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Decline in resting heart rate, its association with other variables, and its role in cardiovascular disease

The Tromsø Study

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Acknowledgements

I conducted this study as a PhD student at the Department of Community Medicine, Faculty of Health Sciences, the University of Tromsø, which later changed its name to UiT – the Arctic University of Norway. After 4 years, my project is almost over; two articles have been published, a third one has been submitted, and the dissertation has been written. I would like to thank the UiT for providing funding for this project and for giving me the opportunity to work in such a nice and encouraging environment. I would also like to thank the Tromsø Study and its participants; without these data, which were collected over several decades, this project would not have been possible.

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Summary

Background Resting heart rate (RHR) is an easily measured cardiovascular parameter that is considered an independent predictor of cardiovascular disease (CVD) and mortality in the general population. However, results on the association between RHR and these outcomes are inconsistent, and studies on longitudinal trends in RHR and the role of long-term changes in RHR in CVD and mortality are scarce.

Objective To investigate secular trends in RHR in the general population of Tromsø, Northern Norway over the last decades, and to explore the role of RHR and long-term changes in RHR in CVD and mortality.

Methods Participants from four surveys of the Tromsø Study conducted between 1986 and 2008 were included in these analyses (n=34 751). RHR in these participants was measured with an automated Dinamap device and resultant data were linked to validated information on incident myocardial infarction, atrial fibrillation, ischemic stroke, cardiovascular death and total death. Statistical methods included mixed models analysis, fractional polynomials, and trajectory analysis.

Main results Over 22 years of observation, the mean age-adjusted RHR declined from 73.4 to 64.7 beats per minute in men, and from 78.3 to 66.4 beats per minute in women; 17.4% of the decline in men and 16.1% of the decline in women was attributable to favourable changes in other cardiovascular risk factors. In men, elevated RHR independently predicted the risk of myocardial infarction, atrial fibrillation, and cardiovascular death. In women, the associations with myocardial infarction and total death were similar to those in men, and we found a J-shaped association with ischemic stroke. Having a constantly elevated RHR or a RHR that increased from moderate to high over 15 years increased the risk of myocardial infarction, cardiovascular death, and total death in men, whereas estimates in women were insignificant.

Conclusions Over the last decades RHR has declined substantially, and this decline has occurred to a large extent independently of other cardiovascular risk factors. RHR independently predicts the risk of CVD and mortality though there are sex differences. Long-term changes in RHR provide additional information for risk assessment. Thus, RHR is as an

independent cardiovascular risk factor, and as such it should be monitored and used in risk assessments by both people themselves and by health professionals. RHR is a modifiable cardiovascular risk factor; however it is unclear whether people could benefit from RHR-lowering interventions.

Abbreviations

BMI – body mass index

BP – blood pressure

bpm – beats per minute

CI – confidence interval

CVD – cardiovascular disease

ECG – electrocardiography

HDL – high-density lipoprotein

HR – hazard ratio

ICD – International Classification of Diseases

LDL – low-density lipoprotein

NHANES – National Health and Nutrition Examination Survey

RHR – resting heart rate

SD – standard deviation

List of Papers

This dissertation is based on the following papers:

Paper I

Sharashova E, Wilsgaard T, Brenn T. **Resting heart rate on the decline: the Tromsø Study 1986-2007.** Int J Epidemiol 2015;44(3):1007-1017. DOI: 10.1093/ije/dyv061.

Paper II

Sharashova E, Wilsgaard T, Mathiesen EB, Løchen ML, Njølstad I, Brenn T. **Resting heart rate predicts incident myocardial infarction, atrial fibrillation, ischaemic stroke and death in the general population: the Tromsø Study.** J Epidemiol Community Health 2016;70(9):902-909. DOI: 10.1136/jech-2015-206663.

Paper III

Sharashova E, Wilsgaard T, Løchen ML, Mathiesen EB, Njølstad I, Brenn T. **Resting heart rate trajectories and myocardial infarction, atrial fibrillation, ischemic stroke and death in the general population: the Tromsø Study.** Eur J Prev Cardiol, submitted.

Chapter 1 Introduction

In 2010-2011, a rough analysis of the data from the Tromsø Study showed that over the last few decades resting heart rate (RHR) has shown a remarkable decline in the general population. Between 1986-1987 and 2007-2008, RHR means dropped by approximately 10 beats per minute (bpm); a little less in men and little more in women. These findings resulted in a project that aimed to describe and explain the decline in RHR, and to explore the role of RHR values in cardiovascular morbidity and mortality.

I have always been interested in research, from my time as a medical student in Northern State Medical University, Arkhangelsk, Russia, and thereafter when I was working as a surgeon. Despite my interest, I lacked the knowledge and the opportunity to conduct a proper study. Soon I was able to enrol in a 2-year master's program in public health at the International School of Public Health in Arkhangelsk. The program was based on the European model and taught by teachers from the University of Tromsø, and it finally gave me the opportunity I was waiting for: to learn how to conduct epidemiological studies, perform statistical analyses, work with real data, and write a scientific paper. I enjoyed statistics and epidemiology a lot, and that is why I became a co-teacher in these subjects. I was eager to continue with research and statistics after graduating from the International School of Public Health in Arkhangelsk.

In June 2011, the day I defended my master thesis, I heard about the RHR PhD project. The topic itself was interesting and attractive to me both as a medical doctor and as a researcher. Moreover, the project was to include comprehensive statistical analyses, as well as opportunities to improve my skills, and to work in a high-level scientific environment with experts in the field. After a year and a half going through the application process, I was accepted as a PhD student at the Department of Community Medicine, the University of Tromsø, which is where the story between me and the decline in RHR in Tromsø begins.

1.1 What this dissertation is about

This dissertation is about RHR in the general adult population and is based on longitudinal data from the Tromsø Study, Northern Norway. This data was linked with validated follow-

up information on cardiovascular disease (CVD) and mortality, which provided a unique opportunity to apply comprehensive statistical methods to thoroughly answer our research questions. This dissertation describes the downward trends in RHR in men and women from the general population of Norway over the last decades (Paper I). The decline was mostly independent: less than one-fifth of the decline was attributable to changes in cardiovascular risk factors other than RHR. The dissertation also shows that single RHR measures can independently predict the risk of CVD and mortality; moreover it demonstrates sex differences in these associations (Paper II). Elevated RHR gradually increases the risk of incident myocardial infarction, atrial fibrillation, cardiovascular mortality, and total mortality in men, and the risk of myocardial infarction, ischemic stroke, cardiovascular mortality, and total mortality in women. However, low RHR in women did not protect against ischemic stroke or cardiovascular death. This dissertation shows that long-term changes in RHR can provide additional prognostic information and are an independent predictor of myocardial infarction, cardiovascular mortality, and total mortality in men (Paper III). Mixed models analysis, fractional polynomials, and trajectory analysis were used to investigate the research question.

1.2 Resting heart rate as a cardiovascular parameter

RHR is defined as the number of heart contractions per minute while at rest,[1] i.e., while a person is awake, in a temperate environment, and has not undergone any recent physical or psychological stimulation. In other words, RHR is the number of heart contractions needed to maintain basic body functions without any physical or psychological activity. Normal RHR in adults is between 60 and 90 bpm. However, people with a high physical activity level, for example professional sportsmen, usually have a RHR lower than 60 bpm.

1.2.1 Measuring resting heart rate

RHR as a clinical parameter has several advantages: it is fast, easy, and cheap to measure; it is easy to interpret; and it is understandable for both clinicians and patients. The measurement of RHR should be strictly standardised, as RHR can be influenced by many factors, such as resting period before measurement, environmental conditions, method of recording, number of readings, duration of measurement, a person's posture, and the nature of the observer.[2] To minimise the effects of these factors, exercise, alcohol consumption,

nicotine, and coffee consumption should be avoided for several hours before the measurement is taken. Before the first RHR measurement is taken, subjects should rest for at least 5 minutes in a quiet room at a comfortable temperature. Subjects should refrain from talking during the procedure. Although there are no objective data on whether one position is better than another, measurements of RHR taken in a sitting position are expected to be 1-2 bpm higher than those taken in the supine position. Moreover, when comparing data from different sources, it is important to know the nature of the observer. Indeed, heart rate increases triggered by one's alarm reaction to the measurement largely depends on this; heart rates tends to be higher when measured by a doctor, intermediate if measured by a nurse, and lower if measured by an automatic device in the absence of an observer.[3]

The most frequently used methods used to measure RHR are: pulse palpation, electrocardiography (ECG), electronic devices, and oscillometry. Pulse palpation is used to measure pulse rate by the counting beats in a set time period (from 15 to 60 seconds) and multiplying that number to get the bpm. Pulse rates can be measured at the radial, carotid, brachial, and femoral arteries, as these arteries are close to the body's surface. If stroke volume varies a lot (for example, in those with atrial fibrillation), some heartbeats can be missed at pulse palpation. In such cases, RHR can be measured directly by heart auscultation. ECG is the most precise way to measure RHR, but implies greater financial costs and may not translate into more meaningful data. RHR measurements from pulse palpation and from ECG are highly correlated ($r>0.9$) and provide similar information.[4]

Electronic pulse meters consist of a transmitter placed over the artery and a receiver attached around the chest or a wrist watch receiver for display. A photo diode or a photo transistor is used to detect pulse rate.[5] Infrared sensors are clipped to finger ends or ear lobes to detect the heart beat using plethysmographic technology. Simple heart rate monitors display pulse rate only. More professional monitors can record time, calculate average and maximum heart rate for a given period, and sound an alarm when a person reaches or exceeds a predetermined target zone. These electronic devices are usually accurate to within 3-4 bpm and are used mostly by athletes.

Oscillometry, or non-invasive automatic blood pressure (BP) monitors such as Dinamap, measure pulse rate and BP using an oscillometric method. This method is based on the principle that pulsatile blood flowing through an artery produces oscillations of the artery wall, which are transmitted to a cuff placed around the upper arm.[6] This method has some advantages over pulse palpation and electronic devices, including lack of observer variation and increased accuracy. Pulse rates derived by the oscillometric method have been reported to be a valid indicator of heart rate.[7] However, when compared to ECG, the oscillometric method measures actual peripheral pulses (pulse rate), not electrical signals or contractions of the heart muscles (heart rate). Thus differences may occur when cardiac electrical signals occasionally fail to produce a peripheral pulse. Differences may also occur in cases of varying stroke volume and/or poor peripheral perfusion.

1.2.2 Regulation of resting heart rate

In normal subjects, heart rate, and RHR in particular, is regulated by the pacemaker activity of the sinoatrial node cells, and constantly changes under the influence of many modifiable and non-modifiable factors.[8] The sinoatrial node is innervated by vagus (parasympathetic fibres) that slows the heart rate and by sympathetic nervous thoracic efferents quicken the heart rate. When these effects are blocked by pharmacologic agents such as sympathetic antagonists (e.g., propranolol and metoprolol) and/or parasympathetic antagonists (e.g., atropine) we can identify the mechanisms of the autonomic regulation of heart rate. Blocking autonomic regulation completely (simultaneous administration of sympathetic and parasympathetic antagonists) allows us to measure intrinsic heart rate, that is, the basal rate of pacemaker cells.

Non-modifiable factors that influence RHR include age (which is a matter of controversy, but some studies have reported a progressive decrease in intrinsic heart rate with age)[9] and sex (women have a higher RHR than men).[10] Physiological determinants of RHR are circadian cycle (lower during sleep compared with waking periods, mediated by neurohormonal factors)[11], posture (lower in supine then in sitting position)[12], and BP (positive association with both systolic and diastolic BP).[4, 8] Modifiable factors that influence RHR include physical activity, mental stress, smoking, alcohol consumption, coffee consumption, and excess body weight. A number of studies have demonstrated that regular endurance physical

activity results in a reduction of RHR,[13-16] which is mediated by a decrease in intrinsic rhythmicity, more predominant parasympathetic activity, and a slight decrease in the sympathetic contribution.[16] Acute mental stress activates the sympathetic nervous system and triggers a consistent increase in plasma catecholamines, thereby increasing RHR and BP.[17] The acute effects of smoking include an increase in heart rate and BP,[8, 18, 19] which is mediated by increased concentrations of plasma catecholamines and stimulation of peripheral adrenergic receptors. These acute changes persist at least for 30 minutes after smoking and occur again at each smoking episode.[18, 20] Smoking also exerts long-term effects in that smokers have higher RHRs than non-smokers.[21] The association between alcohol consumption and RHR is dose-dependent.[8, 21] Moderate daily alcohol consumption decreases RHR, while heavier alcohol consumption causes a sustained increase in RHR and BP. Both coffee and caffeine acutely increase BP and decrease heart rate irrespective of the amount of daily caffeine consumption, caffeine intolerance, or smoking status.[22, 23] As high calorie intake and obesity are associated with higher sympathetic activity, higher body mass index (BMI) was associated with higher RHR.[8, 23]

1.3 Resting heart rate as a cardiovascular risk factor

1.3.1 Epidemiological findings

Unlike factors like elevated levels of total and low-density lipoprotein (LDL) cholesterol, low level of high-density lipoprotein (HDL) cholesterol, elevated BP, excess BMI, physical inactivity, smoking, and family history of heart diseases, RHR is not considered a traditional cardiovascular risk factor.[24, 25] Although evidence on new parameters regarding their contribution to traditional cardiovascular risk assessment and traditional risk scores has been considered and is mentioned in some guidelines, RHR is not mentioned among the new potential parameters, such as cardiorespiratory fitness and high-sensitivity C-reactive protein, that could improve the prediction of CVD.[24, 25]

One potential reason why RHR has not been considered a valid cardiovascular risk factor is that the findings on RHR as an independent risk factor are inconsistent. The role of RHR has been addressed in several large studies like the Framingham Study[10] and the National Health and Nutrition Examination Survey (NHANES),[26] in which RHR appeared to be a strong and independent predictor of cardiovascular morbidity and mortality. These findings correspond

with the results of several studies on RHR and cardiovascular death from other populations[27-30] and with the conclusions of two recent meta-analyses.[31, 32] Another recent meta-analysis on RHR and CVD and non-cardiovascular disease morbidity showed that RHR independently predicted the risk of coronary artery disease, stroke, sudden death, and non-cardiovascular disease in the general population.[33] However, some studies found no independent association between RHR and CVD when looking at both sexes combined[34] or in women alone,[35] between RHR and coronary heart disease,[36, 37] between RHR and mortality from coronary heart disease,[38] or between RHR and stroke.[39, 40]

Moreover, the majority of information on the association between RHR and mortality in the general population has come from observational studies. Thus, another potential reason why RHR is not considered an independent cardiovascular risk factor is the methodological bias associated with population-based studies. However, epidemiological studies "over history paved the way for translation of basic science into successful health interventions".[41]

1.3.2 Pathophysiological mechanisms

The exact mechanism linking heart rate with CVD is still not clear. However, RHR has been shown to fulfil several of the criteria for causality.[27, 42, 43] RHR is regulated by the autonomic nervous system, and the imbalance between sympathetic and parasympathetic activity is one of the important pathogenic components of CVD.[44, 45] High RHR can disturb the balance between myocardial oxygen demand and supply.[46] It has been suggested that heart rate can directly induce atherosclerosis through a local hemodynamic effect of pulsatory blood flow and turbulence on the arterial wall.[45, 46] The pulsatile stress can provoke a proinflammatory response, though this adversely affects the vascular endothelium.[45] Increased RHR is associated with increased systemic inflammation and endothelial dysfunction.[47] Moreover, prolonged elevated RHR increases the risk of hypertension, and is associated with metabolic abnormalities. All these mechanisms contribute to the development and progression of coronary atherosclerosis, facilitate plaque destabilisation, and initiate arrhythmias, leading to acute coronary events and sudden death.[43, 48, 49]

1.3.3 Resting heart rate and other cardiovascular risk factors

RHR correlates with other cardiovascular risk factors such as BP,[27, 29, 44, 50, 51] physical activity,[27, 50-52] atherogenic serum lipid fractions, especially triglyceride levels,[27, 29, 50-53] body weight,[27, 44, 52, 53] and smoking.[27, 29, 52, 53] The presence of more than one cardiovascular risk factor increases the risk of having an elevated RHR.[50] In the Tromsø Study, RHR was positively associated with smoking, total cholesterol, LDL cholesterol, and triglycerides, but inversely correlated with HDL cholesterol and physical activity level.[52] The association between RHR and BMI was U-shaped. It has also been demonstrated that RHR is influenced by mental stress, job strain, alcohol consumption, and genetic determinants.[8, 45, 54]

1.3.4 Trends in resting heart rate and other cardiovascular risk factors in the general population

We found only a few studies that described RHR trends in the general population over the last decades. A study conducted on students of the Queen's University Belfast, the United Kingdom (n=4508) showed a secular decline in RHR from 1949 to 1992 in both sexes, but showed an increase from 2001 to 2004.[55] Another large population-based study from Paris, France (n=226 288) investigated RHR trends among middle-aged participants over 16 years (1992 to 2007).[56] RHR declined by 5 bpm among men, and by 8 bpm among women. Both studies were carried out on cross-sectional data with no information about RHR changes on an individual level. Three studies from Norway demonstrated a decline in RHR over the last decades, although the main aim of these papers was not focused on RHR.[57-59] None of the studies provided an explanation for the observed favourable trend in RHR.

There have been substantial changes in traditional cardiovascular risk factors in the general population over the last decades.[60-63] Total and LDL cholesterol,[61-63] BP,[61-66] and cigarette smoking[60, 61, 63, 64, 66] have declined, whereas physical activity levels,[64-66] BMI, the prevalence of overweight and obesity,[56, 60, 62, 63, 67] and the prevalence of hypertension[56] have increased. However, several studies observed different trends in these risk factors. A substantial decrease in BMI was seen over a 15-year period in Tallinn, Estonia, which was in line with the above-mentioned trends.[64] A decrease in the proportion of physically active people and an approximately stable proportion of smokers was observed from 1992 to 2007 in Paris, France.[56] A decrease in systolic BP and in the prevalence of hypertension between 1980-

1982 and 1995-1997 were paralleled with less favourable trends in total cholesterol, hypercholesterinaemia, and physical activity in the state of Minnesota, United States of America (USA).[60]

A recent paper based on data from the Tromsø Study aimed to estimate associations between trends in modifiable risk factors and a decline in acute coronary heart disease over the last decades.[57] From 1994 to 2008 RHR declined in the general population, and this decline was accompanied by favourable trends in BP, total cholesterol, smoking, and physical activity. In contrast, BMI and the prevalence of diabetes mellitus increased in the population. All these trends in risk factors together accounted for 66% of the decline in the incidence of coronary heart disease; the decline in RHR accounted for 14.5% yielding to the decline in total cholesterol only.

1.4 Cardiovascular mortality in the world and in Norway

CVD is the leading cause of death worldwide.[68] In 2013, one-third of all deaths globally (17.3 million out of 54.9 million deaths) were attributable to CVD. The majority of cardiovascular deaths (84.3%) were caused by ischemic heart disease and stroke. From 1990 to 2013 the total number of cardiovascular deaths increased by 40.8%, mainly due to aging and population growth. However, age-standardised cardiovascular mortality rates from CVD fell by 22.0% between 1990 and 2013. Although mortality rates for ischemic heart disease and stroke fell (by 22.3% and 22.5%, respectively) during the same period, mortality rates for atrial fibrillation and flutter increased by 100.0%. This increase could be due to an increased awareness of atrial fibrillation or to better survival.

Although cardiovascular mortality rates have declined dramatically in the world over the last decades, there are wide variations in the patterns of these rates between regions and countries. In high-income countries, age-standardised cardiovascular mortality rates fell by 43% between 1990 and 2013, whereas the number of cardiovascular deaths did not change during this period.[69] The decline in cardiovascular mortality rates was attributable to favourable changes in risk factors on a population level and to health care improvements. In contrast, in the same period in low- and middle-income countries, age-standardised cardiovascular mortality rates fell by only 13%, and the number of cardiovascular deaths

increased by 66%. The growth and aging of the population increased the proportion of cardiovascular deaths in low- and middle-income countries, and in 2013 this proportion was equal to that in high-income countries.

