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Tuula Tarkiainen

Short-term Heart Rate Dynamics: Methodology and Novel Applications

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TUULA TARKIAINEN

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ABSTRACT

This study evaluated the stability over time and the effect of non-sinus beats on the short-term heart rate (HR) dynamics analyses. In addition, HR variability was used to study the effect of acute carbon monoxide (CO) exposure. Finally, the possibility was assessed of using non-linear HR dynamics to predict the occurrence of postoperative atrial fibrillation (AF) after coronary artery by-pass grafting.

Every two weeks during six months, 131 subjects with stable coronary artery disease (CAD) went through a 40-min ECG recording during a standardised protocol. A set of both conventional and non-linear HR dynamics was analysed; the non-linear HR dynamics with different ways of editing the non-sinus beats. The stability of HR dynamics was analysed by examining the coefficient of variation for repeated measurements. Six subjects with severe CAD underwent simultaneous recordings of ambulatory ECG and CO levels three times with one-week interval. The differences between HR variability preceding and during the CO peaks were analysed. One hundred patients went through a standardised protocol of 10-min rest, paced breathing and head-up tilt one day before coronary surgery. The potential of non-linear HR dynamics to predict the postoperative AF was evaluated.

The results indicated that HR dynamics were stable over a period of three to four months. One exception was noted in the standard deviation of normal-to-normal intervals-parameter. In addition, the stability of short-term scaling exponent of detrended fluctuation analysis (DFA α_1) and approximate entropy remained only moderate. Non-sinus beats remarkably affected non-linear HR dynamics and their stability. The higher levels of acute CO exposure were associated with increased HR variability. The preoperatively reduced DFA α_1 during rest was an independent predictor of postoperative AF after coronary surgery.

In conclusion, most measures of HR dynamics showed acceptable stability among subjects with stable CAD. However, more standardised editing practises are needed. The acute CO altered cardiac autonomic regulation in subjects with severe CAD. Therefore, HR dynamics analysis appears to be feasible for use in air pollution epidemiology. The preoperative non-linear HR dynamics might provide additional information about the pathophysiological factors predisposing to postoperative AF after coronary surgery.

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Medical Subject Headings: Air Pollution; Atrial Fibrillation; Autonomic Nervous System; Cardiac Complexes, Premature; Coronary Artery Disease; Heart Rate; Reproducibility of Results

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TIIVISTELMÄ

Tutkimus määritti lyhytkestoisten sydämen sykevaihtelusuureiden toistettavuutta ja lisälyöntien editointikäytäntöjen vaikutusta analyyseihin. Lisäksi sykevaihtelua käytettiin arvioimaan häkäaltistumisen vaikutuksia. Viimeiseksi selvitettiin epälineaaristen sykevaihtelusuureiden kykyä ennustaa sepelvaltimoiden ohitusleikkauksen jälkeisen eteisvärinän ilmaantumista.

Vakioitujen tutkimuskäyntien aikana tehtiin 40 minuutin kestoinen EKG-rekisteröinti 131 sepelvaltimotautipotilaalle kahden viikon välein kuuden kuukauden ajan. Sekä tavanomaisia että epälineaarisia sykevaihtelusuureita analysoitiin, epälineaarinen sykevaihtelu käyttäen erilaisia lisälyöntien editointimenetelmiä. Sykevaihtelusuureiden toistettavuutta arvioitiin laskemalla toistettujen mittausten variaatiokerroin. Kuuden sepelvaltimotautipotilaan ryhmässä rekisteröitiin samanaikaisesti EKG:ta ja häkäpitoisuuksia kolmesti viikon välein. Häkäaltistusta edeltävää ja sen aikaista sydämen sykevaihtelua verrattiin. Sata potilasta osallistui vakioidulle 10-min levon, tahdistetun hengityksen ja pystyynnoston sisältävälle tutkimuskäynnille päivä ennen sepelvaltimoiden ohitusleikkausta. Epälineaaristen sykevaihtelusuureiden kykyä ennustaa leikkauksen jälkeisen eteisvärinän ilmaantumista arvioitiin.

Tulokset osoittivat sydämen sykevaihtelun olevan toistettavaa kolmen-neljän kuukauden seurantaaikana. Poikkeuksen teki standard deviation of normal-to-normal intervals-suure. Lisäksi detrended fluctuation analysis (DFA) α_1 ja approksimoitu entropia-suureet jäivät toistettavuudeltaan kohtalaisiksi. Lisälyönnit muunsivat merkitsevästi epälineaarisia sykevaihtelusuureita ja heikensivät niiden toistettavuutta. Häkäaltistuminen liittyi sydämen sykevaihtelun lisääntymiseen. Ennen leikkausta levossa alentunut DFA α_1 ennusti itsenäisesti leikkauksen jälkeisen eteisvärinän ilmaantumista.

Johtopäätöksinä voidaan todeta, että useimmat sydämen sykevaihtelusuureet olivat toistettavia vakaata sepelvaltimotautia sairastavilla. Lisälyöntien editoimiseen tarvitaan vakioidumpia käytäntöjä. Akuutti häkäaltistuminen muunsi sydämen autonomista säätelyä vaikeaa sepelvaltimotautia sairastavilla. Näin ollen sydämen sykevaihtelusuureet saattavat osoittautua käyttökelpoisiksi ilmansaaste-epidemiologiassa. Ennen sepelvaltimoiden ohitusleikkausta analysoidut epälineaariset sykevaihtelusuureet saattavat antaa uutta lisätietoa leikkauksen jälkeiselle eteisvärinälle altistavista patofysiologisista tekijöistä.

Yleinen suomalainen asiasanasto: autonominen hermosto; EKG; eteisvärinä; ilman saastuminen; reliabiliteetti; sepelvaltimotauti; syke

To my family

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Mikkeli, January, 2012

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List of the original publications

The dissertation is based on the following original publications.

I Tarkiainen TH, Timonen KL, Tiittanen P, Hartikainen JEK, Pekkanen J, Hoek G, Ibald-Mulli A, Vanninen EJ. Stability over time of short-term heart rate variability. *Clin Auton Res 15:394-399, 2005.*

II Tarkiainen TH, Kuusela TA, Tahvanainen KUO, Hartikainen JEK, Tiittanen P, Timonen KL, Vanninen EJ. Comparison of methods for editing of ectopic beats in measurements of short-term non-linear heart rate dynamics. *Clin Physiol Funct Imaging 27:126-133, 2007.*

III Tarkiainen TH, Timonen KL, Vanninen EJ, Alm S, Hartikainen JEK, Pekkanen J. Effect of acute carbon monoxide exposure on heart rate variability in patients with coronary artery disease. *Clin Physiol Funct Imaging 23:98-102, 2003.*

IV Tarkiainen TH, Hakala T, Hedman A, Vanninen E. Preoperative alterations in correlation properties and complexity of R-R interval dynamics predict the risk of atrial fibrillation after coronary artery bypass grafting in patients with preserved left ventricular function. *J Cardiovasc Electrophysiol 19:907-912, 2008.*

Contents

Abbreviations

AF	Atrial fibrillation
AMI	Acute myocardial infarction
ANS	Autonomic nervous system
ApEn	Approximate entropy
CABG	Coronary artery by-pass grafting
CAD	Coronary artery disease
CI	Confidence interval
CV	Coefficient of variation
CO	Carbon monoxide
DFA	Detrended fluctuation analysis
DFA α_1	Short-term scaling exponent of detrended fluctuation analysis
FD-L	Fractal dimension by curve length
HF	Power in the high frequency range
HR	Heart rate
ICC	Intraclass correlation coefficient
LF	Power in the low frequency range
LF/HF	LF to HF ratio
MeanNN	Mean of the normal-to-normal intervals
MI	Myocardial infarction
MSNA	Muscle sympathetic nerve activity
NN	Normal-to-normal
OR	Odds ratio
PCI	Percutaneous coronary intervention
PM	Particulate matter
$PM_{2.5}$	Particles smaller than 2.5 μm
PM_{10}	Particles smaller than 10 μm
PSD	Power spectral density
r-MSSD	The square root of the mean of the sum of the squares of differences between adjacent normal-to-normal intervals
RM	Return map (Poincaré plot)
RM SD1	Short-term axis of return map
RM SD2	Long-term axis of return map
RSA	Respiratory sinus arrhythmia
SaEn	Sample entropy
SD	Standard deviation
SDANN	Standard deviation of the averages of normal-to-normal intervals in all 5 min segments of the entire recording
SDNN	Standard deviation of normal-to-normal intervals over the selected time interval
SR	Sinus rhythm
SymDyn	Symbolic dynamics
SymDyn En	Entropy of symbolic dynamics
SymDyn FW	Forbidden words of symbolic dynamics
Total power	Variance of all normal-to-normal intervals
TP	Total power
VLF	Power in the very low frequency range

1 Introduction

The assessment of variations in cardiac intervals, i.e. heart rate (HR) dynamics, has become an established method to evaluate cardiac autonomic regulation. The dynamic properties of short-term HR are mostly controlled by the interaction between vagal and sympathetic nervous systems (Bilchick et al. 2006). Therefore, the HR dynamics importantly mirror the entireness of cardiac autonomic regulation (Bilchick et al. 2006) and offer new insights into cardiovascular physiology and pathophysiology.

The interest in HR dynamics truly exploded in 1987, when Kleiger et al. reported that reduced HR variability independently predicted mortality after acute myocardial infarction (AMI). This observation has been verified in a large body of data using both so-called traditional and non-linear HR dynamics measures (Bigger et al. 1992, Fei et al. 1996, Hartikainen et al. 1996, Huikuri et al. 2000, La Rovere et al. 1998, Makikallio et al. 1999, Tapanainen et al. 2002, Zuanetti et al. 1996). Importantly, altered HR dynamics has also been observed to be an early finding in another clinical situation, namely cardiovascular autonomic diabetic neuropathy, and to contain useful prognostic information also in this disease (Laitinen et al. 1999). However, despite much research and abundant data on the predictive power of HR dynamics for predicting cardiovascular mortality at population level (Goldberger et al. 2008), HR dynamics still has not become a method in clinical use. One reason is that the advantage of using HR dynamics as a risk marker in intervention-based studies is still obscure (Hohnloser et al. 2004, Huikuri et al. 2009, Zareba et al. 2003).

Most studies have utilised 24-hour HR dynamics measurements. However, more short-term, i.e. 5- to 15-minute, measurements would be much more feasible for use. Importantly, there is clinical data showing that the predictive power of short-term HR dynamics approaches that of long-term recordings in post-infarction patients (Bigger et al. 1993). Thus, some studies have indicated the short-term recordings to serve as a screening tool before longer recordings (Faber et al. 1996, Fei et al. 1996, Kautzner et al. 1998). Furthermore, in heart failure patients, the short-term HR dynamics have strongly predicted the mortality, and investigators have proposed that these simple bedside methods could become a part of clinical routine in these patients (La Rovere et al. 2003). Reduced 2-min HR variability has predicted the risk of coronary artery disease and mortality also among a general middle-aged population (Dekker et al. 2000). These clinical factors suggest that the short-term HR dynamics have an important subject for research.

In purpose to appropriately standardise the methods used for the assessment of HR dynamics, the European Society of Cardiology and the North American Society of Pacing and Electrophysiology published a Task Force in 1996. Since then, many new measurements, mostly based on non-linear mathematics, have appeared and complemented the information gathered with traditional analysis methods (Huikuri et al. 2009). However, many methodological factors such as the reliability of the measurements, have remained inadequately evaluated (Sandercock 2007). In addition, the possible effects of non-sinus beats on the analyses have been largely ignored.

The HR dynamics are constantly utilised in new research fields. One such area is the research assessing the possible effects of air pollution on cardiovascular regulation (Brook et al. 2004). There is a large body of data showing that air pollution increase both mortality and morbidity, especially in patients with cardiovascular disorders. Therefore, examining the HR dynamics that reflect indirectly the cardiac autonomic regulation might reveal new pathophysiological explanations for the harmful effects of air pollution.

The role of cardiac autonomic regulation in triggering and maintaining atrial fibrillation has been recognised for a long time (Coumel 1994). However, it is not clear whether cardiac autonomic modulation plays an essential role also in the occurrence of atrial fibrillation after cardiac surgery (Hakala et al. 2002, Hogue et al. 1998, Vikman et al. 1999). Therefore HR dynamics might reveal new associations in this respect.

The present study was conducted as collaboration between the Department of Clinical Physiology and Nuclear Medicine at Kuopio University Hospital and University of Eastern Finland and the Department of Environmental Health in the National Institute for Health and Welfare in Kuopio. The study consisted of two parts. The first part focused on the methodological aspects of short-term HR dynamics, such as stability over time and the effects of non-sinus beats on HR dynamics analyses. In the second part, the HR dynamics were used in two novel applications. First, the HR variability measures were used to assess the possible effects of carbon monoxide exposure on cardiac regulation in patients with stable coronary artery disease (CAD). Secondly, the possibility of using short-term non-linear HR dynamics to predict the postoperative atrial fibrillation after cardiac surgery was examined.

2 Review of the literature

2.1 SHORT-TERM HEART RATE DYNAMICS

2.1.1 Assessment of short-term heart rate dynamics

The HR variability – or HR dynamics as it is more often termed in relation to non-linear methods – is a method to assess cardiac autonomic regulation underpinning the cardiac interval variations during sinus rhythm (ESC/NASPE Task Force 1996). For most relevant assessments, one should study the variability of sinus node depolarisations. However, the exact extraction of P-wave onsets from surface ECGs would be technically demanding. Therefore, the potential error in relation to the variation of atrioventricular conduction is accepted and the intervals between consecutive R-peaks, i.e. RR intervals, are used in the HR dynamics analyses (Voss et al. 1996) (Figure 1).

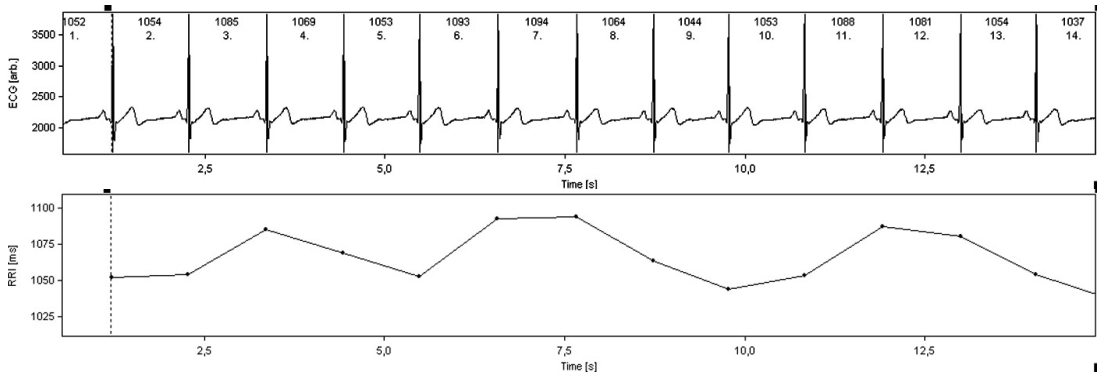


Figure 1. RR intervals analysed from ECG signal

In HR dynamics analysis, the quality of ECG recordings needs to be high to enable an accurate detection of every QRS complexes. Even single misidentifications cause meaningful errors in short-term analyses (Berntson et al. 1998). Therefore, there should be a satisfactory signal-to-noise ratio and reasonable bandwidth in the ECG recording (Bailey et al. 1990) and the digital sampling rate must be adequate (Pinna et al. 1994). An accurate method for the precise localisation of the QRS fiducial point needs to be used (Friesen et al. 1990). However, the automatic analyses often encounter difficulties in QRS identification especially with tall T-waves and low-voltage QRS complexes (Xia et al. 1992). Therefore, the ESC/NASPE Task Force 1996 has proposed visual verification of the analyses. The QRS identification should include the morphological as well as the rhythm information to differentiate sinus beats from ectopic beats and only the sinus beats should be used in the analyses (Malik 2004). New methods are constantly being developed for the classification of QRS complexes (Ince et al. 2009).

The HR dynamics are measured from timeseries of successive RR intervals with methods called time and frequency domain and non-linear methods.

Conventional measurements of heart rate variability

Time domain methods

The time domain methods characterise the variance of either normal-to-normal (NN) RR intervals or differences between NN intervals. These methods include statistical methods that provide results in units of time (ms), and geometrical methods (ESC/NASPE Task Force 1996). The most widely used statistical methods in short-term analyses are the mean of NN intervals (meanNN), the standard deviation of NN intervals (SDNN) and the square root of the mean squared differences of successive NN intervals (r-MSSD). The meanNN is reciprocally related to average HR, however, this relation is not linear. The SDNN characterises all the variability in the signal whereas the r-MSSD is a measure of short-term variation due to the comparisons between RR interval differences (ESC/NASPE Task Force 1996).

The geometrical methods create a geometrical pattern from the existing NN interval series (Malik 2004). The form of the obtained pattern can be assessed visually or alternatively, certain parameters can be calculated. In the return map (RM) analysis (Poincaré plot), each RR interval is plotted against the following RR interval (Gilham 1993) (Figure 3b). The obtained scatterplot has been visually classified (Woo et al. 1992), though this classification is subjective. The most widely used method to quantify the scatterplot is to fit an ellipse into it and then, the width of the ellipse, i.e. the standard deviation (SD1) of the short axis perpendicular to the line-of-identity, corresponds to the instantaneous RR interval variability and the length, i.e. the standard deviation (SD2) of the long axis along the line-of-identity, depicts the continuous variability (Brennan et al. 2001, Huikuri et al. 1996). The return map analysis, in fact, should reflect non-linear processes underneath the instant HR variablity (Woo et al. 1992), however, these measurements from two-dimensional plots are closely related to linear time-domain analyses (Brennan et al. 2001). The return map scatterplot has also been displayed in a three-dimensional way and in this case, the third axis defines the density of the plot (Copie et al. 1996, Hnatkova et al. 1995). It is also possible to calculate the return map with longer lags of 2-10 beats, but in this way, there might be alterations in the physiological correlations (Contreras et al. 2007). In addition, the short-term variability can be divided into two parts to characterise the asymmetry of decelerating or accelerating HR behaviour (Guzik et al. 2007, Piskorski et al. 2007).

In general, the time domain methods are simply methods to be analysed from recordings of sufficient duration; however, many values increase with longer recording times (Malik 2004). Therefore, the recordings of different duration should not be directly compared.

Frequency domain methods

Power spectral density (PSD) analysis makes it possible to represent the magnitude, i.e. power (amplitude squared), of sinusoidal oscillations of RR interval signal and hence, to distribute the variance into certain frequency bands (Akselrod et al. 1981, Malik 2004). The analysis can be based on nonparametric, most often the fast Fourier Transform algorithm or parametric methods such as autoregressive approach (ESC/NASPE Task Force 1996).

These methods provide at least qualitatively comparable assessments of PSD in short-term recordings (Fagard et al. 1998).

The main spectral components calculated in relation to short-term recordings are the very low frequency (VLF) ≤0.04, low frequency (LF) 0.04-0.15 and high frequency (HF) 0.15-0.4 Hz (ESC/NASPE Task Force 1996). The power, i.e. the area under each component is measured and expressed as absolute values (ms^2) (Malliani et al. 1991). The so-called LF to HF ratio (LF/HF) is calculated to reduce the effect of variation in the total power and to assess the relations between vagal and sympathetic autonomic regulation (Pagani et al. 1986; Malliani et al. 1991). Furthermore, it is possible to calculate so called normalised LF and HF, i.e. $LFnu = \frac{LF}{(TotalPower - VLF)} \times 100$, and in addition HFnu in short-term recordings calculated as HFnu=100-LFnu (Malliani et al. 1991).

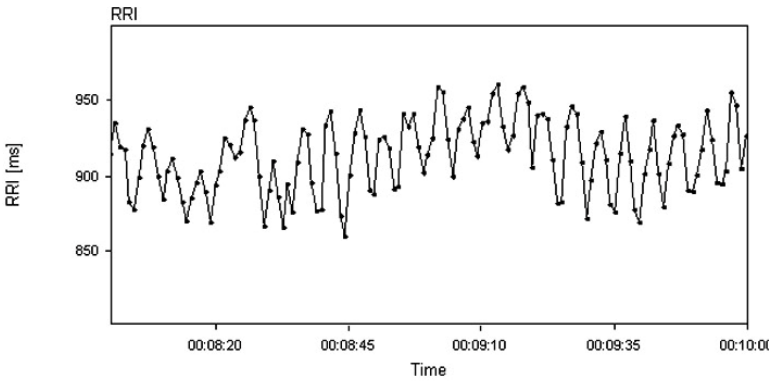


Figure 2a. Periodic oscillations in relation to 0.2 Hz paced breathing

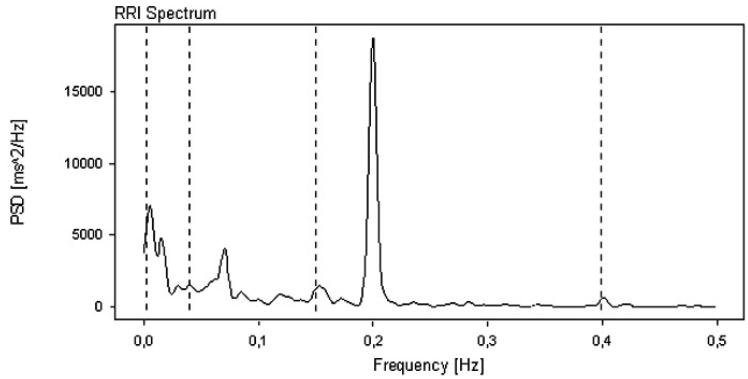


Figure 2b. RR interval FFT spectrum during 5-min 0.2 Hz paced breathing, the same recording as in Figure 2a

In addition to these standard measurements it may be possible to focus on the characteristic frequencies of the spectrum rather than to analyse its power (Korhonen et al. 2001). Nonetheless, the duration of the recording for spectral analysis should be at least ten times the wavelength of the lower frequency bound of the evaluated component (ESC/NASPE Task Force 1996). Thus, the VLF analysis cannot be accurately analysed from

recording lasting under 5 min and for LF approximately 4 min and for HF 1 min recordings are needed. However, in an attempt to standardise the practises between the studies, the ESC/NASPE Task Force 1996 has proposed that the spectral analysis should be performed from 5-min stationary recordings. The stationarity in this case means that the statistical properties of the signal must not change during the analysed period.

With spectral methods, the technical recommendations should be carefully noted. A sufficient sampling frequency of ECG recording (250-500 Hz or higher) is essential since otherwise the HF power might be overestimated, especially in relation to low baseline RR variability (Garcia-Gonzalez et al. 2004, Merri et al. 1990). In practice, however, the sampling rate in an ambulatory ECG recording may be as low as 100 Hz. This creates an error in QRS occurrence estimation by as much as 5 ms, when an acceptable level is assessed as being 1 ms (Bragge et al. 2005). Thus, with a low sampling frequency, one needs an interpolation method to correct R-peak localisation (Bragge et al. 2005, Merri et al. 1990). Editing of the intervals in relation to non-sinus beats or artefacts has been shown to be essential for spectral analysis (Clifford et al. 2005). Furthermore, a detrending can be used to eliminate the effects of baseline trends in RR interval time series (ESC/NASPE Task Force 1996).

With non-parametric methods, an evenly resampled interpolated discrete event series (plot of $R_i - R_{i-1}$ interval versus time) is recommended (ESC/NASPE Task Force 1996). In this case, the resampling rate has to be high enough to avoid the Nyquist frequency within the analysed frequency range (Singh et al. 2004). Clifford and Tarrassenko (Clifford et al. 2005) proposed the resampling rate of 7 Hz, which is capable of analysing the spectrum of HR ≤210 bpm; Singh et al. (Singh et al. 2004) have recommended 4 Hz for most situations. With parametric methods, an RR interval tachogram (RR intervals plotted versus number of progressive beats) can also be used (ESC/NASPE Task Force 1996). Before the analyses, the windowing methods for RR interval signal are used to reduce the spurious HF components due to the limited length of the signal (ESC/NASPE Task Force 1996).

The ESC/NASPE Task Force 1996 recommends that in relation to non-parametric methods, the algorithm of discrete event series interpolation, the resampling rate, the number of samples and the spectral window should be detailed. In relation to parametric methods, the model, the model order, the number of the samples and the central frequency for each spectral band should be detailed and furthermore, appropriate tests used to analyse the suitability of the chosen model.

There are also newer spectral methods that attempt to overcome the non-physiological demands of the signal stationarity. These methods are called time-frequency analyses, which are based on adjustable window lengths for different frequencies and thus, able to optimize time-resolutions for all frequencies (Akselrod 2004).

Non-linear heart rate dynamics measurements

The new methods based on non-linear mathematics do not assess the variance or its distribution in predetermined frequencies as is the case with the conventional HR variability methods rather than focus on the quality properties and dynamics of the RR interval signal (Huikuri et al. 2009). Many of the non-linear measurements quantify the fractal properties, i.e., self-similarity of the signal over multiple time scales (Goldberger

1996). The disappearance of the fractal-like properties or complexity leads either to high regularity or uncorrelated randomness which both mirror a non-adaptable system (Goldberger et al. 2002, Norris et al. 2008, Pikkujamsa et al. 2001). The non-linear measures can be divided into the families of fractal measures, entropy measures, symbolic dynamics measures and occasionally return map (Poincaré plot) analysis (Voss et al. 2009). The return map analysis can, however, also be regarded as a time domain measure.

Fractal measures

Kobayasi et. al. (Kobayashi et al. 1982) found that the RR intervals follow the 1/f fluctuations, i.e. the spectral density is inversely proportional to frequency in a log-log scale for low frequencies. This observation led to the establishment of power law analysis (Saul et al. 1988) that is a method to assess long-term scaling properties of RR interval data. This method was the first non-linear method for obtaining new prognostic information in relation to CAD (Bigger et al. 1996).

Another method, which characterises the intrinsic fractal-like correlation properties of RR interval time series and is suitable also for short-term data is the detrended fluctuation analysis (DFA) (Peng et al. 1995). In the DFA analysis, the root-mean-square fluctuations of integrated and detrended time series are calculated repeatedly in windows of different sizes and then, plotted against the window size on a log-log graph (Peng et al. 1995). A linear relationship on the log-log graph is indicative of self-similarity, i.e. the fluctuations in small boxes are related to the fluctuations in large boxes in a power-law fashion. A scaling exponent α defines the slope of this line (Peng et al. 1995). The α value 1 corresponds to 1/f noise having persistent fractal correlations (Peng et al. 1995). The α can be fitted over two time scales so that α_1 of DFA estimates the intrinsic fractal correlation properties for short-term, e.g. < 11 beats, and α_2 of DFA for long-term, e.g. ≥ 11 beats RR interval data (Makikallio et al. 1999, Peng et al. 1995). The DFA α_1 is most often analysed from periods of 1000 beats (Kleiger et al. 2005).

In the fractal dimension by curve length (FD-L) analysis, the number of segments of various lengths needed to follow the zigzaguing of the timeseries curve is counted (Chau et al. 1993). The curve can be followed better when the length of segments shortens, though more segments are needed (Kuusela et al. 2002). The fractal properties can be shown by this method, if the number of segments needed increases exponentially (Kuusela et al. 2002). The calculation of fractal dimension can be based on other methods such as fractal dimension of dispersion analysis (Bassingthwaighte et al. 1995).

It seems, however, that modulations in HR dynamics are such complex that single non-linear analysis is not able to characterise them (Ivanov et al. 1999). Therefore, multiple scaling exponents might be needed to characterise RR interval timeseries, which is called multifractal analysis (Ivanov et al. 1999).

Entropy measures

The complexity measures are non-linear methods for quantifying the regularity of RR interval timeseries. A presence of repetitive patterns renders timeseries more regular than a timeseries without such patterns (Ho et al. 1997). The approximate entropy (ApEn) is a measure that calculates the logarithmical probability that patterns of length m located close

to each other will be close also on next incremental comparisons m+1 (Pincus et al. 1994). A high regularity and predictability of RR interval time series produce small ApEn values and vice versa. In the calculation, the pattern length m (most often m=2) and the criterion for the similarity r (recommended to be r=0.10-0.25×standard deviation (SD); most often r=0.20×SD) have to be fixed (Pincus et al. 1994). In the ApEn calculation, the number of data points has an influence on the obtained values. However, when the number of data points is larger than 800, the ApEn approaches its final level (Kuusela et al. 2002). The sample entropy (SaEn) is an entropy measure, whose principles are very close to ApEn. However, in the calculation, the so-called self-matches (comparisons to the pattern itself) are not counted (Richman et al. 2000). This difference means that SaEn is more independent of the record length and more consistent with different r-values than the ApEn (Richman et al. 2000).

