HEART RATE VARIABILITY AND BAROREFLEX SENSITIVITY IN SUBJECTS WITHOUT HEART DISEASE
Effects of age, sex and cardiovascular risk factors

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Abstract

Healthy subjects show wide interindividual variation in their heart rate behavior, but the factors affecting heart rate dynamics are not well known. This research was undertaken to evaluate heart rate variability (HRV) and baroreflex sensitivity (BRS) in a large random sample of subjects without evidence of heart disease, and to estimate the relation of heart rate behavior to age, sex and cardiovascular risk factors.

Short-term HRV was analyzed from 15-minute periods of standardized recording in supine and upright positions using time and frequency domain measures, and BRS was calculated using the Valsalva maneuver in an original randomly selected population of 600 hypertensive and 600 control middle-aged subjects. In addition, HRV was analyzed from the same segments using new measures based on fractals and complexity (chaos theory) of R–R interval dynamics in the same random population, and from 24-hour period in 114 healthy subjects aged from 1 to 82 years.

Large interindividual variation was observed in the measures of HRV and BRS in middle-aged subjects; coefficient of variation (CV) of the standard deviation of R–R intervals (SDNN) 39% (54 ± 21 ms) and CV of BRS 49% (9.9 ± 4.9 ms/mmHg). In healthy middle-aged men, SDNN was weakly related to age (r = -0.19, p < 0.01), HDL cholesterol (0.19, p < 0.01), serum insulin (-0.23, p < 0.001) and triglyceride (-0.25, p < 0.001) levels. In women, SDNN was only related to insulin levels (r = -0.23, p < 0.001), BRS was related to systolic blood pressure (r = -0.31 and -0.30, in men and women respectively, p < 0.001 for both) and blood glucose (r = -0.25, p < 0.01) and serum insulin levels (r = -0.34, p < 0.001) in women. Lesser intersubject variation was observed in the non-linear measures of HRV; CV 14% of short-term scaling exponent (a1), a measure of fractal-like correlation properties of HRV, (1.21 ± 0.17) and CV 12% of approximate entropy, a measure of complexity, (1.13 ± 0.14). Neither a1 or ApEn was related to any risk factors. Women had lower overall short-term HRV (p < 0.01) and BRS (p < 0.001), but a higher spectral high-frequency component of HRV, higher ApEn and lower a1 (p < 0.001 for all) compared to men. The impairment in overall HRV was confined to the hypertensive subjects with metabolic features of the insulin resistance syndrome (IRS, n = 69), but the BRS and spectral high-frequency component were also impaired in hypertensive subjects without IRS compared to normotensive subjects. The 24-hour cardiac interbeat interval dynamics changed markedly from childhood to old age. Children showed similar complexity and fractal correlation properties of R–R intervals as young adults. Healthy aging resulted in R–R interval dynamics with higher regularity and predictability and altered fractal scaling.

The traditional measures of HRV and BRS are weakly related to many cardiovascular risk factors in subjects without heart disease, but the interindividual variation of HRV and BRS is only partly explained by these factors, suggesting a genetic background of the intersubject variation in cardiovascular autonomic regulation. The new dynamical measures of HRV show less interindividual variation than the conventional measures of HRV in healthy subjects and are not related to cardiovascular risk variables, suggesting that these dynamical measures quantify the “intrinsic” capacity of a healthy cardiovascular control system without the significant influence of life-style, metabolic or demographic variables. However, there are sex and age-related differences also in the fractal and complexity measures of heart rate behavior.

Keywords: cardiovascular regulation, electrocardiography, non-invasive methods.
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Abbreviations

α1 short-term scaling exponent of fractal-like correlation properties
α2 intermediate-term scaling exponent of fractal-like correlation properties
β slope of the power-law relationship
ApEn approximate entropy
BRS baroreflex sensitivity
CAD coronary artery disease
ECG electrocardiogram, -phic, -phy
HF high frequency
HRV heart rate variability
IRS insulin resistance syndrome
LF low frequency
SDANN standard deviation of average R-R intervals of analyzed segments
SDNN standard deviation of all R-R intervals
ULF ultra low frequency
VLF very low frequency
List of original communications

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


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1. Introduction

Heart rate is controlled most importantly by the autonomic nervous system to maintain cardiovascular homeostasis. A healthy cardiovascular control system is able to adapt to physiologic perturbations and changing conditions. During the past three decades, impairment of autonomic cardiovascular regulation has been observed in many disease states, including hypertension, diabetes and CAD (Airaksinen et al. 1987; Eckberg 1979; Ewing et al. 1981). The relevance of this impairment for clinical cardiology was realized in the late 1980s, when the results of impaired autonomic function were found to independently predict mortality in postinfarction subjects (Kleiger et al. 1987; La Rovere et al. 1988). The clinical importance of assessing cardiac autonomic function so far lies in the risk stratification of postinfarction subjects.

The clinically applicable methods of assessing autonomic function are the measurement of HRV and the evaluation of BRS. HRV analyzed as time and frequency domain reflects primarily the physiological level of tonic autonomic regulation, whereas BRS indicates the capacity of reflex autonomic regulation. New methods based on the complexity and fractal scaling of R-R interval variability have been developed constantly to uncover subtle abnormalities or alterations in time series data that are not otherwise apparent (Lipsitz et al. 1992). Both low HRV and low BRS are associated with increased cardiovascular risk (Kleiger et al. 1987; La Rovere et al. 1988). The new dynamical measures of HRV may be more powerful predictors of adverse outcome in various populations than the conventional measures (Bigger et al. 1996; Huikuri et al. 1998; Mäkikallio et al in press).

Large interindividual variation of autonomic function has been described in healthy subjects (Abdel-Rahman et al. 1994; Huikuri et al. 1990; Kupari et al. 1993; Molgaard et al. 1994), but the factors affecting the autonomic control of heart rate are still largely unknown. It is not known, for instance, whether alterations in HRV and BRS are specifically associated with factors related to cardiovascular risk or determined by genetic or other factors. Interindividual variation or possible relations to the risk factors of the new dynamical measures of HRV have not been studied in detail.

The purpose of the present study was to investigate the interindividual variation and its possible relations to age, sex and cardiovascular risk factors of HRV analyzed with traditional and new dynamical measures and of BRS in subjects without heart disease.
2. Review of the literature

2.1. Anatomy and physiology of cardiovascular autonomic regulation

Heart rate is determined by the depolarization of the cardiac pacemaker, normally the sinoatrial node. To maintain cardiovascular homeostasis, the sinoatrial node discharge rate is carefully controlled by various regulatory systems in close connection with the regulation of blood pressure. Beat-to-beat fluctuations in heart rate and blood pressure reflect the dynamic response of the cardiovascular control systems to naturally occurring physiological changes and challenges. The most important control system is the autonomic nervous system, the function of which is organized on the basis of a reflex arch (Opie 1998). The sensory receptors, which are abundantly found in the carotid sinus and aortic arch, the lungs and the atria, sense the possible occurring perturbations. The afferent pathways from the sensory organs travel within the vagus and sympathetic nerves to the cardiovascular center in the medulla oblongata. The efferent vagal and sympathetic pathways relay impulses from the cardiovascular centre to the effector organs, impinging on both cardiac function (heart rate, atrioventricular conduction, contractility) and the peripheral vasculature (arterial and venous vasomotor tone) (Opie 1998).

The sinus node is richly innervated with postganglionic sympathetic and parasympathetic nerve terminals, which exert a continuous, though varied influence upon heart rate. The two branches of the autonomic nervous system work in a co-ordinated way, usually acting reciprocally, but sometimes synergistically on heart rate. The effects of the vagal and sympathetic systems do not ordinarily summate algebraically, but show complex interactions (Levy 1971; Opie 1998). Under resting conditions like during the night, vagal tone prevails (Levy 1971) and the variations in heart period are largely dependent on vagal modulation (Chess et al. 1975). Vagal stimulation by a release of acetylcholine slows the sinus nodal discharge rate. Adrenergic stimulation mediated by norepinephrine and epinephrine speeds up the sinus discharge rate (Opie 1998). Cardiac responses to brief vagal bursts begin after a slow latency and dissipate quickly (in a few milliseconds); in contrast, responses to sympathetic stimulation both commence and dissipate slowly (in seconds). The rapid onset and offset of the responses to efferent vagal stimulation allow for dynamic vagal modulation of heart rate, whereas the slow
temporal response to sympathetic stimulation precludes any beat-to-beat regulation at high frequencies by sympathetic activity (Berne & Levy 1993).

The cardiovascular regulatory systems take compensatory actions when internal (afferent) stimuli signal a need for regulation. *Respiratory sinus arrhythmia* is the term used to describe the spontaneous fluctuation of R-R interval with respiration. Typically, heart rate accelerates during inspiration and decelerates during expiration. Both the frequency and amplitude of fluctuation vary, depending on the rate and depth of respiration (Hirsch *et al.* 1981). The sensory organs may involve stretch receptors in the lung tissue or the chest wall (Hirsch *et al.* 1981). Respiratory sinus arrhythmia correlates with the vagal efferent traffic (Eckberg 1983; Katona *et al.* 1975). *The Baroreceptor reflex* is a negative feedback system with many control loops, by which transient changes in blood pressure are countered. A rise in blood pressure is sensed by the baroreceptors, which are pressure-sensitive nerve endings mainly found in the wall of the aortic arch and in the carotid sinuses. The baroreceptor reflex is stimulated, which results in a reduction of heart rate and cardiac contractility and, thus, a fall in blood pressure. An initial decrease in blood pressure has opposite effects. The baroreceptor reflex depends mainly on reflex vagal activity (Eckberg *et al.* 1971; Eckberg 1980; Smyth *et al.* 1969). Heart rate fluctuations with lower frequencies, the afferent stimuli of which are somewhat unclear, may be related to thermoregulation and vasomotor tone (Akselrod *et al.* 1981; Hyndman *et al.* 1971; Lindqvist *et al.* 1989; Sayers 1973). However, there are data to suggest that they depend primarily on the presence of vagal outflow (Taylor *et al.* 1998). All circulatory reflexes interact in a complex way and it may thus be difficult to differentiate between the actions of the different control systems (Hainsworth 1998).

### 2.2. Assessment of cardiac autonomic nervous function

Various indirect quantitative measurements of cardiac autonomic function have been developed, since no microneurographic techniques are available for direct measurements of the actual cardiac autonomic nerve traffic. However, all of these indirect measures may be subject to complex interactions with all circulatory reflexes. The development of new technical equipment, i.e. digital, high-frequency, 24-hour potential, multichannel ECG recorders, devices measuring continuous noninvasive blood pressure on a beat-to-beat basis, and microcomputers with sufficient capacity, have enabled the use of more sophisticated measures of cardiac autonomic function, including spontaneous HRV and BRS, in addition to the simple traditional bedside tests.
2.2.1. Measurement of HRV

2.2.1.1. General

The spontaneous fluctuations of R-R intervals (intervals between adjacent QRS complexes resulting from sinus node depolarizations) are estimated using different methods of HRV. The original continuous ECG signals are analogue-to-digital converted into a microcomputer, which stores the length of R-R intervals versus cumulative time. The tachogram generated is the source and basis for all analysis of HRV.

The original ECG data should meet some requirements. Artifact and ectopic beats during the ECG recording can seriously interfere with many measures estimating HRV. Although techniques have been developed to automatically exclude the abnormal intervals, human editing of the tachogram is still usually required. However, none of the methods used to exclude abnormal intervals are optimal. Frequent ectopy or artifacts during the ECG recording may invalidate the HRV measurement either by interfering with the measures or by a loss of normal intervals because of overediting.

The analyses of HRV with many linear (especially spectral) methods require the R-R interval data to be stationary and sufficiently long (Malliani et al. 1991; Öri et al. 1992). However, stationary conditions are unknown to biology, since the mechanisms responsible for heart rate modulation do not remain unchanged for long periods. A practical compromise has to be found between the length of event series and the theoretical mathematical requirements. Linear detrending and filtering are preprocessing techniques that make the data more stationary (Penaz 1978).

HRV can be analyzed from short-term (usually 5-minute) and long-term (usually 24-hour) ECG recordings. The advantage of analyzing short-term HRV from short ECG recordings is the limited duration of ECG monitoring, while the limitations include the possible necessity of interventions and patient co-operation. The measures of short-term HRV may fail to detect low-frequency oscillations and long-term trends that may have clinical importance.

2.2.1.2. Time domain measures of HRV

The time domain measures of HRV are based on statistical or geometric analyses of the lengths of intervals between successive normal complexes (Task Force 1996). The statistical measures using the length of R-R intervals include the mean R-R interval, the standard deviation of all R-R intervals (SDNN), the standard deviation of the average R-R intervals calculated over short periods (SDANN) and the mean of the 5-minute standard deviations of R-R intervals (SDNN index). SDNN reflects all cyclic components in the analyzed period and thus, depends on the length of the period. It is inappropriate to compare SDNN measures obtained from recordings of different durations. The measures derived from differences between R-R intervals (RMSSD, the square root of the mean squared differences of successive R-R intervals, NN50, the number of interval differences of successive R-R intervals greater than 50 ms, and
pNN50, the proportion derived by dividing NN50 by the total number of R-R intervals) estimate the high-frequency variations in heart rate and are correlated closely (Bigger et al. 1989).

The series of R-R intervals can also be converted into a geometric pattern. The variability is judged on the basis of the geometric properties of the resulting pattern by using a basic measurement of the geometric pattern (i.e. quantitative analysis of Poincaré plots (Tulppo et al. 1996)), interpolating the geometric pattern by a mathematically defined shape (i.e. HRV triangular index (Malik et al. 1989)) and classifying the geometric shape into several pattern-based categories (i.e. qualitative analysis of Poincaré plots (Woo et al. 1994)). The major advantage of the geometric methods lies in their relative insensitivity to artifacts and ectopic beats, while the major disadvantage is the need for a reasonable number of R-R intervals and a longer ECG recording.

The methods for expressing overall HRV and its long- and short-term components are not interchangeable. The use of SDNN (an estimate of overall HRV), SDANN (an estimate of the long-term components of HRV) and RMSSD (an estimate of the short-term components of HRV) as time domain measures of HRV are recommended (Task Force 1996).

### 2.2.1.3. Frequency domain measures of HRV

A power spectral density analysis of the tachogram provides the basic information of how power (variance) distributes as a function of frequency. The heart rate signal is decomposed into its frequency components and quantified in terms of their relative intensity (power) (Akselrod et al. 1981; Sayers 1973).

Both nonparametric (fast Fourier transform, FFT) and parametric (autoregressive model estimation) methods are used. In most instances, the two methods provide comparable results. The FFT spectra are characterized by discrete peaks for several frequency components. The advantages of the FFT method are the simplicity of the algorithm used and the high processing speed. However, FFT requires strict periodicity of the data and a priori selection of the number and frequency range of the oscillatory components. The advantages of the autoregressive method include smoother spectral components and easy postprocessing of the spectrum. An accurate estimation of power spectral density even on a small number of samples provides an additional advantage, since the shorter time periods are more likely to meet the requirement of stationarity (Malliani et al. 1991; Öri et al. 1992). It is recommended that the duration of the recording should be at least 10 times the wavelength of the lowest frequency bound of the spectral component investigated (Task Force 1996).