There is also remarkable variation in cardiovascular mortality by country. Between 1985-1989 and 2009-2012, cardiovascular mortality rates steadily declined in most European countries, with stronger declines observed after 1995-1999.[70, 71] The situation is more dire in the Russian Federation and the former Soviet Union, where cardiovascular mortality rates were still extremely high and showed no clear tendency to decline by 2010.[63, 70-72] In Norway, age-adjusted cardiovascular mortality rates decreased between 1991 and 2009, from 430 to 194 deaths per 100 000 inhabitants in men, and from 240 to 129 deaths in women.[73] According to the updated rates from 2012 (179.4 and 117.2 cardiovascular deaths per 100 000 inhabitants in men and in women, respectively), Norway has joined countries with the lowest cardiovascular mortality rates, including France, Portugal, and Spain.[69, 71] There are several possible causes for these wide variations between countries, such as differences in dietary patterns and other risk factors, differences in healthcare systems, political governance, and resulting policy decisions.[69]

1.5 Aims

To investigate secular trends in RHR and other cardiovascular risk factors, as well as the associations between RHR and these factors is important if we are to interpret population changes in cardiovascular morbidity and mortality rates, predict the future burden of CVD, and design effective preventive measures.

The overall aim was to explore secular changes in RHR using data from the Tromsø Study, which included a large study sample from the general population of Tromsø, Northern Norway from 1986 to 2008, and to explore the effect of RHR values on cardiovascular morbidity and mortality. This overall aim was separated into the following main research aims:

- To describe secular changes in RHR in a large sample from the general population over a 22-year period (Paper I).

- To explore individual associations between RHR changes and changes in relevant cardiovascular risk factors and to estimate the contribution of changes in other risk factors to the decline in RHR (Paper I).

- To investigate the effect of single RHR measures on the risk of incident myocardial infarction, incident atrial fibrillation, incident ischemic stroke, cardiovascular death, and total death in men and women (Paper II).

- To identify long-term individual trajectories in RHR and to estimate their effect on the risk of incident myocardial infarction, incident atrial fibrillation, incident ischemic stroke, cardiovascular death, and total death in men and women (Paper III).

- A secondary aim of this project was to apply new and comprehensive methods of statistical analysis, especially longitudinal data analysis (Papers I, II, and III).

Chapter 2 Materials and methods

2.1 The Tromsø Study

2.1.1 Settings, study design, and ethical considerations

The city of Tromsø is the largest urban area in Northern Norway, the third largest city north of the Arctic Circle (following Murmansk and Norilsk), and the eighth largest municipality in Norway. The population has been increasing steadily, from 12 283 residents in 1960, to 50 548 in 1990, to 72 681 in 2015.[74] An excess of births has been a bigger factors than immigration in this population growth. More than 100 nationalities are represented in Tromsø; the more common minorities are the Sami, Russians, and Finns. The climate is subarctic.

The Tromsø Study is a large, single-centre, population-based longitudinal study conducted in the Tromsø municipality. The study was initiated by the University of Tromsø in 1974, and its aim was to investigate the causes of the extremely high cardiovascular mortality in Northern Norwegian men.[75] Later on, the focus of the study expanded to other chronic diseases, and included women as well. At present, six consecutive surveys of the Tromsø Study have been conducted: Tromsø 1 (1974, also referred as the Tromsø Heart Study, it included men only), Tromsø 2 (1979-1980), Tromsø 3 (1986-1987), Tromsø 4 (1994-1995), Tromsø 5 (2001-2002), and Tromsø 6 (2007-2008).

Both whole birth cohorts and random samples of residents of the Tromsø municipality were selected for each survey based on the official population registry.[75] New birth cohorts were successively added to the study population during Tromsø 1-4. Subjects that had been invited to a previous survey were invited to the following survey regardless of their attendance at the previous survey. In contrast to the other surveys, a larger proportion of those selected for participation in Tromsø 5 had participated in Tromsø 4. After adjustment for emigration and deaths, attendance rates were >80% in Tromsø 1-3, 77% in Tromsø 4, 79% in Tromsø 5, and 66% in Tromsø 6. Attendance rates increased with age and were higher in women.

The design of all Tromsø Study surveys was similar. Potential participants were sent an invitation, including information about the study and the examination, and a first questionnaire.[75] Potential participants were allowed to attend whenever convenient within the

survey period, and one reminder was sent to non-attenders. The first questionnaire collected information on self and family history of CVD, physical activity, smoking, and ethnicity; it was completed at home and returned at the time of examination. All examinations were conducted by trained personnel according to standardised protocols, and included a review of the first questionnaire, physical examination, and blood sample collection. In Tromsø 2-6, participants were given a second, more comprehensive questionnaire at the time of examination. This questionnaire collected information on dietary habits, alcohol consumption, history of illnesses, social status, and physiological status; it was to be completed at home and returned by mail in a pre-addressed, stamped envelope. Approximately 90% of participants returned the second questionnaire. In Tromsø 4-6 a large proportion of participants were also invited to a second visit for a more extensive medical examination.

The Tromsø Study was approved by the Data Inspectorate and by the Regional Committee for Medical Research Ethics, North Norway, and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All participants in Tromsø 4-6 provided written informed consent. Participants are allowed to withdraw their consent or give a new one at any time. The invitation letters, questionnaires, consent forms, and thorough information about the data from the Tromsø Study can be found on the Tromsø Study website (www.tromsoundersokelsen.no). Direct weblinks to the invitation letters, questionnaires and informed consent forms for Tromsø 3-6 are presented in the Appendix.

2.1.2 Study population

In the present study, we chose to use data from Tromsø 3-6, as RHR was measured the same way and by the same equipment in these surveys. Male and female participants of these four surveys who were 20 years of age or older at examination and provided informed consent were eligible for inclusion in the present study. As some of the participants did withdraw/give a new consent between 2011 and 2015, the number of participants with consent varied across the papers published over the period (Table 1). In all of the three papers, we excluded survey-specific information on those who were pregnant at the time of

examination and/or had missing RHR values. Other exclusion criteria differed across the papers.

Paper I focused on RHR changes from 1986 to 2008, and therefore included information from Tromsø 3-6. To make the four surveys comparable by age, only those aged 30 to 89 years at the time of examination were included. Therefore the subsample in Paper I comprised 30 699 participants (59 309 visits), 16 406 of whom attended two or more surveys.

In Paper II we analysed associations between single RHR measures and risk of myocardial infarction, atrial fibrillation, ischemic stroke, cardiovascular death, and total death. As Tromsø 4 was the largest of the Tromsø Study surveys, it was chosen as the baseline survey. After exclusion of participants using BP treatment and those with missing information on cardiovascular risk factors, the final subsample for Paper II comprised 24 489 participants aged 25 years and older, 9086 of whom also took part in Tromsø 5 and/or Tromsø 6. Information on all of the considered variables was updated for participants who also took part in Tromsø 5-6, except for those who became pregnant and/or who started BP treatment.

Paper III demonstrated associations between long-term individual trajectories in RHR and the risk of the outcomes mentioned above. Using data from Tromsø 3-5, but not Tromsø 6, allowed for a longer follow-up period. We excluded participants who reported that they had ever used BP treatment. We further excluded information from surveys at which a participant had missing values for any of the considered variables at that survey. In addition, we excluded participants who died or emigrated before 28 August 2001 (median of the Tromsø 5 period – start of follow-up), and those who attended only one survey between Tromsø 3 and Tromsø 5. Following all exclusions, 14 208 participants (31 902 visits) aged 20 to 87 years were included in the subsample for Paper III. For more information on the subsamples used in Papers I-III see Table 1.

Table 1. Overview of subsamples of the Tromsø Study used in Papers I-III.

	Sample size (n) and inclusion criteria		
	Paper I Resting heart rate decline	**Paper II** Resting heart rate and endpoints	**Paper III** Resting heart rate change and endpoints
Number of participants before exclusions[a]:			
- Tromsø 3 (1986-1987)	20 507		20 517
- Tromsø 4 (1994-1995)	26 952	26 966	26 966
- Tromsø 5 (2001-2002)	8 040	8 046	8 046
- Tromsø 6 (2007-2008)	12 980	12 981	
- *Total*	*34 751*	*26 966*	*32 708*
Inclusion criteria[b]:			
- Age (years)	30-89	25-96	20-87
- Not pregnant	+	+	+
- Not using blood pressure treatment	-	+	+
Number of participants after exclusions:			
- Tromsø 3 (1986-1987)	15 053		13 137
- Tromsø 4 (1994-1995)	23 456	24 489	14 072
- Tromsø 5 (2001-2002)	7 993	4 573	4 693
- Tromsø 6 (2007-2008)	12 807	6 572	
- *Total*	*30 699[c]*	*24 489[d]*	*14 208[e]*
Endpoints information	-	+	+

[a]Number of participants who provided informed consent by 5 December 2011 (data for Paper I) and by 11 August 2015 (data for Papers II and III) was different. [b]Those with missing values on RHR or other considered variables were excluded from all papers. [c]Out of 30 699 participants, 14 293 attended only one of the four surveys, 7617 attended two of the surveys, 5374 attended three of the surveys, 3415 attended all of the four surveys, and 16 406 attended at least two surveys. [d]Out of 24 489 participants of Tromsø 4, 9086 attended at least one of the subsequent surveys, and 2059 attended all the three surveys. [e]Every participant attended at least two of the three surveys.

2.1.3 Data collection and resting heart rate measurement

Following standardised protocols, at each examination trained personnel reviewed the first questionnaire, performed a physical examination and collected blood sample. The first questionnaire provided different information depending on the survey. In Tromsø 3-6, it collected information about BP treatment use (yes/no), leisure time physical activity (sedentary, moderate, active, and very active), daily cigarette smoking (yes/no), history of a heart attack (yes/no), history of a cerebral stroke/brain haemorrhage (yes/no), history of diabetes (yes/no), coffee consumption (cups per day). In Tromsø 3-5 the first questionnaire collected information about current pregnancy (yes/no), in Tromsø 4-6 about alcohol consumption (times per month), and in Tromsø 5-6 about dietary habits. The second questionnaire provided information about current pregnancy in Tromsø 6, alcohol consumption (times per month) in Tromsø 3, and dietary habits in Tromsø 3-4.

Weekly average leisure time physical activity for the last year was assigned to one of four levels according to following criteria:

- Sedentary – reading, watching TV, or other sedentary activity;
- Moderate – walking, cycling, or other forms of exercise at least 4 hours per week (include walking or cycling to work, Sunday walk/stroll, etc.);
- Active – participation in recreational sports, heavy gardening, etc. (note: duration of activity at least four hours per week);
- Very active – regular participation in hard training or sports competitions several times per week.

Questions on leisure time physical activity were different in Tromsø 4 and for subjects 70 years of age or older in Tromsø 5: how many hours per week (none, less than 1, 1-2, 3 or more) did you spend on 1) light activity (not sweating/out of breath), and 2) hard physical activity (sweating/out of breath). In order to correspond to the categories used in the other surveys, these answers were regrouped using an algorithm presented in Table 2.[76]

Table 2. Algorithm for regrouping of physical activity questions from Tromsø 4 and for those aged 70 years or older in Tromsø 5 into four levels[76].

Hours of hard physical activity per week	**Hours of light physical activity per week**			
	None	**Less than 1**	**1-2**	**3 or more**
None	Sedentary	Moderate	Moderate	Active
Less than 1	Moderate	Moderate	Moderate	Active
1-2	Moderate	Moderate	Active	Active
3 or more	Active	Active	Active	Highly active

RHR and BP were recorded before blood sampling using an automated, non-invasive, microprocessor-controlled Dinamap device,[77, 78] which uses the oscillometric method. Pulsatile blood flow through an artery produces oscillations of the artery wall that are transmitted to the Dinamap cuff around the upper arm.[6] When the cuff pressure is higher than the systolic BP, the artery is occluded and few oscillations are transmitted to the cuff. When the cuff pressure is lower than systolic BP, the occlusion is partially released, and the pulsating blood flow produces oscillations in the cuff. When the cuff pressure falls below the diastolic BP and partial occlusion of the artery is relieved, oscillations are diminished. These devices inflate and deflate the cuff automatically. Results are displayed on the monitor. This work is controlled by a microprocessor.

When switched on, the Dinamap device starts by pumping the cuff pressure up to 178 mmHg. After this pump-up pressure is reached, the Dinamap begins a stepped deflation. It then determines systolic BP first, then mean arterial pressure, and then diastolic BP and pulse rate from oscillations induced in the cuff at the varied pressure levels. If a subject's systolic BP exceeds the pump-up pressure (the absence of a systolic value), the Dinamap will stop deflation and re-inflate to a higher pump-up pressure (re-inflate maximum is 250 mmHg). The Dinamap deflates the cuff one step each time it detects two pulsations of relatively equal amplitude. If it is unable to find any pulse within 1.6 seconds, it will deflate to the next step. At each step, the microprocessor stores the cuff pressure, the matched pulse amplitude, and the time between successive pulses. The stepped deflation and matched pulse detection

continues until diastolic BP is determined or until the cuff pressure falls below 7 mmHg. Then the Dinamap deflates the cuff to 0 mmHg, analyses the stored data, and presents the results on a display. The maximum pulse rate detected by the monitor is 200 bpm, and the minimum is 40 bpm. For systolic and diastolic BP the maximum/minimum detected values are 245/30 mmHg, and 225/20 mmHg, respectively.

The Dinamap Vital Signs Monitor 1846 (Critikon Inc, Tampa, Florida, USA) was used in Tromsø 3-5,[79] and the Dinamap ProCare 300 (GE Medical Systems Information Technologies, Tampa, Florida, USA) was used in Tromsø 6.[80] The devices were calibrated at regular intervals. The circumference of the upper right arm was measured and the proper cuff size was selected out of four available. After the participants had been seated for 2 minutes with the cuff on, three values were taken at 1-minute intervals.[81] The mean value of the last two RHR measurements (in bpm) and of the last two BP measurements (in mmHg) was used in the present study.

Body weight was measured with an electronic scale. Both body weight and height were measured with light clothing and no shoes, and were used to calculate BMI (kg/m^2).[75] Blood samples were used to determine non-fasting serum total cholesterol, HDL cholesterol and triglycerides (mmol/L). The analyses were carried out at the Department of Clinical Chemistry, Department of Medical Biology, University Hospital of North Norway, Tromsø.[78]

2.2 Follow-up information

Through the unique Norwegian personal identification number, the Tromsø Study participants can be linked to a variety of national and local registries, and can be followed up for a variety of outcomes. In present study participants were followed up for incident non-fatal or fatal myocardial infarction, incident non-fatal or fatal atrial fibrillation, incident non-fatal or fatal ischemic stroke, cardiovascular death, and total death throughout 2012. All cardiovascular outcomes were identified by linkage to the diagnosis registries at the University Hospital of North Norway (outpatient diagnoses included) and the National Causes of Death Registry, through a broad search for the International Classification of Diseases, Ninth Revision (ICD-9) codes 410-414, 427, 428, 430-438, and 798-799 and the ICD, Tenth Revision (ICD-10) codes I20-I25, I46-I48, I50, I60-I69, R96, R98, and R99. The

University Hospital of North Norway is the only hospital that serves the municipality of Tromsø; the next-nearest hospital is located approximately 250 km away by road (148 by air). The National Causes of Death Registry covers individuals registered as living in Norway at the time of their death, without regard to whether the death took place in Norway or abroad.

Following a detailed protocol, an independent endpoint committee validated all possible events that were identified through the broad search. The committee retrieved all available hospital medical records for case validation. They performed manual searches in paper medical records (used until 2001) and electronic text searches of digital medical records for notes on all outcomes in all participants with one or more of the aforementioned ICD codes. Information from the National Causes of Death Registry and from death certificates was used to collect relevant information on fatal events from additional sources such as autopsy reports, nursing home records, ambulance services, and general practitioners.

The definition and ascertainment of incident myocardial infarction was based on a classification algorithm which included clinical symptoms and signs, findings in electrocardiograms, values of cardiac biomarkers, and autopsy reports, when applicable (Paper II, Supplementary Table). In the present study, we included all incident myocardial infarctions classified as definite myocardial infarctions. Stroke was defined according to the World Health Organisation definition as rapidly developing clinical signs of focal or global disturbance of cerebral function, with symptoms lasting 24 hours or longer or leading to death, and with no apparent cause other than vascular origin. Strokes were classified as ischemic when brain imaging and/or autopsy had ruled out primary intracranial haemorrhage. ECG evidence of atrial fibrillation was required. Subjects with transient atrial fibrillation occurring only during acute myocardial infarction or in connection with a cardiac surgical procedure, and persons with atrial fibrillation documented only in the terminal phase of life, here defined as the last 7 days, were not classified as having atrial fibrillation. Participants who had emigrated from Tromsø were identified through the Population Register of Norway.

2.3 Statistical analysis

All analyses were sex-specific and were performed using the SAS program (SAS Institute, Cary, North Carolina, USA) versions 9.3 and 9.4. STATA/MP V.13.0 (StataCorp LP, College Station, Texas, USA) were used to calculate age-adjusted incidence rates.

2.3.1 Paper I

Descriptive statistical methods were used in Paper I to describe secular changes in RHR. RHR means were calculated in the four included surveys, in 10-year age groups and in 10-year birth cohorts. We also calculated survey-specific means (standard deviations, SD) of RHR adjusted for age between sex and surveys using linear mixed models.

Two approaches were applied to explore the associations between individual changes in RHR and changes in other cardiovascular risk factors. The first approach was to estimate the unadjusted association only. Individual changes in RHR between Tromsø 3 and Tromsø 4, between Tromsø 3 and Tromsø 5, and between Tromsø 3 and Tromsø 6 were calculated. The means of these individual changes were presented according to individual changes in other cardiovascular risk factors one by one. BP, blood lipids, and BMI were dichotomised into a survey- and sex-specific median split (yes/no for adverse/favourable level, respectively). The physical activity variable was dichotomised by collapsing sedentary and moderate physical activity levels into "into low physical activity" and active and highly active levels into "high physical activity". For the binary variables there were four combinations of individual change between Tromsø 3 and each of Tromsø 4-6: no-no or yes-yes indicating favourable or adverse levels of a cardiovascular risk factor at both surveys, respectively; no-yes indicating change to adverse, or yes-no indicating change to favourable level of a cardiovascular risk factor.

The second approach was to use data from Tromsø 3-6 as longitudinal, and to explore independent associations between individual changes in RHR and changes in other cardiovascular risk factors. Longitudinal studies are used to investigate the individual development of a certain outcome variable over time, and associations with individual development of other variables.[82] In longitudinal studies, the outcome variable is repeatedly measured in the same individual over time. These repeated measurements are not

independent of each other, and therefore require a special type of statistical analysis to adjust for dependencies. In this study, the linear mixed models analysis was used to assess independent associations between individual changes in RHR and changes in other cardiovascular risk factors.

The mixed models analysis (SAS Proc Mixed with the Repeated statement) has several advantages. First, it does not require complete data and allows the outcome variable to have missing values without affecting other scores from the same individual. Second, time can be continuous, rather than a fixed set of points. Third, possible dependencies between repeated measurements can be controlled for by selecting one of many covariance structures.

In the repeated measures design, two measurements taken at adjacent time points are usually more correlated than two measurements taken several points apart.[83] That is why it is essential to assess the covariance structure of the data. The mixed models analysis consists of two main steps: 1) estimating the covariance structure and 2) fitting the mean model to account for this covariance structure. The covariance parameters are estimated using likelihood-based methods (the restricted maximum likelihood by default). This method obtains estimates of parameters by minimising the likelihood of residuals from fitting the fixed effects portion of the model.

Here is an example of a mixed models equation:

$$Y_{it} = \beta_0 + \sum_{j=1}^{J} \beta_{1j} X_{ij} + \sum_{k=1}^{K} \beta_{2k} Z_{ikt} + \beta_3 t + \varepsilon_{it},$$

where Y_{it} represents measurements of the dependent variable for an individual i at time t, β_0 represents the intercept, X_{ij} is the time-independent covariate j for individual i, β_{1j} is the regression coefficient for time-independent covariate j, J is the number of time-independent covariates, Z_{ikt} is the time-dependent covariate k for individual i at time t, β_{2k} is the regression coefficient for time-dependent covariate k, K is the number of time-dependent covariates, t is time, β_3 is the regression coefficient for time, and ε_{it} is the random error for individual i at time t (within subject covariance structure).

Only those who attended at least two of the Tromsø 3-6 surveys and did not have missing values on considered variables were included in the mixed models analysis. The dependent variable in the model was all subsequent values of RHR after the first attended survey. Time-independent covariates were RHR value, values of other continuous cardiovascular risk factors, and physical activity level (four levels from sedentary to highly active) at the first survey attended, and year of first survey attended (1986, 1994, or 2001). Time-dependent covariates were year of subsequent survey (1994, 2001, or 2007), attained age, changes in other continuous cardiovascular risk factors and physical activity level, calculated as the difference between the subsequent and the first attended survey values, and change in BP treatment and smoking (quit, started, or continued, with 'no' to all as the reference). All continuous independent variables in the model, except age and RHR at first attended survey, were standardised. All continuous independent variables and physical activity variables were centred on their mean value. All the independent variables were included into the model at once, and therefore were mutually adjusted. The unstructured covariance matrix was used to adjust for possible dependencies between repeated observations, as it is the least restrictive of those available. The normality assumption in the linear mixed models analysis was assessed by visual inspection of the residuals.