Costa et al. (Costa et al. 2005) improved the complexity analyses with a method called multiscale entropy that is able to assess the system's complexity over multiple temporal (and spatial) scales.

Symbolic dynamics measures

In the symbolic dynamics (SymDyn) method, the original timeseries are transformed into sequences of few symbols (Voss et al. 1996). This means that a considerable amount of detailed information may disappear but the coarse dynamic features in beat-to-beat variability are maintained (Voss et al. 1996). The dynamics in the signal are divided into four or sometimes six homogenous levels on the basis of mean and standard deviation or the absolute values (Kuusela et al. 2002, Porta et al. 2001, Voss et al. 1996). The symbol indicates at which level an individual RR interval belongs. Thereafter, three symbol words corresponding to certain functional patterns are formed (Voss et al. 1995) The distribution of the words and its complexity can be assessed with the Shannon entropy or the number of so-called forbidden words, which characterises the symbol sequences whose probability is lower than 0.001 (Voss et al. 1996). The lower entropy of SymDyn (SymDyn En) as well as an increasing number of forbidden words of SymDyn (SymDyn FW) characterises higher regularity (Voss et al. 1996).

Porta et al. (Porta et al. 2001) have evaluated an alternative way to analyse SymDyn. The three symbol words are grouped into certain families corresponding to no variation (0V), one variation (1V), two like variations (2LV) in which the three consecutive symbols obtain constantly increasing or decreasing values, and two unlike variations (2UV) in which the second value is larger or smaller than the other two values (Maestri et al. 2007, Porta et al. 2001). Thereafter, the proportions of these families are assessed as percentages.

There are an increasing number of non-linear HR dynamics methods, whose applicability for characterising complex cardiovascular regulation is no longer doubted (Voss et al. 2009). However, there is still debate about which of the methods should be selected in different clinical situations (Voss et al. 2009). Furthermore, the basic prerequisities inherent in the analyses, such as the optimal recording length, are still not as standardised with respect to conventional HR dynamics.

2.1.2 Physiological background of short-term heart rate dynamics

The discharges of sinoatrial node as a primary pacemaker are the basis for HR and its beat-to-beat fluctuations. The sinus node has an intrinsic excitation rate of approximately 100 per minute although it is age and gender dependent (Jose et al. 1970). The short-term modulation of sinus node function is controlled by vagal and sympathetic nervous systems, which are influenced by cardiac reflexes as well as by cortical factors (Hainsworth 2004).

The cardiac autonomic nervous system can be divided into extrinsic and intrinsic components. The extrinsic part consists of the parasympathetic and sympathetic components including brain nuclei, ganglia mostly along the spinal cord and axons to the heart (Hou et al. 2007). The intrinsic component includes interconnecting axons and autonomic ganglia concentrating in epicardial fat pads (Armour et al. 1997). The ganglionate plexus integrates the interactions between extrinsic and intrinsic cardiac autonomic nervous system (Hou et al. 2007).

Parasympathetic effect is delivered through vagal nerves originating from the nucleus ambiguous and dorsal motor nucleus in the brainstem (Spyer 1994). They pass to thorax alongside the carotid arteries and their branches synapse in intrinsic cardiac ganglia and innervate the sinus node, atrio-ventricular node, atrial and probably also the ventricular myocardium (Kapa et al. 1975, Zareba et al. 2001). The target of left parasympathetic neural structures seems to be predominantly the atrio-ventricular conduction and in the right structures it is the sinoatrial node (Hamlin et al. 1968). However, these effects are complexly modulated by the intrinsic cardiac ganglia (Hou et al. 2007). The efferent vagal activation is mediated by release of acetylcholine that has a very short latency period and rapid turnover rate (Levy 1971, Pumprla et al. 2002). Acethylcholine hyperpolarizes the pacemaker cells and diminishes their depolarisation rate and thus, slows the HR (Hainsworth 2004). The rapid effect of vagal activation enables beat-to-beat control of HR. It has been assumed that there is a linear relationship of parasympathetic effect and RR intervals (Katona et al. 1970), which might well be true in strictly defined circumstances. However, more recent experiments have observed a non-linear connection between acetylcholine release and RR interval length (Zaza et al. 2001).

Cardiac sympathetic preganglionic nerves originate in the upper thoracic region of the spinal cord and synapse in the sympathetic ganglia such as stellate ganglion (Janes et al. 1986). The postganglionic sympathetic nerves create a plexus jointly with vagal fibers over the mediastinum and innervate the sinus and atrio-ventricular node and the atrial and ventricular myocardium (Zareba et al. 2001). However, some preganglionic sympathetic nerves synapse on intrinsic cardiac ganglia (Kapa et al. 1975). It seems that the right sympathetic nerves predominantly increase the HR, whereas the left sympathetic nerves have a greater inotropic effect (Furnival et al. 1973). The sympathetic activation elevates the HR above its intrinsic level both via neural release of noradrenaline and release of adrenaline into the circulation (Robertson et al. 1979). The adrenergic activity enhances the depolarisation rate of sinonodal pacemaker cells and thus, directly regulates the RR interval length (Hainsworth 2004). In addition, sympathetic activity increases the atrioventricular conduction and contractility of the heart (Hainsworth 2004). However, it seems that not only is there heterogeneity in the target effects of individual sympathetic nerves but there is also large variability between individuals (Kapa et al. 1975). The effect of sympathetic

activity on HR is not as rapid as the effect of parasympathetic activity: there is a latency of up to 5 s after which HR gradually increases to the new steady level during 20-30 s (Hainsworth 2004).

The sympathetic and vagal systems mainly evoke opposite effects on the cardiovascular system. However, there are complex interactions between the parasympathetic and sympathetic centers in the central nervous system as well as in the periphery (Levy 1971). In the heart, the terminal fibers of the two subdivisions of the autonomic nervous system (ANS) locate near each other (Jacobowitz et al. 1967). Thus, released transmitters can diffuse to the nerve terminals of the other system, myocardium and the intrinsic cardiac ganglion cells (Revington et al. 1990). In fact, Tan et al. (Tan et al. 2006) observed that even one third of intrinsic ganglion cells express both cholinergic and adrenergic phenotypes, which highlights their synergestic role. Therefore, different kinds of interactions are possible. Levy (Levy 1971) described the accentuated antagonism of vagal and sympathetic activation on the heart, in which the response in the other subdivision was larger when the other subdivision was activated.

The normal resting HR is lower than the intrinsic rate of sinoatrial node due to the predominance of vagal tone (Lahiri et al. 2008). This predominance is related both to acetylcholine diminishing the amount of noradrenaline released in relation to sympathetic stimulus as well as weakened response to noradrenaline (Levy 1971). In normal subjects, the provoking agents contributing to sympathetic predominance such as upright tilt, exercise, noradrenaline or isoprenaline infusion or vagal blockade with atropine are observed as increased HR (Goldberger 1999).

The beat-to-beat variations in HR are dynamically modulated by ANS as a response to physiological perturbations (Kleiger et al. 2005). Both parasympathetic and sympathetic fibers carry afferent impulses from the heart to the brain, triggering feedback responses from the autonomic nervous system (Zareba et al. 2001). These responses include various inhibitory and excitatory reflexes modulating the HR and forming complex interactions influenced by cortical regulation (Hainsworth 2004). The arterial baroreceptors are stretch receptors some of which are located in the carotid arteries and aortic arch. The afferent information from these receptors to vasomotor centers in the central nervous system activates reflex adjustements that correct the short-term alterations in blood pressure (La Rovere et al. 2008, Lanfranchi et al. 2002). An increase in blood pressure promotes vagal activation diminishing the HR as well as reduces sympathetic efferent activation, which diminishes the tone of vascular smooth muscles (Raven et al. 2002). A decrease in blood pressure is followed by opposing alterations such as an increase in HR, cardiac contractility, peripheral vascular resistance and venous return (La Rovere et al. 2008).

There are also several other types of cardiac or pulmonary receptors, chemoreceptors and mechanoreceptors (Kapa et al. 1975). The atrial receptors response to increased atrial volume with sympathetic activation causing higher HR as well as water and salt excretion (Hainsworth 2004). This tachycardia caused by hypervolemia has been named the Bainbridge reflex or effect (Hainsworth 1991). This reflex has an opposite effect to the baroreflex (Barbieri et al. 2002). The diving reflex is a unique reflex consisting of trigeminal afferents stimulated by cold face immersion and producing both the enhanced sympathetic and vagal activation resulting in hypertension and bradycardia (Tulppo et al. 2005).

Respiratory sinus arrhythmia

During quiet respiration, the HR accelerates during inspiration and slows down during expiration (Eckberg et al. 1980). This respiratory sinus arrhythmia (RSA) is intended to improve the pulmonary gas exchange via efficient ventilation-perfusion matching (Yasuma et al. 2004). The RSA is a complex result of interactions between the cardiovascular and respiratory systems that include central, mechanical, humoral and neural feedback loops (Grossman et al. 2007).

Eckberg and Orshan (Eckberg et al. 1977) used a neck suction technique to demonstrate that the baroreceptor stimulus was more likely to prolong RR intervals during expiration than inspiration. In addition, the unloading of baroreceptor input was more likely to evoke muscle sympathetic nerve activity (MSNA) bursts during expiration than inspiration (Eckberg et al. 1980). Thus, inspiration suppresses the responses to baroreceptor influences of both vagal and sympathetic efferent activity (Eckberg et al. 1980). To be more precise, the autonomic motoneurone responsiveness is greatest during late inspiration and early expiration (Eckberg et al. 1980). This phenomenon results from the opening of a central gating mechanism according to the respiratory phase (Gilbey et al. 1984, McAllen et al. 1978, Seller et al. 1968), which also explains the RSA via varying vagal effects upon the sinoatrial node (Eckberg et al. 1980).

The muscarinic cholinergic receptor antagonist atropine causes a dose-related reduction or even disappearance of RSA, which implies that the efferent component of RSA should be primarily vagal (Katona et al. 1975, Wheeler et al. 1973). The RSA, however, is a result of the respiratory influences on the phase of vagal activity and not an index of mean vagal tone. Hedman et al. (Hedman et al. 1995) observed in an invasive dog experiment that the same number of vagal bursts occurred irrespective of slow or rapid breathing rates. However, the smaller number of bursts occurring during expiration with rapid than slow breathing explained the reduced magnitude of RSA. In experiments where cardiac vagal activation has attained extreme levels by pharmacological stimulation, the HR reduction has no longer been accompanied with higher RSA (Goldberger et al. 2001). This finding might be related to the saturation of vagal activation across the breathing cycle or alternatively, the vagal activity loses its phasic responses to respiration or the HR reaches such low levels that it is no longer able to fluctuate (Grossman et al. 2007).

There is less consensus about the role of the sympathetic system. During normal breathing rate, i.e. most typically 15/min corresponding to a period of 4 s, the time constant for adrenergic activation has been regarded as being too slow to significantly influence RSA (Eckberg 2000). However, beta-adrenergic blockade enhances RSA (Taylor et al. 2001). Furthermore, in an invasive dog experiment, the direct sympathetic stimulation dimished RSA (Hedman et al. 1995). Thus, the sympathetic activity could attenuate RSA, either due to sympathovagal interactions or via a direct effect on RSA (Grossman et al. 2007).

The RSA is affected by respiratory parameters so that rapid breathing attenuates RSA and larger tidal volume enhances RSA (Hirsch et al. 1981). The RSA is also related to blood pressure level, i.e. at very low blood pressures evoking insignificant baroreceptor stimulation, the RSA is negligible. At higher pressures, the RSA is more prominent, but at very high pressures it may well completely vanish (Eckberg 2000). The blood pressure oscillates according to breathing frequency, as does HR. Thus, the RSA has been related to

baroreceptor responses so that the RR interval fluctuations are suggested to reflect the blood pressure changes provoked by respiration (deBoer et al. 1987, Keyl et al. 2000). Other investigators have suggested that respiration influences in parallel the arterial blood pressure and RR intervals as a third oscillator affecting both sympathetic and vagal cardiac motoneuron pools (Badra et al. 2001, Eckberg 2000).

Effect of posture change

Movement from a supine to an upright posture causes a displacement of blood to lower body parts and thus, a decline in venous return (Hainsworth 2004).

The right and left ventricular volumes dimish and the baroreflex position changes (Hainsworth 2004). One response is a compensatory tachycardia secondary to vagal withdrawal as well as reflex vasoconstriction due to sympathetic activation (Cooke et al. 1999). With head-up tilt, the plasma catecholamine levels increase (Furlan et al. 2000), though the increase is modulated by gender and age (Geelen et al. 2002). The muscle sympathetic nerve activity and its oscillations are enhanced (Cooke et al. 1999). Due to these adaptive changes, the mean blood pressure in the upright position is maintained close to or even above that encountered in the supine position (Furlan et al. 2000). However, the blood pressure variability increases (Cooke et al. 1999). In a large population-based sample, the upright posture independently increased HR and altered measures of HR variability interpreted as consistent with a higher sympathetic tone (Stolarz et al. 2003).

However, the most characteristic feature is a decline in RSA with head-up tilt (Cooke et al. 1999). In fact, head-up tilt consistently decreases the respiratory gating of both vagal and sympathetic responses, which could be attributable to vagal withdrawal as well as enhanced sympathetic stimulation overwhelming the respiratory gating (Cooke et al. 1999).

Exercise

The exercise-induced alterations in autonomic nervous regulation aim to fulfill the metabolic demands of exercising muscles. At the beginning of dynamic exercise, HR increases as a response to vagal withdrawal that is mediated by a 'central command' (Goodwin et al. 1972, Robinson et al. 1966); the increase in HR can ecxeed 30 to 50 beats per minute (Freeman et al. 2006). Thereafter, sympathetic enhancement that is related to the inputs from muscle mechano- and metaboreceptors (exercise pressor reflex) sustain the progressive HR increase as well as increases in blood pressure and cardiac output (Boushel 2010, McCloskey et al. 1972). Both the central command and exercise pressor reflex are involved in baroreflex resetting and this permits the baroreflex to operate during hypertensive stimuli (Raven et al. 2002). The beat-to-beat HR variability is profoundly reduced (Perini et al. 1990). The vagal withdrawal is considered an important mechanism during exercise, however, the parasympathetic withdrawal is never complete (Kannankeril et al. 2004). During the later phase of gradual exercise, the concentrations of plasma catecholamines are significantly increased (Nakamura et al. 1993).

Recovery

At the cessation of exercise, the HR returns to the previous resting level due to the coordinated interaction of vagal reactivation and sympathetic withdrawal (Kannankeril et al. 2004). The rapid return of vagal tone induces a decrease in HR of 30-35 beats/min during the first minute of recovery independently of the exercise intensity (Imai et al. 1994). The sympathetic withdrawal complements a further decrease in HR (Kannankeril et al. 2004). The heart rate recovery during the first minute of recovery has become a new method with which to assess the vagal regulation. A reduction of 12 beats or less during the first cool-down minute following the symptom limited maximal exercise has been associated with increased mortality (Cole et al. 1999).

Physiological correlates of conventional measurements of heart rate variability

Time domain

The SDNN is a measure of overall variability. However, at rest, a large part of this variability is dependent on vagal modulation related to respiratory sinus arrhythmia. The SDNN is dependent on HR and, in fact, the SDNN attains lower values when the average HR increases, even though the relative fluctuations of HR remain similar at different HR levels (Tulppo et al. 2004).

The r-MSSD is a measure based on comparisons between consecutive beats and, thus, it reflects only high-frequency variation (Bilchick et al. 2006). The r-MSSD has been postulated to mirror the vagal modulation of RR intervals driven by ventilation (Kleiger et al. 2005). The SD1 in return map analysis reflects the instantaneous HR beat-to-beat variability as well (Huikuri et al. 1996). Penttilä et al. (Penttila et al. 2001) observed the vagal blockade with glycopyrrolate to diminish r-MMSD 97.0% and SD1 91.3%. However, rapid breathing did not reduce r-MSSD or RM SD1 although the high-frequency variability in the spectral analysis was lowered. Guzik et al. (Guzik et al. 2007) also did not observe any change in r-MSSD or SD1 with an increase in the breathing rate.

The definition of SD1 and SD2 in RM analysis is based on the different time constants of the vagal and sympathetic regulation when they are affecting the RR interval behaviour (Penttila et al. 2001). Thus, the SD2 value in RM analysis has been related to overall variability (Huikuri et al. 1996). It has been observed that RM analysis reflects accurately the cardiac autonomic modulation also during exercise and recovery (Tulppo et al. 2005, Tulppo et al. 2011). Interestingly, the RM SD1 mirrors increased vagal activation during recovery in those subjects who have simultaneously increased sympathetic regulation assessed by MSNA (Tulppo et al. 2011).

The asymmetrical behaviour of RR intervals, i.e. a more rapid increase than decrease during RSA is characterised in the shape of the scatterplot in the RM analysis with left-sided asymmetry (Brennan et al. 2001).

Frequency domain

The total power (TP) from 0 to 0.40 Hz characterises all sinusoidal variability of RR intervals. The TP is closely related to SDNN (Bilchick et al. 2006) and thus, has the same dependency on HR.

The HF component is regarded to reflect mainly respiratory related, vagally mediated effects on RR intervals (Akselrod et al. 1981, Malliani et al. 1991). In fact, breathing is a prerequisite for HF fluctuations in RR intervals, i.e. the holding of breath prevents these oscillations (Badra et al. 2001). One characteristic feature for RR interval spectrum is that the center frequency of HF peak shifts with the ventilatory rate (Novak et al. 1993). If a subject breathes regularly 9-24 breaths/min (2.5- to 6.7 s cycle length), the HF peak is observed at 0.15-0.40 Hz. However, when the breathing frequency is slowlier, the HF peak is located in the LF region. This can occur also during spontaneous breathing, because the breathing rate varies constantly and, thus, the frequencies can spread over a wide range (Pinna et al. 2006). In addition, the varying breathing rate leads to wider HF spectral peak (Penttila et al. 2001). The amplitude of the RR fluctuations is rather small at normal breathing frequencies and obtains its highest values at breathing frequencies of about 0.1 Hz (Hirsch et al. 1981). The meanNN, however, does not change, which signifies that the mean vagal tone has remained constant and the RSA is reflecting the vagal activity within the respiratory cycle (Hedman et al. 1995). Furthermore, the amplitude of RR fluctuations at HF region is proportional to the tidal volume (Hirsch et al. 1981). The HF oscillations are virtually abolished after cholinergic blockade with atropine, which associates these oscillations with vagal modulation (Taylor et al. 1998). However, sympathetic activation seems to have some role, as it dimishes the vagal effect at all breathing frequencies including the usual breathing frequencies near to 0.25 Hz (Taylor et al. 2001).

The LF fluctuations around 0.10 Hz are observed in arterial blood pressure (Mayer waves), MSNA signal as well as RR intervals (Pagani et al. 1997). Therefore, some investigators have postulated that there is a common central mechanism behind these rhythms (Pagani et al. 1997). Breathing at usual frequencies does not alter these rhythms (Badra et al. 2001). Due to the time constants of approximately 10 s for neuronally released noradrenaline, the LF has been claimed to reflect a delayed sympathetic response of the blood pressure alterations on HR (deBoer et al. 1987). Thus, the LF power has been related to arterial baroreflex mechanism (Sleight et al. 1995). However, some researchers have suggested that the baroreflex has only a modulatory role on these rhythms (Cooke et al. 1999).

The head-up tilt typically enhances the LF power, when assessed as normalised units, and attenuates the normalised HF power in both MSNA, systolic blood pressure and RR interval spectrum (Furlan et al. 2000). Thus, the normalised LF and HF or LF/HF-ratio has been interpreted as assessing the sympathovagal balance (Pagani et al. 1997). Cooke et al. (Cooke et al. 1999), however, emphasised that, in fact, during head-up tilt, the most meaningful finding concerns the absolute HF power that linearly attenuates with increasing tilt angle whereas the absolute LF power remains unchanged. Other investigators have emphasised the effect of reduced total power in the interpretation of the data and, thus, favour the proportional or normalised measurements (Furlan et al. 2000). These calculations, however, are based on the assumption that the changes in sympathetic and parasympathetic modulation are reciprocal and equal in magnitude, which does not seem to be the case (Porta et al. 2001). In addition, the results obtained during head-up tilt cannot be generalised to resting conditions. In fact, at rest, the LF power is not related to either MSNA (Notarius et al. 1999) or cardiac noradrenaline spillover (Moak et al. 2007).

Importantly, during heavy exercise, the LF power does not capture the sympathetic activation (Perini et al. 1990, Tulppo et al. 1996). Furthermore, atropine blockade almost abolishes the LF peak in RR interval spectrum, which emphasises the role of vagal modulation for affecting also these oscillations. Thus, it seems that the LF power cannot be regarded as a marker of sympathetic modulation. In fact, the new findings emphasise that LF power depicts an intact baroreflex function (Moak et al. 2007).

The physiological background of VLF oscillations between 25 and 333 s are not well understood. These oscillations bear some relations to the renin-angiotensin-aldosterone mechanism, however, large-dose atropine almost abolishes also them (Taylor et al. 1998). Therefore, it seems that most oscillations in RR intervals are dependent on vagal modulation of the cardiac rhythm.

Physiological correlates of non-linear heart rate dynamics measurements

The physiological correlates of non-linear HR dynamics are only partly understood. However, it has been proposed that the non-linear features enable the system to constantly adapt to varying intrinsic and extrinsic conditions (Goldberger et al. 2002).

Fractal measures

The fractal behaviour in which the same dynamics repeat themselves at different time scales has been proposed to be typical for the fluctuation in healthy subjects' RR intervals (Goldberger 1996). In this case, the short-term scaling exponent of detrended fluctuation analysis would approach a value of 1 (Huikuri et al. 2009). There are, however, also criticisms suggesting that many RR interval time series do not fulfil the expectations of fractality and thus, also the DFA α_1 could not be considered to reliably mirror the autonomic effects on heart rate, at least in individual subjects (Tan et al. 2009). In fact, a complete autonomic blockade has been shown to produce DFA α_1 values near to 1 suggesting that, in fact, the intrinsic pacemaker activity of sinus node would be fractal (Tan et al. 2009).

The DFA α_1 is correlated to the LF/HF ratio in controlled situations (Huikuri et al. 2009), which is explained by mathematical relationship between DFA α_1 being approximately $2\times(\frac{LF}{HF+LF})$ (Francis et al. 2002) Thus, at a group level, situations leading to higher sympathetic activity and vagal withdrawal such as head-up tilt, light dynamic exercise or cold hand immersion, produce increased DFA α_1 values (Hautala et al. 2003, Mourot et al. 2007, Tulppo et al. 2001). However, during intense exercise, the DFA α_1 has decreased (Hautala et al. 2003).

In pharmacological experiments, the parasympathetic blockade by atropine increased DFA α_1, which is a finding congruent with physiological provocations (Perkiomaki et al. 2001, Tulppo et al. 2001). A noradrenaline infusion produced slowing of HR that, however, did not occur in a linear manner but rather as abrupt increases in the RR intervals that could not be explained by respiration. This kind of behaviour was mirrored by a decrease in the DFA α_1 value (Tulppo et al. 1998).

Interestingly, cold face immersion, that produced both increased HF power and MSNA, i.e., a co-activation of both vagal and sympathetic nervous systems, lowered the DFA α_1

values (Tulppo et al. 2005). Mourot et al. (Mourot et al. 2007) have observed the same finding in relation to upright head-out immersion in cold water. The physiological background of low DFA α_1, i.e. a breakdown of fractal dynamics, is a highly interesting subject due to its association with increased cardiovascular mortality (Huikuri et al. 2000, Makikallio et al. 2005, Tapanainen et al. 2002).

The respiratory sinus arrhythmia as a sinusoidal trend seems to affect the DFA α_1 (Perakakis et al. 2009). Thus, it was reported that slow periodic breathing tended to enhance the DFA α_1 whereas the rapid breathing significantly lowered it (Penttila et al. 2001, Perakakis et al. 2009).

In one study, the ability of the fractal dimension to mirror the alterations in HR and blood pressure induced by terbutaline were considered to be minor (Kuusela et al. 2002).

Entropy measures

The complexity measures have been related to non-linear interactions between different regulatory loops; the loss of complexity possibly reflects one of these loops dominating over the others or strengthening of normally mild interactions (Porta et al. 2001, Voss et al. 2009).

There are divergent results about the effects of vagal blockade on ApEn with either no effect (Perkiomaki et al. 2001, Tulppo et al. 1996) or a reduction in ApEn (Penttila et al. 2001), i.e. a loss of complexity having been observed. However, the sympathetic dominance by head-up tilt has decreased corrected ApEn or SaEn (Porta et al. 2007) and tilt, handgrip and high-dose atropine have decreased the SymDyn En (Guzzetti et al. 2005). A low intensity exercise has increased ApEn and SaEn (Lewis et al. 2007, Tulppo et al. 2001).

Thus, at least part of the studies have related the complexity of short-term HR dynamics to cardiac autonomic regulation (Porta et al. 2007). In addition, a lowered breathing rate from 15 to 6 breaths per minute has decreased ApEn significantly (Penttila et al. 2003).

Symbolic dynamics measures

The symbolic dynamics analysis with three beats has been able to characterise the vagal and sympathetic activation alterations during typical experiments or pharmacological activations (Guzzetti et al. 2005). Thus, the sympathetic activation increases 0V and reduces 2V patterns, whereas the vagal activation causes the opposite. Interestingly, this analysis has been able to capture the more advanced role of parasympathetic withdrawal than sympathetic activation at low head-up tilt angles (Porta et al. 2007). Thus, this analysis is suitable for observing both concomitant and reciprocal though different magnitude alterations of parasympathetic and sympathetic regulation (Porta et al. 2007).

Kuusela et al. (Kuusela et al. 2002) have observed that terbutaline infusion could increase SymDyn FW possibly by uncoupling of arterial baroreflex function and diminishing parasympathetic modulation.

Correlations of short-term heart rate dynamics between each other

The correlations of short-term HR dynamics measurements between each other have been studied in very few studies. In long-term recordings, SDNN correlates strongly between all conventional HR variability measures and, in fact, the SDNN and square root of total power are expected to be nearly perfectly correlated due to their mathematical relationship

(Parseval's theorem) (Bilchick et al. 2006). A high correlation between these variables verifies that the editing and spectrum calculations had been performed reliably (Bigger et al. 1992). In long-term recordings, the r-MSSD and HF reflect virtually the same properties of the data (Bigger et al. 1992) and this relation is not expected to change in short-term recordings.

Kuo et al. (Kuo et al. 1999) studied the intrinsic correlations of 5-min frequency domain measures in 1070 middle-aged healthy persons lying down resting and breathing spontaneously. The method for spectrum calculation was fast Fourier transformation. They observed that none of the frequency domain measures correlated strongly with meanNN. However, lnTP correlated strongly with lnLF (0.83) and lnHF (0.78). LnLF and lnHF displayed a high correlation (0.73). Ln(LF/HF) did not have any strong correlations with absolute frequency domain measures.

There are very few studies evaluating the correlations of short-term non-linear HR dynamics between each other. Perkiömäki et al. (Perkiomaki et al. 2002), however, assessed these correlations in nine healthy subjects during 5-min rest with paced breathing. They observed that the DFA α_1 showed either strong or moderate (all $r<-0.51$) inverse correlations with SDNN, r-MSSD and HF power and positive correlations with HR ($r=0.76$). The ApEn, however, did not exhibit any significant correlations with the linear measures.

Maesti et al. (Maestri et al. 2007) evaluated the mutual correlations of a large number of different kinds of non-linear HR dynamics obtained from 24-h recordings in 200 patients with heart failure. They observed that there seemed to be redundancy among the variables, i.e. the parameters characterised the same physiological or pathophysiological properties. Nonetheless, they were able to obtain independent predictive information about heart failure with these new variables.