The power spectrum of healthy subjects consists of four major frequency bands. They do not have fixed periods and the central frequencies may vary considerably. The limits for the spectral components usually used (Task Force 1996) are: HF component 0.15-0.4 Hz, LF component 0.04-0.15 Hz, VLF component 0.003-0.04 Hz and ULF component <0.003 Hz. The components estimate fluctuations with a periodicity of 2.5-7 s, 7-25 s, 25 s-6 min, and > 6 minutes respectively. Total power is represented by the total area under the power spectral curve, and the power of individual frequency components by the area
under the proportion of the curve related to each component. The power of the ULF, VLF, LF and HF components is usually expressed in absolute units (ms²). The ratio between LF and HF components (LF/HF ratio) is also used (Malliani et al. 1991; Pagani et al. 1986). LF and HF components may also be measured in normalized units by dividing the power of the LF and HF components (in ms²) by the total power from which the power <0.04 Hz has been subtracted and then multiplying by 100 (Malliani et al. 1991; Pagani et al. 1986). The normalization tends to minimize the effect of the changes in total power on the values of the HF and LF components. Nevertheless, normalized units should always be quoted with absolute values in order to describe completely the distribution of power in the spectral components.

2.2.1.4. Dynamical analysis of HRV

Chaos theory and fractal behavior. Chaos refers to a system which has characteristics of both periodicity and randomness. Periodic behavior is easily predictable, since it repeats itself over a finite time interval. Random behavior, on the contrary, never repeats itself and is unpredictable and disorganized. Although chaotic behavior looks disorganized, it is really deterministic (Denton et al. 1990). A fractal system is a specific form of chaos. It has the same structure on many measurement scales (i.e. self-similar, scale-invariant structures) (Goldberger 1996). Nonlinear phenomena are involved in the genesis of HRV (Sayers 1973). It has been suggested that healthy heart beat is chaotic and shows a fractal form, which may break down with disease (Goldberger 1996). One of the characteristics of a nonlinear system is that its components interact in a complex way. Multiple mechanisms (sympathetic tone, parasympathetic tone, hormones, preload, afterload), most of which interact and have long feedback loops, control the sinus node. Thus, one may think that a healthy cardiovascular system is a near-perfect substrate for the generation of chaos (Denton et al. 1990).

Nonlinear measures of HRV. The linear inverse power-law relationship of (log) power to (log) frequency describes the distribution of the power spectral density (Bigger et al. 1996; Kobayashi et al. 1982; Saul et al. 1987). The steeper (i.e. more negative) the slope (\( \beta \)) of this power-law relationship is, the greater is the relative power in the lower frequency ranges compared to the higher frequency ranges. \( \beta \) stands for the fractal-like correlation properties of R-R interval data over very-low and ultra-low frequency bands (10⁻³ to 10⁻² Hz).

Detrended fluctuation analysis (DFA) quantifies the fractal-like correlation properties of time series data (Iyengar et al. 1996; Peng et al. 1995). The scaling exponent \( \alpha \), with a value near 1 indicating fractal-like behavior, can also be taken as an indicator of the “roughness” of the time series: the smoother the time series, the larger \( \alpha \). One of the advantages of the DFA method is that it minimizes the noise effects and, by removing local trends remains relatively unaffected by nonstationarities.

Approximate entropy (ApEn) is a measure quantifying the regularity and complexity of time series (Pincus et al. 1992; Pincus et al. 1994). ApEn quantifies the amount of information needed to predict the future state of a system. Lower values of ApEn indicate
a regular (less complex) signal while higher values indicate irregularity (greater complexity).

In addition many other nonlinear methods have been developed for assessing the complexity and fractal-like properties of R-R interval time series (i.e. Lyapunov exponents, Kolmogorov entropy, correlation dimension, Coarse Graining Spectral Analysis). So far, none of the methods has been superior to the others or gained general popularity.

2.2.1.5. Physiological background of different measures of HRV

HRV reflects oscillations in the activity of various regulatory systems associated with a variety of factors, including respiration, baroreceptor reflexes, vasomotor control, and thermoregulatory processes. However, the physiological mechanisms behind the different measures of HRV vary. Especially, by using the spectral components of HRV, it may be possible to discriminate between the effects of various cardiovascular control mechanisms.


The spectral HF component is associated with respiration, and both amplitude and peak frequency vary with respiration (Hirsch et al. 1981; Pagani et al. 1986). Both clinical and experimental studies, including muscarinic receptor blockade, vagotomy and electrical vagal nerve stimulation, have been shown that parasympathetic activity is the major contributor to the HF component (Akselrod et al. 1981; Akselrod et al. 1985; Bailey et al. 1996; Hayano et al. 1991; Pagani et al. 1986; Pomeranz et al. 1985).

Interpretation of the LF component is controversial. Vagal activity largely contributes to the LF component based on the results of parasympathetic blockade (Akselrod et al. 1981; Akselrod et al. 1985; Chess et al. 1975). Experimental and clinical studies with sympathomimetic or sympatholytic agents have yielded variable results. While an association between the LF component and sympathetic activity has been found in some studies (Akselrod et al. 1981; Akselrod et al. 1985; Pomeranz et al. 1985), no correlation has been found in others (Chess et al. 1975). Interventions to increase sympathetic activity, such as postural change, moderate exercise and mental stress, have increased the LF component (Pagani et al. 1986; Pagani et al. 1991; Pomeranz et al. 1985). The LF component may be more interpretable if studied under conditions where activity and body posture are controlled. LF oscillation of heart rate is reduced in subjects with very high sympathetic activity, such as during heavy exercise (Arai et al. 1989; Breuer et al. 1993) and in heart failure (van de Borne et al. 1997). This is speculated to be due to saturation of the LF oscillatory systems caused by the high sympathetic drive or the mechanism to include a central effect of neurohumoral excitation (van de Borne et al. 1997). Some authors have interpreted the LF component to reflect sympathetic activity either in absolute or normalized units and the LF/HF ratio to indicate sympathetic outflow in humans or to mirror sympathovagal balance (Malliani et al. 1991; Pagani et al. 1986). The idea of sympathovagal balance has been seriously criticized recently using
both mathematical and physiological arguments (Eckberg 1997). For instance, changes in LF/HF ratio or in normalized LF and HF power have been interpreted as if sympathetic and parasympathetic activity always change reciprocally. However, since sympathetic and vagal nervous system act in parallel in some situations (i.e. cold immersion of the face), the interpretation may not be physiologically justified in all situations (Eckberg 1997). Changes in baroreceptor activity caused by periodic changes in blood pressure (Madwed et al. 1989; Penaz 1978) are thought to be the predominant determinants of the LF component (Akselrod et al. 1981; Pagani et al. 1986; Taylor et al. 1998). The renin-angiotensin system may play a minor role (Akselrod et al. 1981; Taylor et al. 1998). The VLF and ULF components may also reflect changes in thermoregulation due to peripheral blood flow adjustments (Lindqvist et al. 1989; Sayers 1973).

It must be borne in mind that HRV reflects the fluctuations in autonomic inputs to the heart rather than the mean level of autonomic tone. Thus, both autonomic withdrawal (blockade) and a saturating level of autonomic input lead to diminished HRV, although the effects of the two situations on heart rate are reciprocal (Malik et al. 1993).

The physiological mechanisms behind the fractal-like R-R interval dynamics are still unknown. The finding that denervated hearts are associated with steeper slopes ($\beta$) of the power-law relationship (Bigger et al. 1996) supports the contention that at least this scaling index is substantially influenced by the autonomic input of the heart.

In summary, parasympathetic control is the dominant contributor to all HRV. The other possible mechanisms include the sympathetic nervous system, the renin-angiotensin system, and humoral and thermoregulatory factors.

### 2.2.2. Measurement of BRS

BRS can be calculated with various indirect techniques by measuring the changes in heart rate (in ms) against the changes in blood pressure (in mmHg). Most of the techniques require laboratory conditions and specific interventions. Both blood pressure and heart rate are measured on beat-to-beat basis. Changes in blood pressure are achieved by vasoactive agents (nitroglycerin, phenylephrine), Valsalva maneuver, external neck suction or neck pressure (Bristow et al. 1969; Goldstein et al. 1982; Gribbin et al. 1971). The response of heart rate to these changes is then measured. The slope of the linear relationship between the length of R-R intervals and blood pressure represents BRS. There are no commonly approved recommendations as to which methods should be used,
although invasive testing with phenylephrine has been considered the “gold standard”. Different techniques may measure different aspects of baroreceptor function and hence give variable results. The average correlation coefficient between the different techniques is 0.36 (Goldstein et al. 1982) and that between the phenylephrine and Valsalva methods 0.56 (Airaksinen et al. 1993). Both an inadequate blood pressure rise after the interventions or frequent ectopic beats may invalidate BRS analysis. BRS reflects the capacity of baroreceptor activation to increase vagal efferent activity, i.e. vagal reactivity (Eckberg et al. 1971; Eckberg 1980; Smyth et al. 1969). Also sympathetic activity (plasma norepinephrine levels) have been related to BRS (Hartikainen et al. 1995).

2.2.3. Reproducibility of measures of autonomic function

Intraindividual reproducibility of HRV measurements obtained from two 24-hour ECG recordings is good in both normal subjects and subjects with heart disease (Hohnloser et al. 1992; Huikuri et al. 1990; Kleiger et al. 1991; Van Hoogenhuyze et al. 1991). The observed intraclass correlation coefficients have been high for both time and frequency domain measures of HRV: 0.7-0.9 (mean R-R interval), 0.6-0.9 (SDNN), 0.8-0.9 (HF power), 0.8-0.9 (LF power) (Hohnloser et al. 1992; Kleiger et al. 1991; Van Hoogenhuyze et al. 1991). The intraindividual coefficient of variation for SDNN was 7±6 % and that for the mean R-R interval 5±5% (Huikuri et al. 1990). The reproducibility of BRS measurements have also been high (r=0.73 between two successive tests (Airaksinen et al. 1993), no significant differences emerged between two tests made up to 15 months apart (Gribbin et al. 1971).

2.2.4. Relation to heart rate and correlations between different measures of autonomic function

The time and frequency domain measures of HRV show significant correlations with the mean R-R interval (r between 0.3-0.8 (Bigger et al. 1995; Molgaard et al. 1994; Kleiger et al. 1991; Kupari et al. 1993; Van Hoogenhuyze et al. 1991)). BRS and nonlinear measures of HRV do not significantly correlate with the resting heart rate (Airaksinen et al. 1993; Gribbin et al. 1971; La Rovere et al. 1988; Mäkikallio et al. 1996).

Because of both mathematical and physiological relationships, there are significant correlations between many time and frequency domain variables. The measures of pure tonic vagal activity, i.e. HF, pNN50, RMSSD, correlate strongly (r between 0.94-0.97 (Bigger et al. 1989). The correlations between the other time and frequency domain measures are weaker (r between 0.4 to 0.9 (Bigger et al. 1995; Kleiger et al. 1991)) and those with nonlinear measures of HRV even weaker, if not nonexistent (Bigger et al. 1996; Huikuri et al. 1998; Molgaard et al. 1994). BRS and HRV correlate only moderately (r = 0.6 (Bigger et al. 1989)) or have non-significant correlations (Farrell et al. 1991; Hohnloser et al. 1994). Thus, despite the moderate degrees of correlation, the
different measures of autonomic function may give complementary information and are hence not interchangeable.

The spectral measures of HRV analyzed from short ECG recordings (2 to 15 minutes) correlate well ($r$ between 0.58 and 0.88) with the values analyzed from 24-hour recordings in post-MI patients (Bigger et al. 1993), while there is no data on healthy subjects.

2.3. Autonomic cardiovascular regulation in healthy subjects

2.3.1. Autonomic function in relation to changes in body posture and activity

The cardiovascular regulatory systems of healthy subjects are constantly responding to changes in both internal and external conditions in a highly adaptive way. The changes in the activity of various control systems due to changing conditions are reflected in the measures of autonomic function. Thus, the possible changes in body posture and activity should be considered in the study design when analyzing autonomic function, or at least short-term HRV. On the other hand, the changes in the spectral components of HRV in response to body posture or activity have encouraged the interpretation that various spectral components or their derivates represent the two branches of autonomic system separately. This may be inappropriate, however.

2.3.1.1. Effects of body posture on measures of autonomic function

Alterations in the autonomic function in an upright compared to supine position are generally characterized by a decrease in parasympathetic activity and an increase in sympathetic activity. A marked reduction in the HF component in both absolute and normalized units has been observed in the standing position and during a passive tilt compared to the supine position (Pagani et al. 1986; Pomeranz et al. 1985; Vybiral et al. 1989), but not in all studies (Lipsitz et al. 1990). The LF component in absolute and normalized units and the ratio between the LF and HF components have markedly increased in the upright position (Montano et al. 1994; Pagani et al. 1986; Pomeranz et al. 1985; Vybiral et al. 1989), but, again, not in every study (Lipsitz et al. 1990). The results on total power or total variance have varied even more from a decrease with tilt (Pagani et al. 1986) through no difference with tilt (Vybiral et al. 1989) to an increase with tilt (Lipsitz et al. 1990). Although the results have been inconsistent, some authors have interpreted the increases in the LF component expressed in normalized units or the ratio between the LF and HF components to reflect an increased sympathetic modulation of heart rate or sympathovagal balance (Montano et al. 1994; Pagani et al. 1986; Pagani et al 1991).
2.3.1.2. Acute effects of exercise on measures of autonomic function

A rapid vagal withdrawal occurs upon exercise (Robinson et al. 1966). The HF component of HRV is markedly reduced during exercise (Arai et al. 1989; Breuer et al. 1993; Casadei et al. 1995; Yamamoto et al. 1991), further supporting the vagal origin of the measure. However, the LF component and the total R-R interval variance are also markedly reduced during heavy exercise (Arai et al. 1989; Breuer et al. 1993; Casadei et al. 1995; Yamamoto et al. 1991), suggesting that even these measures are markedly influenced by vagal activity in healthy subjects. Although sympathetic activity certainly increases during exercise (Robinson et al. 1966), neither the LF component in normalized units nor the ratio between the LF and HF components has increased consistently, possibly reflecting the inability of these measures to assess sympathetic tone in healthy subjects (Arai et al. 1989; Casadei et al. 1995). ApEn has been shown to increase during exercise (Tulppo et al. 1996).

2.3.1.3. Circadian rhythm of measures of autonomic function

Autonomic activity shows a circadian rhythm with an increase in sympathetic tone during the day and in parasympathetic tone at night in healthy subjects. The circadian rhythms of different measures of HRV have varied in different studies. Higher values of pNN50 and the HF component (Goldsmith et al. 1992; Molgaard et al. 1991; Molgaard et al. 1994) have been observed at night. LF showed no difference between day and night in some studies (Molgaard et al. 1994), was higher at night in some (Goldsmith et al. 1992) and lower (in normalized units) at night in others (Huikuri et al. 1994). Total HRV was increased at night in some studies (Huikuri et al. 1990; Huikuri et al. 1994; Jensen-Urstad et al. 1997), and decreased in one study (Molgaard et al. 1991). BRS was increased during the nighttime (Hartikainen et al. 1993; Parati et al. 1995; Smyth et al. 1969). In summary, most data suggest that the indexes related to vagal activity are increased at night. The results concerning the indexes possibly measuring sympathetic activity are controversial. The possible effect of circadian variation must be taken into account, especially while cardiac autonomic regulation is assessed from short-term recordings.