Mean RHR change from Tromsø 3 to Tromsø 6 adjusted for other cardiovascular risk factors was estimated as the difference between the intercept of the model (mean RHR at Tromsø 6 whose first survey attendance was at Tromsø 3, and mean values or reference categories for other independent variables) and the RHR mean in Tromsø 3.

2.3.2 Paper II

In Paper II we intended to explore associations between single RHR measures and risk of incident myocardial infarction, incident atrial fibrillation, incident ischemic stroke, cardiovascular death, and total death. To visualise crude and age-adjusted associations we calculated sex-specific crude and age-standardised incidence rates as the number of events per 1000 person-years at risk by 10 bpm RHR groups. We applied direct standardisation using the weights from a reference population (men and women separately) to compute the weighted average of stratum-specific rate estimates in men and women. As the same reference population was used to compute directly standardised estimates for the 10 bpm

RHR groups, the resulting estimates can be compared between the RHR groups within each sex.

Cox proportional hazards regression analysis with time-dependent covariates was used to analyse adjusted associations. However, there were several possible ways to include RHR, the independent variable of interest, into the model: as a continuous variable, as a categorical variable through "dummy variables", and by using fractional polynomials. A continuous approach is easy to perform and to understand, but it is not able to reveal non-linear associations. A categorical approach is capable of that, but the results depend on the number and choice of cutoff points. Moreover, the results falsely postulate that risk suddenly changes as a category cutoff point is crossed and that there is homogeneity within each category. The fractional polynomials approach allows investigators to reveal associations of different forms by avoiding cutoff points. This approach is a simple, data-driven, flexible parametric method of modelling continuous risk factors.[84] Fractional polynomials try out many different curves to find the function that best fits the data, which is then modelled in the main analysis. Thus, to analyse associations between RHR and each of the events, we used fractional polynomials of RHR adjusted for age in a Cox regression model.

To prepare data for analysis, all participants with myocardial infarction, atrial fibrillation, and ischemic stroke before the date of inclusion (attendance of Tromsø 4) were excluded from event-specific analyses. Baseline RHR, as well as information about the other cardiovascular risk factors, was updated if a participant attended Tromsø 5 and/or Tromsø 6 and was not pregnant, and/or not on BP treatment at the time of these surveys. Follow-up started at the date of baseline examination at Tromsø 4 and lasted until the date of the event of interest, date of death, date of emigration, or 31 December 2012, whichever came first.

In sex- and endpoint-specific analyses, we used the following equation to find the best fitting fractional polynomials of RHR adjusted for age both first-degree and second-degree fractional polynomial Cox regression models:

$\ln(h(t)) = \ln(h_0(t)) + b_1 RHR^p + b_2 RHR^q + b_3 Age$ ($b_2 RHR^q = 0$ for first degree models),

where p and q were chosen from -2, -1, -0.5, 0, 0.5, 1, 2, and 3. Thus, 44 models with different combinations of powers (8 first-degree and 36 second-degree models) were fitted,

and sorted by the deviance. The deviance for each model has been subtracted from that of a straight-line model (baseline deviance): gain=baseline deviance – deviance for each model. Positive values of gain indicate an improvement in fit compared with the straight-line model. For each of the events, we chose the best-fitting fractional polynomials using the Akaike information criterion.[85] The first-degree model with a highest gain was chosen over the straight-line model if its gain was ≥2. The second-degree model with the highest gain was chosen over the straight-line model and over the best first-degree model if its gain was ≥6, and 4 or more units higher than the gain for the best first-degree model.

We ran sex-specific Cox regression analyses with time-dependent covariates to estimate hazard ratios (HRs) with 95% confidence intervals (CIs) for each of the five investigated events as a function of RHR and other cardiovascular risk factors. RHR was used as a continuous variable modelled according to the best fitting fractional polynomials form. We estimated HRs for RHRs of 50, 60, 80, 90, and 100 bpm, using 70 bpm as the reference value, using following formula:

$$HR = \exp\left(b_1 * \left(x^p - 7^p\right) + b_2 * \left(x^q - 7^q\right)\right),$$

where x represents the RHR value divided by 10, 7 is the reference RHR of 70 bpm divided by 10, b_1 and b_2 are regression coefficients for RHR in the second-degree model, p and q are degrees in the second degree model. HRs were adjusted for age only, and then for age, systolic BP, total cholesterol, HDL cholesterol, triglycerides, BMI, physical activity, and smoking, simultaneously. Likelihood ratio tests between a model with and a model without fractional polynomial terms of RHR were used to test the associations. The proportional hazard assumption was verified by comparing log minus log of survival curves between RHR groups.

2.3.3 Paper III

In Paper III we aimed to explore associations between long-term changes in RHR and risk of incident myocardial infarction, incident atrial fibrillation, incident ischemic stroke, cardiovascular death, and total death. We had up to four measurements of RHR per subject over the 22-year study period (Tromsø 3-6) and follow-up information on the endpoints of interest throughout 2012. The simplest way to analyse the data would have been to calculate individual changes in RHR between Tromsø 3 and Tromsø 4, and include this RHR change

into the Cox regression model. However, if we had done this we would have lost the RHR information from Tromsø 5 and Tromsø 6. Instead, we applied trajectory analysis (SAS Proc Traj), which allowed us to use RHR information from more than two surveys, and therefore gave a more comprehensive picture of long-term changes in RHR. For the sake of longer follow-up, we ran SAS Proc Traj using RHR information from Tromsø 3-5, but not from Tromsø 6, and then included identified RHR trajectories in the Cox regression analysis as a predictor.

Trajectory analysis (SAS Proc Traj), or latent class models analysis, is designed to identify distinct clusters of individuals, called trajectory groups, who have followed a similar developmental trajectory on a variable of interest over time or age.[86] The program assumes that there are multiple trajectory groups in the population, and fits longitudinal data as a discrete mixture of two or more latent trajectories via maximum likelihood. Proc Traj estimates a regression model for each discrete trajectory group within the population. Proc Traj does not provide any individual-level information on the pattern of time change; subjects are grouped, and it is assumed that every subject in the group follows the same trajectory.[87]

The latent class trajectory function can be specified as follows:

$$\left(y_{it}^{*}\right) = \beta_0^j + \beta_1^j x_{it} + \beta_2^j x_{it}^2 + \beta_3^j x_{it}^3 + \varepsilon_{it},$$

where each trajectory is represented by a latent variable, $\left(y_{it}^{*}\right)$, which is the predicted score of the outcome variable *y* for a given trajectory *j* at a specific time *t*; x_{it}, x_{it}^2, and x_{it}^3 represent time variable in linear, squared, or cubed term, respectively; β_0^j, β_1^j, β_2^j, and β_3^j are the intercept and slopes for a trajectory *j*; ε_{it} is an error tern assumed to be normally distributed with a mean of 0 and a constant SD.[88]

SAS Proc Traj, created by Dr. Bobby Jones, is not part of the standard SAS® program and must be downloaded. The Proc Traj files were downloaded from Dr Jones' website (http://www.andrew.cmu.edu/user/bjones) and copied to the folders on a hard drive as directed on the website. Using SAS Proc Traj, long-term RHR trajectory groups were derived among participants who attended at least two surveys between Tromsø 3 and Tromsø 5. The

censored normal distribution was used for modelling RHR trajectories, as it is appropriate for continuous dependent variables. The year of the survey was used as the time period scale. Using the Bayesian Information Criterion, we tested models with one, two, three, four, five, and six potential trajectories, all in a quadratic form. The final model had five trajectory groups as it fit better than the models with fewer groups, and because increasing the number of trajectory groups led to small group sizes. We compared the model fit of models with five groups with different functional forms (linear or quadratic), but kept all of the five trajectory groups with up to quadratic order terms, as this model was a more sensible representation of the data.[89] The posterior predicted probability of belonging to each of the five RHR trajectory groups was calculated for each individual, and individuals were assigned to a trajectory group based on the highest posterior probability. We named the trajectory groups to describe their visual patterns.

The number (proportion) of participants in the five RHR trajectory groups was calculated. RHR means were plotted according to the three surveys and the RHR trajectory groups. Descriptive characteristics of the RHR trajectory groups were presented as means (SD) and numbers (proportions). Means and percentages were adjusted for age between the surveys and the RHR trajectory groups using linear mixed models or generalised estimating equations, respectively.

Cox regression analysis was used to estimate associations between belonging to a certain RHR trajectory group and the risk of each investigated outcome. In the analysis of morbidity endpoints, we excluded those who had had the event of interest or had moved from Tromsø before the start of follow-up. We calculated HRs and their 95% CIs for each RHR trajectory group, using the low RHR trajectory group as a reference. HRs were adjusted first for the mean age across the surveys alone, and then for the mean values of age and other cardiovascular risk factors (systolic BP, total cholesterol, HDL cholesterol, triglycerides, BMI, leisure time physical activity, and smoking status at the last survey attended) across the surveys.

Chapter 3 Results – Summary of papers

3.1 Paper I "Resting heart rate on the decline: the Tromsø Study 1986-2007"

According to Tromsø 3-6, RHR did not differ with age, and was higher in women than in men. Between 1986 and 2008, mean RHR values declined by approximately 9 bpm in men, and 11 bpm in women. RHR declined in all 10-year age groups and in all birth cohorts. The age-adjusted RHR means decreased from 73.4 (SD 13.0) bpm to 64.7 (SD 10.9) bpm in men, and from 78.3 (SD 12.6) bpm to 66.4 (SD 10.3) bpm in women. More than half of this decline occurred between Tromsø 5 and Tromsø 6.

Univariate analysis showed that RHR declined gradually from survey to survey in all subjects regardless of any individual changes in other cardiovascular risk factors. However, the decline in RHR was more pronounced in those who changed from the adverse to the favourable level of BP, blood lipids, BMI (in men), smoking, and physical activity (in men) than in those who changed in the opposite direction or did not change. RHR declined in all subjects regardless of their change in BP treatment status, except for six men who stopped taking medication during the study period. The decline was less pronounced in those who took BP treatment throughout all the surveys.

When estimated by the linear mixed models analysis, the mutually-adjusted decline in RHR over the study period was 7.19 (95% CI 6.74 to 7.64) bpm in men and 9.99 (95% CI 9.54 to 10.44) bpm in women. Compared to the age-adjusted RHR decline (see above), 17.4% of the decline in men and 16.1% of the decline in women was attributable to individual changes in other cardiovascular risk factors. The strongest predictor of the RHR decline in both men and women was a decline in triglycerides. Thus, a 1-SD more pronounced decline in triglycerides was associated with a more pronounced decline of 1.65 bpm in RHR in men, and with a more pronounced decline of 1.27 bpm in RHR in women, compared to the estimated RHR decline (see above), which was estimated for a person with the mean change in triglycerides. Other strong independent predictors of individual decline in RHR were high initial physical activity level and increase in physical activity over time, decrease in systolic BP, change in smoking status, and a decrease in BMI in men; and initiation of BP treatment, low initial systolic BP,

decrease in systolic BP over time, and change in smoking status in women (arranged in decreasing order of the association strength).

3.2 Paper II "Resting heart rate predicts incident myocardial infarction, atrial fibrillation, ischemic stroke, and death in the general population: the Tromsø Study"

In men, both crude and age-adjusted incidence rates of myocardial infarction, cardiovascular mortality and total mortality increased gradually with RHR, while the association with atrial fibrillation was U-shaped. In women, incidence rates of all the cardiovascular events were elevated at both low and high RHR levels, resulting in U-shaped associations of RHR with myocardial infarction and total mortality, and J-shaped associations of RHR with atrial fibrillation, ischemic stroke, and cardiovascular mortality.

Cox regression analysis with RHR modelled according to the best-fitting fractional polynomials showed that the risk of myocardial infarction, atrial fibrillation, cardiovascular mortality, and total mortality in men increased gradually with RHR in both age-adjusted and mutually-adjusted models. Mutually-adjusted HRs of myocardial infarction, atrial fibrillation, cardiovascular mortality, and total mortality for a RHR of 100 bpm compared to 70 bpm were 1.16 (95% CI 1.04 to 1.30), 1.21 (95% CI 1.03 to 1.43), 1.77 (95% CI 1.49 to 2.10), and 1.72 (95% CI 1.52 to 1.94), respectively. In women, the associations were similar for myocardial infarction (even stronger than in men), cardiovascular mortality, and total mortality, with HRs for a RHR of 100 bpm compared to 70 bpm of 1.37 (95% CI 1.13 to 1.66), 1.63 (95% CI 1.33 to 2.00), and 1.55 (95% CI 1.39 to 1.73), respectively. However, in women we revealed a U-shaped association of RHR with ischaemic stroke: the adjusted HR for a RHR of 50 bpm was 1.31 (95% CI 0.90 to 1.90) and the adjusted HR for a RHR of 100 bpm was 1.32 (95% CI 1.04 to 1.69), with a RHR of 70 bpm as a reference. The positive association between RHR and atrial fibrillation diminished after adjustment for other cardiovascular risk factors.

3.3 Paper III "Long-term resting heart rate trajectories and myocardial infarction, atrial fibrillation, ischemic stroke, cardiovascular and total death in the general population: the Tromsø Study"

Five discrete long-term RHR trajectories were identified among participants who attended at least two surveys between Tromsø 3 and Tromsø 5: low RHR trajectory (those who maintained low RHR in Tromsø 3 through Tromsø 5), moderate RHR trajectory (those who maintained moderate RHR), decreasing RHR trajectory (those who started with high RHR and experienced a decrease to moderate RHR), increasing RHR trajectory (those who started with moderate RHR and experienced an increase to high RHR), and elevated RHR trajectory (those who had elevated RHR throughout). Out of 6898 men, 22.4% were in the low RHR trajectory group, 53.9% in the moderate trajectory group, 11.0% in the decreasing trajectory group, 10.1% in the increasing trajectory group, and 2.7% in the elevated trajectory group. In women (n=7310) the corresponding proportions were 24.6%, 58.6%, 8.6%, 6.9%, and 1.3%.

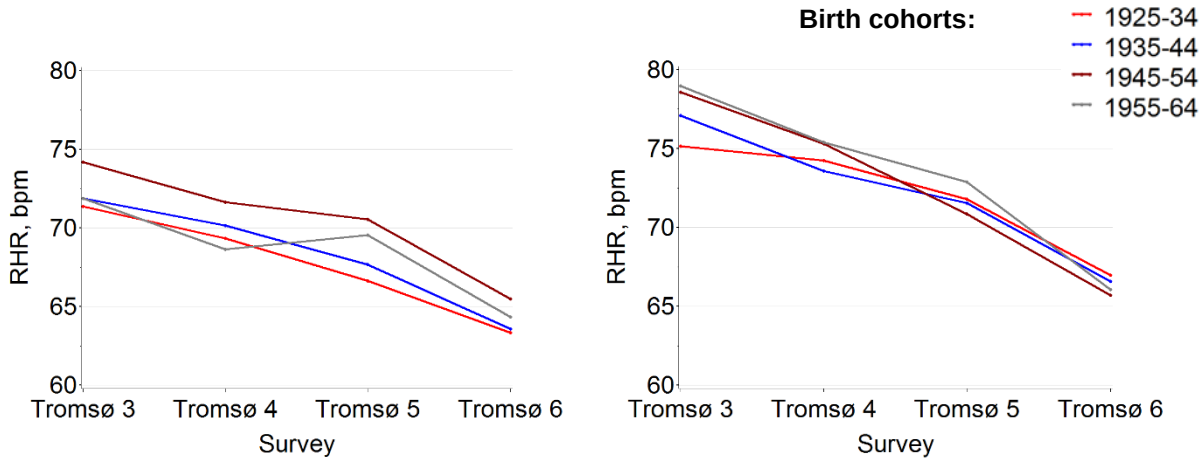
Among men and after adjustment for age, being in the moderate, decreasing, increasing or elevated RHR trajectory groups was associated with a gradually elevated risk of myocardial infarction compared to the low RHR trajectory group. However, only the increasing and the elevated RHR trajectory groups independently predicted myocardial infarction compared to the low RHR trajectory group: HRs were 1.36 (95% CI 0.95 to 1.95) and 1.83 (95% CI 1.11 to 3.02). Among women, associations between RHR trajectories and risk of myocardial infarction were similar to those in men, but adjusted HR did not reach significance. No clear association was found between RHR trajectories and risk of atrial fibrillation or ischemic stroke neither in men nor in women. In men, when compared to the low RHR trajectory, risk of cardiovascular death was independently increased in the increasing (HR 1.86; 95% CI 1.11 to 3.11) and elevated RHR trajectory groups (HR 1.94; 95% CI 0.95 to 3.96). Risk of total death gradually increased from the low to the elevated trajectory groups with HRs of 1.26 (95% CI 1.02 to 1.56) for the moderate, 1.37 (95% CI 1.03 to 1.82) for the decreasing, 1.82 (95% CI 1.40 to 2.38) for the increasing, and 2.44 (95% CI 1.69 to 3.53) for the elevated RHR trajectory groups. We did not reveal associations between RHR trajectories and risk of cardiovascular death or total death in women.

3.4 Additional results

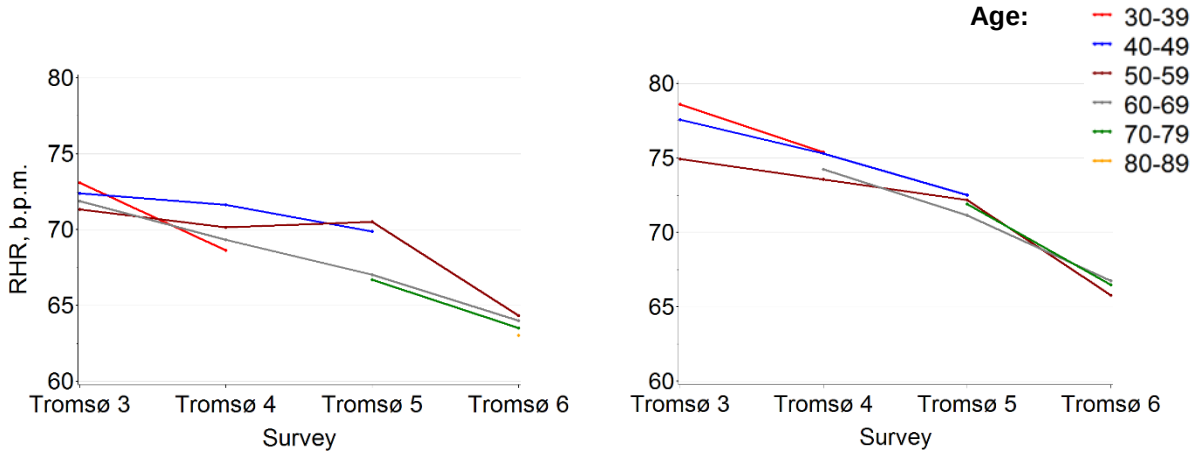
Some of the results were mentioned in the papers, but data were not shown. Figure 1 demonstrates the decline in RHR we observed among those who attended, and had their RHR recorded, at all four surveys. Figure 2 demonstrates RHR means between Tromsø 3 and Tromsø 6, in 10-year age groups and in 10-year birth cohorts among those who attended only one of the surveys. The degree and the pattern of decline in RHR in these groups were similar to the main results presented in Paper I. Figure 3 describes in detail the five RHR trajectory groups used in Paper III. Both individual values and RHR means in the trajectory groups are presented by sex.

