HEART RATE VARIABILITY IN THE GENERAL POPULATION AND ITS DETERMINING FACTORS

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Abstract

Background: As the leading cause of mortality in Switzerland and many other developed countries, cardiovascular disease (CVD) is of high significance in public health: In 2003, CVD was the leading cause of death in Switzerland, accounting for 38% of adult deaths. Heart rate variability (HRV) is a subclinical electrophysiologic marker of cardiac autonomic control and lower HRV is associated with higher CVD morbidity and mortality. Thus altered HRV may signify autonomic dysregulation, and study of factors influencing HRV may inform epidemiologic and clinical evaluation of pathways ultimately leading to cardiac morbidity and mortality. Literature on HRV in the general population and its influencing factors is relatively scarce. Literature on HRV in the general population and its influencing factors is relatively scarce.

Aims: This thesis presents normal values of HRV for the Swiss population and describes its determinants. As an example of an environmental risk factor, we study the influence of passive smoking on HRV and possible mechanisms. A further aim of the thesis is to describe the impact of obesity and a sedentary lifestyle as personal risk factors on HRV and to look at modification of the effect of obesity by physical exercise.

Methods: Between 2001 and 2003, we measured time and frequency domain parameters of HRV in a random sample of the SAPALDIA (Swiss cohort study on Air Pollution and Lung Diseases in Adults) cohort participants aged 50 to 72 using digital 24-hour electrocardiogram recordings. Subjects with recordings of less than 18 hours, atrial fibrillation, recent anaesthesia or myocardial infarction or subjects taking digitalis were excluded, and recordings of insufficient quality discarded, leaving 1742 recordings for analyses. Health status and personal risk factors were assessed in a detailed interview. Blood pressure, height and weight as well as markers of cardiovascular health in the blood were measured. To assess effects of different risk factors on HRV, a structured multivariable linear regression was performed. To estimate percentiles of gender specific HRV parameters as a function of age, subjects with known risk factors (smoking, history of cardiovascular disease, high blood pressure, diabetes, medication) were excluded. In multivariable regression analyses, we determined the effect of environmental tobacco smoke on HRV, heart rate and blood pressure. We estimated the effect of physical activity and BMI on HRV in multivariable regression analyses and investigated the interaction between those two variables.

Results: Taking into account the effects of age, lifestyle factors, variables of cardiovascular health, medication and cardiovascular risk factors in the blood, women had a lower HRV than men. The age dependent decline of HRV differed between the sexes. High blood pressure,

current smoking, higher levels of uric acid, high-sensitive C-reactive protein and non-HDL-cholesterol were independently associated with lower HRV.

Non-smoking subjects exposed to tobacco smoke pollution at home or at work for more than 2 hours/day had lower HRV values than unexposed subjects. We also found a higher heart rate in exposed subjects and a tendency for higher diastolic blood pressure.

Obese subjects exercising regularly 2 hours per week or more had higher HRV than their sedentary peers. Regardless of weight, the improvement of HRV was greatest for those who exercised regularly. Subjects having gained weight over the past 11 years who were exercising at least moderately had increased HRV values compared to subjects not exercising. Exercising regularly significantly modified the relation of obesity with HRV.

Conclusions: HRV shows independent associations with a great variety of known personal and environmental cardiovascular risk factors. These findings help elucidate cardiac autonomic pathways through which factors such as passive smoke exposure and inactivity may adversely influence cardiac morbidity and mortality. As such, the findings strengthen scientific understanding of the etiology of cardiac disease, and may be useful in the development of public health strategies to protect and improve cardiovascular health.

Zusammenfassung

Hintergrund: Kardiovaskuläre Krankheiten (KVK) sind in der Schweiz sowie in vielen anderen Industriestaaten die Todesursache Nummer eins und sind somit äusserst wichtig für Public Health. Bei 38% der im Jahr 2003 in der Schweiz Verstorbenen, waren KVK die Haupttodesursache. Für Herzinfarkt und auch für kardiovaskuläre Krankheiten sind zwar bereits klassische Risikofaktoren beschrieben, doch könnten früher messbare Indikatoren der Herzfunktion für die epidemiologische und gesundheitsbezogene Forschung wertvoll sein und als klinische Hilfsmittel dienen. Die Herzrhythmusvariabilität (HRV) wird durch das autonome Nervensystem reguliert. Frühere Forschungsresultate zeigten, dass tiefere HRV-Werte sind mit erhöhter kardiovaskulärer Morbidität und Mortalität assoziiert sind. Über bevölkerungsbezogene Untersuchungen zur Verteilung von HRV und deren Einflussfaktoren wurden bisher wenig publiziert.

Ziele: Diese Dissertation beschreibt zunächst Normwerte für die HRV in der Schweizer Bevölkerung und analysiert Faktoren, die deren Verteilung beeinflussen. Als Beispiel eines Umweltfaktors untersuchen wir den Einfluss von Passivrauchen auf HRV sowie Mechanismen. Das dritte Ziel der Dissertation ist, den Einfluss von Adipositas und sitzendem Lebensstil als persönliche Risikofaktoren auf HRV zu beschreiben und zu untersuchen, ob körperliche Aktivität die Auswirkung von Adipositas auf HRV verändert.

Methoden: Zwischen 2001-2003 haben wir mit digitalen 24-Stunden-Elektrokardiogramm-aufzeichnungen Zeit- und Frequenzbereichsparameter der HRV in einer Zufallsstichprobe von 50 bis 72 jährigen Teilnehmern der SAPALDIA (Swiss cohort study on Air Pollution and Lung Diseases in Adults / Schweizer Kohortenstudie Luftverschmutzung und Atemwegserkrankungen bei Erwachsenen) Kohorte gemessen. Personen mit einer Aufnahmedauer von weniger als 18 Stunden, Vorhofflimmern, Anästhesie in den vergangenen acht Tagen, Herzinfarkt oder Digitaliseinnahme wurden ausgeschlossen, damit verblieben 1742 Aufzeichnungen. Gesundheitszustand und persönliche Risikofaktoren wurden in einem ausführlichen Interview erfragt. Blutdruck, Körpergrösse und Körpergewicht sowie Blutwerte (Kreatinin, Harnsäure, hochsensitives C-reaktives Protein, Gesamtcholesterin, HDL-Cholesterin und Triglyzeride,) wurden gemessen. Die Auswirkung verschiedener Risikofaktoren auf die HRV wurde mit einer strukturierten multivariablen linearen Regression geschätzt. Für die Berechnung von geschlechtsabhängigen Perzentilenkurven der HRV als Funktion des Alters wurden Personen mit bekannten Risikofaktoren (Hypertonie; Rauchen; > 1 Glas alkoholische Getränke pro Tag; anamnestisch Diabetes; ACE-Hemmer-, Antiarrhythmika- Klasse I oder III, Kalziumantagonisten-, Diuretika- oder Sympathomimetika-Einnahme in den vergangenen 30

Tagen) ausgeschlossen. Die Auswirkung von Passivrauchexposition auf die HRV, Herzfrequenz und den Blutdruck haben wir in multivariablen Regressionsanalysen bestimmt. Zur Berechnungen der Auswirkung von körperlicher Aktivität und BMI auf die HRV haben wir multivariable Regressionsanalysen verwendet, dabei aber auch Interaktionen zwischen diesen Variablen untersucht.

Resultate: Nach Berücksichtigung des Einflusses des Alters, Lebensstilfaktoren, Variablen der kardiovaskulären Gesundheit, Medikamenteneinnahme und kardiovaskulären Risikofaktoren im Blut hatten Frauen eine tiefere HRV als Männer. Die altersabhängige Abnahme der HRV war unterschiedlich bei Männern und Frauen. Hoher Blutdruck, Rauchen, höhere Harnsäurewerte, hoch-sensitives C-reaktives Protein und nicht-HDL-Cholesterin hatten unabhängig voneinander einen Zusammenhang mit tieferer HRV.

Nichtraucher, welche zu Hause oder am Arbeitsplatz für über zwei Stunden pro Tag Zigarettenrauch exponiert waren, hatten tiefere HRV-Werte als nicht exponierte Personen. Erstere hatten auch eine höhere Herzfrequenz und die Tendenz zu einem höheren diastolischen Blutdruck.

Adipöse, welche sich regelmässig mindestens während zwei Stunden pro Woche körperlich betätigen, hatten eine höhere HRV als gleichaltrige ohne körperliche Aktivität. Unabhängig vom Gewicht hatten körperlich Aktive die höchste HRV. Auch körperlich aktive Personen mit Gewichtszunahme in den vergangenen elf Jahren hatten verglichen mit nicht Aktiven eine höhere HRV. Der Einfluss von Adipositas auf die HRV wurde durch regelmässige körperliche Aktivität signifikant verändert.

Schlussfolgerungen: HRV hat einen unabhängigen Zusammenhang mit einer Vielzahl bekannter persönlicher und Umweltrisikofaktoren. Unsere Resultate bestätigen, dass Passivrauchexposition das kardiale Risiko über autonome Dysfunktion erhöht. Negative Effekte von Adipositas auf die HRV können durch regelmässige körperliche Aktivität, welche einen starken positiven Einfluss auf die Funktion des autonomen Nervensystems hat, vollständig aufgehoben werden. Diese Erkenntnisse können der Forschung bei der Interpretation von 24-Stunden-HRV-Werten helfen und sollten Public Health Anstrengungen bei der Entwicklung oder Weiterverfolgung von Strategien unterstützen, um die kardiovaskuläre Gesundheit zu schützen oder zu verbessern.

Abbreviations

ANS	Autonomic nervous system
BMI	Body mass index
CO	Carbon monoxide
CVD	Cardiovascular disease
ECG	Electrocardiogram
ETS	Environmental tobacco smoke
HDL	High density lipoprotein
HF	High frequency power
HRV	Heart rate variability
hs-CRP	High sensitivity C-reactive protein
LF	Low frequency power
SAPALDIA	Swiss Cohort Study on Air Pollution and Lung Diseases in Adults
SDNN	Standard deviation of all normal RR intervals
SOP	Standard operating procedure

1. Introduction

1.1 Background

As the leading cause of mortality in Switzerland (Bundesamt für Statistik 2006) and many other developed (Mathers and Loncar 2006; Murray and Lopez 1997) and increasingly developing countries (Raymond et al. 2006), cardiovascular disease (CVD) is of high significance in public health. In 2003, 41% of women and 35% of men died because of CVD in Switzerland (Bundesamt für Statistik 2006). About 30,000 potential years of life (between the first and 70th years of life) are lost each year in men and women in Switzerland due to CVD (Bundesamt für Statistik 2006). Subjects with CVD have to cope with a lower quality of life and often have to live with disablement, an inability to work, and the loss of autonomy and mobility. CVD is not only a heavy burden for those who suffer from it, but also for the national economy. According to Swiss statistics of medical diagnosis, CVD is the most important reason for 13% of all visits to doctor's offices (2001) (Junker 2004) and for 10% of hospital stays (2003) (Bundesamt für Statistik 2006). Medications for the treatment of CVD were the most commonly sold remedies, taking a market share in Switzerland of 16% (Cueni 2004). While classical risk factors for CVD have been described in relation to higher myocardial infarction or CVD mortality (D'Agostino et al. 2000; Wilson et al. 1998), earlier markers of the functioning of the heart and its autonomic control might help to understand mechanisms of disease development. This would help to develop earlier and better targeted preventive interventions, inasmuch as the evaluation of an individual's risk of experiencing a future cardiovascular event. Such evaluations increasingly forms the basis of clinical guidelines for prevention of cardiovascular diseases worldwide (Smith et al. 2004). They thus might be of importance in epidemiologic and health related research on one hand, but they also might be of significance in assessment of the functioning of the heart and hence be a useful tool in clinical practice.

The Swiss Cohort Study on Air Pollution and Lung Diseases in Adults (SAPALDIA) (Ackermann-Liebrich et al. 1997; Leuenberger et al. 2000; Zemp et al. 1999) has measured markers of cardiovascular risk in its follow-up study. The study, which was originally designed to address the impact of air pollution on respiratory health in Switzerland, examined 9651 participants in a cross-sectional study in 1991. The participants, then 18 to 60 years old, were randomly selected from eight areas of Switzerland representing a broad range of environmental and climatic conditions (Aarau, Basel, Davos, Geneva, Lugano, Montana, Payerne and Wald) (Martin et al. 1997). Between 2001 and 2003, the second round of health examinations of the cohort was accomplished. 8047 participants (83% of the original cohort) were reexamined (Ackermann-Liebrich et al. 2005).

An extensive health examination was conducted in 1991, including a standardised interview, spirometry, methacholine challenge and measurement of endexpiratory carbon monoxide (CO) (Martin et al. 1997). These examinations were repeated and in response to new scientific evidence, demonstrating a direct effect of elevated air pollutant concentration on heart rate (Peters et al. 1999; Pope et al. 1999), heart rate variability (HRV) (Gold et al. 2000; Pope et al. 1999), incidence of cardiac arrhythmia (Peters et al. 2000) and on CVD mortality (Dockery et al. 1993; Pope 2000; Schwartz 1999), additional assessments were done in the second survey, including measurement of cardiovascular risk factors in the blood and of HRV (Ackermann-Liebrich et al. 2005).

While HRV has been used as indicator of cardiovascular health and functioning of the heart, literature on HRV in the general population and its influencing factors is relatively scarce. This thesis uses a population-based sample of 1742 electrocardiograms (ECG) to describe normal values for the Swiss population, personal and environmental risk factors for lowering HRV, in addition to showing the effect of physical activity in normal and obese persons.

1.2 Heart rate variability

Heart rate variability is the temporal variation between sequences of consecutive heartbeats (Figures 1 and 2). On a standard ECG, the maximum upwards deflection of a normal QRS complex is at the peak of the R wave, and the duration between two adjacent R wave peaks is termed the R-R interval. The ECG signal requires editing before HRV analysis can be performed, a process requiring the removal of all non-sinus-node-originating beats. The resulting period between adjacent QRS complexes resulting from sinus node depolarizations is termed the N-N (normal-normal) interval. HRV is the measurement of the variability of the N-N intervals (Reed et al. 2005).

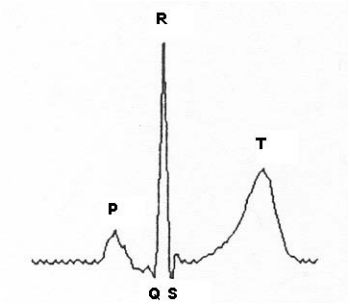

Figure 1. Normal ECG trace, with waves labeled

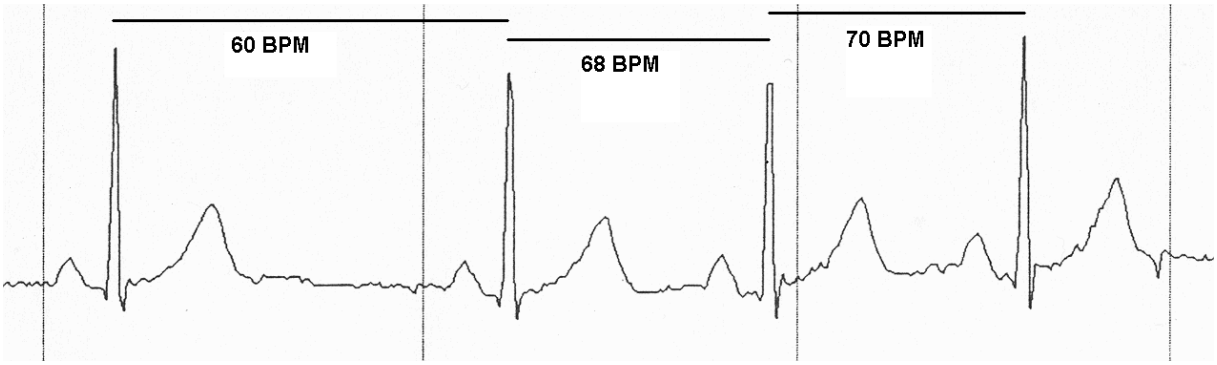

Figure 2. Heart rate variability is a measure of the beat-to-beat changes in heart rate

The cardiovascular concept of homeostasis refers to the tendency of the organism to maintain a relatively regular heart rate and blood pressure in the face of changing environmental conditions. No physiological variable, however, will give a time sequence that is absolutely stationary or periodic. Spontaneous fluctuations can be observed in cardiovascular functions, such as heart rate and blood pressure, even when the environmental parameters are maintained at as constant a level as possible and no perturbing influences can be identified (Makikallio 2000).

Although automaticity is intrinsic to different cardiac tissues with pacemaker properties, the electrical and contractile activity of the myocardium is largely modulated by the autonomic nervous system (ANS). This neural regulation is effected through the interplay of the sympathetic and vagal outflows. In most physiological conditions the efferent sympathetic and parasympathetic branches have opposing actions: the sympathetic system enhances automaticity, whereas the parasympathetic system inhibits it. While the effect of vagal stimulation on the cardiac pacemaker cells is to cause hyperpolarisation and to reduce the rate of depolarization, sympathetic stimulation causes chronotropic effects by increasing the rate of pacemaker depolarization. Both branches of the ANS influence ion channel activity implicated in the regulation of depolarization of the cardiac pacemaker cells (Sztajzel 2004). Vagal mediators exert their influence relatively quickly on the heart, and principally affect the high frequency power (HF) of the HRV spectrum (between 0.15 Hz and 0.4 Hz). Sympathetic mediators appear to exert their influence over longer time periods and are reflected in the low frequency power (LF) (between 0.04 Hz and 0.15 Hz) and the HF power of the HRV spectrum. Thus, the LF/HF ratio is a proxy for the sympatho-vagal balance (North American Society of Pacing and Electrophysiology 1996).

Physiological and pathological processes may influence N-N interval variability. Even at rest, heart rate fluctuates cyclically. High frequency (HF) cyclic fluctuations are modulated by ventilation (respiratory sinus arrhythmia), mediated entirely by changes in vagal outflow. Slower fluctuations occur due to baroreflexes or due to thermoregulation. The greatest variation of heart rate occurs with circadian changes, particularly the difference between

night and day heart rate (Figure 3), mediated by complex and poorly understood neurohormonal rhythms. Exercise and emotion also have profound effects on heart rate (Kleiger et al. 2005) and thus its variability (see Table 1 for physiological correlation of different HRV components).

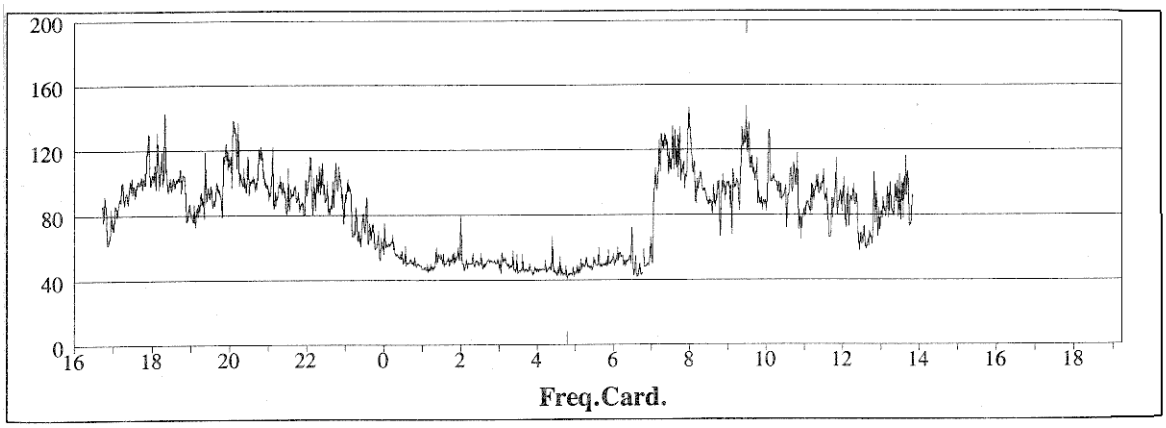

Figure 3. Changing heart rate over 21 hours

Respiratory sinus arrhythmia was described by Hales in 1733 (Hales 1733). Although the temporal fluctuations in cardiovascular signals were noted in ancient times (Bylebyl 1971), physicians have overlooked for a long time the possible significance of beat-to-beat fluctuation of cardiovascular signals. This variability has generally been treated as noise to be either ignored or averaged out. The field in which the potential clinical significance of beat-to-beat variability in cardiovascular signals was first recognised was obstetrics. In 1965, the importance of sinus arrhythmia was described in relation to fetal monitoring. This variability correlated with fetal viability; diminution of beat-to-beat variability indicated fetal compromise (Hon and Lee 1965). In the 1970s, Ewing and colleagues used short-term HRV measurements as a marker of diabetic autonomic neuropathy (Ewing et al. 1985). In 1977, Wolf and colleagues showed that patients with reduced HRV after a myocardial infarction had an increased mortality. This was confirmed by studies showing that HRV is an accurate predictor of mortality after myocardial infarction (Bigger et al. 1993; Kleiger et al. 1987; Malik et al. 1989). The relative risk of mortality was over five times higher in the group with the lowest HRV as compared with the group with the highest HRV (Kleiger et al. 1987).

In 1981, Akselrod and colleagues introduced power spectral analysis of heart rate fluctuations to quantitatively evaluate beat-to-beat cardiovascular control (Akselrod et al. 1981).

Table 1. HRV components and their physiological correlations

HRV component	spectrum	physiological correlation
SDNN[a]	----------	circadian rhythms
Total Power (TP)	≤ 0.40 Hz	----------
High Frequency (HF)	0.15 – 0.40 Hz	vagal activity
Low Frequency (LF)	0.04 – 0.15 Hz	sympathetic and vagal activity, baroreflex sensitivity
LF/HF ratio	----------	Proposed as balance between sympathetic and parasympathetic activities
Very Low Frequency (VLF)	0.0033 – 0.04 Hz	Sympathetic activity + parasympathetic activity + thermoregulation + renin-angiotensin system
Ultra Low Frequency (ULF)	≤ 0,0033 Hz	circadian and neuroendocrine rhythms

[a] Standard deviation of all normal-to-normal RR-intervals

(North American Society of Pacing and Electrophysiology 1996; Sztajzel 2004)

In recent years, alterations in HRV have been found in patients with many conditions: Diabetes and high blood pressure have been described as being associated with impaired autonomic function (Gerritsen et al. 2001; Kaftan and Kaftan 2000; Singh et al. 1998; Tsuji et al. 1996); history of myocardial infarction or congestive heart failure is also associated with lower values of HRV (Casolo et al. 1989; Tsuji et al. 1996); obstructive sleep apnea syndrome (Roche et al. 1999), chronic renal failure (Kurata et al. 2000), depression in patients with stable coronary heart disease (Stein et al. 2000) and even tinnitus (Datzov et al. 1999) showed an association with HRV in various studies. HRV has also been extensively investigated as a tool to predict death and nonfatal cardiovascular events both in survivors of myocardial infarction (Bigger et al. 1993; Kleiger et al. 1987; Tapanainen et al. 2002) and in the asymptomatic population (Tsuji et al. 1994). Pozzati and colleagues showed that HRV decreased significantly in the five minutes before dying in patients with sudden ischemic death during Holter monitoring (Pozzati et al. 1995).

From a functional perspective, the ANS not only includes efferent, but also afferent pathways transmitting information regarding the heart. HRV is therefore also dependent on the state of the heart. It has been shown that HRV is associated with ventricular ejection fraction and it also appears to be related to the size of acute myocardial infarction. It is inversely related to peak myocardial-type creatine kinase (CK-MB) (Odemuyiwa 1995).

Despite all the research done in this field, little is known about 24-hour HRV in a normal population. HRV offers prognostic information independent of and beyond that provided by traditional risk factors. The Task Force of the European Society of Cardiology and the North American Society for Pacing and Electrophysiology stated in 1996 that large population-based studies are needed to establish normal HRV standards for various age and sex subsets (North American Society of Pacing and Electrophysiology 1996). To our knowledge, studies measuring 24-hour HRV in a normal population published since then included up to 300 subjects (Ramaekers et al. 1998; Sosnowski et al. 2002; Umetani et al. 1998), but most studies were restricted to short-term measurements. To date, no such reference values for Switzerland have been published.

HRV is known to be influenced by a great variety of factors, like age, sex, or medication intake. The association between single or combined factors and HRV has been explored in numerous patient studies. To our knowledge, no results about the combined effect of such a wide range of risk factors on HRV in a large population sample have been published yet.

Understanding of the risk factors for cardiovascular disease may yield important insights into the prevention, etiology, course, and treatment of this major public health concern. Exposure to environmental factors such as tobacco smoke pollution is known to be a risk factor for cardiovascular disease. To date, the association between longer-term exposure to environmental tobacco smoke (ETS) and HRV, heart rate, or blood pressure, the alterations of which could potentially be steps in the pathophysiological pathway leading from ETS exposure to cardiopulmonary disease, has not been analysed.

Sedentary lifestyle and obesity are also both well-established risk factors for cardiovascular disease. Different studies have shown an association of either of them with lower HRV. However, it is not known whether the unfavourable effects of obesity would be modified by regular physical activity.

1.3 Aims

The overall aim of this thesis was to study heart rate variability, which is a predictor of death and nonfatal cardiovascular events, and its determining factors.

In particular, the following research questions were addressed:

1. *What is the normal heart rate variability in a general elderly Swiss population and what are its determinants (personal risk factors?)*

   Specific aims:

   I. To determine the influence of cardiovascular risk factors, biomarkers for cardiovascular health and current health status on heart rate variability.

   II. To provide normal ageing curves for healthy men and women between 50 and 70 years of age living in Switzerland for various HRV measurements.

