Raine Virtanen

Association Between Autonomic Regulation and Cardiovascular Risk Factors in Middle-Aged Subjects

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Department of Health and Functional Capacity,
National Public Health Institute, Turku, Finland
and
Department of Medicine, University of Turku, Finland

Turku 2007

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ASSOCIATION BETWEEN AUTONOMIC REGULATION AND CARDIOVASCULAR RISK FACTORS IN MIDDLE-AGED SUBJECTS

ACADEMIC DISSERTATION

To be presented with the permission of the Faculty of Medicine, University of Turku, for public examination in the Auditorium of the Microbiological Institute, on 11th May 2007, at 12 o'clock noon.

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Sulo Virtanen, “Joutsenkaula”, the bow of the Swan of Finland reflected from the water, July 1981

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A merry heart makes a cheerful countenance,
But by sorrow of the heart the spirit is broken.
Proverbs 15:13

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ABSTRACT

Heart rate variability (HRV) and baroreflex sensitivity (BRS) can be used to examine non-invasively autonomic cardiovascular regulation. Although impairments in HRV and BRS are related to adverse outcomes in various populations, the factors that determine these autonomic markers are poorly understood.

The present study applied time and frequency domain analyses of the R-R interval and arterial pressure variability (APV), and cross-spectral analysis of BRS, to examine the associations of these autonomic measures with life style, psychological factors, physical fitness, pulse pressure, hypertension, and other cardiovascular risk factors. The beat-to-beat variability of pulse pressure and its associations with cardiovascular risk factors were also studied.

The results show that HRV is reduced in untreated middle-aged hypertensive subjects. Higher heart rate, advancing age, higher blood pressure, female gender, and higher plasma renin activity are independent determinants of decreased HRV according to multivariate analyses combining hypertensive subjects and healthy controls. In a healthy middle-aged general population, psychological factors, particularly increased hostility and anxiety, and increased ambulatory pulse pressure are associated with reduced BRS and increased APV. Factors pertinent to the metabolic syndrome, such as higher levels of blood pressure, body fat percentage, insulin resistance, and serum triglycerides, are associated with decreased HRV and BRS and with increased APV. The exercise capacity had opposite associations with these parameters. Increased beat-to-beat variability of pulse pressure was related to several risk factors of arterial stiffening and to impaired BRS.

Measures of autonomic cardiovascular regulation are modulated by a complex interplay of several factors, and may also reflect non-autonomic effects, such as those related to arterial stiffening.

Keywords: autonomic nervous system, baroreflex, heart rate, blood pressure

Raine Virtanen, Autonomisen säätelyn ja kardiovaskulaaristen vaaratekijöiden välinen yhteys keski-ikäisillä henkilöillä
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TIIVISTELMÄ

Autonomista kardiovaskulaarista säätelyä voidaan tutkia noninvasiivisesti sykevaihtelun ja baroheijasteherkkyyden avulla. Vaikka sekä sykevaihtelun että baroheijasteherkkyyden heikentymisen on todettu liittyvän epäsuotuisiin kardiovaskulaarisiin päätetapahtumiin useissa eri tutkimuksissa, näitä autonomisen hermoston toimintaa kuvastavia muuttujia määräävät tekijät tunnetaan huonosti.

Tässä tutkimuksessa selvitettiin autonomisten kardiovaskulaaristen muuttujien yhteyksiä elintapoihin, psyykkisiin tekijöihin, fyysiseen kuntoon, pulssipaineeseen, hypertensioon, ja muihin kardiovaskulaarisiin vaaratekijöihin. Menetelmänä käytettiin syke- ja verenpainevaihtelun aika- ja taajuuskenttäanalyysejä sekä ristispektrimenetelmään perustuvaa baroheijasteherkkyyden määritystä. Tutkimuksessa tarkasteltiin lisäksi pulssipaineen lyöntivaihtelua.

Sykevaihtelu todettiin pienentyneeksi keski-ikäisillä hypertensiivisillä henkilöillä. Monimuuttujamallissa sykevaihtelun pienenemistä selittivät itsenäisesti suurentunut syketaajuus, ikääntyminen, kohonnut verenpaine, naissukupuoli sekä suurentunut plasman reniiniaktiivisuus. Väestöotoksessa psyykkiset tekijät, erityisesti vihamielisyys ja ahdistuneisuus, ja ambulatorisen pulssipaineen suureneminen olivat yhteydessä pienentyneeseen baroheijasteherkkyyteen ja suurentuneeseen verenpaineen lyöntivaihteluun. Metaboliselle oireyhtymälle ominaiset tekijät, kuten suurentunut verenpaine, kehon suurentunut rasvaprosentti, insuliiniresistenssi sekä seerumin suurentunut triglyseridipitoisuus, olivat yhteydessä sykevaihtelun ja baroheijasteherkkyyden pienenemiseen sekä verenpaineen lyöntivaihtelun suurenemiseen. Rasituksensiedon yhteys vasteisiin oli vastakkainen. Suurentunut pulssipaineen lyöntivaihtelu liittyi moniin valtimoiden jäykistymiseen yhteydessä oleviin vaaratekijöihin sekä huonontuneeseen baroheijasteherkkyyteen.

Autonomiseen kardiovaskulaariseen säätelyyn vaikuttavat useat keskenään vuorovaikutuksessa olevat tekijät. Todetut yhteydet saattavat kuitenkin selittyä osin muidenkin kuin autonomiseen hermostoon liittyvien vaikutusten kautta.

Avainsanat: autonominen hermosto, baroheijaste, sykevaihtelu, verenpaine

CONTENTS

ABBREVIATIONS

APV	arterial pressure variability
BMI	body mass index
BRS	baroreflex sensitivity
BSI-37	shortened 37-item version of the original 53-item Brief Symptom Inventory
DAP	diastolic arterial pressure
ECG	electrocardiogra|m, -phy, -phic
HDL	high-density lipoprotein
HF	high frequency
HOMA	homeostasis model assessment
HRV	heart rate variability
LDL	low-density lipoprotein
LF	low frequency
MSNA	muscle sympathetic nerve activity
PP	pulse pressure
PWV	pulse wave velocity
RMSSD	square root of the mean of squared differences between adjacent normal R-R intervals (ms)
SAP	systolic arterial pressure
SD	standard deviation
SDNN	standard deviation of the normal-to-normal interval (ms)
STAXI	Spielberger State-Trait Anger Expression Inventory
TAS-26	Toronto Alexithymia Scale
ULF	ultra low frequency
VLF	very low frequency

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original articles referred to in the text by their Roman numerals:

I **Virtanen R, Jula A, Kuusela T, Helenius H, Voipio-Pulkki L-M.** Reduced heart rate variability in hypertension: associations with lifestyle factors and plasma renin activity. *J Hum Hypertens* 2003; 17:(3)171–9.

II **Virtanen R, Jula A, Salminen JK, Voipio-Pulkki L-M, Helenius H, Kuusela T, Airaksinen J.** Anxiety and hostility are associated with reduced baroreflex sensitivity and increased beat-to-beat blood pressure variability. *Psychosom Med* 2003; 65(5):751–6.

III **Virtanen R, Jula A, Huikuri H, Kuusela T, Helenius H, Ylitalo A, Voipio-Pulkki L-M, Kauma H, Kesäniemi YA, Airaksinen J.** Increased pulse pressure is associated with reduced baroreflex sensitivity. *J Hum Hypertens* 2004; 18(4):247–52.

IV **Virtanen R, Jula A, Kuusela T, Airaksinen J.** Beat-to-beat oscillations in pulse pressure. *Clin Physiol Funct Imaging* 2004; 24(5):304–9.

V **Virtanen R, Jula A, Kuusela T, Helenius H, Airaksinen J.** Opposite effects of insulin resistance and exercise capacity on autonomic cardiovascular function. Submitted.

1 INTRODUCTION

It is commonly perceived that a regular heartbeat is a sign of cardiac health. In truth, however, sinus rhythm, the rhythm of a healthy heart, is characterized by significant variability. The purpose of dynamic beat-to-beat responses of heart rate and arterial pressure is to ensure sustained and appropriate perfusion to all tissues despite internal and external perturbations. The autonomic nervous system is one of the main mechanisms regulating circulatory homeostasis. Thus, high beat-to-beat variability of the heart rate is a sign of good adaptability, and implies that the person has well functioning autonomic control mechanisms.

Heart rate variability (HRV) and baroreflex sensitivity (BRS) have been used as simple, non-invasive techniques to examine autonomic cardiovascular nervous function. Depressed levels of HRV and BRS occur in a number of pathological conditions and predict cardiovascular morbidity and mortality (1–4).

A major hurdle to the clinical use of HRV is that healthy subjects show wide interindividual variation in their heart rate behavior (5–7) and, despite more than three decades of research into HRV, the factors affecting heart rate dynamics are still poorly understood. Contradictory observations have been reported in many conditions, also systemic hypertension. There are only few studies examining the link between autonomic cardiovascular regulation and arterial stiffening and there is no data on the association between, on the one hand, pulse pressure (PP) and its beat-to-beat variability and, on the other hand, HRV and BRS.

This thesis was set out to evaluate HRV in newly diagnosed, untreated hypertensive patients and healthy control subjects, and to assess the determinants of HRV in these populations. The present work also studied the relations between the measures of autonomic cardiovascular function and life style, psychological factors, PP, characteristics of insulin resistance, and physical fitness in relatively healthy subjects of the general population. In addition, the beat-to-beat variability of PP and its associations with cardiovascular risk factors were investigated.

2 REVIEW OF THE LITERATURE

2.1 Anatomy and physiology of autonomic cardiovascular regulation

The heart rate is normally determined by depolarization of the sinoatrial node. The autonomic nervous system controls heart rate and blood pressure to maintain cardiovascular homeostasis. The autonomous nervous system can be divided into the sympathetic and the parasympathetic, also called the vagal, branches, which work in a coordinated way, usually reciprocally. Under resting conditions vagal tone prevails. The function of the autonomic nervous system is organized on the basis of a reflex arch, which contains a visceral receptor, an afferent pathway, the central nervous system, an efferent pathway, and the effector organ. Inputs from systemic baroreceptors, cardiopulmonary low-pressure receptors, and chemoreceptors are coupled with a network of several cardiovascular and pulmonary regulation centers in the central nervous system. The efferent autonomic nerves consist of preganglionic and postganglionic neurons, which synapse in autonomic ganglia. The postganglionic fibers innervate the effector organ. The output of this system is further modulated by neurohumoral and local factors. All parasympathetic and preganglionic sympathetic neurons are cholinergic; they release the neurotransmitter acetylcholine. The postganglionic sympathetic neurons are mainly adrenergic and release norepinephrine. The heart receives both cholinergic and adrenergic innervations, whereas the blood vessels are innervated mainly by adrenergic neurons. The autonomic nervous system has effects on heart rate, atrioventricular conduction velocity, myocardial contractility, coronary vasculature, and various cardiac electrophysiological parameters, including refractory periods, fibrillation-defibrillation thresholds, automaticity, and triggered activity after potentials.

2.2 Assessment of autonomic cardiovascular regulation

2.2.1 Assessment of heart rate variability

Quantification of the heart rate period fluctuations over time is termed HRV or R-R interval variability. Standardized electrocardiogram (ECG) acquisition conditions are crucial for the correct assessment of short-term HRV. The respiratory frequency of the subject should either be recorded or the breathing controlled at a frequency of

12–15 breaths per minute. Continuous ECG signals are analogue-to-digital converted into a microcomputer to create a tachogram (Figure 1). Recorded data must be of good quality with minimal artifact. Proper visual inspection and processing of the recordings is essential, since ectopic beats, arrhythmic runs, noise artifacts, and missing data distort the analysis of HRV.

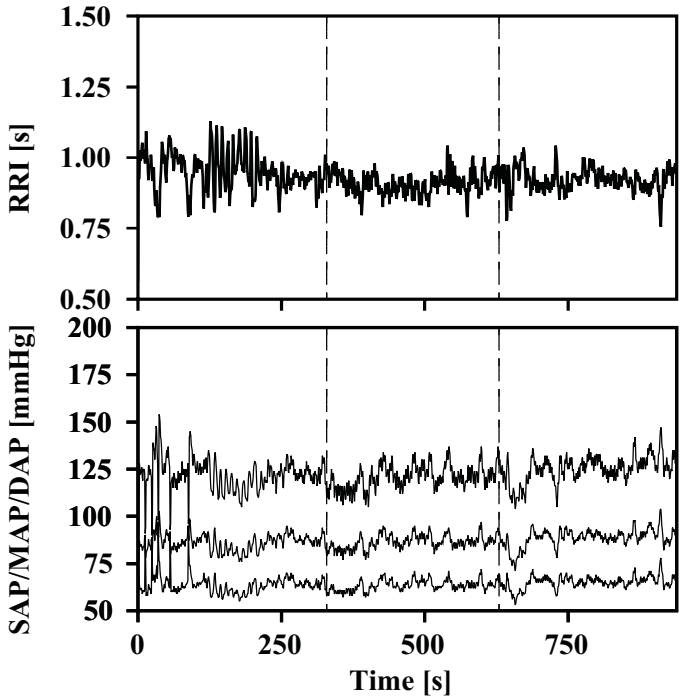

Figure 1. ***Time series of R-R intervals (above) and beat-to-beat systolic (SAP), mean (MAP), and diastolic (DAP) arterial pressures (below) from a woman at age 53 years. A period between the dashed vertical lines has been selected for subsequent spectral analyses which are illustrated in the figures 2, 3, 4, and 8.***

Time domain analysis

Time domain indexes of HRV evaluate oscillations of the heart rate as a function of time. They can be divided into statistical and geometric measurements (Table 1). The former are based on simple statistical analyses of successive R-R intervals or R-R interval differences. The major advantage of the latter is that it is relatively insensitive to artifacts and ectopic beats, while the major disadvantage is that rather long, preferably 24-hour, ECG recordings are needed (8). The most common time domain measure of HRV is the standard deviation of all normal-to-normal R-R

intervals (SDNN). It reflects overall HRV and is mathematically equal to the square root of the total power of HRV. The longer the recording period, the greater the SDNN. Therefore, 24-hour long-term or 5-minute short-term recordings have been preferred (8). In stationary short-term recordings, SDNN reflects mainly the parasympathetic regulation of the heart rate (9). The most common measure derived from interval differences is the square root of the mean squared differences of successive normal-to-normal R-R intervals (RMSSD). This measure of short-term variation reflects fast parasympathetic regulation of the heart rate.

Table 1. ***Selected time domain indexes of HRV***

Statistical measures	
SDNN	standard deviation of all N-N intervals (ms)
SDANN	standard deviation of the averages of N-N intervals in all 5 min segments of the entire recording (ms)
SDNN index	mean of the standard deviations of all N-N intervals for all 5 min segments of the entire recording (ms)
pNN50	percentage of adjacent N-N intervals which differ by at least 50 ms (%)
RMSSD	the square root of the mean of the sum of the squares of differences between adjacent N-N intervals (ms)
Geometric measures	
HRV triangular index	total number of N-N intervals divided by the height of the histogram of all N-N intervals
TINN	triangular interpolation of N-N intervals; width of the least square difference triangular interpolation of the N-N histogram
Poincaré plot	a plot of the current N-N interval (x axis) versus the subsequent N-N interval (y axis); also called Lorenz plot or return map

N-N interval denotes normal-to-normal R-R interval. HRV, heart rate variability.

