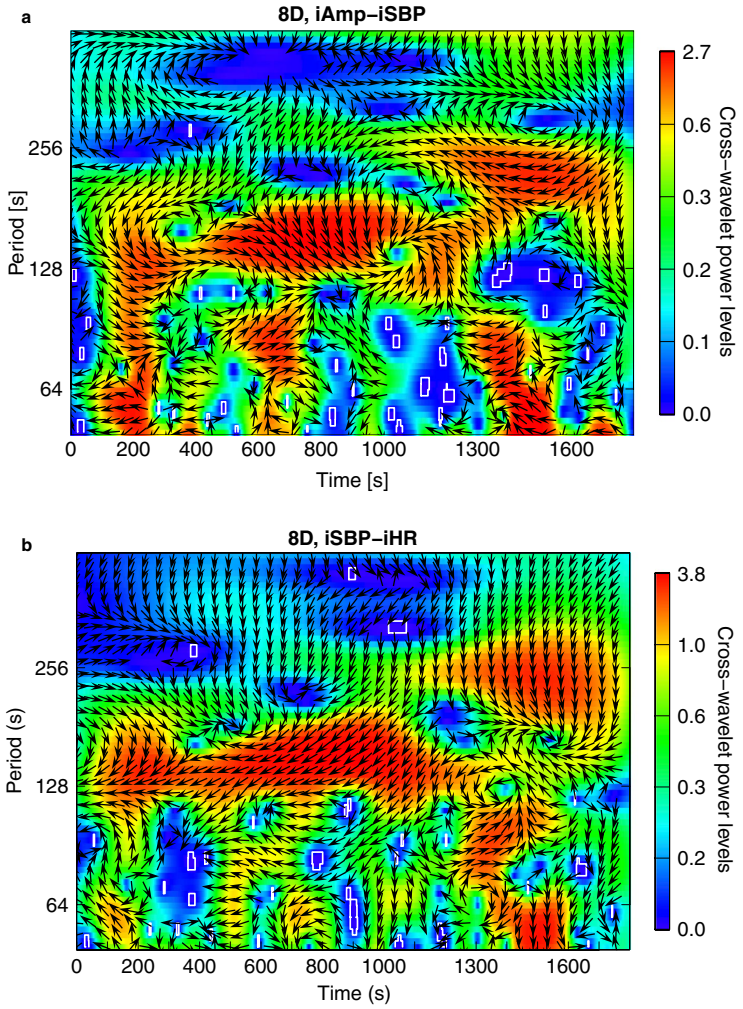


FIGURE 5 Cross-wavelet spectra of the variable pairs iAmp-iSBP and iSBP-iHR of patient 8, situation D. Time (in seconds) is shown on the *x*-axes, and period (in seconds) on a logarithmic scale on the *y*-axes. Power is given by color, according to the scale next to the plots. Significant frequencies are marked by white lines and phase differences by arrows. Both spectra show high power just above 128 s

of 3 months in 2016, whereof two patients were excluded due to non-sinus rhythm. The final data material includes three extracted variables from four situations of eight patients—96 analyzed time series. Being derived from only eight patients, we cannot generalize our findings to the total population of cardiac surgery patients.

The recruited patients are heterogeneous individuals featuring different medical backgrounds, pharmacological profiles, and general health. However, the group altogether holds common features such as high age and coronary heart disease. One could raise the question of selection bias as we recruited patients over a short time period and excluded patients with serious illness such as heart failure, valve disease, and perioperative complications. However, we investigate universal physiological features without performing statistical hypothesis testing or other comparisons on group level. The comparisons we do are only between situations of the perioperative course, and in such cases, the patients serve as their own controls. Interpretation of the results must be done with these aspects in thought, and the results' generalizability should be investigated in bigger study groups.

To minimize autonomic activation and artifacts caused by postural changes, the patients were kept lying during data collection. The data were collected with research hardware and software to secure complete control of filtering and preprocessing algorithms applied to the data. To avoid putting the patients through unnecessary stress by inserting two arterial cannulas prior to surgery, we used different cannulas pre- and postoperatively. A consequence of this could be different absolute values of the BP recordings before and after surgery. However, we believe that the frequency distributions of the signals are unchanged. Vasoactive and analgesic medications, and fluids were administered postoperatively according to the individual

patient's clinical state. Thus, the patients may have received different amounts of medications, with varying contribution to their oscillatory profile.