2. *What is the influence of environmental risk factors on heart rate variability, using exposure to environmental tobacco smoke as an example?*

   Specific aims:

   I. To test the hypothesis that longer-term exposure to environmental tobacco smoke reduces heart rate variability in the general population.

   II. To discuss possible pathways towards reduced HRV

3. *Is there a modification of the negative effect of obesity on heart rate variability by physical activity?*

   Specific aims:

   I. To test the hypothesis that regular exercise improves heart rate variability.

   II. To determine whether adverse effects of obesity and weight gain on heart rate variability would be modified by regular exercise.

In the three papers presented in this thesis, we specifically analysed HRV and its determinants in a healthy population and present normal values for the Swiss population. As an example of an environmental risk factor, we studied the influence of passive smoking on HRV. The impact of obesity and sedentary lifestyle as personal risk factors on HRV and the possibility of enhancing HRV by regular exercise are elucidated in the third paper.

1.4 Methods

SAPALDIA had its baseline assessment in 1991. In 2001-2003, we specifically looked at cardiovascular health. The participants of this study were a random selection of the 4417 SAPALDIA 2 subjects aged ≥ 50. A total of 1846 subjects (955 women, 891 men) took part in the HRV measurements. They underwent 24-hour ECG recordings and filled in a time activity diary. Blood pressure, height and weight as well as cardiovascular risk factors in the blood were measured. Information about cardiovascular risk factors was obtained in an extensive interview. The subsequent chapter describes details of HRV and blood pressure measurements. More information about inclusion criteria, HRV and other measurements and analyses are given in chapters two through four.

24-hour electrocardiogram

24-hour ECG recordings were conducted with digital Holter recorders (Aria, Del Mar Medical Systems, Irvine, CA, USA), using three leads. The placement of the electrodes for V_3 and V_5 was altered, thus resulting in higher R-waves which are desirable for reading HRV. The electrodes for V_1 were customarily placed. After site visits in laboratories in Rochester (USA), Erfurt (Germany) and Saint-Etienne (France), where epidemiological studies of HRV were performed, a standard operating procedure (SOP) was developed (see appendix 1). It was tested in a pilot study with 30 volunteers, where the practicability of the SOP for the field workers, of the instructions for the persons being tested and of their compliance were analysed. The reliability of the devices, the quality of the recordings and the functioning of data transfer were also tested. Part of the pilot study was a double ECG recording in 10 participants, where in two simultaneous ECG recordings the electrodes where placed in slightly different places (results shown in appendix 3). In the pilot study, a time activity diary was also tested, which was adapted for the use of SAPALDIA from the time activity diary of the EXPOLIS study (see appendix 5) (Rotko et al. 2000). A smoothly running logistic was developed during this test period, which included the returning of the devices by the participants by mail, downloading the data from the ECG recorders to a local hard disc and copying the data from there to a compact disc, sending the disc to the coordinating centre and from there to the laboratory in France where the ECGs were analysed. In case of a dangerous condition spotted on the ECG, the coordinating centre and thereby the responsible study physician was notified immediately. The concerning participant was then contacted via telephone by the study physician, who offered to inform the subject's family physician. The results of normal ECGs were sent back from the laboratory to the coordinating centre and were distributed from there to the local centres. Participants then received their report from the local centres in writing if requested. This procedure allowed the

recording of 1846 ECGs using 26 devices. Participants without pathologies received a feedback within approximately four to six weeks.

There were 32 fieldworkers working in the eight SAPALDIA study centres. They were all intensively trained and supervised in order to guarantee a standardized examination of the subjects.

Reading and analyses of electrocardiograms

The ECGs recorded using the Holter recorders were analysed at the laboratory of Dr. Barthélemy at the Université de St. Etienne (Roche et al. 2002; Roche et al. 1999) with programs especially developed for this study (details in appendix 2).

Variability was assessed using time and frequency domain analysis that quantify periodicities in the data captured by a monitor. Standard variables relating to the Holter recording, and two sets of variables for heart rate variability (Fast Fourier transformation and wavelet transformation) were calculated (Pichot et al. 1999). Variables in the analysis include 18 items for heart rate variability for the periods of day and night and the entire recording period for each subject. Recording time, duration of artefacts, frequencies (minimum, mean, and maximum), arrhythmias (number, duration, minimal frequency of bradycardia, number, duration and time of pauses, number, frequency and type of ventricular premature beats including accelerated idioventricular rhythms and ventricular tachycardia, number, frequency and type of supraventricular tachycardia including paroxysmal supraventricular tachycardia) and changes in ST (type, number, lead) were also been measured. Tables 2 and 3 summarize the available time domaine and frequency domaine variables.

Reliability of HRV analyses was tested by analyzing 30 ECG recordings twice with the Holter technician being blindfolded (results shown in appendix 4).

Table 2. HRV time domain parameters

Time domain parameters	entire recording	day	night
Mean RR [ms]	x	x	x
NN50	x	x	x
pNN50 [%]	x	x	x
SDNN [ms]	x	x	x
RMSSD [ms]	x	x	x
SDANN [ms]	x	x	x
SDNN index [ms]	x	x	x
HRV triangular index	x	x	x
TINN [ms]	x	x	x

Table 3. HRV frequency domain parameters

frequency domain parameters	frequency	entire recording		day		night	
		FT	WT	FT	WT	FT	WT
Total Power [ms^2]	$\leq$ 0,40 Hz	x	x	x	x	x	x
ULF [ms^2]	$\leq$ 0,0033 Hz	x	x				
VLF [ms^2]	0,0033 – 0,04 Hz	x	x	x	x	x	x
LF [ms^2]	0,04 – 0,15 Hz	x	x	x	x	x	x
HF [ms^2]	0,15 – 0,40 Hz	x	x	x	x	x	x
Alpha		x					
LF (normalized units)				x	x	x	x
HF (normalized units)				x	x	x	x
LF/HF				x	x	x	x

FT: Fourier transform, WT: Wavelet transform

Measurement of blood pressure

Blood pressure was measured twice with participants at rest and sitting by an automatic device (705CP, OMRON, Tokyo, Japan), which has been tested in two studies and was recommended using the criteria of the British Hypertension Society (BHS) and the US

Association for the Advancement of Medical Instrumentation (Artigao et al. 2000; O'Brien et al. 1996). The devices were tested before their use and shortly before the end of the study according to the statements of conformity EN 1060-1, 1060-2 and 1060-3 (harmonized according to the European Union Medical Device Directive 93/42/EEC) (European Union 1999; European Union 2002). Measurement of blood pressure followed the recommendations of the WHO (see annex 1) (World Health Organization 1994).

The original contribution of the author concerning the methods consisted in the development of the standard operating procedures for the Holter recording and blood pressure measurement for the purposes of SAPALDIA 2 and the training and supervision of the fieldworkers in regular quality control sessions. Statistical analyses presented in this thesis were conducted by the author.

2. Paper 1: Reference values and determining factors of heart rate variability

2.1 Heart rate variability in an ageing population and its association with lifestyle and cardiovascular risk factors: results of the SAPALDIA study

Additional percentile curves are shown in appendix 6.

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Heart rate variability in an ageing population and its association with lifestyle and cardiovascular risk factors: results of the SAPALDIA study

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Aims (i) To report associations between cardiovascular risk factors and heart rate variability (HRV) in a general population and (ii) to provide normal values for various HRV measurements in a healthy European general population sample aged $\geq$50.

Methods and results Twenty-four-hour electrocardiograms were recorded in 1742 randomly selected SAPALDIA (Swiss cohort study on Air Pollution and Lung Diseases in Adults) participants aged $\geq$50. In multivariate regression analyses, women ($n = 895$) had a 6.1% lower standard deviation of all normal RR (NN) intervals (SDNN), a 11.4% lower total power (TP), and a 27.2% lower low-frequency (LF) power than men ($n = 847$). Per unit increase in BMI, SDNN decreased by 0.7% and TP decreased by 1.2%. Persons with high blood pressure had a 9.2% lower LF than normotensive persons and current smokers a 15.5% lower LF than never smokers. Each hour of heavy physical exercise was associated with a 2.0% increase in SDNN, a 3.6% increase in the high frequency (HF) range power and a 4.2% increase in LF power. Higher levels of uric acid, high-sensitive C-reactive protein and non-HDL-cholesterol were associated with lower TP, HF and LF. Percentiles of TP and LF/HF as a function of age were calculated for an asymptomatic subsample of participants ($n = 499$) free of cardioactive medications.

Conclusion Heart rate variability in a general population sample shows expected associations with all known cardiovascular risk factors, although not identically for all HRV domains. Together with our percentile estimates for HRV as a function of age, these findings could assist scientists in interpreting 24 h HRV values and factors influencing them in an ageing population.

Introduction

Heart rate variability (HRV), a measure of cardiac autonomic control (see *Table 1* for physiological correlations of different HRV components), has been established as a strong predictor of death and non-fatal cardiovascular events both in survivors of a myocardial infarction[1–3] and in an asymptomatic population. The Framingham Study has found that reduced HRV in short-term recordings (2 h) predicted new cardiac events, hypertension and hyperglycaemia, in a middle-aged general population,[4–6] and the Whitehall Study showed associations between social position, behavioural factors, components of the metabolic syndrome, and HRV.[7] However, little is known about 24 h HRV in a normal population.[8] The Task Force of the European Society of Cardiology and the North American Society for Pacing and Electrophysiology have identified the need for large prospective population studies to establish normal HRV standards for various age and sex categories.[9]

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Table 1 HRV components and their physiological correlations

HRV component	Spectrum	Physiological correlation
SDNN	–	Circadian rhythms
TP	≤0.40 Hz	–
HF	0.15–0.40 Hz	Vagal activity
LF	0.04–0.15 Hz	Sympathetic and vagal activity, baroreflex sensitivity
LF/HF ratio	–	Proposed as balance between sympathetic and parasympathetic activities
VLF	0.0033–0.04 Hz	Sympathetic activity + parasympathetic activity + thermoregulation + renin–angiotensin system
ULF	<0.0033 Hz	Circadian and neuroendocrine rhythms

From the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology[9] and Sztajzel.[49]

The SAPALDIA (Swiss Cohort Study on Air Pollution and Lung Diseases in Adults) cohort was designed to measure the health effects from long-term exposure to air pollutants in the Swiss adult population.[10,11] In 1991, a random sample of the Swiss population was recruited from eight areas featuring distinct geographical and environmental conditions. In a follow-up examination in 2002, 24 h measurements of HRV were included in those aged ≥50.

In this paper we (i) report the cross-sectional associations of cardiovascular risk factors and cardiovascular disease with HRV in the full sample and (ii) provide normative values for total power and the low to high frequency ratio in a healthy subsample of a large European population aged ≥50. Normal curves for other HRV parameters are available from the authors on request.

Methods

Participants

The design and objectives of the SAPALDIA cohort study have been reported in detail elsewhere.[12] In brief, 9651 participants received intensive health examinations and a detailed health interview in 1991 and have been followed since then. In 2002, we were able to re-examine 8047 of the original participants. A random selection of the 4417 participants aged ≥50 were invited to participate in the HRV assessment and a total of 1846 subjects (955 women, 891 men) participated.

Exclusion criteria were general or spinal anaesthesia in 8 days prior to the ambulatory ECG recording ($n = 5$), having had a myocardial infarction in 3 months prior to the exam ($n = 2$), and taking digitalis ($n = 6$); nobody had an artificial pacemaker. After exclusion of recordings showing atrial fibrillation ($n = 12$), recordings of <18 h (recommendations of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology[9]) ($n = 73$), and 6 recordings of insufficient quality, 1742 subjects were included in this analysis assessing the cross-sectional associations of cardiovascular risk factors or cardiovascular diseases with different parameters of HRV.

HRV measurements and analyses

For the Holter recording, digital devices (Aria, Del Mar Medical Systems, Irvine, CA, USA) with a frequency response of 0.05–40 Hz and a resolution of 128 samples/s were used. Three leads (V_1, altered V_3 with the electrode on the left midclavicular line on the lowest rib, and altered V_5 with the electrode on the left anterior axillary line on the lowest rib) were recorded over 24 h. The mean duration of the Holter recordings was 22.3 (SD 2.1) h.

All recordings were scanned through a StrataScan 563 (Del Mar) and interpreted using the interactive method, with a final visual check on the full disclosure. The length of each RR interval was manually validated during this step. Resampling was made at 4 Hz. Spectral analysis was performed by the fast Fourier transform method using sliding 256 polynomial time approximation scheme (PTAs) windows for day and night periods. For 24 h periods, calculations of RR intervals were made without a sliding window, to allow measurement of ultra-low frequency (ULF) and very low frequency (VLF). Only normal-to-normal intervals were used, with intervals excluded due to ectopy or artefacts being replaced by holding the previous coupling interval level throughout the time interval to the next valid coupling interval. The standard deviation of all normal RR (NN) intervals (SDNN) and the following frequency domain variables have been calculated: Total power (TP) (≤0.40 Hz), ULF power (≤0.0033 Hz), VLF power (0.0033–0.04 Hz), LF power (0.04–0.15 Hz), high frequency (HF) power (0.15–0.40 Hz), and the ratio between LF and HF (LF/HF).

In order to avoid a biased result due to methacholine challenge, which was part of the SAPALDIA lung function testing and which, for practical reasons, was performed before the Holter recording, we excluded the first 2 h of all recordings.

The Holter recordings were made between August 2001 and March 2003. The recorders were placed on participants after a detailed health interview to those giving consent. Participants were asked to follow their regular daily routine and to complete a time-activity diary during the recording period.

Interview

During the interview, information about smoking habits (Questions: 'Have you ever smoked for as long as a year?' 'Do you now smoke, as of 1 month ago?' 'Have you stopped or cut down smoking?'), recent myocardial infarction ('Did you have a myocardial infarction in the past 3 months?'), comorbidities ('Do you have any of the following conditions: ..., hypertension,..., diabetes,...?'), drinking habits ('How often do you normally drink alcoholic beverages like beer, wine, liqueurs, aperitifs, and strong drinks?' 'How often do you normally drink a glass of red wine?'), and amount of physical exercise ('We are talking about physical exercise where you get out of breath at least a little bit, like fast walking, hiking, dancing, gardening, or a variety of sports. On how many days of the week do you do such exercises? On average, how many minutes per day do you do such exercises?' 'How many hours a week do you usually exercise such that you get out of breath or sweat?') had been obtained. Participants were asked to bring all their drugs to the study centre in order to complete a medication list.

Other measurements

Serum creatinine, uric acid, total cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides, and high sensitivity (hs)

C-reactive protein (CRP) were measured in the blood as known cardiovascular risk factors. High density lipoprotein values could only be obtained if the participants had a triglyceride level of $\leq$9.4 mmol/L. Normal values for uric acid were 210–420 μmol/L for men and 150–350 μmol/L for women, for hs-CRP protein RP (should say: hs-CRP) $<$5.0 mg/L, for total cholesterol $<$5.0 mmol/L, and for HDL $>$1.0 mmol/L in both men and women[8]. Low density lipoprotein levels were calculated using the formula of Friedewald for subjects who had fasted for at least 6 h and had triglyceride levels of $\leq$4.7 mmol/L. As a further atherogenic marker, the difference between total cholesterol and HDL (non-HDL-cholesterol) was calculated, as well as the quotient between total cholesterol and HDL.

Blood pressure was measured twice with participants at rest and sitting by an automatic device (705CP, OMRON, Tokyo, Japan) positioned on the left upper arm according to WHO recommendations.[13] Blood pressure values used in the regression models were the arithmetic mean of the two measurements.

High blood pressure was defined as either having a systolic blood pressure $\geq$140 mmHg and/or a diastolic blood pressure $\geq$90 mmHg and/or having answered yes to the question 'Do you have hypertension?'

Body height and weight were measured with the participant not wearing any shoes or coat. End-expiratory carbon monoxide (CO) was measured using an EC50 Micro-Smokerlyzer (BEDFONT, Rochester, UK).

This protocol was approved by the Ethics Committee of the Swiss Academy of Medical Sciences and the responsible Cantonal Ethics Committees.

Statistical analysis

Statistical analysis was performed using the software packages STATA 8.0 (Stata corporation, College Station, TX, USA) and SAS Vers. 8.2 (The SAS System, Cary, NC, USA).

To assess sex differences, in *Tables 2–4*, χ^2 tests (for proportions) and *t*-tests (for means) were used. For comparison of the mean values across different age groups, one-way ANOVA was performed.

Because the distribution of the HRV values was skewed, values were log-transformed before analysis.

Table 2 Baseline characteristics of the total study population

	Men	Women	All
Sex	847 (48.6%)	895 (52.4%)	1742
Age (years)	60.3 (SD 6.1)	60.4 (SD 6.4)	60.4 (SD 6.3)
Lifestyle factors			
Never smokers	277*** (32.7%)	522 (58.5%)	799 (45.9%)
Former smokers	376*** (44.4%)	227 (25.4%)	603 (34.7%)
Current smokers	193** (22.8%)	144 (16.1%)	337 (19.4%)
CO (ppm)	5.6** (SD 9.6)	4.3 (SD 7.6)	4.9 (SD 8.7)
Alcohol[a,b]			
$<$1 glass/day	499*** (59.3%)	728 (81.7%)	1227 (70.8%)
$\geq$1 glass/day	343*** (40.7%)	163 (18.3%)	506 (29.2%)
Red wine[a]			
$<$1 glass/week	318*** (37.8%)	589 (66.1%)	907 (52.3%)
$\geq$1 glass/week	524*** (62.2%)	302 (33.9%)	826 (47.7%)
Light physical activity[a]			
$\leq-$2 days/week	443 (52.7%)	438 (49.2%)	881 (50.9%)
$>$2 days/week	397 (47.3%)	452 (50.8%)	849 (49.1%)
Heavy physical acitivity[a]			
$\leq$1 h/week	575*** (68.8%)	713 (80.5%)	1288 (74.8%)
$>$1 h/week	261*** (31.2%)	173 (19.5%)	434 (25.2%)
Cardiovascular health			
SBP (mmHg)	137.2*** (SD 19.1)	127.2 (SD 18.6)	132.1 (SD 19.5)
DBP (mmHg)	84.3*** (SD 10.5)	79.0 (SD 10.2)	81.6 (SD 10.7)
Self-reported diabetes	44 (5.2%)	30 (3.4%)	74 (4.3%)
BMI[c] (kg/m^2)	27.1*** (SD 3.5)	26.3 (SD 4.9)	26.7 (SD 4.3)
Medication			
ACE-inhibitor	53 (6.3%)	57 (6.4%)	110 (6.3%)
Antiarrhythmic drugs class I and III	5 (0.6%)	2 (0.2%)	7 (0.4%)
Beta-blockers	99 (11.7%)	93 (10.4%)	192 (11.0%)
Calcium channel blockers	43 (5.1%)	38 (4.2%)	81 4.6%
Diuretics	20** (2.4%)	42 (4.7%)	62 3.6%
Sympathomimetics	31 (3.7%)	26 (2.9%)	57 3.3%
Laboratory parameters			
Uric acid (μmol/L)	367.5*** (SD 78.8)	284.6 (SD 70.1)	325.1 (SD 85.2)
hs-C-reactive protein (mg/L)	2.6 (SD 6.3)	2.5 (SD 3.7)	2.6 (SD 5.2)
Non-HDL-cholesterol[d] (mmol/L)	4.8* (SD 1.1)	4.7 (SD 1.1)	4.8 (SD 1.1)

SBP, systolic blood pressure; DBP, diastolic blood pressure.
[a]More categories have been used in the regression analysis.
[b]Alcoholic beverages including red wine.
[c]BMI = body weight/body height^2.
[d]Total cholesterol − HDL-cholesterol.
*$P < 0.05$ (differences between sexes).
**$P < 0.01$ (differences between sexes).
***$P < 0.001$ (differences between sexes).

Table 3 Unadjusted mean[a] HRV values for men and women of different age groups of the total study population

	Age (years)				
	Sex	50–54 ($n_m = 181$; $n_f = 219$)	55–59 ($n_m = 238$; $n_f = 217$)	60–64 ($n_m = 186$; $n_f = 189$)	65–73 ($n_m = 242$; $n_f = 270$)
SDNN (ms)	Men	138.2	129.2	130.8	131.9*
	(95% CI)	(132.5–144.1)	(124.8–133.8)	(126.0–135.7)	(127.0–137.1)
	Women	135.8	134.7	130.6	123.6
	(95% CI)	(131.0–140.7)	(130.4–139.2)	(126.2–135.3)	(119.5–127.9)
TP (ms^2)	Men	3925.2	3616.1	3597.3	3585.8*
	(95% CI)	(3568.6–4317.4)	(3340.5–3914.5)	(3295.8–3926.5)	(3288.4–3910.1)
	Women	4134.0	3876.5	3514.6	3200.5
	(95% CI)	(3818.3–4475.8)	(3600.2–4174.1)	(3223.4–3832.1)	(2973.4–3445.0)
HF (ms^2)	Men	63.6***	59.7***	61.0	68.0
	(95% CI)	(55.4–72.9)	(53.0–67.2)	(53.4–69.7)	(59.4–77.8)
	Women	87.8	81.0	66.1	65.3
	(95% CI)	(79.1–97.4)	(72.6–90.4)	(57.6–75.7)	(58.6–72.8)
LF (ms^2)	Men	318.1**	260.7	228.4**	196.0***
	(95% CI)	(284.4–355.8)	(237.9–285.7)	(205.9–253.4)	(175.7–218.5)
	Women	258.2	234.9	179.0	147.7
	(95% CI)	(238.0–280.0)	(214.5–257.3)	(161.0–199.0)	(135.1–161.4)
LF/HF	Men	5.0***	4.4***	3.7***	2.9***
	(95% CI)	(4.6–5.4)	(4.1–4.7)	(3.4–4.1)	(2.6–3.2)
	Women	2.9	2.9	2.7	2.3
	(95% CI)	(2.7–3.2)	(2.7–3.1)	(2.5–3.0)	(2.1–2.4)
VLF (ms^2)	Men	719.1	641.7	637.9***	563.0***
	(95% CI)	(650.3–795.3)	(589.6–698.3)	(581.0–700.5)	(511.2–620.1)
	Women	668.5	623.7	504.3	450.0
	(95% CI)	(621.6–719.0)	(575.6–675.8)	(461.9–550.6)	(418.7–483.6)
ULF (ms^2)	Men	2653.7	2479.2*	2463.4	2515.2
	(95% CI)	(2390.7–2945.5)	(2273.1–2704.0)	(2232.5–2718.1)	(2293.2–2758.7)
	Women	2928.7	2790.5	2605.8	2384.8
	(95% CI)	(2674.4–3207.1)	(2578.7–3019.8)	(2375.1–2858.8)	(2200.5–2584.6)

n_m, number of male participants; n_f, number of female participants.
[a]Geometric means are shown because values were skewed.
*$P < 0.05$ (differences between sexes).
**$P < 0.01$ (differences between sexes).
***$P < 0.001$ (differences between sexes).

To assess associations of different risk factors with HRV measurements, a structured multivariable linear regression was performed. Because of the known association of HRV with age and sex, these variables were *a priori* included in the model.[14] Variables were then examined by categories (cardiovascular health, alcohol intake, smoking, medication, exercise, laboratory results). Within each category, multiple variables were considered simultaneously, and backwards elimination was used to choose the best predictors of HRV from candidate variables. After the most important representatives of each category of covariates were combined in a full model, backward selection was used in order to identify covariates that might have lost their importance in the multivariable setting. In the final model, all baseline characteristics reported in *Table 2*, as well as dummy variables for the study areas, were included.

Calculation of percentiles

For calculating percentiles of HRV, a 'healthy' subsample of the sample described so far was defined by requiring the absence of each of the following conditions: high blood pressure (systolic blood pressure $\geq$140 mmHg or diastolic blood pressure $\geq$90 mmHg), current smoking, alcohol consumption of more than one glass per day, self-reported diabetes, intake of ACE-inhibitors, antiarrhythmic drugs classes I and III, calcium channel blockers, diuretics, or sympathomimetics in the preceding 30 days. Three hundred and twenty-nine women and 170 men fulfilled these criteria and could be included in the definition of 'healthy individuals'.

Percentiles of power as a function of age in a healthy population have been estimated in the following way: for each of the probabilities $P = 0.05, 0.25, 0.5, 0.75, 0.95$, the p-quantile of ln(TP) for a given age x was assumed to be of the form $y_p(x) = b_0 + b_1 z_p + b_2 x + b_3 z_p x + b_4 x^2 + b_5 z_p x^2$, where z_p denotes the p-quantile of the standard normal distribution.