Frequency domain analysis

A frequency domain analysis, also called power spectral density analysis, of the tachogram provides the basic information on how power, i.e. variance, is distributed as a function of frequency. In other words, the power spectrum reflects the amplitude of the heart rate fluctuations at different oscillation frequencies (10, 11). Frequency domain analysis of HRV is based on the assumption that the signal is stationary, i.e. statistically similar, over long periods. To increase stationarity, linear trends are removed by detrending and filtering the data before calculation of the power spectra. Frequency domain analysis can be performed by non-parametric fast Fourier transform, by parametric autoregressive modelling, and by wavelet decomposition (12). All these methods have their own advantages and disadvantages (8, 12). In practice, fast Fourier transform and autoregressive modelling are the most commonly used algorithms, and they yield practically comparable results (8, 9). Although frequency domain analysis can be used for the assessment of both short-term and long-term

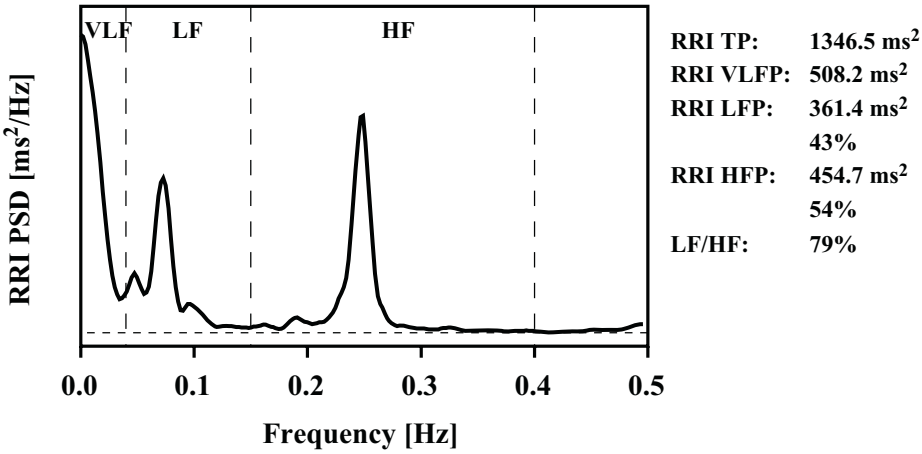

Figure 2. ***A power spectrum of HRV calculated from the tachogram shown in Figure 1. The ECG has been recorded during controlled breathing at a frequency of 0.25 Hz as indicated by a resultant peak in the HF band.***

recordings, frequency domain methods are particularly useful for the assessment of relatively short recordings. The duration of the recording should be at least 10 times the wavelength of the lower frequency bound of the spectral component investigated (8).

An example of a power spectrum of HRV is displayed in Figure 2. The recommended boundaries for the frequency bands are: total power < 0.4 Hz, high frequency (HF) 0.15–0.40 Hz, low frequency (LF) 0.04–0.15 Hz, very low frequency (VLF) 0.003–0.04 Hz, and ultra low frequency (ULF) < 0.003 Hz (8). These components estimate fluctuations with a periodicity of roughly 2.5–6.6 s (HF), 6.6–25 s (LF), 25 s–5 min (VLF), and > 5 min (ULF). Spectral components are reported either as arbitrary, i.e. absolute, units (ms^2) or as normalized units, which usually represent the relative value of each power component in proportion to the total power minus the VLF component (%). The ULF and VLF components account for the majority of the total power of long-term recordings, but the problem of stationarity should be taken into account when analyzing them.

Respiratory sinus arrhythmia is the term used to describe the spontaneous fluctuation of R-R interval with respiration. The heart rate accelerates during inspiration and decelerates during expiration. Respiratory oscillations of the heart rate arise from central mechanisms, and from the function of the baroreflex in response to respiration-synchronous fluctuations in intrathoracic pressure, stroke volume, and arterial pressure. Respiratory sinus arrhythmia both buffers and contributes to respiratory arterial pressure fluctuations.

The HF component of HRV is equivalent to respiratory sinus arrhythmia at respiratory rates > 0.15 Hz (9 breaths/min). There is general agreement that it principally represents vagal modulation of heart rate in response to respiration (9, 11, 13–21). It correlates closely with vagally mediated time domain measures, such as RMSSD. A reduced breathing rate or increased respiratory tidal volume can raise the magnitude of the HF component of HRV (22, 23). However, respiratory sinus arrhythmia is not a purely vagal phenomenon (20, 21, 24, 25). The sympathetic neural outflow restricts respiratory sinus arrhythmia at rapid and at slow breathing frequencies (25), and there appears to be a purely mechanical portion of respiratory sinus arrhythmia (20, 25). A constant or saturated parasympathetic effect on the sinus node may reduce the HF component of HRV (26). Taken together, although the HF component of HRV can be used as an estimate of vagal modulation under standard physiological conditions, changes in these oscillations do not indicate vagal tone.

The LF oscillations of HRV may be generated by baroreflex mechanisms (14, 27) or by a central oscillator control of the autonomic outflow (28, 29). Intrinsic neural rhythmicity may generate LF oscillations in tetraplegic patients (30) and in spinal sectioned decerebrate-vagotomized cats (31). Observations from anesthetized, vagotomized, sinoaortic denervated cats (32) and from patients with severe congestive heart failure treated with a left ventricular assist device (29) further indicate that LF oscillations do not depend on the functional integrity of baroreflex mechanisms, although baroreflex mechanisms account almost entirely for the LF component of HRV at supine rest (33).

The evidence for a link between LF oscillations in HRV and sympathetic excitation comes from experimental studies with denervated animals (16, 18, 34), and from clinical studies with various sympathetic stimuli (15, 16, 35) and direct microneurographic recordings of efferent muscle sympathetic nerve activity (MSNA) (35). However, there is no consistent relationship between LF oscillations and sympathetic outflow. The LF component of HRV is reduced in subjects with very high sympathetic activity, e.g. during strenuous exercise (36, 37) and heart failure (38, 39). Experiments with vagal denervated animals (13, 18) and parasympathetic blockade (11, 14, 15, 40) indicate that parasympathetic activity markedly contributes to the generation of LF variability of HRV. At supine rest, the LF component of HRV is mainly vagally controlled (15). Thus, the LF variability of HRV is mediated jointly by sympathetic and parasympathetic mechanisms.

The VLF component has been linked to vascular α-adrenergic effector mechanisms (41), thermoregulation (10, 42), changes in peripheral chemoreceptor activity (43) and fluctuations in activity of the renin-angiotensin-aldosterone system (11, 14, 40). It is, importantly, determined by vagal cardiac outflow (11, 14, 18, 40). Similar mechanisms to those responsible for the VLF component may be involved in the generation of the

ULF component. In long-term recordings, VLF and ULF fluctuations of HRV depend mainly on physical activity (44).

Some authors have proposed that the LF and HF components of HRV, expressed as normalized units, reflect activities in the sympathetic and parasympathetic limbs of the autonomic nervous system, and that the LF/HF ratio of HRV provides an index of sympathovagal balance (16, 28). However, the sympathetic and parasympathetic nervous systems act in parallel in some situations, e.g. in cold immersion of the face. The idea of sympathovagal balance has been criticized by both mathematical and physiological arguments (45).

Newer methods

Conventional frequency domain methods regard the irregularity present in the heart rate signal as random noise. Fractal and nonlinear approaches have been adopted to evaluate irregular components in heart rate (46, 47). The word "fractal" refers to self-scaling similarity over a wide range of scales. These newer methods are insensitive to changes in the external environment, such as the rate of respiration and physical activity, and they can produce information on heart rate dynamics. The Poincaré plot, or return map, is a graph in which each R-R interval is plotted as a function of the previous R-R interval (46). This geometric method provides a visual and quantitative analysis of R-R intervals. The techniques evaluating the fractal character of heart period include the $1/f^{\beta}$ decay of R-R interval spectral power in the logarithmic scale, detrended fluctuation analysis, and coarse-graining spectral analysis (46, 47). Approximate entropy is one of the many nonlinear techniques which are based on nonlinear system or chaos theories (46, 47). Chaos refers to a system which has the characteristics of both periodicity and randomness. Approximate entropy measures the regularity and complexity of time series data by quantifying the likelihood that runs of patterns that are close to each other will remain close to each other in subsequent comparisons. Several studies suggest that heart rate nonlinear dynamics and fractal analysis may yield superior prognostic information compared to conventional measures of HRV in patients with a recent myocardial infarction or congestive heart failure (46).

In addition to fractal and nonlinear methods, there are other new approaches based on frequency domain analysis, e.g. assessment of the distribution of spectral power within the LF band (prevalent LF oscillation) (48) and of the variability of the phase shift between blood pressure and heart rate fluctuations (49). These methods may help to stratify patients at risk of sudden cardiac death.

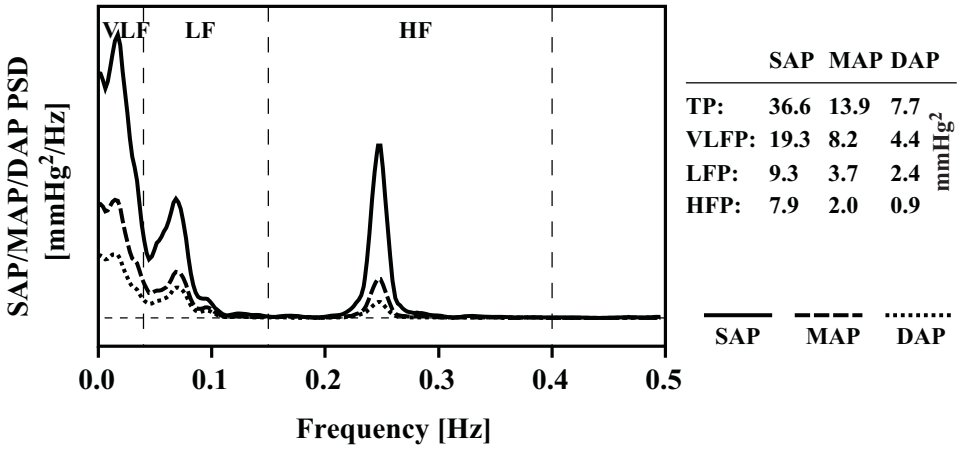

Figure 3. ***Power spectra of beat-to-beat systolic (SAP), mean (MAP), and diastolic (DAP) arterial pressure variabilities calculated from the time series shown in Figure 1. The same spectral peaks as those shown in the power spectrum of R-R intervals (Figure 2) can be detected in the frequency domain analysis of APV.***

2.2.2 Assessment of beat-to-beat arterial pressure variability

Non-invasive beat-to-beat measurement of finger arterial pressure is possible by a photoplethysmographic volume-clamp method (50). As illustrated in Figure 3, the same spectral components as those described for HRV are present in arterial pressure variability (APV) (14, 16, 34).

The HF oscillations of APV are related to respiratory activity. A substantial amount of these oscillations are caused by purely mechanical effects (17, 51), and, to a lesser extent, by vagally mediated R-R interval variability (17, 18, 51).

The LF oscillations of arterial pressure are also known as vasomotor or Mayer waves (21). They are mainly under sympathetic control (18, 34, 35), but arterial baroreflexes also modulate them through a reflex resonance loop involving sympathetic nervous activity and the vasculature (34, 52). The genesis of this variability is thus related to fluctuations in vascular tone and peripheral resistance (53). The LF fluctuations in arterial pressure persist during fixed-rate atrial pacing (51). The structural changes within the vasculature or other mediators of vascular tone, such as angiotensin II, nitric oxide, and endothelial-derived vasodilators, may affect the LF oscillations in arterial pressure (21). The strength of the LF component in arterial pressure does not indicate mean sympathetic tone (21).

The VLF component of APV has been related to α-adrenergic effector mechanisms (41, 53), the renin-angiotensin-aldosterone system (54) and the buffering effect of baroreceptor reflex (52, 55).

2.2.3 Assessment of baroreflex function

The arterial baroreflex buffers abrupt blood pressure changes. The arterial baroreceptors are stretch-sensitive nerve endings located primarily in the arterial wall of the carotid sinuses and the aortic arch. Activation of arterial baroreceptors by an increase in arterial pressure elicits reflex parasympathetic activation and sympathetic inhibition, with subsequent decreases in heart rate, cardiac contractility, vascular resistance, and venous return. Conversely, a decrease in arterial pressure reduces baroreceptor discharge and triggers adjustments that, through increased sympathetic outflow and vagal withdrawal, counteract hypotension.

There are several methods to study baroreflex responsiveness. Most commonly, these methods measure the response of the heart rate to arterial pressure changes, which are either spontaneous or induced by means of vasoactive agents (phenylephrine, sodium nitroprusside, nitroglycerin), the Valsalva maneuvre, external neck suction or neck pressure (56, 57). BRS is the slope of the linear relationship between R-R intervals and systolic arterial pressure (SAP). BRS reflects the capacity of baroreceptor activation to increase vagal efferent activity. There are also newer methods to evaluate baroreflex function, such as the baroreflex effectiveness index (58) and the measurement of baroreflex buffering function (potentiation of the pressor effect of phenylephrine during ganglionic blockade) (59).

Spontaneous fluctuations of arterial pressure and of R-R intervals may be used to assess baroreceptor responsiveness in natural circumstances (57). In the sequence method, sequences of three or more beats, in which SAP spontaneously either increases or decreases and which are followed by parallel changes in the R-R interval, are identified and baroreflex slopes are determined. However, only a minority of progressive beat-to-beat changes in SAP ramps are accompanied by baroreflex-driven parallel changes in R-R interval ramps (58). In cross-spectral analysis of spontaneous baroreceptor responsiveness (27, 60), baroreflex gain is calculated as the square root of the ratio between the R-R interval and SAP variabilities at the frequencies of physiological interest. Although there are several different calculations to estimate cross-spectral BRS (61), this variable is often determined only if the two signals show high coherence (> 0.5) and if arterial pressure changes precede, i.e. phase is negative, R-R interval changes (Figure 4). The drawback of this method is that all subjects do not express coherent fluctuations between R-R intervals and SAP, and it is not known whether this absence of coherence has prognostic implications. While traditional techniques might

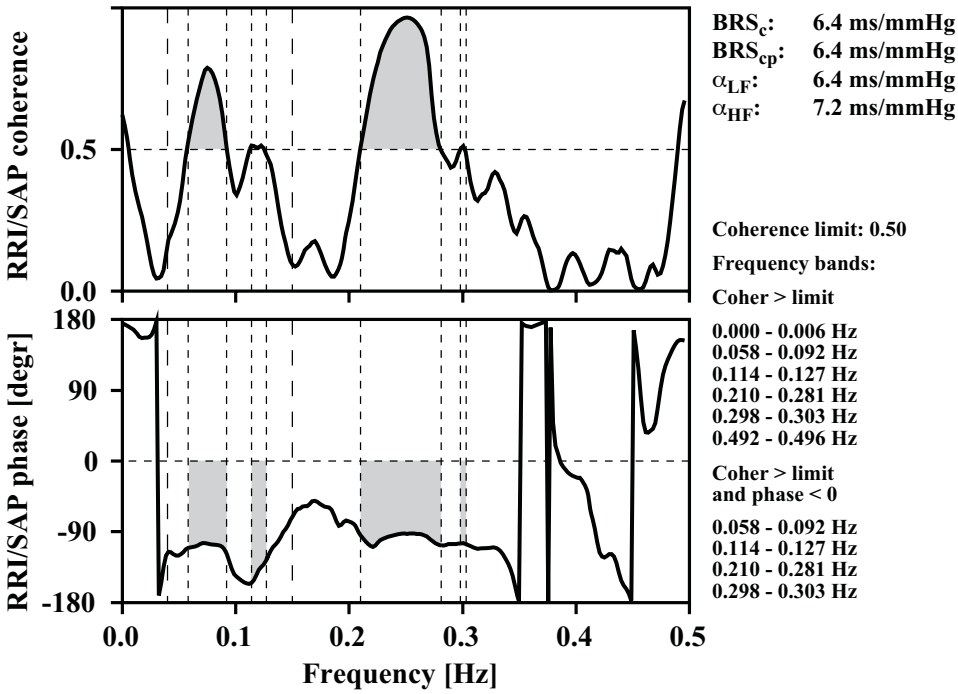

Figure 4. ***A cross-spectral analysis between beat-to-beat R-R interval and SAP variabilities, shown in figures 2 and 3. The shaded areas represent those frequency ranges fulfilling both the coherence and phase limits. The corresponding frequency ranges have also been listed. BRS$_c$, baroreflex sensitivity calculated from frequency ranges showing coherence >0.50; BRS$_{cp}$, baroreflex sensitivity calculated from those frequency ranges which show coherence >0.50 and a negative phase.***

be used to explore the entire stimulus-response curve of baroreceptors, spontaneous techniques provide information on the regulatory influence of baroreflex acting around its set point. Despite this difference, BRS measures calculated with the sequence method (62) and the cross-spectral method (60, 63) correlate reasonably well (*r* from 0.6 to 0.9) with those of phenylephrine method.