Figure 1. Resting heart rate means in those who attended all the surveys under study (Tromsø 3-6, n=3415), the Tromsø Study, 1986-2008

a. By birth cohort and Tromsø Study survey



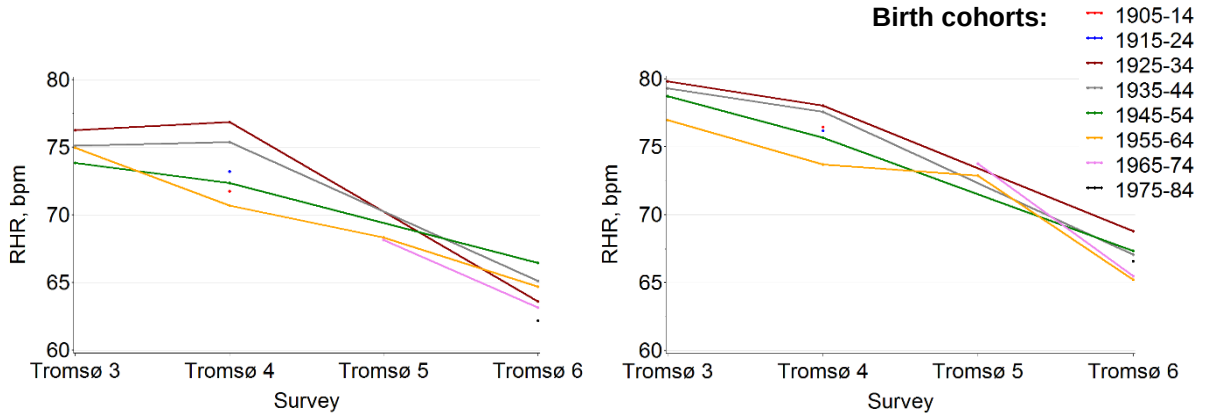
b. By age (years) and Tromsø Study survey



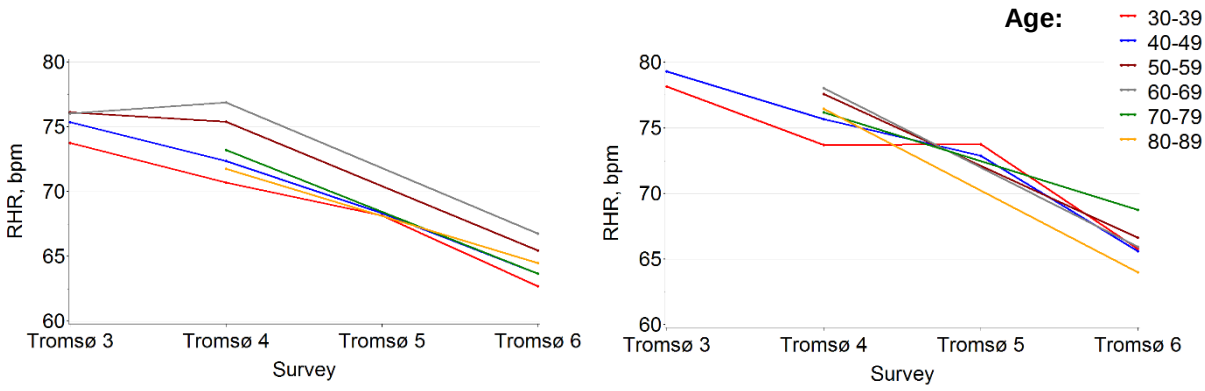
Men **Women**

Figure 2. Resting heart rate means in those who attended one of the surveys under study (Tromsø 3-6, n=10 951), the Tromsø Study, 1986-2008

a. By birth cohort and Tromsø Study survey



b. By age (years) and Tromsø Study survey



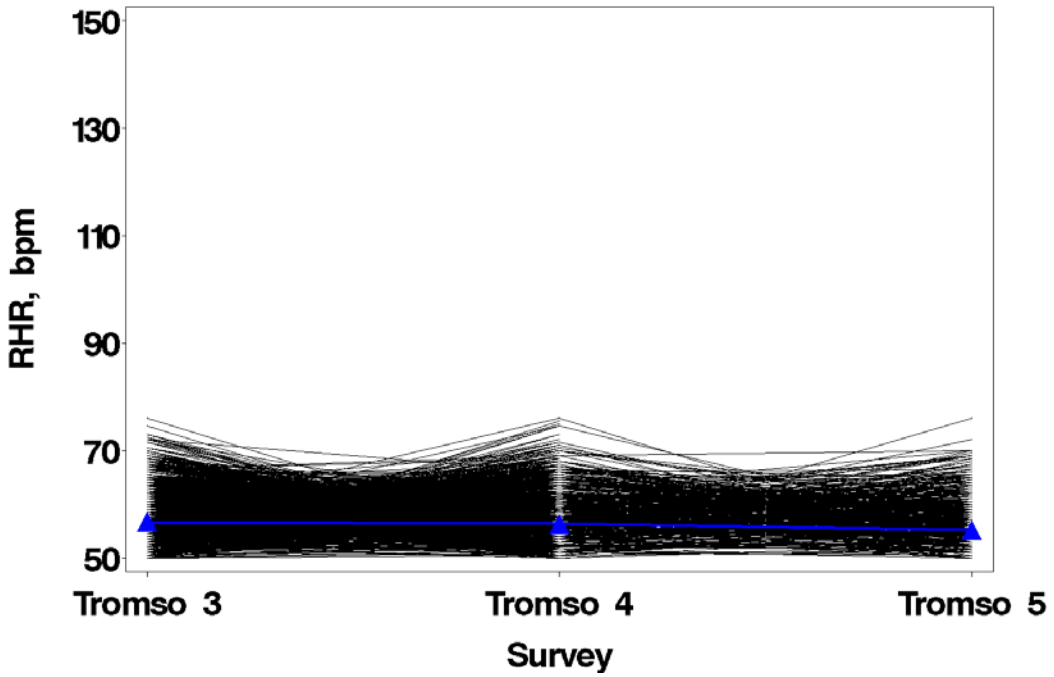
Men **Women**

RHR means for those aged 60-79 years at Tromsø 5 (n=15) and those born between 1915 and 1924 at Tromsø 6 (n=11) are not presented due to small sample size.

Figure 3. Individual values and means (blue triangles) of resting heart rate (RHR) by trajectory groups in men and women, the Tromsø Study, 1986-2001

1. **Men**

a. **Low RHR trajectory group in men**



b. **Moderate RHR trajectory group in men**

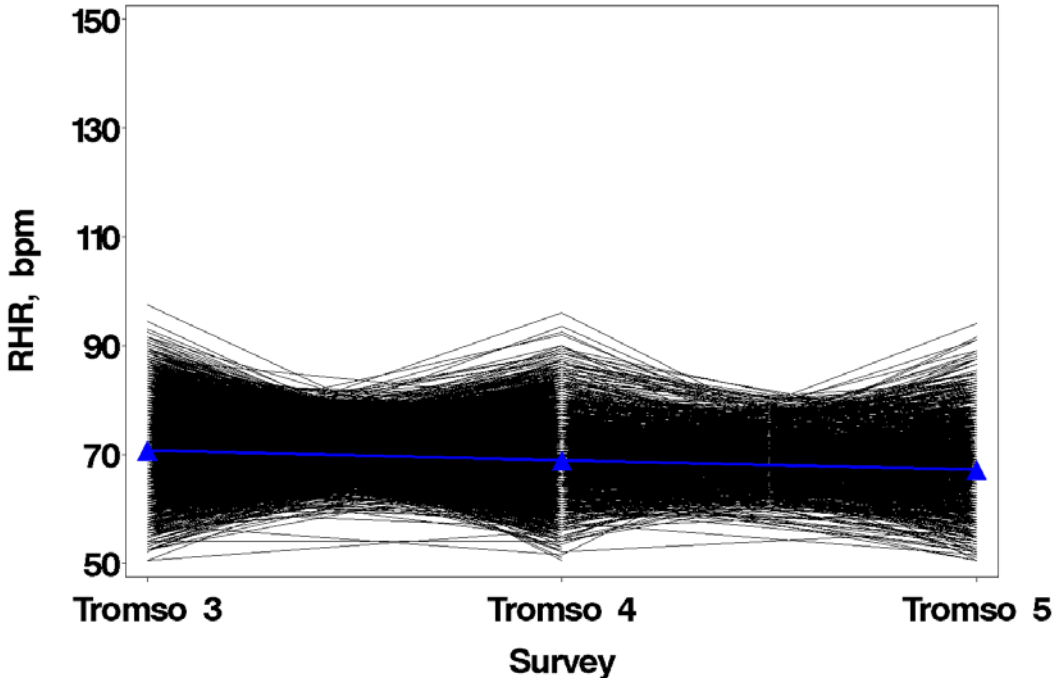
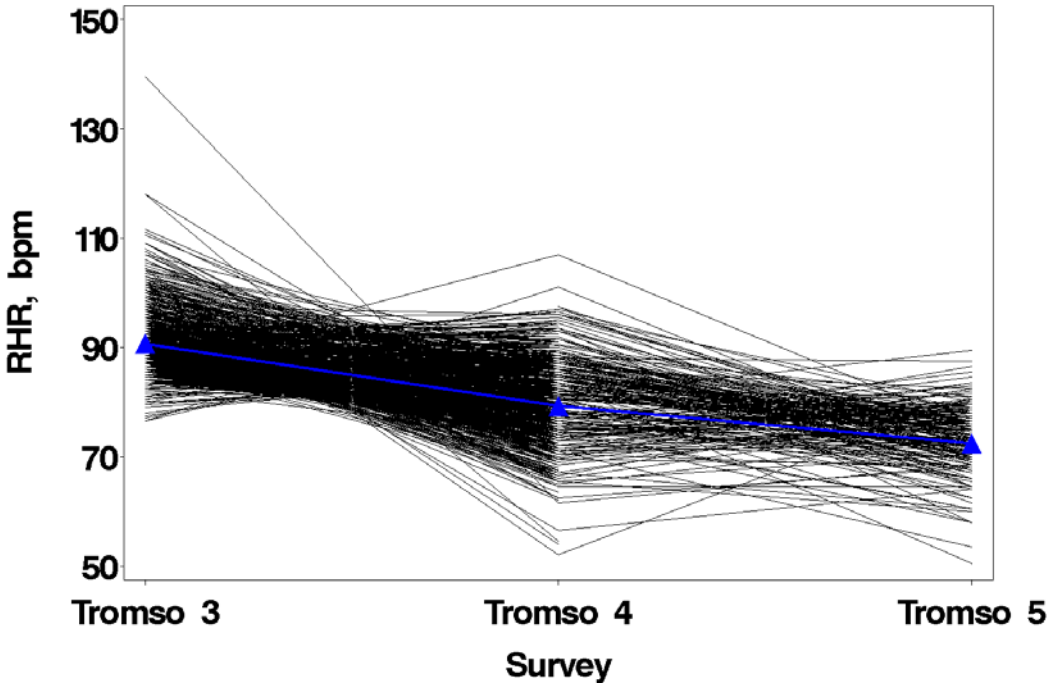


Figure 3. Individual values and means (blue triangles) of resting heart rate (RHR) by trajectory groups in men and women, the Tromsø Study, 1986-2001 (continued)

c. Decreasing RHR trajectory group in men



d. Increasing RHR trajectory group in men

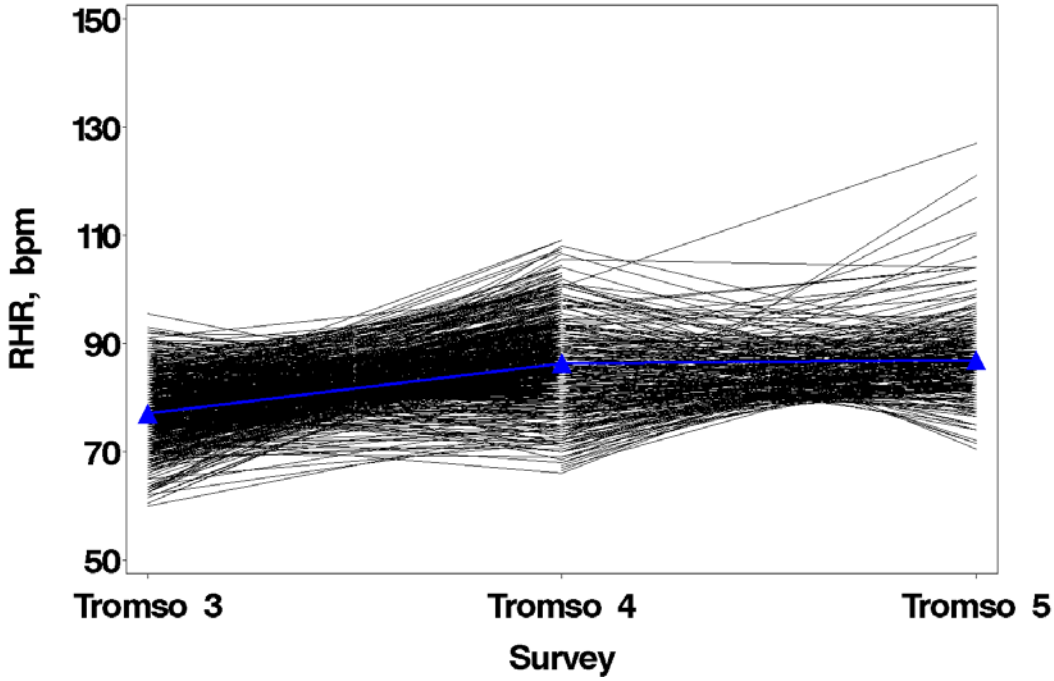
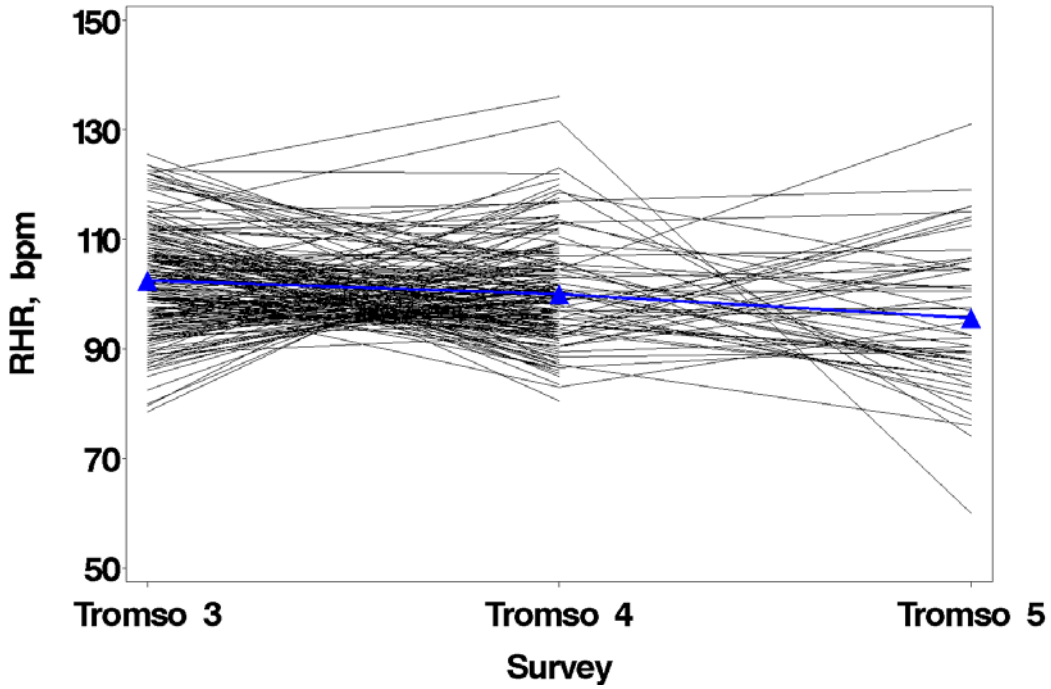


Figure 3. Individual values and means (blue triangles) of resting heart rate (RHR) by trajectory groups in men and women, the Tromsø Study, 1986-2001 (continued)

e. Elevated RHR trajectory group in men



2. Women

a. Low RHR trajectory group in women

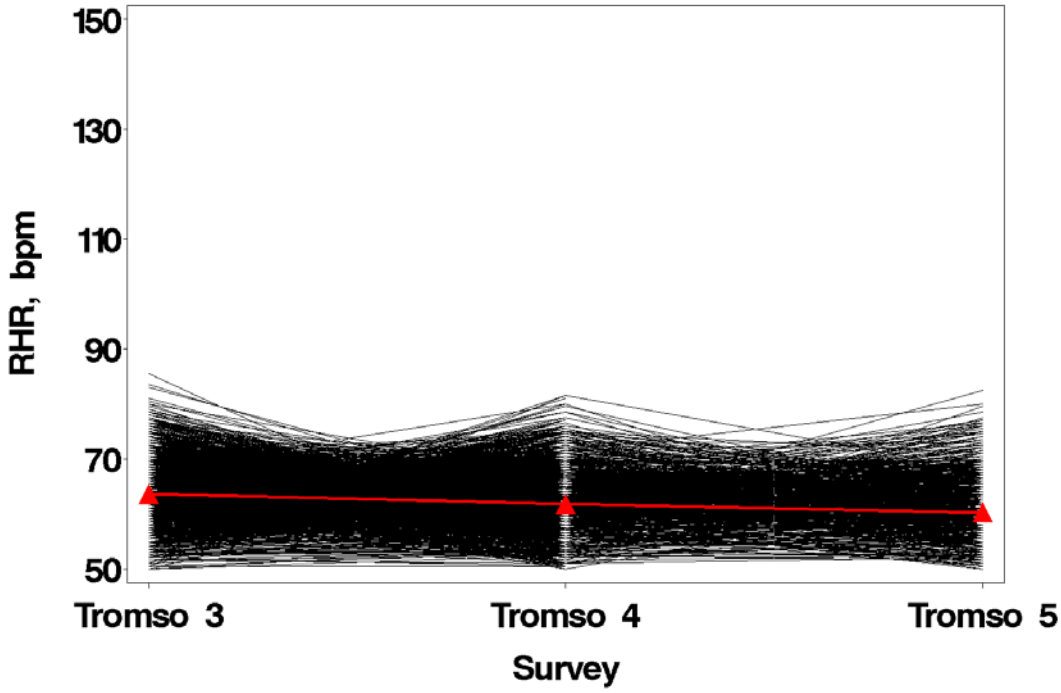
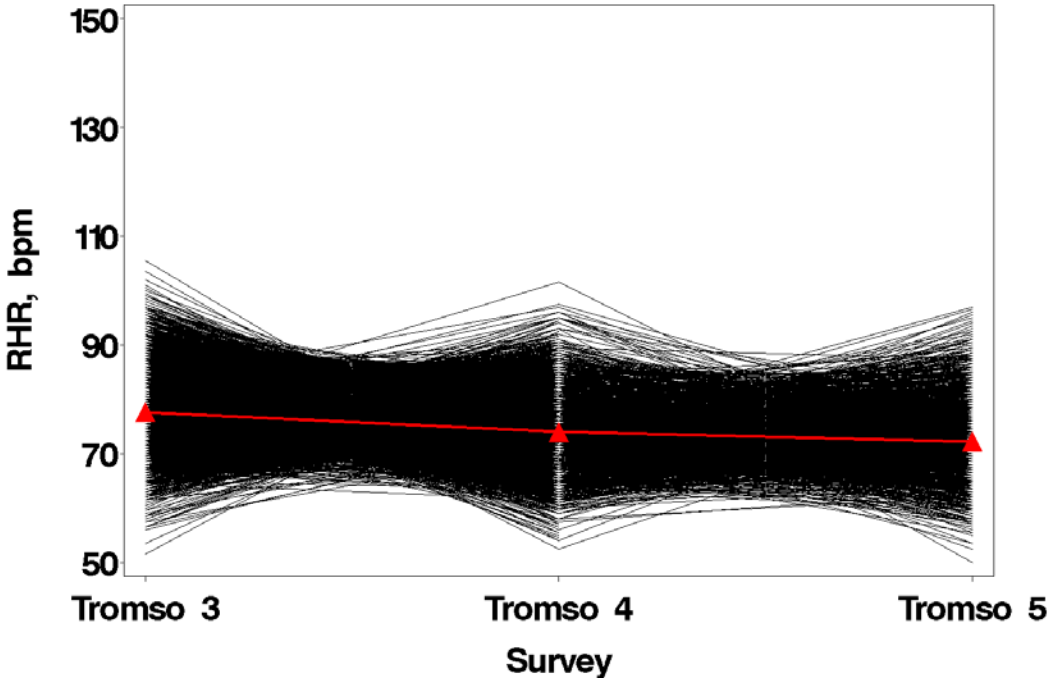