2.3.2. Interindividual variation of autonomic function in healthy subjects

Although healthy subjects are considered to have a highly adaptive cardiovascular control system, they show marked interindividual variation in autonomic function (Abdel-Rahman et al. 1994; Huikuri et al. 1990; Jensen-Urstad et al. 1997; Kupari et al. 1993). The coefficients of variation with different measures of HRV have been 12-15% for the mean R-R interval, 24% for SDANN, 41-155% for LF power, 70-162% for HF power and 20-63% for BRS (Abdel-Rahman et al. 1994; Huikuri et al. 1990; Jensen-
Urstad et al. 1997; Kupari et al. 1993, Töyry et al. 1995). Thus, more intersubject variation has been found in HRV than in the mean heart rate. The reasons and mechanisms of this extensive interindividual variation are not exactly known. Many studies have tested the relations of various clinical, lifestyle and laboratory factors to the alterations in autonomic cardiovascular regulation, usually on a univariate, but sometimes on a multivariate basis.

2.3.2.1. Effects of aging on autonomic function

The effect of age on autonomic function has been uniformly observed in many studies. Increased variation of heart rate occurs during childhood (Finley et al. 1995; Korkushko et al. 1991). On the other hand, increasing age during adult life is associated with a reduction in overall HRV (Bigger et al. 1995; Hayano et al. 1991; Hellman et al. 1976; Korkushko et al. 1991; O’Brien et al. 1986; Shannon et al. 1987), a reduction in BRS (Gribbin et al. 1971; Laitinen et al. 1998), a loss of complexity and altered fractal scaling (Iyengar et al. 1996; Kaplan et al. 1991; Lipsitz et al. 1992; Ryan et al. 1994). Age has also remained an independent determinant of autonomic function in studies with multivariate analysis (Molgaard et al. 1994; Tsuji et al. 1996b). The age-related changes may be due either to structural factors, such as a loss of sinoatrial pacemaker cells in advanced age (Goldberger 1996) or a loss of arterial distensibility (Gribbin et al. 1971) and functional changes, e.g. altered coupling between regulatory components (Goldberger 1996).

2.3.2.2. Sex-related differences in autonomic function

There are several studies on gender-related differences in the autonomic control of heart rate. Women have decreased BRS compared to men (Abdel-Rahman et al. 1994; Laitinen et al. 1998). In studies on HRV, men have usually had higher overall HRV at lower frequencies compared to women (Cowan et al. 1998; Jensen-Urstad et al. 1998; Liao et al. 1995; Molgaard et al. 1994). Women may have higher complexity (ApEn) compared to men (Ryan et al. 1994). The effects of various factors on autonomic function may differ between the sexes (Kupari et al. 1993; Stein et al. 1997). Thus, although younger men have higher overall HRV compared to women, the HRV in older subjects is comparable between the sexes (Stein et al. 1997). The mechanisms of the gender-related differences have not been widely discussed.

2.3.2.3. Effects of lifestyle on autonomic function

Many lifestyle factors may affect autonomic cardiovascular function. The effects of long-term physical activity on autonomic cardiovascular regulation have been addressed in
several studies, but the results have been inconsistent. In some studies, physical training was associated with increased HRV and BRS in healthy subjects (Barney et al. 1988; Dixon et al. 1992; Goldsmith et al. 1992; Molgaard et al. 1991; Molgaard et al. 1994). However, no correlation has been found in some studies (Kupari et al. 1993; Seals et al. 1989). The possible beneficial effects of physical activity on autonomic cardiac regulation are assumed to result from the vagotonic effects and parasympathetic dominance produced by long-term physical training.

Smoking (Hayano et al. 1990; Jensen-Urstad et al. 1998; Molgaard et al. 1994; Kupari et al. 1993) and abundant alcohol consumption (Malpas et al. 1991) have been related to impaired autonomic function. However, the results have been variable between the sexes, showing harmful effects of smoking only in women (Jensen-Urstad et al. 1998) and possible beneficial effects of moderate alcohol consumption in women (Kupari et al. 1993). Subjects expressing features of type A personality show an increased ratio between the LF and HF components compared to type B (Kamada et al. 1992).

2.3.2.4. Clinical and laboratory measures and autonomic function

Many clinical and laboratory measures have also been related to measures of autonomic function in healthy subjects. The results have varied much, depending on the population and the factor tested.

No correlations between blood pressure and HRV measures were found in young healthy adults (Kupari et al. 1993). BMI had no correlations with HRV in a population sample (Kupari et al. 1993), but had a negative correlation with HRV in women (Jensen-Urstad et al. 1998). An inverse relation of R-R interval variation and body fat was found in obese males after beta blockade (Peterson et al. 1988).

Many laboratory values, including serum triglycerides, LDL cholesterol, and leukocyte count, have univariate negative correlations with HRV. HDL cholesterol has been directly related to HRV (Jensen-Urstad et al. 1998; Kupari et al. 1993). No correlations were found with serum triglyceride or insulin levels (Kupari et al. 1993), but total, VLF and LF power had negative univariate correlations with triglycerides (Jensen-Urstad et al. 1998).

2.4. Effect of drugs and disease states on autonomic nervous function

2.4.1. Effect of drugs on HRV and BRS

Many drugs interfere with autonomic function and thus possibly affect the measures of HRV and BRS. Beta-blockers seem to enhance HRV in healthy subjects (Cook et al. 1991; Pagani et al. 1986), in patients with CAD (Niemelä et al. 1994) and in postinfarction patients (Molgaard et al. 1993). The increase is most marked in the measures of pure vagal activity (HF power, RMSSD), while an increase has been also
seen in total and LF power (Cook et al. 1991). Beta-blockers may increase BRS in subjects with mild hypertension (Lucini et al. 1993), while no effect on BRS in subjects with CAD was found (Airaksinen et al. 1994). ACE inhibitors may improve BRS in heart failure patients (Osterziel et al. 1990) and HRV in postinfarction patients (Bonaduce et al. 1994), while no effects on healthy subjects were observed (Kaufman et al. 1993). Amiodarone seems to have no effect on HRV in patients with ventricular arrhythmias, while an attenuation of HRV was seen with propafenone and flecainide (Zuanetti et al. 1991). Digoxin improved HRV in healthy subjects (Kaufman et al. 1993). Transdermal scopolamine increased overall HRV, HF power and BRS in healthy subjects (Dibner-Dunlap et al. 1985; Vybiral et al. 1990), while diltiazem had no effect on HRV (Cook et al. 1991).

### 2.4.2. HRV and BRS in hypertension

Previous studies on HRV and blood pressure have yielded variable results. No differences compared to normotensives were found in some studies (Mancia et al. 1983). Decreased measures of pure parasympathetic control (pNN50, HF power) have been found in unmedicated hypertensive subjects (Langewitz et al. 1994) and in hypertensive subjects with left ventricular hypertrophy (Chakko et al. 1993; Petretta et al. 1995), suggesting decreased vagal tone in hypertension. However, other measures of overall HRV, including SD of R-R intervals (Chakko et al. 1993; Huikuri et al. 1996), LF power (Huikuri et al. 1996; Petretta et al. 1995) and VLF power (Huikuri et al. 1996) in absolute units, have also been decreased in hypertensive subjects, possibly suggesting altered function of other control mechanisms (sympathetic, renin-angiotensin system) as well. A decreased HF component and an increased LF component, expressed as normalized units were found in hypertensive subjects (Guzzetti et al. 1988), which finding was interpreted by the authors as an increased sympathetic tone in hypertensive subjects. Alterations in the circadian rhythm of HRV (Chakko et al. 1993; Dassi et al. 1991) and blunted responses of HRV measures to an upright posture (Guzzetti et al. 1988; Huikuri et al. 1996; Radaelli et al. 1994) have also been observed in hypertensive subjects.

The results concerning the association between BRS and increased blood pressure have been more uniform. An inverse relation between blood pressure and BRS has been observed (Bristow et al. 1969; Gribbin et al. 1971; Sleight 1979). Decreased BRS was seen in unmedicated hypertensive subjects (Eckberg 1979; Bristow et al. 1969; Radaelli et al. 1994) as well as in patients with long-standing drug-treated hypertension (Ylitalo et al. 1997).

In summary, altered cardiovascular autonomic control seems to be common in hypertension. Most of the previous results on HRV and BRS indicate a decrease in both tonic and reflex vagal activity with elevated blood pressure. However, even other mechanisms may be involved in impaired autonomic cardiovascular modulation. There are no studies where the effects on elevated blood pressure vs. the metabolic factors possibly accompanying hypertension on HRV or BRS have been tested.
The alterations of autonomic cardiovascular control characterized by reduced vagal activity and thus resulting in relative sympathetic dominance might contribute to the development of essential hypertension (Eckberg 1979), and hence be one of the pathophysiological mechanisms behind elevated blood pressure. However, the alterations may also be secondary to long-standing hypertension and, for instance, sign of structural alterations of the vessel wall (medial hypertrophy, endothelial damage) associated with long-standing elevated blood pressure (Kingwell et al. 1995; Zanchetti et al. 1991).

### 2.4.3. HRV and BRS in CAD

Many studies have addressed the impaired autonomic modulation of heart rate in CAD. Both HRV (Airaksinen et al. 1987; Hayano et al. 1990; Rich et al. 1988) and BRS (Eckberg et al. 1971) are reduced in patients with CAD, and heart rate dynamics seem to be altered in CAD patients (Mäkikallio et al. 1998). The circadian rhythm of HRV is altered in patients with CAD (Huikuri et al. 1994). The alterations in autonomic function have correlated with the severity of CAD in some studies (Hayano et al. 1991; La Rovere et al. 1988), but not in others (Airaksinen et al. 1987; Rich et al. 1988). BRS and measures of HRV are lower in post-MI subjects (Bigger et al. 1995) and their heart rate dynamics is altered (Mäkikallio et al. 1996). The exact mechanisms underlying this impairment in CAD are not known. Denervation of the afferent and efferent sympathetic or parasympathetic fibers or alteration of their function after ischemic episodes or infarction have been suggested (Zipes 1990).

### 2.4.4. HRV and BRS in other disease states

Impaired autonomic cardiovascular regulation has been observed in various other pathologies of either the heart itself or the autonomic regulatory systems. Decreased HRV has been seen in patients with congestive heart failure (Casolo et al. 1989; Nolan et al. 1992; Saul et al. 1988; Van Hoogenhuyze et al. 1991; van de Borne et al. 1997), in subjects vulnerable to life-threatening arrhythmias (Huikuri et al. 1992; Martin et al. 1987; Myers et al. 1986), post transplantation (Sands et al. 1989), and in patients with diabetes (Ewing et al. 1981), alcoholism (Malpas et al. 1991), chronic renal failure (Cloarec-Blanchard et al. 1992) or one of a variety of neurological disorders, such as stroke (Korpelainen et al. 1996) or brain damage (Lowensohn et al. 1977).

### 2.4.5. Prognostic significance of HRV and BRS

The most important current application of HRV and BRS in clinical cardiology lies in the risk stratification of postinfarction patients. Wolf reported in 1978 that postinfarction subjects with sinus arrhythmia present had better prognosis (Wolf et al. 1978). Kleiger
reported in his classic work in 1987 HRV to be an independent predictor of mortality in patients with acute myocardial infarction. The relative risk of mortality was 5.3 times higher if 24-hour SDNN was <50 ms than if it was >100 ms (Kleiger et al. 1987). The importance of autonomic cardiovascular regulation in predicting mortality after acute myocardial infarction has later been confirmed by many others, using both decreased HRV assessed with various methods (Algra et al. 1993; Bigger et al. 1992; Farrell et al. 1991; Hartikainen et al. 1996; Hohnloser et al. 1994; La Rovere et al. 1988; La Rovere et al. 1998) and decreased BRS (Farrell et al. 1991; La Rovere et al. 1988; La Rovere et al. 1998). Low HRV, altered heart rate behavior and low BRS have predicted total mortality (Bigger et al. 1992; La Rovere et al. 1988; La Rovere et al. 1998), arrhythmic death (Bigger et al. 1992; Farrell et al. 1991; Hartikainen et al. 1996) and nonarrhythmic death (Hartikainen et al. 1996).

There is evidence to support the potential usefulness of HRV and BRS in predicting mortality in various other patient groups as well. Decreased HRV predicts mortality in subjects with angina pectoris without acute myocardial infarction (Rich et al. 1988), and reduced HRV and altered heart rate behavior predict mortality in elderly populations (Huikuri et al. 1998; Tsuji et al. 1994; Tsuji et al. 1996) and in heart failure patients (Brouwer et al. 1996; Ho et al. 1997).
3. Purpose of the present study

The aims of the present study were to examine autonomic cardiovascular regulation assessed by HRV and BRS in relation to age, sex and cardiovascular risk factors in subjects without heart disease. The specific goals were:

1. to evaluate the interindividual variation of HRV and its association with various cardiovascular risk factors in healthy middle-aged subjects (I,V);

2. to study the possible gender-related differences in cardiac autonomic regulation in healthy middle-aged subjects (II,V);

3. to test whether there are differences in cardiac autonomic regulation in hypertensive subjects with and without metabolic features of IRS and normotensive controls (III);

4. to study the effects of aging from early childhood to advanced age on heart rate dynamics in healthy subjects using both conventional measures of HRV and newly derived measures based on complexity and fractal scaling (chaos theory) (IV), and

5. to study the intersubject variation and determinants of short-term correlation properties and complexity of HRV in middle-aged subjects (V).
4. Subjects and methods

4.1. Study populations

The study cohort used in the Oulu Project Elucidating Risk of Atherosclerosis (OPERA) served as the random population of this study (I-III,V). OPERA was a population-based epidemiological study addressing the risk factors and disease end-points of atherosclerotic diseases. The hypertensive cohort comprised 600 subjects (300 males, 300 females) living in the Oulu district. The subjects were randomly selected from the register of the Social Insurance Institution from those receiving reimbursement for antihypertensive medication. The subjects were 40 to 59 years old at the baseline of the study (1990). The randomization was age-stratified, i.e. for each year of birth, 15 males and 15 females were selected. Age- and sex-matched controls (300 males and 300 females) were randomly selected for the hypertensive subjects from the general population of Oulu, excluding those entitled to a refund for antihypertensive medication. The whole study protocol was completed by 471 (79%) hypertensive subjects and 488 (81%) controls. Informed consent was obtained from the subjects, and the protocol was approved by the Ethics Committee of the University of Oulu.

The subjects with symptoms of angina pectoris, cardiac medication, or electrocardiographic evidence of CAD based on the Minnesota codes (Prineas 1982) (35 healthy, 60 hypertensive subjects) were excluded from further HRV and BRS analyses. Known diabetes or fasting blood glucose >6.7 mmol/l (I-III,V) or 2-hour blood glucose >10 mmol/l (III)(16 healthy, 41 hypertensive subjects) also led to exclusion. The subjects in the hypertensive cohort or with recently started antihypertensive or antiarrhythmic medication (37) were excluded from the studies of healthy subjects (I-II,V). Technical artifacts and rhythm abnormalities (atrial fibrillation or frequent ectopic beats) during the ECG-recording invalidated the analyses of HRV in some subjects. Due to an inadequate blood pressure rise after the Valsalva strain release, the BRS analyses of some subjects not otherwise excluded were not possible. The demographic, lifestyle, laboratory and echocardiographic data of the study populations are presented in Table 1 (III,V).