This implies that, for each age x, the distribution of ln(TP) is normal with mean $b_0 + b_2 x + b_4 x^2$ and standard deviation $b_1 + b_3 x + b_5 x^2$. Exploration of the residuals of ln(TP) after regression against age and square of the age indicated that this assumption was tenable. The six parameters of this equation were estimated using weighted L1-regression. Details of this method are given in Braendli *et al.*[15] and Koenker and Basset.[16]

Results

Table 2 gives an overview of the study population's baseline characteristics. Although there were slightly more women than men, their mean age was identical. Men more frequently had an unhealthy lifestyle (smoking, alcohol drinking), more often were overweight and had on average

Table 4 Adjusted estimates[a] of mean per cent changes of HRV associated with different risk factors[b]

	SDNN (%)	TP (%)	HF (%)	LF (%)	LF/HF (%)	VLF (%)	ULF (%)
Female sex	−6.1***	−11.4***	6.0	−27.2***	−31.3 ***	−23.1***	−6.3
Age (year)	−0.3*	−0.9***	−1.4***	−3.2***	−1.9***	−1.9***	−0.5
Lifestyle factors							
Former vs. never smoking	−1.8	−1.7	6.6	4.7	−1.8	−0.1	−3.3
Current vs. never smoking	−4.5*	−15.0**	−10.5	−15.5**	−5.6	−16.6***	−14.9**
CO (ppm)	−0.3**	−0.3	0.0	−0.6*	−0.6**	−0.6*	−0.3
<1 glass alcohol/day vs. 0	−1.0	−6.0	−5.7	−8.8	−3.3	−8.1	−5.8
1 glass alcohol/day vs. 0	−3.0	−15.7*	−19.2	−19.0*	0.3	−17.7*	−13.7
>1 glass alcohol/day vs. 0	−2.0	−13.5	−11.0	−14.6	−4.0	−11.6	−14.6
Exercise heavy vs. never	2.0***	3.4**	3.6***	4.2**	0.6	4.1***	3.2**
Cardiovascular health							
High BP[c]	−1.3	−7.1*	−5.4	−9.2*	−4.0	−6.9*	−7.5*
Self-reported diabetes	−6.7	−14.2	−5.4	−16.1	−11.3	−14.8	−16.5*
BMI (kg/m^2)[d]	−0.7***	−1.2**	0.5	−1.0*	−1.5***	−1.2**	−1.1**
Medications							
ACE-inhibitors	4.1	17.3*	19.1	13.7	−4.5	14.0*	16.5*
Antiarrhythmics	13.2	20.3	206.7**	13.4	−63.0***	−7.6	2.5
Beta-blockers	−6.9***	12.3*	21.5**	7.1	−11.9*	24.2***	10.5
Ca blockers	−0.6	−1.6	20.7	−2.2	−19.0**	−4.0	−1.8
Diuretics	−3.3	−19.9*	−16.0	−25.2**	−11.0	−27.1***	−21.4*
Sympathomimetics	−1.6	−11.9	−12.6	−12.7	−0.1	−10.0	−10.8
Laboratory parameters							
Uric acid (μmol/L)	0.0**	−0.1**	−0.1*	−0.1**	0.0	−0.1**	−0.1*
hs-C-reactive protein (mg/L)	−0.4**	−1.0***	−1.1**	−1.7***	−0.6*	−1.5***	−0.8**
Non-HDL-cholesterol (mmol/L)[e]	−0.2	−2.8*	−7.5***	−3.7*	4.0**	−3.1*	−2.2

[a]Derived from regression model including all factors in Table 2 and study site.
[b]Effect estimates for quantitative covariates refer to one unit increments in these variables. The respective units are given in parentheses.
[c]High blood pressure = systolic blood pressure ≥140 mmHg or diastolic blood pressure >90 mmHg or self-reported high blood pressure.
[d]BMI = body weight/body height^2.
[e]Total cholesterol − HDL-cholesterol.
*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

a higher blood pressure, but more frequently reported heavy physical exercise. Medication intake was similar for both sexes, except for diuretics which were taken twice as often by women. A surprisingly high proportion (11%) of participants were taking beta-blockers; 18.6% of the subjects were on at least one and 5.0% on more than one of the drugs listed in *Table 2*.

Table 3 shows the unadjusted geometric means of HRV values for the 1742 men and women in different age groups. Men of all combined age groups had significantly lower HF than women, but higher LF, LF/HF, and VLF. Looking at each age group separately, women of the oldest age group (65–73 years) had significantly lower SDNN, TP, LF, LF/HF, and VLF than men of the same age group. Across all age groups, women had significantly lower average LF/HF ratios than men, but women between 50 and 59 years had higher HF than men of their age.

Table 4 shows covariate-adjusted estimates of the per cent changes of frequency domain variables of HRV associated with different lifestyle or risk factors.

Even after adjustment for all other factors, women had significantly lower values for all HRV parameters, except for HF and ULF. TP, for example, was 11.4% lower in women than in men. All HRV parameters except ULF decreased with age in both sexes. HF decreased 1.4% per year and LF 3.2% (*Table 4*, first two rows).

Lifestyle factors

Although former smoking showed no influence, we found current smoking to be the strongest predictor for HRV with a decrease mainly in sympathetic activity (SDNN −4.5%, TP −15.0%, LF −15.5%, VLF −16.6%, and ULF −14.9%). Independent of being a current smoker, CO in exhaled air, as a measure of recent tobacco smoke exposure, showed a significant association with decreased SDNN (−0.3%), LF (−0.6%), LF/HF (−0.6%), and VLF (−0.6%). The consumption of one glass of alcoholic beverage per day showed a significant association with decreased TP (−15.7%), LF (−19.0%), and VLF (−17.7%). Heavy physical exercise was generally associated with higher HRV: SDNN, TP, HF, LF, VLF, and ULF increased significantly by ≥2.0% per weekly hour of heavy exercise.

Cardiovascular health

Having high blood pressure as defined above primarily affected sympathetic tone, with decreases in TP, LF, VLF, and ULF, whereas a higher body mass index (BMI) was associated with a decrease in all HRV parameters except HF. On average, TP decreased by 1.2% for each additional kg/m^2 of BMI. Self-reported diabetes decreased ULF significantly by 16.5%.

Medications

Among the drugs examined, antiarrhythmics, as expected, showed the strongest association with HRV, followed by diuretics and beta-blockers. Persons taking diuretics showed significantly diminished TP, LF, VLF, and ULF. Subjects on beta-blockers had an increased vagal tone, with increases in TP of 13%, HF of 22%, and VLF of 25%, but with a 12% decrease in LF/HF. Subjects on ACE-inhibitors had a 18% increase in TP.

Laboratory parameters

All laboratory parameters of the regression model (uric acid, hs-CRP, and non-HDL-cholesterol) consistently showed negative associations with most HRV parameters. The exceptions were the LF/HF ratio for uric acid and SDNN as well as ULF for non-HDL-cholesterol. Both HF and LF decreased by 0.1%/μmol/L increase in uric acid. Per unit increase in hs-CRP, HF decreased by 1.1% and LF by 1.7%. Non-HDL-cholesterol showed a highly significant association with decreased vagal and sympathetic tone (with decreases in HF and LF by 7.5 and 3.7%, respectively).

Normal values

It is often useful to compare subjects or patients' HRV with that found in age- and sex-matched healthy populations. To construct such normal ageing curves for men and women between 50 and 70 years of age, we chose all non-smoking individuals without history of cardiovascular disease, high blood pressure or diabetes, and free of medications, as described in Methods. We then calculated normal ageing curves, which are shown in *Figures 1* and 2.

In men, LF/HF values clearly decrease between 50 and 70 years of age. In women, such a decrease is only observed after age 60.

Discussion

In this study, we have analysed different factors influencing HRV in a population-based sample aged 50–72. We are not aware of many studies about the respective importance of different predictors of HRV, although HRV is known to be influenced by a great variety of factors.[8] Because of our relatively large sample size, we were also able to estimate age-dependent percentile curves for normotensive non-smoking subjects aged $\geq$50 without cardioactive drug intake or moderate to high alcohol consumption. As a result, we are able to show different ageing patterns for HRV in men and women. We found TP to decline clearly with age in men, whereas HF showed this pattern in women. This results in an earlier and more marked age-related decline of LF/HF ratio in men than in women, consistent with the higher life expectancy and the lower risk for cardiovascular diseases of women. This difference might explain why not all previous studies found an age-related decrease in HRV. Age has been described to be negatively associated with frequency domain measures,[17–21] which represent both vagal and sympathetic activity. Some authors[19] found this association only in men. Other authors found a negative association with frequency domain variables in the female sex,[17,18,22] whereas Stein *et al.*[19] describe this association only in subgroups. As stated by Parati and Di Rienzo,[14] age and gender should always be taken into account when quantifying HRV.

Inconsistent findings concerning the effect of smoking on HRV have been reported in the literature.[23,24] In our study, we found both a strong effect of smoking and an independent association with end expiratory CO levels. Although nicotine is a stimulant of the sympathetic nervous system, we found a significant reduction of LF in current smokers. This suggests that either the toxic effect of other components of tobacco smoke is stronger than the increase in the sympathetic tone expected from nicotine alone, leading to an accelerated ageing of the heart, or the continuous stimulation of the sympathetic nervous system eventually leads to the weakening of the response. In the context of all the risk factors considered, we also found an independent negative effect of alcohol consumption on HRV, which has been shown in other studies.[25,26] In contrast, regular exercise has a positive effect on HRV. This has been described by others for both sympathetic and vagal nerve activity.[27–30]

The influence of BMI on HRV is also controversially discussed in the literature.[20,22] We found a small effect of BMI on SDNN, TP, LF, LF/HF ratio, VLF, and ULF. Self-reported

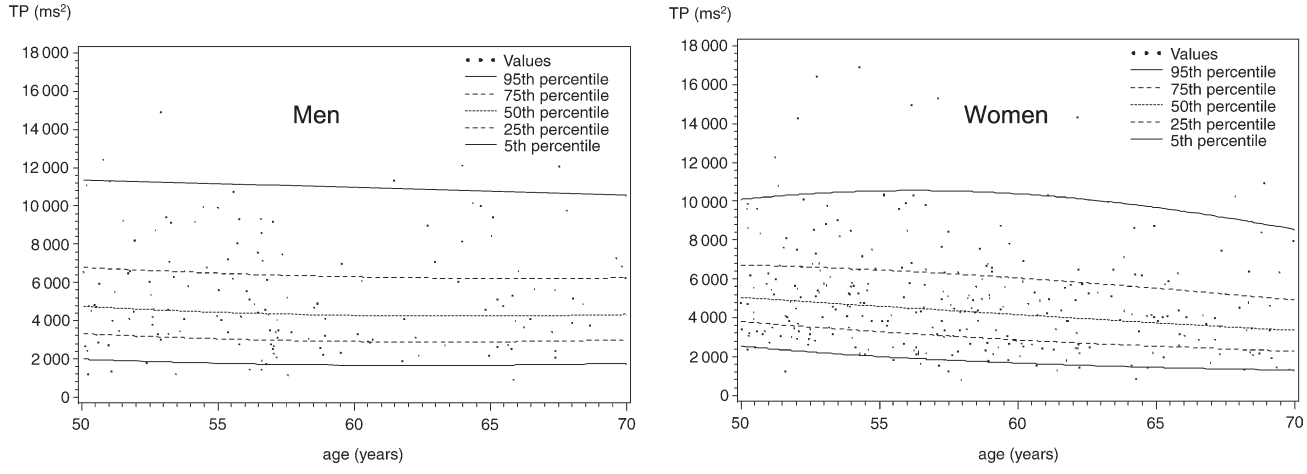

Figure 1 Percentiles of TP for healthy men and women.

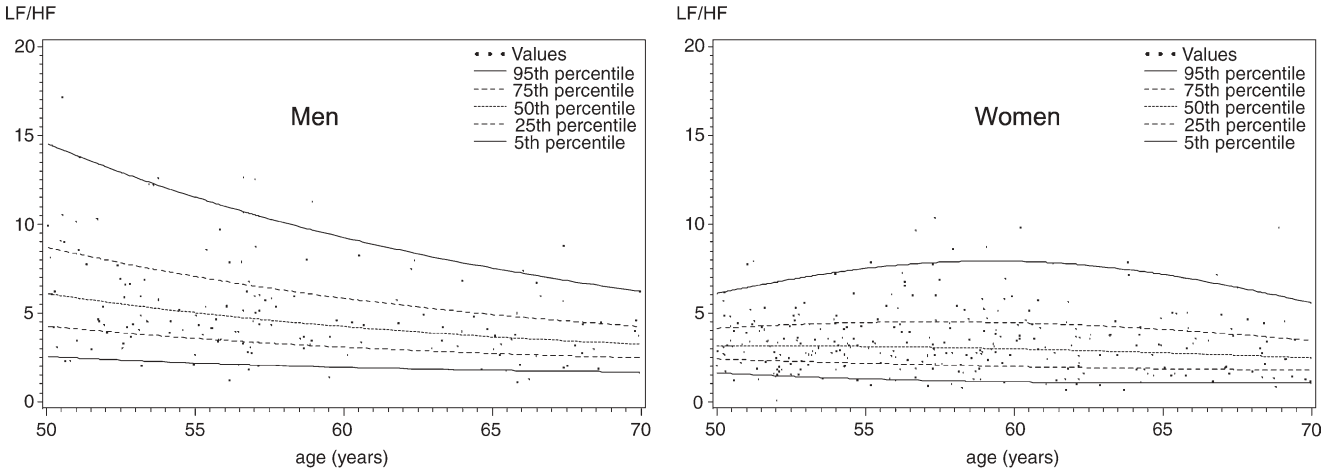

Figure 2 Percentiles of LF/HF for healthy men and women.

diabetes and high blood pressure show weak associations in our study (high blood pressure with TP, LF, VLF, and ULF, diabetes with ULF). Both of these cardiovascular risk factors have also been described as being associated with impaired autonomic function in the literature.[23,31]

We found that persons taking ACE-inhibitors had a higher TP and those taking antiarrhythmics a higher HF, whereas LF/HF was significantly decreased in the latter group. Diuretics had a statistically significant negative effect on TP, LF, VLF, and ULF. Obviously, there is a risk of the confounding factors of between medication commonly taken by the elderly, specifically ACE-inhibitors, antiarrhythmics, and beta-blockers, and the underlying condition requiring these chronic medications influencing the results. In these cases, it can be difficult to separate the effect of a disease from the effect of the drug used to treat it. However, in our study, we could show that the effect of drug intake was opposite to the one of the underlying disease, suggesting that we looked at the true effect and not at an effect confounded by diagnosis. Yet, in our results, the time domain measure SDNN is decreased by beta-blockers. It is possible that this result captures the effect of beta-blockers on sympathetic drive, whereas frequency domain variables, such as TP, may be more sensitive to subtle and fast changes such as enhancement of parasympathetic activity by beta-blockers. Effects from ACE-inhibitors have been demonstrated in smaller clinical studies: increases in HF[32] and LF[32,33] and decreases in LF/HF[34,35] have been described. It is interesting to note that these effects were confirmed in our relatively healthy cohort. Adrenergic beta-antagonists[36–38] and sympathomimetics[39,40] have been widely studied, whereas little has been published about the effects of classes I and III antiarrhythmic agents,[41] calcium channel blockers,[42] or diuretics on frequency domain HRV.[39] We can confirm most of these effects in our population-based sample, even after taking all other cardiovascular risk factors into account.

In our study, the laboratory parameters, uric acid and hs-CRP, were consistently and negatively associated with all HRV parameters but not the LF/HF ratio. In the literature, similar effects of hs-CRP have been described in elderly subjects with no apparent heart disease.[43] However, the causal pathway of this association is not clear. Inflammation may influence autonomic balance or autonomic imbalance may activate inflammation. No information exists in the literature about the association between uric acid and HRV, but it is known that chronic renal failure is associated with impaired autonomic function and reduced HRV.[44,45] Of all the laboratory parameters studied, we found non-HDL cholesterol to have the strongest effect on both HF and LF. We found no evidence for such an association in the literature, whereas studies examining the relation between hypercholesterolaemia and HRV in men without ischaemic heart disease showed non-uniform results.[46,47]

Thus, our findings confirm many of the results of individual smaller studies on the single or combined effects of drugs, lifestyle, or risk factors of cardiovascular disease on HRV.

From a clinical point of view, a shortcoming of this study is the fact that we examined a random sample of the general population not comparable with cardiac patients. Thus, the meaning of our results in clinical settings still needs to be explored.

In this cross-sectional study, we exclusively looked at people aged $\geq$50. In the future, additional age groups need to be examined and longitudinal assessments need to be performed, as stated in the report of the Task Force.[9]

We chose to report SDNN and frequency domain variables. Although the physiological basis for ULF and VLF power are far less clear than that for HF and LF power, they have been shown to be more powerful risk predictors in cardiovascular disease.[48]

To the best of our knowledge, this is the first study simultaneously able to assess the contribution of a wide range of risk factors on HRV and to provide percentile curves for 24 h HRV for an elderly population. We hope that these data will help the interpretation of HRV data in the general population and the clarification of the role of various factors influencing HRV.

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Appendix

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Scientific team at coordinating centres

L. Bayer-Oglesby (exp), D. Felber Dietrich (c), M. Imboden (g), D. Keidel (s), S. Downs (e), P. Städele-Kessler (s), M. Gerbase (p).

(a) medicine of allergy, (c) cardiology, (cc) clinical chemistry, (e) epidemiology, (exp) exposure, (g) genetic and molecular biology, (m) meteorology, (p) pneumology, (s) statistics.

Scientific team at local study sites

C. Burrus, D. Felber Dietrich, U. Egermann, M. Gerbase, R. Gimmi, A. Kick, N. Lutz.

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3. Paper 2: Effects of environmental tobacco smoke on cardiovascular health and pathways

3.1 Effects of passive smoking on heart rate variability, heart rate and blood pressure: an observational study

Supplementary material is shown in appendix 7.

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Effects of passive smoking on heart rate variability, heart rate and blood pressure: an observational study§

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Background Exposure to environmental tobacco smoke (ETS) has been shown to increase the risk for cardiovascular diseases and death, and autonomic dysfunction (specifically, reduced heart rate variability (HRV)) is a predictor of increased cardiac risk. This study tests the hypothesis that ETS exposure reduces HRV in the general population and discusses possible pathways.

Methods This cross-sectional study was conducted between 2001 and 2003 and is part of the SAPALDIA (Swiss Cohort Study on Air Pollution and Lung Diseases in Adults) study.
The analysis included 1218 randomly selected non-smokers aged 50 and above who participated in 24-h electrocardiogram recordings. Other examinations included an interview, investigating health status (especially respiratory and cardiovascular health and health relevant behaviours and exposure to ETS) and measurements of blood pressure, body height and weight.

Results Subjects exposed to ETS at home or at work for more than 2 h/day had a difference of −15% in total power (95%CI: −26 to −3%), low frequency power (−28 to −1%), low/high frequency ratio (−26 to −3%) and −18% (−29 to −4%) in ultralow frequency power of HRV compared with subjects not exposed to ETS at home or work. We also found a 2.7% (−0.01 to 5.34%) higher heart rate during the recording in exposed subjects.

Conclusions Exposure to ETS at home and work is associated with lower HRV and with higher heart rate in an ageing population. Our findings suggest that exposure to ETS increases cardiac risk through disturbances in the autonomic nervous system.

Keywords Tobacco smoke pollution, heart rate variability, autonomic nervous system, heart rate, blood pressure

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Background

Exposure to environmental tobacco smoke (ETS) and reduced heart rate variability (HRV) in middle-aged and elderly subjects,[1] survivors of myocardial infarction,[2] or patients with other cardiovascular diseases[3,4] are both known to be associated with increased cardiovascular morbidity and mortality.[5–10] HRV, a widely used measure of cardiac autonomic control,[11] reflects autonomic modulation of the rhythmic activity of the sinus node.[11] To date, little has been reported on the association between ETS exposure and HRV, heart rate, or blood pressure, the alterations of which may be steps in the pathophysiological pathway leading from ETS exposure to cardiopulmonary disease.

To our knowledge, the effect of longer-term exposures to ETS on HRV has not been analysed. Short-term effects have been described by Pope, who found a reduction in HRV in 16 never smoking subjects equipped with Holter monitors, who were moved from the non-smoking section of an airport to the smoking lounge.[12] Reports from the 1991 SAPALDIA (Swiss Cohort Study on Air Pollution and Lung Diseases in Adults) study[13] describe the effects of ETS on respiratory symptoms in never smokers[14] and on lung function in asthmatics.[15] In the follow-up study (SAPALDIA 2), we also show a higher probability of the development of asthma in subjects exposed to ETS.[16] In SAPALDIA 2, ambulatory 24-h electrocardiograms (ECG) were performed in a random sample of participants age $\geqslant$50. This analysis looks at the effect of ETS exposure on HRV and explores the role of heart rate and blood pressure in this context.

Materials and methods

Participants

The SAPALDIA cohort study was designed to measure the health effects of long-term exposure to air pollutants in the Swiss adult population. Details of its design and objectives have been reported elsewhere.[17,18] In 1991, a random sample of the Swiss population was recruited from eight areas featuring distinct geographical and environmental conditions. SAPALDIA participants were examined in 1991 and 2001–03 for risk factors of cardiovascular disease, respiratory symptoms, pulmonary function evolution and development of lung diseases.

A random sample of 2000 SAPALDIA 2 participants age $\geqslant$50 who agreed to participate were offered a 24-h ECG recording. Data on smoking status were collected during an extensive interview led by trained fieldworkers. Among the 1837 persons having performed the measurements, 1385 reported that they had not smoked cigarettes, pipes, cigarillos or cigars in the last 5 years and were included in this study. Self-reports were confirmed by end-expiratory carbon monoxide (CO) measurements using EC50 Micro-Smokerlyzers (BEDFONT, Rochester, UK); 91 participants were excluded because they had an end-expiratory CO of $\geqslant$7 ppm, indicating that they might be smokers.[19–22] One participant was excluded because of a myocardial infarction in the previous 3 months and four because of digitalis intake in the previous 30 days. None had a cardiac pacer or anaesthesia (narcosis or spinal anaesthesia) in the 8 days prior to the ambulatory ECG recording (Figure 1).

ETS exposure was assessed for different environments by the question 'How many hours per day are you exposed to other people's tobacco smoke (i) at home (ii) at the workplace (iii) in bars and restaurants (iv) elsewhere?' We focused on exposure to ETS at home and work since these two sources dominate overall exposure in most subjects and because it is usually easier to recall routine exposure to ETS at home and at work than in other places. For seven subjects, information on ETS exposure was missing. On the basis of previous work,[14] ETS exposure was categorized into three exposure groups: none, $\leqslant$2 h per day but not none, and >2 h per day.

Cardiovascular risk factors

Blood pressure was measured twice on the left upper arm with the subject sitting and at rest, by an automatic device (705CP, OMRON, Tokyo, Japan) according to WHO recommendations.[23] Blood pressure values used in the regression model were the arithmetic mean of the two measurements. High blood pressure was defined as either a systolic blood pressure (SBP) $\geqslant$140 mmHg, or a diastolic blood pressure (DBP) $\geqslant$90 mmHg, or having answered yes to the question 'Do you have the following condition: Hypertension?'

Body height and weight were measured with participants not wearing any shoes or coats and body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). For two participants, information for calculating BMI was missing.

Data on education, hypertension and current medications were collected during the interview.

The highest degree of education, which is correlated to income,[24] was used as a proxy for social position.

Blood samples were taken from subjects, and several known cardiovascular risk factors were determined by the Institute for Clinical Chemistry of the University Hospital Zürich: A Hitachi Modular Autoanalyser (Rotkreuz, Switzerland); assays from Roche Diagnostics (Mannheim, Germany) were used to measure serum levels of uric acid and total cholesterol (both enzymatic tests) and high-sensitive C-reactive protein (CRP) was measured with a latex-enhanced immunoturbidimetric assay. High-density lipoprotein cholesterol (HDL) was measured with a homogenous assay (Roche diagnostics, Mannheim, Germany) using Roche Cobas Integra (Rotkreuz, Switzerland). HDL values were only used if the participants had a triglyceride level of $\leqslant$9.4 mmol/l. As additional atherogenic markers, the difference between total cholesterol and HDL (non-HDL-cholesterol) and the ratio between total cholesterol and HDL were calculated.

Ethical approval for the study was given by the central ethics committee of the Swiss Academy of Medical Sciences and the Cantonal Ethics Committees for each of the eight examination areas; subjects gave informed consent at the examination. These procedures were in accordance with the recommendations of the Helsinki Declaration.

HRV data

For 24-h ECG (Holter) recording, digital devices (Aria, Del Mar Medical Systems, Irvine, CA) with a frequency response of 0.05–40 Hz and a resolution of 128 samples/s were

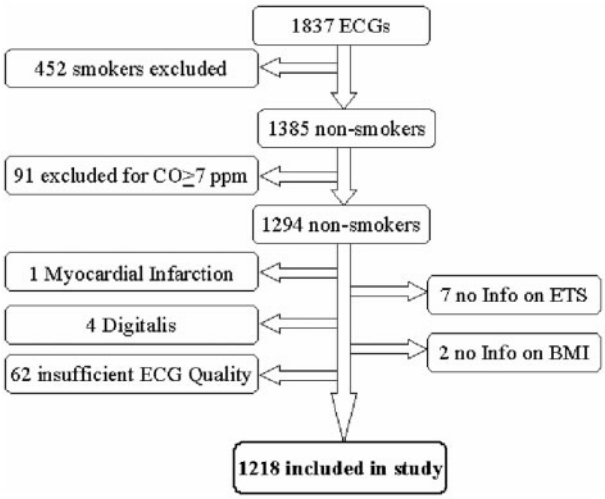

Figure 1 Flow chart of participants and exclusion criteria

used. The recorders were hooked up after the interview. Three leads (V_1, altered V_3 with the electrode on the left midclavicular line on the lowest rib and altered V_5 with the electrode on the left midclavicular line on the lowest rib) were recorded over 24 h. We excluded the first 2 h of recording to avoid bias from the effects of methacholine administered in the preceding broncho-challenge test. Recordings with insufficient quality ($n = 32$) or <18 h ($n = 30$) were not used (Figure 1). Mean duration of the remaining 1218 recordings was 22.3 ± 2.1 h. Participants were asked to follow their regular daily life and to fill in a time-activity diary during the recording time.