Figure 3. Individual values and means (blue triangles) of resting heart rate (RHR) by trajectory groups in men and women, the Tromsø Study, 1986-2001 (continued)

b. Moderate RHR trajectory group in women



c. Decreasing RHR trajectory group in women

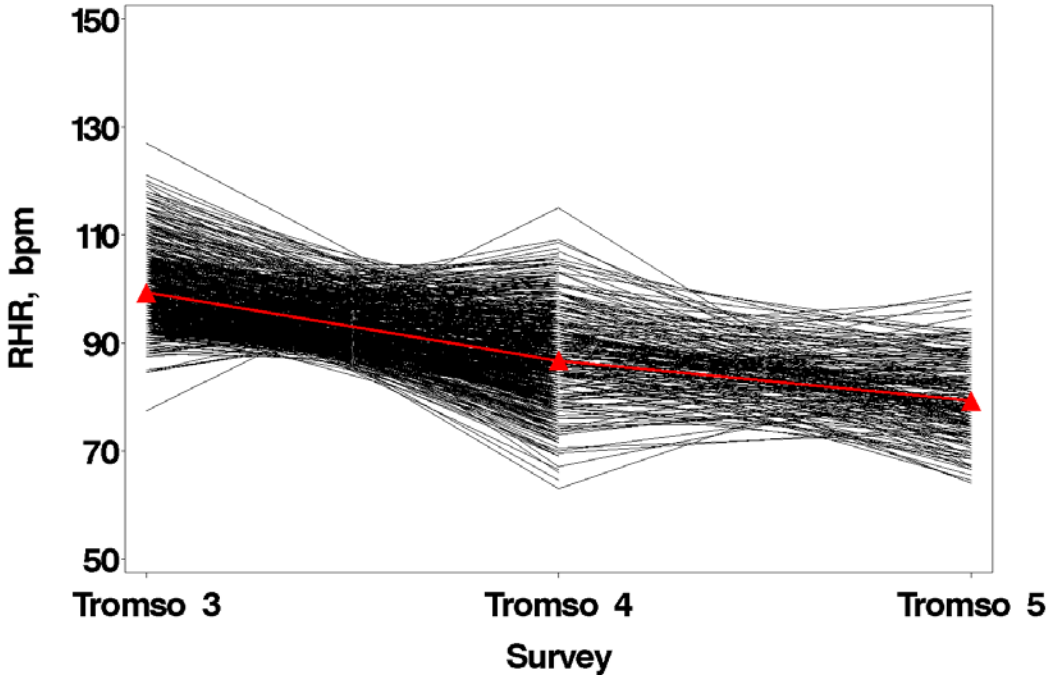
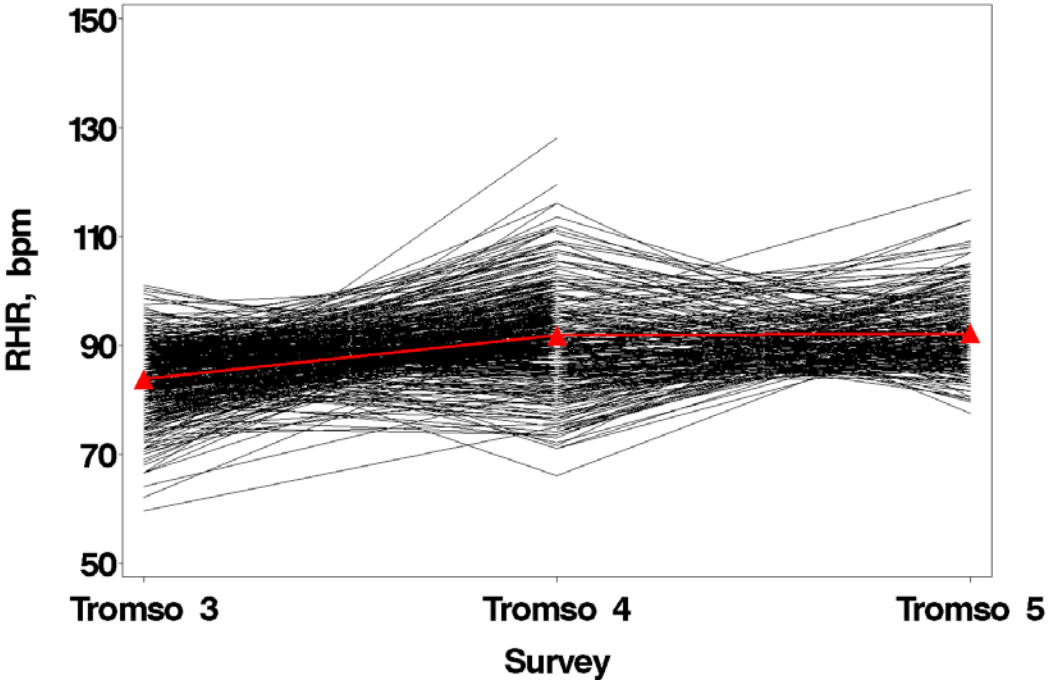
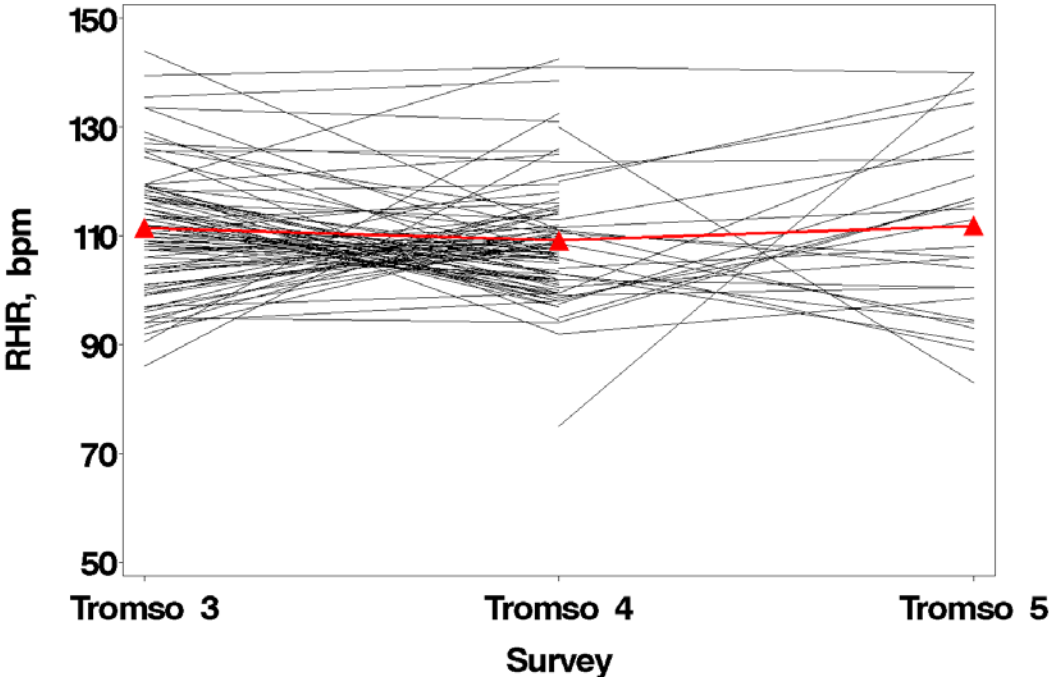


Figure 3. Individual values and means (blue triangles) of resting heart rate (RHR) by trajectory groups in men and women, the Tromsø Study, 1986-2001 (continued)

d. Increasing RHR trajectory group in women



e. Elevated RHR trajectory group in women



Bpm: beats per minute

Chapter 4 Discussion

4.1 Methodological considerations

4.1.1 Study design

This observational study clearly had the potential to answer the research questions stated in the study aims (see 1.4). The study design we used is located toward the top of the hierarchic scale of study designs, following systematic reviews and randomised control trials. Although systematic reviews are considered the best study design for getting precise estimates and drawing reliable conclusions, there are certain disadvantages associated with them, such as heterogeneity, loss of information on important outcomes, and inappropriate subgroup analyses.[90] Moreover, there are not many studies published on RHR changes over time, and none of them included longitudinal information. Randomised control trials would not be relevant for studying secular changes in the general population.

The Tromsø Study is a prospective, observational, population-based study conducted on large representative samples from the general population of the municipality of Tromsø.[67] The Tromsø Study cohort is open, and participants can leave or be added to the cohort over time. We used information from Tromsø 3-6. The fact that many, though not all, participants attended more than one of the these surveys makes the study longitudinal. The main advantage of longitudinal studies over cross-sectional ones is that the outcome variable is measured repeatedly, and the development of this outcome among individuals can be explored over time.[82] Thus, a longitudinal study design with a 22-year study period in a combination with appropriate statistical techniques is a proper way to explore RHR decline in the population in Tromsø 3-6 (Paper I). This approach also allows to update RHR values when studying associations between RHR and cardiovascular morbidity and mortality (Paper II), and to investigate and define long-term RHR trajectories in Tromsø 3-5 (Paper III).

Linking the Tromsø Study data to the diagnostic registries at the University Hospital of North Norway, the National Causes of Death Registry, and the Population Register of Norway through the unique Norwegian personal identification number makes this study a proper cohort study (Papers II and III). None of the participants had the outcome of interest at the beginning of the follow-up period (we excluded those who did using information from the

registries). Those participants who attended several surveys had their baseline information updated during the follow-up period (Paper II). The study cohort was followed up to 2012 for the outcome of interest (Papers II and III). Finally, the incidence rates of the outcome of interest were compared between groups with different exposure variables. This large population-based cohort study is an appropriate study design to answer questions about RHR (Paper II) and long-term individual changes in RHR (Paper III) as risk factors for CVD and mortality.

4.1.2 Selection bias, response rate, and loss to follow-up

Selection bias is a systematic error that can occur during the recruitment of study subjects. When such bias occurs, the results of the study may not be applicable to the general population of interest, as the participants may not be representative of this population. The Tromsø Study is a population-based study. To assure that the study sample was representative of the Tromsø population, entire birth cohorts, as well as random samples of other age groups from the Tromsø municipality were selected and invited based on the official population registry.[67] Thus, the main possible source of selection bias in the Tromsø Study is non-responders, or non-attendees.

The participation rate in the Tromsø Study was relatively high: >75% in Tromsø 3-5 and 66% in Tromsø 6 (adjusted for emigration and deaths). Although selection bias is usually not a big concern in large population-based studies with a high participation rate, some differences between attendees and non-attendees could still lead to selection bias. For example, attendees, especially those who attended several surveys, were more concerned about their health, and could therefore be healthier than people in the general population. This could result in a steeper downward trend in RHR in our Tromsø Study participants than in the general population. Compared to the Tromsø Study participants, non-attendees were younger, more often men and more often single.[67] It is not possible to compare attendees and non-attendees by other parameters, but findings from Tromsø 1 showed that participants who returned the second questionnaire after examination were remarkably similar to those who did not with regard to BP, blood lipids, and BMI.[91] Baseline characteristics of those who attended Tromsø 1[92] and Tromsø 3[93] were similar among those who did and did not participate in subsequent Tromsø Study surveys.

With respect to the endpoints, in cohort studies there is a possibility of differential loss to follow-up. To be differential, subjects who are lost to follow-up should be different from those who remained under observation in respect to the exposure of interest (RHR in Paper II, long-term changes in RHR in Paper III) and to the outcome of interest. If subjects who are lost to follow-up tend to have different risks for the outcome of interest, then only incidence estimates will be biased. If the possibility of being lost to follow-up is different with respect to the exposure of interest, then relative measures of association will be biased. In this dissertation, loss to follow-up is not a major concern because of the unique Norwegian personal identification number, which allows for linkage to national and local registries. The University Hospital of North Norway is the only hospital serving the Tromsø municipality. The National Causes of Death Registry covers individuals registered as living in Norway at the time of their death, without regard to whether the death took place in Norway or abroad. The Population Register of Norway contains information on those who emigrated from Tromsø. Even though there were still some losses to follow-up in our study, they were unlikely to be related to RHR or to the endpoints, and therefore are unlikely to be differential.

4.1.3 Information bias

The long study period is one of the main advantages of this study, but it is also one of the concerns. The study comprises data from the four Tromsø Study surveys conducted between 1986 and 2008 with a break of between 6 to 7 years between surveys. During the 22 years of observation, some changes in questionnaires and measuring techniques did occur. This is an issue of reproducibility, as, although most of these changes were favourable (improved due to scientific and technical progress), they could affect RHR values as well as the values of other variables. This is a potential source of measurement bias, or differential misclassification. In this study, differential misclassification means that the probability of being misclassified differs across the surveys, which could affect long-term changes in RHR and other variables.

During the four surveys RHR and BP were measured by the same Dinamap device, with a new model being introduced in Tromsø 6. In parallel, the most notable decrease in RHR occurred between Tromsø 5 and Tromsø 6. However, the difference in the accuracy of the pulse rate measurement between the two Dinamap models used was only 0.5%, which could

not explain the substantial decline in RHR we found.[79, 80] Moreover, RHR means declined from Tromsø 3, through Tromsø 4, to Tromsø 5, surveys during which the same Dinamap model was used. Finally, a study that aimed to evaluate accuracy of different non-invasive automatic BP monitors showed that the readings given by instruments using the oscillometric method were accurate and reproducible.[6]

The personnel responsible for RHR and BP measurements also changed during the study period. However, all of the surveys used trained female nurses who followed a standardised protocol. Moreover, changes in personnel could not cause a differential bias or result in the downward trend in RHR that we observed. It is very unlikely that newly-employed survey nurses would gradually underestimate RHR. The same logic can be applied to the rooms where these measurements were recorded, including temperature, space, colour of the walls, etc.

Although all of the examination procedures in Tromsø 3-6 were conducted according to standardised protocols, in Tromsø 6 participants underwent a cold water immersion test before RHR and BP measurements were taken. It is again unlikely that this test caused the differences in RHR that we observed across the surveys, as it usually results in an increase in RHR;[94] moreover, participants had time to rest after the test. Finally, RHR went down between Tromsø 3 and Tromsø 5 as well.

The fact that information on physical activity, smoking, BP treatment, diet, coffee consumption, and alcohol consumption was self-reported and that blood tests were non-fasting could also result in misclassification. People can, intentionally or unintentionally, under- or overestimate their physical activity level, misreport their smoking status or use of BP treatment, or underestimate their alcohol consumption. Non-fasting levels of blood lipids are influenced by the type and amount of food consumed, and the time since their last meal. All these potential measurement errors are most likely non-differential with respect to comparisons between the surveys (especially when these questions were asked in the same way), but they can be differential in respect to CVD and mortality.

According to mass media and the Tromsø Study paper on trends in modifiable cardiovascular risk factors, physical activity level increased in the Tromsø population over the last decades.

It reflects new culture, with more training facilities and more people who train.[57, 95, 96] Similar trends can be seen in Norway though there are some discrepancies between the studies.[97] Taking into account the strong negative association between physical activity level and RHR, one can suggest that this new culture is the explanation for the decline in RHR we observed. Nevertheless, our results showed that only a small part of the RHR decline was attributable to individual changes in physical activity. The question on leisure time physical activity in the Tromsø Study questionnaire was introduced by Saltin and Grimby almost 50 years ago and has been widely used in population-based studies ever since.[98] It was validated against various measures. However, instruments that measure a physical activity must not only be valid and reliable, but also sufficiently sensitive to detect relevant changes in patterns of habitual physical activity level. Objective instruments, such as fitness testing or devices to assess movement directly, are usually more sensitive than questionnaires,[99] but they are not yet common in large epidemiological studies due to their cost and the burden they place on respondents. A self-reported instrument such as the Tromsø Study questionnaire may not be sensitive enough to reveal modest, but clinically meaningful, changes in physical activity. There is sparse evidence on the responsiveness to change of common instruments used to measure physical activity. Reeves et al. compared the responsiveness to change of three self-reported measures of physical activity within the context of a broad-reach intervention trial.[100] The authors concluded that brief self-reported measures (2 walking for exercise items from the US National Health Interview Survey; and the 6-item Active Australia Questionnaire) are as responsive to change as more-detailed measures (the 31-item Community Health Activities Model Program for Seniors). Cleland et al. assessed the ability of the Global Physical Activity Questionnaire to detect changes in physical activity.[101] Levels of agreement with objective measurements indicated that the questionnaire was a valid measure of change in moderate-vigorous physical activity, but a less valid measure of change in sedentary behaviour.

Moreover, the physical activity question in our study was able to reveal changes in the duration and probably in the intensity of physical activity, but not in the type or frequency of the activity. Analysis was limited to leisure time physical activity, although physical activity at work may result in less physical activity in leisure time. Knowing these facts it is still

possible that individual change in physical activity played a more important role in the RHR decline than we were able to reveal.

The question on physical activity in Tromsø 4, and partly in Tromsø 5, was asked differently. Although we regrouped the physical activity levels to correspond to the other surveys, the figures in these surveys were quite different from those in other surveys. This could have led to a differential under- or overestimation of physical activity level at Tromsø 4, and partly at Tromsø 5, compared to other surveys, but this would not have affected the overall downward trend in RHR we observed over the study period.

4.1.4 Learning effect and sample attrition

Due to the longitudinal nature of our study, a learning effect may have occurred. A learning effect occurs when individual measurements can be influenced by a changing attitude toward the measurement itself.[82] A learning effect can be positive or negative. RHR is a flexible cardiovascular parameter that is influenced by many external parameters, such as psychological atmosphere, air temperature, light, wall colour, etc. For example, due to test effect, a reduction in the white-coat phenomenon and/or a better awareness among the participants may have made them more relaxed during subsequent surveys, thus lowering their RHR. This is a potential "negative learning effect" that could have contributed to the RHR decline we observed. However, time periods between the surveys were too long to make this theory plausible. Moreover, RHR means of those who attended only one of Trosmø 3-6 went down over the study period as well (Figure 2).

Sample attrition is another potential methodological problem in longitudinal studies that could contribute to the decline in RHR. Participants with an adverse cardiovascular risk profile, including high RHR, might have died before the subsequent survey. However, in our study the cohort was open, and new people were invited to the surveys, and the RHR decline was still of the same magnitude and was present in all age groups, including young people, who are less likely to die. Moreover, when only those who attended all of Tromsø 3-6 were considered, the RHR time-patterns were similar (Figure 1).

4.1.5 Age, time, and cohort effects in longitudinal studies

In longitudinal studies aiming to explore individual changes over time, each measurement of a variable of interest on each subject at a particular time point is influenced by at least three factors: age, period, and birth cohort.[82] The corresponding effects of these factors are referred to as age effects, period effects, and cohort effects, and they must be separated from each other to answer the research question. In this study, RHR changes over time were of interest. The RHR measurement for each subject at each survey could be influenced by the age of the subject at the survey, by the survey itself, and by the subject's year of birth. The aim was to find out whether the RHR decline was due to a period effect, or if it was confounded by age and/or birth cohort effects. A period effect would then be explained by other covariates, such as the development of other cardiovascular risk factors, and/or specific environmental conditions, and/or changes in measurement methods over time, etc.

The multiple longitudinal design, in which repeated measurements are taken in more than one cohort with overlapping ages, is most suitable for studying the development of a variable on an individual level,[82] and the Tromsø Study fits these criteria. Thus, it was possible to distinguish the period effect from the age and cohort effects. As different cohorts were measured at the same time points it was possible to separate period effects from cohort effects; and as several age groups were measured at the same time points it was possible to separate period effects from age effects. To check whether the RHR decline was due to a period effect, all three variables: age, year of survey, and birth year were plotted against each other (Paper I, Figure 2). According to the results, the RHR decline was mostly due to the period effect, with almost no effect of age or birth cohort.

4.1.6 Confounding and interaction

Confounding is a situation in which the association between an exposure and an outcome variable is observed as a result of the influence of a third variable or several variables.[102] In cohort studies, a confounder is a third variable that is unevenly distributed between exposed and unexposed participants and is casually associated with the outcome. Potentially, the associations between RHR changes and changes in other cardiovascular risk factors over time (Paper I) could be confounded by variables such as age, diet, socioeconomic characteristics, climate change, etc. The associations of RHR (Paper II) and RHR change

(Paper III) with CVD and mortality could be confounded by cardiovascular risk factors and other variables.

Effects of age, BP, blood lipids, BMI, smoking, physical activity, and BP treatment on RHR change were mutually adjusted in the mixed models analysis (Paper I). The effect of RHR (Paper II) and RHR trajectories (Paper III) on CVD and mortality were adjusted for the other cardiovascular risk factors in multivariate Cox regression models. We did not have detailed information on treatment, such as type of drugs that interact with RHR, or doses, to properly adjust for this variable in Papers I and II. That is why subjects who used BP treatment were excluded from the analyses. There were other characteristics that could be potential confounders, but they were not measured in the study or were measured differently from survey to survey, which made it difficult/impossible to adjust for them. Such potential confounders were heart rate variability, physical fitness, markers of inflammation, trans fats in food and other dietary features, alcohol consumption, coffee consumption, mental stress, and quality of life.

Interaction is a situation when two or more covariates mutually modify their effects on the outcome variable.[102] When there are theoretical or clinical suspicions, it is recommended to check for an interaction effect between independent variables. Variables that most often interact with biological and health-related parameters are sex and age. We stratified all the analyses by sex and presented results separately for men and women. In order to check and to deal with the possible interaction between age and RHR, we stratified RHR means by 10-year age groups. RHR went down in all age groups, and downward trends were fairly similar across age groups.