2.2 RELIABILITY AND STABILITY OF SHORT-TERM HEART RATE DYNAMICS

The reliability of a method assessing dynamical processes with large within- and between-subject variations such as HR dynamics is a demanding analytical task (Sandercock 2007). In addition, the terms and statistics for reliability analyses have varied and no consensus exists. Therefore, also the practical implications of the results have been lacking. However, recently there has been debate about these matters in relation to the short-term HR dynamics (Pinna et al. 2007, Sandercock 2007).

The reliability is defined as the consistency of repeated measurements, which in practice means that a method has that level of measurement error that can be accepted for practical use (Atkinson et al. 1998). The relative reliability concerns the ability of a method to consistently detect the differences between subjects and therefore, to rank accurately the subjects into certain order or group (Atkinson et al. 1998). The relative reliability assesses the proportion of the within-subject variability to between-subject variability and therefore, is always related to the homogeneity of the data (Atkinson et al. 1998, Pinna et al. 2007). The relative reliability is assessed with methods based on correlation coefficients such as intraclass correlation coefficient (ICC) (Atkinson et al. 1998, Pinna et al. 2007). The calculation of ICC, however, has been based on many formulae and the confidence

intervals (CI) have not necessarily been calculated in all publications. The absolute reliability defines the variability of the repeated measurements of an individual, i.e. within-subject variability (Atkinson et al. 1998, Hopkins 2000). The absolute reliability is assessed with methods such as standard error of measurement, coefficient of variation (CV) or limits of agreement (Atkinson et al. 1998). The results of these statistics can be quantified in actual units or as a proportion of the obtained values (Atkinson et al. 1998).

The measurement error can be divided into systematic bias and random error (Atkinson et al. 1998, Hopkins 2000). The systematic bias mirrors certain trends in the measurements e.g. those that might be attributable to factors such as learning effect (Atkinson et al. 1998, Hopkins 2000). The Bland-Altman method reveals this kind of systematic bias in a test-retest situation (Bland et al. 1986). The systematic bias is, however, normally smaller than the effect of random error (Atkinson et al. 1998). In an attempt to assess random error of HR dynamics, Pinna et al. (Pinna et al. 2007) suggested that the measurements should be made very close to each other. However, even in that situation, the constantly varying physiological state produces within-subject variation in the measurements. This physiological variation cannot be separated from the variation caused by technical factors. However, both physiological variability and technical errors can be assumed to limit the method's ability to discover real changes (Gluer et al. 1995). Previously, many studies have evaluated longer-term day-to-day within-subject variation. In this case, the term reliability is usually replaced with the term stability (Atkinson et al. 1998), or reproducibility.

Techical factors have a clear impact on the reliability of the HR dynamics analyses. Kroll et al. (Kroll et al. 1996) studied the interobserver reproducibility of 24-hour HR variability in 11 recordings containing varying amounts of atrial and ventricular ectopic beats; the interobserver reproducibility coefficient remained <8%. They observed that the differences in the recognition of supraventricular ectopic beats and sinus arrhythmia explained the observer-dependent differences. This factor has a greater effect on the high than on the low frequency measurements. Otherwise the interobserver reproducibility has been assessed in very few studies (Batten et al. 2000, Pardo et al. 1996) and these studies have concerned 24-h and not the short-term HR dynamics.

In the systematic review of the reliability of the short-term HR variability reported by Sandercock et al. (Sandercock 2007), the accurate assessments of the reliability were observed to have been few in number. The study of Sinnreich et al. (Sinnreich et al. 1998) is, however, one such study; they observed CVs to range from 6.0-12% for logarithmically transformed HR variability measurements during spontaneous and 6.1-10.7% during paced breathing in the supine position in 70 healthy volunteers with duplicate measurements made at a two month interval. In clinical populations, the reliability of short-term HR variability has remained at a lower level than in healthy persons (Lord et al. 2001, Salo et al. 1999). The reduced overall HR variability most likely accounts for the poor reliability encountered among patients (Sandercock et al. 2005). In addition, during interventions such as pharmacological stimulation or head-up tilt, the reliability of short-term HR variability is poorer than during rest (Cloarec-Blanchard et al. 1997).

Pinna et al. (Pinna et al. 2007) assessed the test-retest reliability of short-term HR variability measurements thoroughly. They assessed both absolute and relative reliability of 5-min supine recordings during spontaneous and paced breathing in 39 healthy subjects

on consecutive days. They observed large day-to-day random errors (95% limits of random variation): for the best measure, SDNN, the second measurement was 1.9/0.5 times the first one and for the worst measure, LF power, the ratio varied between 3.5/0.3. All the other measures, except meanNN, showed heteroscedasticity, i.e. the error increased with increasing measured values, and thus were logarithmically transformed before the analyses. The within-subject variability represented only a limited proportion of between-subject variability, and therefore, the ICCs were over 0.8 for most of the time and frequency domain measurements. They concluded that due to poor absolute reliability, the HR variability was not suitable for use in a test-retest assessment in individual subjects e.g. if one wished to observe treatment effects. However, due to the fairly good ICCs, the conventional HR variability measures are able to detect real average differences between groups of subjects. Maestri et al. (Maestri et al. 2010) performed comparative analyses among 61 patients who had experienced a previous AMI. The results were practically the same as those found in healthy persons in the study of Pinna et al (Pinna et al. 2007).

Maestri et al. (Maestri et al. 2007) assessed the reliability of the 5-min non-linear measurements in 42 healthy individuals on consecutive days with the same statistics as Pinna et al. (Pinna et al. 2007). With the DFA α_1 value a -0.45 to 0.45 change was needed to observe a real change in an individual and the ICC for DFA α_1 was 0.68. For sample entropy, the change in an individual needed was –0.59 to 0.59 and the ICC was 0.54. Thus, in many non-linear HR dynamics measurements, the absolute reliability was better than with conventional HR variability, which suggests that it may be more suitable for the assessment of the clinical state of an individual. However, the ICCs remained at a lower level.

One important issue for reliability studies are the practical applications of the results obtained. The researchers need to be able to judge whether the measurement is reliable enough for practical purposes (Atkinson et al. 1998). The requirements of reliability can differ extensively depending on the planned use of the measurement, e.g. comparison of changes within an individual patient versus identification of predictors of future cardiovascular events. Reliability calculations are needed if one wishes to estimate the correct sample size (Lehr 1992, Sandercock et al. 2005). However, the sample size estimation of HR rate dynamics is complicated by the large differences in the reported average values of HR dynamics between different studies and the lack of accepted normal values. This makes it difficult to assess any estimation of the expected effect sizes (Sandercock 2007).

2.3 EFFECT OF ECTOPIC BEATS AND EDITING METHODS ON SHORT-TERM HEART RATE DYNAMICS MEASUREMENTS

The beats of non-sinus origin confer major demands of both mathematical signal stationarity and physiological stability as prerequisities for HR dynamics analyses (Vybiral et al. 1990). These beats originate from latent pacemakers in the heart, the supraventricular ectopic beats most typically from pulmorary veins (Haissaguerre et al. 1998). The ectopic beats are usually premature and most often followed by a compensatory pause. Thus, there is a clearly visible artefact in the RR interval tachogram (Vybiral et al. 1990).

The ventricular ectopic beats lead to alterations also in the subsequent sinus rhythm behaviour (Schmidt et al. 1999). This phenomenon, named heart rate turbulence, includes a few interval decrease in the RR interval length after the compensatory pause followed by a more gradual increase of RR intervals before returning to the baseline HR level (Bauer et al. 2008). The physiological background to the immediate HR increase includes a baroreflex-mediated vagal inhibition due to ineffective ventricular contraction and a reduction of systolic blood pressure in relation to ventricular premature beats (Wichterle et al. 2006). The subsequent HR deceleration is caused by early sympathetic activation and an overshoot of arterial pressure, leading to vagal activation (Wichterle et al. 2006). After supraventricular premature beats, the behaviour is different due to sinus node resetting and the following recovery of sinus node automaticity (Bauer et al. 2008). However, both ventricular and supraventricular ectopic beats can affect the sinus rhythm behaviour for up to 20 consecutive beats (Schmidt et al. 1999).

The non-sinus beats often exist even in short-term recordings of healthy subjects as well as in patients with cardiac diseases such as CAD. Therefore, the inclusion of only ectopic-free segments in ECG recordings would cause a selection bias, especially if the autonomic nervous system was causally involved in the appearance of ectopic beats (Lippman et al. 1994). Furthermore, those subjects who display regular ectopic beats might be at a high risk of cardiovascular events and should not be excluded from the clinical studies (Huikuri et al. 1999). Therefore, the usual practice is to edit the ectopic beats with different methods. However, it is important for the accuracy of the HR dynamics method to evaluate both the effects of ectopic beats and the editing of ectopic beats on the HR dynamics measurements.

2.3.1 Effect of ectopic beats

The strong effect of non-sinus beats on HR variability measurements has been demonstrated in several studies. In short recordings of 128 beat duration, even a single ectopic beat has influenced both time and frequency domain analyses (Vybiral et al. 1990). The analysis of HF power was especially distorted: HF obtained values of 588-976% from the original. The length of the coupling interval modulated the effect such that a shorter coupling interval caused a larger error. Berntson and Stowell (Berntson et al. 1998) added both missed beats and spurious R-wave detections to the RR interval time series of 128 beats. They observed that even a single misidentification could dramatically increase power in all frequency bands. The effect of missed beats was larger than that of extra beats. The effects of these artefacts were observed to be so powerful that they could overwhelm the true physiological variability. Clifford and Tarrassenko (Clifford et al. 2005) added one to two ectopic beats with different coupling intervals to a 5-min section of a realistic, though artificial, RR interval tachogram. They noted that the shorter coupling interval was related to increasing values of HF and to decreasing values of LF power. Furthermore, when the number of ectopic beats increased from one to two, an increase in HF and a decrease in LF power were observed. They concluded that the removal of these non-sinus beats would be essential before conducting the spectral analyses.

The effects of non-sinus beats on non-linear HR dynamics have not been extensively studied. However, the DFA analysis is related to spectral analysis such that the effects of ectopic beats on spectral analysis that are previously described most likely also will impact

on the DFA analysis. Peltola et al. (Peltola et al. 2004) added premature beats to recordings of 8000 beat duration and observed that even a small number of premature beats significantly reduced the DFA α_1 values. The baseline HR variability modulated the effect, i.e. there was a more marked effect of ectopic beats seen in relation to low baseline HR variability: e.g. 0.25% of premature beats (20% prematurity) caused an over 25% decrease in DFA α_1 in the recordings of patients with prior AMI whereas in recordings from healthy patients 4% of premature beats were needed to detect such an effect. Vikman et al. (Vikman et al. 1999) added increasing number of short and consecutive long RR intervals, mimicking premature beats, to both real and artificial data. They used both a constant coupling interval of 500 ms and variable coupling intervals from 350 to 800 ms. The DFA α_1 decreased in relation to both constant and variable coupling intervals. The behaviour of ApEn, however, varied with different coupling intervals. With a fixed coupling interval, a constant decrease in ApEn was observed when the number of premature beats increased. However, an unexpected increase in ApEn was observed in relation to very few premature beats. In addition, with variable coupling intervals, the ApEn increased in conjuction with an increasing number of premature beats.

2.3.2 Effect of different editing methods

Due to the severe distortion of the analysis of HR dynamics by the non-sinus beats, there is a need for accurate detection and handling of the ectopic beats. The detection has classically been based on the different morphology of a ventricular QRS complex or an ectopic P-wave or on prematurity in the non-sinus beats (Acar et al. 2000). The arbitrary prematurity limits might, however, exclude normal respiration related variability or alternatively, the non-sinus beats with long coupling intervals might not be recognised and edited (Huikuri et al. 1999). Furthermore, high-risk patients often exhibit sudden changes in the RR interval tachogram that are falsely defined as non-sinus beats (Makikallio et al. 1997). Due to these difficulties, newer mathematical methods are constantly being developed for the detection and correction for these non-sinus beats (Ince et al. 2009).

The geometrical HR dynamic analyses such as the return map offers special benefits in that they display the ectopic beats in a visual manner, i.e. clusters of points generated by non-sinus beats (Brennan et al. 2001) (Figure 3). It is also possible to use the return map-analysis after editing to assess how effective the editing process has been and also, to exclude the distortion of the data due to the editing process (Brennan et al. 2001).

In practice, a certain number of non-sinus beats in the recordings can be tolerated and the RR intervals related to such beats are replaced with different editing methods. Most commonly with the time domain methods, the premature beat related intervals are removed whereas before conducting a spectral analysis the gaps caused by these removed intervals are replaced by means of various methods (Huikuri et al. 1999).

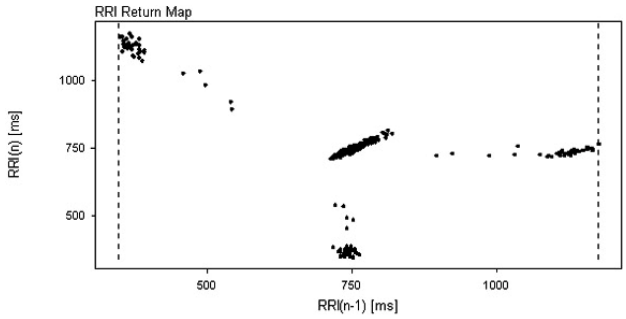


Figure 3a. Return map from 10-min recording during standing. The premature beats (4.5% ventricular and 0.2% supraventricular from all beats) have been left unedited

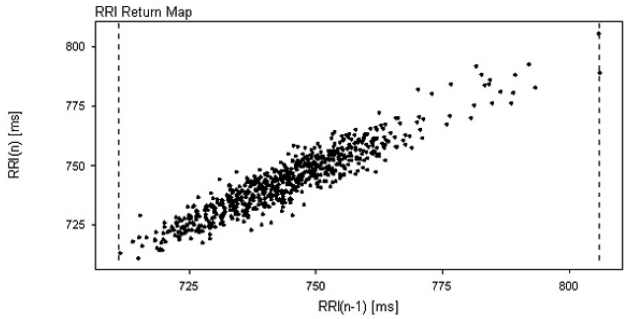


Figure 3b. The same recording as in 3a, but the non-sinus beats have been removed

Lippman et al. (Lippman et al. 1994) evaluated the effects of three editing methods on 5-min recordings with different amounts of added simulated ventricular beats (12-120/h). These beats had caused an overestimation of all HR variability measures, most importantly in short-term variability such as HF power (up to 501% error). The editing methods included the deletion of the RR interval preceding and following the ventricular beats, interpolation with linear or cubic spline method and nonlinear predictive interpolation. With interpolation methods, the number of inserted RR-intervals to replace the ectopy related intervals was assessed first on the basis of ectopy related and sinus intervals preceding and following the ectopic beats. Then, the length of the RR intervals was calculated on the basis of one sinus interval before and after the ectopy by using linear interpolation or on the basis of two preceding and two following sinus RR intervals with cubic splines. Nonlinear predictive interpolation is a method created by these authors to replace the removed RR intervals on the basis of comparisons within the whole ectopy-free data. Lippman et al. found that with time domain methods, all of the editing methods were able to perform reliably, e.g. with variance the error was <2% and with r-MSSD it was <4% when compared to measurements from ectopy-free data. With spectral analysis, the linear and cubic spline interpolation methods evoked significant overestimation of LF and underestimation of HF power when the number of ectopic beats was high. Although the deletion method causes a phase shift, this straightforward method performed as well as or even better than more complicated methods: the errors remained <4%. Lippman et al. also

calculated the CVs between the corrected and original ectopy-free measurements. They observed the time domain measurements to be reliable after correction for non-sinus beats (CVs under 4% even in the presence of high numbers of ectopic beats) whereas in relation to spectral measurements, the CVs exhibited values as high as 23%. Therefore, it was stated that a varying number of ectopic beats in consecutive spectral measurements of an individual would considerably worsen the stability of these analyses.

Salo et al. (Salo et al. 2001) studied the effect of three editing methods on short-term recordings with purely sinus rhythm. The editing methods included deletion, i.e. the removal of RR intervals, interpolation so that the removed intervals were replaced with the average of previous three normal RR intervals (interpolation of degree zero) or with values obtained from a fitted straight line over removable RR intervals (interpolation to degree one). They observed that SDNN was insensitive to a large amount of editing, i.e. up to 30% with any of the methods both in healthy and post-AMI patients did not lead to error values over 5%. The deletion method performed best in this context. The r-MSSD and HF power were highly sensitive to editing with any of the methods. However, the deletion method disturbed these analyses most severely, especially in recordings from healthy individuals. The LF power was also remarkably affected by the editing. In an estimation of the evaluated editing methods, it seemed that the deletion method caused sudden changes in RR interval timeseries, which artificially shortened the wavelength of the oscillations in the signal and increased false HF fluctuations. The interpolation in turn conferred a low pass filtering effect on the data. They concluded that no method was ideal both for time and frequency domain analyses.

Since widely used interpolation methods perform reliably only when the number of ectopic beats is low, new methods have appeared to solve this problem. The Lomb-Scargle periodogram that does not require resampling of uneven RR interval tachogram is not disturbed by even a large amount of removed ectopic beats (Clifford et al. 2005). Other postulated methods are related to the heart timing signal (Mateo et al. 2003, Solem et al. 2006) or use of an adaptive point process algorithm (Barbieri et al. 2006).

Very few studies have compared different editing methods in relation to non-linear HR dynamics. Peltola et al. (Peltola et al. 2004), however, edited the added premature beats before the analysis of DFA with comparable methods as used in the study of Salo et al. (Salo et al. 2001), i.e. deletion, interpolation of degree zero or one and also included cubic spline interpolation. They noted that the interpolation of degree zero achieved the most accurate estimation of the DFA α_1 value. In recordings with high baseline HR variability, the deletion method performed rather well, but in relation to low HR variability, it conferred more error and to the other direction than in high HR variability recordings. Peltola et al. explained this finding by postulating that the deletion of intervals had increased the roughness of the signal when the baseline HR variability was high (decreased DFA α_1) but produced a smoothening effect when the baseline HR variability was low (increased DFA α_1). Nonetheless, with all the editing methods, the error in DFA α_1 remained under ±0.1 when the proportion of premature beats was under or equal to 20%. Thus, the differences between the editing methods remained rather unremarkable, especially when compared to the effect of non-edited premature beats on DFA α_1.

2.4 ACUTE AIR POLLUTION AND SHORT-TERM HEART RATE DYNAMICS

2.4.1 Epidemiology of acute air pollution related to cardiovascular diseases

The American Heart Association (AHA) issued a statement for healthcare professionals in 2004 followed by an update in 2010 on the observed associations between both long-term and acute air pollution exposure and cardiovascular diseases (Brook et al. 2004). The aim was to draw attention to air pollution as an insidious risk factor for cardiovascular events.

The ambient air pollution consists of gaseous pollutants and particulate matter (PM) suspended to the air. The major gaseous pollutants linked to cardiovascular toxicity include carbon monoxide (CO), nitrogen oxides, sulphur dioxide (SO_2) and ozone (Brook et al. 2004). The PM, that is an extensively varying mixture of chemical and organic compounds from different sources (Pope et al. 2006) is, however, regarded as the most harmful component of air pollution with respect to cardiovascular health (Mills et al. 2009). The size of the PM varies from ultrafine particles <0.1 μm to particles up to 100 μm (Brook et al. 2010, Timonen 2005). Larger particles remain in the upper respiratory tract, while particles smaller than 2.5 μm ($PM_{2.5}$) are able to reach lung alveoli (Bhatnagar 2006). Ultrafine particles might be able to penetrate to the circulatory system in the same way as gases (Nemmar et al. 2002). The PM and gaseous pollutants except ozone, correlate with each other and have been claimed to contribute to the effects of co-pollutants (Samet et al. 2000, von Klot et al. 2005). In the industrial world, both gaseous pollutants - except ozone - and fine particles mostly derive from the combustion of fossil fuels (Brook et al. 2004).

The day-to-day variations in daily concentrations of ambient air pollution have been consistently related to cardiac mortality and morbidity. In two large studies: the National Mortality, Morbidity, Air Pollution Study (NMMAPS) in United States and the Air Pollution and Health: a European Approach (APHEA2), the short-term effects of air pollution on mortality were evaluated in more than 100 million inhabitants (Analitis et al. 2006, Dominici et al. 2005, Katsouyanni et al. 2001). In a combined analysis from these studies and including some Canadian studies, the mortality increased 0.2-0.6% for every 10-ug/m^3 elevation in daily ambient particles smaller than 10 μm (PM_{10}); in elderly subjects the effects were greater for cardiovascular than for overall or pulmonary mortality (Samoli et al. 2008). Interestingly, there was no lower-limit threshold for the effect of PM_{10}. The effect of short-term air pollution specifically on cardiovascular mortality has been demonstated in a large number of other epidemiological studies as well (Anderson et al. 2005). Similar results have been observed also in Finland (Halonen et al. 2009, Halonen et al. 2010). The increased number of hospital admissions for cardiovascular diseases such as ischemic events, arrhythmias or heart failure due to temporarily higher ambient PM and/or gaseous pollutant levels has been shown as well (Ballester et al. 2006, D'Ippoliti et al. 2003, Dominici et al. 2006, Lanki et al. 2006, Schwartz 1999, Wellenius et al. 2005); the relationship seems to be especially strong with respect to ischemic heart disease (Brook et al. 2010). The associations between daily variations in several pollutants and the incidence of AMI were summarized in a recent review (Bhaskaran et al. 2009). Particulate air pollution has been related to ST-depression among patients with CAD in ambulatory recordings (Pekkanen et al. 2002). Furthermore, studies on patients with implantable cardioverter defibrillator devices have detected associations between daily increased air pollution levels and the risk

of ventricular tachyarrhythmias (Peters et al. 2000, Rich et al. 2005), though some studies have reported negative results in this respect (Anderson et al. 2010, Metzger et al. 2007).

The air pollution exposure seems to trigger cardiac events also in a more acute manner. The higher concentrations of $PM_{2.5}$ enhanced the risk of the onset of AMI not only within one day but also within a two-hour period (Peters et al. 2001). A transient stay in traffic has also been related to the onset of AMI (Peters et al. 2004). However, Sullivan et al. (Sullivan et al. 2005) detected only a weak association with hourly increases in several pollutants ($PM_{2.5}$, CO and sulfur dioxide) and the onset of AMI. In another study, an increased number of MI deaths were observed within a few hours after being exposed to high concentrations of suspended PM (Murakami et al. 2006). A higher number of ventricular arrhythmias in implantable cardioverter defibrillator patients has been observed to occur within two hours from higher PM_{10} outdoor levels (Ljungman et al. 2008). Mills et al. (Mills et al. 2007) compared the effects of a controlled one-hour dilute diesel exhaust exposure and clean air breathing in patients with previous AMI but who at that particular time had stable CAD. They observed deeper ST-depressions during moderate exercise attributable to exposure when compared to the situation with breathing clean air. Thus, even a relatively brief exposure to traffic might predispose an individual to myocardial ischemia.

Subjects who are susceptible to acute air pollution include the elderly (Samoli et al. 2008) and individuals with existing cardiovascular disease (Pope et al. 2006, von Klot et al. 2005), chronic obstructive pulmonary disease (Rich et al. 2010, Zanobetti et al. 2005) and diabetes mellitus (O'Neill et al. 2005). Some studies have indicated that diabetics suffering from concomitant cardiovascular disease would be especially susceptible (Goldberg et al. 2006), however, some others have observed a smaller impact of diabetes (Rich et al. 2010, Zanobetti et al. 2005).

2.4.2 Air pollution and short-term heart rate dynamics

There are several pathophysiological pathways which may be involved in the cardiovascular effects of air pollution, especially PM exposure (Brook 2008). First, pulmonary inflammation (Ghio et al. 2001) induces systemic inflammation (Ruckerl et al. 2007) and possibly oxidative stress (Chuang et al. 2007, Delfino et al. 2008). Secondly, inflammation and irritation of airways and lungs activates the ANS reflex archs and thus, modifies also cardiac autonomic regulation (Brook et al. 2010, Timonen 2005). Thirdly, pollutants capable of being translocated to the circulatory system are able to directly affect the vasculature and myocardium (Nemmar et al. 2002). These cascades are believed to promote endothelial dysfunction (O'Neill et al. 2005, Schneider et al. 2008), cause arterial vasoconstriction (Brook et al. 2002, Peretz et al. 2008) evoke thrombogenetic changes in blood (Baccarelli et al. 2007, Pekkanen et al. 2002), lead to instability of atherosclerotic plaques (Mills et al. 2007), increase the blood pressure (Dvonch et al. 2009, Zanobetti et al. 2004) and evoke alterations in HR and HR variability (Devlin et al. 2003, Gold et al. 2000, Peters et al. 1999). These reactions could predispose an individual to acute cardiac events such as myocardial ischemia and arrhythmias but possibly also to acceleration of atherosclerosis (Bauer et al. 2010, Hoffmann et al. 2007, Kunzli et al. 2005, Mills et al. 2007).

The alteration in cardiac autonomic regulation is one central hypothesis which could, at least in part, explain the cardiovascular effects of air pollution (Brook et al. 2010). The

response most suspected to be involved is the sympathetic predominance that could account for the susceptibility to cardiac events via increased blood pressure and cardiac work load, altered coronary tone and higher risk of plaque rupture predisposing to myocardial ischemia or electrical instability triggering arrhythmias (Brook et al. 2004). However, parasympathetic activity might also be important in generating electrical instability in heart (Chen et al. 2007). However, the increase in HR and/or declines in short-term HR variability have been the most common findings in relation to increased day-to-day air pollution, mostly PM, among both the general population and the elderly (Adar et al. 2007, Creason et al. 2001, Holguin et al. 2003, Liao et al. 1999, Luttmann-Gibson et al. 2006, Park et al. 2005, Peters et al. 1999, Schwartz 1999). The effect of air pollution has been studied also on an hourly basis either using fixed or personal monitors. It has been observed that increased air pollution levels for one or a few hours during the analysis of HR variability are related to diminished cardiac autonomic regulation in healthy subjects as well as elderly and in patients with cardiovascular or pulmonary disease (Chan et al. 2004, Chang et al. 2007, Gold et al. 2000, Lipsett et al. 2006, Min et al. 2008, Vallejo et al. 2006, Wu et al. 2010, Zanobetti et al 2010). In some studies, however, the effects of daily or hourly variations in air pollution such as fine particles, on HR dynamics have been missing or completely or partly opposite, i.e. increased HR variability mirroring the vagal activation has been observed (Pope et al. 2006, Sullivan et al. 2005). Furthermore, though statistically significant, the findings in HR variability have been minor and their relevance to cardiovascular health is unknown. The acute effects of air pollution have been evaluated also with human exposure studies. A 2 h exposure to PM caused an immediate response of decreased time and frequency domain measurements in elderly subjects (Devlin et al. 2003), providing support for the proposal of sympathetic stress response in relation to air pollution. However, in two diesel exhaust exposure studies, there were no significant effects on HR variability in either patients with CAD or healthy subjects (Mills et al. 2011, Peretz et al. 2008). In fact, in the study of Peretz et al., there was a trend towards increased HF power in the healthy subjects.

The findings with different time-intervals may provide some clues that the effects of air pollution on the autonomic nervous system are activated both by fast neural mechanisms and via more delayed pathways, such as oxidative stress and inflammation. In fact, the relationship between air pollution and HR variability is strongly modified by the presence of obesity or metabolic syndrome as well as genes modulating endogenous oxidative stress and, on the other hand, by the protective factors reducing oxidative stress such as therapy with statins or dietary antioxidants (Baccarelli et al. 2008, Chahine et al. 2007, Chen et al. 2007, Park et al. 2005, Park et al. 2010, Romieu et al. 2005, Schwartz 1999).