2.2.4 Other techniques to evaluate autonomic cardiovascular function

Traditional clinical tests of autonomic cardiovascular function use the response of the heart rate to deep breathing, a Valsalva maneuvre or postural change to examine vagal cardiac function. Cardiovascular sympathetic function is assessed by evaluating

the blood pressure response to active standing, passive tilting, a Valsalva maneuvre, sustained handgrip or cold water immersion (64).

Cardiovascular sympathetic function has also been evaluated with a number of other methods (65), including hemodynamic measurements, adrenergic and ganglionic pharmacological blockade, plasma levels of norepinehrine (66, 67), plasma norepinephrine kinetics (regional norepinephrine spillover measurements) (68, 69), microneurography (69, 70), and radionuclide imaging techniques (71). There is no correlation between the LF component of HRV and cardiac norepinephrine spillover, which provides a cardiac-specific measure of sympathetic nerve firing rate (69). Generally speaking, no single measurement of HRV correlates well with sympathetic activity.

Heart rate turbulence describes the response of the heart rate to the fall in blood pressure that follows a premature ventricular ectopic beat. This results in deceleration of the sinus rhythm after an initial acceleration. Turbulence onset expresses the relative shortening of R-R intervals immediately after the compensatory pause. Turbulence slope expresses the slope of the linear regression line between R-R interval count and R-R interval duration during the subsequent lengthening of R-R intervals (72). In subjects with congestive heart failure, heart rate turbulence correlates strongly with a standard measure of BRS (73). The absence of heart rate turbulence is a strong predictor of mortality of patients who have had a myocardial infarction (74).

2.2.5 Reproducibility of methods evaluating autonomic cardiovascular function on a beat-to-beat basis

Although HRV is commonly regarded as a reliable technique, this assumption may be a gross oversimplification (75). The reproducibility of HRV is dependent on a number of factors, including the measured variable, the study population, the length of the recording, the time of the day, and the stability of the recording conditions. In short-term measurements, it is crucial to standardize the recording conditions and control the breathing rate of the subject to increase the reliability of the results (8). Despite considerable intraindividual day-to-day variations especially among healthy subjects, mean group values for HRV between subsequent recordings correlate well ($r \geq 0.87$) (76). In a study examining the reliability of spectral indexes of HRV from 5-minute recordings obtained 2 months apart in women and men aged 35–65 years (77), the correlation coefficients ranged from 0.64 to 0.82 under metronomic breathing and from 0.65 to 0.75 under free breathing. The spontaneous BRS method is also satisfactorily reproducible (r from 0.54 to 0.87) (78). The repeatability of short-term beat-to-beat APV has not been documented.

2.3 Autonomic cardiovascular regulation in healthy subjects

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

Parasympathetic activity is attenuated upon standing, and this results in reduced arbitrary or normalized HF components of HRV (15, 16, 79, 80). Simultaneous augmentation in sympathetic activity may increase the LF component, expressed in arbitrary or normalized units, and the LF/HF ratio (15, 16, 79, 80). The results concerning the overall variability of HRV in response to standing are variable (16, 79, 81).

Autonomic activity follows a circadian rhythm: the sympathetic tone increases on wakening and the parasympathetic tone at night. In the frequency domain, the total (6, 82) and HF powers (82–84) of HRV are higher during night-time than during daytime. The LF/HF ratio follows also a circadian pattern: values are lowest at night and highest during the day (82, 84).

Traditional measures of HRV do not accurately reflect changes in autonomic modulation during exhaustive exercise. Consistent with the rapid withdrawal of vagal activity, the HF component of HRV decreases at the onset of exercise (36, 37). However, heavy exercise may decrease also the total and LF powers of HRV (36, 37). With increasing exercise intensity, the LF/HF ratio does not increase but rather decreases (37) or remains at a level that is lower than at rest or during recovery (36).

There are also several other factors which may cause intraindividual variation in autonomic activity and which should be controlled during short-term recordings of HRV, e.g. respiration (22, 23), mental and verbal activities (85), food intake (86), and room temperature (87). In menstruating females, cardiac vagal tone is higher in the follicular phase than in the luteal phase (88). In long-term recordings, the time domain indexes and the VLF and ULF components of HRV depend largely on the amount of physical activity (44).

2.3.2 Interindividual variation of autonomic cardiovascular function

Healthy subjects show marked interindividual variation in the measures of autonomic function (5–7). The major determinant of HRV is the basal heart rate (89, 90) which itself is modulated by autonomic cardiovascular regulation. The heart rate is also inversely related to BRS (91, 92).

Aging

HRV gradually increases during childhood (93). Despite lower time and frequency domain measures of HRV, children show similar complexity and fractal correlation properties as young adults (94). Healthy aging is associated with a decline in HRV (90, 93–98), a loss of fractal scaling properties, and reduced complexity of heart rate dynamics (94). Parasympathetic indexes of HRV decline earlier and more rapidly (93, 96, 97). Age is an independent determinant of HRV in studies with multivariate analysis (83, 89, 98). Also BRS decreases with aging (91, 92, 99–101). The relationship between age and beat-to-beat APV is less clear (101), although some associations with age have been observed (102, 103).

Gender

Overall, the HRV (90, 95, 98, 104) and BRS (91, 92, 105) of females is lower than of males. The LF power of HRV, expressed as arbitrary or normalized units, (80, 90, 95, 97, 98, 105, 106) and the LF/HF ratio (80, 90, 95, 97, 98, 105, 106) are lower, and the HF power, expressed as arbitrary or normalized units, is higher (80, 97, 105, 106) or similar (90) in women compared to men. The circadian profile of the LF component of HRV has been reported to differ between sexes, men having the highest level in the morning and afternoon, and women in the afternoon and evening (107). The gender influences on HRV disappear after age 40–60 years (90, 96, 97, 106), and BRS may even be higher in women after age 60 years (92). The mechanisms of gender-related differences are not precisely known, but seem to be related to female sex hormone production.

Lifestyle

Many lifestyle factors affect autonomic cardiovascular function. An acute intake of alcohol (108, 109) or caffeine (110, 111), and smoking a cigarette (112) reduce parasympathetic measures of HRV and BRS. Results from the studies evaluating the long-term influences of smoking, coffee drinking, and alcohol consumption on autonomic function are inconsistent and may be modified by the environmental and cultural factors (80). In some cross-sectional studies, alcohol consumption (7, 89, 98), coffee intake (80, 89), and smoking (7, 83, 89, 98, 104, 113) have been related to HRV changes implying reduced vagal or increased sympathetic modulation. There is an improvement in HRV after cessation of smoking (114). Higher alcohol consumption and smoking have also been linked with a decrease in BRS (92). Alcoholics appear to have reductions in principally parasympathetic indexes of HRV (108) and in BRS (115).

Trained athletes have high parasympathetic activity, which is partly responsible for the sinus bradycardia so common among athletes. If overtraining conditions are excluded (116), the vagally mediated measures of HRV are higher in athletes than in

non-competitive healthy subjects (12). Some cross-sectional studies have related HRV indexes with functional capacity or physical training level (83, 97, 98, 117). Fitness has also been related to higher BRS (100, 118). Although there are discrepancies (12), exercise training seems to increase HRV, to augment baroreflex function, and to decrease MSNA in sedentary healthy subjects (119–121).

There is no conclusive information on associations between salt intake and autonomic function, although one would expect such associations to exist. Omega-3 fatty acid supplementation increases the HF component of HRV in patients with a history of myocardial infarction (122).

Clinical and laboratory measures

Many other clinical and laboratory parameters have been related to autonomic cardiovascular function in healthy subjects. Serum triglycerides (104, 113, 117, 123), total cholesterol (124) and low-density lipoprotein (LDL) cholesterol (7, 124) are inversely related to HRV, whereas a direct association may exist between high-density lipoprotein (HDL) cholesterol and HRV (104, 123). Uric acid (98) and microalbuminuria (117) have also been associated with impaired autonomic cardiovascular function. Recent studies have related reduced HRV to increased levels of inflammation markers, such as leukocyte count (104), C-reactive protein concentration (98, 113, 125), interleukin-6 (126, 127), and tumor necrosis factor (128), in general population or in apparently healthy subjects (98, 104, 113) and in patients with stable coronary heart disease (126), acute coronary syndrome (125), or congestive heart failure (127, 128). Some other associations are mentioned in the context of hypertension and insulin resistance in this review.

Heritability

There are racial differences in autonomic cardiovascular function (129, 130). In a large study involving 772 healthy twins and singleton siblings, multivariate genetic analyses suggested that genetics explain 35–48% of the variance in time domain HRV parameters (131). In the Framingham heart study, heritability was estimated to account for 13–23% of the variation among HRV measures (132). BRS is also strongly influenced by genetic factors (133, 134).

2.4 Psychological factors and autonomic cardiovascular regulation

The interest of scientists in the relationship between psychological factors and autonomic nervous system function originates in an effort to identify the physiologic parallels of psychiatric illness, and to discover possible mechanisms how psychological conditions

affect the pathogenesis and progression of cardiovascular diseases. Anxiety, anger, depression, and possibly even other negative psychological states have been linked to the development of hypertension (135) and to the risk of coronary heart disease (136, 137). Depression has also been related to increased cardiovascular mortality and morbidity among patients with coronary heart disease (138). While exact mechanisms underlying these associations are poorly understood, both direct biological and indirect behavioral effects are involved. In addition to autonomic nervous dysfunction, other direct mechanisms that may be involved include neuroendocrine activation, platelet and blood coagulation abnormalities, and vascular effects, e.g. inflammatory activation and endothelial dysfunction (136).

Anxiety disorders

Research into the relationship between anxiety disorders and autonomic cardiovascular function has mostly evaluated patients with panic disorder. Mixed results have been reported, including no changes (67, 139, 140), decreases in overall variability (141–143) and vagal (142, 144) indexes of HRV, and increases in sympathetic indexes of HRV (142, 144, 145). Further proof against sympathetic activation comes from studies showing that norepinehrine spillover and MSNA values are within the reference ranges (144, 146, 147). One of these studies evaluated also the neuronal reuptake of noradrenaline in the heart and found that it is impaired in panic disorder patients (144). However, the studies performed outside panic attacks cannot exclude the possibility that patients with panic disorder might display a state of sympathetic overactivity during acute episodes. Relatively few studies in patients with generalized anxiety disorder (148), blood phobia (142), or posttraumatic stress disorder (145) suggest that the overall variability or vagal indexes of HRV may be reduced in these disorders, possibly, with a concomitant relative increase in sympathetic modulation in the posttraumatic stress disorder.

Depressive disorders

While one study observed reduced vagal indexes compared to controls in patients with major depressive disorder (149), others have not reported differences (139, 150–155) in HRV at supine rest. Similarly, BRS is impaired in patients with major depressive disorder according to some (150, 156) but not all (153, 157) studies. Contrary to these mainly negative results in major depressive disorder, most studies in various coronary heart disease populations indicate that HRV and BRS are lower among depressed than non-depressed patients (158–163). No differences have been detected in the beat-to-beat variability of SAP between depressive and non-depressive patients (150, 157, 158).

Psychological factors in subjects with no psychiatric disorders

Subjects with no psychiatric disorders have been examined with regard to any relationships between psychological factors and autonomic cardiovascular regulation. Both positive and negative emotions affect measures of HRV in short-term recordings (164). The indexes that mainly reflect vagal modulation are lower in subjects with anxiety than in healthy persons (165–168). In hypertensive subjects, anxiety has been related to a decrease in the HF component of HRV and to an increase in left ventricular mass (168, 169). Studies examining hostility, anger expression, or type A personality imply that there is an increased sympathovagal balance (170, 171) or a reduction in the parasympathetic measures of HRV (171, 172). One study found an association between coping style, the way people cope with stressors, and HRV in men but not in women (173). Perceived psychosocial stress has been related to increased sympathovagal balance (174, 175). Asymptomatic coronary artery calcification correlated independently in postmenopausal women with a greater reduction in the HF component of HRV in response to a psychosocial stressor (176). Alexithymia, a marked difficulty in describing and expressing feelings, has been related to an increase in the LF/HF ratio (177). Finally, depressive symptoms have been related to a decrease in HRV among college students (178) and postmenopausal women (179). There are no population-based studies on the relationship between psychological factors and autonomic activity.

The data on the relationship between psychological factors and BRS or beat-to-beat APV are scant. The few studies that are available suggest that anxious subjects may have lower BRS (166) and a higher LF component of SAP variability than non-anxious controls (167). Psychosocial stress may also decrease BRS and increase the LF component of SAP variability (175).

2.5 Effects of drugs and somatic illness on autonomic cardiovascular regulation

2.5.1 Effects of drugs on autonomic cardiovascular regulation

Many drugs interfere with autonomic cardiovascular function either directly or indirectly. Some drugs affect central sympathetic outflow, alter baroreflex control of sympathetic motoneurons or interfere with norepinephrine release at sympathetic neuroeffector junctions (180). The indirect drug effects may be secondary to drug actions on arterial wall properties or hemodynamics, or, in the long term, result from the reversion of structural changes in the large arteries or from the regression of left

ventricular hypertrophy. The cardioprotective effects of some drugs may at least partly be based on their effects on autonomic cardiovascular control.

β-receptor blocking agents enhance HRV and BRS also in healthy subjects (98, 181, 182). Their effect is most marked on the measures of vagal activity. Despite some initial observations on dogs (14) and subsequent findings in studies on healthy young subjects (40) according to which blockade of angiotensin-converting enzyme (ACE) induces a modest increase in the VLF power of HRV, there are no uniform data on the effects of ACE-inhibitors and angiotensin 1 receptor antagonists on HRV and BRS (98, 183–185). The effects of ACE inhibitors and angiotensin 1 receptor antagonists on autonomic measures may depend on the state of activation of the renin-angiotensin-aldosterone system. For example, BRS is improved in sodium-depleted but not in sodium-replete subjects (185–187). The indirect mechanisms related to hemodynamic improvement and the regression of left ventricular hypertrophy are probably crucial for the effects of ACE inhibitors and angiotensin 1 receptor antagonists on autonomic measures (188, 189).

Other drugs that produce favorable changes in HRV or BRS include aldosterone antagonists (190), digoxin (183), verapamil (191), statins (192), estrogen replacement therapy (193), and anticholinergic muscarinic blockers at low doses (194). On the other hand, class IC antiarrhythmic drugs (195), short-acting nifedipine (196), nitroglycerin (197), and diuretics (98) are associated with a decrease in HRV or BRS. Tricyclic antidepressants reduce HRV markedly (149, 151, 198). Although selective serotonin reuptake inhibitors are usually considered safe drugs for the heart (198), one study reported that they might reduce HRV (155). Diltiazem, slow-releasing nifedipine formulations, and dihydropyridine derivatives with a long half-life (181, 196, 199, 200) do not have major effects on HRV according to most studies. Concerning the effects of centrally acting antihypertensive drugs (α_2- and imidazoline (I1)-agonists) and α_1-adrenergic receptor antagonists, results are inconsistent or lacking.

2.5.2 Autonomic cardiovascular regulation in systemic hypertension

Research on the role of the autonomic nervous system for the development of hypertension was stimulated by observations that early in hypertension hemodynamics are hyperkinetic (201). Early research also indicated that increased sympathetic tone is coupled with reduced parasympathetic activity in borderline hypertension (202). Thereafter, studies with several experimental methods, including plasma norepinehrine levels (66), direct microneurographic recordings (70), and norepinephrine spillover (68), have suggested that at least a subgroup of hypertensive subjects has increased sympathetic tone. HRV studies suggest that a low HRV predicts a greater risk of incident hypertension (203–205).