Interindividual variation and relation to cardiovascular risk factors of HRV using ECG recordings with supine, sitting and walking periods were analyzed in 172 healthy middle-aged males (I). Since most of the technical artifacts were seen during the walking period
or were related to changes in body posture, more subjects were included when HRV was
analyzed only during the lying and sitting periods (II, V). The time and frequency domain
measures of HRV and BRS were studied in 188 healthy men and 186 women (HRV) and
151 men and 152 women (BRS) (II), respectively. The correlation properties and the
complexity of R-R intervals were studied in 192 men and 202 women (V). Women had
lower blood pressure (p<0.001 for both systolic and diastolic blood pressure), higher
Framingham psychosocial scores (p<0.001) and included fewer drinkers (p<0.001).
Women had lower fasting blood glucose and insulin values (p<0.001 for both), serum
lipid values (p<0.001) and left ventricular mass (p<0.001) compared to men. No other
significant differences in the baseline variables were found between men and women
(Table 1).

Sixty-nine hypertensive subjects (50 males, 19 females) with IRS were found, when
classified according to the following criteria: (1) fasting serum insulin ≥ 12 mU/l, (2)
serum triglycerides ≥ 2.0 mmol/l and (3) either established long-standing hypertension
with antihypertensive medication or systolic blood pressure >160 mmHg or diastolic
blood pressure >90 mmHg (criteria modified from references (WHO 1959, Consensus
hypertensive controls without IRS were selected from the original study subjects, who
met the hypertension criteria, but not the two metabolic criteria and age- and sex-matched
normotensive controls were selected from the subjects who met none of the three IRS
criteria. Blood pressure was significantly higher in both hypertensive groups compared to
normotensives (p<0.0001 for both systolic and diastolic blood pressure), while BMI,
waist/hip ratio, fasting and 2-hour blood glucose and insulin and the lipid values (total
cholesterol, low-density and very-low-density lipoprotein cholesterol and serum
triglycerides) were significantly higher and high-density lipoprotein cholesterol lower in
the hypertensive subjects with IRS than in the other two groups (p<0.0001 for all, <0.05
for LDL cholesterol) (Table 1). The antihypertensive medication used in the hypertensive
groups is listed in Table 2.
Table 1. Clinical, lifestyle and laboratory characteristics of the study populations.

<table>
<thead>
<tr>
<th></th>
<th>Healthy middle-aged males (n=192) (V)</th>
<th>Healthy middle-aged females (n=202) (V)</th>
<th>Normotensive subjects (n=69) (III)</th>
<th>Hypertensive subjects without IRS (n=69) (III)</th>
<th>Hypertensive subjects with IRS (n=69) (III)</th>
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</thead>
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<tr>
<td>Age (years)</td>
<td>50 ± 6</td>
<td>51 ± 6</td>
<td>49 ± 5</td>
<td>51 ± 6</td>
<td>49 ± 6</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>26.4 ± 3.5</td>
<td>25.6 ± 4.0</td>
<td>24.5 ± 2.8</td>
<td>25.8 ± 3.1</td>
<td>30.4 ± 4.1</td>
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<td>Waist/hip ratio</td>
<td>0.91 ± 0.06</td>
<td>0.78 ± 0.05</td>
<td>0.84 ± 0.08</td>
<td>0.87 ± 0.07</td>
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<tr>
<td>Systolic blood pressure</td>
<td>147 ± 19</td>
<td>138 ± 20</td>
<td>130 ± 12</td>
<td>161 ± 14</td>
<td>161 ± 18</td>
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<tr>
<td>Diastolic blood pressure</td>
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<td>82 ± 12</td>
<td>78 ± 7</td>
<td>97 ± 8</td>
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<tr>
<td>No smoking</td>
<td>123 (64%)</td>
<td>150 (74%)</td>
<td>49 (71%)</td>
<td>49 (71%)</td>
<td>39 (57%)</td>
</tr>
<tr>
<td>Moderate smoking (&lt;20/d)</td>
<td>57 (30%)</td>
<td>49 (24%)</td>
<td>17 (25%)</td>
<td>16 (23%)</td>
<td>25 (36%)</td>
</tr>
<tr>
<td>Heavy smoking (&gt;20/day)</td>
<td>12 (6%)</td>
<td>3 (2%)</td>
<td>3 (4%)</td>
<td>4 (6%)</td>
<td>5 (7%)</td>
</tr>
<tr>
<td>Duration of smoking</td>
<td>16 ± 14</td>
<td>5 ± 10</td>
<td>10 (15%)</td>
<td>12 (17%)</td>
<td>8 (12%)</td>
</tr>
<tr>
<td>(years when smoked 20</td>
<td>Median 15</td>
<td>Median 0</td>
<td>50 (72%)</td>
<td>36 (52%)</td>
<td>38 (55%)</td>
</tr>
<tr>
<td>cigarettes/day)</td>
<td>(0-46 years)</td>
<td>(0-57 years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No drinking</td>
<td>24 (13%)</td>
<td>37 (18%)</td>
<td>10 (15%)</td>
<td>12 (17%)</td>
<td>8 (12%)</td>
</tr>
<tr>
<td>1-100g/week</td>
<td>96 (50%)</td>
<td>162 (80%)</td>
<td>50 (72%)</td>
<td>36 (52%)</td>
<td>38 (55%)</td>
</tr>
<tr>
<td>&gt;100g/week</td>
<td>72 (37%)</td>
<td>3 (2%)</td>
<td>9 (13%)</td>
<td>21 (30%)</td>
<td>23 (33%)</td>
</tr>
<tr>
<td>Leisure time physical activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No activity</td>
<td>6 (3%)</td>
<td>7 (3%)</td>
<td>15 (22%)</td>
<td>4 (6%)</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>Mild activity</td>
<td>57 (30%)</td>
<td>44 (22%)</td>
<td>27 (39%)</td>
<td>14 (20%)</td>
<td>35 (51%)</td>
</tr>
<tr>
<td>Moderate activity</td>
<td>68 (35%)</td>
<td>69 (34%)</td>
<td>22 (32%)</td>
<td>23 (33%)</td>
<td>21 (30%)</td>
</tr>
<tr>
<td>Heavy activity</td>
<td>61 (32%)</td>
<td>82 (41%)</td>
<td>5 (7%)</td>
<td>28 (41%)</td>
<td>11 (16%)</td>
</tr>
<tr>
<td>Personality type</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Framingham</td>
<td>26 ± 5</td>
<td>28 ± 5</td>
<td>10 ± 15</td>
<td>12 ± 17</td>
<td>8 ± 12</td>
</tr>
<tr>
<td>Bortener</td>
<td>22 ± 3</td>
<td>23 ± 3</td>
<td>50 ± 72</td>
<td>36 ± 52</td>
<td>38 ± 55</td>
</tr>
<tr>
<td>Hostility</td>
<td>7 ± 3</td>
<td>7 ± 3</td>
<td>9 ± 13</td>
<td>21 ± 30</td>
<td>23 ± 33</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/l)</td>
<td>4.4 ± 0.5</td>
<td>4.3 ± 0.4</td>
<td>4.1 ± 0.4</td>
<td>4.3 ± 0.5</td>
<td>4.8 ± 0.6</td>
</tr>
<tr>
<td>2-hour blood glucose (mmol/l)</td>
<td>5.1 ± 1.5</td>
<td>5.1 ± 1.3</td>
<td>4.6 ± 0.9</td>
<td>5.0 ± 1.1</td>
<td>6.1 ± 1.6</td>
</tr>
<tr>
<td>Fasting serum insulin (mU/l)</td>
<td>13 ± 9</td>
<td>9 ± 6</td>
<td>6.8 ± 2.3</td>
<td>7.4 ± 2.1</td>
<td>22.7 ± 13.9</td>
</tr>
<tr>
<td>2-hour serum insulin (mU/l)</td>
<td>55 ± 53</td>
<td>54 ± 45</td>
<td>31.2 ± 15.4</td>
<td>41.3 ± 22.5</td>
<td>116.9 ± 97.9</td>
</tr>
<tr>
<td>Total serum cholesterol (mmol/l)</td>
<td>5.78 ± 1.11</td>
<td>5.49 ± 1.00</td>
<td>5.4 ± 1.1</td>
<td>5.5 ± 0.9</td>
<td>6.2 ± 1.1</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.24 ± 0.30</td>
<td>1.57 ± 0.38</td>
<td>1.45 ± 0.44</td>
<td>1.44 ± 0.35</td>
<td>1.05 ± 0.22</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>3.75 ± 0.95</td>
<td>3.30 ± 0.90</td>
<td>3.38 ± 1.00</td>
<td>3.50 ± 0.89</td>
<td>3.84 ± 1.01</td>
</tr>
<tr>
<td>VLDL-cholesterol (mmol/l)</td>
<td>0.43 ± 0.29</td>
<td>0.28 ± 0.20</td>
<td>0.26 ± 0.15</td>
<td>0.27 ± 0.13</td>
<td>0.85 ± 0.32</td>
</tr>
<tr>
<td>Serum triglycerides (mmol/l)</td>
<td>1.51 ± 0.76</td>
<td>1.13 ± 0.60</td>
<td>1.01 ± 0.33</td>
<td>1.07 ± 1.31</td>
<td>2.73 ± 0.84</td>
</tr>
<tr>
<td>Left ventricular mass #</td>
<td>221 ± 53</td>
<td>154 ± 35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left ventricular mass index #</td>
<td>112 ± 26</td>
<td>91 ± 19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fractional shortening (%) #</td>
<td>34 ± 6</td>
<td>35 ± 5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The values are means ± SD or the numbers of subjects in each group when appropriate.
# n=193 women, n=166 men. BMI = body mass index, HDL = high-density lipoprotein, IRS = insulin resistance syndrome, LDL = low-density lipoprotein, VLDL = very-low-density lipoprotein.
Table 2. Antihypertensive medication of hypertensive subjects with and without IRS (III).

<table>
<thead>
<tr>
<th>Medication</th>
<th>Hypertensive subjects without IRS (n=69)</th>
<th>Hypertensive subjects with IRS (n=69)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No medication</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>Monotherapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β blocker</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Diuretic</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Calcium antagonist</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Combination therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β blocker + calcium antagonist</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>β blocker + ACE inhibitor</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>β blocker + diuretic</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>β blocker + ACE inhibitor + diuretic</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Calcium antagonist + ACE inhibitor</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Calcium antagonist + diuretic</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>ACE inhibitor + diuretic</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>ACE inhibitor + calcium antagonist + diuretic</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>β blocker + calcium antagonist + ACE inhibitor</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>β blocker + calcium antagonist + ACE inhibitor + diuretic</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>ACE inhibitor + diuretic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other antihypertensive medication</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Values are numbers of subjects in each group. ACE = angiotensin converting enzyme. For other abbreviations, see Table 1.

One-hundred and fourteen healthy subjects ranging in age from 1 to 82 years were included in the study of 24-hour heart rate dynamics (IV). The subjects were divided into four groups: (1) children, age under 15 years (mean 8 ± 5 years), (2) young adults, age 15-40 years (mean 28 ± 6 years), (3) middle-aged, age 40-60 years (mean 50 ± 6 years) and (4) elderly, age over 60 years (mean 71 ± 5 years). There were 15 males and 12 females in the group of children and 17 males and 12 females in all the other groups. The children and young adults were apparently healthy with no history or symptoms of heart disease, hypertension or diabetes, and with normal findings in the clinical examination. The middle-aged subjects were selected from the OPERA population. The elderly subjects were selected from a sample of 480 elderly people living in Turku, who had been randomly selected from the register of the Social Insurance Institution (Räiha et al. 1994). All middle-aged and elderly subjects underwent a physical examination with blood pressure measurements, a standard 12-lead ECG, a chest x-ray and laboratory tests, including blood glucose measurements. No subjects with a previous history, symptoms or clinical evidence (including the analysis of a 12-lead ECG with the Minnesota code) of ischemic heart disease, diabetes, hypertension or any other medical disorder were
included. Fasting blood glucose was < 6.7 mmol/l and blood pressure <160/90 mmHg in all subjects. None of the subjects included in this study were taking any medication.

4.2. Collection of clinical data

The study subjects (I-III,V) were interviewed by a physician, and a standardized health questionnaire of past medical history, medication used, cardiac symptoms (angina pectoris, dyspnea), smoking habits, alcohol consumption, physical activity and personality type was filled in. Alcohol intake was converted into grams of absolute ethanol per week. On the basis of leisure-time physical activity, four groups were formed, using a modification of the method described by Grimby (Grimby 1986). The personality type was scaled by three different methods: (1) Framingham type A behavior pattern scale (Haynes et al. 1978), (2) Bortner’s short rating scale of behavior pattern (Bortner 1969), and (3) hostility scaling (Koskenvuo et al. 1988). Women were classified as postmenopausal if at least six months had passed since their latest menstruation.

All the subjects went through a clinical examination, including height, weight and waist and hip circumference measurements. Blood pressure was measured and a standard 12-lead ECG and chest x-ray were taken. ECGs were evaluated using the Minnesota Code (Prineas 1982). A wide range of laboratory tests, echocardiographic measurements, a 45-minute or a 24-hour ECG-recording and tests to evaluate BRS were performed.

4.3. Blood pressure measurements

Blood pressure was measured with an automatic oscillometric blood pressure recorder (Dinamap model 18465X, Criticon Ltd., Inc., Tampa, FL, USA). The subject was seated for at least 5 minutes, after which blood pressure was measured three times at one-minute intervals from the right arm. The mean of the three measurements was used in the analysis.

4.4. Laboratory methods

All the laboratory test samples were drawn after a 12-hour requested fast. A two-hour oral glucose tolerance test (75 g) was performed by determining the 0- and 2-hour glucose (a glucose-oxidase method, Diagnostica, Merck, Darmstadt, Germany) and insulin (a two-site immunoenzymometric assay, Tosoh Corp., Tokyo, Japan) concentrations.

To determine the lipoprotein fractions, the VLDL fraction was first separated from plasma by ultracentrifugation. Then the HDL fraction was determined from the VLDL-free fraction and the plasma LDL cholesterol concentration was calculated by subtracting the cholesterol concentration in HDL from that in the VLDL-free fraction (Kervinen et
The concentrations of total cholesterol and triglycerides were determined in the plasma and lipoprotein fractions by enzymatic colorimetric methods (kits of Boehringer Diagnostica, Mannheim GmbH, Germany) using a Kone Specific analyser (Kone Spesific, Selective Chemistry Analyser, Kone Instruments, Espoo, Finland).

4.5. Echocardiographic measurements

A Hewlett-Packard 77020A ultrasound color system (Hewlett-Packard, Andover, MA, USA) was used for M-mode, two-dimensional and Doppler-echocardiographic studies of each subject (I-III,V). All studies were performed by the same experienced cardiologist blind to the clinical data of the subjects. Standard techniques were used. The M-mode measurements were made according to the American Society of Echocardiography guidelines (Sahn et al. 1978), and left ventricular mass was calculated with the formula of Troy (Troy et al. 1972). Fractional shortening was calculated by dividing the difference between the left ventricular internal dimensions in diastole and systole by the diastolic dimension and then multiplying it by 100. Measurements of the left ventricle were technically inadequate in some subjects, mostly due to obesity. Thus, the echocardiographic data of 166 middle-aged males and 193 females (V) were used.