Some studies have related changes in the inflammatory or thrombogenic components in the blood to alterations in HR variability (Graff et al. 2009, Pope et al. 2006, Samet et al. 2009, Yeatts et al. 2007). In the study of Chuang et al. (Chuang et al. 2007) done in healthy students, a higher daily ambient air pollution caused simultaneous formation of markers of inflammation and oxidative stress, prothrombotic changes and a reduction in HR variability. Among healthy policemen, the in-vehicle exposure to $PM_{2.5}$ enhanced the inflammatory and prothrombotic markers in blood as well, but caused an opposite finding to the cardiac autonomic regulation, i.e. a decrease in HR and increases in HR variability

few hours after the exposure (Riediker et al. 2004). Thus, it seems clear that the effects of acute variations in air pollution on cardiac autonomic regulation are integrated with other pathophysiological pathways which may explain the effects of ambient air pollution.

Baseline health seems to modulate responses to air pollution. Those few studies which have detected increases in HR variability have been performed in young healthy subjects (Peretz et al. 2008, Riediker et al. 2004), whereas in patients with cardiovascular diseases or diabetes, the finding has consistently been an attenuation in HR variability (Chuang et al. 2007, Lipsett et al. 2006, Schneider et al. 2010, Wheeler et al. 2006, Whitsel et al. 2009, Zanobetti et al. 2010). Medication might be one factor alternating the effects of air pollution, e.g. beta-blockers seem to protect for some effects of the pollutants (Folino et al. 2009). The different pollutants as well as varying components of PM might provoke different pathophysiological mechanisms with varying time-intervals (Chuang et al. 2005). In an occupational setting, e.g., Magari et al. (Magari et al. 2002) observed increased short-term SDNN in relation to some specific metal components of PM, whereas the main influence of PM was a decrease in SDNN (Magari et al. 2001).

The acute effects of ambient air pollution on HR dynamics have been shown in several studies and they are likely to explain part of the acute cardiovascular effects of air pollution. On the other hand, the long-term importance to cardiac health of such short-term changes in HR dynamics still remains unclear. In addition, the details of the pathophysiological mechanisms responsible for the effects of air pollution and the identification of the most harmful components in the air pollutants need more clarification. When one considers the serious effects of air pollution, this is clearly a major challenge for medical research and policymakers.

2.5 ATRIAL FIBRILLION AFTER CARDIAC SURGERY AND SHORT-TERM HEART RATE DYNAMICS

Atrial fibrillation (AF) is most frequent complication of coronary artery by-pass grafting (CABG) affecting around one third of patients (Attaran et al. 2011, Mathew et al. 2004). The incidence of postoperative AF after cardiac surgery has remained constant despite advances in anaesthetic and surgical procedures. The problem most likely is attributable to the aging of the patient population (Mathew et al. 2004).

Postoperative AF is often regarded as a short-lived and self-limiting consequence of cardiac surgery. It occurs most often during the second postoperative day and mostly converts to sinus rhythm during the following postoperative weeks (Mathew et al. 2004). However, it leads to a higher risk of perioperative mortality and morbidity (Mathew et al. 2004, Villareal et al. 2004). The risk of thromboembolic complications such as stroke is elevated three-fold (Attaran et al. 2011, Creswell et al. 1993, Lahtinen et al. 2004). Thus, postoperative AF after CABG creates a need for additional treatment and lengthens the hospital stay and, therefore, increases the cost of the surgical procedure (Mathew et al. 2004, Villareal et al. 2004). Although transient in nature, the postoperative AF seems to have an independent effect also on long-term mortality after CABG (Attaran et al. 2011, El-Chami et al. 2010, Villareal et al. 2004). There is, however, some controversy about whether

the reduced survival is explained by the complications of postoperative AF or whether it actually associates with more severe pre-existing cardiac disease.

The prevention of postoperative AF after CABG includes therapy with beta-blockers and in high-risk patients with amiodarone (Echahidi et al. 2008, Halonen et al. 2006). Biatrial pacing can effectively reduce the occurrence of postoperative AF, but its complexity limits its applicability (Burgess et al. 2006). Moreover, the anti-inflammatory agents such as corticosteroids as well as statins and polyunsaturated fatty acids and even magnesium have been claimed to provide extra benefits (Calo et al. 2005, Halonen et al. 2007, Miller et al. 2005, Patti et al. 2006). However, despite all efforts, postoperative AF remains a serious complication of cardiac surgery and the factors predisposing to this state are only partly understood (Attaran et al. 2011).

2.5.1 Pathophysiological mechanisms of postoperative atrial fibrillation

The major clinical risk factors for postoperative AF after cardiac surgery include previous episodes of AF, advanced age, left atrial enlargement and valvular surgery (Echahidi et al. 2008, Hakala et al. 2002, Mathew et al. 2004, Osranek et al. 2006). There are, however, also new recognised clinical risk factors such as visceral obesity and metabolic syndrome (Echahidi et al. 2007, Zacharias et al. 2005). The roles of the inflammatory response, oxidative stress or genetic differences as predisposing factors have been a subject for intense research recently (Gaudino et al. 2003, Ramlawi et al. 2007).

In particular, the aging causes degenerative and inflammatory modifications in atrial anatomy such as dilation, fibrosis or lipid deposition (Mariscalco et al. 2006). An additional anatomical substrate for postoperative AF consists of several intra- and postoperative factors such as operative trauma or atrial ischemia (Echahidi et al. 2008). These kinds of structural changes are associated with altered electrophysiological states (Echahidi et al. 2008, Hogue et al. 2005). The shortening of the atrial refractory period and decreased conduction velocity are two key factors in atrial electrophysiology, shortening the wavelength for re-entry (Allessie et al. 2001). In addition, the dispersed atrial refractoriness facilitates the existence of the multiple re-entry depolarisation wavelets needed to initiate and maintain the AF (Moe et al. 1959). When an arrhythmogenic substrate is present, an atrial premature beat arising typically from pulmory veins most often serves as a trigger, initiating the postoperative AF (Po et al. 2005).

2.5.2 Prediction of postoperative atrial fibrillation

Several algorithms combining pre-, intra- and/or early postoperative indicators have been developed to predict postoperative AF after cardiac surgery (Hakala et al. 2002, Magee et al. 2007, Mathew et al. 2004, Osranek et al. 2006). The predictive capability of these algorithms has, however, remained poor. In the multicenter study of Mathew et al. (Mathew et al. 2004) the preoperative factors in a model included age, previous AF episodes, chronic pulmonary disease, valve surgery, withdrawal of beta-blockers or angiotensin converting enzyme inhibitors combined with a few intra- and postoperative factors, whereas the study of Osranek et al. (Osranek et al. 2006) included only preoperative left atrial enlargement and advanced age. These studies provided comparable predictive powers: the area under receiver operating characteristic curve was 0.77 in both studies.

Other investigators have attempted to capture the altered electrophysiological state with the analysis of signal-averaged P-wave analysis and combined these measures with clinical risk factors, however, with no better success (Zaman et al. 2000). Thus, the characterisation of high-risk individuals for postoperative AF remains a challenge.

2.5.3 Postoperative atrial fibrillation and short-term heart rate dynamics

Clinical and experimental studies as well as studies utilising HR dynamics have indicated that autonomic activation has an impact on the initiation of both paroxysmal and postoperative AF. The autonomic activity modifies atrial electrophysiological properties in a way that predisposes to AF; both sympathetic and vagal nervous systems seem to be involved (Chen et al. 2007).

Clinical studies

Coumel (Coumel 1994) was the first to emphasise the role of cardiac autonomic regulation in the initiation of AF. He described the vagally mediated AF occurring among healthy, young males during sleep, after meals or alcohol consumption, and which terminated spontaneously. The sympathetically mediated AF was postulated to occur during stress in older patients with structural heart diseases. However, Coumel also emphasised that there might be interactions between the two limbs of ANS.

The AF after cardiac surgery could be considered attributable to sympathetic activation both in relation to the underlying severe heart disease and operative stress. This is supported by the observation that beta-blockers reduce the occurrence of postoperative AF. In fact, continuation of beta-blocker therapy is an established routine after CABG (Echahidi et al. 2008). However, the preventive effect of beta-blocker medication is somewhat limited: postoperative AF occurred in 17% of patients despite intravenous metoprolol administration (Halonen et al. 2006).

The targeted vagal denervation in relation to pulmonary vein isolation has been postulated as a new way to treat AF (Pappone et al. 2004) but the results have been conflicting. Therefore, new methods targeting the cardiac autonomic regulation such as low-level electrical stimulation of vago-sympathetic trunks are constantly being developed (Scherlag et al. 2011), but their effectiveness remains to be clarified.

Experimental studies

Sharifov et al. (Sharifov et al. 2004) studied dogs with rapid chronic pacing and noted that a direct infusion of adrenergic agents into the sinus node artery induced AF in 21% of dogs. However, with acetylcholine infusion, the inducibility was elevated to 100%. Interestingly, in combination with sympathetic agents, the threshold for acetylcholine to induce AF was lowered. Therefore, it was postulated that most likely a dual sympathovagal discharge could be most profibrillatory.

In dogs, the straight stimulation of autonomic ganglia in the heart has been shown to convert pulmonary focal ectopies into AF (Scherlag et al. 2005). New findings have shown that the stimulation of intrinsic cardiac autonomic ganglia can induce pulmonary vein ectopy and atrial fibrillation also in humans (Lim et al. 2011).

Tan et al. (Tan et al. 2008) provided completely new information with a method to monitor the left stellate ganglion and vagal nerve activity in ambulatory dogs. First they induced paroxysmal AF and atrial tachycardia episodes with intermittent rapid left atrial pacing over several weeks. Thereafter, there was a concomitant sympathovagal activity, which preceded the onset of atrial arrhythmias in 73% of episodes. In contrast, sinus tachycardia was preceded with sympathetic activation and vagal withdrawal. The causality between concomitant sympathovagal excitation and AF was shown when the ablation of extrinsic cardiac sympathovagal nerves completely abolished the episodes of paroxysmal AF. The cryoablation, however, did not prevent the development of sustained AF, i.e. the cardiac autonomic nerve activity was not the only determinant of AF. However, the role of concurrent sympathetic and vagal activation as a specific triggering factor for paroxysmal AF was shown convincingly.

The mechanisms of sympathovagal activation have been related to the vagal stimulation reducing the conduction velocity in atrial tissue and shortening the atrial effective refractory period nonuniformly and, thus, increasing the dispersion of the effective refractory period, whereas sympathetic stimulation promotes ectopic beat activity and also might abbreviate the atrial refractory period, although uniformly (Liu et al. 1997, Oliveira et al. 2011). There are, however, new hypotheses relating the sympathovagal activation to an abbreviated atrial action potential duration, i.e. late phase three early afterdepolarisations and activity triggered due to prolonged intracellular calcium transient (Burashnikov et al. 2003, Patterson et al. 2006).

HR dynamics and atrial fibrillation

HR dynamics measurements have been used to indirectly assess the cardiac autonomic regulation before the initiation of both paroxysmal and postoperative AF. There have been some findings pointing to a vagal prevalence preceding the initiation of paroxysmal AF (Herweg et al. 1998, Vincenti et al. 2006). However, in many studies it has not been possible to designate HR variability as being either vagally or sympathetically driven; both types have been recognised among subjects (Fioranelli et al. 1999, Huang et al. 1998, Lombardi et al. 2004, Tomita et al. 2003). In the postoperative situation, the findings have been suggestive of sympathetic activation (Dimmer et al. 1998). However, there are also findings indicative of concomitant sympathovagal activation. An increase in HR mirroring the enhanced sympathetic activity combined with an increase in HR variability, reflecting a concomitant vagal resurgence has been observed before the onset of both paroxysmal and postoperative AF (Amar et al. 2003, Bettoni et al. 2002).

Vikman et al. (Vikman et al. 1999) evaluated non-linear HR dynamics during six 20-minute periods before paroxysmal AF episodes in patients without structural heart disease. Traditional time and frequency domain HR variability measures did not reveal any significant changes before the onset of AF. However, a progressive decrease occurred both in ApEn and DFA α_1. Shin et al. (Shin et al. 2006) also reported a reduction in ApEn and SaEn before the onset of AF. In addition, they observed a trend towards short-term decrease in the DFA α_1 value in patients having transiently increased HF and decreased LF/HF ratio, i.e. a vagal enhancement, before the initiation of paroxysmal AF. In patients with transiently decreased HF and an increased LF/HF ratio, i.e. a finding indicative of

sympathetic predominance, the DFA α_1 showed a trend to increase transiently. In the postoperative situation, Hogue et al. (Hogue et al. 1998) performed three 20-minute HR dynamics analyses before the onset of 24 episodes of postoperative AF in 18 patients. They observed a transient increase in HR and a decrease in ApEn preceding the initiation of AF. In traditional HR variability evaluations, the findings were not consistent since both increased and decreased HR variability were seen. Therefore, a reduced complexity seems to be the most typical finding shortly before the initiation of AF in these studies.

The relationships between cardiac autonomic regulation and AF have been studied also with methods other than HR dynamics such as HR turbulence after atrial premature beats (Vikman et al. 2005) or HR recovery after exercise (Maddox et al. 2009). The turbulence method has the advantage that it is not disturbed by the various editing practices used to eliminate non-sinus beats. The finding in the study of Vikman et al. was suggestive of transient enhancement of a parasympathetic regulation preceding the initiation of AF. The HR recovery after exercise has been reported to be blunted, which indicates that an autonomic dysfunction could be a predisposing factor to AF (Maddox et al. 2009).

The short-term HR dynamics could have a role as a part of the algorithms to identify those patients with high-risk for postoperative AF though few studies have examined this problem. Hakala et al. (Hakala et al. 2002) utilised short-term HR variability one-day before CABG, but these measurements did not identify the patients who would develop postoperative AF. The increased preoperative time domain measures in 24-h recordings, however, independently predicted the occurrence of post-operative AF (Kinoshita et al. 2011). Furthermore, the study of Chamchad et al. (Chamchad et al. 2006) suggested that the peak point correlation dimension from 10-min ECG recordings could predict the postoperative AF with OR 3.95/unit, p=0.096. The predictive capabilities of other preoperative short-term HR dynamics measurements are not known. However, if there were precise tools for risk-stratification, the available therapeutic preventive strategies could be accurately targeted to those susceptible patients likely to develop postoperative AF after cardiac surgery.

3 Aims of the study

The purpose of the present study was to investigate the methodological aspects of the short-term measurements of HR dynamics and to evaluate their applicability in two novel situations. The specific aims of the individual studies were to evaluate:

1. stability over time of short-term heart rate dynamics in subjects with stable coronary artery disease (Studies I and II)
2. effects of non-sinus beats and different editing methods for handling non-sinus beats on short-term non-linear heart rate dynamics (Study II)
3. whether there is an association between acute carbon monoxide exposure and short-term heart rate variability in subjects with stable coronary artery disease (Study III)
4. whether the preoperative non-linear heart rate dynamics predict the occurrence of postoperative atrial fibrillation after coronary artery by-pass grafting (Study IV)

4 Subjects and methods

4.1 STUDY DESIGN AND SUBJECTS

4.1.1 Heart rate dynamics in stable coronary artery disease (Studies I and II)

The data for studying the HR dynamics among elderly patients with stable CAD were obtained in the framework of the ULTRA (Exposure and risk assessment for fine and ultrafine particles in ambient air) study, which was a multi-center study investigating the effects of exposure to ambient air particulate matter of different sizes and chemical compositions in patients with stable CAD. The methods used in the ULTRA study have been described in detail elsewhere (Pekkanen et al. 2000).

The ULTRA study was a prospective study carried out during the winter of 1998 to 1999 in three study centers in Amsterdam in the Netherlands, Erfurt in Germany and Helsinki in Finland. The inclusion criteria were self-reported physician diagnosed CAD (e.g. a history of typical angina pectoris, previous myocardial infarction, percutaneous coronary intervention or coronary artery by-pass surgery), non-smoking, age >50 years and ability to perform the study protocol. The exclusion criteria included a recent, i.e. less than three months cardiac event such as myocardial infarction, stroke or by-pass surgery, unstable angina, a cardiac pacemaker, type I diabetes or poor co-operation. The subjects were asked to fill in a detailed questionnaire.

The study design included a follow-up for six to eight months with clinical visits made every two weeks. The visits were on the same weekday and at the same time of the day for every subject whenever possible. An ambulatory ECG recording during a standardised 40-minute protocol consisting of 5-minute periods of rest, paced breathing and standing, a 6-minute submaximal exercise and a 10-minute recovery was performed during the visits. During the rest and recovery periods, the subjects were in the supine position breathing freely. During the paced breathing period, the subjects were also in the supine position, but were asked to breathe at a frequency of 0.2 Hz (5 s breathing cycles) in time with a metronome. The exercise was performed with a bicycle ergometer; the HR was aimed to be 90-100 bpm for five minutes.

There was a total of 131 subjects with stable CAD. The characteristics of the subjects are described in Table 1. Their medication was not changed for the visits. During the study period, the medication use was mostly stable, i.e. in only 14 of the 131 subjects the medication was changed or stopped during the study period.

Table 1. Characteristics of the 131 subjects with stable coronary artery disease

	No	Range	%
Sex			
Male	91		69
Mean age (years)	68	(40-84)	
Angina pectoris	80		61
CCS criteria			
CCS I	54		41
CCS II	47		36
CCS III	7		5
CCS IV	3		2
Past AMI	86		66
CABG/PCI	74		56
Q-wave evidence of AMI*	33		26
Diabetes mellitus	14		11
COPD or asthma	10		8
Daily medication			
Beta-blockers	79		60
Calcium antagonist	42		32
Aspirin	94		72
Nitroglycerin	43		33
ACE-inhibitor or AT-blocker	47		36
Digitalis	18		14
Inhaled beta-agonist	6		5

CCS I Ordinary physical activity does not cause angina;
CCS II Slight limitation of ordinary activity due to angina;
CCS III Marked limitation of ordinary physical activity due to angina;
CCS IV Inability to carry on any physical acitivity without discomfort, anginal syndrome may be present at rest (Campeau 1976); COPD, chronic obstructive pulmonary disease; ACE, angiotensin-converting enzyme; AT, angiotensin receptor; *Both diagnostic (1-1-x) and major criteria (1-2-x except 1-2-6 and 1-2-8) in Minnesota coding (Prineas *et al.* 1982)

For the analyses of the stability over time of conventional HR variability (Study I), the subjects were selected who had at least seven HR variability measurements during the first nine consecutive visits. With this selection criterion, the evaluated time period became three to four months. There were 21 female and 68 male subjects in the 89 subjects included. The mean age was 67.3 (SD 8.4, range 40-83 years). A percutaneous coronary intervention (PCI) or CABG was reported by 57 subjects and 58 subjects had experienced a previous AMI. Fifty-five subjects were receiving beta-blocker medication.

For the analysis of the effects of ectopic beats and stability over time of non-linear HR dynamics (Study II), the subjects who had at least two non-linear HR dynamics analysis from recordings with ectopic beats were selected and therefore, the number of patients became 104.

Before the actual study, the interobserver reproducibility of short-term HR variability was evaluated from 17 ECG recordings from 15 test-subjects: five males and ten females of mean age of 35.4 (SD 9.2) years, range 22-50 years. The subjects were either institute staff or their relatives and friends at the three ULTRA research centers. Ten subjects were healthy and five had CAD. Three recordings were taken from one healthy woman on different days.

4.1.2 Acute effects of carbon monoxide on heart rate dynamics (Study III)

To study the acute effects of CO among subjects with stable CAD, the study protocol included 24-h ambulatory ECG recordings with simultaneous continuous personal CO exposure monitoring performed three times within a one-week interval. The recordings started between 11.50 and 12.35 always on the same day of the week. The study was conducted in Kuopio, Finland, between October 1997 and May 1998.

Ten male subjects who underwent coronary angiography because of suspected CAD were originally recruited into the study. All subjects had undergone coronary angiography within three months, most of them within two weeks of the study. Of the eight subjects with angiographically significant CAD, six (age range 55-68 years) had both technically acceptable personal CO exposure measurements and high-quality ECG data with the proper time notes in the ambulatory recordings, and were included in the analysis.

The coronary angiography revealed three-vessel coronary artery disease in five subjects and a single-vessel disease in one subject. All the subjects were referred to either CABG (n=4) or PCI (n=1) after the study, except for one subject, who had had a PCI two months before the study.

During the first visit, the subjects were asked about their health, medication, smoking habits and socioeconomic status. Four subjects had experienced a previous AMI and two subjects had hypertension. All six subjects were taking medication for CAD. Five subjects were receiving beta-blocker medication, but two gradually terminated and one reduced the beta-blocker medication by half for the study. Five subjects had stopped smoking, three recently and one subject was still a regular smoker.

The subjects followed their usual daily routines during each monitoring session. They were also asked to fill in a diary about their location (outside, inside or in a vehicle), physical activity, symptoms and passive or active smoking for every 15-min periods during the monitoring sessions. They were asked to evaluate their physical activity subjectively as low, moderate or high.

4.1.3 Postoperative atrial fibrillation and heart rate dynamics (Study IV)

The study population originally included 100 patients. The inclusion criteria were a first elective CABG without accompanying procedures and preoperative sinus rhythm. The exclusion criteria included a previous episode of AF, an AMI less than one month preoperatively and a reduced left ventricular ejection fraction less than 50% at the time of cardiac catheterization. All of their medications were continued until surgery. Due to presence of severe CAD, all the patients were receiving beta-blocker medication.

All the patients went through similar surgical and anaesthetic procedures. The anaesthesia was opioid-based with supplementary pancuronium and isoflurane. A mild systemic hypothermia (venous temperature 34° C) and intermittent cold crystalloid cardioplegia delivered through the antegrade route were used. On the first postoperative day, the beta-blocking agents were continued in all patients. Postoperative ECG monitoring was conducted during the whole hospital stay to identify all episodes of AF. The continuous monitoring was checked daily in order to note every episode of AF. The AF was defined as an irregular rhythm lasting over five minutes with no P-wave before the QRS complex. The first occurrence of AF was an endpoint of the study.

One day before the coronary surgery, all patients had a continuous ECG recording during a standardised situation consisting of three 10-min periods: spontaneous and paced breathing at 0.2 Hz frequency in the supine position and a passive head-up tilt at 70 degree angle after a 2-minute rest. This data was used to evaluate the possible predictive power of HR dynamics measures for postoperative AF.

4.2 METHODS

4.2.1 Heart rate dynamics

In the methodological part of the present study (Studies I and II) two-channel ambulatory ECG recordings were conducted with analogous Medilog MR63 recorders (Oxford Instruments, Abington, U.K.) with standard electrode positions for modified lead V_5 and V_1 in the three research centers. Thereafter, the recordings were sent weekly to the Department of Clinical Physiology and Nuclear Medicine at Kuopio University Hospital.

In the study of acute effects of CO (Study III) three-channel ambulatory ECG recordings (Marquette 8500, Marquette Inc., Milwaukee, WI, USA) were taken with standard electrode positions as recommended by the manufacturer.

In the study on the prediction of postoperative AF (Study IV), a commercial software package (CAFTS, version 3.3.9 Medikro Inc, Kuopio, Finland) was used for data acquisition.

Time and frequency domain measurements (Study I and III)

In the study of stability of short-term HR variability, experienced nurses interactively edited the ambulatory ECGs and performed the HR variability analyses with a commercial Exel Medilog II V7.5 system (Oxford Instruments, Abington, U.K.). The analyses were performed from selected 5-min epochs with as high signal quality as possible, i.e. as few ectopic beats and artefacts as possible.

From the 131 subjects in the Study I, one subject was excluded from the HR variability analysis because of a second-degree atrioventricular block, six because of atrial fibrillation and two because of the continuous presence of frequent supraventricular ectopic beats (Figure 6). Therefore, the number of patients was reduced to 122 subjects in whom there were 1297 ECG recordings. Finally, those recordings in which the number of ectopic beats exceeded 10% of the total were excluded (n=23). For the 40-minute measurements the recordings were excluded in which the exercise had not been performed (n=111) or had been stopped for any reason or in whom there was low-quality ECG (n=44). The reason for not performing the exercise was mainly some cardiorespiratory or other symptom. Furthermore, only recordings having high-quality ECG were included. In summary, the number of recordings was 1106 for time and frequency domain measurements during the 40-min recording, 1261 for time domain and 1250 for frequency domain during rest, 1266 for time and 1254 for frequency domain during paced breathing, 1262 for time domain and 1252 for frequency domain during standing, 1092 for time domain and 1085 for frequency domain during exercise, 1117 for time domain and 1102 for frequency domain measurements during recovery.

The recordings were digitised with a sampling rate of 128 Hz. Due to the low sampling rate, an interpolation algorithm was used by the software to localise the R-wave fiducial

point. The software annotations (normal, supraventricular, ventricular, unclassified or artefact) were manually checked and, when necessary, edited. A premature beat was identified if the preceding RR interval was more than 20% shorter than the following RR interval. Before the analysis, a triple-beat filter was used and only normal-to-normal intervals between 300 and 2000 ms accepted. An interpolated tachogram was used in the HR variability analysis. Linear detrending, i.e. a least sum of squares best fit straight line, was generated and subtracted from the data prior to the spectral analysis. Furthermore, the Hanning window function was applied before performing spectral analysis based on the fast Fourier transformation method.

The time and frequency domain analyses were performed from the whole 40-min ECG recordings as well as from periods of 5-min rest, paced breathing, standing, exercise and recovery (Figure 4). In the recordings, there were event marks at the beginning and at the end of each period and, the time points were also marked in a clinical visit logbook. The time domain measurements included the meanNN (ms), SDNN (ms) and r-MSSD (ms) and in the spectral measurements there were the total power (TP; 0-0.40 Hz), very low frequency (VLF; 0.003-0.04 Hz), low frequency (LF; 0.04-0.15 Hz) and high frequency (HF; 0.15-0.40 Hz). The VLF was analysed only from the 40-min period. The integrals under respective power spectral density functions were calculated and expressed in absolute units (ms^2). In addition, the LF and HF ratio was calculated.

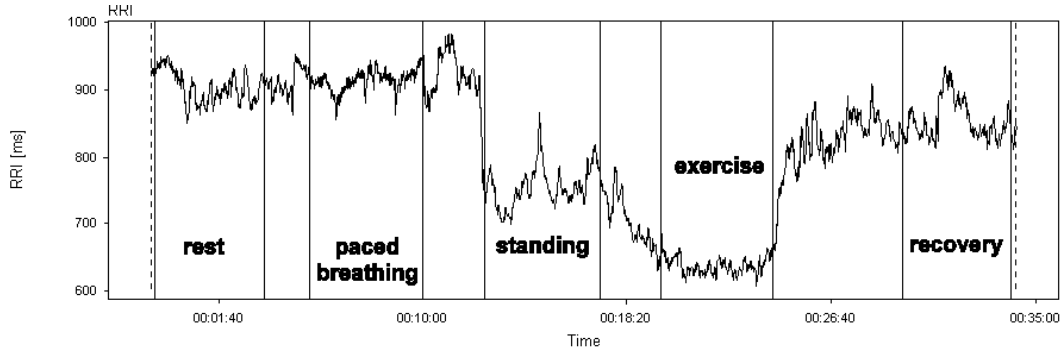


Figure 4. Example of RR interval tachogram during the 40-minute period

For the assessment of interobserver reproducibility, 17 ECG recordings were analysed and HR variability measurements performed independently by six nurses using the same methods as in the actual study.

In the study of the acute effects of CO (Study III), the time domain HR variability measurements were also analysed with Exel Medilog II V7.5 system (Oxford Instruments, Abington, U.K.). Only NN intervals between 300 and 2000 ms with NN ratios between 0.8 and 1.2 were included. The calculated measures included the meanNN, SDNN and r-MSSD analysed for two 5-min segments with every single episode of CO exposure. The baseline was measured from 7 to 2 minutes before the beginning of CO exposure and the acute effect from 2 to 7 minutes after the beginning of CO exposure (Figure 5). However, when the CO exposure contained multiple peaks, the baseline was measured from the 5-min

segment before the entire CO exposure period but the acute effect from 2 to 7 minutes after the beginning of each separate peak.