In the rather few studies on HRV in borderline hypertensive subjects, there have been no differences between borderline hypertensive and normotensive subjects (206–209). Despite similar HRV, the VLF component (208) or all the components (207) of beat-to-beat APV have been increased in borderline hypertensive subjects compared with normotensive controls (207, 208) and hypertensive subjects (208). Borderline hypertensive subjects had have attenuated night-day variation in the VLF component of APV (208). Their median frequency of LF oscillations may be shifted to lower frequencies (210), which may predict progression to sustained hypertension (211). A reduction in BRS occurs in individuals with high normal blood pressure (212).

HRV in patients with sustained essential hypertension varies. The early study of Pagani and coworkers found that hypertensive subjects had a higher peak in the LF band at rest, and they were unable to further increase it with tilting (213). Later, scientists at the same department published an article where they did not report spectral powers in arbitrary units at all, but claimed that untreated hypertensive subjects had a decreased HF component and an increased LF component of HRV, expressed as normalized units, and an increased LF/HF ratio (206). These findings were interpreted by the scientists as suggesting increased sympathetic activity in essential hypertension. Subsequent studies comparing untreated hypertensive and normotensive individuals have found that the normalized LF component of HRV is similar (214, 215), higher (216–218) or age-dependent (219), i.e. higher in young and middle-aged but not in elderly people. Similarly, the LF/HF ratio has been reported to be higher (216–218, 220), similar (203, 204, 214, 221), or age-dependent (219) in hypertensive subjects.

Data concerning the parasympathetic cardiovascular regulation in hypertension are more concordant. Although there are studies that have not demonstrated differences in HRV between untreated hypertensive and normotensive subjects (208), most studies have found either decreases in the vagal indexes of HRV (218, 220) or an overall reduction in HRV (203–205, 215, 217, 219, 222). The circadian rhythm of HRV is altered in hypertensive subjects (223, 224), and the changes in HRV are blunted in response to an upright position (206, 214, 216, 221, 225). However, these findings have not been confirmed in all studies (208, 226, 227).

Ambulatory blood pressure variability is greater in hypertensive than normotensive subjects (228). Explored by frequency domain, beat-to-beat APV has been similar (208, 214) or increased in the total (175), LF (175, 222, 229) and HF powers (175). Similarly, both normal responses (208, 214) and blunted increases in the LF component of APV (229) in response to an upright posture have been reported in hypertensive subjects. The etiology and severity of hypertension, and secondary adaptive processes during the course of hypertension may influence beat-to-beat APV. Increased beat-to-beat APV was not observed in patients with left ventricular hypertrophy (222). Severe renovascular hypertension has been associated with a decrease in beat-to-beat APV

(230). BRS is decreased in hypertensive subjects (133, 222, 231–234), and it may be one of the mechanisms which result in increased fluctuations of SAP variability.

2.5.3 Autonomic cardiovascular regulation in insulin resistance

In the general population, the body mass index (BMI) and the waist-to-hip ratio correlate negatively with HRV (104, 117, 235) and BRS (92, 100, 235), and positively with the LF/HF ratio of HRV (80). Obese normotensive subjects have reduced HRV (236–239) and BRS (238, 240, 241), and their beat-to-beat APV (237) and MSNA (242–244) are increased. In euglycemic-hyperinsulinemic clamp studies, the LF/HF ratio of HRV has been higher in obese subjects than in lean subjects at baseline, and it remained unchanged or increased less after insulin in obese subjects than lean subjects (236, 245). Weight gain reduces the HF component of HRV (246). Conversely, weight loss increases HRV (239, 247), augments baroreflex function (248), and reduces MSNA (248), which is consistent with augmented parasympathetic and suppressed sympathetic control of the heart rate.

In the general population, fasting insulin, glucose, or glycosylated hemoglobin (117, 249, 250) correlate inversely with HRV. These factors are also negatively associated with BRS in healthy volunteers (100). Autonomic dysfunction deteriorates as the impairment of glucose metabolism advances (235, 250, 251). Also, subjects with low levels of HRV have an increased risk of diabetes (252).

Diabetes mellitus, even uncomplicated, is associated with reductions in all spectral components of HRV (89, 249, 250, 253), an increase in the LF component of SAP variability, and a decrease in BRS (253). Overt clinical neuropathy results in further impairment of autonomic cardiovascular function, and measures of HRV and BRS have been applied for the early detection of autonomic neuropathy associated with diabetes (254, 255). Impaired HRV predicts coronary heart disease (256) and increased mortality (257) among diabetics. Improvements in HRV have been reported after strict glycemic control, lifestyle modification, and metformin administration (258–260).

The metabolic syndrome, the insulin resistance syndrome, or syndrome X is a cluster of coronary heart disease risk factors with insulin resistance as the common denominator. It is associated with hypertension, visceral obesity, glucose intolerance, a low level of HDL cholesterol, and a high triglyceride level. Although insulin resistance is generally considered to be linked with enhanced sympathetic drive, it is not known whether insulin resistance represents the initial defect that activates the sympathetic nervous system or whether the primary defect is an increase in sympathetic nervous system activity.

Enhanced sympathetic drive may contribute to several components of the metabolic

syndrome. Insulin shifts cardiac sympathovagal balance towards sympathetic predominance. It stimulates a decrease of the HF and LF components expressed in arbitrary units (236) and an increase in the LF/HF ratio of HRV (236, 245) and in MSNA (261). Also leptin has been related to enhanced sympathetic drive (262). In hypertensive subjects, insulin sensitivity has been related to HRV (263). One study found that changes in HRV and BRS emerged only in those hypertensive subjects who also had the metabolic syndrome (264). Research on abdominal-to-peripheral fat distribution suggests that HRV and BRS are reduced more (241, 265) and MSNA is increased more (242, 244) in visceral obesity than peripheral obesity. Insulin resistance is associated with autonomic dysfunction also in subjects with normal glucose tolerance (235, 251).

Risk factors that are associated with insulin resistance cluster around a reduction in HRV (117). HRV is decreased (266) and MSNA is increased (267, 268) in patients with the metabolic syndrome. Higher levels of fasting insulin and insulin resistance, calculated by the homeostatis model assessment (HOMA), are inversely related with HRV and BRS (269, 270) and directly to MSNA (262, 268). HRV and BRS are inversely associated with the number of components of the metabolic syndrome (269, 270). In agreement with this, the presence of hypertension and obesity (240) or the metabolic syndrome in the same subject (267, 268) is associated with a further increase in MSNA compared with either condition alone. This suggests a greater sympathetic activation when multiple components of the metabolic syndrome are present.

2.5.4 Autonomic cardiovascular regulation in cardiac diseases

Autonomic cardiovascular function is abnormal in various cardiac diseases. HRV is reduced in asymptomatic patients with coronary artery calcification (117). HRV and BRS are decreased in patients with coronary heart disease (4, 271), especially after myocardial infarction, and in patients with congestive heart failure (39, 271, 272). Reduced HRV may predict the development and progression of coronary heart disease (4). Several parameters reflecting the severity of congestive heart failure (273, 274) and the angiographic severity of coronary heart disease (275) are related to HRV. Changes in autonomic nervous cardiac activity may precede ventricular tachyarrhythmias (276, 277). Most importantly, impaired HRV may predict mortality, particularly in patients who have had a myocardial infarction (1, 4) and in patients with congestive heart failure (272, 278). BRS has also been used to risk stratify postinfarction patients (279).

Alterations in autonomic cardiovascular function are also related to various other cardiac diseases, including cardiac syndrome X (280), vasospastic angina (281), chronic severe mitral regurgitation (282), and idiopathic dilated cardiomyopathy

(283). HRV is markedly reduced after cardiac transplantation, but cardiac sympathetic nerve function is partially restored over time (284). Autonomic modulation may be associated with initiation and recurrence of atrial fibrillation and atrial flutter (285–288). HRV does not improve the predictive accuracy of established arrhythmic risk factors in patients with hypertrophic cardiomyopathy (289).

Despite several studies confirming that HRV and BRS provide information on the risk of subsequent death in various populations, including the general population (2, 3, 290), autonomic tests are not applied in clinical practices. This is due to several circumstances. First, the mechanisms of this association are largely unknown and there is, secondly, no specific therapy available to improve the prognosis. Thirdly, there is no consensus on the best way to measure HRV for clinical purposes. Further, no single test is alone satisfactory for predicting the risk of major arrhythmic events. And fifthly, the value of autonomic risk markers for predicting sudden death has become somewhat blurred with the widespread use of statins and medications resulting in a more comprehensive blockade of β-adrenergic and renin-angiotensin-aldosterone systems (291).

2.5.5 Autonomic cardiovascular regulation in non-cardiac diseases

In addition to structural heart diseases, there are numerous reports linking altered HRV to various non-cardiac diseases, e.g. end stage renal disease (292), chronic liver disease (293), hypoxia in chronic respiratory disease (294), obstructive sleep apnea (215, 295), disorders of the nervous system (296), anemia (297), systemic diseases like amyloidosis (298), and severe infections (299).

2.6 Pulse pressure and arterial stiffening

PP arises from the interaction between the left ventricular stroke volume and the characteristics of the arterial circulation that determine compliance and wave reflection (300). PP is a surrogate marker for arterial stiffness. Elevated PP is related to increased left ventricular and arterial wall thickness and to cardiovascular events and mortality (300). There is a vicious cycle between PP and atherosclerosis: a high PP promotes atherosclerosis, which results in large-vessel stiffening and increased wave reflection, thus further amplifying PP. Although there is high colinearity between PP and systolic blood pressure, PP can be a superior predictor of cardiovascular risk compared with systolic, diastolic, or mean blood pressures, at least in the elderly (301).

Aging is associated with a loss of arterial elasticity. Arterial stiffness is also related to several cardiovascular risks, such as hypertension, obesity, metabolic syndrome, and

diabetes (302). Several devices and methods have been developed to quantify arterial stiffening in humans (303). The intima-media thickness, arterial compliance, and arterial distensibility can be determined by sonographic evaluation of the common carotid artery. While arterial compliance denotes absolute diameter or area change for a given pressure increment, arterial distensibility refers to the corresponding relative changes. Special instrumentation can be used to quantify pulse transit time and to analyze the wave contour of the arterial pulse. Pulse wave velocity (PWV) (i.e. the velocity of the pulse wave along the arterial tree) increases as the stiffness of the arterial system increases. Increases in PWV may also be due to increased sympathetic tone (152). Arterial stiffness affects pulse wave reflection and pulse wave analysis can be used to detect resultant alterations in pressure wave contour. For example, young subjects have high pressure wave augmentation, which results in progressive elevation of systolic blood pressure in peripheral arteries. In older subjects, the more rapid propagation of the pulse wave reduces pressure wave augmentation and this results in nearly identical central and peripheral blood pressures (302).

There are only few studies that have examined associations between autonomic cardiovascular function and arterial stiffening. The PWV correlates positively with the LF/HF ratio and negatively with the HF component of HRV in young male subjects (304). In middle-aged healthy men, PWV correlates inversely with HRV (305). PWV and arterial compliance measurements are also related to HRV in type 1 diabetics (306, 307). A lower pulse transit time and a higher PWV have been associated with reduced BRS in stroke and chronic hemodialysis patients (308, 309).

2.7 Summary

HRV and BRS convey prognostic information, especially of subjects who have had a myocardial infarction. However, there is substantial interindividual variation in the measures of autonomic function, and apart from cardiac disease, numerous lifestyle-related, physiologic, psychological, and hereditary factors, as well as medications affect these indexes. A complex interplay among these factors that is currently not well understood may explain a part of the conflicting results from studies evaluating determinants of autonomic cardiovascular regulation. This thesis is an effort at clarifying the above issues.

3 AIMS OF THE STUDY

The aims of the present study were to examine the abnormalities of autonomic cardiovascular regulation of patients with untreated hypertension and to evaluate the determinants of the beat-to-beat R-R interval, SAP, diastolic arterial pressure (DAP), and PP variabilities, and of BRS, in middle-aged subjects. The specific goals were:

1 to compare the measures of HRV in newly diagnosed, untreated hypertensive patients and control subjects, and to assess the determinants of HRV in these populations (I)

2 to study the associations between psychological factors and autonomic cardiovascular regulation in a healthy middle-aged population through spectral analyses of the beat-to-beat R-R interval, SAP, and DAP variabilities, and BRS (II)

3 to test whether PP is associated with autonomic cardiovascular regulation in two apparently healthy middle-aged populations as assessed by the beat-to-beat R-R interval and SAP variabilities and BRS (III)

4 to examine the beat-to-beat PP variability and its associations with PP and risk factors for atherosclerosis and arterial stiffening in a healthy middle-aged population (IV)

5 to evaluate the effects of glucose and lipid metabolism, body fat composition, lifestyle factors, and physical fitness on autonomic cardiovascular regulation in a healthy middle-aged population (V)

4 MATERIALS AND METHODS

Three populations were examined (Figure 5). Populations 1 and 2 included randomly chosen age- and gender-stratified apparently healthy subjects. Population 3 consisted of patients with recently diagnosed, untreated hypertension.

4.1 Study populations

4.1.1 Population 1

Population 1 consisted of an age- and gender-stratified random sample of 45 subjects of both genders and each 10-year age group (35–44, 45–54, and 55–64), selected from the national population register of inhabitants residing in the vicinity of Turku in southwestern Finland. Subjects with coronary artery disease, congestive heart failure, previous cerebrovascular event, claudication, hemodynamically significant valvular disease, severe anemia (hemoglobin < 110 g/l for men and < 100 g/l for women), chronic alcoholism, diabetes mellitus, or confounding medication were excluded based on medical history, clinical examination, routine biochemical tests, exercise EGG, and echocardiographic examination. Medications resulting in exclusion included all ordinary cardiovascular drugs, β-blocker eye drops, teophyllamine, β_2-sympathomimetics, and antineoplastic drugs, and in studies II–V, additionally, antidepressants and neuroleptics. For the control group of newly diagnosed hypertensive patients (i.e. population 3) in study I, subjects aged 35–54 were selected, and those with a systolic or diastolic blood pressure of ≥ 140 or ≥ 90 mm Hg, respectively, were excluded. After further exclusion of subjects who did not complete the studies, inadequate ECG, or ambulatory blood pressure recordings, the final analyses included 105 subjects (56 women and 49 men) in study I, 149 subjects (78 women and 71 men) in study III, and 150 subjects (79 women and 71 men) in studies II, IV, and V. The studies were conducted following the Second Declaration of Helsinki and were approved by the Ethics Committee of the Social Insurance Institution of Finland. All subjects gave written informed consent.