All recordings were scanned through a StrataScan 563 (Del Mar) and interpreted with the interactive method, with a final visual check of the full disclosure. Mean heart rate per minute was derived from Holter measurements. The length of each RR interval was manually validated during this step. Re-sampling was done at 4 Hz. Spectral analysis was performed by the fast Fourier transform method using sliding 256 PTAs windows for night periods. For 24-h periods, calculations of RR intervals were made without sliding window, to allow measurement of ultralow frequency power (ULF) and of very low frequency power (VLF). Only normal to normal (NN) intervals were used, with intervals excluded due to ectopy or artefacts being replaced by holding the previous coupling interval level throughout the time interval to the next valid coupling interval. The standard deviation of all normal to normal RR intervals (SDNN) and the following frequency domain variables were calculated: total power (≤0.40 Hz), ULF (≤0.0033 Hz), VLF (0.0033–0.04 Hz), low frequency (LF) power (0.04–0.15 Hz), high frequency (HF) power (0.15–0.40 Hz) and the ratio between LF and HF (LF/HF).

Statistical analysis

Because an initial inspection suggested that the distribution of the residuals was skewed, the HRV values were log-transformed before further analysis and the results are presented as percent differences between exposure groups.

We evaluated the effect of ETS on HRV, heart rate and blood pressure using a multivariable regression model adjusting for study site, sex, age and age squared, education, BMI, self-reported diabetes and beta-blocker intake in the previous 30 days (core model). Sensitivity analysis for additional potential confounders (hypertension, physical activity, alcohol drinking, cardioactive medication and cardiovascular risk factors in the blood) and additional analyses for the period during sleep at night to exclude shorter-term effects were carried out. To check whether former smoking would have a residual effect on cardiac autonomic function, we controlled for smoking status in a further analysis.

To assess differences of proportions and means in the three ETS exposure groups in Table 1, Chi-squared-tests and the Kruskal–Wallis test were performed.

Statistical analysis was performed using the software package Stata 9.2 (Stata corporation, College Station, TX).

Results

Among the 1218 never smokers and former smokers included in the final study group, 184 (15%) were regularly exposed to ETS at home or at work: 104 (8.5%) ≤2 h/day, and 80 (6.6%) >2 h/day.

Table 1 gives an overview of the study population's characteristics, categorized by ETS exposure. Subjects without ETS exposure were slightly older, had a lower BMI and drank less alcohol.

Table 2 shows geometric means of HRV measures, adjusted for the covariates of the core model, according to ETS exposure. In subjects exposed >2 h/day to ETS at home or at work, total power (95% CI 26 to −3%), LF (CI −28 to −1%) and LF/HF (CI −26 to −3%) were 15% lower than in unexposed subjects. ULF was 18% (CI −29 to −4%) lower. Although results for HF, VLF and SDNN showed no substantial difference between exposed and unexposed subjects, the trend was the same as for the other outcome variables. Similar results emerged when we stratified for sex (Supplementary data are available at *International Journal of Epidemiology* online). In order to test possible pathways, we analysed heart rate and blood pressure: the results are shown in Table 3 as arithmetic means adjusted for the covariates of the core model. Heart rate was 2.7% (CI 0.1 to 5.5%; $P = 0.049$) higher in subjects exposed >2 h/day to ETS than in unexposed subjects. Systolic blood SBP was similar in the two groups and DBP was 1.9% (−3.0 to 2.9%; $P = 0.174$) higher in subjects exposed to ETS > 2 h/day. Unadjusted results are presented as online supplementary material.

Figure 2 shows the relationship between ETS exposure and percent difference in LF, heart rate and DBP with control for all factors of the core model. There were trends for a lower LF with increasing ETS exposure and for higher heart rate and DBP with higher ETS exposure at home or work.

Sensitivity analyses

In order to guard against confounding by factors not considered in the core model, we conducted sensitivity analyses by including complementary categories of covariates in the model each at a time: additional control for physical exercise causing sweating and physical exercise causing slight shortness of breath did not change the difference in total power between the reference group and the highest ETS exposure group

Table 1 Overview of the study population baseline characteristics by exposure group

ETS exposure	None	⩽2 h/day	>2 h/day
n	1034 (84.9%)	104 (8.5%)	80 (6.6%)
[a]Female	556 (53.8%)	53 (51.0%)	41 (51.3%)
[a]Age (years)	61.0 (SD 6.4)	59.8 (SD 6.2)	58.4 (SD 5.0)
Blood pressure			
systolic (mmHg)	132.7 (SD 19.7)	133.7 (SD 21.0)	131.5 (SD 18.6)
diastolic (mmHg)	81.7 (SD 10.8)	82.4 (SD 10.4)	83.5 (SD 11.3)
[a]BMI (kg/m^2)	26.5 (SD 4.3)	27.8 (SD 4.8)	27.4 (SD 4.3)
[a]Self-reported diabetes	44 (4.3%)	4 (3.9%)	3 (3.8%)
[a]Education			
Primary	93 (9.0%)	15 (14.4%)	4 (5.0%)
Secondary	660 (63.8%)	69 (66.4%)	59 (73.8%)
Tertiary	281 (27.2%)	20 (19.2%)	17 (21.3%)
Light physical activity			
⩽2 d/w	496 (48.1%)	55 (52.9%)	42 (52.5%)
>2 d/w	536 (51.9%)	49 (47.1%)	38 (47.5%)
Heavy physical activity			
⩽1 h/w	763 (74.2%)	77 (74.8%)	54 (67.5%)
>1 h/w	266 (25.9%)	26 (25.2%)	26 (32.5%)
Alcohol			
<1 glass/day	776 (75.1%)	66 (63.5%)	56 (70%)
⩾1 glass/day	258 (25.0%)	38 (36.5%)	24 (30.0%)
[a]Beta-blocker medication	123 (11.9%)	17 (16.4%)	8 (10.0%)
ACE-inhibitor	64 (6.2%)	6 (5.8%)	6 (7.5%)
Antiarrhythmic drugs class I + III	4 (0.4%)	0 (0.0%)	1 (1.3%)
Calcium channel blocker	45 (4.4%)	7 (6.7%)	5 (6.3%)
Diuretics	43 (4.2%)	6 (5.8%)	2 (2.5%)
Sympathomimetics	36 (3.5%)	5 (4.8%)	0 (0.0%)
Uric acid [mol/l]	321.1 (SD 81.4)	332.6 (SD 79.0)	331.7 (SD 93.4)
Hs-CRP [mg/l]	2.6 (SD 5.9)	2.4 (SD 4.8)	2.2 (SD 3.2)
Non-HDL-cholesterol [mmol/l]	4.7 (SD 1.1)	4.8 (SD 1.2)	4.9 (SD 1.2)

[a] Covariates of the core model.

(Supplementary data are available at *International Journal of Epidemiology* online). Also, no sizeable change of the results was seen with additional control for hypertension. The difference in total power between the reference group and the highest exposure group slightly increased with additional control for consumption of red wine and alcoholic beverages or for serum levels of uric acid, high-sensitive CRP and non-HDL cholesterol or when subjects taking ACE inhibitors, antiarrhythmic medication, calcium channel blockers, diuretics or sympathomimetics were excluded. Moreover, results were not sensitive to excluding education from the model. We could also see no difference in the influence of educational level on HRV between men and women (data not shown). Additional analyses of HRV restricted to the sleep period according to diary information showed results similar to the analyses of the 24-h measures. On average, LF power at night was 15.1% (CI: −25 to −4%; $P = 0.009$) lower in subjects exposed to ETS ⩾2 h/day than in the reference group. Additional controlling for smoking status showed little evidence for a residual effect of former smoking. The strongest effect of former smoking was seen for ULF with a 5% lower ULF (CI −13 to 2%) in former smokers than in never smokers.

Discussion

In recent years, there has been increasing evidence that ETS exposure affects cardiovascular health.[5–10,25–27] Our study provides further evidence that ETS exposure is associated with cardiac autonomic dysregulation, which may be an intermediate step in the pathway to cardiac instability.[4,28] Our observations are in agreement with the findings of Pope, who described a short-term decrease in all HRV domains after acute exposure to ETS, but with relatively high standard errors in HF.[12]

LF, which is considered to represent both sympathetic and parasympathetic activities,[29] was lower in subjects with higher ETS exposure. We also observed ETS-associated increases in heart rate and, more weakly, in DBP, consistent with increases in sympathetic stimulation.

Since few people are exposed to ETS during sleep, we restricted analyses to the sleep period, when acute exposure can be excluded and found results similar to those of the 24-h measures. Therefore, we think that our findings do not reflect acute responses.

Table 2 Adjusted[a] geometric mean HRV according to ETS exposure

HRV variable	No current ETS exp.	ETS ⩽2 h/day	ETS >2 h/day
n	1034	104	80
SDNN [ms]	134.0 (131.8, 136.1)	134.0 (127.4, 140.9)	129.3 (122.1, 136.9)
Total Power [ms^2]	3866.7 (3729.6, 4008.8)	3712.1 (3308.78,4164.5)	3270.1 (2868.7, 3727.7)
HF [ms^2]	71.3 (67.5, 75.2)	61.5 (51.8, 73.1)	71.2 (58.5, 86.6)
LF [ms^2]	233.6 (224.0, 243.7)	210.3(183.8, 240.6)	197.7 (169.6, 230.5)
LF/HF	3.3 (3.2, 3.4)	3.4 (3.0, 3.8)	2.8 (2.4, 3.2)
VLF [ms^2]	626.1 (603.4, 649.7)	598.9 (532.5, 673.7)	560.0 (489.8, 640.3)
ULF [ms^2]	2741.3 (2634.1, 2852.8)	2671.6 (2352.9, 3033.6)	2256.3 (1952.4, 2607.6)

Values in parentheses are 95% CIs.

[a] Adjusted for study site, gender, age, education, BMI, diabetes and beta-blocker intake.

Table 3 Adjusted[a] geometric means of heart rate and blood pressure according to ETS exposure

Variable	No current ETS exp.	ETS ⩽2 h/day	ETS >2 h/day
Heart rate [bpm]	73.4 (72.9, 73.9)	73.4 (71.8, 75.1)	75.3 (73.5, 77.2)
SBP [mmHg]	132.7 (131.6, 133.8)	133.5 (130.1, 136.9)	132.6 (128.7, 136.5)
DBP [mmHg]	81.7 (81.1, 82.3)	82.1 (80.2, 84.0)	83.3 (81.1, 85.5)

Values in parentheses are 95% CIs.
SBP: systolic blood pressure, DBP: diastolic blood pressure.
[a] Adjusted for study site, gender, age, education, BMI, diabetes and beta-blocker intake.

ETS may affect autonomic control of the heart through activation of neural receptors of the respiratory tract. On the other hand, gaseous components, soluble fractions of the particulate component and ultrafine particle components of ETS may be absorbed in the lung and have additional systemic effects. In the experimental setting, chronic ETS exposure has been shown to increase proinflammatory cytokines and arterial resistance, to decrease concentrations of antioxidants and to increase lipid peroxidation.[30] We found no evidence of ETS-associated increases in inflammation as measured by CRP and other causal mechanisms may predominate with low-grade chronic exposure. Recent work by Bartoli and colleagues[31] suggests that particle exposures alter barometric reflexes, a pathway through which ETS exposure might also influence HRV.[31] Ultrafine particles are associated with oxidative stress,[32] as well as with reduced HRV.[33]

To better understand why exposure to ETS is associated with lower HRV, it is interesting to compare these effects with those of active smoking on HRV. In a recent publication, we showed that active smokers had lower overall and low to ULF HRV measures than never smokers, which reflect the dynamic features of sympathetic cardiovascular modulation, analogous to the results described in this work. The effect of current smoking compared with never smoking was also of magnitude similar to the effect shown in this study.[17] Thus, we could deduce that active smoking and passive smoking harm the cardiovascular system through similar toxic substances.

The consequence of autonomic dysfunction caused by pollutants such as ETS, air pollution and other factors can be an increased risk for ventricular arrhythmia in vulnerable patients or a contribution to the instability of a vascular plaque, eventually leading to cardiac death.[34–36]

We measured exposure to ETS using questionnaire data and used CO measures to validate smoking status. We believe that self-reporting gives a better estimate for exposure to ETS during the past year than measurement of biomarkers, which reflect the level of exposure to passive smoking in the immediate past with half-lifes of only several hours.[37] In the literature, measurement of exhaled CO in combination with self-reporting has been described to provide an acceptable degree of discrimination between smokers and non-smokers.[37]

Reporting bias might have occurred if participants with lower HRV had been more likely to report passive exposure to cigarette smoke. However, this is very unlikely since HRV is not readily apparent to the participant. Moreover, exposure to ETS is generally associated with respiratory symptoms and to a much lesser degree with cardiovascular health. In addition, we observed a 13% increase of individuals not exposed to ETS compared with 1991—which would point towards under—rather than overreporting of ETS.

A recent study has shown that social position expressed as low employment grade is associated with low HRV.[38] In our study, the effect of ETS exposure on the HRV parameters did not change

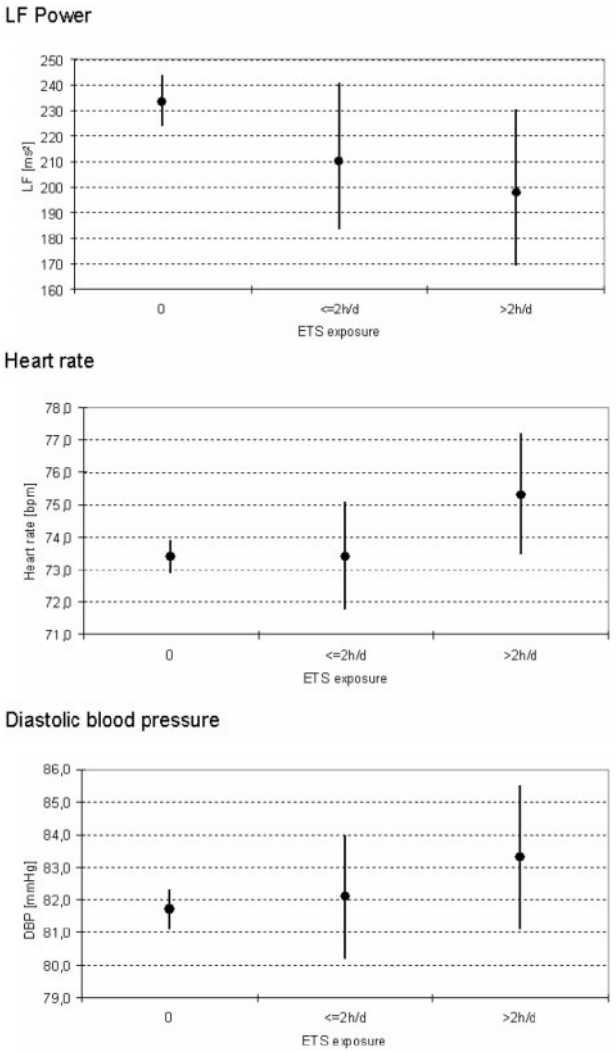

Figure 2 Hours of environmental tobacco smoke exposure at home and at work and low frequency heart rate variability, heart rate and diastolic blood pressure and respective 95% confidence intervals, adjusted for study site, sex, age, education, BMI, diabetes and beta-blocker intake

when we did not account for level of education, indicating that these results are not likely to be confounded by social position.

In conclusion, our results contribute to the evidence that exposure to second-hand smoke increases cardiac risk through cardiac autonomic dysfunction. Health benefits can be expected if people are protected from passive smoking.

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Local fieldworkers:

Aarau: M Broglie, M Bünter, D Gashi, Basel: R Armbruster, T Damm, U Egermann, M Gut, L Maier, A Vögelin, L Walter, Davos: D Jud, N Lutz, Geneva: M Ares, M Bennour, B Galobardes, E Namer, Lugano: B Baumberger, S Boccia Soldati, E Gehrig-Van Essen, S Ronchetto, Montana: C Bonvin, C Burrus, Payerne: S Blanc, AV Ebinger, ML Fragnière, J Jordan, Wald: R Gimmi, N Kourkoulos, U Schafroth.

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Holter system technicians: M Victoire, D Maudoux.

Mathematical HRV engineering: V Pichot.

Administrative staff: N Bauer, R Nilly.

KEY MESSAGES

- ETS has been shown to be a risk factor for cardiovascular disease, but so far little is known about possible mechanisms.
- We show that subjects exposed to environmental tobacco smoke ETS for over 2 h/day at home and/or at work had a reduced heart rate variability compared with unexposed subjects.
- Exposed subjects also had a higher heart rate and a tendency for higher diastolic blood pressure, suggesting a possible pathway of increased cardiac risk through disturbances in the autonomic nervous system.

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4. Paper 3: Effect of exercise on cardiovascular health of the obese

4.1 Effect of physical activity on heart rate variability in normal weight, overweight and obese subjects: results from the SAPALDIA study

Supplementary material is shown in appendix 8.

The original publication is available at http://www.springerlink.com/

Effect of physical activity on heart rate variability in normal weight, overweight and obese subjects: results from the SAPALDIA study

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Abstract Many studies have demonstrated an association of both a sedentary lifestyle and a high body mass index (BMI) with greater risk for cardiovascular disease. Within the prospective SAPALDIA cohort (Swiss cohort study on Air Pollution and Lung Diseases in Adults), we investigated whether regular exercise was protective against reduced heart rate variability (HRV), a clinically relevant predictor of cardiovascular morbidity and mortality, and whether adverse effects of obesity and weight gain on HRV were modified by regular exercise.

24-hour electrocardiograms were recorded in 1712 randomly selected SAPALDIA participants aged ≥50, for whom BMI was assessed in the years 1991 and 2001-2003. Other examinations included an interview investigating health status (especially respiratory and cardiovascular health and health relevant behaviours including physical activity) and measurements of blood pressure, body height and weight. The association between regular physical activity and HRV and interactions with BMI and BMI change was assessed in multivariable linear regression analyses.

Compared to sedentary obese subjects, SDNN (standard deviation of all RR intervals) was 14% (95% CI: 8-20%) higher in sedentary normal weight subjects; 19% (CI: 12-27%) higher in normal weight subjects exercising regularly ≥ 2h/week; and 19% (CI:11 - 28%) higher in obese subjects exercising regularly ≥ 2h/week.

Compared with sedentary subjects who gained weight, those who gained weight but did exercise regularly had a 13% higher SDNN (CI: 7-20%).

Regular physical exercise has strong beneficial effects on cardiac autonomic nervous function and thus appears to offset the negative effect of obesity on HRV.

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Key words Heart rate variability; Autonomic nervous system; Body mass index; Obesity; Body weight change; Exercise

Introduction

Obesity and weight gain are imposing a growing threat to world health, as in many countries 20-30% of adults are categorized as clinically obese, and their number is still increasing (World Health Organization 1999; World Health Organization 2002). Many studies have demonstrated an association of both a high body mass index (BMI) and a sedentary lifestyle with greater risk for cardiovascular disease (Fang et al. 2003; Wei et al. 1999).

In the SAPALDIA (Swiss cohort study on Air Pollution and Lung Diseases in Adults) study of a large sample of Swiss adults, we confirmed the relation of reduced heart rate variability (HRV) with increased BMI (Felber Dietrich et al. 2006). Heart rate variability refers to the beat-to-beat variation in heart rate and is a marker of cardiac autonomic control (Kleiger et al. 2005). Reduced HRV predicts increased risk of cardiovascular disease and mortality in longitudinal studies (Bigger et al. 1993; Kleiger et al. 1987). Other studies have noted that increased BMI is associated with reduced HRV, which has been attributed to decreased adreno-receptor responsiveness, withdrawal of parasympathetic tone and/or increased sympathetic activity (Fraley et al. 2005; Ramaekers et al. 1998). Likewise, weight changes have been associated with changes in HRV (Hirsch et al. 1991; Poirier et al. 2003). There is also evidence from the literature, that regular moderate endurance training as opposed to strength training has a positive effect on HRV (Hottenrott et al. 2006). Based on these findings, we wanted to test the hypothesis within the prospective SAPALDIA cohort, that regular exercise is associated with improved HRV, and that adverse effects of obesity and weight gain on HRV can be modified by regular exercise.

Methods

This study is part of the SAPALDIA cohort study, which was primarily designed to assess health effects from long-term exposure to air pollutants in the Swiss adult population. Details of its design and objectives have been reported elsewhere (Ackermann-Liebrich et al. 2005; Martin et al. 1997). In brief, a random sample of the Swiss population was recruited in 1991 from eight areas featuring distinct geographical and environmental conditions. 9651 participants received intensive health examinations and a detailed health interview in 1991. In 2001-2003, we were able to re-examine 8047 of the original participants and to assess HRV in a random selection (n=1846: 955 women, 891 men) of the 4417 participants aged ≥50 years by 24-hour ECGs. Exclusion criteria were general or spinal anaesthesia within eight days prior to the ambulatory ECG recording (n=5), having had a myocardial infarction within 3 months prior to the examination (n=2), and taking digitalis (n=6); nobody had an artificial pacemaker. Exclusion of recordings showing atrial fibrillation (n=12), recordings of <18h (recommendations of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (North American Society of Pacing and Electrophysiology 1996)) (n=73), and recordings of insufficient quality (n=6) left 1742 datasets. We had complete information on 1718 subjects for analysis of HRV involving BMI and exercise and on 1712 subjects for analysis involving weight gain from baseline to follow-up. For statistical reasons, 15 subjects being underweight at follow-up were excluded from analyses where BMI was used as a

categorical variable, leaving 1703 records for analyses.

HRV measurements and analyses

For the Holter recording, digital devices (Aria, Del Mar Medical Systems, Irvine, CA, USA) with a frequency response of 0.05–40Hz and a resolution of 128 samples/s were used. Three leads (V_1, altered V_3 with the electrode on the left midclavicular line on the lowest rib, and altered V_5 with the electrode on the left anterior axillary line on the lowest rib) were recorded over 24 hours. The mean duration of the Holter recordings was 22.3 (SD 2.1) hours.

All recordings were scanned through a StrataScan 563 (Del Mar) and interpreted using the interactive method, with a final visual check on the full disclosure. The length of each RR interval was manually validated during this step. Resampling was made at 4Hz. Spectral analysis was performed by the fast Fourier transform method. Only normal-to-normal intervals were used, with intervals excluded due to ectopy or artefacts being replaced by holding the previous coupling interval level throughout the time interval to the next valid coupling interval. The standard deviation of all normal RR (NN) intervals (SDNN), which is a summary measure of HRV and the following frequency domain variables have been calculated: Total power (TP) (≤0.40 Hz), LF power (0.04–0.15Hz), high frequency (HF) power (0.15–0.40Hz) and the LF/HF ratio. HF power is an index of the parasympathetic modulation of heart rate, whereas LF power is an index of the combined parasympathetic and sympathetic modulation of heart rate. LF/HF represents the sympatho-vagal balance (North American Society of Pacing and Electrophysiology 1996; Sztajzel 2004).

In order to avoid a biased result due to methacholine challenge, which was part of the SAPALDIA lung function testing and which, for practical reasons, was performed before the Holter recording, we excluded the first two hours of all recordings (van der Woude et al. 2004).

Holter recordings were made between August 2001 and March 2003. Recorders were placed on participants having given consent after a detailed health interview. Participants were asked to follow their regular daily routine and to complete a time-activity diary during the recording period.

Other measurements

Body height and weight were measured with participants not wearing any shoes or coats and body mass index was calculated as weight (kg) divided by height squared (m^2). Four BMI groups were distinguished according to the WHO definitions (World Health Organization 1999): underweight (< 18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25.0-29.9 kg/m^2) and obese (≥ 30kg/m^2). In the 1991 cross-sectional study, body weight was asked. Weight gain was defined as being in a higher BMI category in the follow-up study compared to the first survey.

The amount of physical activity (Question: 'How many hours a week do you usually exercise such that you get out of breath or sweat?') (The European Community Respiratory Health Survey II 2000) and data on smoking, education, diabetes, level of daytime sleepiness (Epworth sleepiness scale) (Johns 1991; Johns 1993) and current medications status were assessed during a standardized interview which was led by trained fieldworkers. The participants were divided into tertiles of exercise: no regular physical exercise, ½ to 1 hour and ≥ 2 hours of regular physical exercise.

Blood pressure was measured twice at rest in the sitting position on the left upper arm by an automatic device (705CP, OMRON, Tokyo, Japan) according to WHO recommendations(World Health Organization 1996). Blood pressure values used in the regression model were obtained

by averaging the two measurements. Having high blood pressure was defined as either having a systolic blood pressure ≥ 140 mmHg, or a diastolic blood pressure ≥ 90 mmHg, or having answered yes to the question "Do you have the following condition: Hypertension?".

The highest degree of education was used as a proxy for social position.

Blood samples were taken from the subjects and known cardiovascular risk factors have been determined by the Institute for Clinical Chemistry of the University Hospital Zürich: A Hitachi Modular Autoanalyser (Rotkreuz, Switzerland) and assays from Roche Diagnostics (Mannheim, Germany) have been used to measure serum levels of uric acid, triglycerides and total cholesterol (all enzymatic tests). High sensitive C-reactive protein (CRP) was measured using a latex-enhanced immunoturbidimetric assay. High-density lipoprotein cholesterol (HDL) was measured with a homogenous assay (Roche diagnostics, Mannheim, Germany) using Roche Cobas Integra (Rotkreuz, Switzerland). HDL values were only used if the participants had a triglyceride level of 9.4 mmol/l or lower. As additional atherogenic markers, the difference between total cholesterol and HDL (non-HDL-cholesterol) and the ratio between total cholesterol and HDL were calculated.

Ethical approval for the study was given by the central Ethics Committee of the Swiss Academy of Medical Sciences and the Cantonal Ethics Committees for each of the eight examination areas and subjects signed an informed consent at the examination.