Another variable that could be considered as a potential effect modifier is BP treatment, which was self-reported. Details on the type of drugs that interact with RHR and their doses were not available. However, excluding those taking BP treatment from the analysis had virtually no effect on the downward trend in RHR (Paper I). When only those on BP treatment were considered, the downward trend in RHR disappeared. For the same reasons we excluded those taking BP treatment from the analyses with follow-up information (Papers II and III).

4.1.7 Statistical analysis

Keeping in mind the unique longitudinal data we have in the Tromsø Study, one of the unspoken aims of this project was to probe novel and comprehensive statistical methods to answer the research questions. Many epidemiological and clinical studies are being conducted worldwide, and more and more data, including longitudinal data, are available for answering different research questions. The Tromsø Study consists of six repeated surveys conducted from 1974 to 2008, and Tromsø 7 is almost complete, providing an opportunity to explore long-term trends in cardiovascular risk factors and many other health-related characteristics, to explore associations between the development of these characteristics over time on an individual level, and their effects on different outcomes. Thus, new elaborate methods of statistical analysis are needed to properly adjust for dependencies of repeated measurements within a subject, to be able to reveal different shapes of associations between a continuous predictor and outcome, to take into account the time-dependency of a variable, and finally to reveal common long-term trajectories of a parameter with repeated measurements.

In order to decide on how to analyse multilevel, or hierarchical data, one should distinguish between clustered data, repeated measures, and longitudinal data.[103] In clustered data, the dependent variable is measured only once per subject, but the subjects are somehow clustered; and there is no ranking of subjects within clusters. In repeated measures data, the dependent variable is measured more than once per subject, and these measurements are taken under different conditions (a separate independent variable). In longitudinal data, the dependent variable is measured several times per subject over a certain, relatively long time period, and time itself is an important independent variable. Missing observations or dropouts is one of the main challenges in longitudinal studies, and data can be both clustered and longitudinal at the same time.

Three main approaches can be used to analyse such multilevel data: repeated measures multivariate ANOVA/GLM, the marginal multilevel model (generalised estimated equations), and the linear mixed models.[104] ANOVA, or multivariate approach is usually used for analysing repeated measures data. Multiple measurements are included in the analysis as multiple dependent variables. This method is conceptually simple, but has many

assumptions, such as balanced design and equal correlation among dependent variables. It does not allow post-hoc tests for the repeated measures factor.

In the marginal level model there is only one dependent variable, and another variable indicates the condition or time of a measurement. In the marginal model, the correlations among each subject's residuals can be estimated directly. There are several common patterns that residuals can correlate. For example, an auto-regressive structure, in which some repeated measurements are closer to each other than others (over either time or space), has higher correlations. This could be relevant for longitudinal data, but is not an issue for purely clustered data. In a situation when the assumptions of equal variances and equal correlations are not met, a marginal model is preferred over repeated measures multivariate ANOVA. Another advantage is the possibility to perform post-hoc tests for the repeated measures factor.

Similar to the marginal model, the linear mixed models control for dependency between observations, but in a different way. It adds one or more random effects for subjects to the model in the form of additional residual terms with estimated variances. The mixed models are flexible and can handle both clustered data and repeated measures in one model. Another advantage is that data can be unbalanced.

We tried several options when analysing the associations between single RHR measures and the outcomes. RHR as a main predictor was included in the Cox regression analysis as a continuous and as a categorical variable with different cutoff points. The continuous approach would not allow us to reveal non-linear associations, and the categorical approach was fraught with many subjective decisions on RHR categorisation, which had an effect on the results. Finally, it was decided to apply fractional polynomials. This approach allowed us to reveal possible non-linear associations and to use RHR in a continuous form. This method also provided the possibility to derive risk estimates for extremely low and extremely high RHR values. Using the Cox regression analysis with time-dependent covariates allowed us to update RHR and other risk factors whenever it was possible.

Finally, we applied statistical analyses that allowed us to identify detailed RHR trajectories during the 15-years of observation. One way to construct categories of developmental

trajectories for more than two repeated measurements was to assign these categories based on subjective criteria. Although this method is reasonable, there are some important limitations, such as an *a priori* assumption of the existence of distinct developmental trajectories. Moreover, an individual's group membership cannot be quantified in the form of probabilities.[86] The trajectory analysis has the capacity to identify qualitatively distinct developmental progressions and to distinguish variations due to chance from real differences across individuals. As recommended, in order to choose the number of trajectories in the final models, we used both a careful weighting of formal statistical criteria against explanatory power and usability in the analyses.[86] Figure 3 demonstrates clearly that the trajectory analysis managed very well to assign individuals to the five trajectory groups.

4.2 Discussion of main results

4.2.1 Secular trends in resting heart rate

This study found that in the 22 years from 1986 to 2008, RHR means declined in the general population of Tromsø, Norway by approximately 9 bpm in men, and 11 bpm in women. Later, and based on the same Tromsø Study data, Mannsverk et al. reported that the age- and sex-adjusted proportion of those with an RHR of 80 bpm or higher declined from 30% (95% CI 29 to 31) in Tromsø 4 to 11% (95% CI 10 to 11) in Tromsø 6.[57] The National Health Screening Service has been offering regular cardiovascular risk factor screening to men and women aged 40-42 years from all Norwegian counties except Oslo since 1985. The National Health Screening Service reported a decline in RHR from 72.5 bpm in 1994-96 to 70.6 bpm in 1997-99 in men and from 76.7 bpm to 74.9 bpm in women.[58] Another recently published study from Norway, the Nord-Trøndelag Health Study, showed that from 1984-86 to 2006-08 RHR means declined from 74.9 (SD 12.6) to 70.2 (SD 11.5) in men and women from the general population aged 20 years and older.[59]

A substantial decline in RHR has also been described in general populations from other countries.[56, 105] The NHANES carried out in the USA collected RHR data in 1971-75 (NHANES I) and 1999-2008 (NHANES 1999-2008) in order to report and update population-based reference ranges for different age groups.[105] Mean RHR values declined from 78.6 bpm to 70.0 bpm in White men aged 25-74 year during NHANES I, and among those 20 years and older during NHANES 1999-2008. The comparable RHR values in

women declined from 81.0 bpm to 74.5 bpm. Although the authors explained the difference in RHR between the two surveys by systematic differences in methodology, I think the decline in mean values of 8.7 bpm in men and of 6.5 bpm in women over the three decades is too pronounced to be explained by a change in measuring technique. A downward trend in RHR values has also been reported in Paris, France,[56] where RHR means declined between 1992 and 2007 by 5 bpm in men and by 7 bpm in women of all ages (mean age 44.7 years, SD 12.7) from the general population.

The first published study on RHR trends was conducted on first-year students (16-24 years) of Queen's University Belfast, United Kingdom.[55] From 1949 to 1992 RHR means declined by 9 bpm in men and 10 bpm in women. However, from 2001 to 2004 RHR means increased by 5 bpm in men and 8 bpm in women. This J-shape pattern does not correspond to our results, or to the results of the studies mentioned above. However, participants in the study from the United Kingdom were younger and the sample size smaller. Moreover, the authors mention that the values from 2001 could be influenced by the fact that recruitment after 2001 was voluntary.

Although the methodology of the studies on RHR trends in general populations was different (aims of the studies, recruitment procedures, cross-sectional or longitudinal design, RHR measurement technique), all the studies, with the exception of the study from the United Kingdom,[55] demonstrated a consistent and substantial decline in RHR values in both men and women over the last decades. In general there is a perception that low RHR is associated with a healthy lifestyle and therefore a lower CVD risk compared to elevated RHR, and many studies have confirmed that.[44, 106, 107] RHR is modifiable, and people with a higher physical activity level, lower BP, and non-smokers have a lower RHR. Thus, when one hears about a decline in RHR over the last decades, one may believe strongly that it is due to BP treatment, increased physical activity, and/or a lower proportion of smokers in society. Although positive trends in BP[Paper I, 57-59], blood lipids,[Paper I, 56, 57, 59] physical activity,[Paper I, 57, 59] smoking cessation,[Paper I, 57, 59] and BP treatment[Paper I, 57, 59] are present in populations, none of the above-mentioned studies proved that the decline in RHR could be explained by changes in traditional cardiovascular risk factors.

The two Norwegian studies that reported a decline in RHR focused on decline in BP.[58, 59] However, as RHR and BP are correlated, the reasons behind the decline in both RHR and BP could be similar. Tverdal et al. found no reasonable explanation for the decline in BP, but the cross-sectional study design and absence of information on most of the other cardiovascular risk factors would not allow for that.[58] The authors confirmed that the BP decline was not related to methodological aspects.

Holmen et al. analysed the data on BP and other cardiovascular risk factors from 74 549 participants, 27 605 of whom attended all the three Nord-Trøndelag Health Study surveys conducted in 1984-1986, 1995-1997, and 2006-2008.[59] Thus, the authors were able to look at the individual associations between change in BP and change in other cardiovascular risk factors. When restricting the analysis to those who reported they had never taken BP treatment, the authors found that the observed diastolic BP decrease was associated with increased age, decreased BMI, decreased total cholesterol, and decreased RHR in both men and women. The fact that individual decreas in BP was associated with individual decreas in RHR led the authors to conclude that a possible explanation for both could be increased parasympathetic activity. One of the suggested explanations was a reduction in the white coat phenomenon. In my opinion, though it could be partly true, the white coat phenomenon cannot explain such a substantial decline in RHR or BP. Indeed, we analysed data from participants who attended only one Tromsø Study survey, and we observed a similar decline in RHR (Figure 2).

The study from Paris had cross-sectional data and could not analyse associations between change in RHR and change in other parameters on an individual level, so they used stratification.[56] The authors concluded that the decline in RHR they observed could not be accounted for by traditional cardiovascular risk factors, including physical activity and CVD treatment. Similarly, Black et al. concluded that RHR trends in students could not be accounted for by physical activity or smoking status.[55] We had longitudinal data, a large amount of participants who attended two or more of the Tromsø 3-6 surveys, and comprehensive information on other cardiovascular risk factors, and we found that only one-fifth of the decline we observed was attributable to individual changes in other cardiovascular risk factors and BP treatment (Paper I).

Thus, none of the studies that reported a decline in RHR in the general population over the last decades could find the reasons behind this trend. However, it is debatable whether such epidemiological studies are able to find the explanation. RHR varies a lot within individuals, even during the day, and is highly dependent on many internal and external factors as well as interactions between them. Moreover, not all the factors can be properly measured in large samples, and not all the factors can be measured on an individual level. One possible explanation could be changes in diet and eating patterns.[Paper I, 59]

Diet, alcohol consumption, and coffee consumption influence RHR and other cardiovascular risk factors (Paper I). Over the last decades dietary habits in Norway have changed substantially: energy intake and consumption of trans fats, potatoes, and eggs have decreased, while consumption of fruits, vegetables, cereals, fish, meat, cheese, unsaturated fat, and potassium have increased.[Paper I, 59] However, it is difficult to prove whether these changes could have caused the decline in RHR we observed, especially on an individual level. We did analyse associations between change in RHR and change in some of the dietary variables, alcohol consumption, and coffee consumption, but we did not find any associations. This could be because information on diet was self-reported and was asked differently from survey to survey. We also found that an individual decrease in blood levels of triglycerides was the strongest predictor of RHR decline in both men and women. Non-fasting levels of triglycerides are influenced by food eaten recently, and therefore could approximate dietary habits (Paper I). On the other hand, population-based dietary and lifestyle interventions could explain the non-linearity of the RHR decline we observed (Paper I). It could also explain why the RHR decline in France occurred several years earlier,[56] as some positive dietary changes were applied there earlier (Paper I).

Plichart et al. suggested that the decline in RHR could be a consequence of environmental changes such as outdoor temperature and pollution.[56] Unfortunately, it is difficult to measure such exposures on an individual level, especially over long periods. Air concentrations of ozone (O_3), nitrogen dioxide (NO_2) and sulfur dioxide (SO_2) can influence cardiac autonomic function, but Norwegian trends of these concentrations were not consistent with the RHR trends we found (Paper I).

Decreases in emotional, cognitive, and physical stress levels in society could be another possible explanation for the decline in RHR we observed. Mental stress results in an autonomic imbalance, increased sympathetic activity, and plasma catecholamines, thereby increasing the heart rate.[8] Job strain and low job control have also been shown to be associated with elevated RHR.[54] Stress level may have decreased in society over the last decades, resulting in the RHR decline, but we do not have data to confirm this.

4.2.2 Resting heart rate as a cardiovascular risk factor

RHR is not considered a traditional cardiovascular risk factor, and it is not part of the cardiovascular risk assessment in American or European guidelines.[24, 25] However, RHR levels have declined in the general population over the last decades,[Paper I, 56, 58, 59, 105] and this trend was accompanied by changes in other cardiovascular risk factors and a decline in cardiovascular morbidity and mortality. The key question is: is RHR an independent predictor of CVD and should it be considered a separate cardiovascular risk factor? Although many studies have been conducted on the association between RHR and CVD and mortality, the results were inconsistent.[37] We had data that could potentially overcome the limitations of previous studies and found that elevated RHR independently increased cardiovascular risk, while low RHR had a protective effect on the risk of incident myocardial infarction, incident atrial fibrillation, cardiovascular death, and total death in men. In women, elevated RHR independently increased the risk of myocardial infarction, ischemic stroke, cardiovascular death, and total death; however low RHR values had no protective effect in relation to ischemic stroke and cardiovascular death (Paper II).

The association between RHR and incident myocardial infarction, atrial fibrillation, and ischemic stroke in the general population has not been well investigated or described in the literature. We found a strong, independent, positive gradual association between RHR and incident myocardial infarction in both men and women, and it was stronger in women than in men (Paper II). There are few studies on the association between RHR and myocardial infarction, but those that do exist are in agreement with our findings, especially in women.[27, 39, 40] A recently published meta-analysis based on 30 cohort studies (1 227 511 participants and 18 364 cases) that used coronary artery disease as an outcome demonstrated the mutually adjusted relative risks by category of RHR (<60 bpm – reference category, 60-70 bpm, 70-80

bpm, and >80 bpm) of 0.99 (95% CI 0.93 to 1.04), 1.08 (95% CI 1.01 to 1.16), and 1.30 (95% CI 1.19 to 1.43), respectively in both men and women from the general population.[33] The independent risk of coronary artery disease increased significantly by 12% for every 10 bpm increment in RHR. As myocardial infarction is one of the major components of coronary artery disease and shares the same pathogenesis (coronary atherosclerosis and myocardial ischemia), these figures correspond well to our findings.

A recently published study based on Tromsø Study data estimated that 66.1% (95% CI 47.6 to 96.8) of the decline in the incidence of acute coronary heart disease from 1995 to 2010 was accounted for by trends in modifiable cardiovascular risk factors.[57] The decline in RHR over the study period contributed 14.5% (95% CI 6.9 to 24.0); 13.6% (95% CI 6.3 to 24.1) in men and 17.6% (95% CI 1.5 to 45.2) in women. The only cardiovascular risk factors that contributed to the decline in coronary heart disease incidence more than to the decline in RHR were total cholesterol in both sexes and systolic BP in women.

Elevated heart rate contributes to an imbalance between myocardial oxygen demand and to a decrease in coronary blood supply.[46] Experimental evidence supports the role of heart rate in endothelial dysfunction, atherosclerosis progression, and plaque rupture, resulting in coronary thrombosis.[46, 108, 109] Based on these considerations, it is plausible that elevated RHR is an independent and self-sufficient risk factor for coronary heart disease, and myocardial infarction in particular. Thus, several criteria for validating RHR as a risk factor for myocardial infarction are fulfilled: plausibility (pathophysiological mechanisms), strength, and gradation of the association.

Our findings of a positive, gradual association between RHR and atrial fibrillation in men (in women the association was weaker and insignificant) contradict other population-based studies that reported an increased risk of atrial fibrillation in those with low RHR.[110-112] This inconsistency may be explained by the ascertainment of the outcome, lone atrial fibrillation (Paper II). Lone atrial fibrillation is defined as the occurrence of atrial fibrillation before the age of 60/65 years in the absence of underlying disorders such as hypertension, heart failure, myocardial infarction, valvular heart disease, diabetes, and hyperthyroidism. The underlying mechanism for lone atrial fibrillation is different from that of atrial fibrillation with the

underlying conditions.[Paper II, 113] In our study, only a small proportion (6%) of the participants had lone atrial fibrillation. O'Neal et al. included participants aged 65 years or more and found an increased risk of general atrial fibrillation in those with an RHR of 60 bpm or lower.[111] However, RHR was measured by wrist palpation, and ascertainment of atrial fibrillation was different, meaning that cases of non-permanent atrial fibrillation could have been missed, which was noted by the investigators. Participants taking BP treatment were not excluded from the analysis.

One recent paper based on the same Tromsø Study data showed opposite results, i.e., that the risk of atrial fibrillation increased with decreasing RHR.[112] The adjusted HR for each 10 bpm increase in RHR was 0.92 (95% CI 0.86 to 0.98), and an RHR of less than 50 bpm was an independent risk factor for atrial fibrillation compared to an RHR of 60 bpm or higher. The authors were intrigued by the fact that RHR and physical activity had a different relationship with atrial fibrillation (they found J-shaped association between physical activity and risk of atrial fibrillation with the lowest risk at moderate activity levels). In my opinion, these two Tromsø Study-based papers showed different results because Morseth et al. did not exclude participants who reported BP treatment as we did. Instead, Morseth et al. adjusted for BP treatment in the models. Participants talking BP treatment are at a higher risk of atrial fibrillation, as hypertension is an important risk factor. At the same time, some of these medications affect RHR. Detailed information on treatment (type of drugs or doses) was not available to properly adjust for this variable, so we excluded participants taking BP treatment at baseline and did not update information for those who started to take these medications at later surveys. Our results for atrial fibrillation were not stable when including those on BP treatment at baseline in the analysis and adjusting for BP treatment, or when only updating information on those who started to take medications later and adjusting for BP treatment.

Data from the Copenhagen ECG study demonstrated a J-shaped association between RHR and risk of incident atrial fibrillation in 281 451 primary care patients with a median follow-up time of 8.4 years.[114] Detailed information on heart rate-lowering medications was available so that proper adjustment could be done, and adjusted HRs of atrial fibrillation for RHRs of 30-51 bpm and of 95-120 bpm compared to a RHR of 66-72 bpm were 1.16 (95% CI 1.06 to 1.27) and 1.36 (95% CI 1.26 to 1.46), respectively. However, in the lone atrial

fibrillation subgroup analysis (164 064, 58.3% were eligible, 1631 developed lone atrial fibrillation) the association was accentuated. A recent meta-analysis on genome-wide associations reported that the genetic variants that increase and decrease RHR were associated with an increased risk of atrial fibrillation.[115] This could explain the strong J-shaped association between RHR and the risk of lone atrial fibrillation, as genetic reasons for atrial fibrillation are more typical among relatively young people without comorbidities. As mentioned above, we had a small number of cases of lone atrial fibrillation compared to the Copenhagen ECG study.

Using lone atrial fibrillation as the outcome, Grunndvold et al. also revealed the highest incidence rates for atrial fibrillation among healthy middle-aged men with very low or very high RHR levels, demonstrating a possible U-shaped relation.[116] However, the RHR variable was dichotomised (<50 bpm/≥ 50 bpm) in the multivariate Cox regression model, which made it impossible to reveal non-linear associations. The authors concluded that low RHR (<50 bpm) increases the risk of lone atrial fibrillation in men compared to RHR of 50 bpm or higher: adjusted HR 1.69 (95% CI 0.79 to 3.21).

Considering all this evidence, RHR should be considered an independent risk factor for atrial fibrillation. Extremely low RHRs seem to increase the risk of lone atrial fibrillation compared to moderate RHRs.[110, 114] Elevated RHR seems to increase the risk of both lone atrial fibrillation[114] and general atrial fibrillation (Paper II), though studies are not consistent.[110-112] These inconsistencies are most probably due to methods of atrial fibrillation ascertainment, and how BP treatment is dealt with. The difference in the associations between RHR and lone atrial fibrillation and atrial fibrillation with underlying disorder can be explained by the different pathophysiological mechanisms of these types of atrial fibrillation. The second type is “substrate related” and occurs due to diseased and dilated atria with stretch and fibrosis.[113] Lone atrial fibrillation is more related to electrophysiological triggers (including genetic factors) in structurally normal atria.