4.6. ECG recordings for HRV analysis

Both short-term ECG recordings in controlled conditions and ambulatory 24-hour ECG recordings were used in this study. The short-term ECG recordings were obtained for 45 minutes with an ambulatory ECG recorder (Dynacord Holter Recorder, Model 420, DM Scientific, Irvine, CA, USA) with a sample frequency of 256 Hz, and each subject was monitored for 15 minutes while quietly lying down and breathing normally, for 15 minutes in the sitting position and for 15 minutes while walking (I-III,V). The length of the short-term ECG recordings was chosen after a pilot study on 37 normotensive and 40 hypertensive subjects to calculate the shortest period (including lying, sitting and walking) that would give a reasonable correlation (r>0.6) with the 24-hour measures of HRV (Huikuri et al. 1996). These short-term ECG recordings were performed between 7 a.m. and 3 p.m. The mean values of the three periods (supine, sitting, walking) were used in the studies I and III. Most of the technical artifacts were found during the walking period. Thus, the decision to use the ECG data of only the supine and sitting periods made it possible to include more subjects (II,V). In this summary, the interindividual variations and the relations of measures of HRV to sex and risk factors in healthy subjects are presented based on the results of these studies with controlled supine and sitting periods (II,V). Any subject with the ECG data of the analyzed period including less than 85% sinus beats was excluded from further analysis.

The 24-hour ambulatory ECG recordings were made while the subjects were performing usual everyday activities out of hospital (IV). All the subjects had at least 18 hours (mean 23 ± 1 hours) of ECG data, including at least 85% normal sinus beats.
4.7. Measurement of HRV

The digitized ECG data were transferred from the Del Mar Avionics ECG scanner (Model 500, Del Mar Avionics, Irvine, CA, USA) to a microcomputer for the analysis of HRV with the use of a custom-made program (Hearts, Heart Signal co, Kempele, Finland). The program automatically detects and labels each QRS complex. Premature beats and noise were excluded automatically and manually from the computer-generated tachograms. The gaps were either refilled with an average value computed in the local neighborhood (I-III) or deleted (IV,V). Segments with >85% qualified beats were included in the final analysis.

4.7.1. Time and frequency domain measures

A linear detrend was used for segments of 512 consecutive beats to make the data more stationary. A straight line was first fitted to a segment by the standard least squares method and then subtracted from the sample value. An autoregressive model was used to estimate the power spectral densities from short-term ECG recordings (I-III). The value 20 was used as the order of the model. Four frequency bands (total power <0.4 Hz, HF power 0.15-0.4 Hz, LF power 0.04-0.15 Hz and VLF power 0.005-0.04 Hz) were calculated from successive segments of 512 beats. Average values of these segments giving the spectral power in absolute units (ms²) during the period analyzed were used. The mean R-R interval and SDANN (I-III) were used as time domain measures. Furthermore, the ratio between the LF and HF components (LFHF ratio) and the normalized units of the LF and HF components (e.g. LF or HF spectral power in absolute units divided by total power from which the VLF power had been subtracted) were calculated (Huikuri et al. 1994). Time and frequency domain measures of HRV were calculated with linear detrending from a segment of the middle 13 minutes of the 15-minute supine and sitting periods, respectively (V), to avoid the possible confounding effects of the nonstationarities and artifacts in the ECG data resulting from changes in body posture, but by otherwise using the above mentioned methods.

The power spectrum densities were estimated using the fast Fourier method from the 24-hour recordings (IV). ULF (<0.0033 Hz) and VLF power (0.0033-0.04 Hz) were calculated from the entire 24-hour segment. LF power (0.04-0.15 Hz), HF power (0.15-0.4 Hz) and nighttime and daytime VLF power were calculated from 1-hour segments of the 24-hour recording. The average value of these segments was used. The mean R-R interval and SDNN calculated from the entire 24-hour period were used as time domain measures.
4.7.3. Fractal scaling and complexity measures

The measures of R-R interval dynamics were calculated from the entire 24-hour recording and also separately from the hours representing the nighttime (0 - 6 am) and the daytime (9 am - 6 pm), to assess the possible differences between day and night.

**Power-law relationship analysis.** The slope ($\beta$) of the linear inverse power-law relationship of (log) power to (log) frequency was calculated from the frequency range of $10^{-4}$ to $10^{-2}$ Hz (period approximately 3 hours (10 000 seconds) to 1.5 minutes (100 seconds)) from the 24-hour ECG recordings (IV). The point power spectrum was logarithmically smoothed in the frequency domain, and the power was integrated into bins spaced 0.0167 log(Hz) apart. A robust line-fitting algorithm of log(power) on log(frequency) was then applied to the power spectrum between $10^{-4}$ and $10^{-3}$ Hz, and the slope of this line was calculated. This range was chosen because of the typically linear relationship between log (power) and log (frequency) in this frequency band (Bigger et al. 1996).

**Detrended fluctuation analysis** The DFA technique was used to quantify the fractal-like scaling properties of R-R interval data. The root-mean-square fluctuation of the integrated and detrended data was measured in observation windows of varying sizes and then plotted against the size of the window on a log-log scale. The scaling exponent $\alpha$ represents the slope of the line which relates (log) fluctuation to (log) window size. In this study (IV), both $\alpha_1$, the short-term (4 to 11 beats) and $\alpha_2$, the intermediate-term (>11 beats to minutes) scaling exponents were calculated. The scaling exponents were calculated from segments of 8000 beats of the 24-hour ECG recording with the method earlier described (Iyengar et al. 1996; Peng et al. 1995), and average values of these segments were used. $\alpha_1$ and $\alpha_2$ were also calculated from segments of 4000 beats in 3-hour periods (0-3 am, 3-6 am, 9-12 am, 12 am -3 pm, 3-6 pm), to obtain the nighttime (0-6 am) and daytime (9 am-6 pm) average values. The short-term scaling exponent $\alpha_1$ was also calculated from a block of the middle 13 minutes of the 15-minute supine and sitting periods, respectively, in the short-term ECG recordings (V), using otherwise the abovementioned methods.

**Approximate entropy** was used to measure the complexity of the time series data. ApEn was calculated from segments of 8000 beats of the 24-hour ECG recording (IV) with fixed input variables $m=2$ and $r=20\%$ with a method described earlier (Pincus et al. 1992; Pincus et al. 1994) and using the average values of these segments. ApEn was also calculated from segments of 4000 beats in 3 hour periods (0-3 am, 3-6 am, 9-12 am, 12 am -3 pm, 3-6 pm), to obtain the nighttime (0-6 am) and daytime (9 am-6 pm) average values. ApEn was also calculated from a block of the middle 13 minutes of the 15-minute supine and sitting periods, respectively, in short-term ECG recordings (V), using otherwise the abovementioned methods.

4.8. Measurement of BRS

BRS was measured using the Valsalva method (Airaksinen et al. 1993). The study subjects performed the Valsalva maneuver in the sitting position by blowing into a rubber
tube connected to an aneroid manometer and maintaining a pressure of 40 mmHg for 15 seconds. The R-R intervals were obtained from the surface ECG and fed into the analog-to-digital converter (DAP 1200, Microstar Laboratories, Inc., USA) of a microcomputer. Non-invasive arterial pressure was measured on a beat-to-beat basis using the Finapres finger-cuff method. The analysis of BRS was accomplished with a menu-driven software package (CAFTS, Medikro Oy, Kuopio, Finland). The tachograms of beat-to-beat R-R intervals and blood pressure values were viewed and optionally edited on the screen. The time window of the rapid blood pressure rise after the strain release was defined for the calculation of the BRS. This time window ranged from the beat on which the systolic blood pressure exceeded the systolic blood pressure level at the end of the Valsalva strain to the beat following the maximum systolic blood pressure overshoot. BRS was calculated as the slope of the linear relationship between the length of the R-R interval (in ms) and the preceding systolic blood pressure value (in mmHg) within the defined time window using linear least-mean-squares fitting.

Only regression lines with a correlation coefficient greater than 0.8 or with a significant p-value (<0.05) and with a blood pressure change = 15 mmHg were accepted for analysis. The test was repeated three times. The BRS value used in this study was calculated as the mean value of two accepted tests with the best correlation coefficients of the regression lines.

BRS analyses could not be performed if the blood pressure rise after strain release was inadequate or if frequent ectopies or artifacts were present during the ECG recording.

4.9. Statistics

Continuous variables are expressed as mean ± standard deviation, unless specified otherwise. p-values <0.05 (I-IV) and <0.01 (V) were considered statistically significant. A logarithmic transformation to the natural base was made on the variables with highly skewed distributions. Parametric statistical methods were chosen based on the results of the Kolmogorov-Smirnov test (Z < 1.0) of normal distribution (IV).

The associations between different variables were analyzed with univariate techniques (Pearson’s bivariate correlation, one-way ANOVA). A stepwise linear multiple regression analysis was performed by including all the variables which had a significant univariate relation to the dependent variable in question (I,III). The results of the stepwise linear multiple regression analysis are given as standardized multiple regression correlates (β) coefficients and multiple R and R² of the model.

The comparisons between two samples or groups were performed using the Mann-Whitney two-sample test (II), the χ² test (II) and the independent samples t-test (III, IV,V) and those between more than two groups using the one-way ANOVA followed by the Bonferroni correction for multiple comparisons (III,IV). The differences between supine vs. upright positions were tested with paired samples t-test (V). The significant differences in the baseline variables between the groups were taken into account by means of analysis of covariance (ANCOVA ) (II, IV,V).
5. Results

5.1. Time and frequency domain measures of HRV in healthy middle-aged subjects

5.1.1. Interindividual variation in measures of HRV

Wide interindividual variation compared to the variation in the mean R-R interval was observed in SDANN and the spectral measures of HRV as analyzed in both absolute and normalized units (n=394, Table 3) (V). The interindividual variations were larger in the controlled supine compared to the upright (sitting) position.

5.1.2. Sex-related differences in measures of HRV

The measures of R-R interval dynamics in men (n=192) and women (n=202) are shown in Table 4. (V). Women had lower SDANN, higher ApEn and lower α1 compared to men (p<0.001 for all), while the mean R-R interval, LF or HF power in absolute units did not differ. The LFHF ratio (p<0.001) and normalized LF power (p<0.01) were lower in women, while the normalized HF power was higher (p<0.001). When the observed differences in the baseline variables (blood pressure, serum lipid values, fasting blood glucose and insulin values, left ventricular mass, alcohol consumption and the Framingham psychosocial score, Table 1.) between men and women were taken into account in ANCOVA by using clinical and laboratory variables as covariates, α1 was still lower (F=7.9, p<0.01), ApEn higher (F=8.8, p<0.01) and SDANN lower (F=28.7, p<0.001) in women compared to men. The significant sex-related differences in the LFHF ratio (p<0.01) and normalized HF power (p<0.001) also remained.

SDANN (62 ± 24 vs. 48 ± 18 ms, p<0.01), HF power (350 ± 290 vs. 260 ± 310 ms², p<0.05) and LF power (710 ± 110 vs. 410 ± 340 ms², p<0.01) in absolute units were significantly higher in postmenopausal women on estrogen replacement therapy (n=46) than in age-matched postmenopausal women without hormone therapy (II).
Table 3. Interindividual variation in the measures of R-R interval dynamics in healthy middle-aged subjects (n=394).

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Range</th>
<th>Coefficient of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean R-R interval (ms)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>supine+sitting</td>
<td>865 ± 122</td>
<td>535 - 1324</td>
<td>14 %</td>
</tr>
<tr>
<td>supine</td>
<td>891 ± 131</td>
<td>573 - 1402</td>
<td>15 %</td>
</tr>
<tr>
<td>sitting</td>
<td>839 ± 121</td>
<td>428 - 1260</td>
<td>14 %</td>
</tr>
<tr>
<td><strong>SDANN (ms)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>supine+sitting</td>
<td>54 ± 21</td>
<td>18 - 174</td>
<td>39 %</td>
</tr>
<tr>
<td>supine</td>
<td>49 ± 21</td>
<td>13 - 168</td>
<td>43 %</td>
</tr>
<tr>
<td>sitting</td>
<td>59 ± 24</td>
<td>16 - 180</td>
<td>41 %</td>
</tr>
<tr>
<td><strong>LF power (0.04-0.15 Hz)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ms²) supine+sitting</td>
<td>736 ± 630</td>
<td>36 - 4331</td>
<td>86 %</td>
</tr>
<tr>
<td>supine</td>
<td>655 ± 655</td>
<td>35 - 5941</td>
<td>100 %</td>
</tr>
<tr>
<td>sitting</td>
<td>817 ± 729</td>
<td>26 - 5961</td>
<td>89 %</td>
</tr>
<tr>
<td>(nu) supine+sitting</td>
<td>66 ± 15</td>
<td>14 - 98</td>
<td>23 %</td>
</tr>
<tr>
<td>supine</td>
<td>63 ± 18</td>
<td>11 - 98</td>
<td>28 %</td>
</tr>
<tr>
<td>sitting</td>
<td>69 ± 17</td>
<td>8 - 98</td>
<td>25 %</td>
</tr>
<tr>
<td><strong>HF power (0.15-0.4 Hz)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ms²) supine+sitting</td>
<td>354 ± 450</td>
<td>20 - 5074</td>
<td>127 %</td>
</tr>
<tr>
<td>supine</td>
<td>381 ± 585</td>
<td>10 - 7231</td>
<td>154 %</td>
</tr>
<tr>
<td>sitting</td>
<td>326 ± 385</td>
<td>10 - 3870</td>
<td>118 %</td>
</tr>
<tr>
<td>(nu) supine+sitting</td>
<td>28 ± 11</td>
<td>8 - 63</td>
<td>38 %</td>
</tr>
<tr>
<td>supine</td>
<td>31 ± 14</td>
<td>3 - 72</td>
<td>44 %</td>
</tr>
<tr>
<td>sitting</td>
<td>25 ± 11</td>
<td>2 - 59</td>
<td>43 %</td>
</tr>
<tr>
<td><strong>LFHF-ratio</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>supine+sitting</td>
<td>2.93 ± 1.78</td>
<td>0.50 -11.9</td>
<td>61%</td>
</tr>
<tr>
<td>supine</td>
<td>2.64 ± 2.04</td>
<td>0.24 - 17.10</td>
<td>77%</td>
</tr>
<tr>
<td>sitting</td>
<td>3.62 ± 3.31</td>
<td>0.45 - 13.24</td>
<td>91%</td>
</tr>
<tr>
<td><strong>α1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>supine+sitting</td>
<td>1.21 ± 0.17</td>
<td>0.71 - 1.61</td>
<td>14 %</td>
</tr>
<tr>
<td>supine</td>
<td>1.15 ± 0.20</td>
<td>0.50 - 1.68</td>
<td>17 %</td>
</tr>
<tr>
<td>sitting</td>
<td>1.26 ± 0.18</td>
<td>0.61 - 1.67</td>
<td>14 %</td>
</tr>
<tr>
<td><strong>ApEn</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>supine+sitting</td>
<td>1.13 ± 0.14</td>
<td>0.56 - 1.45</td>
<td>12 %</td>
</tr>
<tr>
<td>supine</td>
<td>1.14 ± 0.18</td>
<td>0.31 - 1.49</td>
<td>16 %</td>
</tr>
<tr>
<td>sitting</td>
<td>1.11 ± 0.19</td>
<td>0.48 - 1.48</td>
<td>17 %</td>
</tr>
</tbody>
</table>

α1 = short-term scaling exponent, ApEn = approximate entropy, HF = high frequency, LF = low frequency, nu = normalized units, SDANN = standard deviation of R-R intervals
Table 4. Measures of R-R interval dynamics in healthy middle-aged men and women.