Statistical methods

Because an initial inspection suggested that the distribution of the residuals was skewed, the HRV-values were log-transformed for further analysis and the results are presented as percent difference between the exposure groups as well as geometric means.

To calculate the effect of BMI and physical activity on HRV, we used a multivariable regression model including variables known from a previous study to influence HRV(study site, sex, age and age squared, education, self-reported diabetes, hypertension, smoking status and beta-blocker intake in the previous 30 days) (Felber Dietrich et al. 2006).

Table 1 Baseline characteristics of the study population (n= 1703)

BMI	normal weight		overweight		obese	
n	636	(37%)	723	(43%)	344	(20%)
Female sex	400	(63%)	291	(40%)	180	(52%)
Current smokers	135	(21%)	139	(19%)	58	(17%)
Tertiary education	178	(28%)	189	(26%)	67	(20%)
Diabetes	10	(2%)	27	(4%)	34	(10%)
Beta-blocker intake	40	(6%)	88	(12%)	58	(17%)
Higher BMI cat. in follow-up	17	(3%)	348	(48%)	223	(65%)
no regular exercise	264	(42%)	295	(41%)	152	(44%)
½ - 1 h/week exercise	204	(32%)	243	(34%)	113	(33%)
≥2 h/week exercise	168	(26%)	185	(26%)	79	(23%)
Symptoms of chronic bronchitis	97	(15%)	115	(16%)	60	(17%)
Diuretic intake	8	(1%)	25	(4%)	27	(8%)
Sympathomimetic intake	11	(2%)	29	(4%)	13	(4%)
Calcium channel blocker	13	(2%)	37	(5%)	31	(9%)
ACE inhibitor intake	23	(4%)	49	(7%)	36	(11%)
Mean heart rate (bpm)	74	(SD 9.7)	74	(SD 9.0)	75	(SD 9.1)
Systolic blood pressure (mmHg)	125	(SD 19.0)	136	(SD 18.6)	138	(SD 17.0)
Diastolic blood pressure (mmHg)	78	(SD 10.2)	84	(SD 10.1)	85	(SD 10.0)
Age (years)	59.4	(SD 6.3)	60.8	(SD 6.2)	61.3	(SD 6.1)
Non-HDL cholesterol (mmol/L)	4.6	(SD 1.1)	4.9	(SD 1.2)	4.8	(SD 1.0)
Uric acid (µmol/L)	288.1	(SD 75.1)	341.8	(SD 79.9)	359.8	(SD 84.1)
C-reactive protein (mg/L)	1.9	(SD 4.5)	2.5	(SD 5.5)	3.9	(SD 5.5)
Epworth sleep score	6.3	(SD 3.6)	6.4	(SD 3.5)	7.0	(SD 3.8)

Table 2 Adjusted[a] geometric mean (GM) of HRV in different exercise categories

BMI		Normal weight		Overweight		Obese	
	Exercise (h/week)	GM	95% CI	GM	95% CI	GM	95% CI
SDNN (ms)	None	131.8	(127.5, 136.3)	128.8	(124.9, 132.8)	115.9	(111.0, 121.1)
	½ - 1	135.1	(130.2, 140.2)	131.3	(127.0, 135.8)	127.0	(120.9, 133.5)
	≥ 2	138.3	(132.8, 144.0)	137.3	(132.1, 142.8)	137.8	(129.9, 146.2)
Total power (ms^2)	None	3733.2	(3458.6, 4029.6)	3459.4	(3223.6, 3712.6)	3018.3	(2732.1, 3334.5)
	½ - 1	3816.4	(3504.8, 4155.7)	3689.6	(3415.7, 3985.4)	3517.4	(3141.0, 3938.8)
	≥ 2	4081.3	(3717.0, 4481.4)	3725.3	(3407.3, 4072.9)	3904.0	(3410.0, 4469.7)
HF (ms^2)	None	65.1	(58.0, 73.1)	68.6	(61.6, 76.3)	62.0	(53.3, 72.0)
	½ - 1	67.1	(59.0, 76.3)	64.4	(57.4, 72.4)	71.0	(59.8, 84.2)
	≥ 2	69.1	(60.0, 80.0)	77.1	(67.4, 88.2)	79.8	(65.1, 97.9)
LF (ms^2)	None	206.6	(188.9, 225.9)	217.7	(200.4, 236.4)	167.4	(148.9, 188.1)
	½ - 1	235.2	(212.9, 259.9)	223.2	(203.9, 244.3)	207.4	(181.6, 236.8)
	≥ 2	247.4	(221.7, 276.0)	240.0	(216.2, 266.5)	211.9	(180.9, 248.4)
LF/HF	None	3.2	(2.9, 3.4)	3.2	(3.0, 3.4)	2.8	(2.5, 3.1)
	½ - 1	3.5	(3.2, 3.8)	3.5	(3.2, 3.8)	2.9	(2.6, 3.3)
	≥ 2	3.6	(3.3, 3.9)	3.1	(2.8, 3.4)	2.7	(2.3, 3.0)

[a] adjusted for sex, age, age squared, study site, education, diabetes, hypertension, beta-blocker intake and smoking status

We investigated modification of the effect of BMI by physical activity by introducing according interactions terms between exercise and BMI categories into the multivariable regression model.

Sensitivity analyses screening for additional potential confounders (Epworth sleepiness score >10 vs ≤6, diuretics, sympathomimetics, calcium-channel blockers and angiotensin converting enzyme inhibitor intake during the previous 30 days; non-HDL-cholesterol, uric acid and high sensitive CRP levels) were carried out.

Statistical analyses were performed using Stata 9.2 (Stata corporation, College Station, Texas).

Results

Mean age of the population was 60 years (50-73 years). Baseline characteristics of the 1703 study participants by exercise category are given in Table 1. 37% of the study population were normal weight, 43% were overweight, and 20% obese, with a homogeneous distribution across exercise categories. Less than 1% of the study participants were morbidly obese. Between the first and the second survey, 35% had gained weight so that they changed into a higher BMI category. There was a higher proportion of women and current smokers in the normal weight group, but fewer subjects with diabetes or subjects taking beta-blockers, diuretics or angiotensin converting enzyme (ACE) inhibitor. In that same group, subjects had lower average heart rate and systolic and diastolic blood pressure, and they were on average better educated and slightly younger; they also had lower levels of non-HDL-cholesterol, uric acid or C-reactive protein.

Table 2 exhibits the adjusted means of HRV parameters for different BMI and exercise categories. Within each BMI category, subjects regularly exercising ≥ 2 hours per week had higher HRV than sedentary subjects. Even obese subjectsexercising ≥ 2 hours had a higher HRV than sedentary normal weight subjects.

Using a cubic model for BMI (continuous), subjects who exercise regularly had higher SDNN than their sedentary peers (figure showing curves of adjusted geometric means for the three exercise categories shown in annex 8). As can be deducted from these curves, SDNN in physically active subjects (≥ 2 hours of exercise per week) did not depend on BMI.

Figure 1 compares SDNN for obese subjects not exercising (reference group) to other BMI and exercise categories. Compared to obese subjects not exercising regularly, overweight subjects not

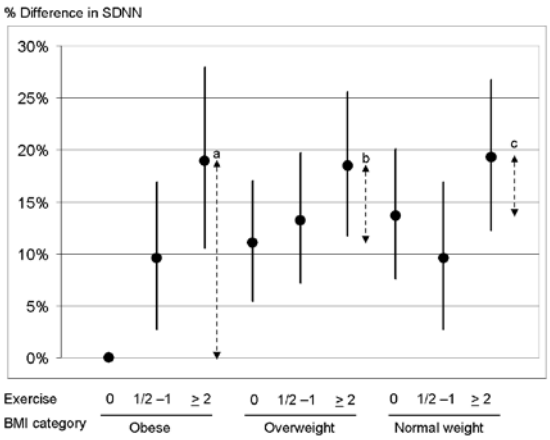

Fig. 1 Compares SDNN for obese subjects not exercising (reference group) to other subject groups stratified by BMI category (normal, overweight, obese) and exercise status. Normal weight is defined as 18.5≤ BMI < 25.0 kg/m ^{2}; overweight 25.0≤ BMI < 30.0 kg/m ^{2}; obese 30.0≤ BMI kg/m^2). Effect estimates and 95% confidence intervals. *a* Difference to sedentary obese subjects. *b* Difference to sedentary overweight subjects (95% CI: -0.196, -0.022). *c* Difference to sedentary normal weight subjects (95% CI: -0.214, -0,036)

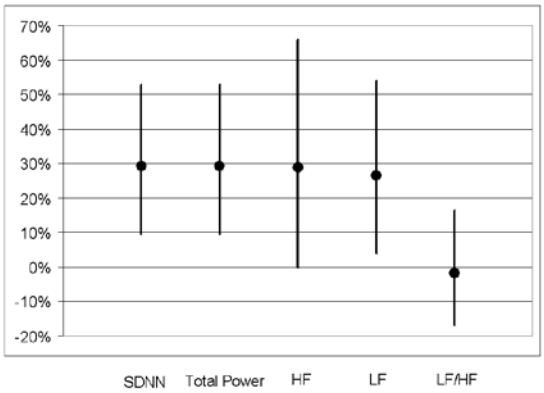

Fig. 2 Average percent increase and 95% confidence interval in measures of HRV (SDNN, total power, HF, LF) in obese subjects exercising ≥ 2 h/week, compared to sedentary obese subjects.

exercising had an 11% [95% confidence interval (95% CI: 5 to 17%)] higher SDNN and normal weight subjects not exercising had a 14% (95% CI: 8 to 20%) higher SDNN.

Regardless of weight, the improvement in SDNN was greatest for those who exercised regularly≥ 2 hours per week. Compared to obese subjects not exercising, among those who exercised regularly, SDNN was 19% (95% CI: 12 to 27%) higher in normal weight subjects, 18% (95% CI: 12 to 26%) higher in overweight subjects, and 19% (95% CI: 11 to 28%) higher in obese subjects. The difference in SDNN within each BMI category between subjects exercising regularly≥ 2 hours and sedentary subjects was highest in obese subjects. Regular exercise modified the relation of obesity with SDNN (95% CI: -0.214 to -0.036 for the difference in the effect of obesity compared to normal weight between subjects exercising regularly (i.e., ≥ 2 hours) and subjects with a sedentary lifestyle) (supplementary Table 1 shown in annex 9).

Compared to sedentary obese subjects, obese subjects exercising regularly also had 29% (95% CI: 9 to 53%) higher total power, 29% (95% CI: 0 to 66%) higher HF, 27% (95% CI: 4 to 54%) higher LF (Figure 2) and a 2% (95% CI: -17 to 16%) lower LF/HF.

Next, we analysed the influence of weight gain between the first survey and the follow-up survey on HRV. Weight gain is defined as changing into a higher BMI category—e.g., becoming overweight or obese. Compared with subjects who gained weight and did not exercise regularly, those who gained weight but did exercise regularly had a 13% higher SDNN (95% CI: 7 to 20%), 16% higher total power (95% CI: 2 to 32%), 25% higher HF (95% CI: 3 to 52%), 24% higher LF (95% CI: 6 to 44%) and a 1% (95% CI: -14 to 12%) lower LF/HF (Figure 3).

These associations were not due to a reduction in heart rate with exercise as there was no evidence for a change when heart rate was introduced into the models (Table 3). Mean 24-hour heart rate in physically active was 74 bpm (95% CI: 72 to 76 bpm) as opposed to 76 bpm (95% CI: 74 to 77 bpm) in inactive obese.

Sensitivity analyses

We tested the robustness of the results by including additional potential confounders

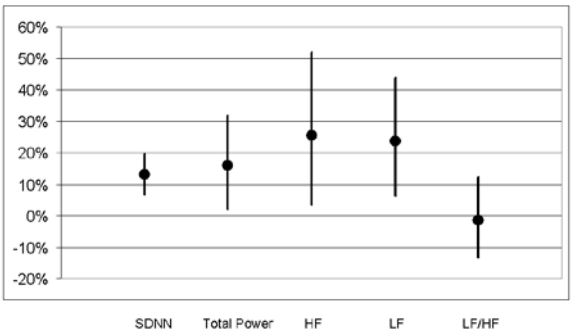

Fig. 3 Average percent increase in measures of HRV (SDNN, total power, HF, LF) in subjects with weight gain exercising ≥ 2 h/week, compared to sedentary subjects with weight gain. Weight gain is defined as changing to a higher weight category (e.g., becoming overweight or obese)

into the model. Table 3 shows that daytime sleepiness, symptoms of chronic bronchitis, heart rate, cardioactive medication intake or cardiovascular risk markers in the blood did not sizeably change the effect estimates.

We also evaluated whether daytime activity was responsible for HRV differences between populations with higher levels of exercise compared to populations with low levels of exercise. Although the estimates for the sleeping periods at night were different to the ones for the 24-hour periods, the relation between the different groups was similar to the one of the 24-hour periods, which include active periods (supplementary Table 2 shown in annex 9).

Discussion

This study shows that middle-aged and elderly obese subjects who were regularly physically active had a higher HRV than their sedentary peers even after taking into account the effects of sex, age, study site, education, diabetes, hypertension, beta-blocker intake and smoking status. In addition, the improvement in HRV associated with exercise was similar for obese and normal weight subjects. Thus, our data suggest that exercise improves autonomic function as measured by HRV. Analogously to the recently published results of Sui et al. of their study on cardiorespiratory fitness and adiposity as mortality predictors in older adults (Sui et al. 2007), we found that lower autonomic activity among inactive subjects was related to body mass index: inactive subjects who were normal weight had lower HRV than normal weight exercising subjects, but higher than inactive obese subjects.

Several potential sources of bias were considered in this study. Smoking, diabetes or hypertension, all known to have an impact on HRV (Felber Dietrich et al. 2006), were controlled for in the baseline analysis. Obesity is also associated with obstructive sleep apnoea syndrome (OSAS) (Resta et al. 2001), which in turn, has a strong influence on the autonomic nervous system (Narkiewicz and Somers 2003). Even more, obese patients with OSAS who loose weight experience an improvement in the severity of OSAS, as well as in blood pressure and cardiac autonomic regulation (Kansanen et al. 1998). We addressed this issue by additionally controlling for daytime sleepiness in sensitivity analyses but found no indication of such confounding. Furthermore, we were able to demonstrate that the observed differences in HRV between groups were not caused by differences in health status represented by symptoms of chronic bronchitis, intake of

Table 3 Sensitivity analyses adding different variables to the baseline model with the natural logarithm of SDNN as outcome variable

	Effect estimate[a]	**95% CI**
Baseline analysis	19%	11-28%
+ Daytime sleepiness[b]	23%	12-35%
+ Symptoms of chronic bronchitis[c]	19%	10-28%
+ Heart rate	16%	9-24%
+Medication[d]	19%	10-28%
+ Cardiovascular risk markers in the blood[e]	20%	11-29%

[a] Estimated %-increase in SDNN associated with regular physical (as compared to a sedentary way of life) exercise in obese, adjusted for sex, age, age squared, study site, education, diabetes, hypertension, beta-blocker intake and smoking status

[b] Epworth sleepiness score >10 vs. ≤6

[c] questionnaire data: "regular cough or phlegm, during the day or at night"

[d] diuretics, sympathomimetics, calcium channel blockers, angiotensin converting enzyme inhibitors

[e] non-HDL-cholesterol, uric acid, high sensitive c-reactive protein

cardioactive medication or cardiovascular risk markers in the blood. We also excluded the first two hours of all recordings in order to avoid a biased result due to methacholine challenge, after which full recovery without application of a beta-agonist takes 33.6 minutes (1-75 minutes) (van der Woude et al. 2004). Being a muscarinic receptor agonist, methacholine would lead to an increase in high frequency power.

Our findings are consistent with results from intervention trials comparing the beneficial effects of exercise and weight loss. Oberbach and colleagues have found that increases in adiponectin levels after four weeks of physical training disproportionally exceeded the beneficial effects of reduced percent body fat and increased fitness level (Oberbach et al. 2006). In other studies, three-month aerobic exercise intervention in overweight and obese subjects increased tissue-type plasminogen activator without evidence of changes in body mass or adiposity (Van Guilder et al. 2005), and 12 weeks of aerobic training improved insulin sensitivity in overweight and obese girls without change in body weight, percent body fat and concentrations of inflammatory markers (Nassis et al. 2005).

There are several limitations of this study. One part of the study was cross-sectional in design - and hence causality cannot be inferred from existing associations. However, we were able to assess weight gain prospectively in the whole population and the fact that weight gain is showing the same effect strengthens the argument for a causal relationship.

Our physical activity data give an overview of the subjects' activity during a relatively recent period; they do not cover lifetime or adulthood exercise. Moreover, they may include anaerobic exercise. The data on physical activity were self-reported, as in most epidemiological studies (US Department of Health and Human Services 1996). Over-reporting of physical activity, the only conceivable form of reporting bias in this context, would have led to an underestimation of the effect size. Nonetheless, the physical activity questionnaire that we used has been previously validated (Washburn et al. 1990) and used in other epidemiological studies. The comparative study concluded that self-reported sweat hours are suitable for distinguishing active from inactive subjects in epidemiological surveys. Our three activity categories allowed for such a distinction. In the European Community Respiratory Health Survey physical activity level assessed by the same tool was predictive of bronchial hyperresponsiveness (Shaaban et al. 2007). Other studies have used other exercise categories; e.g. the Nurses Health Study used four categories from zero, moderate, to vigorous exercise, to ≥4 hours/week of vigorous exercise, showing that women who exercised ≥4 hours/week had a reduced risk for sudden cardiac death compared to those not being physically active (Whang et al. 2006). The Women's Health Study showed that even as little as 1 to 1.5 hours of light-to-moderate activity are associated with lower coronary heart disease rates (Lee et al. 2001), which puts more emphasis on our results.

A given BMI may not correspond to the same degree of fatness in different people. In addition, the percentage of body fat mass is higher in women than in men for equivalent BMI (Ross et al. 1994). However, different studies have shown that BMI does sufficiently coincide with the degree of body fatness when adjusting for age and sex (Gallagher et al. 1996; Movsesyan et al. 2003). Moreover, the discriminatory power for body fat and lean mass increases with the value of BMI and is high in obese people for which our results are most pronounced (Romero-Corral et al. 2006).

A major strength of the present study was the large number of participants. Additionally, detailed information was available for numerous cardiovascular risk factors, allowing for control of potential confounders.

In conclusion, our results suggest that regular physical exercise has strong beneficial effects on cardiac autonomic nervous function, a clinically relevant predictor of cardiovascular morbidity and mortality and that exercise may offset the negative effect of obesity.

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5. Summary of main findings

5.1 Determining factors and reference values

Prevalence of cardiovascular risk factors in male and female participants of the study

In this study, we could determine the prevalence of cardiovascular risk factors in male and female participants of the SAPALDIA cohort study and their influence on measurements of HRV. Men were more frequently current smokers (23% vs. 16%), drank on average more alcoholic beverages (41% vs. 18% ≥ 1 glass of alcohol/day) and had on average a higher body mass index (BMI) (27.1 kg/m^2 vs. 26.3 kg/m^2) than women, but they more frequently reported heavy physical activity (31% vs. 20% > 1 hour/week). Men also had on average a higher blood pressure than women (137/84 mmHg vs. 127/79 mmHg). 19% of all participants took at least one cardioactive drug (angiotensin converting enzyme inhibitor, anriarrhyhtmic drugs class I or III, beta-blockers, calcium channel blockers, diuretics or sympathomimetics), 5% of the participants took even more than one of those drugs. Almost twice as many women than men took diuretics (4.7% vs. 2.4%). Mean level of uric acid in the blood was 325 µmol/L, of high sensitivity C-reactive protein (hs-CRP) 2.6 mg/L and of non high density lipoprotein cholesterol (non-HDL-cholesterol) 4.8 mmol/L. Both uric acid and non-HDL-cholesterol levels in the blood were significantly higher in men than in women. In a separate paper (not included in this thesis), we could also show a higher prevalence of renal malfunction in the female population (Nitsch et al. 2006).

Influence of cardiovascular risk factors, biomarkers for cardiovascular health and current health status on the results of heart rate variability

Women showed on average lower HRV. Moreover, increasing age was associated with lower HRV. This finding was controversially discussed in the literature so far (Cowan et al. 1994; Jensen-Urstad et al. 1997; Stein et al. 1997). This might be due to the fact that ageing curves differ between the sexes. We also found lower HRV in subjects with known cardiovascular risk factors like smoking, sedentary lifestyle, higher BMI, high blood pressure, high levels of uric acid, hs-CRP or non-HDL-cholesterol. Subjects who took beta-blockers also had on average lower HRV and HF was elevated in subjects taking antiarrhythmic drugs or beta-blockers.

Normal ageing curves for healthy men and women for various HRV measurements

We calculated normal ageing curves for men and women between 50 and 70 years of age who were non-smoking individuals without history of cardiovascular disease, high blood pressure or diabetes, and were free of medications. In men, LF/HF values clearly decrease between 50 and 70 years of age. In women, such a decrease is only observed after age 60.

5.2 Environmental tobacco smoke

Longer-term exposure to environmental tobacco smoke reduces heart rate variability

Subjects who had been exposed to environmental tobacco smoke at home or at work for more than two hours per day over the preceding year had a lower 24-hour and night-time HRV than subjects not exposed to second-hand smoke in these places. The elevated night-time values point toward a long-term effect of passive smoking on HRV.

Possible pathways for the reduced heart rate variability in association with exposure to environmental tobacco smoke

In order to test possible pathways of reduced HRV in association with exposure to environmental tobacco smoke, we also analysed heart rate and blood pressure. Both heart rate and diastolic blood pressure were higher in subjects exposed for more than two hours per day at home or at work as opposed to subjects not exposed to tobacco smoke pollution in these places. These findings together with the observation of a decreased LF power are consistent with increases in sympathetic stimulation.

5.3 Exercise and body mass index

Regular exercise improves heart rate variability

In a multivariable regression analysis, obese subjects (BMI $\geq$ 30 kg/m^2) exercising regularly $\geq$ 2 hours per week had a 19% higher standard deviation of all normal RR (NN) intervals (SDNN) than obese sedentary subjects. Within each BMI category, subjects exercising $\geq$ 2 hours per week had higher HRV values than subjects not exercising. Compared with subjects who gained weight and did not exercise regularly, those who gained weight but did exercise regularly had a 13% higher SDNN.

Adverse effects of obesity and weight gain on heart rate variability are modified by regular exercise

Exercising regularly significantly modified the relation of obesity with SDNN. The interplay terms between body mass index and ≥ 2 hours per week of physical activity in their effect on the natural logarithm of SDNN were all statistically significant. HRV in physically active persons did not depend on BMI.

6. General discussion

6.1 Discussion

In this thesis, we described normal values of HRV for a healthy population, personal and environmental determinants of HRV and modification of the effect of obesity as a risk factor for lower HRV by exercise, based on a population-based sample of 1742 24-hour ECG recordings.

As HRV is known to be associated with cardiovascular disease risk and mortality, it could serve clinically as an early marker for cardiovascular disease. To be an appropriable diagnostic tool, population-based reference values are needed (North American Society of Pacing and Electrophysiology 1996). Up to the present, such normal values based on large samples have not been published, as far as is known. We were able to present age-dependent percentile curves for 24-hour HRV in a middle-aged to elderly population, showing different ageing patterns for men and women. In men between 50 and 70 years of age, the ratio between LF and HF decreased linearly, whereas in women such a decline was only manifest after the age of 60.

To our knowledge, we were also the first to show the combined effect of a large variety of risk factors on HRV in a normal population. Independent of other risk factors, current smoking showed a strong negative effect on HRV. Higher BMI proved to be an independent risk factor for lower HRV in our data. This allows speculations on mechanisms and observations on the effect of behaviours that are known to improve cardiovascular health (Buchheit et al. 2004; Tulppo et al. 2003).

The main goal of the SAPALDIA study was to look at the effect of air pollution, including environmental tobacco smoke, on human health. Results from SAPALDIA about the association between ETS and respiratory symptoms (Leuenberger et al. 1994), respiratory function (Kunzli et al. 2000) as well as the role of bronchial hyperreactivity in the development of respiratory symptoms in subjects exposed to ETS (Gerbase et al. 2006)

have been published. In this thesis we looked at the effect of ETS on HRV and at possible pathways. We were able to demonstrate that exposure to passive smoking is associated with lower HRV, and we showed that not only 24-hour HRV was lower in exposed subjects, but also night-time values of HRV, thereby suggesting a longer-term effect of second-hand smoke on the autonomic nervous system. To date, the only other study which has been published on the association between exposure to ETS and HRV showed acute effects (Pope et al. 2001). The distribution of the HRV frequency and a concurrent association between exposure to ETS and higher heart rate as well as a tendency for lower blood pressure allowed us to draw conclusions on the mechanism of the risk factor on the cardiovascular system.

In a furder step, we then were interested to see if the negative effect of a risk factor could be modified by advantageous behaviour. We were able to show that regular exercise improves HRV in normal weight, overweight and obese subjects. We also demonstrated that being physically active can override the inauspicious effects of obesity on cardiac autonomic control and that HRV in physically active persons does not depend on BMI, a finding which has not been previously described.

6.2 Limitations

Causality

There are several limitations of this study. According to Bradford Hill's considerations of causality, exposure must precede illness (Hill 1965). The cross-sectional character of this study makes causal inference difficult. However, we know the weight gain in a prospective way and our cross-sectional analyses are in line with results from cohort studies where such information is available (Gulli et al. 2003; Hirsch et al. 1991; Jurca et al. 2004).