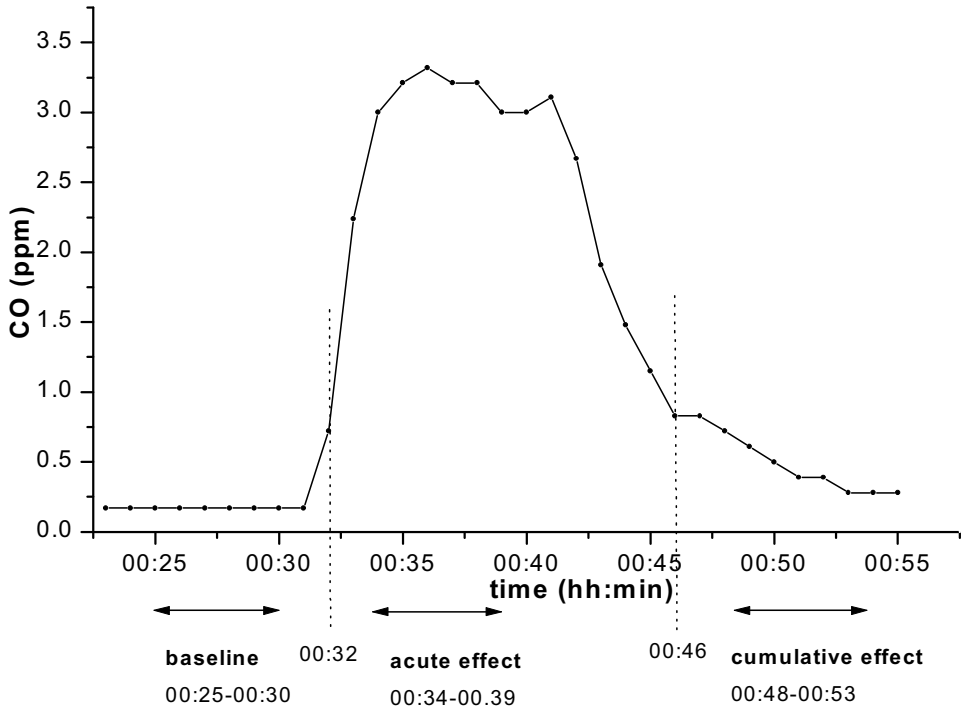


Figure 5. The baseline and acute effect HR variability measurements in relation to one example CO exposure episode

Non-linear heart rate dynamics measurements (Studies II and IV)

For the analysis of the effects of ectopic beats and stability of non-linear HR dynamics the data in ASCII format was transferred to a computer and the analyses performed with a custom software package (WinCPRS; Absolute Aliens Oy; Turku, Finland).

Before the non-linear HR dynamics analysis, an additional four patients were excluded because of frequent ectopic beats and, thus, there were 118 patients (Figure 6). Recordings with more than 10% ectopic beats had been excluded in relation to HR variability analyses. In relation to the non-linear HR dynamics, the 40-min recordings and 5-min exercise and recovery periods were not analysed if the exercise period contained more than 10% ectopic beats (n=25). In addition, the non-linear HR dynamics were not analysed if any of the 5-minute periods were missing or were assessed to be of poor quality after careful visual assessment (n=43). Some recordings could not be opened after data transfer. Altogether, the number of recordings was 989 during the 40-min recording, 1140 during rest, 1144 during paced breathing, 1147 during standing, 1018 during exercise, 1007 during recovery.

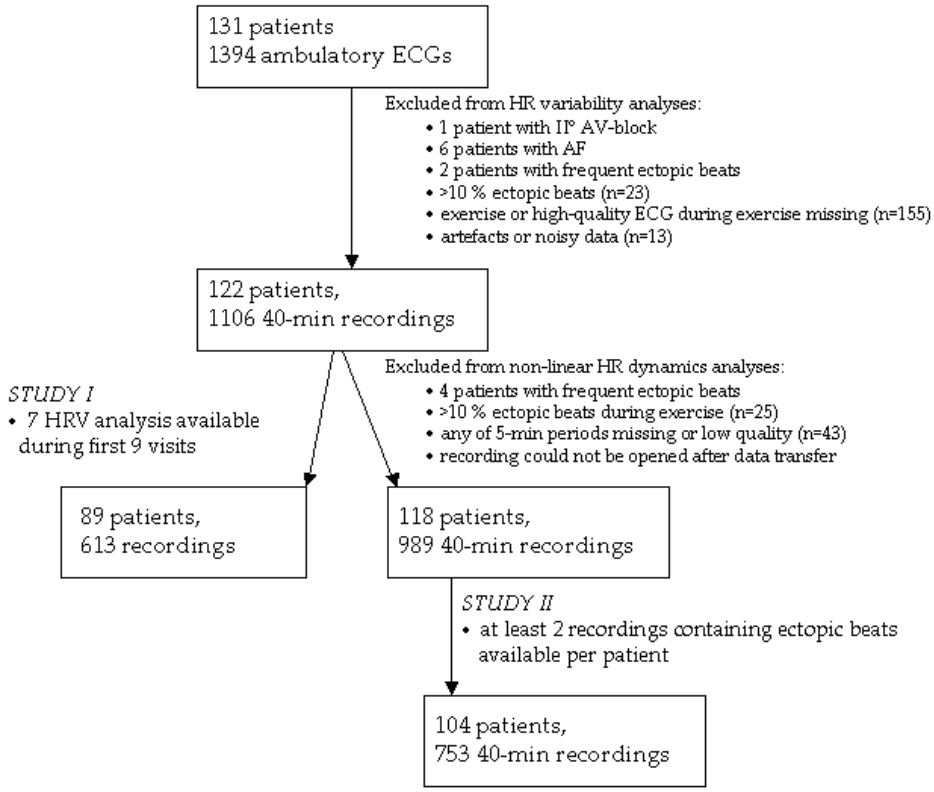


Figure 6. Study exclusion flow chart

During the analysis, the ectopic beats were recognised both on the basis of the morphological annotations in Oxford Medilog analyses and with the criteria for prematurity, i.e. the RR interval remained 20% shorter than the mean of three previous intervals. The non-linear HR dynamics were analysed in three settings: First, the non-edited recordings that included up to 10% ectopic beats were analysed. Secondly, the premature beats were edited with the interpolation method before the HR dynamics analyses. Both the premature beat and the adjacent beat were replaced with interpolation according to the previous normal and the next correct RRI values. Thirdly, an RR interval before and after the premature and ventricular ectopic beats was completely deleted before the HR dynamics analyses. The editing process of the entire signal was performed only once, which might have meant that some RR intervals related to closely located or consecutive ectopic beats remained unedited.

The non-linear HR dynamics measurements included the approximate and sample entropy, fractal dimension by curve length, return map in terms of SD1 and SD2, short-term scaling exponent (α_1) of detrended fluctuation analysis and forbidden words and entropy of symbolic dynamics analysed from a 40-min period as well as from a 5-min period. However, the ApEn was not analysed from the 5-min period because 5-min is too short a time to do this kind of analysis. The fractal dimension by curve length was not analysed from the 5-minute standing period.

The ApEn was computed with the fixed threshold level, i.e., r=0.20×SD and m=2. With the detrended fluctuation analysis, the minimum box size was four beats, the maximum box size 300 beats and the box number was 20. With the SymDyn method, the mean and SD were used to generate four symbols, which were interpreted as three symbol words. The complexity of the word distribution was measured with Shannon's entropy of symbolic dynamics (SymDyn En).

To study the relation of non-linear HR dynamics to postoperative AF (Study IV), 33 out of 100 patients were excluded before the analysis of non-linear RR interval dynamics. The most important exclusion criterion was diabetes mellitus (n=16) since this is known to severely affect the non-linear RR interval dynamics due to possible clinical or subclinical autonomic neuropathy. Two patients were excluded because they had to undergo an unscheduled cardiac procedure with beating heart due to calcification of ascending aorta and two patients due to unplanned mitral valve repair. Otherwise, the recordings were analysed only when all three recorded periods of ECG were of high quality. One patient was excluded due to sinus pauses and one because of nearly continuous ventricular bigeminia. In two patients, the recordings had lasted less than nine minutes. In three patients, the editing of frequent ectopic beats (7% ectopic beats and a short paroxysmal supraventricular tachycardia in one patient and 10-11% ectopic beats of all beats in two patients) shortened the recordings so that they were less than nine minutes. In one patient the editing algorithms could not detect correctly very frequent ectopic beats. Furthermore, the data of five patients could not be transferred due to software incompatibilities.

Nonetheless, all the recordings were also analysed so that no non-sinus beats were edited. Then, those three patients were included whose recordings had shortened too much with the editing as well as including the recording of one patient with editing difficulties. Therefore, the final number of these patients reverted to 71.

The measures of HR dynamics included meanNN, DFA α_1, ApEn and SymDyn En and were analysed with the same software and with the same options as in the study of the effects of ectopic beats (Study II). One exception was that before the ApEn analysis, the linear trend was first removed. In the editing, the ventricular ectopic beats were recognised visually. In addition, a beat was considered as being premature if the RR interval preceding the beat was ≥20% shorter than five previous beats. The RR interval preceding and following an ectopic beat was removed during the editing process.

Furthermore, in Study IV, 39 10-min recordings containing non-sinus beats during paced breathing and 30 10-min recordings during upright tilt were selected to study the effect of ectopic beats.

4.2.2 Carbon monoxide concentration monitoring

In the study of the acute effects of CO (Study III), the CO concentrations were measured continuously with personal exposure monitors (Langan Model T15 High Resolution Personal Exposure Measurer, Langan Products Inc., San Francisco, CA, USA). The monitors were placed in a waist bag under the outer clothing for outdoor wear. Otherwise the subjects were asked to keep the monitors either in the waist bag or to be placed on a table or a chair in the same or next room, not on the floor.

One investigator visually selected the periods of increased CO levels from the graphic output, an example is shown in Figure 7. Since there are always small amounts of CO in ambient air, only those periods with clearly increased levels of CO were defined as CO exposure. The durations of CO exposures were defined from the time of the first increased CO value to the time that the CO level returned back to baseline. The CO exposures were quantified as the maximum CO value (ppm) during the CO. In some cases, the CO exposures contained more than one peak close to each other. In such instances each peak was regarded as being separate.

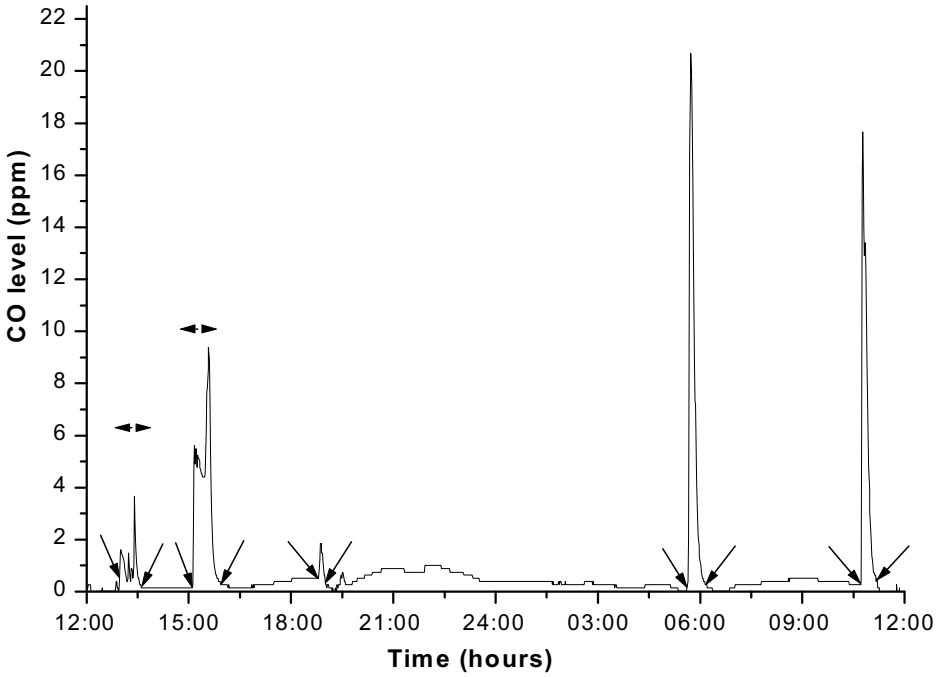


Figure 7. The monitoring of carbon monoxide levels during example of 24 hour monitoring. → the start time and end of CO exposure and ↔ episodes of CO exposure with multiple peaks

The data of the hourly outside temperature was obtained from the measurements of City of Kuopio. When the subject was inside during the CO exposure, inside temperature was assumed to be 20 ˚C.

4.3 STATISTICAL ANALYSIS

All the analysis were performed using the SPSS 9.0-11.0 software package for Microsoft Windows (SPSS Inc., Chigaco, Ill., USA)

The coefficient of variation was used when the stability over time of short-term conventional HR variability was to be assessed (Study I). Glüer et al. (Gluer et al. 1995) has defined the formulas for the accurate assessment of short-term precision and these were

used in the calculation of CV as well as in the estimation of the CI of CV. The key point was that the short-term precision error (expressed as CVs) was calculated as root-mean-square averages of standard deviations of repeated measurements and not by the arithmetic mean of individual subjects' precision errors, because that can lead to underestimation of the true imprecision.

Both frequency domain measurements and r-MSSD were non-normally distributed and were normalised with logarithmic transformation and therefore, they were used as such in the analyses. The LF/HF ratio as percentages was also logarithmically transformed.

The correlation between the increase in HR during submaximal exercise, i.e. change in meanNN from rest to exercise, and SDNN was assessed with the Spearman correlation coefficient, since it is insensitive to skewed distributions. There were many consecutive measurements from each subject and therefore, the effect of individual differences had to be taken into account and adjusted. Thus, the differences between each measure and the average of the consecutive measures of the subject were used in the analysis.

The interobserver reproducibility was assessed with CVs evaluated from the parallel HRV measurements made by the six nurses. In addition, the analysis of variance for repeated measures (general linear model) was used to assess whether there were systematic differences between the nurses who had performed the analyses.

When the effect of ectopic beats on non-linear HR dynamics was being evaluated (Study II), the means and SDs for non-linear HR dynamics obtained from differently edited data were calculated and the distributions compared. The correlations between the proportion of ectopic beats and non-linear HR dynamics from differently edited data were analysed with the Spearman correlation. The effect of individual differences due to several measurements from each subject was adjusted in the same way as in Study I. Bland-Altman plots were used to demonstrate the effects of editing on ApEn, RM SD1 and DFA α_1.

In Study IV, the differences between non-linear HR dynamics measures obtained from differently edited data were analysed with paired samples t-test.

The stability of non-linear HR dynamics obtained from differently edited data was assessed as in Study I by calculating the coefficient of variation. The RM SD1 and SD2 showed highly skewed distribution and were corrected towards normal by a logarithmical transformation prior to the analyses. The test developed by Feltz and Miller (Feltz et al. 1996) was used to test the equality of CVs obtained with three editing methods.

The correlations between different conventional and traditional HR dynamics were assessed by Spearman correlation coefficient. The effect of individual differences was adjusted in the same way as in Study I and II.

In the evaluation of the acute effects of CO (Study III), the statistical analyses were conducted by the analysis of variance for repeated measures (general linear model). The episodes of CO exposure were divided into two groups according to the median of the maximum CO values (2.7 ppm). Due to the skewness in the data, the HR variability measures were analysed after logarithmic transformation and were placed as within-subject factors in the model. The subjects had different numbers of CO exposure episodes during the monitoring and therefore, the analyses were adjusted for the interindividual variance so that the subject's identification number was placed as a between-subject variable in the model.

In Study IV, the analysis of variance for repeated measures (general linear model) was used to compare the RR interval dynamics obtained during three standardised situations and for between-group comparisons. The demographic variables between the groups remaining in sinus rhythm (SR) or developing AF after CABG were compared with the independent samples t-test for continuous variables and with χ^2-test for the categorized variables. The Fisher's exact test was used in the case of small groups not suitable for χ^2-test. The Mann-Whitney U-test was used to compare the duration of the hospital stay between the groups. The intrinsic correlations between the non-linear RR interval measurements were analysed with the Pearson correlation coefficients.

Thereafter, a logistic regression model was used to assess the relationship between preoperative non-linear HR dynamics and postoperative AF. The differences in the non-linear HR dynamics between the groups were largest during spontaneous breathing and therefore, these measurements were used in the analysis. The basic analyses were conducted without any other covariates. Thereafter, the meanNN and age were entered as covariates into the model to adjust for the possible effects of HR or age on the associations between the risk of postoperative AF and RR interval dynamics. Then, a multivariate logistic regression analysis was used (backward, stepwise (likelihood ratio)) including DFA α_1, SymDyn En, age, preoperative hemoglobin value, body mass index and chronic pulmonary obstructive disease as covariates. The odds ratio (OR) of AF was calculated for an interquartile change (increase from 1st to 3rd quartile) of DFA α_1 and SymDyn En during spontaneous breathing. The linearity of the observed associations between the risk of AF and DFA α_1 or SymDyn En were validated so that the analyses were repeated after the data had been divided into groups, ordered on the continuous DFA α_1 or SymDyn En variable.

A P-value<0.05 was considered to be statistically significant in all of the analyses.

4.4 ETHICS APPROVAL

The subjects provided a written informed consent before participating in the studies and the local ethics committees approved the study protocols. The investigations conform to the principles outlined in the Declaration of Helsinki.

5 Results

5.1 SHORT-TERM HEART RATE DYNAMICS IN STUDIES I AND II

The conventional HR variability and non-linear HR dynamics measurements in the whole study population are presented in Table 2. The differences between the HR dynamics during the periods were not tested statistically and therefore, the data is only descriptive.

The HR was lowest during the paced breathing period and rest and increased during standing and even more during exercise, and returned to near to the resting level during recovery. The paced breathing seemed to reduce the HR variability with respect to SDNN, TP and LF, but in r-MSSD there was no clear change and HF seemed to increase. The LF/HF ratio decreased with paced breathing and increased during standing.

The RM showed the same kind of trend from one period to the next as r-MSSD. The DFA α_1 behaved concurrently as the LF/HF ratio, however, during exercise it obtained lower values than during paced breathing which was not the case with LF/HF ratio. The entropy measures (SaEn and Symdyn En) seemed to increase during paced breathing; SymDyn En reduced with standing, and both remained low with exercise and increased again with recovery. The SymDyn FW behaved oppositely to SymDyn En except than during exercise, when both of these measures remained lower than those obtained during rest.

The correlations between time and frequency domain and non-linear HR dynamics measures are presented in Tables 3 and 4.

Table 2. Heart rate dynamics of subjects with stable coronary artery disease

	5-min rest **n=1261/ 1250/1140 mean (SD)**	**5-min paced breathing** **n=1266/ 1254/1144 mean (SD)**	**5-min standing** **n=1262/ 1252/1147 mean (SD)**	**5-min exercise** **n=1092/ 1085/1018 mean (SD)**	**5-min recovery** **n=1117/ 1102/1007 mean (SD)**	**40-min whole recording** **n=1106/ 1106/989 mean (SD)**
HR (bpm)	63.8 (9.6)	63.3 (9.5)	69.0 (10.6)	83.4 (10.9)	65.2 (9.3)	68.5 (8.9)
meanNN (ms)	962 (147)	969 (148)	891 (143)	734 (112)	939 (144)	892 (124)
SDNN (ms)	43.8 (22.1)	36.2 (22.1)	50.1 (21.3)	35.2 (17.4)	45.6 (23.3)	106.0 (42.3)
r-MSSD (ms)	32.4 (28.4)	31.8 (30.2)	28.3 (21.6)	24.5 (17.5)	33.0 (30.5)	33.0 (22.7)
lnr-MSSD	3.2 (0.6)	3.2 (0.7)	3.1 (0.6)	3.0 (0.6)	3.3 (0.6)	3.3 (0.6)
TP (ms^2)	1929 (2085)	1314 (1777)	2241 (2092)	789 (984)	2053 (2362)	2407 (1731)
lnTP	7.2 (0.9)	6.6 (1.0)	7.4 (0.8)	6.2 (0.9)	7.2 (1.0)	7.6 (0.7)
VLF (ms^2)	NA	NA	NA	NA	NA	1354 (967)
lnVLF						7.0 (0.7)
LF (ms^2)	503 (732)	329 (704)	487 (616)	157 (324)	563 (831)	427 (497)
lnLF	5.6 (1.1)	4.9 (1.3)	5.6 (1.1)	4.3 (1.2)	5.7 (1.2)	5.9 (1.0)
HF (ms^2)	351 (685)	474 (907)	239 (466)	139 (257)	344 (628)	290 (429)
lnHF	4.9 (1.3)	5.2 (1.4)	4.6 (1.2)	4.1 (1.3)	4.9 (1.3)	5.0 (1.1)
LF/HF	2.9 (2.6)	1.3 (1.4)	4.0 (4.0)	2.0 (2.2)	3.1 (2.9)	2.4 (1.8)
ln(LF/HF %)	5.3 (0.9)	4.3 (1.0)	5.6 (1.0)	4.8 (1.1)	5.3 (0.9)	5.2 (0.8)
ApEn	NA	NA	NA	NA	NA	0.75 (0.28)
SaEn	1.41 (0.43)	1.51 (0.45)	NA	1.28 (0.48)	1.37 (0.43)	0.60 (0.26)
FD-L	1.65 (0.48)	1.89 (0.56)	NA	1.87 (0.61)	1.66 (0.46)	1.45 (0.17)
RM SD1	26.1 (23.0)	25.5 (23.5)	24.3 (20.9)	21.6 (20.4)	26.1 (23.4)	27.4 (18.8)
RM SD2	91.5 (24.8)	91.3 (24.9)	84.0 (23.2)	64.5 (20.5)	88.8 (25.4)	42.7 (17.1)
DFA α_1	1.05 (0.33)	0.85 (0.30)	1.14 (0.34)	0.81 (0.34)	1.06 (0.32)	1.00 (0.27)
SymDyn En	4.13 (0.56)	4.28 (0.62)	3.86 (0.52)	3.99 (0.72)	4.11 (0.54)	3.17 (0.50)
SymDyn FW	47.8 (13.1)	45.1 (13.5)	52.7 (11.7)	45.9 (16.1)	48.1 (12.8)	61.2 (8.8)

Premature beats has been edited with interpolation method before non-linear heart rate dynamics analyses; n = number of time domain/frequency domain/non-linear heart rate dynamics measures

Table 3. Correlations between time and frequency domain and non-linear heart rate dynamics during 40-min recordings
Previously unpublished data

	SDNN	**r-MSSD**	**TP**	**VLF**	**LF**	**HF**	**LF/HF**	**ApEn**	**SaEn**	**FD-L**	**RM SD1**	**RM SD2**	**DFA α_1**	**Sym Dyn FW**	**Sym Dyn En**
meanNN	0.38	0.35	0.44	0.39	0.32	0.35	-0.20	0.02	0.04	-0.05	0.30	0.52	-0.08	-0.05	0.03
SDNN		0.40	0.59	0.58	0.32	0.33	-0.15	-0.37	-0.39	-0.27	0.32	0.42	-0.08	0.09	-0.19
r-MSSD			0.55	0.34	0.53	0.80	-0.41	0.23	0.12	0.32	0.74	0.70	-0.45	-0.42	0.35
TP				0.87	0.59	0.53	-0.06	-0.03	-0.09	-0.01	0.41	0.49	-0.01	-0.12	0.05
VLF					0.36	0.27	-0.01	-0.11	-0.14	-0.12	0.25	0.35	0.08	-0.01	-0.05
LF						0.63	0.14	0.12	0.05	0.16	0.39	0.42	0.06	-0.26	0.18
HF							-0.41	0.23	0.13	0.29	0.61	0.60	-0.31	-0.37	0.34
LF/HF								-0.14	-0.10	-0.15	-0.34	-0.32	0.54	0.17	-0.17
ApEn									0.94	0.64	0.15	0.11	-0.14	-0.36	0.59
SaEn										0.55	0.06	0.03	-0.08	-0.26	0.52
FD-L											0.09	0.04	-0.06	-0.41	0.59
RM SD1												0.90	-0.66	-0.49	0.27
RM SD2													-0.53	-0.41	0.20
DFA α_1														0.35	-0.22
SymDyn FW															-0.46
SymDyn En															

n=1106 for time domain and frequency domain, n=980 for non-linear HR dynamics measures
P<0.05 for all correlations, when r>0.07 or <-0.07
Non-linear HR dynamics are obtained after deletion of previous and following RR interval after ectopic beats

Table 4. Correlations between time and frequency domain and non-linear heart rate dynamics during 5-min rest with spontaneous breathing
Previously unpublished data

	SDNN	**r-MSSD**	**TP**	**LF**	**HF**	**LF/HF**	**SaEn**	**FD-L**	**RM SD1**	**RM SD2**	**DFA α_1**	**Sym Dyn FW**	**Sym Dyn En**
meanNN	0.27	0.34	0.26	0.26	0.27	-0.12	0.11	0.01	0.28	0.79	-0.11	-0.13	0.17
SDNN		0.62	0.92	0.58	0.51	0.06	-0.31	-0.14	0.42	0.47	0.06	0.16	-0.24
r-MSSD			0.55	0.52	0.80	-0.30	0.06	0.13	0.64	0.52	-0.32	-0.36	0.24
TP				0.62	0.49	0.11	-0.29	-0.17	0.37	0.43	0.08	0.15	-0.22
LF					0.53	0.27	-0.10	-0.17	0.34	0.38	0.15	-0.06	-0.02
HF						-0.37	0.06	0.10	0.52	0.43	-0.23	-0.30	0.19
LF/HF							-0.19	-0.30	-0.21	-0.13	0.40	0.32	-0.28
SaEn								0.42	-0.21	-0.10	-0.04	-0.44	0.60
FD-L									-0.11	-0.14	-0.16	-0.39	0.56
RM SD1										0.65	-0.56	-0.16	-0.08
RM SD2											-0.28	-0.06	-0.04
DFA α_1												0.33	-0.16
SymDyn FW													-0.80
SymDyn En													

n=1261 for time domain, n=1250 for frequency domain, n=1140 for non-linear HR dynamics measures
P<0.05 for all correlations, when r>0.06 or <-0.06
Non-linear HR dynamics are obtained after interpolation of previous and following RR interval after ectopic beats

Table 5. Correlations between meanNN and different non-linear heart rate dynamics during 10-min rest with spontaneous breathing, Study IV. Previously unpublished data

	meanNN	**ApEn**	**RM SD1**	**RM SD2**	**DFA α_1**	**SymDyn FW**	**SymDyn En**
meanNN		0.30	0.52	0.15	-0.44	-0.56	0.57
ApEn			0.21	-0.18	-0.50	-0.59	0.74
RM SD1				0.65	-0.49	-0.64	0.55
RM SD2					0.08	-0.08	-0.07
DFA α_1						0.76	-0.78
SymDyn FW							-0.91

n=67
P<0.05 for all correlations, when r>0.26 or <-0.26

5.2 STABILITY OVER TIME OF SHORT-TERM HEART RATE DYNAMICS (STUDY I AND II)

In the interobserver reproducibility analysis, the CVs remained under 3.9% for time domain measures (meanNN, SDNN and lnr-MSSD) and <6.0 % for logarithmically transformed frequency domain measures. During 40-min recordings, the CVs were less than 1.2 %. There were no systematic differences between the nurses who had performed the analyses.

The stability over time of short-term HR dynamics measures is presented in Table 6. The CVs varied between 6.1-29.9 % during the 5-min rest and 6.0-37.1 % during the 5-min paced breathing in the time domain (r-MSSD logarithmically transformed) and 7.2-14.7 % during 5-min rest and 9.9-16.5 % during 5-min paced breathing in the frequency domain measures. During the 40-min recording, the CVs varied between 5.1-16.7 % in the time domain (r-MSSD logarithmically transformed) and 4.4-11.0 % in the frequency domain measures.

The CVs for non-linear HR dynamics remained ≤10% for FD-L, RM and SymDyn, whereas the ApEn had CV of 21.9% and DFA α_1 of 16.5%. These results were obtained when the ectopic beats had been edited with the interpolation method similarly as done in the time and frequency domain measurements.

The SDNN was observed to have a very high correlation (r=0.84) between change in meanNN from rest to submaximal exercise (Figure 8).