4.1.2 Population 2

Population 2 (III) consisted of an age- and gender-stratified random sample of 300 men and 300 women, aged 40–59 years, selected from the register of the Social Insurance Institution of the inhabitants of Oulu in northern Finland for OPERA (Oulu Project

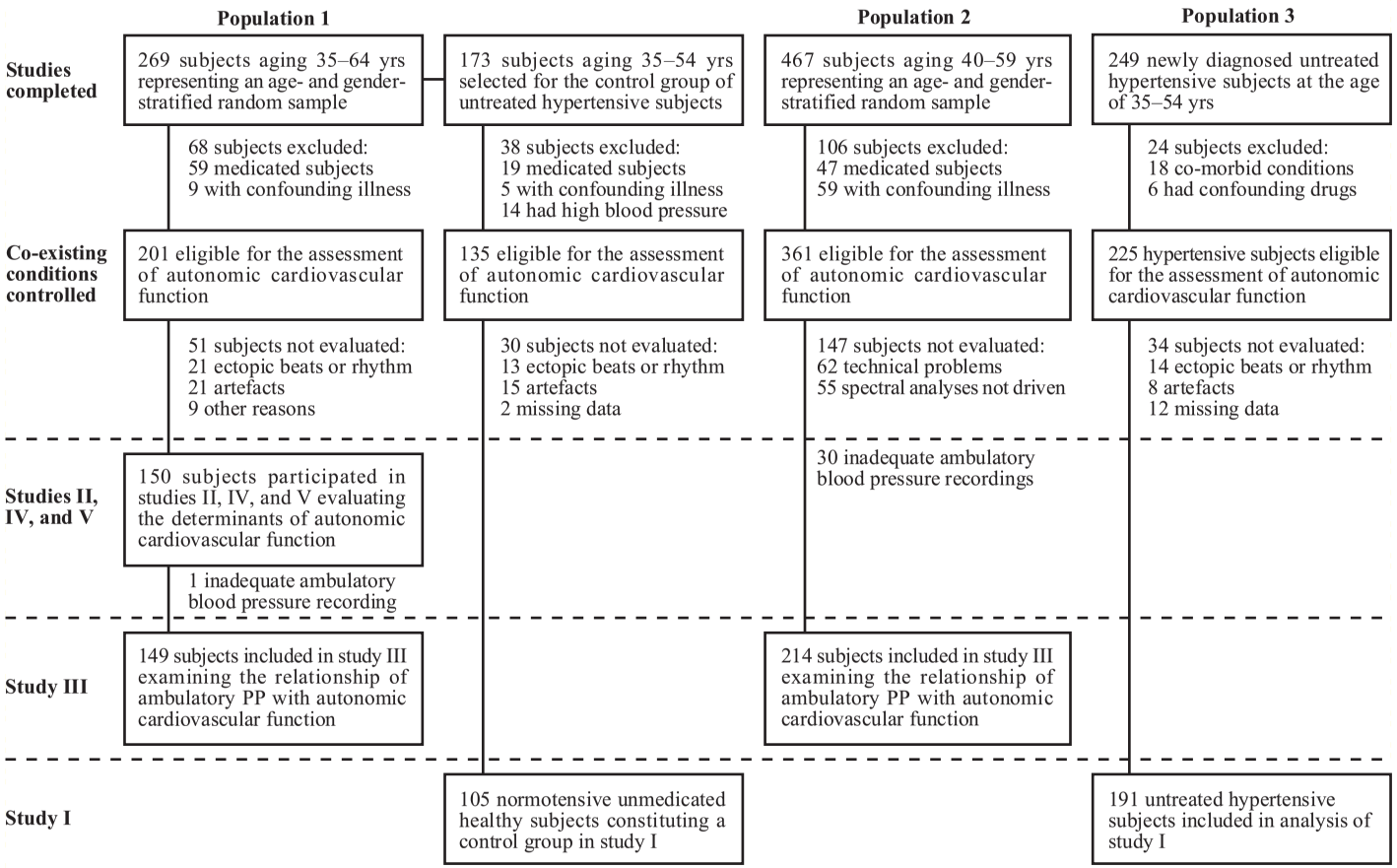

Figure 5. *A flow chart illustrating the evolution of study samples in studies I–V.*

Elucidating Risk of Atherosclerosis), an epidemiological study of cardiovascular risk factors (19). This population was examined only in study III. After application of exclusion criteria, similar to those used for population 1, the final analyses included 214 subjects (126 women and 88 men). Informed consent was obtained of each subject, and the protocol was approved by the Ethics Committee of the University of Oulu.

4.1.3 Population 3

Population 3 (I) was drawn from the city of Turku and three neighboring municipalities in southwestern Finland. It consisted of 249 moderately to severely hypertensive white men and women, aged 35–54 years. They had newly diagnosed, untreated hypertension. The inclusion criteria were a systolic or diastolic blood pressure consistently in the range of 180–220 or 100–120 mm Hg, respectively, as measured within primary health care. Except for high blood pressure, the same exclusion criteria were adopted as above, and this resulted in the exclusion of 24 patients. Of the eligible patients, 34 were excluded because of a lack of sufficient uninterrupted sinus rhythm for the analysis of HRV. The final analyses included thus data on 191 (82 women and 109 men) hypertensive patients.

4.2 Heart rate and continuous arterial pressure signal acquisition

In populations 1 and 3, the recordings for the assessment of HRV, APV, and BRS were carried out between 8.30 a.m. and 12.00 a.m. in an isolated examination room at a stable temperature between 20 and 22 °C. The subjects were requested to avoid coffee, tea, cola drinks, and smoking for 12 hours, and alcoholic beverages for 24 hours before the investigations. A light breakfast was allowed not later than 2 hours before these investigations. The ECG was recorded after a 10-minute supine rest for 5 minutes while subjects were resting supine and breathing freely (I) or at a controlled frequency of 0.25 Hz (15 breaths per minute) (II–V). R-R intervals were measured from chest leads V_1–V_6 of the ECG. The signal was digitized and fed into a computer.

In population 2, ECG was recorded for 45 minutes with an ambulatory ECG device (Dynacord Holter Recorder, Model 420, DM Scientific, Irvine, CA, USA) with a sampling frequency of 256 Hz. Each subject was monitored for 15 minutes in a supine posture, for 15 minutes in a sitting posture while breathing quietly at a frequency of 0.25 Hz, and for 15 minutes while walking. The duration of the recordings was chosen after a pilot study involving 37 normotensive and 40 hypertensive subjects by calculating the shortest period that would give a reasonable correlation (r = 0.6) with the 24-hour measures of HRV (225). The ECG recordings were performed between

7 a.m. and 3 p.m., and the data were transferred from the Del Mar Avionics scanner (Model 500, Del Mar Avionics, Irvine, CA, USA) to a computer for the analysis of HRV.

A photoplethysmographic technique (Finapres 2300, Ohmeda, Englewood, CO, USA) was applied to record the continuous arterial pressure signal. The reliability of this technique has been assessed (50). In population 1, a beat-to-beat arterial pressure tracking was obtained from the middle phalanx of the third finger of the left hand. In population 2, it was recorded from the second finger of the left hand. The finger with a carefully fitted cuff was held at heart level. The automatic servo-adjustment option of the Finapres device was disabled during the recordings. Whereas the same time series were used in population 1 to assess all the measures of autonomic cardiovascular function, separate stationary segments without ectopic beats of about 5 minutes were used in population 2 to calculate beat-to-beat SAP variability and cross-spectral BRS.

4.3 Heart rate variability

For a part of populations 1 and 3, subjects were excluded from the analyses if unstationarities, artifacts, or arrhythmias, even a single premature beat, made it impossible to obtain at least 4 minutes of continuous sequence. In population 2, premature beats and noise were excluded automatically and manually, and the gaps were refilled with an average value computed from data of the local neighborhood. Segments with > 85% qualified beats were included in the analysis. The time domain measures of HRV evaluated in this study were SDNN (I, III, IV) and RMSSD (I).

Before spectral analyses, for a part of populations 1 and 3, time series were detrended, linearly interpolated, resampled at 5 Hz, and subjected to a Partzen window. Correspondingly, in population 2, a linear detrend was applied to the R-R interval data in segments of 512 samples to make it more stationary. The power density spectra of HRV were computed using either a fast Fourier transform algorithm with a triangular smoothing (populations 1 and 3) or an autoregressive model (population 2). The frequency bands for the assessment of total, HF, LF, and VLF powers of HRV were < 0.4 Hz, 0.15–0.4 Hz, 0.04–0.15 Hz, and < 0.04 Hz, in respective order. The spectral measures of HRV were analyzed as absolute units (ms^2), and the LF and HF components also as normalized units (nu, %; I, III).

A custom-built version of the CPRS software (CardioPulmonary Research Software, Absolute Aliens Ltd, Turku, Finland), specifically intended for computation of the power spectra of beat-to-beat PP, was used to calculate all the measures of autonomic cardiovascular function in populations 1 and 3. In population 2, another custom-made program was used (Hearts, Heart Signal Co, Kempele, Finland). Measurements were performed in compliance with the standards of the Task Force (8).

4.4 Beat-to-beat arterial pressure variability

In population 1, the same time series, frequency bands, and spectral methods as described above for the assessment of HRV were used to calculate the beat-to-beat APV of SAP (II–V), DAP (II, IV, V), and PP (IV). In population 2, a fast Fourier algorithm was applied to calculate the beat-to-beat SAP variability in frequency bands including total power, LF power (0.04–0.06 Hz), medium frequency power (0.07–0.14 Hz), and HF power (0.15–0.40 Hz). In study IV, standard deviations (SD) of beat-to-beat SAP, DAP, and PP were calculated to describe the overall variability of each signal.

4.5 Baroreflex sensitivity

BRS was determined by cross-spectral analysis between R-R and SAP variabilities provided that the coefficient of coherence was > 0.50, and that the phase angle was negative (Figure 4). The cross-spectral BRS was calculated either from the LF band (0.04–0.15 Hz, population 1) or from the combination of medium frequency and HF bands (0.07–0.40 Hz, population 2). Data of 7 and 17 subjects in populations 1 and 2, respectively, did not meet the coherence and phase criteria but were used to calculate the power spectra of HRV and APV.

4.6 Blood pressure

In studies I, II, IV, and V, blood pressure and heart rate were estimated by a trained nurse and averaged of 4 duplicate measures (310). Blood pressure was recorded with the subject sitting. A mercury sphygmomanometer was used, and the measurements were always made between 8 and 10 a.m. according to the guidelines of the American Society of Hypertension (311).

Ambulatory blood pressure recording was used in study III with an auscultatory device (Accutracker II, Suntech Medical Instruments, Raleigh, NC, USA) in population 1 (312) and with Space-Labs 90207 oscillometric unit (SpaceLabs Inc., Redmond, WA, USA) in population 2. PP was defined in both populations as the mean difference of all corresponding ambulatory 24-hour systolic and diastolic blood pressure readings. However, in study IV, PP was calculated from the 4 duplicate blood pressure readings.

4.7 Lifestyle variables

For body weight the subjects were weighed in light clothing without shoes at an accuracy of 0.1 kg, and height was measured at an accuracy of 1 cm. Information on smoking and alcohol consumption was obtained by questionnaires. Smoking was expressed on a dichotomized scale (smokers or non-smokers). The alcoholic drinks were converted into grams of absolute ethanol per week. Alcohol intake was analyzed as a continuous variable (I) or as a categorical variable (teetotalers, and tertiles of women and men consuming alcohol; III, V). Dietary salt intake was estimated by urinary 24-hour sodium excretion.

4.8 Psychological factors

Three self-report questionnaires (II) were used for the assessment of psychological factors: a shortened 37-item version (BSI-37) of the original 53-item Brief Symptom Inventory (313), the shortened version of the Spielberger State-Trait Anger Expression Inventory (STAXI) (314), and the Toronto Alexithymia Scale (TAS-26) (315). The total score of the BSI-37 divided by the number of items, known as the general severity index, was used as an indicator of psychological distress. Symptom dimensions for somatization, depression, anxiety, hostility, and phobicity were derived from the BSI-37. The total score of the TAS-26 was used as an indicator of alexithymia.

The TAS-26 consists of four factors which describe difficulty in identifying and distinguishing between feelings and bodily sensations (Factor 1), difficulty in describing feelings (Factor 2), reduced daydreaming (Factor 3), and externally oriented thinking (Factor 4). The STAXI consists of 31 items which reflect the intensity of feelings of anger (state anger), the disposition to experience anger (trait anger), behaviorally expressed anger (anger-out), suppressed anger (anger-in), and self-control of anger behavior (anger control).

4.9 Laboratory analyses

Laboratory tests were conducted after a 12-hour requested fasting. The serum samples were frozen and stored at –70 °C until assayed. Urinary 24-hour sodium (I, V) and potassium (V) were analyzed by emission flame photometry. The urinary collections were judged to be complete in over 90% of subjects (310). Radioimmunoassay was used for the determination of plasma renin activity (Phadebas Angiotensin I test, Pharmacia Diagnostics, Stockholm, Sweden) and plasma aldosterone (Aldosterone RIA, Abbott Laboratories, Chicago, IL, USA) (I). Enzymatic methods were used for the determinations of serum cholesterol, triglyceride, glucose (Merck Diagnostica,

Darmstadt, Germany), and HDL cholesterol (Boehringer Mannheim, Germany) (IV, V). HDL cholesterol was analyzed after magnesium-phosphotungstate precipitation of very-low-density lipoprotein and LDL cholesterol. The LDL cholesterol content was estimated by the Friedewald formula. Serum insulin was determined by microparticle enzyme immunoassay (Abbott Laboratories, Dainabot, Tokyo, Japan) (IV, V). The HOMA formula was used to assess insulin resistance (316) (IV, V).

4.10 Exercise capacity

Exercise stress testing with a bicycle ergometer was used to assess maximal oxygen uptake (V). The staged protocol was started at 3 minutes of pedaling at a power output of 20 W for women and 30 W for men. Thereafter the work rate was increased by a uniform amount every 2 minutes, with increments ranging from 10 to 20 W for women and 15 to 30 W for men, depending on a patient's expected performance. Maximal oxygen uptake was calculated with a linear regression method based on maximal work capacity.

4.11 Body fat composition

BMI was calculated as the body weight in kilograms divided by the square of the height in meters. The body fat composition was determined with an RJL impedance meter (model BIA-101A/S & Spectrum, RJL Systems, Mt. Clemens, MI, USA) (V). The BIA equation of Lukaski $((0.734 \cdot (\text{height})^2 / \text{resistance}) + (0.116 \cdot \text{weight}) + (0.096 \cdot \text{reactance}) + (0.878 \cdot \text{sex (Female=0, Male=1)}) - 4.03)$ was used to estimate the fatfree mass, and fat mass was calculated as weight minus fatfree mass (317).

4.12 Statistical analyses

Before statistical analyses, the variables with skewed distributions were transformed logarithmically. The summary statistics are given as mean (SD) or mean (s.e.) for indexes of HRV. Group differences were compared with a two-way analysis of variance (ANOVA) (I), the non-parametric Mann-Whitney test (I), unpaired t-tests (III–V), or, for categorical variables, the χ^2-test. Associations between continuous variables were studied with Pearson's correlation coefficients (I–IV), adjusted partial correlation coefficients (I, IV, V), and linear regression (III).

Multivariate stepwise linear regression analyses were used to evaluate the independent associations of dependent variables. The effects of age and gender were controlled in these analyses (II, III, V). In study II, heart rate, systolic, and diastolic blood

pressures were finally forced into the model to evaluate whether the associations between psychological factors and dependent variables result from their relations to heart rate or blood pressure. In study V, after the observation of a preferential role of blood pressure over other characteristics of insulin resistance, multivariate regression analyses were repeated without systolic and diastolic blood pressures to test whether their removal brings out another component of the metabolic syndrome. In study III, the regression analyses were carried out with standardized variables for comparability of the regression coefficients of different variables between the two populations. In study I, the adjusted means of HRV variables were finally calculated for hypertensive and normotensive women and men after adjustment for statistically significant covariates.

Statistical analyses were performed with the SPSS software (SPSS Inc., Chicago, IL, USA). P-values, derived from 2-tailed tests, less than 0.05 were considered statistically significant.

Table 2. ***Characteristics of study populations***

	Population 1			Population 2	Population 3
	Stydy I	Study III	Studies II, IV and V	Study III	Study I
Number of subjects	105	149	150	214	191
Gender (female / male)	56 / 49	78 / 71	79 / 71	126 / 88	82 / 109
Age (yr)	44 (5)	48 (8)	48 (8)	51 (6)	46 (5)
Systolic blood pressure (mm Hg)	117 (10)	120 (13)	121 (13)	126 (13)	144 (12)
Diastolic blood pressure (mm Hg)	74 (7)	73 (8)	75 (9)	78 (8)	94 (7)
Heart rate (beats/min)[I, II, IV, V]	70 (8)		71 (8)		76 (9)
Pulse pressure (mm Hg)[III, IV]		47 (8)	45 (8)	48 (8)	
Body mass index (kg/m^2)[I, III-V]	25.4 (3.7)	26.0 (4.0)	26.0 (4.0)	25.9 (3.8)	27.4 (4.1)
Smoking (%)[I, III-V]	33.7	29.5	29.3	30.4	24.1
Alcohol intake (g/week)[I, III, V]	91 (120)	75 (94)	74 (94)	48 (72)	105 (128)
Cholesterol (mmol/l)[IV, V]			5.6 (1.1)		
HDL-Cholesterol (mmol/l)[IV, V]			1.3 (0.3)		
Triglycerides (mmol/l)[IV, V]			1.5 (1.1)		
LDL-Cholesterol (mmol/l)[IV, V]			3.7 (1.0)		
Glucose (mmol/l)[IV, V]			5.2 (0.5)		
Insulin (mU/l)[IV, V]			7.4 (4.0)		
HOMA (mmol·mU/l^2)[IV, V]			1.72 (1.05)		
PRA (ngAI/ml·h)[I]	1.430 (1.122)				0.999 (1.003)
Plasma aldosterone (pmol/l)[I]	172.2 (101.6)				163.6 (73.6)
24-h urinary sodium (mmol)[I, V]	135 (50)		136 (50)		166 (72)
PRA/24-h urinary sodium (ngAI/l·h·mmol)[I]	12.2 (12.2)				7.2 (7.4)
Fat mass (kg)[V]			20.2 (8.8)		
Fat in percentages (%)[V]			26.6 (8.4)		
Maximal oxygen uptake index (ml/kg/min)[V]			31.5 (7.6)		
24-h urinary potassium (mmol)[V]			77.4 (23.9)		

Values are mean (SD). HOMA, the homeostasis model assessment; PRA, plasma renin activity. The roman numerals in superscript indicate the respective original articles.