Questionnaire data

Some risk factors which have been used in this study were assessed in the interview. This makes the information less reliable than if measured in a less subjective way. This problem is most obvious for physical activity, which has been assessed by four different questions. Those questions have been validated (Washburn et al. 1990) and already been used in other studies (The European Community Respiratory Health Survey II 2000). In addition, we have data on physical activity from the time activity diary for validation (data not yet appropriable).

Measurements

Because the interpretation of HRV is to a high degree dependent on the study population and the circumstances of recording, comparability with other studies is limited.

If in future HRV should also clinically be used as a marker of cardiovascular health, we also have to be alert to several difficulties. Its measurement requires normal sinus rhythm and reasonable signal quality. In patients with atrial fibrillation, sinoatrial dysfunction, and >20% ectopic complexes its use is precluded (Kleiger et al. 2005), whereas in the normal population such arrhythmias are not very frequent (Aronow 2006). Signal quality and elimination of background noise requires great care in the preparation of the skin and the applying of the electrodes. Careful editing of the recording is necessary to exclude ectopic complexes and artifacts from the calculations and clinical scanning is not usually adequate for detailed HRV analysis. The best predictors of cardiovascular mortality require rather long recording periods in order to include both night-time and day-time periods, inducing a considerable amount of work for the editing.

Another problem when measuring HRV is of accurately locating the successive R-wave peaks on the ECG. This requires a robust R-wave detector algorithm. The more accurate the R-wave detector, the less error in the analysed HRV spectrum. A completely missed R wave will cause greater error than a slightly miscorrected R wave, and this error is reflected more in the HF than in the LF of the HRV spectrum (Reed et al. 2005).

HRV variables

Although research has made considerable advances in the field, it is still unclear as to which HRV variable suits best as a predictor for cardiovascular mortality; conventional time domain, baroreflex sensitivity, heart rate turbulence, spectral measures, geometric measures, and a variety of nonlinear variables reflect different aspects of HRV and have all been significantly associated with outcome without clear, consistent superiority for any of the variables (Kleiger et al. 2005).

6.3 Strengths

A major strength of this study was the large number of participants, who were a random selection of the population aged ≥ 50 of eight different areas of Switzerland. To our knowledge, no other study has presented 24-hour HRV results from a population-based sample of this size. The collected data allowed us to detect sex differences in the age-related

decline of HRV and to study independent associations of a variety of cardiovascular risk factors with HRV.

6.4 Outlook

Implications for public health practice

This study adds to the evidence of desirable or disadvantageous effects of different factors on cardiovascular health. The implications from the described results for public health practice should be to develop or pursue strategies of cardiovascular prevention, i.e. among other things prevention of tobacco smoke exposure, of obesity and of sedentary lifestyle, especially in obese persons. Public health policies must focus on prevention of all major risk factors simultaneously, using lifestyle approaches from early ages onwards to reduce population cardiovascular risk. The prevention strategies should aim to be effective on different levels, from primordial over primary and secondary to tertiary on an individual as well as on a community level. Primordial prevention, i.e. social, legal and other activities which may lead to a lowering of risk factors, seems to be especially suited for the prevention of exposure to environmental tobacco smoke. Non-smokers should be prevented from exposure to ETS, if necessary by legal ways. Ticino was the first canton in Switzerland to adopt a law for smoke-free restaurants, which will come into force in April 2007. A scientific evaluation of the health consequences of the affected persons would bring more insight into topics discussed in this thesis. The implementation of such laws might also lead to a decrease in active smoking, another important risk factor of cardiovascular disease.

HRV might in future play a role in cardiovascular disease risk stratification of the asymptomatic population for targeted prevention. As stated in the guidelines for prevention by the World Heart and Stroke Forum, a combination of risk factors should be considered for assessing total CVD risk (Smith et al. 2004). Several tools have been developed for risk estimation, most of which are derived from the Framingham Study (Anderson et al. 1991; D'Agostino et al. 2000; Wilson et al. 1998). HRV could be a measure which, in addition to traditional cardiovascular risk factors, helps to identify subjects for which primary prevention is especially effective. Since HRV is influenced over pathways involving the autonomic nervous system, it can be speculated that in subjects with low HRV, preventive measures should aim at improving the autonomic balance. Further research is needed to answer these questions.

HRV measurements could be an eligible tool for secondary cardiovascular prevention, i.e. screening of symptomatic patients, and for the control of the success of tertiary prevention. It

could also serve to detect those at greatest risk (individual approach) and to determine the effectiveness of prevention programs.

Outlook for public health research

The original and main reason for SAPALDIA was to study the consequences of exposure to air pollution on human health. This question is still unanswered for cardiovascular health. A next step will be to look at the effect of exposure to different air pollutants on HRV. In this context, there are further questions which are of interest, for example the association of polymorphisms in blood coagulation genes and HRV, and their interaction with air pollution.

As mentioned in the discussion, the present work has a cross-sectional design. In order to be able to answer the question of causality, a further follow-up study is needed. In this follow-up study, the following research questions could perhaps be answered:

- Is HRV usable as an early marker of cardiovascular health in the normal Swiss population?
- Which other cardiovascular risk factors have to be considered for a risk stratification of the asymptomatic population?
- Which part of cardiovascular risk is explained by HRV and which part by other risk factors?
- What is the influence of longer-term exposure to environmental risk factors on HRV?
- Is there a modification of the effect of longer-term exposure to environmental risk factors on HRV by genetic markers?

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8. Appendices

8.1 Instructions for fieldworkers

8.1.1 Standard operating procedure for the ECG recording

1. Ziel:
Das Ziel dieser Untersuchung ist eine kontinuierliche Aufzeichnung der Herzstromkurve während mindestens 20 Stunden.

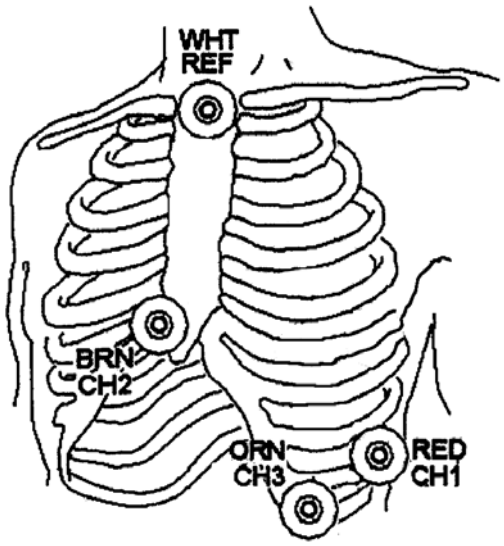
2. Information für Fieldworker:
Die Aufzeichnungen werden an unseren Partner in Frankreich (Dr. JC Barthélémy, Université de St-Etienne) gesandt, welcher die Analyse der Herzrhythmusvariabilität und von ST-Segment-Senkungen vornimmt.

3. Informationen für Probanden:
Die Probanden erhalten ein Informationsblatt mit den wichtigen Informationen (siehe Anhang).
Die Probanden dürfen während der 20-stündigen EKG-Aufzeichnung bis auf Duschen, Baden oder Sauna alle Aktivitäten ausüben; sie sollen ihrem normalen Tagesablauf nachgehen. Es ist wichtig, dass die Probanden nur im Notfall (z.B. bei allergischer Reaktion auf die Elektroden) das EKG vor Ablauf der 20 Stunden abnehmen.

4. Aus- und Einschlusskriterien:
Ausschlusskriterien:
- Träger von Herzschrittmachern
- Narkose oder Spinalanästhesie innerhalb der vergangenen 8 Tage
- Probanden und Probandinnen, welche jünger als 50 Jahre alt sind (Einschluss ab dem 50. Geburtstag)

5. Vorbereitung:
1. Ableitungskabel aus dem EKG-Rekorder ziehen
2. Kartonschachtel für den Rückversand bereit machen
   mit CHF 3.60 frankieren
   mit „A Priority“ kennzeichnen
   Adressettikette prüfen
   Ersatzelektroden, Klebstreifen und Polstermaterial in die Schachtel legen
3. Tasche für das Tragen des EKG Rekorders um den Hals aus TubeGaze vorbereiten

6. Untersuchungsablauf:
1. Die Elektroden werden nach der Blutentnahme befestigt.
2. Der Proband soll auf der Untersuchungsliege liegen und den Oberkörper entkleidet haben (auch BH).
3. Auffinden der vier Stellen, wo die Elektroden befestigt werden, durch genaue Palpation. Die rote und orange Elektrode sollen möglichst auf der untersten Rippe zu liegen kommen. Falls jedoch durch Kleidungsstücke (z.B. Hosenbund) Aufzeichnungsstörungen verursacht werden können, müssen diese Elektroden auf einer Rippe weiter kranial platziert werden.*



Weiss:	Mitte des Manubriums
Rot:	linke vordere Axillarlinie, **auf** der untersten Rippe*
Braun:	2 cm rechts des Processus xyphoideus am Rippenrand
Orange:	Medioclavicularlinie, **auf** der untersten Rippe*

4. Sehr gründliches Reinigen der Haut an den 4 Stellen mit einem Alkoholtupfer.
5. Rasieren eines kleinen Areals über den vier Stellen falls nötig.
6. Abwischen der Haut mit trockener Gaze
7. Aufkleben der Elektroden an den entsprechenden Stellen. Nicht auf das Zentrum der Elektrode drücken, da sonst der Kontaktgel unter die Klebefläche quillt.
8. Anschliessen der Ableitungen gemäss Schema.
9. Ableitungskabel gemäss Farbcode fest in den EKG-Rekorder einstecken.
10. Ankleben der Ableitungen, damit Bewegungen des Probanden möglichst keine Störungen bei der Aufzeichnungen verursachen.
11. Einlegen der Batterie ins Batteriefach des Rekorders. Es ertönt ein Piepston.
12. Langes Drücken auf den Knopf mit dem Herz-Symbol bis mehrere kurze Piepstöne und ein langer Piepston zu hören sind. Damit beginnt die EKG-Aufzeichnung.
13. Einlegen des Rekorders in die Tasche und Umlegen um den Hals
14. Notieren der Rekorder-Nummer auf dem Tagebuch und der Startzeit auf dem Laufblatt
15. Notieren der Zeit auf dem Tagebuch, wann das Gerät frühestens entfernt werden darf (= aktuelle Zeit + 20 Stunden; aufrunden auf nächste volle Viertelstunde)
16. Erklären von Tagebuch und Fragebogen. Abmachen mit der Probandin/dem Probanden, ob sie das Gerät selbst entfernen oder ob sie dafür ins Zentrum kommen wollen.

Entfernen des Rekorders

1. Entfernen der Batterie aus dem Rekorder. Die Batterie reicht nur für eine Aufnahme und soll sofort in die Recycling-Box geworfen werden (Ausnahme bei Verwendung von wiederaufladbaren Akkus), damit sie nicht fälschlicherweise nochmals gebraucht wird.
2. Notieren der genauen Zeit auf dem Laufblatt
3. Vorsichtiges Entfernen der Klebestreifen
4. Lösen der Ableitungen von den Elektroden
5. Lösen der Elektroden von der Haut. Die Elektroden werden nach einmaligem Gebrauch weggeworfen.
6. Reinigen der Haut von Kleb- oder Kontaktgelrückständen
7. Reinigung des Gerätes und der Ableitungen mit Desinfektionsmittel, wobei kein Desinfektionsmittel in das Gerät gelangen darf
8. Bei Verwendung von Akkus Wiederaufladen derselben

7. <u>Rückgabe:</u>

1. Überprüfen, ob die Rückgabe komplett ist: Rekorder mit 4 Ableitungskabeln, Tagebuch ausgefüllt, Kardio-Fragebogen ausgefüllt, wiederaufladbarer Akku
2. Überprüfen, ob Batterie/Akku aus dem Gerät entfernt wurde
3. Reinigung des Gerätes und der Ableitungen mit Desinfektionsmittel, wobei kein Desinfektionsmittel in das Gerät gelangen darf

Die Kartonschachtel, in der die Rückgabe erfolgt, kann mehrmals verwendet werden.

8. Laden der Daten auf den Computer:

1. Wahl von Windows 98 beim Aufstarten des PCs
2. Doppelklicken auf dem Desktop auf das Icon HOLTER 98
3. Drücken der Taste „F2“ oder Klicken auf „F2 NEUER PAT.“.
4. Eingeben der Probanden-Nummer (z.B. 9999/160) bei NAME, REPORT NUMMER und ID #.
5. Eingeben der Startzeit der EKG-Aufzeichnung bei START TIME, des Anfangsdatums bei TEST DATE, des aktuellen Datums bei REPORT DATE, des Alters, des Geschlechts bei SEX (weiblich: f; männlich: m), der Fieldworker-Nummer (z.B. 160/02) bei PHYSICIAN und wählen von ARIA bei RECORDER TYPE.
6. "ESC" und danach "YES" wählen
7. Entfernen der Ableitungen durch senkrechtes Ziehen an den farbigen Steckern.
8. Einstecken des Downloadkabels in den Rekorder.
9. Wieder am PC: markieren des gewünschten Probanden durch anklicken. Als Hintergrund erscheint ein blauer Balken.
10. Drücken der Taste „F3“ oder auf „F3 LADEN“ drücken.
11. Wenn das rote Fenster „ECG Data Download Aria Recorder“ erscheint, die Taste “ESC” drücken
12. Beim nächsten Fenster „1“ wählen
13. Beim nächsten Fenster mit „Y“ bestätigen. Der Download beginnt
14. Kontrollieren, ob beim entsprechenden Probanden bei Status „LAD“ steht. Falls das nicht der Fall ist, ist ein Fehler unterlaufen. In diesem Fall nochmals Punkte 8 bis 13 wiederholen.
15. zum Verlassen des Programmes dürfen keine Patienten ausgewählt sein (kein hellblauer Balken).

15.1 „F8“
15.2 „Exit to Desktop“ anklicken
15.3 Bestätigen mit „j“

16 Einstecken der Ableitungen in den Rekorder gemäss Farbcode.

Überspielen der Daten auf CD-ROM

17 Die EKG-Aufzeichnungen werden 1x wöchentlich auf einen CD-Rohling überspielt und ans ISPM Basel gesandt.
18 Wahl von Windows 98 beim Aufstarten des PCs
19 Einlegen einer unbeschriebenen CD in das CD-Laufwerk
20 Formatieren der CD-R
20.1 im Fenster von directCD „CD formatieren“ wählen
20.2 im Fenster "Kennung" CD mit Ort und aktuellem Datum benennen (z.B. "Basel 121101" für eine CD, welche am 12.11.01 in Basel gebrannt wurde)
20.3 auf „Formatierung beginnen“ klicken
20.4 im Fenster „CD bereit“ auf „OK“ klicken
21 Auf dem Desktop auf „Holter98“ doppelklicken
22 Drücken der Taste „F8“ oder „F8 EXIT“ anklicken
23 „Build Optical Disk“ wählen
24 mit “ESC” zurück zur Patientenauswahl
25 Auswählen der Daten, welche kopiert werden sollen durch anklicken der entsprechenden Zeile (Auswahl wird durch hellblauen Hintergrund markiert)
26 „ALT" drücken und ohne loszulassen "F8" drücken: Die Daten werden auf CD gebrannt
27 zum Verlassen des Programmes dürfen keine Patienten ausgewählt sein (kein hellblauer Balken).
27.1 „F8“
27.2 „Exit to Desktop“ anklicken
27.3 Bestätigen mit „j“
28 Beim Auswurf der CD erscheint ein Dialogfeld: „unverändert lassen“ wählen

9. Resultate

Die Probanden erhalten in der Regel ca. 4 Wochen nach der Untersuchung ihre Resultate schriftlich (Vgl. Anhang „Probandeninformation“). Falls jedoch ein gravierender pathologischer Befund entdeckt würde, werden sie schnellstmöglich informiert.

8.1.2 Standard operating procedure for the measurement of blood pressure

1. Ziel: Zweimaliges Messen des systolischen und des diastolischen Blutdruckes in Ruhe.

2. Information für Fieldworker:
Der OMRON 705CP ist ein vollautomatisches Blutdruckmessgerät, welches mit der oszillometrischen Methode misst. Nach dem Aufpumpen der Manschette bis zum Verschluss der Arteria brachialis wird der Druck automatisch kontinuierlich verringert. Mit sinkendem Manschettendruck öffnet sich die Arterie, wobei sich Druckschwankungen verstärken, welche schliesslich wieder auf kleinere Oszillationsamplituden abfallen. Diese Schwingungen werden vom Gerät registriert und der systolische und diastolische Wert daraus errechnet[1].
Gemäss WHO sollten die Blutdruckwerte idealerweise < 120/80 mmHg liegen. Normaler Blutdruck wird mit Werten < 130/85 mmHg definiert, Hypertonie mit Blutdruckwerten ≥ 140/90 mmHg. Weil der Blutdruck grossen Schwankungen unterliegt, sollte die Diagnose „Hypertonie" erst nach wiederholten Messungen gestellt werden[2].
Eine Internetsite der WHO liefert gute Hintergrundinformation zum Thema Hypertonie: http://www.who.int/ncd/cvd/PracticeGuidelinesSlideset2/index.htm

3. Information für Probanden:
In einigen internationalen Studien wurden Hinweise für einen Zusammenhang zwischen Luftverschmutzung und Gesundheit von Herz und Kreislauf gefunden. In SAPALDIA 2 messen wir unter anderem den Blutdruck, um solche Zusammenhänge zu erforschen.

4. Aus- und Einschlusskriterien

Einschlusskriterien:
Der Blutdruck wird bei allen Probanden gemessen.

5. Vorbereitung:

Zeitpunkt der Untersuchung
Die Blutdruckmessungen finden nach dem Interview statt. (Der Proband muss mindestens 10 Minuten gesessen haben und sich an die Umgebung gewöhnt haben. Idealerweise findet die Blutentnahme nicht vor dieser Messung statt.)

1. Messung am nackten Oberarm links
   (Ausnahme: Strahlentherapie oder Lymphknotenausräumung in der Axilla z.B. nach Mamma-Carcinom, Shunt am linken Arm bei Dialysepatienten, Lähmung oder Kontrakturen am linken Arm: Vermerk „rechts" unter Problem-Feld).
   Hochgekrempelte Bekleidung darf den Arm nicht einschnüren. Allenfalls ist die Messung über einem dünnen Kleidungsstück möglich.
2. Der Proband soll bequem sitzen und den linken Arm so auf dem Tisch aufstützen (eventuell mit Kissen), dass sich die Ellenbeuge auf Herzhöhe (vierter Interkostalraum) befindet.
3. Messen des Oberarmumfanges links an der dicksten Stelle mit dem Massband.
   Umfang 22-32 cm: Standardmanschette / Umfang 32-42 cm: extragrosse Manschette (protokollieren bei Verwenden der extragrossen Manschette bei „Probleme").
4. Einschalten des Blutdruckmessgerätes (Taste „O/I)
   Es leuchten alle Symbole auf der Anzeige während etwa 2 Sekunden, was zur Überprüfung der Anzeige dient. Dann erlöschen alle Symbole, und das Luftablass-Symbol (⇩) beginnt zu blinken. Wenn die Messvorbereitungen abgeschlossen sind, erscheint das Messbereitschafts-Symbol (♥) auf der Anzeige.

6. Untersuchungsablauf:

[1] Gebrauchsanweisung OMRON 705CP.
[2] http://www.who.int/ncd/cvd/PracticeGuidelinesSlideset2/index.htm

1. Manschette so am linken Oberarm anlegen, dass Unterkante 2-3 cm oberhalb der Ellenbeuge liegt und sich die grüne Markierung über der Arteria brachialis befindet. Die Manschette soll satt anliegen aber den Arm nicht einschnüren.
2. Der Proband wird aufgefordert, während der Messung nicht zu sprechen und sich nicht zu bewegen.
3. Drücken der Start-Taste (◇).
   Das Gerät pumpt nun automatisch bis zum Erreichen des gewählten Druckwertes auf (erweist sich der eingestellte Wert als nicht ausreichend, pumpt das Gerät automatisch weiter auf, bis der Druck für die Messung ausreicht), wonach die Luft aus der Manschette automatisch abgelassen wird. Das Druckabfall-Symbol (⇩) zeigt den Entlüftungsvorgang an. Sobald der Pulsschlag erkannt ist, beginnt das Symbol (♥) zu blinken, und gleichzeitig ertönt ein Piepen. Wenn die Luft vollständig entwichen ist, erscheint das Symbol (♥) auf der Anzeige, und es werden Blutdruck und Puls abwechselnd ca. 5 Minuten lang angezeigt.
4. Ausdrucken der Messwerte durch drücken der Taste „P“
5. Wiederholung der Punkte 7.-9. nach mindestens 3 Minuten.
   Idealerweise sollten die Messungen nicht viel länger als 3 Minuten auseinander liegen. Die Messzeiten sind auf dem Ausdruck ersichtlich.
6. Aufkleben der ausgedruckten Resultate auf die vorgesehene Stelle auf dem Laufblatt.
7. Nochmaliges Ausdrucken der letzten Messwerte, zusammen mit dem Blatt „Blutdruckmessung“ dem Probanden mitgeben.

7. <u>Qualitätskriterien:</u>

Errormeldung:

Falls bei einer Messung eine Errormeldung erscheint, Messung nach mindestens 3 Minuten wiederholen.

- Falls nach 2 Versuchen keine Messung erfolgreich war, durch Palpation des Pulses überprüfen, ob eine Arrhythmie vorliegt. Bei Bestätigung einer Arrhythmie Eintrag „Arrhythmie“ unter Probleme und Information des Probanden (Empfehlung einer Konsultation beim Hausarzt, falls Problem nicht schon bekannt ist).
- Bei Wiederholung der Messung wegen Errormeldung Vermerk unter Probleme, z.B. „1. Messung Errormeldung“

„Unwahrscheinliche“ Messresultate

Der Blutdruck unterliegt einer grossen Variabilität, auch wenn zwei Messungen innerhalb von nur einigen Minuten erfolgen. (Varianz bei zwei Messungen innerhalb von 5 Minuten: 26.5 mm^2Hg beim systolischen Blutdruck, 11.8 mm^2Hg beim diastolischen[3]).

Falls die Messwerte in einem „unwahrscheinlichen“ Bereich liegen (Proband sagt zum Beispiel, dass er sonst ganz andere Blutdruckwerte habe):

- Wurde die richtige Manschettengrösse verwendet?
- Liegt eine Arrhythmie vor (vgl. oben)?

Es werden immer nur die zwei ersten Messergebnisse nach korrekter Messung an das Laufblatt geheftet. Falls aus irgendeinem Grund (wenn z.B. der Proband beunruhigt ist und eine zusätzliche Messung wünscht) eine dritte Messung erfolgt, wird dieses Resultat nicht zu den Unterlagen genommen.

Bei anderen Problemen mit dem Gerät die Gebrauchsanleitung konsultieren.
Lieferant: Advance AG, Wädenswil: 01 782 68 78

Qualitätskontrolle

Die verschiedenen Geräte sind nummeriert und die Studienleitung hat die Übersicht über den Standort und Prüfstatus der einzelnen Geräte.
Die Geräte wurden vor dem 1. Gebrauch auf ihre Genauigkeit geprüft. Diese Prüfung wird im Laufe der Studie wiederholt, wobei das Untersuchungszentrum eine Aufforderung erhält, das zu prüfende

[3] Klungel OH, de Boer A, Paes AHP, Nagelkerke NJD, Seidell JC, Bakker A. Estimating the prevalence of hypertension corrected for the effect of within-person variability in blood pressure. Journal of Clinical Epidemiology 2000; 53: 1158-63.

Gerät einzusenden und für diese Zeit ein Ersatzgerät erhält. Bei Verwenden des Ersatzgerätes auf dem Papierstreifen mit den Resultaten Gerätenummer notieren.