5 | CONCLUSION

In this study, we decomposed BP and ECG recordings from eight cardiac surgery patients to time series of SBP, HR, and R-wave amplitude. Four 30-min selections, representing key events of the perioperative course, were analyzed with the CWT and average wavelet power spectra were used to illustrate the patients' circulatory frequency distributions. We identified oscillatory components in all variables, patients, and situations, and they were more or less randomly distributed through the examined frequency range. The high variety in circulatory oscillations seems to represent inter-individual variations, more than factors of the performed surgery. Linking the circulatory frequency distributions to the overall complexity of circulatory signals, no reduction of such is identified. Considering the heterogeneity of our findings, we have not presented information that is suitable for use in any monitoring device or other clinical decision tools. The study is limited by challenges regarding noise-handling, and generalizability due to small sample size.

ACKNOWLEDGMENTS

We thank Bjørn Gardsjord Lio and Fredrik Einar Tobias Axelsson for assistance in collecting data, and Tord Åsnes for designing Figure 1.

CONFLICT OF INTEREST

All authors declare that they have no competing interests.

ORCID

Kathrine Knai https://orcid.org/0000-0002-7586-8029

REFERENCES

Addison, P. S. (2005). Wavelet transforms and the ECG: A review. *Physiological Measurement*, *26*(5), R155–R199. https://doi.org/10.1088/0967-3334/26/5/R01

Akselrod, S., Gordon, D., Ubel, F. A., Shannon, D. C., Berger, A. C., & Cohen, R. J. (1981). Power spectrum analysis of heart rate fluctuation: A quantitative probe of beat-to-beat cardiovascular control. *Science*, *213*(4504), 220–222. https://doi.org/10.1126/science.6166045

Alfons, A.. (2016). *robustHD: Robust Methods for High-Dimensional Data [Internet]*. Available from: https://CRAN.R-project.org/package=robustHD

Bengtsson, H. (2016). R.matlab: Read and Write MAT Files and Call MATLAB from Within R [Internet]. Available from: https://github.com/HenrikBengtsson/R.matlab

Bracic, M., & Stefanovska, A. (1998). Wavelet-based analysis of human blood-flow dynamics. *Bulletin of Mathematical Biology*, *60*(5), 919–935. https://doi.org/10.1006/bulm.1998.0047

Brody, D. A. (1956). A theoretical analysis of intracavitary blood mass influence on the heart-lead relationship. *Circulation Research*, *4*(6), 731–738. https://doi.org/10.1161/01.RES.4.6.731

Claydon, V. E., & Krassioukov, A. V. (2008). Clinical correlates of frequency analyses of cardiovascular control after spinal cord injury. *American Journal of Physiology-Heart and Circulatory Physiology*, *294*(2), H668–H678. https://doi.org/10.1152/ajpheart.00869.2007

Cleveland, W. S., & Devlin, S. J. (1988). Locally weighted regression: An approach to regression analysis by local fitting. *Journal of the American Statistical Association*, *83*(403), 596–610. https://doi.org/10.1080/01621459.1988.10478639

Fugal, D. L. (2009). *Conceptual wavelets in digital signal processing: An in-depth, practical approach for the non-mathematician* (p. 382). Space & Signals Technical Pub.

Goldberger, A. L. (1996). Non-linear dynamics for clinicians: Chaos theory, fractals, and complexity at the bedside. *The Lancet*, *347*(9011), 1312–1314. https://doi.org/10.1016/S0140-6736(96)90948-4

Goldberger, A. L., Moody, G. B., & Costa, M. D. (2012). *Variability vs. Complexity [Internet]*. Physionet.org. Available from: https://archive.physionet.org/tutorials/cv/

Goldstein, B., Fiser, D. H., Kelly, M. M., Mickelsen, D., Ruttimann, U., & Pollack, M. M. (1998). Decomplexification in critical illness and injury: Relationship between heart rate variability, severity of illness, and outcome. *Critical Care Medicine*, *26*(2), 352–357. https://doi.org/10.1097/00003246-199802000-00040

Hogue, C. W., Stein, P. K., Apostolidou, I., Lappas, D. G., & Kleiger, R. E. (1994). Alterations in temporal patterns of heart rate variability after coronary artery bypass graft surgery. *Anesthesiology*, *81*(6), 1356–1364. https://doi.org/10.1097/00000542-199412000-00009

Johnson, N. (2009). *Simply complexity: A clear guide to complexity theory* (p. 202). Oxford, UK: Oneworld Publications.