Our findings demonstrated sex differences in the association between RHR and ischemic stroke. We found no independent association in men, and a J-shaped association with insignificantly increased risk in extremely low RHR values and a gradually increasing risk

with increasing RHRs starting from 70 bpm in women. Most of the other studies on the association between RHR and ischemic stroke analysed men and women combined, lacked a standardised protocol for RHR measurement, lacked endpoint verification and/or classification procedures, or did not adjust for important potential confounders such as physical activity and BP.[36, 39, 40, 117] None of these studies demonstrated sex differences in their associations or J-shaped associations. However, the Women's Health Initiative study reported an independently increased risk of any stroke in women with a RHR higher than 76 bpm, which is in agreement with our results.[39] The Kailuan Study showed that elevated RHR is a marker, but not an independent predictor, of ischemic stroke in a study sample that was 80% male,[40] which corresponds to our findings in men. A recent meta-analysis showed that the risk of any stroke increased by 5% (95% CI 1 to 8) for every increment in RHR of 10 bpm in both men and women combined (based on 20 cohort studies); however when RHR was categorised (<60 bpm, 60-70 bpm, 70-80 bpm, and >80 bpm) this positive association disappeared.[33]

The REasons for Geographic And Racial Differences in Stroke study included 24 730 participants with RHR measured by ECG, had a median follow-up of 7.6 years, and had all first-time ischemic stroke events adjudicated by a physician.[118] The authors found an increased risk of ischemic stroke for each 10 bpm increase in RHR in men and women combined: the adjusted HR was 1.10 (95% CI 1.02 to 1.18). The increased risk was observed in the middle (61-70 bpm) and upper (>70 bpm) tertiles of RHR compared with the lower tertile (<61 bpm): HRs were 1.29 (95% CI 1.06 to 1.57) and 1.37 (95% CI 1.12 to 1.67), respectively. However, in a subgroup analysis by sex, the linear association between RHR and ischemic stroke in women disappeared: HR for 10 bpm increase in RHR in women was 1.07 (95% CI 0.97 to 1.19) and 1.12 (95% CI 1.02 to 1.24) in men. This could be evidence of a non-linear association between RHR and ischemic stroke in women. Contradictions with our findings in men (positive association disappeared after adjustment) could be due to a lack of adjustment for physical activity, which is a very important potential confounder.

Thus, elevated RHR in women increases the risk of ischemic stroke, whereas low RHR levels are not beneficial compared to moderate levels. In men, increased RHR is a marker for increased risk of ischemic stroke. Several mechanisms can explain the association between

RHR and ischemic stroke, and they are similar to those for myocardial infarction: elevated RHR is associated with oxidative stress, endothelial dysfunction, and progression of atherosclerosis.[119, 120] However, atrial fibrillation is a known risk factor for ischemic stroke, and it is possible that atrial fibrillation mediates the association between RHR and ischemic stroke. As discussed above, low RHR increases the risk of lone atrial fibrillation,[110, 114] and therefore can increase the risk of subsequent ischemic stroke.

Our findings are in agreement with a recent, large meta-analysis of prospective cohort studies on the association between RHR and all-cause and cardiovascular mortality in the general population.[32] The authors included 46 studies involving 848 320 participants and 25 800 cardiovascular deaths, and 1 246 203 participants and 78 349 deaths from all causes. In this meta-analysis, elevated RHR independently increased both the risk of all-cause and cardiovascular mortality. Compared to the lowest RHR, the multivariate-adjusted relative risk of cardiovascular mortality was 1.08 (95% CI 0.99 to 1.17) for those with a RHR of 60-80 bpm and 1.33 (95% CI 1.19 to 1.47) for those with a RHR of higher than 80 bpm; the adjusted relative risk for every 10 bpm increase was 1.08 (95% CI 1.06 to 1.10). For all-cause mortality the corresponding relative risks were 1.12 (95% CI 1.07 to 1.17), 1.45 (95% CI 1.34 to 1.57) and 1.09 (95% CI1.07 to 1.12), respectively. The associations were even stronger among participants not taking RHR-lowering medications. The dose-response analysis showed that, compared to 45 bpm, the risk of cardiovascular mortality increased significantly at 90 bpm, while the risk of all-cause mortality increased significantly with increasing RHR in a linear relation. Although the authors did not stratify their analyses by sex, their results correspond to ours. We found a strong, positive, gradual association between RHR and cardiovascular mortality in men, and between RHR and total mortality in both men and women (Paper II). The risk of cardiovascular death in women significantly and gradually increased with increasing RHR after 70 bpm.

Another meta-analysis on the association between RHR and cardiovascular mortality was based on 20 studies (all included in the meta-analysis described above).[31] Its main findings were similar to those of the above-mentioned meta-analysis. The authors conducted a subgroup analysis for sex and found a positive association between RHR and the risk of cardiovascular mortality in both men and women. However, the association in men was

stronger than that in women: the relative risk of cardiovascular mortality for a RHR higher than 80 bpm compared to the lowest RHR was 1.60 (95% CI 1.03 to 2.50) in men and 1.41 (95% CI 1.12 to 1.78) in women. Relative risks for each 10 bpm increase were 1.08 (95% CI 1.05 to 1.12) in men and 1.03 (95% CI 1.01-1.06) in women. This could be because the association between RHR and cardiovascular mortality in women is positive, but not linear: low levels of RHR are not protective when compared to moderate levels. That was the case in our study, in which we were able to reveal potential non-linear associations and provide risk estimates for a wide range of RHR values (Paper II).

Another recently published study based on a general population without known coronary artery disease showed that elevated RHR was an independent predictor of total death, but not of incident coronary events (non-fatal myocardial infarction or coronary death) in those without heart rate-lowering medication.[37] The authors also reported that RHR was not associated with total mortality risk in those taking heart rate-lowering medications; however mortality rates and coronary event rates were higher among these subjects compared to those without hart rate-lowering medications. Thus, heart rate-lowering medications can attenuate risk, but not enough to achieve the risk levels of healthy individuals.

Thus, taken together, our study confirms that RHR is an independent predictor of CVD and mortality in both men and women, as reported by others, including the recent meta-analyses. However, findings on the associations between RHR and atrial fibrillation and ischemic stroke are contradictory.

4.2.3 Individual long-term changes in resting heart rate and risk of cardiovascular disease and death

Single RHR measures independently predict CVD and mortality. Over the last decades, RHR means declined considerably in the general population. Do individual changes in RHR over time provide additional information for CVD and mortality risk assessment? There are few studies on individual time changes in RHR and their associations with CVD and mortality,[121-124] and most of them[121-123] used the difference between two RHR recordings as the measure of change in RHR over time.

Three studies reported an adverse effect of individual increases in RHR over time on total mortality[121, 123] and ischemic heart disease mortality[122] in the general population, using no change in RHR as the reference. However, findings on the effect of RHR decrease over time were inconsistent: Jouven et al. found a favourable effect of RHR decrease on total mortality, whereas in Hartaig's paper the effect of RHR decrease on total mortality was not different from the effect of stable RHR. Nauman et al. showed insignificant U-shaped association between RHR change and ischemic heart disease mortality, with a risk of ischemic heart disease that was 1.80 times (95% CI 1.10 to 3.10) higher in those with RHR increases of more than 25 bpm and 1.30 (95% CI 0.60 to 2.60) times higher in those with a RHR decrease of 25 bpm or more.[122]

Jouven et al. included men only;[121] whereas Nauman et al. and Hartaigh et al. found no interaction between RHR and sex and therefore analysed men and women together.[122, 123] All three studies used the difference in RHR at two time points as an approximation of temporal changes in RHR and adjusted for RHR at baseline. This approach does not provide a comprehensive picture of long-term changes in RHR.[125, 126] Moreover, there is an interaction between RHR at baseline and RHR over time.[121, 122]

A recent study analysed the associations between variation in RHR over 4 years and risk of incident myocardial infarction and mortality among adults aged 65 years or older.[124] The RHR of the Cardiovascular Health Study participants was measured by ECG at the first five annual study visits. For each participant, linear regression was used to estimate mean, trend (the slop), and variation (the SD of the five residuals) in RHR. The authors found that RHR mean and variations in RHR, but not trend in RHR were positively associated with mortality risk, and none of the RHR variables were associated with myocardial infarction, though the number of myocardial infarction events was low. A continuous distribution of RHR trend derived from multiple measurements in this study is a better approximation of RHR change over time than the difference in RHR at two time points. However it is still not sensitive enough to draw a comprehensive picture of RHR change over time. The effect of RHR trend was adjusted for RHR mean and variation in RHR, but interaction between RHR trend and the other two RHR variables was neither checked nor taken into consideration in the analysis. Moreover, the study population was older than in our study and in the studies described

above. Limited power, especially for the myocardial infarction analysis, could also be a reason why this study failed to replicate the findings of other studies on the association between RHR trends and the risk of myocardial infarction or total death. For example, the fully adjusted HR of myocardial infarction for RHR trend (2 bpm per year) was 1.08 (95% CI 0.95 to 1.23), which is quite high for a 4-year period of RHR change. In comparison, fully-adjusted HRs of myocardial infarction for RHR mean (10 bpm) and RHR variation (2 bpm) were 0.99 (95% CI 0.85 to 1.16) and 1.01 (95% CI 0.90 to 1.14).

To describe individual long-term trends in RHR, we used three RHR measurements over a 15-year period and applied latent class models (SAS Proc Traj) to determine RHR trajectory groups, and we assigned participants to these groups based on the highest posterior predicted probability. This allowed us to avoid the problem of interaction between RHR at baseline or at the end of the study period and RHR change over time, as both parameters were taken into accounts in the form of trajectories. We found that men with constantly high levels of RHR during adulthood were at the highest risk of myocardial infarction and total death. Another RHR trajectory with adverse effect on cardiovascular death and total death in men was having an RHR that increased over the years. Men with consistently low RHR levels had the lowest risk. We did not observe any independent associations in women, but this is most probably due to limited power, as the point estimates for myocardial infarction and total death in women were similar to those in men. Point estimates for a decrease from high to moderate levels RHR trajectory were similar to point estimates for a trajectory with constantly moderate RHR levels, and were not significantly different from the low RHR trajectory in both men and women.

Thus, our findings correspond well with Mannsverk's findings on the important role of RHR decline in the declining incidence of acute chronic heart disease in the population over the last decades.[57]

Chapter 5 Conclusion and future research

RHR mean values declined over the last decades in the general population, which corresponds well with positive trends in cardiovascular morbidity and mortality. At the same time, the decline could not be explained by individual changes in traditional cardiovascular risk factors. Moreover, RHR itself and individual long-term changes in RHR independently predict CVD and mortality in both men and women. These findings suggest that RHR must be considered as a major cardiovascular risk factor. As RHR is a simple and easily measured cardiovascular parameter, it can be used to monitor CVD risk.

As over the last decades, RHR declined substantially in the general population, thus new definitions of normal RHR may be needed, and the reasons behind the decline in RHR in the general population need to be further elucidated. Increased use of beta blockers and calcium channel blockers for reasons other than hypertension, use of selective I_f inhibitors, and increased use of lipid-lowering drugs should be explored in further studies as possible explanations of the RHR decline. Though there is enough evidence on independent associations of RHR with myocardial infarction, cardiovascular mortality and total mortality, associations of RHR with other outcomes need to be clarified. The association between RHR and the risk of atrial fibrillation and ischemic stroke in the general population needs to be further investigated. The effect of low RHR levels on the risk of atrial fibrillation in men and on the risk of ischemic stroke in women, as well as the independent association between RHR and ischemic stroke in men, are of particular interest. As RHR is a modifiable parameter, it may be possible to use it for CVD prevention. However, studies (including randomised control trials) on the effect of RHR-lowering interventions such as lifestyle interventions and RHR-lowering drugs on CVD and mortality risk in the general populations are warranted. A prediction algorithm needs to be developed to consider RHR, individual change in RHR, and traditional cardiovascular risk factors in order to allow clinicians to use RHR in their practice.

References

1. American Heart Association http://www.heart.org/HEARTORG/Conditions/More/MyHeartandStrokeNews/All-About-Heart-Rate-Pulse_UCM_438850_Article.jsp#.V92Gq_l95aS (20 September 2016, date last accessed).
2. Palatini P, Benetos A, Grassi G, Julius S, Kjeldsen SE, Mancia G, et al; European Society of Hypertension. Identification and management of the hypertensive patient with elevated heart rate: statement of a European Society of Hypertension Consensus Meeting. J Hypertens 2006;24(4):603-610.
3. Mancia G, Parati G, Pomidossi G, Grassi G, Casadei R, Zanchetti A. Alerting reaction and rise in blood pressure during measurement by physician and nurse. Hypertension 1987;9:209-215.
4. Erikssen J, Rodahl K. Resting heart rate in apparently healthy middle-aged men. Eur J Appl Physiol Occup Physiol 1979;42(1):61-69.
5. Wilhelm FH, Roth WT, Sackner MA. The lifeShirt. An advanced system for ambulatory measurement of respiratory and cardiac function. Behav Modif 2003; 27:671-691.
6. Kawahara M. Evaluation of the accuracy of non-invasive automatic blood pressure monitors. Anesth Prog 1990;37(5):244-247.
7. Cullen PM, Dye J, Hughes DG. Clinical assessment of the neonatal Dinamap 847 during anesthesia in neonates and infants. J Clin Monit 1987;3(4):229-234.
8. Valentini M, Parati G. Variables influencing heart rate. Prog Cardiovasc Dis 2009;52(1):11-19.
9. Christou DD, Seals DR. Decreased maximal heart rate with aging is related to reduced beta-adrenergic responsiveness but is largely explained by a reduction in intrinsic heart rate. J Appl Physiol 2008;105(1):24-29.
10. Kannel WB, Kannel C, Paffenbarger RS Jr, Cupples LA. Heart rate and cardiovascular mortality: the Framingham Study. Am Heart J 1987;113(6):1489-1494.
11. Ben-Dov IZ, Kark JD, Ben-Ishay D, Mekler J, Ben-Arie L, Bursztyn M. Blunted heart rate dip during sleep and all-cause mortality. Arch Intern Med 2007;167(19):2116-2121.
12. Smith JJ, Porth CM, Erickson M. Hemodynamic response to the upright posture. J Clin Pharmacol 1994;34(5):375-386.
13. Borresen J, Lambert MI. Autonomic control of heart rate during and after exercise: measurements and implications for monitoring training status. Sports Med 2008;38(8):633-646.
14. Levy WC, Cerqueira MD, Harp GD, Johannessen KA, Abrass IB, Schwartz RS, et al. Effect of endurance exercise training on heart rate variability at rest in healthy young and older men. Am J Cardiol 1998;82(10):1236-1241.
15. Wilmore JH, Stanforth PR, Gagnon J, Rice T, Mandel S, Leon AS, et al. Heart rate and blood pressure changes with endurance training: the HERITAGE Family Study. Med Sci Sports Exerc 2001;33(1):107-116.
16. Smith ML, Hudson DL, Graitzer HM, Raven PB. Exercise training bradycardia: the role of autonomic balance. Med Sci Sports Exerc 1989;21(1):40-44.

17. Carter JR, Ray CA. Sympathetic neural responses to mental stress: responders, nonresponders and sex differences. Am J Physiol Heart Circ Physiol 2009;296(3):H847-853.
18. Groppelli A, Giorgi DM, Omboni S, Parati G, Mancia G. Persistent blood pressure increase induced by heavy smoking. J Hypertens 1992;10(5):495-499.
19. Trap-Jensen J. Effects of smoking on the heart and peripheral circulation. Am Heart J 1988;115(1 Pt 2):263-267.
20. Grassi G, Seravalle G, Calhoun DA, Bolla GB, Giannattasio C, Marabini M, et al. Mechanisms responsible for sympathetic activation by cigarette smoking in humans. Circulation 1994;90(1):248-253.
21. Zhang J, Kesteloot H. Anthropometric, lifestyle and metabolic determinants of resting heart rate. A population study. Eur Heart J 1999;20(2):103-110.
22. Stolarz K, Staessen JA, Kuznetsova T, Tikhonoff V, State D, Babeanu S, et al; European Project on Genes in Hypertension (EPOGH) Investigators. Host and environmental determinants of heart rate and heart rate variability in four European populations. J Hypertens 2003;21(3):525-535.
23. Whitsett TL, Manion CV, Christensen HD. Cardiovascular effects of coffee and caffeine. Am J Cardiol 1984;53(7):918-922.
24. Goff DC Jr, Lloyd-Jones DM, Bennett G, Coady S, D'Agostino RB Sr, Gibbons R, et al; American College of Cardiology/American Heart Association Task Force on Practice Guidelines. 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol 2014;63(25 Pt B):2935-2959.
25. Authors/Task Force Members, Piepoli MF, Hoes AW, Agewall S, Albus C, Brotons C, Catapano AL, et al. 2016 European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice. Eur J Prev Cardiol 2016;23(11):NP1-NP96.
26. Gillum RF, Makuc DM, Feldman JJ. Pulse rate, coronary heart disease, and death: the NHANES I Epidemiologic Follow-up Study. Am Heart J 1991;121(1 Pt 1):172-177.
27. Cooney MT, Vartiainen E, Laatikainen T, Juolevi A, Dudina A, Graham IM. Elevated resting heart rate is an independent risk factor for cardiovascular disease in healthy men and women. Am Heart J 2010;159(4):612-619.
28. Jensen MT, Marott JL, Allin KH, Nordestgaard BG, Jensen GB. Resting heart rate is associated with cardiovascular and all-cause mortality after adjusting for inflammatory markers: the Copenhagen City Heart Study. Eur J Prev Cardiol 2012;19(1):102-108.
29. Johansen CD, Olsen RH, Pedersen LR, Kumarathurai P, Mouridsen MR, Binici Z, et al. Resting, night-time, and 24 h heart rate as markers of cardiovascular risk in middle-aged and elderly men and women with no apparent heart disease. Eur Heart J 2013;34(23):1732-1739.
30. Seccareccia F, Pannozzo F, Dima F, Minoprio A, Menditto A, Lo Noce C, et al; Malattie Cardiovascolari Aterosclerotiche Istituto Superiore di Sanita Project. Heart rate as a predictor of mortality: the MATISS project. Am J Public Health 2001;91(8):1258-1263.