8. <u>Datentransfer</u>

Anhang

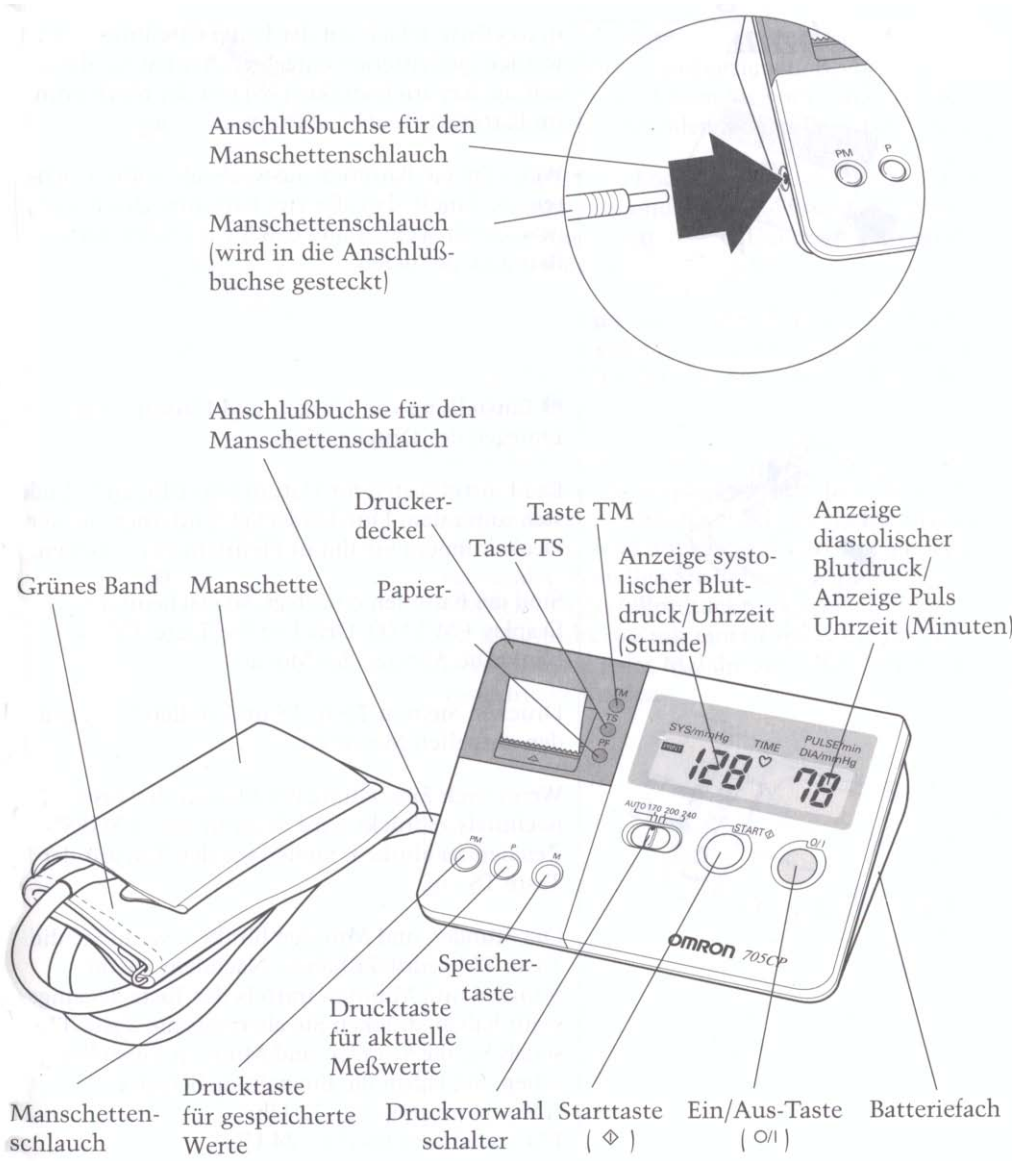
Inbetriebnahme des Blutdruckmessgerätes:

1. Anschliessen des Messgerätes an Stromquelle mit Netzteil (separates Zubehör).
2. Einstellen von Uhrzeit und Datum:

Drücken der Taste „TM“. Die Anzeige des Monats beginnt zu blinken.
Mehrfaches drücken der Taste „TS“ zur Einstellung des aktuellen Monats.
Drücken der Taste „TM“. Die „TAGES“-Zeichen beginnt zu blinken.
Mehrfaches Drücken der Taste „TS“ zur Einstellung des Tages.
Drücken der Taste „TM“. Die Anzeige von Stunden und Minuten beginnt zu blinken.
Mehrfaches drücken der Taste „TS“ zur Einstellung der aktuellen Zeit.
Drücken der Taste „TM“.
Kontrollieren von Datum und Uhrzeit: AM = vormittags (0-12 Uhr), PM = nachmittags (12-24 Uhr). Erneute Kontrolle zu Beginn jeder Woche (Achtung: Wechsel Sommer-/Winterzeit!)

3. Druckerpapier einlegen

Entfernen des Klebestreifens von der Rolle und Geradeschneiden des Papierendes mit einer Schere.
Einführen des Papierstreifenendes in den Papierschlitz.
Drücken Sie der Papiertransporttaste „PF“ und Herausführen des Papiers bei der Abrisskante.
Einlegen der Papierrolle in das Fach.
Durch die Öffnung im Druckerdeckel ziehen des Streifens und Schliessen des Druckerdeckels.
Einstellen Druckvorwahlschalter auf 170 mmHg.


Anschlußbuchse für den Manschettenschlauch
Manschettenschlauch (wird in die Anschlußbuchse gesteckt)
PM
P
Anschlußbuchse für den Manschettenschlauch
Drucker-deckel
Taste TM
Taste TS
Anzeige diastolischer Blutdruck/ Anzeige Puls
Grünes Band
Manschette
Papier-
Anzeige systolischer Blutdruck/Uhrzeit (Stunde)
Uhrzeit (Minuten)
SYS/mmHg
TIME
PULSE/min
DIA/mmHg
128
78
OMRON 705CP
Speicher-taste
Drucktaste für aktuelle Meßwerte
Manschetten-schlauch
Drucktaste für gespeicherte Werte
Druckvorwahl schalter
Starttaste ( )
Ein/Aus-Taste (O/I)
Batteriefach

8.2 Procedure for analyses of electrocardiograms

by Jean-Claude Barthélémy and Frédéric Roche, Université Jean Monnet, Saint-Etienne, France, 2004

Standard 24-hour Holter Monitoring

Standard 3-channel Aria® ECG Holter recorders were used to acquire the data (Del Mar, Irvine, CA). All recordings were scanned through a StrataScan 563 (Del Mar) and read using the interactive method, followed by a final visual check of the full disclosure. The electrocardiographic Holter system allowed to extract the list of RR intervals with a precision of 1/128 second. The length of each RR interval was manually validated during this step.

Arrhythmia analysis:

- *Pauses*. An R-R interval was considered to be a pause when it reached or exceeded 3.5 seconds.
- *Bradycardia*. Bradycardia was defined as a rhythm below 40 beats per minute.
- *Ventricular ectopic activity*. Isolated and grouped ventricular ectopic activities were analysed. Non-sustained ventricular tachycardia was defined as 3 or more consecutive ventricular ectopic complexes, at a rate faster than 120 beats per minute and lasting less than 30 seconds. The severity of ventricular arrhythmias was evaluated by the mean number of ventricular premature complexes (VPCs) per hour (threshold value : „ 30/h).
- *Supraventricular ectopic activity.* Isolated and grouped supraventricular ectopic activities were analyzed separately. Supraventricular tachycardia (SVT) was defined as more than 6 consecutive supraventricular ectopic complexes, at a rate higher than 120 beats per minute. Sustained SVT was defined as more than 30 seconds of supraventricular ectopic complexes at a rate faster than 120 beats per minute.

Three recording periods were defined: a "nocturnal" period, a "diurnal" period and "full recording" period from the beginning to the end of the recording.

Heart rate variability analysis

Time Domain Analysis:

The r-MSSD (root mean square successive differences, the square root of the mean of the sum of the squares differences between adjacent normal RR intervals) and pNN50 (percent of adjacent normal RR intervals which differ by more than 50 msec computed over the entire recording) indices were considered as the parasympathetic tone component. The SDNN (standard deviation of all normal RR intervals in the entire recording), SDANN (standard deviation of the mean of all 5-minute segments of the normal RR intervals) and SD Index (mean of the standard deviations of all normal intervals for all the 5-minutes segments of the all ECG recordings) indices were also computed.

Frequency Domain Analysis:

Spectral analysis was performed by the Fast Fourier Transform method using sliding 256 PTAs Hanning windows. Only normal-to-normal intervals were used, with intervals excluded due to ectopy or artefacts being replaced by holding the previous coupling interval level throughout the time interval to the next valid coupling interval

Power spectrum indices were calculated as recommended by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. The high frequency peak of the spectrum (HF, 0.15 to 0.40 Hz) is known to represent parasympathetic activity, while the low frequency (0.04 to 0.15 Hz) represents both parasympathetic and sympathetic activities. Additional calculations were included: the very low frequency power (0 to 0.04 Hz); the ratio LF/HF, which represents an evaluation of the autonomic nervous system balance (sympathetic/parasympathetic); the normalized low and high frequency power (LF nu and HF nu) as 100*LF/(total power - VLF) and 100*HF/(total power - VLF), respectively; and the total frequency power (Ptot).

Wavelet Transform:

Then, autonomous nervous system activity was evaluated using time-frequency analysis of the RR variability signal. This method and the meaning of the usual indices have been described and validated in published studies.

Unlike Fourier, Wavelet transform is devoted to the analysis of non-stationary signal. Thus, there is no prerequisite regarding the stability of the frequency content along the signal analysed. This analysis is devoted to the extraction of characteristic frequencies, contained along a signal which, in this case, was composed by consecutive intervals between RR interval series. The decomposition of a signal by Wavelet transform requires a function adequately regular and localised, named Mother function. Starting from this initial function, a

family of functions is built by dilatation and translocation, which constitutes the so-called Wavelet frame.

The analysis amounts to sliding a window of different weights (corresponding to different levels) containing the Wavelet function, all along the signal. The calculation gives a serial list of coefficients named Wavelet coefficients, which represent the evolution of the correlation between the signal f and the chosen Wavelet at different levels of analysis (or different ranges of frequencies) all along the signal f.

In the present analysis, we used the Daubechies 4 Wavelet transform. For each record, the Wavelet coefficients were calculated on sets of 256 RR intervals, giving seven separate levels of analysis named 2, 4, 8 ... 128. Then, we calculated the variability power, level by level, as the sum of squares of the coefficients. Thus, we obtained, for each recordings, the variability power for each level.

The sum of Wavelet power coefficients at levels 2, 4 and 8 (HFWavelet), approximately corresponds to the Fourier High Frequencies (an index of parasympathetic activity); Wavelet power coefficients at levels 16 and 32 (LFWavelet) roughly corresponds to the Fourier Low Frequencies; Wavelet power coefficients at levels 64 and 128 (VLFWavelet) to the Fourier Very Low Frequencies; and the ratio LFWavelet/HFWavelet to the Fourier ratio.

Pertinent HRV variables for the autonomic status were calculated for daytime, night-time and full recording periods.

8.3 Results of simultaneous ECG recordings

Table of one-way analysis of variance for the simultaneous recordings (n=20)

Variable	Standard deviation of participant effect	Standard deviation of measurement error	Intraclasscorrelation
SDNN	41.4	3.6	0.996
Total Power	1236.8	597.7	0.895
HF	83.3	8.1	0.995
LF	244.9	8.0	1.000
VLF	178.1	12.5	0.998
ULF	2450.4	604.2	0.971

8.4 Results of double ECG analyses

Table of one-way analysis of variance for the double recordings (n=9)

Variable	Standard deviation of participant effect	Standard deviation of measurement error	Intraclasscorrelation
SDNN (24h)	26.7	0.5	1.000
SDNN (day)	25.9	4.6	0.984
SDNN (night)	23.0	10.7	0.903
Total Power (24h)	2277.9	59.3	1.000
Total Power (day)	509.0	98.5	0.981
Total Power (night)	648.7	185.4	0.961
HF (24h)	31.4	8.7	0.963
HF (day)	30.5	13.7	0.908
HF (night)	64.5	10.8	0.986
LF (24h)	104.6	3.8	0.999
LF (day)	125.2	11.1	0.996
LF (night)	115.5	19.7	0.986
VLF (24h)	342.8	9.6	1.000
VLF (day)	400.9	68.7	0.986
VLF (night)	498.1	160.5	0.951
ULF (24h)	1948.9	43.1	1.000

8.5 Time activity diary

Langzeit-EKG

Fragebogen und Tagebuch

Hier Etikette mit Probanden-ID aufkleben

Recorder-Nr.: _____

Ende frühestens _____

Information

Das Elektrokardiogramm (EKG)
Der EKG-Rekorder zeichnet den elektrischen Strom auf, welcher im Herzen generiert wird. Dieser Strom ist dafür verantwortlich, dass sich die Herzmuskulatur rhythmisch zusammenzieht und somit das Blut zirkulieren lässt.

Das Langzeit-EKG (Holter-EKG)
Das Langzeit-EKG wird üblicherweise verwendet, um Herzrhythmusstörungen oder Durchblutungsstörungen des Herzmuskels zu erkennen, wenn ein Verdacht auf eine solche Krankheit vorliegt. In SAPALDIA 2 zeichnen wir die Herzstromkurve während 20 Stunden auf und beurteilen anschliessend den Herzrhythmus sowie dessen Variabilität und wir suchen nach Anzeichen einer Durchblutungsstörung im Herzen. Diese Analysen werden von unserem Forschungspartner in Frankreich, einem Spezialisten auf dem Gebiet der Herz- und Lungenphysiologie, gemacht, weshalb Sie im Normalfall ungefähr 4 Wochen warten müssen, bevor Sie von uns das Resultat dieser Untersuchung erhalten.

Was Sie während der EKG-Aufzeichnung beachten müssen
Sie dürfen während der EKG-Aufzeichnung Ihrem gewohnten Tagesablauf nachgehen – einzig das Baden oder Duschen müssen Sie während dieser Zeit unterlassen. Behalten Sie während der Nacht das Gerät in der Tragetasche um den Hals.

Wie Sie das Gerät selber abstellen und entfernen
Falls wir mit Ihnen vereinbart haben, dass Sie das Gerät selber entfernen, gehen Sie bitte nach der auf dem Tagebuch notierten Zeit so vor:

1. Nehmen Sie das Gerät aus dem Umhängebeutel und entfernen Sie die Batterie aus dem Batteriefach auf der Rückseite des Gerätes (Öffnen des Batteriefaches durch Schieben des Deckels nach links).
2. Schreiben Sie die Zeit auf das Tagebuch.
3. Entfernen Sie die Klebestreifen.
4. Lösen Sie vorsichtig die farbigen Druckknöpfe von den auf die Haut geklebten Elektroden. Lassen Sie die Ableitungskabel im Gerät stecken.
5. Lösen Sie die Elektroden von Ihrer Haut. Sie können die Elektroden wegwerfen.
6. Reinigen Sie Ihre Haut gründlich aber sanft. Das Auftragen einer milden Körperlotion kann helfen, einen Juckreiz zu verhindern oder zu lindern.
7. Bringen Sie uns Gerät, Kabel und Batterie zurück oder senden sie es uns im frankierten und adressierten Karton möglichst rasch zurück.

Unvorhergesehene Fälle

- **Falls sich eine Elektrode von der Haut löst**, was bei starkem Schwitzen vorkommen kann, kleben Sie eine neue Elektrode aus dem Notfallset auf dieselbe Stelle und befestigen Sie das Ableitungskabel an der Elektrode.
- **Falls sich ein Ableitungskabel von der Elektrode löst**, so bringen Sie dieses wieder an.
- In seltenen Fällen kann es vorkommen, dass die Haut **allergisch auf die Klebestreifen** reagiert. Entfernen Sie in diesem Fall das Gerät, indem Sie wie oben beschrieben (Punkt 1. bis 7.) vorgehen. Die Haut kann in einem solchen Fall mehrere Tage gerötet sein und jucken.

Legen Sie die Batterie keinesfalls wieder in das Gerät ein, wenn sie einmal entfernt wurde oder heraus fiel!

1. Teil Fragebogen

Bitte beantworten Sie die ersten 3 Fragen möglichst rasch.

Wie viele Tassen Kaffe haben Sie während den 4 Stunden vor der EKG-Untersuchung getrunken?	__ Tassen
Wie viele Tassen Schwarz- oder Grüntee haben Sie während den 4 Stunden vor der EKG-Untersuchung getrunken?	_ _ Tassen
Wie viele Gläser Alkohol haben Sie während den 24 Stunden vor der EKG-Untersuchung getrunken?	_ _ Gläser

Leiden Sie an folgender Krankheit, hatten Sie folgende Operation?	**Ja**	**Nein**
• Herztransplantation	☐	☐
• Sudeck'sche Krankheit (Algodystrophie, sympathische Reflexdystrophie, komplexes regionales Schmerzsyndrom)	☐	☐
• hyperreaktiver Karotissinus-Reflex	☐	☐
• Amyloidose	☐	☐
• Kollagenose	☐	☐
• Schilddrüsenüberfunktion (Hyperthyreose, Thyreotoxikose)	☐	☐
• Leberzirrhose oder hepatozelluläre Insuffizienz	☐	☐
• Anorexia nervosa (Magersucht) oder andere Ernährungsverhaltensstörungen (Bulimie)	☐	☐
• HIV-Infektion (AIDS)	☐	☐
Hatten Sie im vergangenen Monat gelegentlich das Gefühl eines unregelmässigen Herzrhythmus?	☐	☐
Hatten Sie in den vergangenen 8 Tagen eine Narkose (Allgemeinanästhesie) oder eine Spinalanästhesie (durch Injektion in den Rückenmarkskanal)?	☐	☐
Haben Sie zur Zeit eine Sauerstofftherapie?	☐	☐
Konsumieren Sie Kokain?	☐	☐

Anleitung zum Tagebuch

Anleitung:

Was Sie beachten sollten, bevor Sie das Tagebuch auszufüllen beginnen:

- Führen Sie das Tagebuch 5 mal am Tag nach, z.B. wenn Sie jetzt wieder zur Arbeit kommen, am Abend, wenn Sie von der Arbeit nach Hause kommen, bevor Sie zu Bett gehen, nach dem Aufstehen und bevor sie uns das Aufnahmegerät zurücksenden.
- Kreuzen Sie die Kreise an: O
- Wenn Sie eine Tätigkeit länger als 15 Minuten ausführen oder sich länger an einem Ort aufhalten, können Sie die Kreise mit Linien verbinden.

  O
  O
  O
- Fahren Sie nach 24:00 Uhr auf der ersten Seite des Tagebuches weiter.

Erläuterungen:

Tätigkeit:
- Als Erinnerungsstütze können Sie in der dafür vorgesehenen Kolonne Ihre Tätigkeit kurz beschreiben. Dies ist jedoch freiwillig.

Aktivitätsgrad:
Kreuzen Sie wenigstens einen Aktivitätsgrad an.
- Sehr anstrengend: Sie schwitzen und sind erschöpft, z.B. Aerobics, schnelles Velofahren, Jogging
- Mässig anstrengend: z.B. Haushalten, Gehen, gemütliches Velofahren
- Wenig anstrengend: z.B. Stehen, Büroarbeit, Sitzen, Telefonieren, Autofahren
- Ruhe: Liegen oder Schlafen

Ort:
Kreuzen Sie wenigstens einen Ort an. Wenn Sie während eines 15-Minuten-Abschnittes sowohl draussen als auch drinnen waren, dann kreuzen Sie beides an.

Konsum:
Tragen Sie die Anzahl Tassen oder Gläser des konsumierten Getränkes in die entsprechende Kolonne ein.

Rauchen:
- Kreuzen Sie das Feld „selber" an, falls Sie während diesem Zeitabschnitt geraucht haben (Zigarette, Zigarre, Pfeife etc.)
- Kreuzen Sie das Feld „gleicher Raum" an, falls jemand in Ihrer Umgebung in einem geschlossenen Raum eine Zigarette, Zigarre, Pfeife etc. raucht.

Medikamente:
Tragen Sie in dieser Kolonne den Namen und die Dosierung der eingenommenen Medikamenten ein.

Zeit		Tätigkeit	Ort		Aktivitätsgrad				Konsum			Rauchen		Medikamente
Zeit		Beschreiben Sie kurz Ihre Tätigkeit	Drinnen	Draussen	sehr anstrengend	mässig anstrengend	wenig anstrengend	Ruhe	Kaffe (Tässen)	Schwarz-/Grüntee	Alkohol (Gläser)	selber	gleicher Raum	Name, Dosierung
12	00		O	O	O	O	O	O				O	O	
	15		O	O	O	O	O	O				O	O	
	30		O	O	O	O	O	O				O	O	
	45		O	O	O	O	O	O				O	O	
13	00		O	O	O	O	O	O				O	O	
	15		O	O	O	O	O	O				O	O	
	30		O	O	O	O	O	O				O	O	
	45		O	O	O	O	O	O				O	O	
14	00		O	O	O	O	O	O				O	O	
	15		O	O	O	O	O	O				O	O	
	30		O	O	O	O	O	O				O	O	
	45		O	O	O	O	O	O				O	O	
15	00		O	O	O	O	O	O				O	O	
	15		O	O	O	O	O	O				O	O	
	30		O	O	O	O	O	O				O	O	
	45		O	O	O	O	O	O				O	O	
16	00		O	O	O	O	O	O				O	O	
	15		O	O	O	O	O	O				O	O	
	30		O	O	O	O	O	O				O	O	
	45		O	O	O	O	O	O				O	O	
17	00		O	O	O	O	O	O				O	O	
	15		O	O	O	O	O	O				O	O	
	30		O	O	O	O	O	O				O	O	
	45		O	O	O	O	O	O				O	O	
18	00		O	O	O	O	O	O				O	O	
	15		O	O	O	O	O	O				O	O	
	30		O	O	O	O	O	O				O	O	
	45		O	O	O	O	O	O				O	O	
19	00		O	O	O	O	O	O				O	O	
	15		O	O	O	O	O	O				O	O	
	30		O	O	O	O	O	O				O	O	
	45		O	O	O	O	O	O				O	O	
20	00		O	O	O	O	O	O				O	O	
	15		O	O	O	O	O	O				O	O	
	30		O	O	O	O	O	O				O	O	
	45		O	O	O	O	O	O				O	O	
21	00		O	O	O	O	O	O				O	O	
	15		O	O	O	O	O	O				O	O	
	30		O	O	O	O	O	O				O	O	
	45		O	O	O	O	O	O				O	O	
22	00		O	O	O	O	O	O				O	O	
	15		O	O	O	O	O	O				O	O	
	30		O	O	O	O	O	O				O	O	
	45		O	O	O	O	O	O				O	O	
23	00		O	O	O	O	O	O				O	O	
	15		O	O	O	O	O	O				O	O	
	30		O	O	O	O	O	O				O	O	
	45		O	O	O	O	O	O				O	O	

Zeit		Tätigkeit	Ort		Aktivitätsgrad				Konsum			Rauchen		Medikamente
Zeit		Beschreiben Sie kurz Ihre Tätigkeit	Drinnen	Draussen	sehr anstrengend	mässig anstrengend	wenig anstrengend	Ruhe	Kaffee (Tässen)	Schwarz-/Grüntee	Alkohol (Gläser)	selber	gleicher Raum	Name, Dosierung
0	00		O	O	O	O	O	O				O	O	
	15		O	O	O	O	O	O				O	O	
	30		O	O	O	O	O	O				O	O	
	45		O	O	O	O	O	O				O	O	
1	00		O	O	O	O	O	O				O	O	
	15		O	O	O	O	O	O				O	O	
	30		O	O	O	O	O	O				O	O	
	45		O	O	O	O	O	O				O	O	
2	00		O	O	O	O	O	O				O	O	
	15		O	O	O	O	O	O				O	O	
	30		O	O	O	O	O	O				O	O	
	45		O	O	O	O	O	O				O	O	
3	00		O	O	O	O	O	O				O	O	
	15		O	O	O	O	O	O				O	O	
	30		O	O	O	O	O	O				O	O	
	45		O	O	O	O	O	O				O	O	
4	00		O	O	O	O	O	O				O	O	
	15		O	O	O	O	O	O				O	O	
	30		O	O	O	O	O	O				O	O	
	45		O	O	O	O	O	O				O	O	
5	00		O	O	O	O	O	O				O	O	
	15		O	O	O	O	O	O				O	O	
	30		O	O	O	O	O	O				O	O	
	45		O	O	O	O	O	O				O	O	
6	00		O	O	O	O	O	O				O	O	
	15		O	O	O	O	O	O				O	O	
	30		O	O	O	O	O	O				O	O	
	45		O	O	O	O	O	O				O	O	
7	00		O	O	O	O	O	O				O	O	
	15		O	O	O	O	O	O				O	O	
	30		O	O	O	O	O	O				O	O	
	45		O	O	O	O	O	O				O	O	
8	00		O	O	O	O	O	O				O	O	
	15		O	O	O	O	O	O				O	O	
	30		O	O	O	O	O	O				O	O	
	45		O	O	O	O	O	O				O	O	
9	00		O	O	O	O	O	O				O	O	
	15		O	O	O	O	O	O				O	O	
	30		O	O	O	O	O	O				O	O	
	45		O	O	O	O	O	O				O	O	
10	00		O	O	O	O	O	O				O	O	
	15		O	O	O	O	O	O				O	O	
	30		O	O	O	O	O	O				O	O	
	45		O	O	O	O	O	O				O	O	
11	00		O	O	O	O	O	O				O	O	
	15		O	O	O	O	O	O				O	O	
	30		O	O	O	O	O	O				O	O	
	45		O	O	O	O	O	O				O	O	

2. Teil Fragebogen

Bitte beantworten Sie diese Fragen am Morgen nach dem Aufstehen.