5 RESULTS

The examined variables and the characteristics of study populations are presented in Table 2.

5.1 Heart rate variability and its determinants in newly diagnosed untreated systemic hypertension (I)

All measures of HRV were reduced in hypertensive subjects as compared to their normotensive controls (Table 3). However, there were no differences in the normalized

Table 3. ***Indexes of HRV between hypertensive and normotensive subjects***

	Men		Women		
	Hypertensive (n = 109)	Normotensive (n = 49)	Hypertensive (n = 82)	Normotensive (n = 56)	p*
Total power					
ms^2	1562.6 (138.5)	2977.4 (364.1)	1374.0 (118.8)	2392.1 (263.3)	
$ln(ms^2)$	7.00 (0.08)	7.67 (0.13)	6.92 (0.09)	7.49 (0.10)	< 0.001
VLF power					
ms^2	832.6 (79.1)	1424.9 (254.9)	711.2 (67.4)	1094.7 (123.5)	
$ln(ms^2)$	6.31 (0.09)	6.79 (0.14)	6.22 (0.10)	6.72 (0.10)	< 0.001
LF power					
ms^2	488.8 (54.2)	1017.8 (130.7)	372.2 (39.5)	721.8 (105.1)	
$ln(ms^2)$	5.79 (0.09)	6.52 (0.14)	5.52 (0.10)	6.13 (0.13)	< 0.001
nu	68.8 (1.4)	65.9 (2.3)	57.9 (1.8)	59.1 (2.4)	< 0.657
HF power					
ms^2	221.8 (29.1)	509.6 (90.5)	272.4 (38.8)	541.2 (93.9)	
$ln(ms^2)$	4.83 (0.10)	5.71 (0.16)	5.09 (0.12)	5.63 (0.16)	< 0.001
nu	28.8 (1.3)	32.1 (2.2)	39.1 (1.7)	37.6 (2.3)	< 0.622
LF/HF ratio					
%	327.6 (20.6)	304.7 (35.8)	199.5 (16.5)	226.6 (25.0)	
ln(%)	5.56 (0.07)	5.42 (0.11)	5.04 (0.08)	5.11 (0.11)	< 0.696
RMSSD					
ms	22 (1)	35 (2)	23 (1)	33 (3)	
ln(ms)	2.94 (0.05)	3.42 (0.08)	3.01 (0.06)	3.35 (0.07)	< 0.001
SDNN					
ms	37.9 (1.4)	55.7 (3.1)	36.2 (1.6)	49.3 (2.6)	
ln(ms)	3.56 (0.04)	3.93 (0.07)	3.51 (0.05)	3.83 (0.05)	< 0.001

HF, high frequency; HRV, heart rate variability; LF, low frequency; RMSSD, the square root of the mean of squared differences between adjacent normal R-R intervals; SDNN, the standard deviation of normal-to-normal R-R intervals; VLF, very low frequency.
Values are mean (s.e.). There were no two-way interactions between gender and group factor (1 = hypertension, 2 = normotension). Gender was not significant as a main-effect term except for LF power (p = 0.004), normalized LF power (p < 0.001), normalized HF power (p < 0.001), and LF/HF ratio (p < 0.001).
*p indicates the significance level of group factor as a main-effect term in the ANOVA model.

components of HRV and the LF/HF ratio. Age, heart rate, blood pressure, and BMI showed negative crude or adjusted correlations with the indexes of HRV. There were consistent inverse relationships between PRA and the HF component of HRV and RMSSD (Figure 6; I, Table 3), and between PRA and SDNN (I, Table 3).

In multivariate regression analyses (Table 4), except for the LF component, absolute measures of HRV were independently associated with heart rate, age, and mean arterial pressure. The LF component was also explained by gender, women having lower LF powers than men. Decreased RMSSD and the HF component of HRV were also predicted by a high PRA. Even after adjustment for the independent predictors (age,

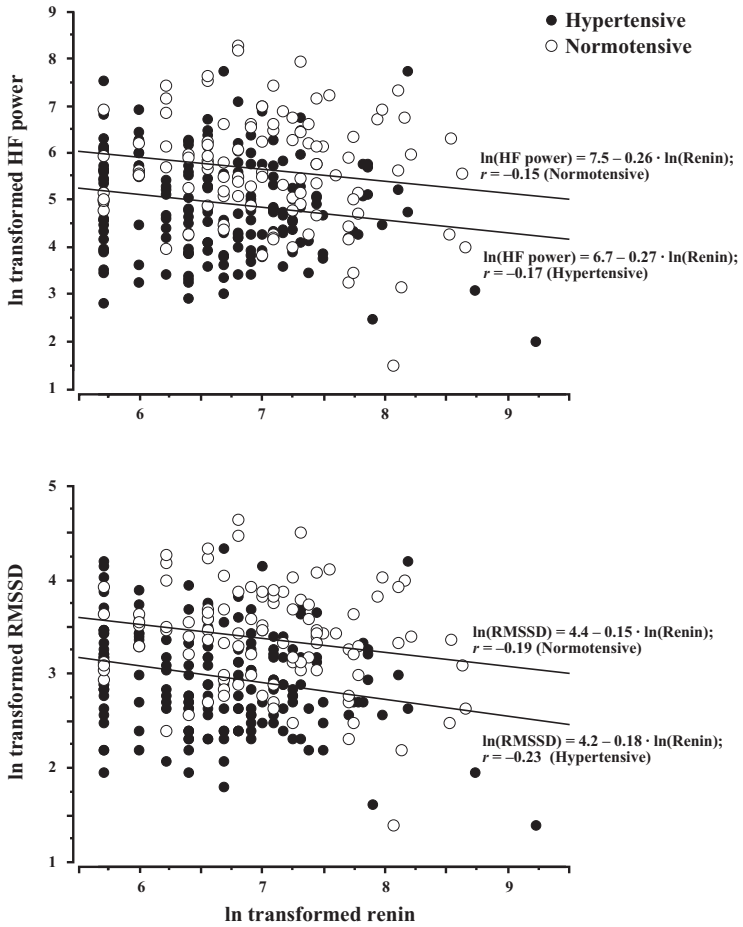

Figure 6. ***Scatterplots showing the HF component of HRV and RMSSD in relation to plasma renin activity. Reproduced with permission from the Nature Publishing Group, J Hum Hypertens 2003; 17:(3)171–9.***

Table 4. ***Independent correlates of HRV in hypertensive and normotensive subjects according to multivariate stepwise linear regression analyses***

	Total power ln(ms^2)	VLF power ln(ms^2)	LF power ln(ms^2)	HF power ln(ms^2)	SDNN ln(ms)	RMSSD ln(ms)
Heart rate/10 beats						
B	–0.44	–0.48	–0.39	–0.43	–0.21	–0.28
s.e.	0.05	0.06	0.06	0.07	0.03	0.03
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Age/10 yr						
B	–0.31		–0.36	–0.57	–0.15	–0.27
s.e.	0.09		0.11	0.12	0.05	0.06
p	0.001		0.001	<0.001	0.001	<0.001
Mean arterial pressure/10 mm Hg						
B	–0.08		–0.13	–0.16	–0.06	–0.08
s.e.	0.04		0.04	0.05	0.02	0.02
p	0.035		0.004	0.001	0.002	0.001
Gender 1 = men, 2 = women						
B			–0.30			
s.e.			0.11			
p			0.005			
PRA/ln(ngAI/ml·h)						
B				–0.19		–0.11
s.e.				0.08		0.04
p				0.026		0.007
Model R^2	0.28	0.20	0.26	0.29	0.30	0.36

HF, high frequency; HRV, heart rate variability; LF, low frequency; PRA, plasma renin activity; RMSSD, the square root of the mean of squared differences between adjacent normal R-R intervals; SDNN, the standard deviation of normal-to-normal R-R intervals; VLF, very low frequency. Potential independent variables in every stepwise analysis were age, gender, body mass index, mean arterial pressure, heart rate, smoking, PRA, 24-h urinary sodium, and PRA to 24-h urinary sodium ratio. B is the regression coefficient and s.e. its standard error.

heart rate, and PRA), total ($p = 0.007$), VLF ($p = 0.015$), and LF ($p = 0.011$) powers in hypertensive women, and LF ($p = 0.028$) and HF ($p = 0.006$) powers in hypertensive men were lower when compared with their normotensive counterparts (I, Figure 2).

5.2 Psychological factors and autonomic cardiovascular regulation (II)

Psychological measures were strongly interrelated (Table 5). Only increased behaviorally expressed anger (anger-out) correlated with increased LF power of HRV ($r = 0.18$, $p = 0.032$). According to multivariate regression analyses, none of the psychological factors predicted HRV.

Higher scores of somatization, depression, anxiety, hostility, phobicity, and the general severity index of the BSI-37, and state anger of the STAXI were associated with higher LF power of APV (range of Pearson's r from 0.16 to 0.31, Table 6) Multivariate regression analyses showed that increased anxiety was associated with increased LF power of SAP variability (standardized regression coefficient, $\beta = 0.26$, $p = 0.001$) and increased hostility with increased LF power of DAP variability ($\beta = 0.30$, $p < 0.001$), independently of age, gender, and other psychological factors. Most importantly, these associations remained essentially unchanged when heart rate, systolic, and diastolic blood pressures were added to the model (Table 7).

A higher score of somatization ($r = 0.23$) and a lower score of anger-in ($r = -0.16$) were associated with increased HF power of SAP variability. Multivariate analysis showed that reduced anger-in ($\beta = -0.19$, $p = 0.020$) and increased hostility ($\beta = 0.18$, $p = 0.033$) were associated with increased HF power of SAP variability independently of age, gender, other psychological factors, heart rate, systolic, and diastolic blood pressures (model $R^2 = 0.13$, $p = 0.005$). None of the psychological variables were associated with the HF power of DAP variability.

Higher scores of somatization, depression, anxiety, hostility, phobicity, and the general severity index of the BSI-37 were associated with reduced BRS (range of Pearson's r from -0.19 to -0.29, Table 6). Multivariate regression analysis showed that higher age ($\beta = -0.39$, $p < 0.001$), higher anxiety ($\beta = -0.29$, $p < 0.001$), and a lower total score of the TAS-26 ($\beta = 0.16$, $p = 0.050$) were associated with reduced BRS independently of age, gender, and other psychological factors (model $R^2 = 0.25$, $p < 0.001$). The association between a higher score of anxiety and lower BRS remained unchanged, but the association between the total score of the TAS-26 and BRS disappeared after heart rate, systolic, and diastolic blood pressures were added to the model (Table 7).

Table 5. ***Correlation matrix of selected psychological measures***

	Depression	Anxiety	Hostility	Phobicity	General severity index	Total TAS-26	State anger	Anger-out	Anger-in
Somatization	0.47***	0.60***	0.43***	0.48***	0.76***	0.30***	0.32***	0.05	0.11
Depression	—	0.66***	0.55***	0.52***	0.83***	0.29***	0.26**	0.22**	0.31***
Anxiety		—	0.63***	0.68***	0.87***	0.32***	0.45***	0.24**	0.18*
Hostility			—	0.41***	0.74***	0.13	0.29***	0.41***	0.13
Phobicity				—	0.69***	0.37***	0.32***	0.14	0.12
General severity index					—	0.38***	0.41***	0.22**	0.26**
Total TAS-26						—	0.11	–0.16	0.29***
State anger							—	0.11	0.25**
Anger-out								—	–0.14

TAS-26, Toronto Alexithymia Scale. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table 6. ***Pearson's correlation coefficients between selected psychological measures and the indexes of beat-to-beat APV and BRS***

	LF power of SAP variability ln(mm Hg)^2	HF power of SAP variability ln(mm Hg)^2	LF power of DAP variability ln(mm Hg)^2	HF power of DAP variability ln(mm Hg)^2	BRS[a] ln(ms/mm Hg)
Somatization	0.19*	0.20*	0.16*	0.12	–0.21*
Depression	0.19*	0.11	0.17*	0.09	–0.21*
Anxiety	0.26**	0.08	0.29***	0.03	–0.29***
Hostility	0.22**	0.16	0.31***	0.15	–0.19*
Phobicity	0.15	0.02	0.16*	–0.01	–0.22**
General severity index	0.23**	0.13	0.24**	0.09	–0.27**
Total TAS-26	0.04	–0.11	0.00	0.06	0.03
State anger	0.12	–0.02	0.16*	–0.04	–0.07
Anger-in	0.06	–0.16*	0.07	0.05	–0.09

APV, arterial pressure variability; BRS, baroreflex sensitivity; DAP, diastolic arterial pressure; HF, high frequency; LF, low frequency; SAP, systolic arterial pressure; TAS-26, Toronto Alexithymia Scale. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. [a]$n = 143$, otherwise $n = 150$.

Table 7. ***Final multivariate regression models testing independent associations of psychological measures with the LF components of SAP and DAP variabilities and BRS***

	LF power of SAP variability ln(mm Hg^2)	LF power of DAP variability ln(mm Hg^2)	BRS[a] ln(ms/mm Hg)
Age (yr)	0.13 (0.106)	–0.08 (0.364)	–0.36 (<0.001)
Gender (1 = men; 2 = women)	–0.02 (0.797)	0.05 (0.595)	–0.09 (0.233)
Anxiety	0.25 (0.002)	—	–0.27 (0.001)
Hostility	—	0.29 (0.001)	—
Heart rate (beats/min)	0.03 (0.699)	0.02 (0.836)	–0.18 (0.031)
Systolic blood pressure (mm Hg)	0.23 (0.088)	0.09 (0.626)	–0.05 (0.655)
Diastolic blood pressure (mm Hg)	–0.01 (0.963)	0.05 (0.381)	–0.14 (0.250)
Model R^2	0.16	0.11	0.34
Model p	<0.001	0.010	<0.001

Variable values are β (p). β, regression coefficient corresponding standardized variable; p, significance of the variable in the model; R^2, R square. BRS, baroreflex sensitivity; DAP, diastolic arterial pressure; HF, high frequency; LF, low frequency; SAP, systolic arterial pressure.
[a]n = 143, otherwise n = 150.

5.3 Ambulatory pulse pressure and autonomic cardiovascular regulation (III)

Associations between 24-hour ambulatory PP and autonomic cardiovascular regulation were studied in two separate populations. PP correlated inversely with the HF power of HRV in population 1 ($r = -0.18$, $p = 0.025$), and with the LF power of HRV in population 2 ($r = -0.14$, $p = 0.039$). However, correlations between PP and HRV were not significant after controlling for age and gender.

In both populations, PP correlated inversely with BRS ($r = -0.33$, $p < 0.001$, population 1; $r = -0.29$, $p < 0.001$, population 2), and according to multivariate regression analyses, independently of age and gender ($\beta = -0.28$, $p < 0.001$, population 1; $\beta = -0.22$, $p = 0.003$, population 2). By multivariate analyses with age, gender, 24-hour ambulatory diastolic blood pressure, BMI, alcohol intake, and smoking increased 24-hour ambulatory PP was still an independent determinant of decreased BRS (Table 8).