Table 6. Stability over time of short-term heart rate dynamics, coefficient of variation with 95% confidence intervals (Study I and II)

	5-min rest	**5-min paced breathing**	**40-min whole recording**
	CV % (CI)	**CV % (CI)**	**CV %**
Time domain measurements n=623			
meanNN	6.1 (5.8-6.5)	6.0 (5.6-6.3)	5.1 (4.8-5.4)
SDNN	29.9 (28.2-31.8)	37.1 (35.0-39.5)	16.7 (15.8-17.8)
r-MSSD	48.9 (46.2-52.1)	52.1 (49.1-55.4)	31.1 (29.3-33.1)
lnr-MSSD	*11.0 (10.3-11.7)*	*12.7 (12.0-13.5)*	*7.8 (7.4-8.3)*
Frequency domain measurements n=623			
LnTP	7.2 (6.8-7.7)	9.9 (9.3-10.5)	4.4 (4.2-4.7)
lnVLF	NA	NA	4.9 (4.6-5.2)
lnLF	11.3 (10.7-12.0)	16.5 (15.5-17.5)	8.6 (8.1-9.1)
lnHF	14.7 (13.9-15.7)	14.9 (14.0-15.8)	11.0 (10.4-11.7)
ln(LF/HF ratio)	11.5 (10.9-12.3)	16.1 (15.2-17.2)	7.3 (6.9-7.8)
Non-linear heart rate dynamics n=753			
ApEn	NA	NA	21.9 (20.8-23.1)
FD-L	NA	NA	7.4 (7.1-7.9)
ln(RM SD1)	NA	NA	10.4 (9.9-11.0)
ln(RM SD2)	NA	NA	4.9 (4.7-5.2)
DFA α_1	NA	NA	16.5 (15.6-17.4)
SymDyn FW	NA	NA	9.7 (9.2-10.2)
SymDyn En	NA	NA	9.2 (8.7-9.7)

NA, not available

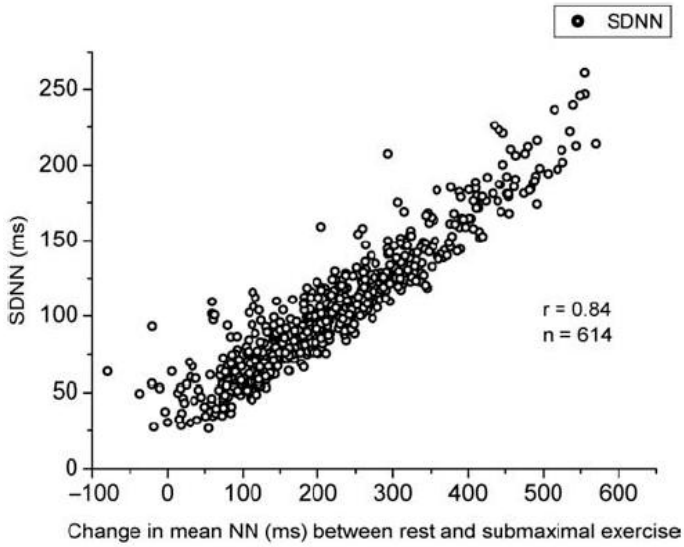


Figure 8. The correlation between SDNN of the whole 40-minute recording and HR increase during 5-minute submaximal exercise

5.3 ECTOPIC BEATS IN THE ASSESSMENT OF NON-LINEAR HEART RATE DYNAMICS (STUDY II AND IV)

On average, there were 0.7% ventricular or premature supraventricular ectopic beats (range from one ectopic beat to 8.2%) in the 753 40-min ECG recordings examined in Study II.

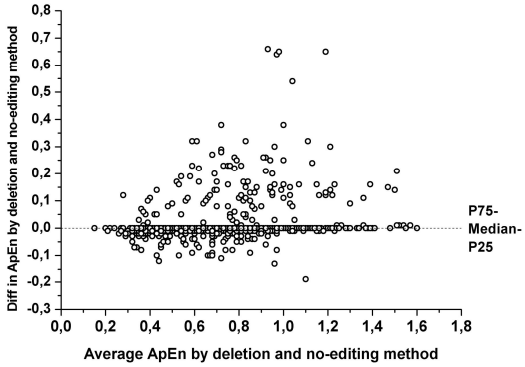
The distributions of RM, DFA α_1 and SymDyn FW changed considerably when the method used to edit the ectopic beats was changed (Table 7). The proportion of ectopic beats correlated linearly and strongly with measures of RM and moderately with DFA α_1 and SymDyn FW. However, the editing of ectopic beats changed the correlations into moderate or weak for RM and to weak for DFA α_1 and SymDyn FW.

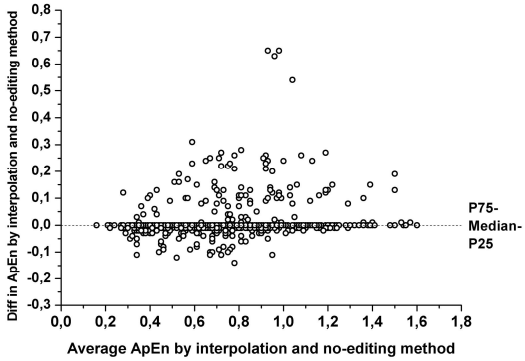
Table 7. Non-linear heart rate dynamics obtained with different editing methods

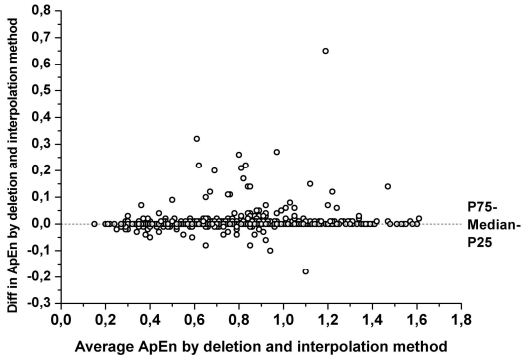
	mean	SD	range	Correlation with % of ectopic beats r
ApEn				
ectopic beats not edited	0.73	0.27	0.16-1.60	-0.01
ectopic beats interpolated	0.75	0.28	0.16-1.60	0.03
ectopic beats deleted	0.75	0.29	0.15-1.62	0.05
FD-L				
ectopic beats not edited	1.44	0.23	0.81-2.16	0.26
ectopic beats interpolated	1.45	0.18	0.81-2.09	0.09
ectopic beats deleted	1.45	0.17	0.79-2.12	-0.01
RM SD1				
ectopic beats not edited	42.7	30.1	7-226	0.73
ectopic beats interpolated	29.3	19.8	7-173	0.50
ectopic beats deleted	25.9	16.4	6-129	0.33
RM SD2				
ectopic beats not edited	55.0	27.4	20-230	0.68
ectopic beats interpolated	43.7	17.8	17-178	0.42
ectopic beats deleted	41.4	15.0	16-134	0.27
DFA α_1				
ectopic beats not edited	0.77	0.28	0.18-1.60	-0.55
ectopic beats interpolated	0.96	0.27	0.25-1.60	-0.38
ectopic beats deleted	1.00	0.26	0.28-1.63	-0.22
SymDyn FW				
ectopic beats not edited	57.1	10.6	9-78	-0.54
ectopic beats interpolated	60.6	9.4	5-78	-0.36
ectopic beats deleted	61.9	8.6	5-78	-0.21
SymDyn En				
ectopic beats not edited	3.20	0.49	1.61-4.83	0.14
ectopic beats interpolated	3.19	0.50	1.59-5.17	0.11
ectopic beats deleted	3.18	0.50	1.56-5.23	-0.10

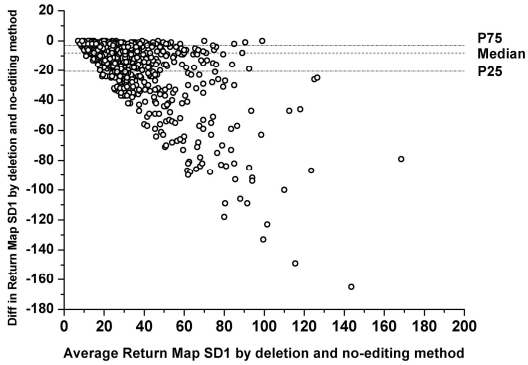
n= 753; for correlations P<0.05, if r>0.09 or <-0.09

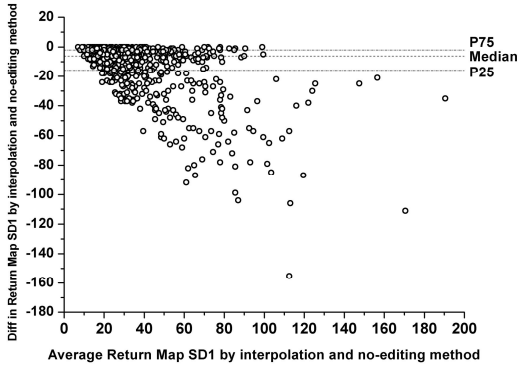
In the Bland-Altman plots (Figure 9), it was observed that for ApEn, the effect of editing was unpredictable, thus in some measurements there were large upward changes though in general the effects were minor. On the contrary, with RM the changes were large with editing and almost invariably downwards in the measures. The effects of editing were largest with the high values; the deletion seemed to operate most efficiently. With DFA α_1, the effect of editing was almost entirely upwards with both methods.


Diff in ApEn by deletion and no-editing method
Average ApEn by deletion and no-editing method
P75-
Median-
P25


Diff in ApEn by interpolation and no-editing method
Average ApEn by interpolation and no-editing method
P75-
Median-
P25


Diff in ApEn by deletion and interpolation method
Average ApEn by deletion and interpolation method
P75-
Median-
P25


Diff in Return Map SD1 by deletion and no-editing method
Average Return Map SD1 by deletion and no-editing method
P75
Median
P25
20
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
0
20
40
60
80
100
120
140
160
180
200


Diff in Return Map SD1 by interpolation and no-editing method
Average Return Map SD1 by interpolation and no-editing method
P75
Median
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20
0
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-100
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-160
-180
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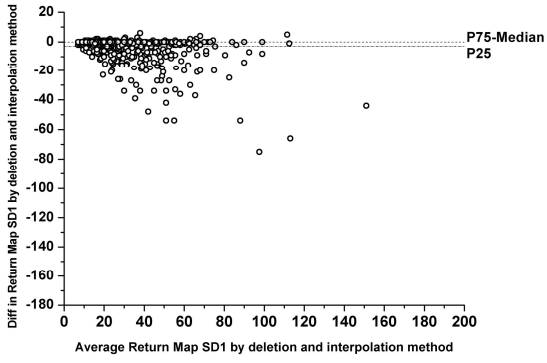
140
160
180
200


Diff in Return Map SD1 by deletion and interpolaion method
Average Return Map SD1 by deletion and interpolation method
P75-Median
P25
20
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
0
20
40
60
80
100
120
140
160
180
200

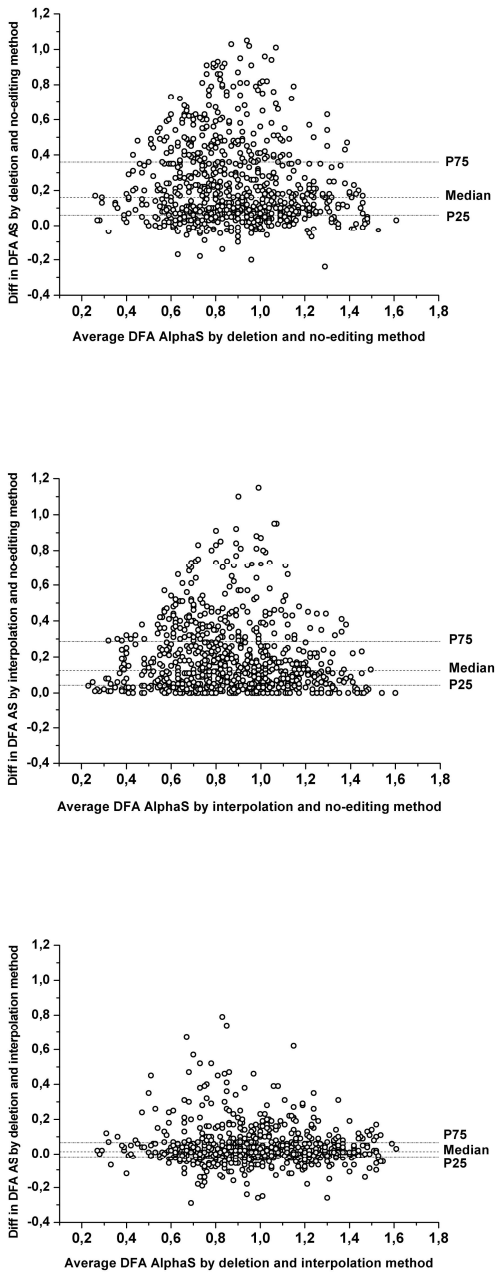


Figure 9. Effect of editing on ApEn, RM SD1 and DFA α_1. The difference (y-axis) represents the change in measurements when either the deletion or interpolation method was used or when the deletion method was used instead of the interpolation method. P75 = upper quartile; P25 = lower quartile.

The number of consecutive measurements per subject was on average 7.2 (range 2-15) and the times during which these measures were collected varied between one to eight months. The stability of FD-L, RM SD2, DFA α_1 and SymDyn FW was affected by the choice of editing method: the lowest stability was obtained with no-editing method and highest with the deletion method (Figure 10). The choice between the different editing methods did not affect the stability of the entropy measurements.

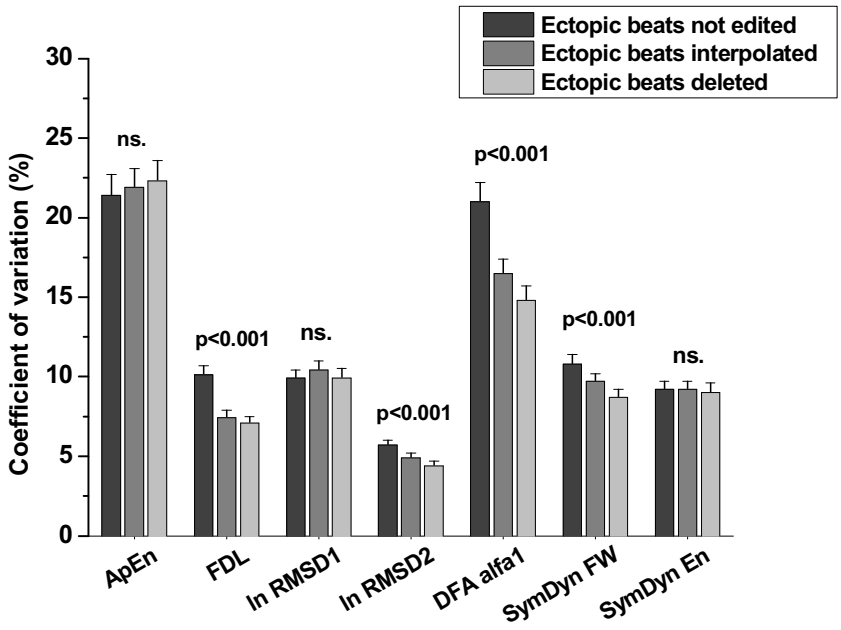


Figure 10. The effect of editing on the stability of non-linear HR dynamics

The effects of ectopic beats were evaluated also in Study IV. Then, among 39 recordings during paced breathing and 30 recordings during head-up tilt, the proportion of non-sinus beats was on average 0.8% and 1.4%.

The meanNN did not differ significantly whether analysed from edited or unedited data (Table 8). With the remaining ectopic beats, the entropy values were lower and the number of forbidden words of SymDyn higher than in recordings with deletion of ectopic beats related RR intervals. The return map values decreased with editing. The values of DFA α_1 were lower, when the ectopic beats were left unedited.

Table 8. The effect of editing of ectopic beats in Study IV, unpublished data

	Paced breathing n=39			**Upright tilt n=30**		
	Non-sinus beats unedited mean (SD)	**Non-sinus beats removed mean (SD)**	**P-value**	**Non-sinus beats unedited mean (SD)**	**Non-sinus beats removed mean (SD)**	**P-value**
meanNN	994 (164)	995 (164)	NS	886 (147)	891 (148)	NS
SaEn	1.08 (0.42)	1.45 (0.31)	<0.001	0.70 (0.33)	1.06 (0.39)	<0.001
RM SD1	48.9 (29.6)	18.9 (13.1)	<0.001	48.4 (41.1)	12.8 (8.8)	<0.001
RM SD2	58.6 (26.6)	46.7 (22.0)	<0.001	65.4 (41.1)	47.5 (21.4)	<0.001
DFA α_1	0.57 (0.25)	0.91 (0.22)	<0.001	0.69 (0.36)	1.16 (0.36)	<0.001
SymDyn En	3.66 (0.82)	4.32 (0.64)	<0.001	3.04 (0.66)	3.60 (0.73)	<0.001
SymDyn FW	48.8 (14.2)	41.8 (14.8)	<0.001	56.9 (11.9)	52.4 (14.4)	<0.001

5.4 ACUTE EFFECTS OF CARBON MONOXIDE ON HEART RATE DYNAMICS (STUDY III)

During 13 24-h monitoring periods, there were 62 episodes of CO exposure. One episode was excluded because of the simultaneous non-sinus rhythm. Nineteen episodes contained multiple peaks. The mean of the maximum CO levels was 4.6 ppm (range 0.5-27.4 ppm). The duration of 55 episodes was on average 17 min (SD 8 min, range 5-47 min). The duration of six CO exposures could not be exactly measured.

The ambient temperature ranged from –20 °C to 20 °C during the CO exposures, with the mean temperature being 6.5 °C. The traffic was related to 12 of 31 (39%) of low and 23 of 30 (77%) of high CO exposures. Smoking explained two cases of low as well as two cases of high CO exposures. During one of the low CO exposures, the physical stress was assessed as being high, otherwise the physical stress was always either low or moderate. No cardiorespiratory symptoms were reported during the CO exposures.

The meanNN did not change during the CO exposure significantly (Table 9). Furthermore, SDNN did not show any significant change, thus, no change in overall HRV was found. However, during the high CO exposure, r-MSSD increased on average by 2.4 ms (p=0.034) after adjusting for the patient. When the ambient temperature was included as a covariate in the model, the change was still 2.4 ms (p=0.052).

Table 9. Time domain measures of heart rate variability before and during CO episode

	Before CO exposure mean (SD)	**During CO exposure mean (SD)**	**P-value***
meanNN (ms)			
CO exposure ≤2.7 ppm	915 (182)	880 (172)	NS
CO exposure >2.7 ppm	775 (129)	778 (114)	NS
SDNN (ms)			
CO exposure ≤2.7 ppm	51 (29)	49 (19)	NS
CO exposure >2.7 ppm	55 (36)	54 (30)	NS
r-MSSD (ms)			
CO exposure ≤2.7 ppm	31 (15)	27 (8)	†
CO exposure >2.7 ppm	22 (6)	24 (7)	0.034/0.052‡

*statistical analyses made with heart rate variability measurements log-transformed
†P-value is not relevant because the patients as between-subjects variable in the model differed from each other statistically significantly
‡ambient temperature as a covariate in the model
NS, not significant

5.5 POSTOPERATIVE ATRIAL FIBRILLATION AND HEART RATE DYNAMICS (STUDY IV)

In the analyses there were 67 patients of whom 19 (28%) experienced a postoperative AF and 48 remained in SR. The patients developing AF after CABG tended to be older and heavier and have preoperatively lower hemoglobin values (g/L) than patients who remained in SR (Table 10). There was more diagnosis of chronic obstructive pulmonary disease among patients with postoperative AF. There were no significant differences in the other preoperative clinical characteristics between patients remaining in SR and developing postoperative AF. All patients were receiving beta-blocker medication at the time of ECG recording.

Table 10. Clinical characteristics of the patients with sinus rhythm or atrial fibrillation after coronary artery by-pass grafting

	AF Patients n=19	**SR Patients n=48**	
	mean (SD)	**mean (SD)**	**P-value**
Preoperative Characteristics			
Age, years	64 (9)	60 (10)	0.12
Gender (men/women)	15/4	38/10	NS*
Body mass index	28 (4)	27 (3)	0.10
Unstable angina	2	4	NS*
History of MI	7	19	NS
History of claudication	1	3	NS*
Hypertension	10	20	NS
Digoxin use	0	1	NS*
Current tobacco use	4	6	NS*
COPD	4	2	0.05
Serum creatinine µmol/l	93 (16)	91 (16)	NS
Hemoglobin value g/l	136 (13)	141 (12)	0.09
Perioperative Characteristics			
Cardiopulmonary bypass time, min	99 (43)	89 (27)	NS
Cross-clamp time, min	86 (34)	80 (24)	NS
Number of by-pass grafts	4 (1)	4 (1)	NS
Serum CK-MBm, µg/l	32 (21)	30 (20)	NS

CK-MBm, first postoperative MB isoentzyme of creatinine kinase; COPD, chronic obstructive pulmonary disease; MI, myocardial infarction; NS, not significant; *Fisher's Exact test

There were no postoperative deaths in the study group. However, in patients with SR, there were two and in the AF group one perioperative AMI (new Q-wave in 12-lead ECG). In the AF group, two patients required prolonged ventilation (< 24 h). In the SR group, there was the appearance of one new atrioventricular conduction defect. In the AF group, there was one perioperative stroke, which was diagnosed with a computer tomography scan.

The postoperative AF appeared after a median of 54 (range 12-115) hours from the surgery. The postoperative AF was related to a longer hospital stay: median length of hospital stay 6 vs. 5 days, p=0.019.

RR interval dynamics

In patients developing postoperative AF, the DFA α_1 was constantly lower than in patients remaining in SR (p=0.016). This difference was most notable during spontaneous breathing (Table 11).

Table 11. Heart rate dynamics in patients with sinus rhythm and atrial fibrillation after coronary artery by-pass grafting

	Rest, spontanous breathing mean (SD)	Rest, paced breathing mean (SD)	Upright Tilt mean (SD)	P-value
Heart rate				tested as meanNN
SR	61.5 (9.7)	63.1 (9.4)	70.1 (11.2)	
AF	57.4 (6.9)	58.2 (7.3)	67.0 (10.6)	
meanNN				0.095*
SR	997 (142)	970 (135)	876 (131)	
AF	1059 (121)	1045 (121)	916 (133)	
DFA α_1				0.016*
SR	1.13 (0.24)	1.02 (0.26)	1.39 (0.25)	
AF	0.93 (0.33)	0.90 (0.34)	1.26 (0.38)	
SymDyn En				0.001†; during spontaneous breathing: 0.012‡
SR	4.36 (0.51)	4.42 (0.53)	3.48 (0.63)	
AF	4.72 (0.51)	4.35 (0.75)	3.26 (0.70)	
ApEn				
SR	1.27 (0.13)	1.27 (0.11)	0.97 (0.27)	
AF	1.29 (0.09)	1.20 (0.18)	0.94 (0.29)	

*Between-group comparisons and †interaction and ‡pairwise comparison in analysis of variance for repeated measures (general linear model)

The behaviour of SymDyn En during the protocol was different in patients developing AF than in patients remaining in SR (p=0.001). The SymDyn En was higher during spontaneous breathing in patients with AF than in patients with SR (p=0.012). In ApEn, there was a similar trend as found in SymDyn En, though, this was not statistically significant. There was also a trend towards higher meanNN in patients with postoperative AF than those remaining in SR.

In the logistic regression analysis, both the DFA α_1 and SymDyn En during spontaneous breathing, when analysed independently, were associated with the risk of postoperative AF (OR 0.31 for an interquartile increase in DFA α_1, 95% CI 0.13-0.78, p=0.012, and OR 3.16 for an interquartile increase in SymDyn En, 95% CI 1.23-8.10, p=0.017) (Table 11). Adjustment for age did not affect these associations. The adjustment of meanNN did not affect the association when DFA α_1 was concerned, however, the association weakened slightly with SymDyn En (OR 2.95, 95% CI 0.97-8.95, p=0.057). In multivariate logistic regression including DFA α_1, SymDyn En, age, preoperative hemoglobin value, body mass index and chronic obstructive pulmonary disease, the lower DFA α_1 was observed to be an independent risk factor for postoperative AF. Since the DFA α_1 and SymDyn En had intrinsic correlations (r=-0.78, p<0.001), the multivariate logistic regression analysis was

repeated without DFA α_1 and then the SymDyn En was the only independent covariate to associate with the risk of postoperative AF.

The low DFA α_1 had a positive predictive value of 53% for postoperative AF, but high negative predictive value 80%. When the low DFA α_1 (<0.89, lower quartile) and high SymDyn En (>4.83, upper quartile) were used together to predict the risk of postoperative AF, the area under receiver operating curve was 0.70. These cut-off values identified 81% patients with a negative predictive value of 80%.

The non-linear HR dynamics were also analysed without editing of ectopic beats (Table 12). In 74 recordings out of 203, there were ectopic beats, however, their proportion was low, i.e. 0.9%. The number of non-sinus beats during the 10-min periods did not differ significantly between the patients who developed AF and those who remained in SR. The results were quite comparable to those obtained with the editing of non-sinus beats: the DFA α_1 value was consistently lower (p=0.002) and SymDyn En tended to exhibit a different behaviour (p=0.054) in patients developing postoperative AF than in patients with SR. The DFA α_1 had predictive power for postoperative AF (OR 0.39 for an interquartile increase in DFA α_1, 95% CI 0.17-0.90, p=0.043), though the relationship was not linear as assumed by the analysis method. The SymDyn En lost its predictive power.

Table 12. Heart rate dynamics obtained from nonedited recordings in patients with sinus rhythm and atrial fibrillation after coronary artery by-pass grafting

	Rest, spontanous breathing mean (SD)	**Rest, paced breathing mean (SD)**	**Upright Tilt mean (SD)**	**P-value**
Heart rate				tested as meanNN
SR	61.6 (9.6)	63.2 (9.4)	70.2 (11.1)	
AF	56.6 (8.0)	57.1 (8.4)	65.9 (10.8)	
meanNN				0.022*
SR	996 (141)	969 (134)	875 (130)	
AF	1081 (160)	1073 (166)	933 (151)	
DFA α_1				0.002*
SR	0.98 (0.34)	0.91 (0.34)	1.28 (0.38)	
AF	0.78 (0.34)	0.71 (0.36)	0.96 (0.45)	
SymDyn En				0.054†
SR	4.03 (0.79)	4.15 (0.70)	3.33 (0.68)	
AF	4.20 (0.88)	4.01 (0.89)	3.10 (0.70)	
ApEn				
SR	1.17 (0.24)	1.20 (0.20)	0.92 (0.30)	
AF	1.12 (0.29)	1.10 (0.28)	0.84 (0.29)	
Ectopic beats (%)				
SR	0.25 (0.68)	0.24 (0.71)	0.36 (1.58)	
AF	0.42 (0.80)	0.54 (1.37)	0.70 (2.05)	

*Between-group comparisons and †interaction in analysis of variance for repeated measures (general linear model)

6 Discussion

The number of studies utilising the methods of HR dynamics is increasing exponentially (Sandercock et al. 2005). However, the impact of many methodological factors, such as the reliability of the measures, has been poorly evaluated. Therefore, this study focused on its methodological part on the stability of short-term HR dynamics over time in subjects with stable CAD as well as the effects of non-sinus beats on the non-linear HR dynamics analyses. Thereafter, the methods for examining short-term HR dynamics were applied to evaluate the associations between air pollution and cardiac autonomic regulation as well as to predict the risk of postoperative atrial fibrillation after CABG.

6.1 SHORT-TERM HEART RATE DYNAMICS

Analysis of short-term heart rate dynamics

The short-term recordings are feasible for use and convenient for the patient as compared to long-term recordings. Furthermore, they are inexpensive to perform (Kautzner et al. 1998). An important methodological advantage is that the short-term recordings can easily be visually checked for any incorrect R-peak annotations and technical artefacts, as done also in the present studies by experienced technicians.

In Studies I-III the ambulatory ECG recordings were performed with analogous recorders as have most of studies published in recent years. A digital recording would, however, have been more recommendable to avoid possible mechanical difficulties with the tapes. Irregular tape revolution can lead to erroneously high HR variability measures in 24-h recordings (Körber et al. 2000), especially when the recordings do not include a separate time track record (Simula et al. 1998). Most likely the short-term recordings are not as prone to experiencing mechanical difficulties. The analog recordings, however, also demand an analog-to-digital conversion. Previously the sampling frequency of ambulatory ECG recordings has generally been kept low as in the present study, i.e. 128 Hz, to hasten the speed of the analysis and to limit the memory capacity required (Hejjel et al. 2004). A finite sampling frequency might lead to an erroneously high HF component in the spectral analysis (Garcia-Gonzalez et al. 2004). However, the software in the present study used an interpolation method to refine the QRS fiducial point, which is believed to assure reliable results in relation to the low sampling rates (Merri et al. 1990).