Wann sind Sie eingeschlafen? *(Möglichst genaue Zeitangabe)*	_ _ : _ _ Uhr
Wann sind Sie aufgewacht? *(Möglichst genaue Zeitangabe)*	_ _ : _ _ Uhr
Wie lange haben Sie vergangene Nacht gebraucht um einzuschlafen?	_ _ _ Minuten
Wie oft sind Sie nachts erwacht?	_ _ _ Mal
Wie lange sind Sie heute Nacht insgesamt wach gelegen (ohne Einschlafzeit)?	_ _ _ Minuten

Wie würden Sie die Qualität des Schlafes im Vergleich zu Ihrem gewöhnlichen Schlaf einschätzen? *(Kreisen Sie das Zutreffende an. 1: sehr viel schlechter als gewöhnlich, 5: wie gewöhnlich, 10: sehr viel besser als gewöhnlich)*

Unruhiger als gewöhnlich	1 2 3 4 5 6 7 8 9 10	Ruhiger als gewöhnlich
Mehr Wachphasen als gewöhnlich	1 2 3 4 5 6 7 8 9 10	Weniger Wachphasen als gewöhnlich

Bewerten Sie, wie stark der Verkehrslärm während der vergangenen Nacht Ihren Schlaf gestört hat *(1: keine Störung; 10 extreme Störung)*

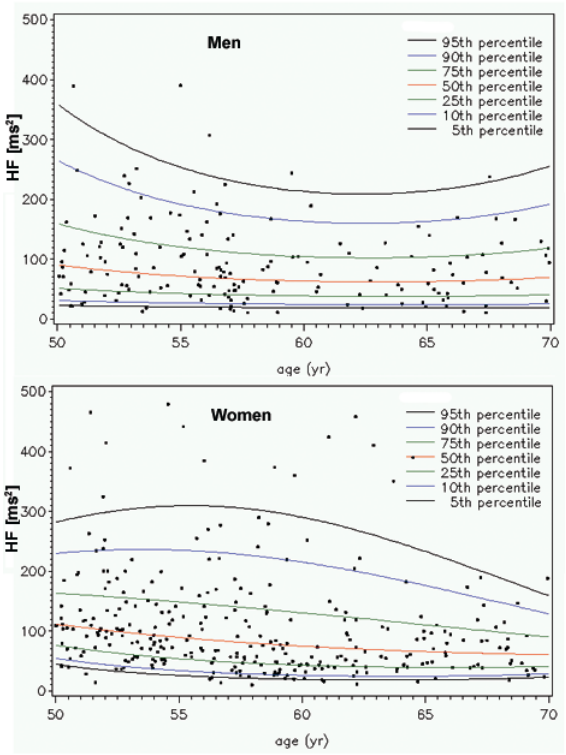
1 2 3 4 5 6 7 8 9 10

Wann haben Sie die EKG-Aufzeichnung beendet? _____ Uhr

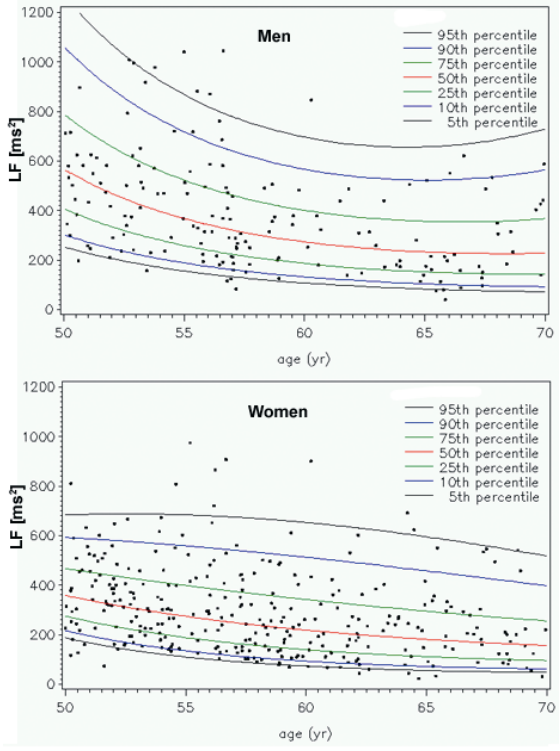
Besten Dank fürs Mitmachen!

8.6 Percentile curves

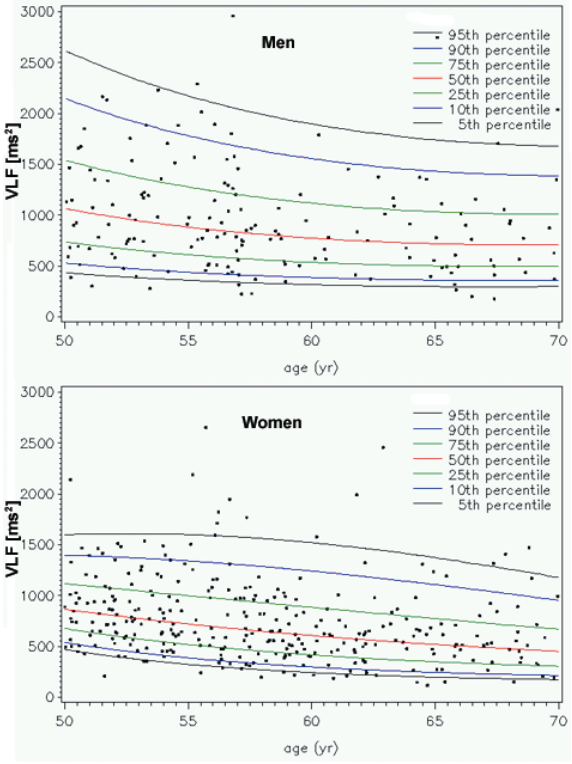
Percentile of HF for healthy men and women



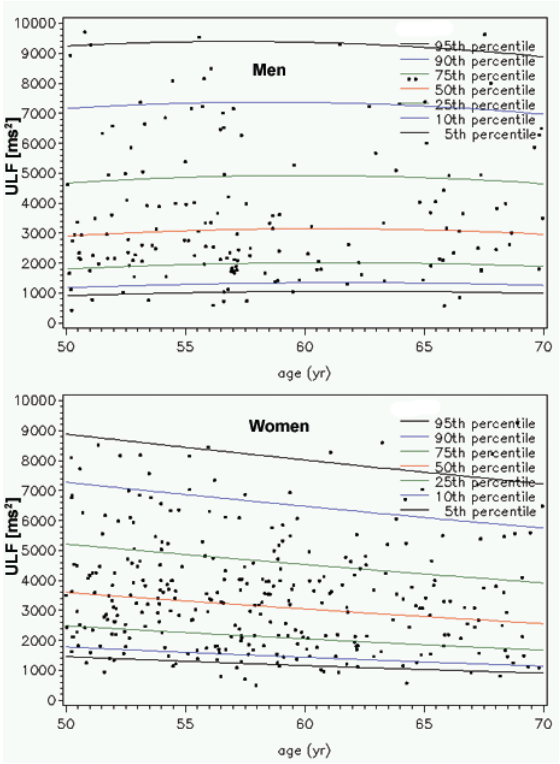
Percentile of LF for healthy men and women



Percentile of VLF for healthy men and women



Percentile of ULF for healthy men and women



Supplementary material to paper 2

Supplementary Table 1. Unadjusted geometric mean HRV according to ETS exposure

HRV variable	no current ETS exp.	ETS ≤ 2 hrs/d	ETS > 2 hrs/d
n	1034	104	80
SDNN [ms]	134.1 (131.9, 136.3)	132.5 (125.8, 139.5)	129.3 (121.9, 137.2)
Total Power [ms^2]	3874.1 (3732.7, 4020.9)	3621.5 (3220.8, 4072.0)	3294.4 (2882.1, 3765.6)
HF [ms^2]	71.1 (67.3, 75.1)	62.8 (52.8, 74.6)	71.9 (59.1, 87.6)
LF [ms^2]	231.8 (221.3.0, 242.8)	214.9 (185.8, 248.7)	212.7 (180.1, 251.2)
LF/HF	3.3 (3.1, 3.4)	3.4 (3.0, 3.9)	3.0 (2.6, 3.4)
VLF [ms^2]	624.1 (600.0, 649.2)	602.5 (532.1, 682.2)	579.8 (503.2, 668.0)
ULF [ms^2]	2751.4 (2641.6, 2865.7)	2576.1 (2265.8, 2929.0)	2255.6 (1948.5, 2611.1)

Values in parentheses are 95% CIs.

Supplementary Table 2. Adjusted[a] geometric mean HRV according to ETS exposure, stratified by sex

HRV variable	no current ETS exp.		ETS ≤ 2 hrs/d		ETS > 2 hrs/d	
	Men	Women	Men	Women	Men	Women
n	479	557	51	53	39	41
SDNN [ms]	136.9 (133.7, 140.2)	131.5 (128.7, 134.3)	137.4 (127.6, 147.9)	130.3 (121.6, 139.6)	127.9 (117.6, 139.2)	130.9 (121.0, 141.6)
Total Power [ms^2]	3981.9 (3766.7, 4209.4)	3775.8 (3603.2, 3957.9)	3549.5 (2985.8 ,4219.7)	3798.4 (3254.1, 4433.7)	3556.28 (2921.7, 4328.7)	3038.3 (2548.3, 3622.6)
HF [ms^2]	68.3 (62.9, 74.2)	74.2 (69.1, 79.7)	53.6 (41.4, 69.3)	68.5 (54.2, 86.6)	69.3 (51.7, 92.9)	71.3 (54.6, 93.1)
LF [ms^2]	270.6 (253.7, 288.7)	205.7 (194.6, 217.5)	230.1 (188.2, 281.3)	192.8 (160.6, 231.4)	230.9 (183.8, 290.2)	172.8 (140.4, 212.7)
LF/HF	4.0 (3.8, 4.2)	2.8 (2.6, 2.9)	4.3 (3.6, 5.1)	2.8 (2.4, 3.3)	3.3 (2.7, 4.1)	2.4 (2.0, 2.9)
VLF [ms^2]	698.2 (658.6, 740.2)	570.7 (544.7, 598.1)	638.0 (531.9, 765.3)	554.4 (475.4, 646.4)	670.9 (545.7, 825.0)	474.8 (398.7, 565.4)
ULF [ms^2]	2723.5 (2560.3, 2897.1)	2761.3 (2622.1, 2907.89	2442.6 (2015.2, 2960.7)	2843.7 (2399.5, 3370.2)	2368.0 (1903.1, 2946.6)	2172.7 (1791.0, 2635.7)

[a]Adjusted for study site, age, education, BMI, diabetes, and beta-blocker intake.
Values in parentheses are 95% CIs.

Supplementary Table 3. Unadjusted means of heart rate and blood pressure according to ETS exposure

Variable	no current ETS exp.	ETS ≤ 2 hrs/d	ETS > 2 hrs/d
heart rate [bpm]	73.4 (72.8, 73.9)	73.3 (71.6, 75.0)	75.8 (73.8, 77.7)
SBP [mmHg]	132.7 (131.5, 133.9)	133.7 (129.9, 137.5)	131.5 (127.1, 135.8)
DBP [mmHg]	81.7 (81.0, 82.3)	82.4 (80.4, 84.5)	83.5 (81.1, 85.9)

Values in parentheses are 95% CIs.
SBP: systolic blood pressure, DBP: diastolic blood pressure.

Supplementary Table 4. Adjusted[a] geometric means of heart rate and blood pressure according to ETS exposure, stratified by sex

Variable	no current ETS exp.		ETS ≤ 2 hrs/d		ETS > 2 hrs/d	
	Men	Women	Men	Women	Men	Women
heart rate [bpm]	72.3 (71.4, 73.1)	74.3 (73.7, 75.0)	72.4 (69.8, 74.9)	74.5 (72.3, 76.6)	74.1 (71.2, 77.0)	76.4 (73.9, 78.8)
SBP [mmHg]	136.9 (135.2, 138.7)	129.0 (127.6, 130.5)	138.0 (132.8, 143.2)	129.6 (125.0, 134.2)	137.5 (131.5, 143.4)	128.7 (123.4, 134.0)
DBP [mmHg]	84.0 (83.1, 85.0	79.8 (78.9, 80.6)	84.8 (81.9, 87.6)	79.8 (77.1, 82.4)	85.0 (81.7, 88.2)	82.1 (79.1, 85.1)

Values in parentheses are 95% CIs.

SBP: systolic blood pressure, DBP: diastolic blood pressure.

[a] Adjusted for study site, age, education, BMI, diabetes, and beta-blocker intake.

Supplementary Table 5. Sensitivity analyses including or excluding additional variables into the model, with the natural logarithm of total power as outcome variable

	Effect estimate[a]	**95% CI**	**P**
Baseline analysis[b]	-9.1%	-17.4%−-0.1%	0.048
including exercise[c]	-8.8%	-17.0%−0.3%	0.058
including alcohol[d]	-9.0%	-17.3%−0.2%	0.054
including hypertension	-8.7%	-16.9%−0.4%	0.06
incl. cardiovascular risk markers in the blood[e]	-8.5%	-16.8%−0.6%	0.067
including CO	-9.1%	-17.4%−-0.1%	0.048
excluding education	-9.3%	-17.5%−-0.3%	0.044
excluding subjects with medication intake[f]	-10.2%	-18.7%−-0.9%	0.033

[a] Estimated %-increase in total power associated with exposure to ETS at home or at work (as compared to no exposure to ETS at home or at work), adjusted for sex, age, age squared, study site, education, diabetes and beta-blocker

[b] adjusted for sex, age, age squared, study site, education, diabetes and beta-blocker

[c] physical exercise causing sweating and physical exercise causing slight shortness of breath

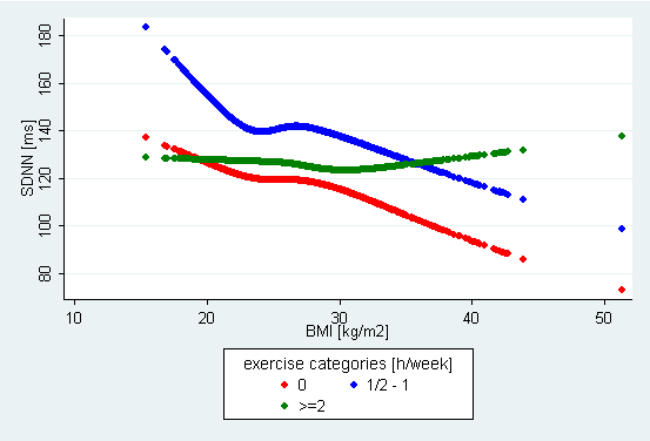
[d] consumption of red wine and alcoholic beverages

[e] serum levels of uric acid, high sensitive C-reactive protein and non-HDL-cholesterol

[f] ACE inhibitors, antiarrhythmic medication, calcium channel blockers, diuretics or sympathomimetics

8.7 Supplementary material to paper 3

Supplementary Figure. Curves of adjusted geometric means of SDNN for the three exercise categories with BMI as independent variable being continuous[a]



[a] adjusted for sex, age, age squared, study site, education, diabetes, hypertension, beta-blocker intake and smoking status in a cubic model for BMI

Supplementary Table 1. Interaction terms between body mass index and physical activity in their effect on the natural logarithm of SDNN

BMI	normal weight		overweight		obese	
	Coefficient	P	Coefficient	P	Coefficient	P
no regular exercise	0.128	0.000	0.105	0.000	Ref.	-
½ - 1 h/week exercise	-0.067	0.104	-0.072	0.071	0.092	0.006
≥2 h/week exercise	-0.125	0.006	-0.109	0.014	0.173	0.000

Supplementary Table 2. Adjusted[a] geometric mean (GM) of night-time HRV in different exercise categories

BMI		normal weight		overweight		obese	
	Exercise [h/week]	GM	95% CI	GM	95% CI	GM	95% CI
SDNN [ms]	None	79.3	(76.4, 82.2	81.2	(78.5, 84.1)	77.0	(73.6, 80.6)
	½ - 1	83.9	(80.5, 87.4)	81.8	(78.8, 85.0)	82.5	(78.1, 87.2)
	≥ 2	85.8	(82.0, 89.8)	86.7	(83.0, 90.5)	88.6	(82.9, 94.6)
Total power [ms^2]	None	1304.4	(1208.2, 1408.3)	1353.7	(1261.2, 1453.0)	1206.4	(1098.8, 1324.6)
	½ - 1	1462.7	(1342.9, 1593.1)	1421.5	(1315.7, 1535.7)	1423.6	(1270.9, 1594.6)
	≥ 2	1450.8	(1321.0, 1593.3)	1516.3	(1386.6, 1658.2)	1578.9	(1378.6, 1808.3)
HF [ms^2]	None	104.3	(92.9, 117.0)	114.8	(103.2, 127.7)	108.8	(94.5, 125.2)
	½ - 1	106.3	(93.4, 120.9)	106.7	(94.9, 119.9)	131.9	(111.1, 156.4)
	≥ 2	104.0	(90.3, 119.8)	132.0	(115.4, 151.1)	150.2	(122.4, 184.2)
LF [ms^2]	None	261.0	(238.1, 286.0)	281.2	(258.4, 306.1)	241.5	(215.9, 270.0)
	½ - 1	313.7	(283.2, 347.4)	290.7	(265.0, 318.9)	286.0	(249.7, 327.5)
	≥ 2	305.1	(272.8, 341.4)	314.7	(282.8, 350.3)	309.8	(263.4, 364.4)
LF/HF	None	3.2	(2.9, 3.4)	3.2	(3.0, 3.4)	3.0	(2.7, 3.3)
	½ - 1	3.7	(3.4, 4.0)	3.4	(3.2, 3.7)	2.8	(2.5, 3.2)
	≥ 2	3.7	(3.4, 4.1)	3.1	(2.8, 3.3)	2.6	(2.3, 3.0)

[a] adjusted for sex, age, age squared, study site, education, diabetes, hypertension, beta-blocker intake and smoking status

9. Curriculum Vitae

BIOGRAPHICAL INFORMATION

Name:	Denise FELBER DIETRICH
Nationality	Swiss and U.S.
Place and date of birth:	Ravensburg (Germany), 12 November 1971

ACADEMIC TRAINING

1992 – 1994	Medical studies	University of Fribourg
1994 – 1999	Medical studies *Federal medical diploma*	University of Berne
2003 – 2007	M.D. Ph.D. in Medical Sciences, Public Health studies and thesis, supervised by Prof. Dr. Ursula Ackermann-Liebrich: "Heart rate variability in the general populationa and its determining factors".	University of Basel
2003 - 2008	Master of public health studies *Master of public health*	Universities of Basel, Berne and Zurich

AWARDS AND GRANTS

2005	Best poster presentation award for *"Environmental tobacco smoke and heart rate variability: results from the SAPALDIA 2 study"*	Swiss Society of Public Health
2006	Young Investigators award Population Sciences for "*Regular exercise corrects altered heart rate variability in obese people*"	European Society of Cardiology
2006	Grant for participation at "*HRV 2006*" at Harvard Medical School/Beth Israel Deaconess Medical Center, Boston	Reisefonds, University of Basel
2008	Grant for scientific visit at Harvard School of Public Health, Boston	Swiss National Science Foundation

WORK EXPERIENCE

07/1991 – 06/1992	*Fantasy Destinations* *Representative, Tour Guide*	Orlando, FL (USA)
01/2000 – 10/2000	*Zieglerspital Bern, Surgical department* *Resident*	Berne (BE)
11/2000 – 09/2001	*Institute of Social- und Preventive Medicine, University of Basel* *Research fellow*	Basel (BS)
10/2001 – 12/2002	Swiss Cohort Study on Air Pollution and Lung Disease in Adults (SAPALDIA 2) *Study physician (80%)*	Payerne (VD)
	Institute of Social- und Preventive Medicine, University of Basel *Research fellow (20%)*	Basel (BS)
01/2003 – 01/2008	Institute of Social- und Preventive Medicine, University of Basel *Research fellow*	Basel (BS)
02/2008 - 03/2008	Harvard School of Public Health *Visiting scientist*	Boston, MA (USA)
04/2008 – present	Institute of Social- und Preventive Medicine, University of Basel *Research fellow*	Basel (BS)

TEACHING EXPERIENCE

2004, 2007	*Basics of medical statistics*, tutor for 2nd year medical students	University of Basel, Medical Faculty
2007	*Problem based teaching*, psycho-social medicine, tutor for 2nd year medical students	University of Basel, Medical Faculty
2007	*Environmental epidemiology*, "The Barcelona exercise"	Universities of Basel, Berne and Zurich, Master of Public Health studies
2007	*Reading epidemiological papers*, tutor for 6th year medical students	University of Basel, Medical Faculty
2008	*Epidemiology of chronic disease,* lecture for 6th year medical students	University of Basel, Medical Faculty
2008	*Practical training in psycho-social medicine,* course organizer for 3rd year medical students	University of Basel, Medical Faculty

PUBLICATIONS AND PRESENTATIONS

Peer-reviewed publications	Ackermann-Liebrich U., Kuna-Dibbert B., Probst-Hensch NM, Schindler C., Felber Dietrich D., Zemp Stutz E., Bayer-Oglesby L., Baum F., Brändli O., Brutsche M., Downs SH, Keidel D., Gerbase MW, Imboden M., Keller R., Knöpfli B., Künzli N., Nicod L., Pons M., Staedele P., Tschopp JM, Zellweger JP, Leuenberger P.: *Follow-up of the Swiss Cohort Study on Air Pollution and Lung Diseases in Adults (SAPALDIA 2) 1991-2003: Methods and characterization of participants*	Soz Praventivmed. 2005; 50:1-19.
	Nitsch D., Felber Dietrich D., von Eckardstein A., Gaspoz JM., Downs SH., Leuenberger P., Tschopp JM., Brändli O., Keller R., Gerbase MW., Probst-Hensch NM., Zemp Stutz E., Ackermann-Liebrich U.: *Prevalence of renal impairment in a general population: results of the Swiss SAPALDIA cohort study*	Nephrol Dial Transplant. 2006;21(4):935-44.
	Felber Dietrich D, Schindler Ch, Schwartz J, Barthelemy JC, Tschopp JM, Roche F, von Eckardstein A, Brändli O, Leuenberger Ph, Gold DR, Gaspoz JM, Ackermann-Liebrich U: *Heart rate variability in an ageing population: results of the SAPALDIA study*	Europace. 2006;8:521-529.
	Felber Dietrich D., Schwartz J., Schindler C., Gaspoz JM., Barthélémy JM., Tschopp JM., Roche F.,, von Eckardstein A., Brändli O., Leuenberger Ph., Gold DR., Ackermann-Liebrich U. and SAPALDIA-team *Effects of passive smoking on heart rate variability, heart rate and blood pressure: an observational study*	Int J Epidemiol. 2007;36(4):834-40.
	Felber Dietrich D, Ackermann-Liebrich U, Schindler C, Barthélémy JC, Brändli O, Gold DR, Knöpfli B, Probst-Hensch NM, Roche F, Tschopp JM, von Eckardstein A, Gaspoz JM and SAPALDIA Team *Effect of physical activity on heart rate variability in normal weight, overweight and obese subjects: results from the SAPALDIA study.*	Eur J Appl Physiol. 2008; DOI 10.1007/s00421-008-0800-0
	Felber Dietrich D., Gemperli A., Gaspoz JM., Schindler C., Liu LJ., Gold DR., Schwartz J., Rochat T., Barthélémy JC., Pons M., Roche F., Probst-Hensch NM., Bridevaux PO., Gerbase MW., Neu U., Ackermann-Liebrich U. and SAPALDIA Team *Differences in heart rate variability associated with longterm exposure to NO2*	Environ Health Perspect. 2008; in press
Book section	Ackermann-Liebrich U, Felber Dietrich D *Umweltfaktoren*	in: Battegay E, Riesen WF, Noseda G; Atherosklerosepräven tion. Diagnostik und Therapie von Risikofaktoren, Bern 2007.

Reports	Eichler K., Felber Dietrich D., Bachmann L., Steurer J., Quinto C., Zemp Stutz E.: *State of the art der Prävention, Diagnose und Behandlung der Osteoporose und der nichtmedikamentösen Prävention von Frakturen im Alter*	Bundesamt für Sozialversicherung, Forschungsbericht Nr. 25/03, Bern 2003
	Eichler K., Felber Dietrich D.: *Evidenz aus der Literatur zu Prävention, Diagnose und Behandlung der Osteoporose und der nichtmedikamentösen Prävention von Frakturen im Alter*	in: Osteoporose und Stürze im Alter. Bundesamt für Gesundheit, Bern 2004
Oral presentations	*Luftbelastung und Herzerkrankungen*	Fachtagung Schwebestaub und Gesundheit, Universität Basel 18.10.2001
	Felber Dietrich D., Eichler K.: *Prävention, Diagnose und Behandlung der Osteoporose und nichtmedikamentöse Prävention von Frakturen im Alter*	Osteoporose und Stürze im Alter, Arbeitstagung BSV Bern, 4.9.2003
	Felber Dietrich D.; Gaspoz JM.; Schindler C.; Schwartz J.; Gold DR.; Barthélémy JC.; Tschopp JM.; von Eckardstein A.; Brändli O.; Leuenberger P.; Ackermann-Liebrich U. and SAPALDIA Team: *Regular exercise corrects altered heart rate variability in obese people*	Annual meeting of the Swiss Society of Cardiology Basel 8.6.2006. Among the 15 best abstracts.
	Felber Dietrich D; Gaspoz JM; Schindler C; Schwartz J; Gold DR; Barthélémy JC; Tschopp JM; Ackermann-Liebrich U and SAPALDIA Team: *Regular exercise corrects altered heart rate variability in obese people*	World Congress of Cardiology, Barcelona, 3.9.2006.
	Felber Dietrich D *Heart rate variability in SAPALDIA and its association with exposure to NO_2*	Invited presentation at the Institute for medical informatics, biometrics and epidemiology, Universitätsklinikum Essen, 6.6.2007
	Felber Dietrich D, U Ackermann-Liebrich, C Schindler, DR Gold, JC Barthélémy, JM Gaspoz: *A healthy lifestyle is associated with higher heart rate variability*	Swiss Public Health Conference, Olten, 22.6.2007
	Felber Dietrich D *Long-term effect of NO_2 exposure on heart rate variability: results from the Swiss Cohort Study on Air Pollution and Lung Diseases in Adults (SAPALDIA)*	Invited presentation at the Harvard School of Public Health, 27.2.2008
	Felber Dietrich D *Heart rate variability in SAPALDIA: Associations with air pollution and effect of physical activity in normal weight and obese subjects*	Invited presentation at the Harvard School of Public Health, 4.3.2008

Poster presentations	Kuna-Dibbert B., Ackermann-Liebrich U., Bayer-Oglesby L., Probst-Hensch NM, Schindler C., Staedele-Kessler P., Felber Dietrich D., Leuenberger P.: *SAPALDIA Kohortenstudie: Methoden und Beteiligung*	Annual meeting of the Swiss Society of Public Health Basel 2003
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