<table>
<thead>
<tr>
<th></th>
<th>Men (n=192)</th>
<th>Women (n=202)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean R-R Interval</td>
<td>864 ± 136</td>
<td>866 ± 107</td>
<td>ns</td>
</tr>
<tr>
<td>ApEn</td>
<td>1.10 ± 0.14</td>
<td>1.15 ± 0.14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ρ</td>
<td>1.25 ± 0.17</td>
<td>1.17 ± 0.16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SDANN (ms)</td>
<td>58 ± 24</td>
<td>50 ± 16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LF (ms²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>805 ± 714</td>
<td>671 ± 532</td>
<td>ns</td>
</tr>
<tr>
<td>(nu)</td>
<td>68 ± 17</td>
<td>64 ± 14</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>HF (ms²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>357 ± 541</td>
<td>351 ± 344</td>
<td>ns</td>
</tr>
<tr>
<td>(nu)</td>
<td>25 ± 11</td>
<td>31 ± 10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LFHF-ratio</td>
<td>3.4 ± 2.0</td>
<td>2.5 ± 1.5</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

For abbreviations, see Table 3.

5.1.3. Relation of HRV to demographic, lifestyle and cardiovascular risk factors

Tables 5 and 6 list the significant bivariate correlations between the time and frequency domain measures of HRV and the demographic and laboratory variables in men (n=192) and women (n=202) (V). The time and frequency domain measures had significant inverse correlations with age and serum insulin levels in both men and women and additionally with systolic blood pressure and serum triglyceride levels in men. They also correlated directly with HDL cholesterol in men. The LFHF ratio and normalized spectral HF power and LF power were not related to any measure in either men or women. No significant relations were found between any measure of HRV and current smoking habits, alcohol consumption, leisurtime physical activity, personality type or echocardiographic parameters in either men or women. When the measures of HRV were analyzed in supine or upright positions separately, the relations to the abovementioned factors were similar.

Table 5. Significant relationships of measures of R-R interval dynamics to demographic and laboratory parameters in healthy middle-aged men (n=192).

<table>
<thead>
<tr>
<th></th>
<th>αl</th>
<th>ApEn</th>
<th>SDANN</th>
<th>LF</th>
<th>HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>ns</td>
<td>ns</td>
<td>-0.19 *</td>
<td>-0.29 **</td>
<td>-0.19 *</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>-0.21 *</td>
</tr>
<tr>
<td>2-hour serum insulin</td>
<td>ns</td>
<td>ns</td>
<td>-0.23 **</td>
<td>-0.23 *</td>
<td>-0.29 **</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>ns</td>
<td>ns</td>
<td>0.19 *</td>
<td>0.20 *</td>
<td>ns</td>
</tr>
<tr>
<td>Serum triglycerides</td>
<td>ns</td>
<td>ns</td>
<td>-0.25 **</td>
<td>-0.21 *</td>
<td>-0.23 **</td>
</tr>
</tbody>
</table>

* p<0.01, ** p<0.001, ns = non-significant. For other abbreviations, see Tables 1 and 3.
Table 6. Significant relationships of measures of R-R interval dynamics to demographic and laboratory parameters in healthy middle-aged women (n=202).

<table>
<thead>
<tr>
<th></th>
<th>$\alpha_1$</th>
<th>ApEn</th>
<th>SDANN</th>
<th>LF</th>
<th>HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>-0.24 **</td>
<td>-0.21 *</td>
</tr>
<tr>
<td>Blood glucose</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>-0.18 *</td>
</tr>
<tr>
<td>Fasting serum insulin</td>
<td>ns</td>
<td>ns</td>
<td>-0.23 **</td>
<td>-0.18 *</td>
<td>-0.19 *</td>
</tr>
<tr>
<td>2-hour serum insulin</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>-0.18 *</td>
</tr>
</tbody>
</table>

For abbreviations, see Tables 1, 3 and 4.

5.2. BRS in healthy middle-aged subjects

5.2.1. Interindividual variation and relations to demographic, lifestyle and cardiovascular risk factors of BRS

Large interindividual variation in BRS was observed in both men (n=151) (10.5 ± 4.6 ms/mmHg, coefficient of variation 58%) and women (n=152) (8.0 ± 4.6 ms/mmHg, coefficient of variation 44%) (II). BRS was significantly related to systolic blood pressure ($r=-0.31$ and $r=-0.30$, in men and women, respectively, $p<0.001$ for both) (unpublished). In addition BRS was related to blood glucose ($r=-0.25$, $p<0.01$) and 2-hour serum insulin ($r=-0.34$, $p<0.001$) in women (unpublished). No other significant relations to demographic, laboratory, lifestyle or echocardiographic factors were found.

5.2.2. Sex-related differences in BRS

BRS was significantly lower in women (n=152) than in men (n=151) (8.0 ± 4.6 ms/mmHg vs. 10.5 ± 4.6 ms/mmHg, $p<0.001$) and the difference remained significant after adjustment for the differences in baseline variables (Table 1) ($F=22.8$, $p<0.001$) (II). BRS (10.4 ± 5.0 vs. 6.8 ± 3.2 ms/mmHg, $p<0.01$) was significantly higher in postmenopausal women on estrogen replacement therapy (n=46) than in age-matched postmenopausal women without hormone therapy (II).
5.3. HRV and BRS in hypertensive subjects with and without insulin resistance syndrome

5.3.1. Overall HRV and BRS in hypertensive subjects with and without metabolic features of insulin resistance syndrome and normotensive controls

All the time and frequency domain measures of HRV analyzed in absolute units, with the exception of the HF component, were significantly lower in hypertensive subjects with IRS (n=69) compared to hypertensive subjects without IRS and normotensive subjects (Table 7) (III). On the other hand, hypertensive subjects without IRS (n=69) did not differ significantly from normotensive subjects with respect to total or LF power or SDANN. The HF power of HRV and BRS were significantly higher in normotensive subjects than hypertensive subjects, but did not differ between the two hypertensive groups. The average heart rate, LFHF ratio and normalized LF and HF power were not significantly different between any of the groups.

Hypertensive subjects without IRS and antihypertensive medication (n=23) did not differ significantly from the normotensive group, but the LF component (p<0.05), HF, total power and SDANN (p<0.001 for all) were significantly lower in the hypertensive subjects with IRS and without medication (n=23) than in the normotensive controls. The measures of HRV or BRS did not differ when the subjects with and without β-blockers, ACE inhibitors, calcium antagonists or diuretics were compared in each group.

5.3.2. Relationships of HRV and BRS to features of insulin resistance syndrome

In the group of hypertensive subjects with and without IRS (n=138), all measures of HRV had significant negative correlations with systolic blood pressure (r between -0.20 and -0.28, p<0.05), fasting blood glucose (r between -0.17 and -0.31, p<0.05) and 2-hour glucose levels (r between -0.20 and -0.31, p<0.05). All measures except HF power had significant correlations with the fasting serum insulin (-0.23 and -0.24, p<0.01) and 2-hour insulin levels (r between -0.23 and -0.23, p<0.05), serum triglyceride levels (-0.27 and -0.31, p<0.01) and serum VLDL cholesterol levels (-0.21 and -0.28, p<0.05). Total power and SDANN also correlated with body mass index (r between -0.17 and -0.18, p<0.05). No significant correlations emerged for total, low-density or high-density lipoprotein cholesterol. BRS was only related to systolic blood pressure (r=-0.32, p<0.01). In the multiple regression analysis, all measures of HRV and BRS were significantly predicted by systolic blood pressure (Table 8). Total and LF power and SDANN were also independently predicted by the serum triglyceride level, which was an even more powerful predictor of total and LF power and SDANN than systolic blood pressure.
### Table 7. Mean R-R interval, HRV and BRS of hypertensive subjects with and without IRS and normotensive controls.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normotensive subjects (n=69)</th>
<th>Hypertensive subjects without IRS (n=69)</th>
<th>Hypertensive subjects with IRS (n=69)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean R-R interval (ms)</td>
<td>848 ± 101</td>
<td>836 ± 125</td>
<td>815 ± 139</td>
<td>ns</td>
</tr>
<tr>
<td>SDANN (ms)</td>
<td>65 ± 18</td>
<td>59 ± 20</td>
<td>50 ± 19§</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HF power ms²</td>
<td>322 ± 320</td>
<td>231 ± 202</td>
<td>208 ± 221</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ln</td>
<td>5.46 ± 0.76§</td>
<td>5.13 ± 0.80</td>
<td>5.01 ± 0.76</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>nu</td>
<td>32 ± 11</td>
<td>29 ± 10</td>
<td>34 ± 11</td>
<td>ns</td>
</tr>
<tr>
<td>LF power ms²</td>
<td>659 ± 499</td>
<td>573 ± 423</td>
<td>414 ± 354</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ln</td>
<td>6.25 ± 0.69</td>
<td>6.05 ± 0.86</td>
<td>5.72 ± 0.81§</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>nu</td>
<td>68 ± 11</td>
<td>71 ± 10</td>
<td>66 ± 11</td>
<td>ns</td>
</tr>
<tr>
<td>Total power ms²</td>
<td>2885 ± 1943</td>
<td>2386 ± 1628</td>
<td>1834 ± 1613</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ln</td>
<td>7.78 ± 0.61</td>
<td>7.53 ± 0.77</td>
<td>7.23 ± 0.74§</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LFHF ratio</td>
<td>2.57 ± 1.53</td>
<td>2.82 ± 1.38</td>
<td>2.33 ± 1.25</td>
<td>ns</td>
</tr>
<tr>
<td>BRS (ms/mmHg) #</td>
<td>11.1 ± 4.8§</td>
<td>9.2 ± 6.0</td>
<td>7.7 ± 4.0</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are mean ± SD. # n=46, 40 and 30 subjects in the groups. BRS = baroreflex sensitivity, HRV = heart rate variability, ln = natural logarithm. p-value from one-way ANOVA. § indicates that the group differed in the Bonferroni post hoc analysis from the other two groups, ns indicates difference between normotensive subjects and hypertensive subjects with IRS. For other abbreviations, see Tables 1, 3 and 4.

### Table 8. Results of the multiple regression analysis of the different measures of HRV and BRS and the features of IRS in hypertensive subjects with and without IRS (n=138).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SDANN</th>
<th>Total power</th>
<th>LF power</th>
<th>HF power</th>
<th>BRS #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure</td>
<td>β</td>
<td>-0.22</td>
<td>-0.26</td>
<td>-0.27</td>
<td>-0.32</td>
</tr>
<tr>
<td>p-value</td>
<td></td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Serum triglycerides</td>
<td>β</td>
<td>-0.28</td>
<td>-0.27</td>
<td>-0.30</td>
<td>ns</td>
</tr>
<tr>
<td>p-value</td>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Multiple R</td>
<td></td>
<td>0.37</td>
<td>0.39</td>
<td>0.42</td>
<td>0.20</td>
</tr>
<tr>
<td>R²</td>
<td></td>
<td>0.13</td>
<td>0.15</td>
<td>0.17</td>
<td>0.04</td>
</tr>
</tbody>
</table>

ns = factor did not enter the model, # n=70. For other abbreviations, see Tables 3 and 7.
5.4. Fractal correlation properties and complexity of HRV

5.4.1. Interindividual variation and relations to demographic, lifestyle and cardiovascular risk factors in short-term scaling exponent and approximate entropy

Both the short-term scaling exponent $\alpha_1$ and ApEn showed only slight interindividual variation (coefficients of variations 14% and 12%, respectively) in controlled conditions ($n=394$, Table 3)(V). Women ($n=202$) had higher ApEn (1.15 ± 0.14 vs. 1.10 ± 0.14, $p<0.001$) and lower $\alpha_1$ (1.17 ± 0.16 vs. 1.25 ± 0.17, $p<0.001$) compared to men ($n=192$) (Table 4)(V). $\alpha_1$ and ApEn were not significantly related to any other demographic, lifestyle, laboratory or echocardiographic factors in middle-aged subjects (V).

5.4.2. Effects of age on 24-hour fractal correlation properties and complexity of HRV

Representative examples of R-R interval time series, 24-hour power spectra, power-law relationship analyses and detrended fluctuation analyses (DFA) of 7-year, 29-year and 76-year old healthy males are shown in Figures 1 and 2. Different measures of HR dynamics as a function of age are plotted in the Figures 3-5 (IV).
Figure 1. Representative examples of R-R interval time series and 24-hour power spectra of healthy males aged 7 years (left), 29 years (middle) and 76 years (right).
Figure 3. Time domain measures of heart rate variability in relation to age in 114 healthy subjects.
Figure 3. Time domain measures of heart rate variability in relation to age in 114 healthy subjects.
Figure 4. Frequency domain measures of heart rate variability in relation to age in 114 healthy subjects.
Figure 5. Measures of complexity and fractal scaling of R-R interval dynamics in relation to age in 114 healthy subjects.
The complexity (ApEn) and the short-term ($\alpha_1$) and longer-term temporal correlation properties ($\alpha_2$ and $\beta$) of R-R intervals did not differ between children (n=27) and young adults (n=29) (Table 9). However, the total variance and all the power spectral measures were lower in children than in young adults. An age-related linear decrease of ApEn ($r=-0.69$) and $\beta$ ($r=0.60$) and an corresponding increase of $\alpha_2$ ($r=0.63$) took place in middle age and old age ($p<0.001$ for all) (Figure 5, Table 9). Middle-aged (n=29) and elderly subjects (n=29) had significantly lower values for $\beta$ and ApEn and higher values for $\alpha_2$ than the two younger groups (Table 9). The short-term scaling exponent, $\alpha_1$, did not differ between the three adult groups. A decrease of all time and frequency domain measures also took place over age in adults (Table 9). The differences in various indices between the age groups were similar during the daytime and the nighttime (Table 9) (IV).

To find out if a decrease in total HR variability explains the changes in the dynamical measures of R-R interval variability with increasing age, an analysis of covariance (ANCOVA) was performed using SDNN and age-group as explanatory variables and each of the four dynamical measures of R-R interval variability as dependent variables. The significant differences in ApEn, $\beta$, $\alpha_1$ and $\alpha_2$ between the groups remained after adjustment for SDNN ($p<0.001$ for each).

In all age groups, ApEn was higher ($p<0.001$ in each group), $\alpha_1$ lower ($p<0.001$ in children and young adults, $p<0.05$ in middle-aged and elderly) and all spectral measures higher ($p<0.01$ for all in each group) during the sleeping times than in the daytime (Table 9).

$\alpha_1$ was significantly lower ($1.10 \pm 0.13$ vs. $1.18 \pm 0.16$, $p<0.01$), $\beta$ was slightly steeper ($-1.29 \pm 0.21$ vs. $-1.21 \pm 0.19$, $p<0.05$) and VLF slightly lower ($7.14 \pm 0.70$ vs. $7.45 \pm 0.78$, $p<0.05$) in females (n=48), while no other differences were observed compared to males (n=66). Similar age-dependencies of the different measures of R-R interval dynamics were observed in both males and females.
### Table 9. Measures of 24-hour R-R interval dynamics in healthy children, young, middle-aged and elderly subjects (n=114).