Elevated PP was related to increased beat-to-beat SAP variability. By multivariate analyses, higher 24-hour ambulatory PP was associated in population 1 with higher total, VLF, LF ($\beta = 0.40, 0.37, 0.31$, respectively, $p < 0.001$ for all), and HF ($\beta = 0.28$, $p = 0.001$) powers of SAP variability, and in population 2, with higher total, LF, and medium frequency powers of SAP variability ($\beta = 0.16, 0.15$ and 0.16, respectively, $p < 0.05$ for all).

Table 8. ***Ambulatory pulse pressure as a determinant of BRS***

	Population 1			Population 2		
	β	*CI*	*p*	β	*CI*	*p*
Age (yr)	–0.28	–0.43 to –0.13	<0.001	–0.23	–0.38 to –0.09	0.002
Gender (female vs male)	–0.25	–0.43 to –0.08	0.005	0.03	–0.13 to +0.18	0.746
Body mass index (kg/m^2)	–0.20	–0.36 to –0.04	0.015		—	
24-h pulse pressure (mm Hg)	–0.20	–0.37 to –0.04	0.018	–0.16	–0.30 to –0.01	0.033
24-h diastolic blood pressure (mm Hg)		—		–0.23	–0.37 to –0.09	0.002

Population 1: Model $p < 0.001$, $R^2 = 0.27$, $n = 149$
Population 2: Model $p < 0.001$, $R^2 = 0.16$, $n = 214$

BRS, baroreflex sensitivity. β, regression coefficient corresponding standardized variable (except gender); *CI*, 95% confidence interval for the regression coefficient; *p*, significance of the variable in the model; R^2, R square. Dependent variable in the analysis was natural logarithmic corrected BRS. Age and gender were forced in the model. Ambulatory diastolic and pulse pressures, body mass index, alcohol intake, and smoking were tested in a stepwise manner.

5.4 Power spectral analysis of beat-to-beat pulse pressure variability (IV)

Like the power spectra of beat-to-beat R-R interval, SAP, and DAP variabilities, the power spectrum of beat-to-beat PP variability showed a large interindividual variation, a skewed distribution, and similar frequency peaks (IV, Table 2; Figure 7).

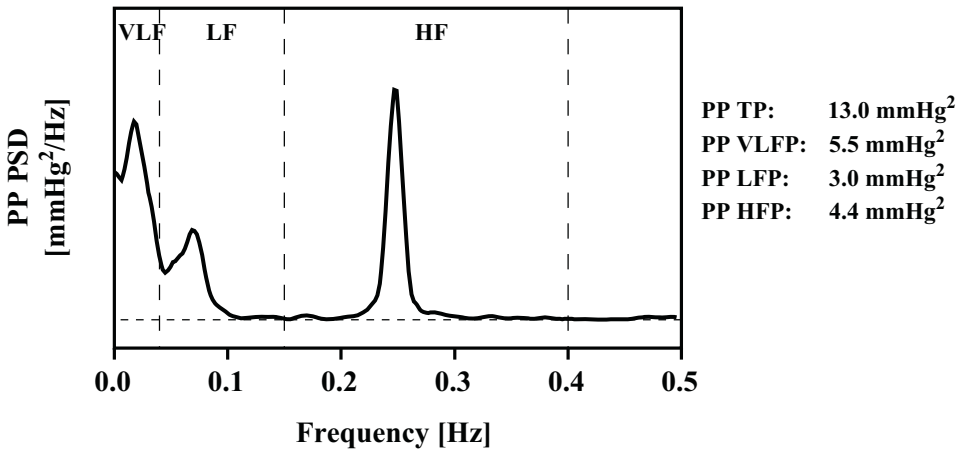

Figure 7. ***A power spectrum of beat-to-beat PP variability from the same period of time and subject as shown previously in figures 1, 2, and 3. The HF component of PP variability is proportionally greater than those of systolic, mean, and diastolic APV, and in this case even greater than the HF component of HRV.***

An unexpected finding was that the proportion of HF to total power was higher in PP than in SAP and DAP variabilities (Figure 8).

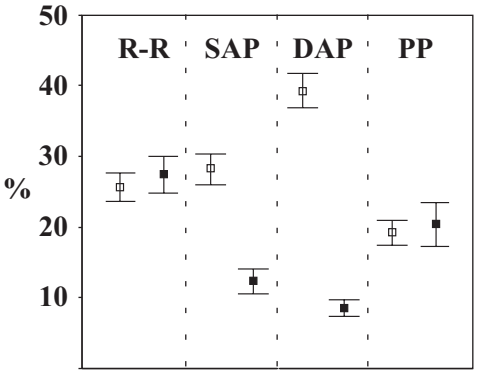

Figure 8. ***LF (open boxes) and HF (black boxes) components in percentages of the overall variability of R-R interval (R-R), systolic (SAP), diastolic (DAP), and pulse (PP) pressure variabilities. Mean values and their 95% confidence intervals are shown. Reproduced with permission from the Blackwell Publishing, Clin Physiol Funct Imaging 2004; 24(5):304–9.***

The SD of beat-to-beat PP correlated closely with the LF component of PP variability ($r = 0.76$, $p < 0.001$), but only weakly with the HF component of PP variability ($r = 0.18$, $p < 0.032$). There was no significant correlation between the HF and LF components of PP variability. Measures of PP variability were closely related to the respective measures of SAP variability and less closely to those of DAP variability (Table 9). A higher HF component of PP variability was associated with a higher HF component of HRV, but the LF component of PP variability did not correlate with the respective component of HRV.

Table 9. ***Correlation coefficients of beat-to-beat PP variability with the measures of SAP, DAP, and R-R interval variabilities and BRS***

	LF power of PP variability ln(mm Hg^2)	HF power of PP variability ln(mm Hg^2)	SD of beat-to-beat PP ln(mm Hg)
SBP variability			
LF power, ln(mm Hg^2)	0.89***	0.13	0.61***
HF power, ln(mm Hg^2)	0.22**	0.86***	0.24**
SD, ln(mm Hg)	0.74***	0.17*	0.92***
DBP variability			
LF power, ln(mm Hg^2)	0.55***	0.08	0.24**
HF power, ln(mm Hg^2)	0.16*	0.33***	0.16
SD, ln(mm Hg)	0.44***	0.19*	0.46***
RR interval variability			
LF power, ln(ms^2)	0.13	0.09	0.03
HF power, ln(ms^2)	–0.13	0.30***	–0.07
SD, ln(ms)	–0.09	0.18*	–0.06
BRS, ln(ms/mm Hg)[a]	–0.48***	–0.07	–0.32***

BRS, baroreflex sensitivity; DAP, diastolic arterial pressure; HF, high frequency; LF, low frequency; PP, pulse pressure; SAP, systolic arterial pressure; SD, standard deviation.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.
[a]$n = 143$, otherwise $n = 150$.

5.5 Determinants of beat-to-beat pulse pressure variability (IV)

The SD of beat-to-beat PP ($r = 0.21$, $p = 0.010$) and the LF component ($r = 0.17$, $p = 0.041$) of PP variability increased with age. SD was greater in men than in women (mean ± s.e. 4.4 ± 0.2 vs. 3.9 ± 0.2, $p = 0.045$), but for the spectral powers, no significant gender differences were found.

Age- and gender-adjusted correlations (Table 10) showed that the SD of beat-to-beat PP and the LF component of PP variability increased with PP, systolic blood pressure, and BMI. A higher LF component of PP variability was also associated with higher levels of serum insulin, HOMA, and triglycerides. The HF component of PP variability correlated only with PP.

Multivariate stepwise regression analysis showed that elevated PP ($\beta = 0.24$, $p = 0.003$) and higher BMI ($\beta = 0.19$, $p = 0.020$) were associated with an increased LF component of beat-to-beat PP variability independently of age, gender, insulin resistance, serum triglycerides, and smoking (Model $R^2 = 0.15$, $p < 0.001$).

Table 10. ***Age- and gender-adjusted correlation coefficients between the indexes of beat-to-beat PP variability and cardiovascular risk factors***

	LF power of PP variability ln(mm Hg^2)	HF power of PP variability ln(mm Hg^2)	SD of beat-to-beat PP ln(mm Hg)
Systolic blood pressure (mm Hg)	0.28**	0.14	0.24**
Diastolic blood pressure (mm Hg)	0.15	–0.04	0.05
Pulse pressure (mm Hg)	0.27**	0.26**	0.31***
Heart rate (beats/min)	0.10	–0.06	0.06
Body mass index (kg/m^2)	0.22**	0.05	0.22**
Cholesterol (mmol/l)	0.03	0.09	–0.03
HDL-Cholesterol (mmol/l)	–0.14	–0.11	–0.08
Triglycerides ln(mmol/l)	0.16*	0.10	0.07
LDL-Cholesterol (mmol/l)	0.03	0.08	–0.03
Glucose (mmol/l)	0.11	–0.10	0.09
Insulin ln(mU/l)	0.17*	0.15	0.11
HOMA ln(mmol·mU/l^2)	0.18*	0.14	0.12

HOMA, homeostasis model assessment. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

5.6 Association of beat-to-beat pulse pressure variability with baroreflex sensitivity (IV)

Cross-spectral BRS correlated inversely with the LF component of PP variability ($r = -0.48$, $p < 0.001$) and with the SD of beat-to-beat PP ($r = -0.32$, $p < 0.001$). To test an independent role of short-term PP fluctuation as a determinant of BRS, multivariate

models were made with static blood pressure readings, age, and gender as explanatory variables. Age, gender, and systolic blood pressure alone turned out to explain 24.5%, and together with the LF component of PP variability 41.0%, of the variation in BRS. Age, gender, and diastolic blood pressure explained 25.7% of the variation in BRS, whereas the predictive value of these variables combined with the LF component of PP variability was 43.7%.

5.7 Effects of insulin resistance and exercise capacity on autonomic cardiovascular regulation (V)

Factors pertinent to the metabolic syndrome, e.g. elevated levels of blood pressure, serum triglycerides, insulin, insulin resistance, BMI, and body fat mass were inversely associated with the LF and HF components of HRV and with BRS (Table 11). The same factors correlated positively with the HF components of beat-to-beat SAP and DAP variabilities, but, except for blood pressure, not with their LF components. The maximal oxygen uptake index was positively associated with the LF and HF components of HRV and with BRS, and negatively with the HF components of beat-to-beat SAP and DAP variabilities. Smoking, alcohol consumption, and urinary sodium and potassium excretion were not significantly associated with HRV, beat-to-beat APV, or BRS.

Multivariate regression analyses showed that higher age ($\beta = -0.31$, $p < 0.001$) and higher diastolic blood pressure ($\beta = -0.29$, $p < 0.001$) were independent determinants of a reduced HF component of HRV (model $R^2 = 0.22$, $p < 0.001$). Higher age ($\beta = -0.28$, $p = 0.001$) and a lower maximal oxygen uptake index ($\beta = 0.25$, $p = 0.011$) were independent correlates of decreased LF component of HRV (model $R^2 = 0.21$, $p < 0.001$). Correspondingly, a lower level of maximal oxygen uptake index was associated with increased HF components of beat-to-beat SAP ($\beta = -0.46$, $p < 0.001$) and DAP ($\beta = -0.30$, $p = 0.006$) variabilities. Higher age ($\beta = -0.33$, $p < 0.001$), higher diastolic blood pressure ($\beta = -0.22$, $p = 0.007$), and a higher proportion of fat of the total body weight ($\beta = -0.19$, $p = 0.030$) were associated with reduced BRS (model $R^2 = 0.28$, $p < 0.001$). There was a reasonable correlation between diastolic blood pressure and HOMA ($r = 0.47$, $p < 0.001$), and between diastolic blood pressure and the serum triglyceride concentration ($r = 0.49$, $p < 0.001$). Multivariate regression analyses without blood pressure showed that increased HOMA was a determinant of reduced HF component of HRV ($\beta = -0.19$, $p = 0.021$), and a higher level of serum triglycerides ($\beta = -0.22$, $p = 0.007$) was as a determinant of reduced BRS.

Table 11. ***Age- and gender-adjusted correlation coefficients of autonomic cardiovascular indexes with cardiovascular risk factors and maximal oxygen uptake index***

	LF power of HRV ln(ms^2)	HF power of HRV ln(ms^2)	LF power of SAP variability ln(mm Hg^2)	HF power of SAP variability ln(mm Hg^2)	LF power of DAP variability ln(mm Hg^2)	HF power of DAP variability ln(mm Hg^2)	BRS [a] ln(ms/mmHg)
Systolic blood pressure (mm Hg)	–0.12	–0.21*	0.25**	0.20*	0.17*	0.16*	–0.27**
Distolic blood pressure (mm Hg)	–0.17*	–0.28**	0.18*	0.11	0.14	0.18*	–0.29***
Cholesterol (mmol/l)	–0.03	–0.09	0.09	0.16*	0.06	0.05	–0.07
HDL-cholesterol (mmol/l)	0.08	0.06	–0.16	–0.16*	–0.11	–0.17*	0.19*
Triglycerides ln(mmol/l)	–0.20*	–0.21*	0.16	0.24**	0.07	0.14	–0.29***
Glucose (mmol/l)	–0.07	–0.11	0.10	0.12	0.06	0.05	–0.14
Insulin ln(mU/l)	–0.17*	–0.19*	0.12	0.24**	0.10	0.24**	–0.23**
HOMA ln(mmol·mU/l^2)	–0.17*	–0.20*	0.14	0.24**	0.10	0.23**	–0.23**
Body mass index (kg/m^2)	–0.21*	–0.16*	0.14	0.22**	–0.02	0.24**	–0.30***
Fatmass (kg)	–0.21*	–0.16	0.11	0.20*	–0.03	0.21*	–0.27**
Fat in percentages (%)	–0.16	–0.14	0.15	0.23**	0.02	0.21*	–0.26**
Maximal oxygen uptake index (ml/kg/min)	0.28**	0.23**	–0.00	–0.35***	0.09	–0.26**	0.23**

BRS, baroreflex sensitivity; DAP, diastolic arterial pressure; HOMA, homeostasis model assessment; HF, high frequency; HRV, heart rate variability; LF, low frequency; SAP, systolic arterial pressure.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.
[a] $n = 143$, otherwise $n = 150$.

6 DISCUSSION

6.1 Heart rate variability in essential hypertension (I)

Divergent results from studies examining HRV in essential hypertension can be ascribed to a number of factors. Whereas differences in measurement conditions and inconsistency in the expression of the indexes of HRV may explain some of the differing findings, methodological differences regarding the computation of the HRV measures play a minor role (218). Differences between the examined populations account, perhaps, for a large part of the divergence in results. Studies differ in respect to the selection criteria of hypertensive subjects and have handled various confounding factors, e.g. medication and co-morbidities, differently. Although studies with treated hypertensive individuals mainly support the finding of decreased HRV (225, 318), there are data suggesting that long-term antihypertensive treatment might in part correct abnormalities of HRV (205). Studies reporting HRV separately for untreated and treated hypertensive subjects (203, 218) indicate that the latter ones, in general, have more severely reduced HRV. Only few studies have used a population-based design (204, 218, 225).

Even after careful exclusion of medications and illnesses affecting autonomic cardiovascular function, factors remain that are inherent to hypertension; such factors may contribute to the divergent results. These include insulin sensitivity (263), a clustering of hypertension with other components of the metabolic syndrome (264, 269), sodium sensitivity (319–321), left ventricular hypertrophy (222, 322, 323), and presence or absence of a nocturnal fall in blood pressure (324, 325). High sodium intake enhances HRV and APV in hypertensive and in normotensive subjects (319). Sodium sensitivity is inversely related to BRS and HRV in hypertensive subjects (321), and sodium sensitive hypertensive subjects have a blunted capability to increase BRS or the HF component of HRV in response to a high sodium intake (319, 320). Among hypertensive patients with left ventricular hypertrophy, the circadian variation in HRV is blunted (326) and HRV is decreased both in comparison to normotensive subjects (222, 322, 323, 326) and hypertensive patients without left ventricular hypertrophy (222). Left ventricular mass is inversely correlated to the LF (222, 322, 323) and HF (322, 323) components of HRV. The absence of a nocturnal fall in blood pressure, i.e. non-dipping phenomenon, is related to decreased HRV (324, 325), to blunted circadian variation in HRV (325), and to an abnormal response of HRV to change of posture (324). Studies on the white-coat effect have resulted in conflicting findings (327, 328).