Kaplan, D. T., Furman, M. I., Pincus, S. M., Ryan, S. M., Lipsitz, L. A., & Goldberger, A. L. (1991). Aging and the complexity of cardiovascular dynamics. *Biophysical Journal*, *59*(4), 945–949. https://doi.org/10.1016/S0006-3495(91)82309-8

Kleiger, R. E., Miller, J. P., Bigger, J. T., & Moss, A. J. (1987). Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. *The American Journal of Cardiology*, *59*(4), 256–262. https://doi.org/10.1016/0002-9149(87)90795-8

Knai, K., Kulia, G., Molinas, M., & Skjaervold, N. K. (2017). Instantaneous frequencies of continuous blood pressure a comparison of the power spectrum, the continuous wavelet transform and the Hilbert-Huang transform. *Advances in Data Science and Adaptive Analysis*, *09*(04), 1750009.

Kuo, C.-D., Chen, G.-Y., Lai, S.-T., Wang, Y.-Y., Shih, C.-C., & Wang, J.-H. (1999). Sequential changes in heart rate variability after coronary artery bypass grafting. *The American Journal of Cardiology*, *83*(5), 776–779. https://doi.org/10.1016/S0002-9149(98)00989-8

Lipsitz, L. A., & Goldberger, A. L. (1992). Loss of "complexity" and aging: Potential applications of fractals and chaos theory to senescence. *JAMA*, *267*(13), 1806. https://doi.org/10.1001/jama.1992.03480130122036

Nahiyan, K. M. T., & Amin, A. A. (2017). Removal of ECG baseline wander using Savitzky-Golay filter based method. *Bangladesh*

Journal of Medical Physics, *8*(1), 32–45. https://doi.org/10.3329/bjmp.v8i1.33932

Pomeranz, B., Macaulay, R. J., Caudill, M. A., Kutz, I., Adam, D., Gordon, D., … Cohen, R. J.. (1985). Assessment of autonomic function in humans by heart rate spectral analysis. *American Journal of Physiology-Heart and Circulatory Physiology*, *248*(1), H151–H153. https://doi.org/10.1152/ajpheart.1985.248.1.H151

R Foundation for Statistical Computing. (2018). *R: A Language and Environment for Statistical Computing [Internet]*. Vienna, Austria: R Foundation for Statistical Computing. Available from: https://www.R-project.org

Riordan, W. P., Norris, P. R., Jenkins, J. M., & Morris, J. A. (2009). Early loss of heart rate complexity predicts mortality regardless of mechanism, anatomic location, or severity of injury in 2178 trauma patients. *Journal of Surgical Research*, *156*(2), 283–289. https://doi.org/10.1016/j.jss.2009.03.086

Roesch, A., & Schmidbauer, H. (2018). *WaveletComp: Computational Wavelet Analysis [Internet]*. Available from: https://CRAN.R-project.org/package=WaveletComp

Shaffer, F., McCraty, R., & Zerr, C. L. (2014). A healthy heart is not a metronome: An integrative review of the heart's anatomy and heart rate variability. *Frontiers in Psychology*, *5*, 1040. https://doi.org/10.3389/fpsyg.2014.01040

Signal developers. (2013). *signal: Signal processing [Internet]*. Available from: http://r-forge.r-project.org/projects/signal/

Takahashi, A. C. M., Porta, A., Melo, R. C., Quitério, R. J., da Silva, E., Borghi-Silva, A., … Catai, A. M.. (2012). Aging reduces complexity of heart rate variability assessed by conditional entropy and symbolic analysis. *Internal and Emergency Medicine*, *7*(3), 229–235. https://doi.org/10.1007/s11739-011-0512-z

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. (1996). Heart rate variability standards of measurement, physiological interpretation, and clinical use. *Circulation*, *93*(5), 1043–1065.

Umetani, K., Singer, D. H., McCraty, R., & Atkinson, M. (1998). Twenty-four hour time domain heart rate variability and heart rate: Relations to age and gender over nine decades. *Journal of the American College of Cardiology*, *31*(3), 593–601. https://doi.org/10.1016/S0735-1097(97)00554-8

Wolf, M. M., Varigos, G. A., Hunt, D., & Sloman, J. G. (1978). Sinus arrhythmia in acute myocardial infarction. *Medical Journal of Australia*, *2*(2), 52–53.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Knai K, Aadahl P, Skjaervold NK. Cardiac surgery does not lead to loss of oscillatory components in circulatory signals. *Physiol Rep*. 2020;8:e14423. https://doi.org/10.14814/phy2.14423