<table>
<thead>
<tr>
<th></th>
<th>Children</th>
<th>Young adults</th>
<th>Middle-aged</th>
<th>Elderly</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;15 years</td>
<td>15-39 years</td>
<td>40-60 years</td>
<td>&gt;60 years</td>
</tr>
<tr>
<td></td>
<td>(n=27)</td>
<td>(n=29)</td>
<td>(n=29)</td>
<td>(n=29)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>8 ± 5</td>
<td>28 ± 6</td>
<td>50 ± 6</td>
<td>71 ± 5</td>
</tr>
<tr>
<td>Mean R-R interval (ms)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-hour</td>
<td>678 ± 105 *</td>
<td>875 ± 121</td>
<td>876 ± 88</td>
<td>829 ± 96</td>
</tr>
<tr>
<td>0 am–6 am</td>
<td>614 ± 91 *</td>
<td>1074 ± 117</td>
<td>1050 ± 127</td>
<td>954 ± 119</td>
</tr>
<tr>
<td>9 am–6 pm</td>
<td></td>
<td>811 ± 146</td>
<td>796 ± 81</td>
<td>730 ± 168</td>
</tr>
<tr>
<td>SDNN (ms)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-hour</td>
<td>140 ± 46?</td>
<td>196 ± 39</td>
<td>169 ± 39</td>
<td>138 ± 32?</td>
</tr>
<tr>
<td>0 am–6 am</td>
<td>95 ± 41</td>
<td>135 ± 36 *</td>
<td>92 ± 28</td>
<td>73 ± 16</td>
</tr>
<tr>
<td>9 am–6 pm</td>
<td>80 ± 23</td>
<td>118 ± 33 *</td>
<td>98 ± 21</td>
<td>76 ± 18</td>
</tr>
<tr>
<td>HF (ln)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-hour</td>
<td>6.83 ± 1.12</td>
<td>7.35 ± 0.94</td>
<td>6.10 ± 0.72</td>
<td>5.06 ± 0.61*</td>
</tr>
<tr>
<td>0 am–6 am</td>
<td>7.27 ± 1.35</td>
<td>7.82 ± 0.97</td>
<td>6.51 ± 0.89</td>
<td>5.27 ± 0.73*</td>
</tr>
<tr>
<td>9 am–6 pm</td>
<td>6.20 ± 1.03</td>
<td>6.69 ± 1.04</td>
<td>5.55 ± 0.76</td>
<td>4.77 ± 0.59*</td>
</tr>
<tr>
<td>LF (ln)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-hour</td>
<td>6.85 ± 0.97</td>
<td>7.74 ± 0.50 *</td>
<td>6.66 ± 0.74</td>
<td>5.73 ± 0.63 *</td>
</tr>
<tr>
<td>0 am–6 am</td>
<td>7.03 ± 1.09</td>
<td>7.98 ± 0.55 *</td>
<td>6.90 ± 0.93</td>
<td>6.01 ± 0.73 *</td>
</tr>
<tr>
<td>9 am–6 pm</td>
<td>6.66 ± 0.98</td>
<td>7.49 ± 0.65 *</td>
<td>6.44 ± 0.66</td>
<td>5.39 ± 0.65 *</td>
</tr>
<tr>
<td>VLF (ln)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-hour</td>
<td>7.19 ± 0.75</td>
<td>8.07 ± 0.53 *</td>
<td>7.30 ± 0.58</td>
<td>6.72 ± 0.45 *</td>
</tr>
<tr>
<td>0 am–6 am</td>
<td>7.60 ± 0.76</td>
<td>8.52 ± 0.39 *</td>
<td>7.84 ± 0.71</td>
<td>7.17 ± 0.48 *</td>
</tr>
<tr>
<td>9 am–6 pm</td>
<td>7.05 ± 0.67</td>
<td>7.90 ± 0.62 *</td>
<td>7.22 ± 0.59</td>
<td>6.46 ± 0.51 *</td>
</tr>
<tr>
<td>ULF (ln) 24-hour</td>
<td>9.39 ± 0.81</td>
<td>10.02 ± 0.66 *</td>
<td>9.54 ± 0.56</td>
<td>9.25 ± 0.43 *</td>
</tr>
<tr>
<td>β</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-hour</td>
<td>-1.15 ± 0.18</td>
<td>-1.12 ± 0.19</td>
<td>-1.32 ± 0.14</td>
<td>-1.38 ± 0.17 ‡</td>
</tr>
<tr>
<td>α1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-hour</td>
<td>1.06 ± 0.11 §</td>
<td>1.15 ± 0.16</td>
<td>1.19 ± 0.14</td>
<td>1.19 ± 0.16</td>
</tr>
<tr>
<td>0 am–6 am</td>
<td>0.91 ± 0.18 §</td>
<td>1.01 ± 0.21</td>
<td>1.13 ± 0.21</td>
<td>1.26 ± 0.20</td>
</tr>
<tr>
<td>9 am–6 pm</td>
<td>1.13 ± 0.13</td>
<td>1.20 ± 0.16</td>
<td>1.24 ± 0.14</td>
<td>1.15 ± 0.16</td>
</tr>
<tr>
<td>α2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-hour</td>
<td>0.98 ± 0.06</td>
<td>1.00 ± 0.08</td>
<td>1.07 ± 0.07 †</td>
<td>1.14 ± 0.07 *</td>
</tr>
<tr>
<td>0 am–6 am</td>
<td>0.96 ± 0.10 §</td>
<td>0.99 ± 0.10</td>
<td>1.06 ± 0.10</td>
<td>1.12 ± 0.11</td>
</tr>
<tr>
<td>9 am–6 pm</td>
<td>0.95 ± 0.10</td>
<td>0.98 ± 0.09</td>
<td>1.06 ± 0.08 †</td>
<td>1.12 ± 0.10 ‡</td>
</tr>
<tr>
<td>ApEn</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-hour</td>
<td>1.26 ± 0.12</td>
<td>1.21 ± 0.14</td>
<td>1.01 ± 0.16 †</td>
<td>0.88 ± 0.16 *</td>
</tr>
<tr>
<td>0 am–6 am</td>
<td>1.37 ± 0.13</td>
<td>1.34 ± 0.19</td>
<td>1.27 ± 0.17</td>
<td>1.06 ± 0.15 *</td>
</tr>
<tr>
<td>9 am–6 pm</td>
<td>1.18 ± 0.14</td>
<td>1.12 ± 0.12</td>
<td>0.98 ± 0.13 †</td>
<td>0.91 ± 0.17 ‡</td>
</tr>
</tbody>
</table>

Values are mean ± SD. β = slope of the power-law relationship of HRV; α2 = intermediate-term scaling exponent; SDNN = standard deviation of R-R intervals, ULF = ultra low frequency power, VLF = very low frequency. For other abbreviations, see Table 3. The symbols indicate the difference between groups in one-way ANOVA followed by Bonferroni post hoc analysis with a confidence level of p<0.05. * = group differed from the other three groups, † = middle-aged subjects differed from children and young adults, ‡ = elderly subjects differed from children and young adults, § = children differed from middle-aged and elderly subjects, ? = group differed from young adults and middle-aged subjects.
Table 10. Correlation matrix of the relationship between heart rate and measures of 24-hour R-R interval dynamics in healthy subjects (n=114).

<table>
<thead>
<tr>
<th></th>
<th>Mean R-R interval</th>
<th>SDNN</th>
<th>HF</th>
<th>LF</th>
<th>VLF</th>
<th>ULF</th>
<th>β</th>
<th>α1</th>
<th>α2</th>
<th>ApEn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean R-R</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDNN</td>
<td>1.00</td>
<td>0.58</td>
<td>#</td>
<td>0.14</td>
<td>#</td>
<td>0.01</td>
<td>0.37</td>
<td>#</td>
<td>0.04</td>
<td>0.19</td>
</tr>
<tr>
<td>HF</td>
<td>1.00</td>
<td>0.48</td>
<td>#</td>
<td>0.70</td>
<td>#</td>
<td>0.80</td>
<td>#</td>
<td>0.24</td>
<td>*</td>
<td>0.13</td>
</tr>
<tr>
<td>LF</td>
<td>1.00</td>
<td>0.72</td>
<td>#</td>
<td>0.52</td>
<td>#</td>
<td>0.50</td>
<td>#</td>
<td>0.36</td>
<td>#</td>
<td>-0.48</td>
</tr>
<tr>
<td>VLF</td>
<td>1.00</td>
<td>0.81</td>
<td>#</td>
<td>0.65</td>
<td>#</td>
<td>0.46</td>
<td>#</td>
<td>-0.15</td>
<td>-0.56</td>
<td>0.52</td>
</tr>
<tr>
<td>ULF</td>
<td>1.00</td>
<td>0.71</td>
<td>#</td>
<td>0.47</td>
<td>#</td>
<td>-0.06</td>
<td>*</td>
<td>-0.21</td>
<td>#</td>
<td>0.36</td>
</tr>
<tr>
<td>β</td>
<td>1.00</td>
<td>0.24</td>
<td>§</td>
<td>-0.04</td>
<td>§</td>
<td>-0.26</td>
<td>*</td>
<td>0.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α1</td>
<td>1.00</td>
<td>-0.6</td>
<td></td>
<td>-0.33</td>
<td>#</td>
<td>0.50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α2</td>
<td>1.00</td>
<td>0.24</td>
<td>*</td>
<td>-0.55</td>
<td>#</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ApEn</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* = p<0.05, § = p<0.01, # = p<0.001. For other abbreviations, see Tables 3, 7 and 9.
6. Discussion

6.1. Interindividual variation of HRV and BRS in healthy middle-aged subjects

Large interindividual variation in HRV and BRS has been described in healthy subjects, including relatively small samples of subjects without evidence of heart disease (Abdel-Rahman et al. 1994; Huikuri et al. 1990; Kupari et al. 1993; Molgaard et al. 1994). In agreement with the results of the previous smaller studies, wide interindividual variation was observed in the traditional nonspectral and spectral measures of HRV in this large cohort of middle-aged subjects (II,V). The interindividual variation of HRV was more than twofold compared to the variation in the mean heart rate. Large interindividual variation was also observed in BRS and in the normalized spectral components of HRV. These results show that both tonic autonomic regulation, expressed as HRV, and reflex regulation, expressed as BRS, vary markedly between healthy subjects. The interindividual variation in autonomic function in healthy subjects may indicate that some subjects have a better capacity to adapt to changing conditions than some others. It is not known whether these differences in autonomic function between healthy middle-aged subjects have any prognostic significance.

6.2. Relations of HRV and BRS to demographic, lifestyle and laboratory factors in healthy middle-aged subjects

The observed interindividual variation in the autonomic function (Abdel-Rahman et al. 1994; Huikuri et al. 1990; Kupari et al. 1993; Molgaard et al. 1994) has been suggested to partly depend on the effect of various risk factors on HRV and BRS. Although HRV has been found to be related to many clinical, lifestyle and laboratory factors (Dixon et al. 1992; Goldsmith et al. 1992; Hayano et al. 1990; Jensen-Urstad et al. 1998; Kupari et al. 1993; Molgaard et al. 1994; Tsuji et al. 1996), the effects of various factors on HRV may nevertheless differ between the sexes (Jensen-Urstad et al. 1998; Kupari et al. 1993; Stein et al. 1997). On the basis of the results of this study, most importantly age and
glucose metabolism in both men and women and additionally blood pressure and lipid values in men, appear to have great relevance in relation to autonomic function. BRS was related to blood pressure in both men and women and also to values of glucose metabolism in women.

Advancing age during adult life is associated with a reduction in overall HRV (Bigger et al. 1995; Hellman et al. 1976; Korkushko et al. 1991; Lipsitz et al. 1990; O'Brien et al. 1986; Shannon et al. 1987) and a reduction in BRS (Gribbin et al. 1971; Laitinen et al. 1998). Age has also remained an independent determinant of autonomic function in studies with multivariate analysis (Molgaard et al. 1994, Tsuji et al. 1996). Here, too, the time and frequency domain measures were related to age in both men and women.

HRV and BRS have been suggested to be related to certain personality features, lifestyle and physical activity in healthy subjects (Barney et al. 1988; Dixon et al. 1992; Goldsmith et al. 1992; Molgaard et al. 1991; Molgaard et al. 1994). In this large sample of middle-aged subjects, lifestyle factors did not correlate with autonomic function. This is contradictory to the previous smaller studies (Dixon et al. 1992; Goldsmith et al. 1992; Hayano et al. 1990; Jensen-Urstad et al. 1998; Malpas et al. 1991; Molgaard et al. 1991; Molgaard et al. 1994; Kupari et al. 1993). Few of the present subjects were either physically inactive or well trained, which may partly explain the differences in the effects of physical activity (Dixon et al. 1992; Goldsmith et al. 1992; Molgaard et al. 1991). A trend towards lower values of HRV was also observed in smokers, but it did not reach statistical significance.

Both HRV and BRS showed inverse correlations with glucose and insulin levels. These findings agree with several previous studies on the effects of glucose metabolism on cardiovascular autonomic regulation.

There are various speculative mechanisms on how risk factors may affect autonomic function. The autonomic function may be altered in any part of the reflex arch, i.e. the sensory receptors, the afferent pathways, the central connections, the efferent pathways and the effector organs. Since all cardiovascular reflexes interact in a complex manner, it is difficult to study the exact mechanisms of altered autonomic function. The altered autonomic function with aging has been suggested to be due to both structural factors (e.g., loss of sinoatrial pacemaker cells) and functional changes (e.g., altered coupling between regulatory components) (Goldberger 1996). Arterial distensibility and thus altered baroreceptor function may be associated with aging, elevated blood pressure and atherogenic lipid metabolism (Bonyhay et al 1996; Kingwell et al. 1995; Kupari et al. 1993; Zanchetti et al. 1991). Altered glucose metabolism and extensive alcohol consumption may have effects on the neural pathways, as in diabetic or alcoholic neuropathy (Ewing et al. 1981; Malpas et al. 1991), while smoking may affect various parts of the autonomic system (Hayano et al. 1990b). Various factors may also alter heart rate more directly, which in turn may have effects on HRV measures.

The normalized units of the HF and LF spectral power of HRV were not related to age, lifestyle or risk factors. Recent studies using direct muscle sympathetic nerve activity as a reference index have suggested that the LF and HF spectral components analyzed in normalized units may provide information on sympathetic and vagal outflow, respectively, in subjects without structural heart disease (Montano et al 1998; Pagani et al. 1997). Although the interpretation of the physiologic background of normalized spectral units has been challenged (Eckberg 1997), the present data may suggest that
although the magnitude of overall HRV is related to age and laboratory variables, the specific autonomic input, which may be mediated by central mechanisms (Pagani et al. 1997), is not related to these variables. Thus, it may be speculated that the normalized units of HF and LF power reflect the intrinsic autonomic modulation of heart rate independently of cardiovascular risk factors.

In summary, relatively weak relations were observed here between the traditional measures of HRV or BRS and demographic variables, lifestyle, personality type or laboratory variables. The differences in various clinical and laboratory factors have also explained only a small proportion of the large interindividual variation in HRV in the previous smaller studies (Molgaard et al. 1994). Thus, genetic or some other unmeasured factors may explain most of the large interindividual variation in overall HRV and BRS in healthy subjects. The large interindividual variation among healthy children (IV) similarly suggests that genetic factors may largely determine autonomic function.