After careful exclusion of subjects with confounding factors, the present study (I) compared the indexes of HRV in newly diagnosed, untreated middle-aged hypertensive men and women with healthy counterparts of the general population. The observation that decreased SDNN, RMSSD, and all absolute frequency domain measures of HRV differentiated untreated hypertensive subjects as a group separate of their normotensive control group is in line with findings in previous population-based (204, 218, 225) and other studies (203, 234). Compared with the present study, the hypertensive subjects in these studies were older (203, 204, 218, 225, 234), exclusively male (225), or a subgroup (203, 218), or all (225, 234) had received (234), or were receiving (203, 218, 225) antihypertensive treatment. Only one of these studies (218) reported an increase in the LF/HF ratio of HRV in untreated hypertensive subjects. However, this increase was secondary to a reduction in the absolute HF component of HRV, and the LF component of HRV in arbitrary units was not increased. Thus, these studies and the present one did not support the concept of increased sympathetic activity in essential hypertension, but did consistently show attenuation of vagal modulation of HRV.

Study I shows that higher age, heart rate, and blood pressure were independent determinants of decreased measures of HRV in the combined sample of hypertensive subjects and healthy controls. A new finding was that the association of blood pressure with HRV is independent of heart rate. In accordance with previous studies involving hypertensive subjects (234, 318), higher age was associated with reduced HRV. Study I also confirmed the previous findings that female gender is related to an increase in normalized HF component and to decreases in arbitrary or normalized LF components and LF/HF ratio of HRV (234). The most interesting finding of the study was that increased plasma renin activity was an independent determinant of decreased vagal modulation of heart rate, as indicated by RMSSD and the HF component of HRV. Although no obvious explanation presents itself for this finding, it might be related to sodium sensitivity (321).

6.2 Determinants of heart rate variability in healthy subjects (II, III, V)

Age, gender, heart rate, and blood pressure affect HRV in an important way. The present studies were not primarily focused on these variables, but attempted rather to control the effects of age and gender (II, III, and V), and of heart rate and blood pressure (II) by running multivariate regression analyses of the data. Although reported only in the study V, advanced age was related to decreased HRV in all studies. Gender differences were also identified particularly in the normalized components of HRV. In study V, higher diastolic blood pressure was an independent determinant of reduced HF component of HRV in multivariate regression analysis that tested several factors

related to insulin resistance. Although study III revealed an association between PP and BRS, PP was not independently associated with any measure of HRV. This suggests that PP does not have any significant effect on tonic autonomic cardiovascular control and that it affects baroreflex-mediated heart rate fluctuations.

Research on the relationship between psychological factors and autonomic cardiovascular regulation has yielded inconsistent findings, and the majority of data originates from small case-control studies. Although many studies have linked depression with autonomic cardiovascular regulation in coronary heart disease (158–163), depression might be associated with the severity of coronary heart disease, which, in turn, could affect HRV – an association that is often overlooked. Only seldom is there comprehensive information on left ventricular systolic function or severity of coronary heart disease, based on angiographic, stress echocardiographic, or scintigraphic evaluation (158–162). Two studies that controlled carefully for the severity of coronary heart disease yielded negative results (329, 330).

Study II was the first to report the effects of psychological factors on HRV, APV, and BRS in a general population. The findings suggest that the LF and HF components of short-term HRV under supine resting conditions do not reflect symptoms of anxiety, hostility, and depression in middle-aged healthy men and women. These negative findings may be explained by the population-based setup and the strict exclusion of medicated patients with psychiatric disorders. The effects of psychological factors on HRV may only be identified in recordings that are sufficiently long to produce transactions between individuals and their environments (171). Posture may also modify results, as persons with panic disorder or anxiety have been found to have altered HRV only when standing, not supine (67, 139, 141, 150). These findings might indicate lower sympathetic reserve, because there are no differences in BRS between panic disorder patients and healthy subjects at rest (147). Thus, prudence is necessary when drawing conclusions from the present findings.

The findings in study V are in harmony with previous studies showing that a disturbance in glucose metabolism, measured by fasting glucose, insulin, or glycosylated hemoglobin (117, 249, 250), higher levels of triglycerides (104, 113, 117, 123), blood pressure (235, 269), and BMI (104, 117, 235) are associated, alone or in combination (269, 270), with a reduction of HRV. Previous studies have included diabetics and subjects treated for hypertension or other cardiovascular diseases (117, 235, 250, 269). The observations in study show that the characteristics of the metabolic syndrome relate to reduced LF and HF components of HRV also in a middle-aged healthy general population. In accordance with previous studies (12), higher HF and LF components of HRV were associated with an increment in maximal oxygen uptake index suggesting that a high level of physical fitness has a counteractive effect on HRV (V).

6.3 Determinants of beat-to-beat arterial pressure variability in healthy subjects (II, III, V)

Beat-to-beat APV has been the subject of only little research. One obvious reason for this is that this measure lacks prognostic implications. Although there are no longitudinal studies on beat-to-beat APV, the ambulatory blood pressure variability has been shown to carry prognostic information (331).

In the present study (II), a higher score of state anger and higher symptom scores of depression, somatization, anxiety, hostility, and phobicity were related to increased LF power of APV. Anxiety and hostility were the strongest determinants of increased LF powers of SAP and DAP variabilities. Interestingly, they explained beat-to-beat APV over age, gender, heart rate, and blood pressure, which are the main predictors of most measures of autonomic function. The second important finding was that the multivariate models used explained only a small fraction of the variation in the LF component of APV. Thus, the vast majority of the short-term beat-to-beat APV is explained by factors not evaluated in study II. Psychological factors were not associated with the HF component of DAP variability, and the associations between the HF component of SAP variability and anger-in or hostility were only weak. This absence of relation is logical because the HF component of APV reflects principally respiratory mechanics (332).

In two separate population-based samples of middle-aged women and men (III) increased 24-hour ambulatory PP was associated with increased beat-to-beat SAP variability independent of age, gender, diastolic blood pressure, BMI, smoking, and alcohol intake. Arterial stiffening might increase beat-to-beat APV through its effects on pulse wave reflection. On the other hand, arterial stiffening may impair the buffering function of the baroreceptor reflex, which may lead into increased APV. Consistent with the conclusion that arterial stiffening may, in part, explain increased oscillations in SAP, a recent study in newly diagnosed untreated hypertensive patients found that higher ambulatory blood pressure variability was a predictor of increased PWV (333).

An increase in the LF power of SAP variability has been reported in subjects with insulin dependent diabetes (253). Study V could not demonstrate significant relations between the characteristics of insulin resistance and LF components of SAP or DAP variabilities. On the other hand, higher HF components of SAP and DAP variabilities were consistently associated with higher levels of serum insulin, triglycerides, HOMA, BMI, and body fat content, and with a lower level of HDL cholesterol. Another new finding was that a higher level of physical fitness is associated with decreased HF components of beat-to-beat SAP and DAP variabilities. Because the HF component of beat-to-beat APV results principally from rhythmic changes of intrathoracic pressure

owing to respiratory mechanics (332), these findings may merely reflect indirect effects of physical fitness.

6.4 Determinants of baroreflex sensitivity in healthy subjects (II–V)

Across many studies, the main predictors of BRS are age (91, 92, 99–101), gender (91, 92, 105), blood pressure level (91, 92), and heart rate (91, 92). The effects of age and gender were controlled for in studies II, III, and V, and also the effects of heart rate and blood pressure in study II when the multivariate regression analyses were run. Consistent with previous studies, increased age was the most important determinant of reduced BRS by multivariate regression (II, III and V). Gender remained an independent determinant of BRS only for the part of population 1 in study III. The lack of an association in population 2 was not unexpected, because, in contrast to uniformly observed influence of age, gender has not been found to have an effect on BRS in every study. On the other hand, the lack of an independent association in studies II and V of population 1 suggests that gender effects are partly intermixed with the other studied variables, e.g. psychological factors, characteristics of insulin resistance, and physical fitness.

Study II and previous studies (153, 166) suggest that, when using short-term measurements from recumbent subjects, BRS reflects more sensitively than HRV the effects of psychological factors on autonomic cardiovascular function. Depression, increased anxiety, hostility, phobicity, and the general severity index of the BSI-37 were related to a reduction in BRS. Anxiety was the strongest psychological determinant of decreased BRS, independently of age, gender, other psychological factors, heart rate, and blood pressure.

Study III involved two separate, apparently healthy population-based samples of middle-aged women and men. Here, increased ambulatory PP was associated with a decreased BRS independent of age, gender, diastolic blood pressure, BMI, alcohol intake, and smoking. Although enhanced sympathetic drive may result in simultaneous attenuation of BRS and increased PP, it is more likely that a common denominator, e.g. increased arterial stiffness, accounts for these changes. Stiffness of the aorta might reduce the stretch of baroreceptors in response to a rise in arterial pressure. Concurrent with this idea, BRS has been correlated inversely with the measures of arterial stiffness in patients with recent stroke or chronic hemodialysis (308, 309). Carotid artery distensibility has been related to BRS even in healthy young people (334).

A recent population-based study found that BRS correlated negatively with waist circumference, BMI, insulin, HOMA, glycosylated hemoglobin, fasting glucose,

triglycerides, total and LDL cholesterol, and systolic blood pressure (335). Concordant with this and previous studies (100, 235), study V showed that BRS correlates inversely with BMI, insulin, HOMA, triglycerides, systolic, and diastolic blood pressures. There was a negative association with fat mass and with the proportion of body fat. Although a low level of HDL cholesterol is one of the characteristic features of the metabolic syndrome, a recent study did not find a significant association between BRS and HDL cholesterol (335). In contrast to that study, and in accordance with clinical expectations and a previous study (234), study V showed a positive univariate association between BRS and HDL cholesterol.

In study V, the maximal oxygen uptake index showed a positive univariate association with BRS, implicating that good physical fitness has a protecting effect on baroreflex function. This finding is in line with previous studies showing a correlation between BRS and exercise capacity in healthy subjects (91, 118) and an increase in BRS after exercise training (121).

Multivariate regression analysis (V) showed that higher age, higher diastolic blood pressure, and a higher body fat percentage were independent determinants of decreased BRS. An important role of blood pressure among the factors associated with insulin resistance is in accordance with a recent finding that blood pressure had a predominant effect over other components of metabolic syndrome on the arterial intima-media thickness and PWV (336). On the other hand, a recent population-based study that evaluated many factors associated with insulin resistance, showed that the only multivariate predictors of BRS were age and waist circumference (335).

An interesting finding in study IV was that increased LF component of beat-to-beat PP variability was an approximately twice as powerful determinant of decreased BRS as systolic and diastolic blood pressures alone and together, and was able to totally replace them as a determinant of BRS. This finding may indicate that short-term fluctuation of PP, rather than a static blood pressure level, modulates BRS. Alternatively, impaired baroreflex function may lead to increased fluctuation in PP. It is also possible that an increase in beat-to-beat PP variability and a decrease in BRS may parallel a common denominator such as vascular stiffening. The present study did not clarify the mechanisms behind these associations.

6.5 Beat-to-beat pulse pressure variability in healthy subjects (IV)

The novel hypothesis was that arterial stiffening leads to increased beat-to-beat variability of PP. Using custom-built software, specifically intended for computation of beat-to-beat time series and power spectra of PP, the present study showed that the frequency distribution in the power spectral density of beat-to-beat PP bears

remarkable similarity to those in R-R interval, SAP, and DAP variabilities. A finding of a prominent respiratory peak in PP variability is in accordance with similar findings in rats (337). Thus, although PP variability closely correlates with SAP variability, the HF component of PP variability resembles more that of HRV. However, in contrast to the respective component of HRV (V), the HF power of PP variability (IV) did not correlate with BMI, serum insulin, HOMA, or serum triglycerides. The absence of these associations might suggest that non-autonomic mechanisms are principally responsible for respiratory oscillations of PP in recumbent humans.

There was an association between increased beat-to-beat PP variability and elevated PP in a middle-aged general population (IV). The initial hypothesis was also supported by the observed associations between beat-to-beat PP variability and clinical risk factors of arterial stiffening. The increased LF component of beat-to-beat PP variability was associated with advancing age and factors pertinent to insulin resistance including higher BMI, higher levels of serum insulin, HOMA, and triglycerides. The absence of a relation between PP variability and serum cholesterol concentration is in accordance with studies showing a lack of or even a negative association between serum cholesterol and aortic stiffness (338, 339).

6.6 Limitations

Although consistent with the Task Force (8), the short-term recordings of beat-to-beat oscillations in R-R interval and arterial pressure of recumbent subjects cause some obvious limitations. The results regarding the VLF component should be dealt with caution, as durations of time series were inadequate to fully reflect this spectral band. Additionally, no information is available on the cardiovascular reactivity to various challenges present in real life. For example, it is possible that short-term recordings in recumbent subjects lead to an underestimation of the relations between psychological factors and HRV. Although a respiratory rate of 15 per minute (0.25 Hz), used in studies II–V, may increase the HF component, controlled breathing importantly reduces the confounding effects of different breathing patterns on the results (22, 23). Because different methods were used for the evaluation of autonomic nervous function in the two study populations in study III, comparisons between the populations were avoided and attention was focused on the congruent observations from these carefully controlled materials.

There are some concerns regarding the generalization of the results. Firstly, a substantial number of subjects were excluded because of measurement inadequacy. However, this concern was tested in study I which showed that the resultant study groups were representative samples of the initial cohorts of subjects applicable to the study. Secondly, these findings may be limited to subjects in an age group of

35–54 years (I) or 35–64 years (II–V). This concern may be justified, because there is some evidence that the association between psychological factors and autonomic cardiovascular regulation is dependent on age (171). Finally, strict criteria were used to exclude subjects with any confounding medications or illnesses. Therefore the resultant samples, originated from population-based cohorts of subjects, represent exceptionally healthy individuals. This may moderate the results regarding associations between autonomic cardiovascular function and its determinants. For example, the exclusion of subjects using selective serotonin reuptake inhibitors might have affected the variation in depression scores among the study subjects contributing to the negative findings.

Because this study was cross-sectional, the data do not allow temporal analyses or conclusions regarding cause-effect relations. No definite conclusions can be drawn from the mechanisms accounting for the observations. A major limitation is that no direct measurements of arterial compliance or arterial stiffness were used. Further studies are indicated to corroborate the present findings on a linkage between arterial stiffening and autonomic cardiovascular regulation, particularly with respect to BRS and beat-to-beat PP variability.

7 CONCLUSIONS

1. Absolute measures of HRV (power spectral components, RMSSD and SDNN) are reduced in untreated middle-aged hypertensive subjects. Higher heart rate, advanced age, higher blood pressure, female gender, and higher plasma renin activity were independent determinants of decreased HRV when hypertensive patients and healthy controls were combined.

2 Psychological factors, particularly increased hostility and anxiety, are related to reduced BRS and to increased LF oscillations of APV in a healthy middle-aged general population.

3 Increased ambulatory PP is associated with reduced BRS and increased SAP variability in a healthy middle-aged general population.

4 Beat-to-beat variability of PP is composed of similar frequency peaks observed in the spectral analyses of R-R interval, SAP, and DAP variabilities, but has relatively larger HF component than those of SAP and DAP variabilities.

5 An increased beat-to-beat oscillation of PP is related to several risk factors of arterial stiffening, including age, PP, BMI, serum insulin concentration, insulin resistance, and serum triglyceride concentration. It is also related to impaired BRS in a healthy middle-aged general population. These associations suggest that increased beat-to-beat oscillation of PP may be determined by arterial stiffening and impaired baroreflex function.

6 Factors related to the metabolic syndrome, e.g. hypertension, increased body fat percentage, insulin resistance, and high levels of serum triglycerides, are associated with decreased HRV and BRS and with increased HF power of APV in a healthy middle-aged population. A higher exercise capacity has opposite effects on the same parameters.

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