The methodological substudies (Studies I-II) had a multi-center approach, which is a challenge for quality control (Yi et al. 2000). Therefore, standard operation procedures as published in http://www.ktl.fi/ultra were established to standardise the methods between the centers. In order, to guarantee the high quality of the ambulatory ECG recordings, the importance of proper preparation of the skin and good contact with electrodes was emphasised during the training of the technicians. All the HR dynamics analyses were performed in one core laboratory whose technicians immediately informed the three research centers about any quality deviations in the ECG recordings. Nonetheless, in 5% of the 40-min recordings included in the conventional HR variability analyses, some of the 5-min periods did not achieve high quality and therefore, also the whole 40-min periods were judged to have to remain omitted from the analyses. Most difficulties for the quality of the

ECGs were encountered during the exercise period. With non-linear HR dynamics analysis, the recordings were analysed also a second time, in fact, the recordings were more carefully checked than normally. This led to more exclusion due to supraventricular ectopic beats. In the last clinical study (Study IV), which assessed the relationships between the HR dynamics and postoperative AF, the recordings were also analysed such that no editing for ectopic beats was performed and in this way, selection bias due to supraventricular ectopic beats was avoided.

Study I used an interpolated tachogram, i.e. a tachogram in which the rejected ectopic beats had been replaced with the interpolation method, in both the time domain and spectral analysis with a fast Fourier transformation method. In the time domain analysis, the raw tachogram without replacement of removed intervals would have been the simplest choice with no artificial information then being added to the signal. Nonetheless, the impact of this effect on the values obtained is most likely to be insignificant (Lippman et al. 1994). In the spectral analysis, the efficient resampling of the tachogram at a high sampling rate would have been the most recommendable choice (ESC/NASPE Task Force 1996). Resampling makes the signal evenly sampled for fast Fourier transformation analysis, but also allows its synchronisation with other physiological signals (Singh et al. 2004). However, this possibility was not needed in the present study. The use of the tachogram without resampling might cause warping of the frequency axis even after rescaling the frequency axis from $\frac{1}{beat}$ to Hz with the average RR interval duration (Bilchick et al. 2006). In addition, inaccuracies might appear in the spectrum especially in relation to large RR interval fluctuations (Singh et al. 2004). However, the use of interpolation for ectopic beats and resampling of the tachogram might also cause errors, i.e. an overestimation of LF power (Clifford et al. 2005). However, it is difficult to assess the effect of such factors in the present study, many kinds of techniques have been used among different studies. There is also a method called Lomb-Scargle periodogram for spectrum calculation, which is suitable for an unevenly sampled signal such as the tachogram. However, this method has not been widely used and is not included in those described by the ESC/NASPE Task Force 1996. In all cases, each method provides only an estimation of the true spectrum and, in fact, even in optimal situations with simulated data, the statistical error in relation to frequency domain measures has been observed to be large when compared to time domain measures (Kuss et al. 2008).

At the moment, there are no such standardised recommendations for non-linear HR dynamics as are for time and frequency domain analysis as given in the ESC/NASPE Task Force 1996. For example, the optimal time period for these recordings is not well defined. Here 40-min analyses were used to assess the effect of ectopic beats and the stability of the measures. The 40-min protocol was planned to simulate the normal daily activities of elderly patients. Therefore, it was intended to mirror the cardiac autonomic responses to changing activity levels, though, in standardised conditions. In Study IV the 10-min standardised periods of different activity were chosen as the best way to provide most stationary data. Furthermore, were HR dynamics to be used as a screening method, bedside analysis would be the preferred choise. The 10-min DFA α_1 and ApEn have been observed to correlate with the 24-h measurements, though the absolute values differ significantly

(Perkiomaki et al. 2001). In addition, both before and after cardiac surgery, the correlations between 15-min and 24-h measurements of DFA α_1 and FD have been strong or moderate (Ksela et al. 2009) and it has been reported that series lengths over or equal to 256 points gave acceptably accurate fractal analyses (Delignieres et al. 2004). However, the length of the recording is always a compromise between the requirement of stationarity of the signal and the time needed to gather the necessary information with the method used.

Most of the non-linear methods have the advantage that they do not have a priori assumptions of the signal properties or dynamics and differ in this respect from the spectral methods that characterise the sinusoidal oscillations (Huikuri et al. 2009). Some of these measures such as ApEn and FD-L depend only vaguely on the absolute variability in the data (Kuusela et al. 2002). However, this does not mean, that these measures are insensitive to unstationarity of the data as is sometimes postulated. For example ApEn is very sensitive to linear trends (Kuusela et al. 2002, Voss et al. 2009). The ApEn calculation is based on the real values in the coordinate and therefore, a trend causes the regular signal to be assessed as more unpredictable than it really is (Kuusela et al. 2002). Therefore, linear detrending was used in Study IV. The trends might also create difficulties for the evaluation of symbol strings in SymDyn analysis (Voss et al. 2009). Missed beats, artefacts and ectopic beats all affect the analysis of DFA, entropy measures and SymDyn (Voss et al. 2009). With short-term recordings, many of these caveats can be avoided by visual inspection of the data.

Occasionally the basis for the analysis might vary between studies, such as the methods used to generate the symbols in SymDyn analysis. These can be based on absolute values or mean and standard deviation. Here the latter option was chosen to provide the measurements, which would not be as dependent on absolute values. Some of the non-linear analyses might be difficult to interpret, such as the shape of the scatterplot in RM analysis or the distribution of the words in SymDyn analysis (Kuusela et al. 2002). Thus, some concurrent and improved ways have been devised to interpret the same analysis, and these might sometimes be confusing. The non-linear HR dynamics can be divided into certain families and in the present studies, the measurements of every family were used (Voss et al. 2009). However, newer methods based on multifractal or multiscale analyses were not included. In general, one difficulty with applications based on the non-linear methods might be the increasing number of new and complex parameters, which would be largely unfamiliar to clinicians.

The short-term recordings in the present Studies I-II and IV were performed under physiologically controlled conditions. Paced breathing is often regarded as the most physiologically stable situation for HR dynamics analysis, but in fact, it might be affected by the ability of a subject to co-operate and the stress caused with breathing to the beat of a metronome signal (Malliani 2005). Grossman et al. (Grossman et al. 2007), however, found the paced breathing to be a simple and reliable way to adjust the measurements for the effects of breathing. The exercise performed in Studies I-II remained lighter than intended, i.e. the HR increased on average from 63.8 to 85.4 bpm. The symptoms during the exercise were experienced only in a few cases (shortness of breath in 9% and chest pain in 3% of the exercises, data not shown). However, most of the subjects had symptoms only on moderate or severe exertion (Table 1). Although the 40-min protocol was much more stable for analysis of HR dynamics than a free-running 24-h period, the problem with 40-min spectral

analysis still was that the spectral measures reflect certain modulations in cardiac autonomic regulation and the averaging of these modulations is regarded as uninformative (ESC/NASPE Task Force 1996).

Relationships of short-term heart rate dynamics to autonomic regulation

The HR dynamics in Study I-II were analysed during rest, paced breathing, standing, light steady-state exercise and recovery in subjects with stable CAD (Table 2). We did not test statistically the differences between the HR dynamics during these periods, i.e. the data is only descriptive.

The HR was lowest during rest periods mirroring the vagal prominence. The HR increased during standing and exercise, which reflects the vagal withdrawal and sympathetic predominance. During recovery, the HR returned to near to the resting level as a sign of vagal reactivation and sympathetic withdrawal.

The paced breathing was performed with 0.2 Hz pacing that corresponds to a breathing rate of 12 times per minute. This breathing rate is rather typical for humans so the pacing of respiratory rate was not expected to cause large changes in HR dynamics. Previously Pinna et al. (Pinna et al. 2006) observed that pacing with 0.25 Hz did not cause significant changes in spectral indices among those subjects, who breathed regularly during non-paced breathing. However, they recommended using pacing to standardise the measurements to avoid spectral leakage of respiration to the LF band.

In the present study, the meanNN did not seem to vary between paced breathing and spontaneous breathing period, which is in accordance with the proposal that the paced breathing did not affect the vagal tone (Hedman et al. 1995). However, some reduction in SDNN and TP was observed most likely mirroring a reduction in the range of RR intervals. The LF/HF ratio was reduced and HF seemed to increase, which might reflect a resurgence of vagal modulation. The changes in tidal volume might have affected the results, even though there are several studies claiming that the effects of paced breathing on tidal volumes are negligible (Pagani et al. 1986, Pinna et al. 2006). This is important because controlling of only the breathing rate is much more feasible than contolling the tidal volume at the same time (Pinna et al. 2006). The r-MSSD or RM SD1 did not capture any change. Previously, the r-MSSD and RM SD1 have been observed to be insensitive to changes in breathing rate (Penttila et al. 2001). The DFA α_1 behaved similarly as LF/HF ratio as expected (Francis et al. 2002). The FD-L did not evoke any changes that could be interpreted as characteristic of sympathovagal alterations during the paced breathing or other periods. In the entropy measures only slight increases occurred during paced breathing.

Standing up slightly increased the HR, however, the HR variability did not decrease as would be expected; the RM SD2 was, however, reduced to some extent. The r-MSSD, HF and RM SD1 seemed to decrease which is most likely a reflection of the reduced RSA during the standing. The only entropy measure analysed, i.e. SymDyn En, seemed to be decreased as has been observed previously in relation to upright tilt (Guzzetti et al. 2005).

The exercise period used was a steady-state regime with a bicycle ergometer, so it was not possible to assess any trends during exercise. It seems that in the present study the most prominent finding was a total power reduction. Total power typically dimishes most at the

beginning of the exercise (Perini et al. 1990), i.e. during low-intensity exercise as in our study. This reduction most likely is attributable to the vagal withdrawal that is the major autonomic mechanism at the beginning of exercise. The DFA α_1 value increased during standing and light exercise in the study of Tulppo et al. (Tulppo et al. 2001), however, in our study, the DFA α_1 seemed to increase during standing but to be reduced during exercise. The present study group, however, consisted of patients with CAD and thus, their responses might differ from those of healthy subjects. Furthermore, if the relationship of DFA α_1 to spectral LF/HF ratio is noticed, the alterations in either high- or low-frequency variability might produce unexpected large changes in DFA α_1 (Tan et al. 2009).

During the recovery that was assessed 5 to 10 minutes after the cessation of exercise, almost all HR dynamics had returned to near the baseline level. Previously, five minutes has been shown to be sufficient to return the HR variablility to control values after submaximal exercise (Perini et al. 1990).

Relationships of SDNN to physical activity

In Study I a high correlation was found between 40-min SDNN and the change in meanNN from rest to exercise mirroring the HR increase during 5-min exercise (Figure 8). Therefore, the physical activity seems to impact on the SDNN measure via HR changes.

Roach et al. (Roach et al. 2004) evaluated the effects of physical activity on the standard deviation of the averages of NN intervals in all 5 min segments of the entire recording (SDANN) that is known to display a high correlation with SDNN (Malik 2004). They observed that the SDANN increased as the range of RR intervals increased due to physical activity. The periods during which there were the largest deviations in meanNN from the average meanNN contributed most to the SDANN value. For example, in the controlled situation, a 10-min treadmill walk contributed to the 4-hour SDANN by 8.0 ms/5 min whereas the 90-min supine rest added only 1.2 ms/5 min. Their main aim was to evaluate why the patients with impaired ventricular function had such low SDANN values. They observed the same coefficient of variation among patients and healthy subjects in controlled situations, thus, the higher HRs among patients led to reduced SDANN. However, under uncontrolled conditions, the dimished SDANN was related not only to higher HRs but also to a reduced range of physical activity.

Hautala et al. (Hautala et al. 2010) did not detect any correlation with 30-min SDNN when they simultaneously measured different levels of physical activity but a correlation between mean HR, RM SD1, SaEn and physical activity was found. Here the relationship between other measures than SDNN and changes in HR during the exercise was not analysed. In short-term recording, the SDNN is, however, linked to high-frequency variation and, thus, the present finding might be in accordance with the study of Hautala et al. who did detect assocations between RM SD1 and physical activity.

Here there was no comparison made between the actual level of activity producing the responses in HR. However, one can say that in this kind of protocol, even a light 5-min exercise causes the RRI intervals to deviate from the average meanNN so clearly that it determines the SDNN. Thus in the light of this and other observations (Osterhues et al. 1997, Roach et al. 2004), it seems that the effects of functional capabilities might be one factor behind the predictive capacity of SDNN.

Relationship of short-term heart rate dynamics to normal values

There are unexpectedly large variations in the average HR dynamics values in the different studies (Sandercock 2007). Therefore, it has been recommended that new HR dynamics measurements should always be compared to the average values from large-scale studies (Sandercock 2007) since this would be one way to assure the accuracy of the analysis and also make the findings of the studies more practical.

The most comparable reference study to the present study is that of Sinnreich et al. (Sinnreich et al. 1998). They presented the average values in unselected 294 people aged 35-65 years during 5-min spontaneous and paced breathing at supine rest. The values obtained were lnr-MSSD 3.3, lnTP 6.5, lnLF 5.3 and lnHF 5.0 during spontaneous and lnr-MSSD 3.3, lnTP 6.3, lnLF 5.0 and lnHF 5.1 during paced breathing, which seem to be rather near the values obtained in this study (Table 2). Unfortunately, Sinnreich et al. did not present the meanNN or raw measures of HR variability, which would make the comparisons even more appropriate. However, the HR variability seems to be rather similar although the present study population did not consist of healthy subjects but had patients with CAD and previous AMI in 66% of cases (Table 1). In fact, the present values were somewhat higher than correspoinding values obtained in the study of Kuo et al. (Kuo et al. 1999), where lnLF was 4.60 ± 0.03 (mean ± standard error) and lnHF 4.06 ± 0.04 during the 5-min rest in 1140 healthy persons. However, the HR in that study was higher, i.e. meanNN was 792 ± 4 ms corresponding to HR 75.8 bpm, which most likely explains the difference. A large proportion of the subjects in the present study, i.e. 60%, had beta-blocking medication (Table 1), which might have affected the HR and HR variability. The beta-blockade is known to be associated with preserved HR variability (Feldman et al. 2010).

There are very few population-based studies concerning the normal values of non-linear HR dynamics. Pikkujämsä et al. (Pikkujamsa et al. 2001) studied a random population of 389 healthy middle-aged people during 13-min supine rest and sitting. The DFA α_1 during supine rest in that study was 1.15 ± 0.20, whereas here values of 1.05 ± 0.33 were obtained. In Study IV, which assessed the predictive capability of preoperative non-linear HR dynamics to postoperative AF, the DFA α_1 was 1.13 ± 0.24 among those who remained in sinus rhythm postoperatively, i.e. practically the same as in the random population in the study of Pikkujämsä et al. (Pikkujamsa et al. 2001).

Correlations of short-term heart rate dynamics between each other

Though increasing numbers of different methods are available to analyse the HR dynamics, there is the possibility that many of the measures refer to the same physiological or pathophysiological information despite the different algorithms used (Maestri et al. 2007). Therefore, possible intrinsic relations need to be evaluated.

In the present study in subjects with stable CAD the meanNN had a high correlation only with RM SD2 (Table 3 and 4). Kuo et al (Kuo et al. 1999) observed also that no spectral measurements correlated with meanNN in healthy subjects. This is somewhat unexpected in the light of the study of Zaza and Lombardi (Zaza et al. 2001) observing a close connection between acetylcholine, meanNN and its variability at the cellular level. The SDNN showed a very high correlation with total power during 5-min rest as expected (r=0.92), but for some reason not during the 40-min recording (r=0.59). The most likely

explanation is that the non-stationarity of the 40-min protocol somehow affected the spectral measurements. Otherwise, the correlation with the 40-min SDNN and other HR variability remained somewhat lower than the level in the 24-h recordings in the study of Bigger et al. in patients after AMI (Bigger et al. 1992). However, as in that study, the correlations between TP and VLF (r=0.87) were very strong. In addition, the r-MSSD and HF (r=0.80) can be interpreted as reflecting the same properties also in the present study. During 5-min recordings, the TP displayed some weaker correlations with LF and HF than in the study of Kuo et al., however, the LF/HF ratio did not exhibit any strong correlations with absolute frequency domain measures in either of these studies.

The RM SD1 and SD2 during 40-min were positively correlated with each other (r=0.90) and with many linear measures such as r-MSSD (r=0.74 and 0.70) and HF (r=0.61 and 0.60). The relationship between RM SD1 and HF variability is known and mathematically explained (Brennan et al. 2001). However, the very close relation with RM SD1 and SD2 as noted in this study was unexpected. In long-term recordings, the length of the scatterplot has been related to long-term HR variability; it most likely reflects the variation from highest to lowest values (Copie et al. 1996, Maestri et al. 2007). However, it seems, that in this 40-min protocol, the short-term variability overwhelms the long-term variability and accounts for the high intrinsic correlation. However, in 5-min recordings in Studies I-II and in 10-min recordings in patients with severe CAD in Study IV (Table 5), the intrinsic correlations of RM SD1 and SD2 were strong, but not as high as observed during the 40-min recordings (r=0.65 in both studies).

The entropy measures ApEn, SaEn or SymDyn En did not correlate strongly with meanNN or any conventional HR variability measures suggesting that the complexity measures describe different features in HR dynamics than the spectral analysis methods in this patient population. This finding is, however, in accordance with the study of Perkiömäki et al. (Perkiomaki et al. 2002) who did not observe significant correlations between ApEn and conventional HR variability in healthy subjects.

The DFA α_1 during 40-min recordings had only very weak correlations with linear indices other than r-MSSD (r=-0.45), HF (r=-0.31), RM (SD1 r=-0.66, SD2 r=-0.53) and LF/HF (r=0.54). Thus, it seems that the DFA α_1 displayed an inverse correlation only with the parameters characterising vagal regulation in subjects with CAD. Perkiömäki et al. (Perkiomaki et al. 2002) observed the DFA α_1 to correlate inversely with SDNN, r-MSSD and HF power but also positively with HR (r=0.76) in healthy subjects. Furthermore, in the study of Maestri et al. (Maestri et al. 2007), though assessing 24-hour HR dynamics, there were moderate correlations with DFA α_1 and SDNN, LF and VLF. These differences are most likely related to the different patient populations. Maestri et al. performed their study among subjects with heart failure and this is known to remarkably affect the LF power (La Rovere et al. 2003). The DFA α_1 has a close theoretical relation to spectral ratios (Francis et al. 2002) and therefore, the alterations in either low- or high-frequency variability might affect the DFA α_1 (Francis et al. 2002). The SymDyn FW had moderate negative correlations with r-MSSD and RM measures during 40-min recordings, i.e. it seems that this measure displayed inverse relationship to high-frequency HR variability in this patient population.

The non-linear HR dynamics showed correlations also within each other. Not unexpectedly, the entropy measures ApEn, SaEn and SymDyn En seemed to reflect the

same physiological or pathophysiological factors. However, also FD-L showed at least moderate positive correlations with these measures (Tables 3 and 4). The SymDyn FW correlated very strongly in an inverse manner with SymDyn En, during 5- to 10-min recordings. In patients with severe CAD (Study IV), the DFA α_1 correlated very strongly with SymDyn measures, which was not the case in elderly patients with stable CAD (Study I-II). Therefore, it is clear that the intrinsic correlations differ in different patient populations. Generally, the intrinsic correlations of non-linear HR dynamics seemed to be stronger during the 40-min than the 5-min recordings, which might indicate that the external stimuli such as physical activity changed the values towards the same direction in 40-min recordings.

6.2 STABILITY OVER TIME OF SHORT-TERM HEART RATE DYNAMICS

The aim of the present study was to characterise the stability of both conventional and non-linear HR dynamics measures in subjects with stable CAD. The scope was the absolute reliability (Atkinson et al. 1998, Hopkins 2000), i.e. within-subject variability of repeated measures over a longer time span. There were multiple consecutive measurements and therefore, the calculation of coefficient of variation and its confidence intervals were selected as most appropriate statistics. These CV calculations were applied to compare the stability of different HR dynamics measurements not only in the present study but also to permit comparison against measurements from other studies. In addition, they can be applied for sample size calculations. In fact, the evaluation of the reliability of a new method such as non-linear HR dynamics should be the first analytical task before these methods are used more widely.

Most 40-min conventional HR variability measures obtained CV values under 10% (Table 6). The CV for 40-min SDNN was somewhat higher, i.e. 16.7%. Unexpectedly, the stability of 5-min HR variability measurements remained at a less satisfactory level; e.g. the CV for SDNN during paced breathing was 37.1%. The stability of 40-min non-linear HR dynamics was the same or even better than the stability of conventional HR variability measures when SymDyn, FD-L and logarithmically transformed RM were considered, i.e. all of these parameters obtained CVs equal to or under 10%. The DFA α_1 was as stable as conventional HR variability measurements (CV 16.5%), but the stability of ApEn remained somewhat lower (CV 21.9%).

There are very few studies assessing the stability of HR dynamics that fulfil the recommendations that the sample size should contain at least 50 individuals with three or more repeated measurements (Hopkins 2000). In the study of Sinnreich et al. (Sinnreich et al. 1998) where there were duplicate measurements with a two months interval in 70 subjects, the CVs remained under 12.1% for 5-min frequency domain measures analysed during spontaneous and paced breathing whereas the present values for seven measurements over three to four months remained less than 16.5%. The CVs for r-MSSD in their study remained under 8.0% and in the present study they were less than 12.7%. They calculated the CVs for logarithmically transformed SDNN, which explains the large difference between the obtained values (6.1% vs. 37.1% during paced breathing). Generally, however, the SDNN has been used without logarithmic transformation. The somewhat

better stability in the study of Sinnreich et al. is most likely related to their population of healthy persons whereas here the patients suffered from stable CAD. Previously, the stability of HR variability has been lower among subjects with cardiopulmonary diseases than in healthy subjects (Salo et al. 1999). In the study of Maestri et al. (Maestri et al. 2010) of 61 post-AMI patients with a one-day interval, the value of the logarithmically transformed SDNN was observed to be the most reliable measure, which is in contrast to the present study. However, also the SDNN exhibited extensive day-to-day within-subject variation (CI for random variation –41-61%) in the study of Maestri et al. and therefore, they concluded that the short-term HR variability is not able to clinically monitor the state of the patient.

The lower stability for 5-min than 40-min conventional HR variability was unexpected. One would predict that the 5-min paced breathing period should be the most physiologically stable period during the 40-min protocol simulating normal daily activities of elderly subjects. The finding of better stability of 40-min HR variability could, however, be related to the observed close relation between SDNN and exercise level (Figure 8). The exercise level was kept constant during the repeated measures. Does this, in fact, mean that long-term HR variability loses its ability to accurately mirror subtle changes in cardiovascular state due to these close relationships with the changes in activity?

Pinna et al. (Pinna et al. 2007) recommended that the reliability measurements should be performed close to each other to obtain the estimate of the random error. Pinna et al. performed the measurements with a one-day interval. Sometimes it is recommended that the reliability measurements should be performed during a single day, but in that situation, the circadian variation might affect the assessements (Atkinson et al. 1998). However, the most accurate assessments for random error of short-term HR dynamics would be obtained from adjacent measurements. Freed et al. (Freed et al. 1994) evaluated the measurement error of two adjacent 10-min frequency domain measurements at rest in elderly patients waiting for subsequent surgical operation: the CVs varied from 9% (Total Power) to 15% (LF). Thus, the level was surprisingly similar to those in the present study with a long time span, but again the different study population might explain the finding. The time frame of three to seven months in the present study was, however, large and therefore, the CVs contained the physiological variation typical in elderly patients with stable CAD such as myocardial ischemia. In fact, the physiological variation most likely represents the largest part of the instability of the measures, though technical features are unavoidably mixed with physiological variation in the analyses. The technical inaccuracies include observer dependent errors such as mistakes in the detection and handling of artefacts and the selection of which time intervals should be included in the analyses. Though, the interobserver reproducibility in the present study with conventional HR variability was high (CVs 0.1-1.2% for 40-minute and 0.2-6.0% for 5-minute HRV, data not shown). Another technical factor evaluated in Study II was the effect of different editing practises of ectopic beats, which was observed to be significant for the stability of several of the non-linear HR dynamics measures. Nonetheless, both the physiological variability and technical errors can be assumed to affect the method's ability to detect real changes (Gluer et al. 1995).

The results in the present study can be applied for the sample size evaluation in a follow-up study of a comparable duration with a similar patient group (Hopkins 2000). The

sample size is proportional to the square of the absolute reliability statistics (standard error of measurement or CV) (Hopkins 2000, Lehr 1992). Therefore, according to the formula presented by Lehr (Lehr 1992) for the same effect size one would need 3.4 times larger sample size with 40-min SDNN than with SymDyn En (upper limit of 95% CI for CV 17.8 vs 9.6%) for each sample. Therefore, the reliability of the measurement parameter has a remarkable impact when the experiment is being planned. Even in this case, the obtained sample size is regarded as a minimum and e.g. the effects of possible differences in equipments or characteristics of the patients should be considered (Hopkins 2000). Furthermore, in intervention studies, the intervention might cause different responses in different participants, which might impact the previously assessed reliability of the measurements (Hopkins 2000). However, a serious limitation in any sample size calculation is the lack of normative values of HR dynamics. This makes it difficult to reliably estimate clinically relevant effect sizes, which are needed for sample size calculations (Sandercock 2007).

Before these findings are applied to other studies, it should be noted that they only characterise the stability of short-term HR dynamics among elderly patients with stable CAD. In the present patient population, there were more males than females, a large proportion of the patients had undergone a coronary intervention and was receiving beta-blocker medication, i.e. in fact, they represented a rather typical sample of patients with CAD. However, diabetics requiring insulin treatment had been excluded because diabetes has a major effect on autonomic nervous regulation. Furthermore, smokers were excluded because the smoking would have interfered the aim of the ULTRA study to assess the effects of ambient air pollution. In these respects, the patient population cannot be regarded as typical.

Although this study had a large population and many repeated measurements, there are also some limitations. First, we could have expressed our findings in actual units, but the ratio scale had the advantage to make comparisons between different parameters. Second, the possibility of systematic bias, e.g. due to the learning effect, was not assessed. In addition, formulas for the analysis of spesifically short-term precision were used, which might have caused some overestimation of the instability of HR dynamics. The normality was assessed the most common way, though the analysis of the normal distributions of several repeated measures done in a single individual would be a difficult task. The possible heteroscedasticity of the data was not specifically evaluated. Heteroscedasticity defines the phenomenon where a larger error is related to larger values (that is also a basis for the use of CV) but also to some subgroups such in the present study the patients receiving and not receiving beta-blocker medication, leading to different magnitude of measurement error (Hopkins 2000). In the test-retest situation, the evaluation of heteroscedasticity could be done with Bland-Altman plots, but it is not so simple with multiple consecutive measurements (Atkinson et al. 1998). Especially the finding of the instability of SDNN might be partly caused by heteroscedasticity that could have been corrected with logarithmical transformation. This could also explain the discrepancy between the present study and that of Pinna et al. (Pinna et al. 2007) in healthy persons and Maestri et al. (Maestri et al. 2010) in patients after AMI. In those studies, the SDNN was

observed to be most reliable measure of 5-min conventional HR variability in the test-retest situation.

In conclusion, most short-term conventional and non-linear HR dynamics measures in the present study seemed to be stable over a period of few months. However, the stability of SDNN, but also ApEn and DFA α_1 remained at a lower level, which might reflect the influences of variation due to pathophysiological state e.g. due to myocardial ischemia in this specific study population with CAD. These stability evaluations could be applied especially for the assessment of the sample size in a similar patient population in a comparable study.

6.3 ECTOPIC BEATS IN THE ASSESSMENT OF SHORT-TERM NON-LINEAR HEART RATE DYNAMICS

The main finding of the present study was that measurements of non-linear HR dynamics were sensitive to the presence of non-sinus beats in the recordings. The editing corrected the effects of ectopic beats on absolute values and also, improved the stability over time of these measurements.