6.3. Sex-related differences in HRV and BRS

Previous studies have revealed sex-related differences in HRV and BRS (Abdel-Rahman et al. 1994; Cowan et al. 1998; Jensen-Urstad et al. 1998; Laitinen et al. 1998; Liao et al. 1995; Molgaard et al. 1994). In these studies men have usually had higher BRS and higher overall HRV, particularly at lower frequencies, compared to women, but women have shown lower LFHF ratios. The results of this study (II) also indicate that there are sex-related differences in cardiovascular autonomic regulation. The heart rate response to an abrupt rise in blood pressure and the LF modulation of heart rate analyzed in normalized units are lower in women than in men, whereas the HF modulation of heart rate is higher in women. The heart rate response to the overshoot phase of the Valsalva maneuver reflects the reflex vagal activity in response to a rapid rise of arterial blood pressure, and the LF component of HRV is determined by spontaneous oscillation of blood pressure, also reflecting the baroreflex-mediated control of heart rate. Consistently with the previous studies (Abdel-Rahman et al. 1994; Laitinen et al. 1998) these observations show that the baroreflex responsiveness is attenuated, whereas the tonic vagal activity is augmented in women compared to men. The mechanisms of the sex-related differences in R-R interval dynamics are not known. Possible effects of sex hormones (Ryan et al. 1994) and differences in baseline variables, such as blood pressure (Ryan et al. 1994) have been speculated. After adjustment for differences in several baseline variables, the observed sex-related differences in HRV remained unchanged, suggesting that the mechanisms behind gender-related differences are probably more closely related to hormonal or genetic factors than to differences in lifestyle or laboratory values. BRS and total HRV were higher in the postmenopausal women who were on estrogen replacement therapy compared to those without hormone therapy, suggesting that hormonal factors may partly explain the sex-related differences in autonomic modulation of heart rate.
6.4. HRV and BRS in hypertensive subjects with and without insulin resistance syndrome

Previous studies have shown that both HRV and BRS are impaired in patients with systemic hypertension (Bristow et al. 1969; Chakko et al. 1993; Dassi et al. 1991; Eckberg 1979; Guzzetti et al. 1988; Huikuri et al. 1996; Langewitz et al. 1994; Mancia et al. 1983; Petretta et al. 1995; Radaelli et al. 1994; Sleight 1979; Ylitalo et al. 1997). The possible effects of metabolic features have not been studied in patients with hypertension, although a large proportion of hypertensive subjects are known to have IRS (hypertension with insulin resistance, hyperinsulinemia, atherogenic lipid abnormalities and central body obesity) (Reaven 1988).

The results of this study (III) show that the impairment of overall HRV is confined to the hypertensive subjects with metabolic features of IRS (hyperinsulinemia and hypertriglyceridemia), while BRS and respiratory modulation of heart rate are impaired even in hypertensive subjects without IRS. In multiple regression analysis, both hypertension and metabolic features of IRS predicted the HRV values, but BRS only inversely related only to systolic blood pressure. Thus, metabolic aspects and elevated blood pressure have independent roles in the impairment of total HRV. The observed abnormalities in autonomic function might be related to the increased cardiovascular risk of subjects with IRS (Casassus et al. 1992; Despres et al. 1994; Pyörälä et al. 1985).

The HF component of HRV and BRS mainly reflect the tonic and reflex vagal outflow, respectively. Thus, cardiac vagal function seems to be impaired in systemic hypertension even in the subjects without features of IRS. There are some disagreements as to whether the LF component represents either sympathetic or vagal outflow in humans (Eckberg 1997; Liao et al. 1995; Malik et al. 1993). Studies on patients with heart failure (van de Borne et al. 1997) and on healthy subjects during exercise (Arai et al. 1989; Breuer et al. 1993) have consistently revealed a reduction in LF power, which has been attributed to increased sympathetic activity. This is speculated to be due to saturation of the LF oscillatory systems caused by the high sympathetic drive or the mechanism that includes the central effect of neurohumoral excitation (van de Borne et al. 1997). Insulin resistance is assumed to be associated with altered sympathetic nerve discharge (Arauz-Pacheco et al. 1996; Facchini et al. 1996; Reaven et al. 1996; Stern et al. 1992; Troisi et al. 1991). Insulin-resistant subjects have increased plasma and urinary concentrations of norepinephrine (Arauz-Pacheco et al. 1996; Troisi et al. 1991) and an impaired ability of insulin to stimulate sympathetic activity and muscle blood flow (Laakso et al. 1990; Vollenweider et al. 1994; Vollenweider et al. 1995). Although the underlying mechanisms of this impairment are unknown, these factors may also be linked to the impairment of LF modulation of heart rate.
6.5. Dynamical measures of HRV in healthy subjects

6.5.1. Dynamical analysis of R-R intervals

New dynamical methods of R-R interval variability based on fractals (“chaos theory”) and complexity have been used in conjunction with the traditional measures, since they may give complementary information of HR dynamics by revealing “hidden” abnormalities or alterations in time series data that are not otherwise apparent (Ho et al 1997; Hogue et al 1998, Lipsitz & Goldberger 1992; Mäkikallio et al 1996, 1997, 1998). Recently, dynamical analysis of HR variability with the detrended fluctuation method has provided better prognostic information than the traditional methods of HR variability in various populations (Mäkikallio et al 1999, in press), and the complexity measure, approximate entropy (ApEn), has been shown to predict the onset of atrial fibrillation (Vikman et al). The mathematical background of the new dynamical measures of R-R interval variability used in this study has been described in detail previously (Bigger et al. 1996; Huikuri et al. 1998; Iyengar et al. 1996; Peng et al. 1995; Pincus et al. 1992; Pincus et al. 1994; Saul et al 1987). Briefly, ApEn indicates the predictability or complexity of time series data (Mäkikallio et al. 1996; Pincus et al. 1992; Pincus et al. 1994), while the slope of the power-law relationship represents the fractal-like correlation properties of R-R interval data over very-low and ultra-low frequency bands (10^{-4} to 10^{-2} Hz)(Bigger et al. 1996; Huikuri et al. 1998; Saul et al 1987), and α1 and α2 obtained by detrended fluctuation analysis (DFA) stand for the correlation properties of short-term and intermediate-term R-R interval fluctuations, respectively (Iyengar et al. 1996; Peng et al. 1995). The fluctuations of a time series can also be described by comparing their behavior to various types of “noise” with different dynamics. White noise represents time series where no correlations are found, and the data points are completely random. The frequency spectrum is flat, because all frequencies are represented in equal densities. The slope of the power-law relationship (β) is 0 and the fractal scaling exponent (α) is 0.5. Brownian noise (random walk or 1/f^2 noise), can be observed upon integration of a white noise signal, and it is characterized by the frequency spectrum of a curve rapidly decaying (power inversely proportional to frequency squared) with β=-2 and α=1.5. For 1/f noise, the frequency curve is smooth with fluctuations inversely proportional to frequency (1/f) and with β=-1 and α=1. Values of ApEn are higher for 1/f noise compared to 1/f^2 noise. 1/f noise represents an example of a fractal-like process characterized by fluctuations that display scale invariance (self-similarity) and long-range correlations. Such processes generate irregular and complex fluctuations over multiple time scales.

6.5.2. Effect of aging from childhood to advanced age on R-R interval dynamics

In this study, children had similar complexity (ApEn) and similar wide-range temporal correlation properties of HR behavior (α1, α2 and β) as young adults. Both children and
young adults showed R-R interval dynamics resembling that of 1/f behavior ($\alpha_1$ and $\alpha_2 \sim 1.0$, $\beta \sim -1.0$) consistent with a system with fractal-like, scale-invariant correlations. Our findings confirm the reduced frequency domain measures in young children vs. young adults obtained previously (Finley et al. 1995; Korkushko et al. 1991).

A steeper $\beta$ (slope of power-law relationship), a decrease of ApEn (complexity), and an increase of $\alpha_2$ (intermediate-term scaling exponent) were observed upon increasing age, suggesting that the longer-term R-R interval dynamics change from 1/f behavior towards 1/f^2 behavior. These findings are consistent with the lower complexity (higher regularity and predictability) of R-R interval dynamics seen with increasing age. All time and frequency domain measures of HRV also decrease during adult life with increasing age, as evidenced by the lower total variance and smaller power seen at all frequencies. These observations are consistent with the previous findings showing decreased total variance (Hellman et al. 1976; Lipsitz et al. 1990; O'Brien et al. 1986; Shannon et al. 1987), decreased spectral power of VLF, LF and HF (Bigger et al. 1995), steeper slopes of the power-law relationship (Lipsitz et al. 1990) and reduced ApEn values (Kaplan et al. 1991; Ryan et al. 1994) in old age. The values of the short-term scaling exponent, $\alpha_1$, did not change significantly during healthy adulthood in this study, although children had somewhat lower values than elderly subjects.

The findings of increased ApEn, decreased $\alpha_1$ and increased spectral components in the nighttime indicate increased variance and complexity of HR dynamics at night. The age-dependence of different measures of R-R interval dynamics was similar regardless of whether they were analyzed from 24-hour or nighttime recordings. Thus, the differences in physical activity between the different age-groups (children vs. elderly) during the daytime do not explain the observed age-related changes in R-R interval dynamics, since the nighttime (0 - 6 am) can be considered a time period when physical activity is likely to be most comparable between subjects.

6.5.3. Determinants of correlation properties and complexity of R-R intervals in healthy middle-aged subjects

According to the results of this study (V), the fractal and complexity measures of HRV show smaller interindividual variation compared to the traditional measures of HRV among healthy subjects and are thus within a narrow range in the subjects without evidence of structural heart disease. The dynamical measures were not related to any demographic, lifestyle or laboratory values. However, a sex-difference was observed. Women had a lower short-term scaling exponent, $\alpha_1$, and a higher ApEn compared to men, despite the decreased overall variance, confirming that the R-R interval dynamics of healthy middle-aged women are characterized by lower total variance but more complex, fractal-like behavior compared to men. The sex-difference persisted even after adjustment for differences in several baseline variables, suggesting that the mechanisms of the observed difference probably involve hormonal or genetic factors. These measures may quantify the “intrinsic” capacity of the healthy cardiovascular control system, which only breaks down when disease states develop.
6.5.4. Physiological interpretation of R-R interval dynamics

It has been suggested that scale invariance may be a central organizing principle of physiological structure and function. The breakdown of this scale-invariant fractal organization could lead to either totally uncorrelated randomness or highly predictable (single-scale) behavior, both of which may result in a less adaptable system (Goldberger 1996). Thus, changes from $1/f$ scale-invariant behavior towards behavior resembling either random fluctuations (white noise) or $1/f^2$ behavior with less complexity might be physiologically deleterious. Such changes seem to occur upon physiologic aging. In contrast, children already show a “mature” pattern of R-R interval dynamics comparable to that of healthy young adults, with complex fractal dynamics suggesting a highly adaptive cardiovascular regulatory system.

The age-related changes in different measures of R-R interval dynamics are probably markers of the various physiological mechanisms affecting these measures, especially neuroautonomic inputs (Akselrod et al. 1985; Bigger et al. 1996). The finding that children showed a similar slope of the power-law relationship of R-R interval dynamics compared to young adults despite the reduced power of various spectral components, indicates that these indexes are differentially regulated and cannot be used as surrogates for “complexity” measures. Further studies are needed to address the physiological background of the dynamical measures of HRV.

6.6. Limitations of the study

Due to the primary selection criteria of the large original study population, which only excluded the subjects with refund for antihypertensive medication from the control group, some subjects were excluded from the studies on healthy subjects (I,II,V) because of diabetes, CAD or chronic arrhythmias. Thus, our population may not represent a truly random sample of healthy middle-aged subjects. Since exercise tolerance test was not used to evaluate CAD, some asymptomatic subjects with CAD may have been included, although those with symptoms, medication, or electrocardiographic evidence of CAD were excluded.

Because of the large original study population (I-III,V), 24-hour ECG recording to could not be used to assess HRV. According to our pilot study, reasonably good correlations (r between 0.61 and 0.81) were found between 24-hour HRV and HRV analyzed from our specific standard 45-minute recording (Huikuri et al. 1996). The dynamical measures from short-term ECG recordings may show less correlation to the 24-hour values, however. Artifacts and nonstationarities in the ECG were mostly found during the 15-minute walking period. Heart rate behavior was studied in more controlled and stationary conditions, including supine and sitting periods in the studies II and V. Thus, more subjects were included and the effects of nonstationarities on the results were possibly better eliminated in these studies. However, the frequency and depth of breathing were not controlled, which means that the conditions were not completely standardized. 24-hour ECG recordings during normal activities have been recommended by the Task Force (1996) for HRV testing for risk stratification because of the better
reproducibility of long-term vs. short-term recordings. We therefore tested the R-R interval dynamics of 24-hour recordings of healthy subjects despite the potential confounding effects of nonstationarities on R-R interval data (IV).

The “golden standard” method, i.e. the phenylephrine test, was not used in this study of a large population to assess the BRS because of its invasive nature. The validity and reproducibility of the Valsalva maneuver in the assessment of BRS have been previously described (Airaksinen et al. 1993), but the inadequate increase in blood pressure after the release of the Valsalva strain unfortunately limits its use in a certain proportion of subjects, as also found in our study.

Our criteria for IRS were chosen not to underestimate the incidence of IRS in the population sample (III). The criteria seemed to be able to distinguish between the groups in terms of all major features of the syndrome, i.e. hypertensives with IRS were more obese, had poorer glucose tolerance with higher insulin levels and had an atherogenic lipid profile (Reaven 1988). However, the euglycemic clamp technique was not used, and the actual insulin resistance of the subjects therefore remains open. Due to the large original study population, the antihypertensive medication could not be discontinued (III). We therefore matched the hypertensive subjects with and without IRS according to the medication, to make the groups comparable. Medication may influence the results, although theoretically, based on previous studies (Lucini et al. 1993; Niemelä et al. 1994), medication might rather increase than decrease the HRV and BRS of hypertensive subjects.

This study included multiple analyses between various factors and autonomic function and multiple comparisons between different groups. Although Bonferroni adjustments and multivariate analysis were used, the problem of multiplicity exists. This cross-sectional study was an observational one. Thus, although significant associations of age, sex and cardiovascular risk factors with autonomic function were observed, the results do not actually give information on causality. It remains to be ascertained whether autonomic cardiovascular function can be modified with interventions concerning various risk factors.
7. Conclusions

1. A large interindividual variation is observed in the measures of autonomic cardiovascular function in healthy middle-aged subjects. The measures of overall HRV are related to several cardiovascular risk factors, most importantly to age and factors of glucose metabolism in both men and women and, in addition, to blood pressure and lipid metabolism in men. BRS is related to blood pressure in both men and women. However, demographic and metabolic factors only explain a small portion of the interindividual variation in autonomic function.

2. There are sex-related differences in autonomic cardiovascular function. Overall HRV and BRS are attenuated in middle-aged women compared to men, but the HF component of HRV is augmented.

3. In hypertensive subjects, the impairment of overall HRV is confined to those with metabolic features of IRS (hyperinsulinemia and hypertriglyceridemia), but the BRS and respiratory modulation of heart rate are also impaired in hypertensive subjects without IRS. Both hypertension and metabolic features of IRS predict the HRV values, but BRS is only related to systolic blood pressure.

4. There are gradual significant changes in R-R interval dynamics from childhood to old age in healthy subjects. Children show comparable complexity and fractal correlation properties of R-R interval time series as young adults, suggesting a highly adaptive cardiovascular regulatory system despite the lower overall HRV. Healthy aging results in R-R interval dynamics with higher regularity and predictability and altered fractal scaling consistent with a possibly harmful loss of complex variability.

5. Healthy middle-aged subjects show little interindividual variation in their fractal correlation properties and complexity of heart rate behavior, suggesting that these dynamical measures quantify the “intrinsic” capacity of a healthy cardiovascular control system without a significant influence of life-style, metabolic or demographic variables. However, there are sex-related differences in fractal correlation properties and complexity of heart rate behavior.
8. References