31. Li Y. Association between resting heart rate and cardiovascular mortality: evidence from a meta-analysis of prospective studies. Int J Clin Exp Med 2015;8(9):15329-15339.
32. Zhang D, Shen X, Qi X. Resting heart rate and all-cause and cardiovascular mortality in the general population: a meta-analysis. CMAJ 2016;188(3):E53-63.
33. Zhang D, Wang W, Li F. Association between resting heart rate and coronary artery disease, stroke, sudden death and noncardiovascular diseases: a meta-analysis. CMAJ 2016. DOI: 10.1503/cmaj.160050 [Epub ahead of print]
34. Tverdal A, Hjellvik V, Selmer R. Heart rate and mortality from cardiovascular causes: a 12 year follow-up study of 379,843 men and women aged 40-45 years. Eur Heart J 2008;29(22):2772-2781.
35. Benetos A, Rudnichi A, Thomas F, Safar M, Guize L. Influence of heart rate on mortality in a French population: role of age, gender, and blood pressure. Hypertension 1999;33(1):44-52.
36. Woodward M, Webster R, Murakami Y, Barzi F, Lam TH, Fang X, et al; from the Asia Pacific Cohort Studies Collaboration. The association between resting heart rate, cardiovascular disease and mortality: evidence from 112,680 men and women in 12 cohorts. Eur J Prev Cardiol 2014;21(6):719-726.
37. Custodis F, Roggenbuck U, Lehmann N, Moebus S, Laufs U, Mahabadi AA, et al. Resting heart rate is an independent predictor of all-cause mortality in the middle aged general population. Clin Res Cardiol 2016;105(7):601-612.
38. Legeai C, Jouven X, Tafflet M, Dartigues JF, Helmer C, Ritchie K, et al. Resting heart rate, mortality and future coronary heart disease in the elderly: the 3C Study. Eur J Cardiovasc Prev Rehabil 2011;18(3):488-497.
39. Hsia J, Larson JC, Ockene JK, Sarto GE, Allison MA, Hendrix SL, et al; Women's Health Initiative Research Group. Resting heart rate as a low tech predictor of coronary events in women: prospective cohort study. BMJ 2009;338:b219.
40. Wang A, Chen S, Wang C, Zhou Y, Wu Y, Xing A, et al. Resting heart rate and risk of cardiovascular diseases and all-cause death: the Kailuan study. PLoS One 2014;9(10):e110985.
41. Nauman J. Why measure resting heart rate? Tidsskr Nor Laegeforen 2012;132(11):1314.
42. Palatini P. Elevated heart rate: a "new" cardiovascular risk factor? Prog Cardiovasc Dis 2009;52(1):1-5.
43. Fox K, Borer JS, Camm AJ, Danchin N, Ferrari R, Lopez Sendon JL, et al; Heart Rate Working Group. Resting heart rate in cardiovascular disease. J Am Coll Cardiol 2007;50(9):823-830.
44. Piwońska A, Piotrowski W, Broda G, Drygas W, Głuszek J, Zdrojewski T, et al. The relationship between resting heart rate and atherosclerosis risk factors. Kardiol Pol 2008;66(10):1069-1075.
45. Verrier RL, Tan A. Heart rate, autonomic markers, and cardiac mortality. Heart Rhythm 2009;6(11 Suppl):S68-75.
46. Palatini P. Heart rate as an independent risk factor for cardiovascular disease: current evidence and basic mechanisms. Drugs 2007;67 Suppl 2:3-13.
47. Nanchen D, Stott DJ, Gussekloo J, Mooijaart SP, Westendorp RG, Jukema JW, et al; PROSPER Group. Resting heart rate and incident heart failure and cardiovascular

mortality in older adults: role of inflammation and endothelial dysfunction: the PROSPER study. Eur J Heart Fail 2013;15(5):581-588.
48. Palatini P. Elevated heart rate in cardiovascular diseases: a target for treatment? Prog Cardiovasc Dis 2009;52(1):46-60.
49. Cook S, Togni M, Schaub MC, Wenaweser P, Hess OM. High heart rate: a cardiovascular risk factor? Eur Heart J 2006;27(20):2387-2393.
50. Inoue T, Oshiro S, Iseki K, Tozawa M, Touma T, Ikemiya Y, et al. High heart rate relates to clustering of cardiovascular risk factors in a screened cohort. Jpn Circ J 2001;65(11):969-973.
51. Morcet JF, Safar M, Thomas F, Guize L, Benetos A. Associations between heart rate and other risk factors in a large French population. J Hypertens 1999;17(12 Pt 1):1671-1676.
52. Bønaa KH, Arnesen E. Association between heart rate and atherogenic blood lipid fractions in a population. The Tromsø Study. Circulation 1992;86(2):394-405.
53. Leistner DM, Klotsche J, Palm S, Pieper L, Stalla GK, Lehnert H, et al; DETECT-Study Group. Resting heart rate as a tool for risk stratification in primary care: does it provide incremental prognostic information? Eur J Prev Cardiol 2012;19(2):275-284.
54. Eriksson P, Schiöler L, Söderberg M, Rosengren A, Torén K. Job strain and resting heart rate: a cross-sectional study in a Swedish random working sample. BMC Public Health 2016;16:228.
55. Black A, Murray L, Cardwell C, Smith GD, McCarron P. Secular trends in heart rate in young adults, 1949 to 2004: analyses of cross sectional studies. Heart 2006;92(4):468-473.
56. Plichart M, Thomas F, Empana JP, Bean K, Périer MC, Celermajer DS, et al. Gender-specific trends in heart rate in the general population from 1992-2007: a study of 226,288 French adults. Eur J Prev Cardiol 2013;20(1):61-72.
57. Mannsverk J, Wilsgaard T, Mathiesen EB, Løchen ML, Rasmussen K, Thelle DS, et al. Trends in Modifiable Risk Factors Are Associated With Declining Incidence of Hospitalized and Nonhospitalized Acute Coronary Heart Disease in a Population. Circulation 2016;133(1):74-81.
58. Tverdal A. Significant decline in blood pressure levels after 1996-fact or artefact? Tidsskr Nor Laegeforen 2001;121(15):1821-1825. [Article in Norwegian]
59. Holmen J, Holmen TL, Tverdal A, Holmen OL, Sund ER, Midthjell K. Blood pressure changes during 22-year of follow-up in large general population - the HUNT Study, Norway. BMC Cardiovasc Disord 2016;16:94.
60. Arnett DK, McGovern PG, Jacobs DR Jr, Shahar E, Duval S, Blackburn H, et al. Fifteen-year trends in cardiovascular risk factors (1980-1982 through 1995-1997): the Minnesota Heart Survey. Am J Epidemiol 2002;156(10):929-935.
61. Kanjilal S, Gregg EW, Cheng YJ, Zhang P, Nelson DE, Mensah G, et al. Socioeconomic status and trends in disparities in 4 major risk factors for cardiovascular disease among US adults, 1971-2002. Arch Intern Med 2006;166(21):2348-2355.
62. Gregg EW, Cheng YJ, Cadwell BL, Imperatore G, Williams DE, Flegal KM, et al. Secular trends in cardiovascular disease risk factors according to body mass index in US adults. JAMA 2005;293(15):1868-1874.

63. Ezzati M, Obermeyer Z, Tzoulaki I, Mayosi BM, Elliott P, Leon DA. Contributions of risk factors and medical care to cardiovascular mortality trends. Nat Rev Cardiol 2015;12(9):508-530.
64. Abina J, Volozh O, Solodkaya E, Saava M. Blood pressure and contributing factors in inhabitants of Estonia: 15-year trends. Blood Press 2003;12(2):111-121.
65. Ikeda N, Gakidou E, Hasegawa T, Murray CJ. Understanding the decline of mean systolic blood pressure in Japan: an analysis of pooled data from the National Nutrition Survey, 1986-2002. Bull World Health Organ 2008;86(12):978-988.
66. Björkelund C, Andersson-Hange D, Andersson K, Bengtsson C, Blomstrand A, Bondyr-Carlsson D, et al. Secular trends in cardiovascular risk factors with a 36-year perspective: observations from 38- and 50-year-olds in the Population Study of Women in Gothenburg. Scand J Prim Health Care 2008;26(3):140-146.
67. Jacobsen BK, Njølstad I, Thune I, Wilsgaard T, Løchen ML, Schirmer H. Increase in weight in all birth cohorts in a general population: The Tromsø Study, 1974-1994. Arch Intern Med 2001;161(3):466-472.
68. GBD 2013 Mortality and Causes of Death Collaborators. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2015;385(9963):117-171.
69. Roth GA, Huffman MD, Moran AE, Feigin V, Mensah GA, Naghavi M, et al. Global and regional patterns in cardiovascular mortality from 1990 to 2013. Circulation 2015;132(17):1667-1678.
70. Bertuccio P, Levi F, Lucchini F, Chatenoud L, Bosetti C, Negri E, et al. Coronary heart disease and cerebrovascular disease mortality in young adults: recent trends in Europe. Eur J Cardiovasc Prev Rehabil 2011;18(4):627-634.
71. Nichols M, Townsend N, Scarborough P, Rayner M. Cardiovascular disease in Europe 2014: epidemiological update. Eur Heart J 2014;35(42):2950-2959.
72. Levi F, Chatenoud L, Bertuccio P, Lucchini F, Negri E, La Vecchia C. Mortality from cardiovascular and cerebrovascular diseases in Europe and other areas of the world: an update. Eur J Cardiovasc Prev Rehabil 2009;16(3):333-350.
73. Pedersen AG. Sammenligninger av dødsårsaker i Norge og Europa. Hva dør man av i EU – og i Norge? [Comparisons of causes of death in Norway and Europe. What dies one of in the EU – and in Norway?] Samfunnsspeilet 2011;2:82–87. Statistisk sentralbyrå [Statistics Norway] http://www.ssb.no/helse/artikler-og-publikasjoner/hva-dor-man-av-i-eu-og-i-norge (20 September 2016, date last accessed). [Article in Norwegian]
74. Folkemengdens bevegelse. Tromsøstatistikk – befolkning. [Population movement. Tromso statistics – population.] Tromsø kommune 2016:3-4. http://www.tromso.kommune.no/fakta-om-tromsoe-kommune.241052.no.html (20 September 2016, date last accessed). [Article in Norwegian]
75. Jacobsen BK, Eggen AE, Mathiesen EB, Wilsgaard T, Njølstad I. Cohort profile: the Tromso Study. Int J Epidemiol 2012;41(4):961-967.
76. Morseth B, Ahmed LA, Bjørnerem Å, Emaus N, Jacobsen BK, Joakimsen R, et al. Leisure time physical activity and risk of non-vertebral fracture in men and women aged 55 years and older: the Tromsø Study. Eur J Epidemiol 2012;27(6):463-471.

77. Wilsgaard T, Arnesen E. Body mass index and coronary heart disease risk score: the Tromsø Study, 1979 to 2001. Ann Epidemiol 2007;17(2):100–105.
78. Emaus A, Degerstrøm J, Wilsgaard T, Hansen BH, Dieli-Conwright CM, Furberg AS, et al. Does a variation in self-reported physical activity reflect variation in objectively measured physical activity, resting heart rate, and physical fitness? Results from the Tromso study. Scand J Public Health 2010;38(5 Suppl):105-118.
79. DinamapTM adult/pediatric and neonatal vital signs monitor model 1846. Operation manual. Tampa, FL: Circon Inc., 1984.
80. Dinamap® ProCare Monitor. Service manual, revision B. Tampa, FL: GE Medical Systems Information Technologies, 2004.
81. Nyrnes A, Mathiesen EB, Njølstad I, Wilsgaard T, Løchen ML. Palpitations are predictive of future atrial fibrillation. An 11-year follow-up of 22,815 men and women: the Tromsø Study.Eur J Prev Cardiol 2013;20(5):729-736.
82. Twisk JWR. Applied Longitudinal Data Analysis for Epidemiology. A Practical Guide. Cambridge, UK: Cambridge University Press, 2003.
83. Littell RC, Stroup WW, Milliken GA, Wolfinger RD, Schabenberger O. SAS for Mixed Models, Second Edition. Cary, NC: SAS Institute Inc, 2006.
84. Royston P, Ambler G, Sauerbrei W. The use of fractional polynomials to model continuous risk variables in epidemiology. Int J Epidemiol 1999;28(5):964-974.
85. Royston P, Altman DG. Regression using fractional polynomials of continuous covariates: parsimonious parametric modelling. Appl Stat 1994;43(3):429–467.
86. Nagin DS, Odgers CL. Group-based trajectory modeling in clinical research. Annu Rev Clin Psychol 2010;6:109-138.
87. Arrandale VH, Koehoorn M, MacNab Y, Kennedy SM. Longitudinal analysis of respiratory symptoms in population studies with a focus on dyspnea in marine transportation workers. Int Arch Occup Environ Health 2009;82(9):1097-1105.
88. Andruff H, Carraro N, Thompson A, Gaudreau P. Latent class growth modeling: a tutorial. Tut Quant Meth Psych 2009;5(1):11-24.
89. Zheng H, Tumin D, Qian Z. Obesity and mortality risk: new findings from body mass index trajectories. Am J Epidemiol 2013;178(11):1591-1599.
90. Gopalakrishnan S, Ganeshkumar P. Systematic reviews and meta-analysis: understanding the best evidence in primary healthcare. J Family Med Prim Care 2013;2(1):9-14.
91. Jacobsen BK, Thelle DS. The Tromsø Heart Study: responders and non-responders to a health questionnaire, do they differ? Scand J Soc Med 1988;16(2):101-104.
92. Wilsgaard T, Brenn T, Arnesen E. Tracking of blood pressure in adult men: the Tromsø Study 1974-1986. J Epidemiol Biostat 1998;3(3):269-276.
93. Wilsgaard T, Schirmer H, Arnesen E. Impact of body weight on blood pressure with a focus on sex differences: the Tromsø Study, 1986-1995. Arch Intern Med 2000;160(18):2847-2853.
94. Mishra S, Manjareeka M, Mishra J. Blood pressure response to cold water immersion test. IJBPAS. 2012;1(10):1483-1491.
95. Haakensen T. Look to Tromsø! iTromsø, 2015. http://www.itromso.no/nyheter/2015/12/28/iLook-to-Troms%C3%B8i-11970600.ece (26 September 2016, date last accessed). [Article in Norwegian]

96. Høydal HF. Ny norsk studie vekker oppsikt: Halvert sjanse for hjerteinfarkt. [New Norwegian study attracts attention: Halved chance of heart attack.] VG, 2015. http://www.vg.no/forbruker/helse/ny-norsk-studie-vekker-oppsikt-halvert-sjanse-for-hjerteinfarkt/a/23586028/ (26 September 2016, date last accessed).
97. Søgaard AJ, Bø K, Klungland M, Jacobsen BK. En oversikt over norske studier – hvor mye beveger vi oss i fritiden? [An overview of Norwegian studies – how much are we moving in spare time?] Tidsskr Nor Laegeforen 2000;120(28):3439-3446. [Article in Norwegian]
98. Morseth B, Emaus N, Wilsgaard T, Jacobsen BK, Jørgensen L. Leisure time physical activity in adulthood is positively associated with bone mineral density 22 years later. The Tromsø study. Eur J Epidemiol 2010;25(5):325-331.
99. Shephard RJ. Limits to the measurement of habitual physical activity by questionnaires. Br J Sports Med 2003;37(3):197-206.
100. Reeves MM, Marshall AL, Owen N, Winkler EA, Eakin EG. Measuring physical activity change in broad-reach intervention trials. J Phys Act Health 2010;7(2):194-202.
101. Cleland CL, Hunter RF, Kee F, Cupples ME, Sallis JF, Tully MA. Validity of the global physical activity questionnaire (GPAQ) in assessing levels and change in moderate-vigorous physical activity and sedentary behaviour. BMC Public Health 2014;14:1255.
102. Szklo M, Nieto FJ. Epidemiology: beyond the basics. Third edition. Jones and Bartlett Publishers: Burlington, MA, USA, 2014.
103. West BT, Welch KB, Galecki AT. Linear Mixed Models – A Practical Guide Using Statistical Software. London: Chapman & Hall/CRC, 2006.
104. Grace-Martin K. Approaches to Repeated Measures Data: Repeated Measures ANOVA, Marginal, and Mixed Models; The Analysis Factor. http://www.theanalysisfactor.com/repeated-measures-approaches/ (20 September 2016, date last accessed)
105. Ostchega Y, Porter KS, Hughes J, Dillon CF, Nwankwo T. Resting pulse rate reference data for children, adolescents, and adults: United States, 1999-2008. Natl Health Stat Report 201124;(41):1-16.
106. Ferrières J, Ruidavets JB. Association between resting heart rate and hypertension treatment in a general population. Am J Hypertens 1999;12(6):628-631.
107. Palatini P, Julius S. The physiological determinants and risk correlations of elevated heart rate. Am J Hypertens 1999;12(1 Pt 2):3S-8S.
108. Giannoglou GD, Chatzizisis YS, Zamboulis C, Parcharidis GE, Mikhailidis DP, Louridas GE. Elevated heart rate and atherosclerosis: an overview of the pathogenetic mechanisms. Int J Cardiol 2008;126(3):302-312.
109. Heidland UE, Strauer BE. Left ventricular muscle mass and elevated heart rate are associated with coronary plaque disruption. Circulation 2001;104(13):1477-1482.
110. Thelle DS, Selmer R, Gjesdal K, Sakshaug S, Jugessur A, Graff-Iversen S, et al. Resting heart rate and physical activity as risk factors for lone atrial fibrillation: a prospective study of 309,540 men and women. Heart 2013;99(23):1755-1760.
111. O'Neal WT, Almahmoud MF, Soliman EZ. Resting heart rate and incident atrial fibrillation in the elderly. Pacing Clin Electrophysiol 2015;38(5):591-597.

112. Morseth B, Graff-Iversen S, Jacobsen BK, Jørgensen L, Nyrnes A, Thelle DS, et al. Associations between physical activity and atrial fibrillation: Is resting heart rate a Mediator? The Tromsø Study. Med Sci Sports Exerc 2016;48(5 Suppl 1):223.
113. Schoonderwoerd BA, Smit MD, Pen L, Van Gelder IC. New risk factors for atrial fibrillation: causes of 'not-so-lone atrial fibrillation'. Europace 2008;10(6):668-673.
114. Skov MW, Bachmann TN, Rasmussen PV, Olesen MS, Pietersen A, Graff C, et al. Association Between Heart Rate at Rest and Incident Atrial Fibrillation (from the Copenhagen Electrocardiographic Study). Am J Cardiol 2016;118(5):708-713.
115. den Hoed M, Eijgelsheim M, Esko T, Brundel BJ, Peal DS, Evans DM, et al. Identification of heart rate-associated loci and their effects on cardiac conduction and rhythm disorders. Nat Genet 2013;45(6):621-631.
116. Grundvold I, Skretteberg PT, Liestøl K, Erikssen G, Engeseth K, Gjesdal K, et al. Low heart rates predict incident atrial fibrillation in healthy middle-aged men. Circ Arrhythm Electrophysiol 2013;6(4):726-731.
117. Mao Q, Huang JF, Lu X, Wu X, Chen J, Cao J, et al. Heart rate influence on incidence of cardiovascular disease among adults in China. Int J Epidemiol 2010;39(6):1638-1646.
118. O'Neal WT, Qureshi WT, Judd SE, Meschia JF, Howard VJ, Howard G, et al. Heart rate and ischemic stroke: the REasons for Geographic And Racial Differences in Stroke (REGARDS) study. Int J Stroke 2015;10(8):1229-1235.
119. Custodis F, Baumhäkel M, Schlimmer N, List F, Gensch C, Böhm M, et al. Heart rate reduction by ivabradine reduces oxidative stress, improves endothelial function, and prevents atherosclerosis in apolipoprotein E-deficient mice. Circulation 2008;117(18):2377-2387.
120. Kröller-Schön S, Schulz E, Wenzel P, Kleschyov AL, Hortmann M, Torzewski M, et al. Differential effects of heart rate reduction with ivabradine in two models of endothelial dysfunction and oxidative stress. Basic Res Cardiol 2011;106(6):1147-1158.
121. Jouven X, Empana JP, Escolano S, Buyck JF, Tafflet M, Desnos M, et al. Relation of heart rate at rest and long-term (>20 years) death rate in initially healthy middle-aged men. Am J Cardiol 2009;103(2):279-283.
122. Nauman J, Janszky I, Vatten LJ, Wisløff U. Temporal changes in resting heart rate and deaths from ischemic heart disease. JAMA 2011;306(23):2579-2587.
123. Hartaigh Bó, Allore HG, Trentalange M, McAvay G, Pilz S, Dodson JA, et al. Elevations in time-varying resting heart rate predict subsequent all-cause mortality in older adults. Eur J Prev Cardiol 2015;22(4):527-534.
124. Floyd JS, Sitlani CM, Wiggins KL, Wallace E, Suchy-Dicey A, Abbasi SA, et al. Variation in resting heart rate over 4 years and the risks of myocardial infarction and death among older adults. Heart 2015;101(2):132-138.
125. Hutcheon JA, Chiolero A, Hanley JA. Random measurement error and regression dilution bias. BMJ 2010;340:c2289.
126. MacMahon S, Peto R, Cutler J, Collins R, Sorlie P, Neaton J, et al. Blood pressure, stroke, and coronary heart disease. Part 1, Prolonged differences in blood pressure: prospective observational studies corrected for the regression dilution bias. Lancet 1990;335(8692):765-774.

Appendix

Links to the Tromsø Study invitation letters, questionnaires and informed consent forms

Tromsø 3:

Invitation: http://uit.no/Content/271752/T3_Invitation.pdf

Questionnaire 1: https://uit.no/Content/271762/T3_Q1.pdf

Questionnaire 2: https://uit.no/Content/271763/T3_Q2.pd

Tromsø 4:

Invitation: http://uit.no/Content/271754/T4_Invitation.pdf

Questionnaire 1: http://uit.no/Content/271764/T4_Q1.pdf

Invitation phase 2: http://uit.no/Content/271753/T4_Information_brochure_Phase2.pdf

Questionnaire 2:

- Less than 70 years of age: http://uit.no/Content/430574/T4_Q2_U70.pdf
- 70 years of age or more: http://uit.no/Content/271765/T4_Q2_O70.pdf

Consent form: http://uit.no/Content/70750/samtykkerklaeringer-pdf

Tromsø 5:

Invitation: http://uit.no/Content/271757/T5_Invitation.pdf

Questionnaire 1:

- Less than 70 years of age: http://uit.no/Content/430584/T5_Q1_U70.pdf
- 70 years of age or more: http://uit.no/Content/430586/T5_Q1_O70.pdf

Invitation phase 2: http://uit.no/Content/271756/T5_Information_brochure_Phase2.pdf

Questionnaire 2: http://uit.no/Content/430588/T5_Q2.pdf

Consent form: http://uit.no/Content/70750/samtykkerklaeringer-pdf

Tromsø 6:

Information brochure: http://uit.no/Content/100340/Forespoersel_om_deltakelse_t6.pdf (In Norwegian only)

Questionnaire 1: http://uit.no/Content/401052/Questionnaire_T6_1.pdf

Questionnaire 2: http://uit.no/Content/401053/Questionnaire_T6_2.pdf

Consent form: http://uit.no/Content/111929/samtykke%20Tr6.pdf