Effect of ectopic beats on non-linear heart rate dynamics measurements

The present study evaluated the effects of real ventricular and supraventricular beats on non-linear HR dynamics measurements. A strong effect on the distributions of non-linear HR dynamics measurements was observed in relation to small amount of non-sinus beats. The small number of non-sinus beats is explained by the same inclusion criterion as used in many clinical studies, i.e. less than 10% of non-sinus beats in the recordings. The values of RM, DFA α_1 and SymDyn FW correlated linearly and strongly with the number of ectopic beats (Table 7). Howewer, the entropy measurements seemed to be more stable and only weak correlations were observed.

The effects of non-sinus beats on non-linear HR dynamics have not been extensively studied. In the study of Vikman et al. (Vikman et al. 1999), however, the ApEn displayed a peculiar behaviour in relation to added ectopic beats in RR interval data. The ApEn progressively decreased with an increasing number of non-sinus beats, however, in situations with very few ectopic beats or ectopic beats with varying coupling intervals, the effect could be the opposite. In the present study, the distribution of ApEn did not seem to vary with the editing of non-sinus beats, however, in Bland-Altman plots, large changes were observed in some measurements (Figure 9). The varying effect of non-sinus beats on ApEn and possibly SaEn is most likely explained by the criterion for similarity r in the analysis that is based on the standard deviation. The standard deviation is affected by the large RR interval variations (short coupling interval and long compensatory pause) in relation to existing non-sinus beats. Therefore, commercial software should allow the r to be calculated from a period not containing non-sinus beats.

The DFA α_1 values have been observed to be reduced in relation to non-sinus beats (Peltola et al. 2004, Vikman et al. 1999) and this kind of a reduction was observed here as well. In fact, in Study IV, during short-term stable periods, it was noted that even a single non-sinus beat could reduce DFA α_1. The only exceptions to this rule were due to the

consecutive atrial ectopic beats or interpolated ventricular beats, which occasionally led to higher DFA α_1 values than obtained from edited data (data not shown). In fact, there were 28 recordings with non-sinus beats and 43 recordings with pure sinus beats during the spontaneous breathing. Without editing, the DFA α_1 values were 0.67 (0.21-1.37) whereas with the removal of ectopic beats the DFA α_1 was 1.03 (0.44-1.60). With pure sinus beats, the DFA α_1 values were 1.08 (0.36-1.60). Therefore, it seems that the values attained with editing of ectopic beats were close to those expected. Furthermore, the effect of ectopic beats was strong when compared to the effect caused by paced breathing or upright tilt (Table 11) and this would likely be able to obscure the findings of physiological alterations in cardiac autonomic regulation.

Peltola et al. examined that the effect of non-sinus beats on DFA α_1 varied between healthy subjects and patients, most likely due to different baseline levels of HR variability (Peltola et al. 2004). Thus, the effect of ectopic beats would be difficult to predict. A reduction in DFA α_1 values, i.e. a breakdown in fractal dynamics, has been related to an increased risk for cardiovascular events (Makikallio et al. 1997, Vikman et al. 1999) or mortality (Huikuri et al. 2000, Tapanainen et al. 2002). In relation to risk stratification, Peltola et al. proposed that the recordings might not need to be edited. In study IV, the non-edited measurements also predicted the postoperative AF successfully. Naturally, if it were possible to omit the troublesome editing, this would make the HR dynamics more suitable for use. However, in risk stratification, one should be able to identify the pathophysiological factors increasing the risk. Therefore, if the HR dynamics simply reflect the already well-established risk associated with a high number of ectopic beats, they might not be providing any relevant new information. However, it has been suggested that ectopic beats should be included in the analysis as such because they represent the real dynamics of HR (Huikuri 2008). It is, indeed, a fascinating possibility that the ectopic beats might simply increase the risk of cardiovascular events due to the breakdown of fractal properties or reduced complexity of cardiac autonomic regulation (Huikuri 2008). A practical solution to this question could be to express the non-linear HR dynamics both with and without editing of the ectopic beats.

The strong effect of ectopic beats on RM is explained by its nature as a HR variability measure. The premature RR interval preceding and the compensatory pause following the non-sinus beats create higher beat-to-beat variability and therefore, lead to higher return map values. This same phenomenon increases the range and standard deviation of the RR interval data and thus, might reduce the number of different patterns obtained in the SymDyn analysis which might be interpreted as reduced complexity (Guzzetti et al. 2005). In the present study, however, the non-sinus beats had only minor effects on SymDyn En, although the SymDyn FW was more extensively affected, but to an unexpected direction.

Effect of different editing methods

The effects of different editing methods on non-linear HR dynamics have been evaluated in only very few studies. Peltola et al (Peltola et al. 2004) compared different methods in relation to DFA analyses. They noted that the interpolation method in which the ectopy related RR intervals were replaced with a local average of previous sinus intervals seemed to perform most reliably. However, when the number of premature beats was under 10%,

the differences between the editing methods remained small. However, with the deletion method it seemed that the effect of editing on HR dynamics changed its direction in relation to the variations in the baseline HR dynamics. In the present study, the interpolation also altered the DFA α_1 values more consistently upwards than the deletion method (Figure 9). There are also additional problems with the deletion method such as the sudden changes in the RR interval signal caused by the removal of intervals, corruption of cumulative time making impossible the synchronisation with other physiological signals and the data loss. One important expected difficulty with the deletion method is the phase shift affecting the oscillatory patterns in the RR interval tachogram. However, in some studies, the effects on spectral analyses that estimate these oscillatory patterns have remained smaller than expected (Lippman et al. 1994). With interpolation methods, there are no such sudden changes caused in the signal and the effects on oscillatory patterns are smaller than with deletion method. This means that the interpolation could be most preferred choice for editing. On the other hand, interpolation has caused a low pass filtering effect in the spectral analysis (Salo et al. 2001) and, thus, when the relationships between DFA and frequency domain analysis are considered, it might be that the interpolation is also not a perfect method, especially when the number of non-sinus beats is high. In the present study the deletion method seemed to perform most efficiently with respect to many of the non-linear HR dynamics measurements. The deletion method included the morphological recognition of ventricular beats and in practice, the preceding and following RR intervals next to ventricular beats were deleted whether they were premature or not. The definition of prematurity is always arbitrary and leads to exclusion of normal breathing related variability and also, fails to recognise all non-sinus intervals. Therefore, the included morphological annotations might partly explain the superiority of the deletion method.

The editing methods thus far have covered only the nearest RR intervals in relation to the ectopic beats. However, the well known longer lasting effects of non-sinus beats, called HR turbulence, raises the question of the optimal number of RR intervals to be edited (Huikuri 2008). However, it could be expected that the effect of immediate large fluctuations caused by ectopic beats has a greater impact on the values of HR dynamics than the HR turbulence. In fact, in one study with 24 h recordings, the editing of HR turbulence also did not significantly change the DFA α_1 values (Peltola et al. 2011). Furthermore, the deletion or any kind of editing of 6-20 intervals naturally is quite a serious interference with the data and possibly increases the significance of single ectopic beats in the HR dynamics analysis. However, the effect of HR turbulence on the HR dynamics is a virtually unexplored topic and is worthy of future evaluations.

The effects of editing of ectopic beats on the stability of the non-linear HR dynamics were also evaluated (Figure 10). As expected, editing improved the stability of those measures that had strongly been affected by non-sinus beats such as DFA α_1, RM SD2 and SymDyn FW. However, the stability of FD-L also improved although the effects on the absolute values of FD-L had been minor.

In conclusion, the presence of ectopic beats altered the non-linear HR dynamics remarkably when compared to the physiological alterations. The effect of non-sinus beats was unpredictable in relation to some measures such as approximate entropy. Furthermore,

most stable measurements over time were obtained with properly edited recordings. These findings mandate the editing of non-sinus beats in relation to HR dynamics analyses especially when the purpose is to characterise the cardiac autonomic regulation. However, it would be important to standardise the editing practices within and between the studies and consider the possibility to express the values obtained with and without the editing. The deletion of intervals closely related to both ventricular and premature supraventricular beats performed most efficiently in the present study and seemed to be that most recommended. However, the deletion method cannot be considered as a perfect method, due its inherent shortcomings such as data loss and distortion of synchronisation to other physiological signals.

6.4 ACUTE EFFECTS OF CARBON MONOXIDE ON SHORT-TERM HEART RATE DYNAMICS

In the present study acute CO exposure was related to altered short-term HR variability in elderly subjects with stable CAD. More precisely, the personally monitored CO exposure >2.7 ppm associated with acutely increased 5-min r-MSSD (Table 9), which was considered to reflect increased vagal modulation. MeanNN did not show any significant change which excludes that the change in HR would explain the observed finding. However, there was no significant change in SDNN and thus no effect was observed in overall HR variability.

The aim of the present study was to evaluate the acute response to personally monitored CO exposure. Thus, there was a focus on CO exposure that has recently not been as intensively studied as the effects and pathophysiological mechanisms of PM pollution. However, Burnett et al. (Burnett et al. 1998) stated that in an ambient air pollution mixture, the daily CO virtually alone explained the increased mortality with the PM contributing only some additional risk. The significance of CO pollution was shown again in a large European database of APHEA2, in which the daily CO level independently associated with total and cardiovascular mortality (Samoli et al. 2007). In the review of Maitre et al. (Maitre et al. 2006) CO, PM and nitrogen oxides were concluded to be those pollutants that have impact on CAD. In the large study of Stieb et al. (Stieb et al. 2009) a same-day 0.7 ppm increase in CO augmented the risk of hospitalisation for ischemic events by 2.6% (95% CI 0.0-4.2%). In another large study of Bell et al. (Bell et al. 2009), a 1 ppm increase in the same-day 1-hour maximum CO increased the risk for hospital admission due to cardiovascular disease by 0.96%; the risk was observable even at CO levels <1 ppm. Thus, the pathophysiological cascades originating from low level CO exposure among patients with CAD as examined in the present study are relevant subjects for study.

The present study differed from its predecessors also in that its focus was on the acute effects, i.e. the response appearing within a few minutes from the beginning of CO exposure. The relationships between CO and cardiac autonomic regulation have been studied previously, but with longer time lags. Dales (Dales 2004) observed the daily increased CO levels as well as average $PM_{2.5}$ simultaneously to increase the SDNN in patients with CAD, but only if the patients were not being treated with beta-blocker medication. Liao et al. in 2004 found reduced short-term HR variability in relation to daily increased levels of multiple ambient pollutants (PM_{10}, CO, nitrogen dioxide, sulfur dioxide)

especially in subjects with cardiovascular diseases. Min et al. (Min et al. 2008) found decreased 5-min SDNN and HF values 25-48 h after CO exposure in subjects with metabolic syndrome, whereas in subjects without metabolic syndrome, there were no associations. Chuang et al. (Chuang et al. 2007) observed that the daily variations in CO level did not affect the inflammative, oxidative, prothrombotic factors or HR variability measurements in healthy students. Also in the study of Park et al. (Park et al. 2005) though there were correlations between other daily and hourly increased pollutant levels and HR variability, the associations with CO remained completely negative. Thus, the effects of daily increased CO on cardiac autonomic regulation in these studies seem to be divergent and related to most likely to patient characteristics, baseline health, medication and even to other modulating factors not yet recognised.

Very few studies have evaluated the immediate effect of CO with personal monitors. Riojas-Rodrígues et al., however, studied the relations between personally monitored $PM_{2.5}$ and CO and simultaneous continuous 5-min HR variability assessments in patients with previous AMI (Riojas-Rodriguez et al. 2006). Thus, their study protocol was rather comparable. Both studies had a patient group with severe though stable CAD, but the proportion of patients receiving beta-blocking medication was somewhat higher, i.e. 76% in the study of Riojas-Rodrígues et al. vs. 50% in the present study. The baseline level of CO was higher in their study, i.e. the average 5-min CO exposure was 2.9 ppm throughout the 11 hours of the recording period. They observed the $PM_{2.5}$ to associate with decreased HF whereas the CO was related to diminished LF; the HR was adjusted in the analyses. With $PM_{2.5}$, the effect was similar whether or not this was analysed simultaneously or with a 5-min lag, but with CO, the clearest effect was observed simultaneously and weakened strongly with a 10-min lag. In the present study, the time-interval from the beginning of CO exposure to 5-min HR variability period was two minutes. The observation in relation to $PM_{2.5}$ in the study of Riojas-Rodrígues et al. can be considered as expected and to reflect a withdrawal of parasympathetic modulation. However, the decrease in LF in relation to the CO exposure is more difficult to explain, since the LF is considered to be affected both by sympathetic and vagal activity. However, Hausberg et al. (Hausberg et al. 1997) conducted a controlled human exposure study and found that CO exposure evoking a modest increase in carboxyhemoglobin level (8.3%) did not exert any acute sympathetic effects such as an increase in HR, blood pressure, muscle sympathetic nerve activity or forearm blood flow in healthy subjects. The increase in r-MSSD found in the present study is suggestive of increased vagal modulation and is therefore not consistent with the study of Riojas-Rodrígues et al.

The CO is an exceptional pollutant as its physiological effects with high-level exposures are well known (Samoli et al. 2007). CO is a tasteless, odourless and colourless toxic gas. After being absorbed through lungs, it forms carboxyhemoglobin and this impairs the oxygen carrying capacity of erythrocytes and affects the delivery of oxygen to tissues (Samoli et al. 2007, Townsend et al. 2002). Thus, not only brain but also the myocardial oxygen supply might be compromised. In fact, CO has been observed to shorten the time before exercise-induced angina pectoris and ischemic ST-depressions when the carboxyhemoglobin level has been elevated from 1.5 to 3% during an acute controlled CO exposure (Kleinman et al. 1989). In order to reach such carboxyhemoglobin levels, e.g. 2.5%,

a 26.1 ppm exposure for 1 hour is needed (Townsend et al. 2002). At such high CO exposures, the hypoxemia is the primary pathway affecting cardiac function though the possible effects on thrombus formation and vasoconstriction might supplement the hypoxia (Baccarelli et al. 2007, Mittleman 2007). CO exposure has also been related to oxidative stress and inflammation although the evidence is scanty and partly conflicting (Chuang et al. 2007, Delfino et al. 2008). Thus, though the association between very low-level CO exposure and cardiovascular morbidity has been demonstated (Bell et al. 2009), the pathophysiology behind this association remains poorly understood (Townsend et al. 2002).

In the study of Riojas-Rodrígues et al. (Riojas-Rodriguez et al. 2006), the personally monitored $PM_{2.5}$ and CO displayed high interrelationships. No particle data was available in the present study, which is a limitation. However, it is known that there are strong correlations between pollutants such as fine particles, nitrogen oxides and CO derived from combustion (Delfino et al. 2008). In the present study, most of the higher CO peaks (77%) were related to the individual's presence in traffic. Thus, one cannot exclude the possibility that the observed finding would reflect the effects of some other pollutant or a mixture of several pollutants derived from traffic or other factors related to traffic, such as noise or psychological stress. However, the stress would most likely have caused increase in HR and this was not observed. Previously, a trend of increased HF and decreased LF/HF ratio mirroring a vagal effect has been observed among healthy subjects during diesel exhaust exposure (Peretz et al. 2008). However, these findings are contrary to what would be expected to be detrimental to patients with cardiovascular diseases although at least theoretically, a vagal effect could predispose to cardiac electrical instability (Chen et al. 2007). For some to the pollutants other than CO, such as PM or sulfur dioxide, direct irritation of the pulmonary tissues could be a potential mechanism leading to symptoms in the respiratory tract, central modulation of breathing and cardiovascular system (Brook et al. 2010, Tunnicliffe et al. 2001). Therefore, the continuous monitoring of respiratory parameters such as respiratory frequency and tidal volume in ambulatory conditions might improve our understanding of the possible mechanisms of ambient air pollution. Hopefully in the future this will become technically feasible.

The personal measurement of pollutant levels is a new method to characterise the exposure of an individual. Until now, especially the definition of causal mechanisms behind the deleterious effects of ambient air pollution have been limited due to the inexact measurements of exposure (Mills et al. 2009). In particular, the concentrations of pollutants derived from traffic vary highly over short distances (Adar et al. 2007) and for CO, this is especially important, i.e. personal monitorings conducted in the city of London identified local traffic to be the most important source for personal exposure to CO (Kaur et al. 2009). Thus, part of the effects of very low-level exposure related to mortality and cardiovascular morbidity might be, in fact, explained by the differences between measured ambient exposure levels and real personal exposures. This could be the case if an individual is living near to major roads, where the CO levels will be much higher than the ambient background levels (Townsend et al. 2002). In addition, personal activities naturally affect the real exposure to pollutants (Kaur et al. 2009). The personal measurements are a more laborious and costly way to assess the pollution exposures. However, they could be utilised in

representative samples of individuals and applied in dynamical models to assess the real exposure of the general population (Jerrett et al. 2005).

The major limitation of the present study was the small size of the study population. However, the number of CO exposure periods was large enough to allow appropriate statistical analyses. The clinical history of the patients with angiographically verified CAD was well characterised and the patients formed a rather homogenous group representing a potential risk group for ambient air pollution. In fact, this is the first study to utilise the personal monitoring of air pollution to evaluate the effects of acute air pollution on cardiac autonomic regulation. Therefore, larger studies on the acute effects of both carbon monoxide and other relevant air pollutants will need to confirm these findings. However, this study does suggest that the assessment of HR dynamics might be useful when one wishes to evaluate the effects of ambient air pollution on cardiovascular regulation.

6.5 POSTOPERATIVE ATRIAL FIBRILLATION AND HEART RATE DYNAMICS

One novel finding in the present study was a constant preoperative reduction in DFA α_1 reflecting a breakdown in fractal RR interval dynamics, and the increased complexity of RR intervals during spontaneous breathing in patients experiencing postoperative AF after CABG (Table 11). Both of these preoperative short-term measurements predicted the occurrence of postoperative AF independently from other clinical risk factors.

Previous studies utilising short-term HR dynamics have observed transient alterations shortly before the onset of postoperative AF after cardiac surgery (Amar et al. 2003, Hogue et al. 1998). However, the present study aimed to assess whether the HR dynamics are altered already preoperatively and whether these alterations provide information for the prediction of postoperative AF after CABG. The previous study using conventional HR variability measurements among the same study population did not support their usefulness for risk-stratification (Hakala et al. 2002). However, the new non-linear measurements characterising the fractal properties and complexity of RR interval dynamics were postulated to possibly reveal the kinds of abnormalities that are not observed with the conventional HR variability analyses.

The occurrence of postoperative AF among the present study population, i.e. 28%, was at a typical level. The incidence of postoperative AF has remained constant or might even be increasing due to older age of patients now undergoing the CABG surgery (Creswell et al. 1993, Mathew et al. 2004). In the present population, the well-known risk factors such as advanced age, higher body mass index and chronic obstructive pulmonary disease were more common among those patients developing postoperative AF (Table 10). However, altered non-linear HR dynamics was a predictor of postoperative AF independently of these clinical risk factors.

The reduction in fractal RR interval dynamics that is captured by lower DFA α_1 is now considered an interesting phenomenon due to its clinical associations. The reduced DFA α_1 has been proposed to predict the cardiac mortality among the general elderly population (Makikallio et al. 2001). It has also predicted mortality after AMI better than the conventional HR variability measures among patients with preserved and lowered left

ventricular function (Huikuri et al. 2000, Makikallio et al. 2005, Tapanainen et al. 2002). Furthermore, reduced DFA α_1 associates with different kinds of morbidity after CABG (de Godoy et al. 2009). In ambulatory recordings, a transient reduction in DFA α_1 has preceded the onset of paroxysmal AF in patients without structural heart disease (Shin et al. 2006, Vikman et al. 1999) and importantly, a constantly reduced DFA α_1 in 24-h recordings has predicted the new-onset AF after AMI (Jons et al. 2010)

The major pathophysiological mechanism in cardiac autonomic regulation predisposing to postoperative AF has been claimed to be excessive sympathetic stimulation due to existing severe heart disease and the stressful perioperative state (Coumel 1994). The benefit obtained from continuous beta-blocker medication supports this proposal. However, the effect of beta-blockers has, in fact, remained limited even though the importance of the intravenous administration route has been recognised (Halonen et al. 2006). The experimental studies convincingly emphasise the significance of the synergistic role of vagal and sympathetic cardiac regulation in the initiation of AF (Sharifov et al. 2004, Tan et al. 2008). However, the HR variability reflects the effects of cardiac autonomic regulation on the sinus node, which might differ from the effects on other parts of the heart. Nevertheless, in the postoperative situation, the HR variability alterations have mirrored the parasympathetic enhancement competing with the high background sympathetic activity before the onset of AF (Amar et al. 2003), which is in accordance with the experimental findings. Interestingly, the pathophysiological background of lowered DFA α_1 has also been related to a coactivation of vagal outflow during noradrenaline infusion as well as to physiological concomitant sympathetic and vagal activation during cold face immersion in healthy persons (Tulppo et al. 2001, Tulppo et al. 2005). In the present work, the study population consisted of clinical patients receiving beta-blocking medication and, thus, the mechanisms behind the reduced DFA α_1 values might vary from those encountered in healthy persons. It seems, however, that the preoperatively constantly decreased DFA α_1 in the present study most likely reflected a tendency to increase vagal modulation concomitantly with the high prevailing sympathetic background activity and that this tendency predisposed to the postoperative AF. The trend towards lower HR in patients developing postoperative AF is support for this proposal.

The second finding of preoperatively constantly increased SymDyn En during spontaneous breathing associating with postoperative AF was somewhat unexpected since previous studies have observed a transient decrease in entropy measurements preceding the onset of both postoperative and paroxysmal AF (Hogue et al. 1998, Shin et al. 2006, Vikman et al. 1999). It could be that reduced complexity before the initiation of postoperative AF is a transient rather than a constant phenomenon. In the study of Ovrieu et al. (Ovrieu et al. 2008) the postoperative ApEn analysed from recordings lasting 92 min to 28 hours before the initiation of AF did not differ between patients experiencing AF or those remaining in SR. Bauernschmitt et al. (Bauernschmitt et al. 2007)) observed the complexity of RR interval dynamics to reduce postoperatively when compared to the preoperative state only in patients remaining in SR. Preoperatively, the complexity was somewhat lower in patients developing AF, i.e. the preoperative finding was not in agreement with that found in the present study.

The physiological background of SymDyn En has been related to the sympathovagal balance. Guzzetti et al. (Guzzetti et al. 2005) studied the utility of SymDyn to characterise the cardiac sympathetic and parasympathetic regulation in healthy persons. They also estimated SymDyn En and observed that higher sympathetic modulation caused a decrease in SymDyn En. Also in the present study, vagal withdrawal caused by the upright tilt evoked a decrease in SymDyn En both in the patients developing AF and those remaining in SR. In terms of parasympathetic activation, the findings in the study of Guzzetti et al. were not significant. Therefore, the cause of the increase in SymDyn En during spontaneous breathing remains speculative in the present study, as is the impact of other contributing factors such as breathing rate and volume.

Non-sinus beats are frequent in patients experiencing AF, however, there was no significant difference in the proportion of these beats among those patients experiencing postoperative AF and those remaining in SR (Table 12). This is important because the DFA α_1 is affected by non-sinus beats. In the present study, the DFA α_1 values were also lower when the non-sinus beats were left unedited as compared to the situation when non-sinus beats had been removed. However, the decision to edit or not to edit the ectopic beats before the analysis of DFA α_1 did not affect the predictive capability of this new measure, which provides support for its possible applicability.

One limitation of the present analysis was that patients with diabetes mellitus had to be excluded. Diabetes affects the cardiac autonomic regulation and would have largely obscured the present findings. This is a shortcoming due to the ubiquitouness of diabetes; e.g. 16% of the patients in the original study population were diabetics. In a meta-analysis of more than 100 000 patients, the diabetes was related to increased mortality and morbidity after CABG although diabetes did not predispose subjects to postoperative AF (Zhang et al. 2011).

Currently, there are few tools available to identify the patients with a risk of postoperative AF after cardiac surgery. Even the multifactorial models combining pre-, intra- or postoperative risk factors have not obtained areas under the receiver operating characteristic curve more than 0.69-0.78 (Hakala et al. 2002, Magee et al. 2007, Mathew et al. 2004). The corresponding value in the present study was 0.70. Therefore, the predictive capability of these two new bedside measurements alone seemed to be high. The constant alteration in HR dynamics found in the present study suggests that there is a preexisting condition predisposing to postoperative AF that most likely also includes other predictors of AF in addition to altered autonomic state. Therefore, it would be interesting to combine the non-linear HR dynamics measures with other useful predictors of postoperative AF such as left atrial enlargement (Osranek et al. 2006) or with newly recognised risk factors such as obesity, metabolic syndrome or parameters characterising inflammation and/or oxidative stress (Echahidi et al. 2007, Gaudino et al. 2003). Hopefully, these kinds of analyses will clarify the pathophysiological mechanisms behind postoperative AF.

It would also be a fascinating view that these novel short-term HR dynamics analyses would help target accurately the preventive strategies such as pharmacological or surgical prophylaxis to high-risk patients. However, although there is abundant data on the predictive power of HR dynamics for cardiovascular morbidity and mortality, the breakthrough to clinical decision making with these methods has still been lacking.

Therefore, more prospective studies are needed to show, whether these measurements are able to direct the interventions so that it leads to improved outcome (Huikuri et al. 2009).

In conclusion, the preoperatively reduced fractal RR interval dynamics and the increased complexity of RR interval dynamics predicted the occurrence of postoperative AF independently of clinical factors. Therefore, short-term non-linear RR interval dynamics might provide valuable additional information about the pathophysiological factors increasing the risk for postoperative AF after CABG.

7 Conclusions

Most conventional short-term heart rate variability measurements showed acceptable stability over a few months period, the evidence of low physiological variation in subjects with stable coronary artery disease. However, the standard deviation of normal-to-normal intervals-parameter was an exception, since its stability remained at a less satisfactory level. Furthermore, the SDNN was highly affected by heart rate changes caused by physical activity. With respect to short-term non-linear heart rate dynamics, the fractal dimension by curve length, return map and symbolic dynamics-measures showed high stability, whereas the stability of short-term scaling exponent of detrended fluctuation analysis and approximate entropy was only moderate. However, the observation of acceptable stability over time of most short-term heart rate dynamics indicate that these measurements can be applied to characterise cardiac autonomic modulation, especially in follow-up studies in subjects with stable coronary artery disease.

Non-sinus beats affected the measures of return map, short-term scaling exponent of detrended fluctuation analysis and forbidden words of symbolic dynamics remarkably. The entropy measurements seemed to be less sensitive. The deletion method, in which the immediate effect of both ventricular and supraventricular ectopic beats was removed, corrected for the effect of non-sinus beats most efficiently. However, the interpolation method performed nearly as efficiently and to some extent even more consistently. In addition, when editing was performed with either method, this improved the stability of many of the non-linear heart rate dynamics measurements. Therefore, standardised practices for the editing within and between the studies would improve the reliability of the observations in relation to non-linear heart rate dynamics.

The simultaneous measurements of heart rate variability and, for the first time, personal carbon monoxide exposure revealed increased short-term heart rate variability during high carbon monoxide peaks. Therefore, this represents a novel attempt to link the acute effects of air pollution to cardiac autonomic regulation and may represent a way of investigating the possible link between air pollution and increased cardiovascular mortality.

The occurrence of postoperative atrial fibrillation after coronary artery by-pass grafting was independently associated with preoperatively altered fractal and complexity heart rate dynamics measurements. Therefore, one could speculate that these bedside short-term heart rate dynamics measurements might provide new additional information about the pathophysiological factors that increase the risk of postoperative arrhythmia.

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Tuula Tarkiainen

Short-term Heart Rate Dynamics: Methodology and Novel Applications

Most measures of short-term heart rate dynamics were shown to be stable over a few months period. However, non-sinus beats remarkably affected non-linear heart rate dynamics and their stability. Therefore, more standardised practices for the editing are needed. The simultaneous measurements of heart rate variability and personal carbon monoxide exposure revealed altered cardiac autonomic regulation during carbon monoxide exposure. The preoperative non-linear heart rate dynamics independently predicted the occurrence of atrial fibrillation after coronary surgery.

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