Long-Term Predictors of Cardiovascular Disease (CVD) and CVD Related Mortality in Healthy Middle-Aged Norwegian Men

Ph.D. Thesis

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Oslo, 2018

Series of dissertations submitted to the
Faculty of Medicine, University of Oslo

ISBN 978-82-8377-442-9

Cover: Hanne Baadsgaard Utigard.
Print production: Reprosentralen, University of Oslo.

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Acknowledgments

First, I would like to thank, MD Ph.D. Per Torger Skretteberg, who is my principal supervisor and introduced me to the working methods of the Oslo Ischemia Study group. He helped me grasp the statistical approaches used throughout this work, showed me how to write a scientific abstract based on our findings and develop this into a full scientific paper. His input along the way has also been crucial when developing the hypotheses that form the content of this thesis and critically revising manuscripts.

I wish to thank Professor emeritus Jan Emil Erikssen in the most profoundly. He created a long-term follow-up study, now labeled the Oslo Ischemia Study, during the early 1970s and has generously shared his outstanding work with all members of our study group for more than four decades. The exceptional level of precision and accountability of his inclusions, examinations, data handling, and research methodology formed the basic premise for all further research on this cohort. The unique benefit of having Erikssen as a co-author and advisor has given invaluable insight and intellectual input to the present work.

I would also like to thank my co-supervisor, Professor Sverre Erik Kjeldsen for his steady guidance throughout the process from becoming a Ph.D.-candidate to the completion of the research papers and the final thesis. His positive attitude and loyalty, seemingly unlimited capacity and efficiency, generosity and immense routine have driven this project forward, and the present work would not have been possible without his help and support. My co-supervisor, MD Ph.D. Johan Bodegård has also put in a significant contribution. He possesses an in-depth knowledge of the epidemiological method and has an outstanding overview of the study design and end-point collection procedures, and I wish to thank him for his precise and always apt influences.

All my co-researchers and co-authors also deserve my gratitude. All members of the study group have put in relevant and valuable contributions to many different aspects of the work, and this group effort has molded the present work into its final form. In particular, I would like to thank MD Ph.D. Irene Grundvold for her efforts in leading the study group and Professor Knut Liestøl for his invaluable and reassuring statistical guidance.

A Ph.D.-scholarship from the South Eastern Norway Health Authority (Helse Sørøst) funded the present work. I am very grateful for this contribution, which has enabled me to take part-time leave from clinical practice to perform the research precipitating the present thesis.

While working on the present thesis, I have also been able to carry out my work as a clinician at the Department of Acute Medicine, Oslo University Hospital, Ullevaal. Parallel to writing my Ph.D.-thesis, I am also finalizing my specialty in Internal Medicine. I am indebted by this opportunity and would like to thank my brilliant and supportive leaders and colleagues very much.

I would also like to thank the staff members at Section for Cardiovascular and Renal Research at Oslo University Hospital, Ullevaal, and MD Ph.D. Fadl El Mula Mohamed Fadl El Mula in particular, for introducing me to clinical research. I had the privilege to spend some of the early months of my Ph.D. period working part-time as a co-investigator for the Oslo RDN study giving me valuable experience in the meticulous process of patient inclusion for a randomized clinical trial.

My parents, Elin and Kjell-Ove, and my sister Katrine deserve my gratitude. They have always emphasized that I could reach my goals through hard work and belief in my abilities. The road to becoming a medical doctor and a Ph.D.-candidate has not always been straightforward, and the support from family throughout life has been invaluable to me.

Finally, I wish to thank my wife Marit and our three children, Agnes, Herman, and Ulrik for their love, support and care. You guys! You are the pillars of my life, and none of this would mean anything if I couldn't share my achievements with the four of you!

List of papers

I. Engeseth K, Hodnesdal C, Grundvold I, Liestøl K, Gjesdal K, Erikssen G, Kjeldsen SE, Erikssen JE, Bodegard J, Skretteberg PT.
Heart rate reserve predicts cardiovascular death among physically unfit but otherwise healthy middle-aged men: A 35-year follow-up study
European J Prev Cardiol. 2016;23:59-66

II. Engeseth K, Hodnesdal C, Grundvold I, Liestøl K, Gjesdal K, Kjeldsen SE, Erikssen JE, Bodegard J, Skretteberg PT.
Temporal reduction in chronotropic index predicts risk of cardiovascular death among healthy middle-aged men: A 28-year follow-up study
J Am Heart Assoc. 2016;23:e004555

III. Engeseth K, Prestgaard EE, Mariampillai JE, Grundvold I, Liestøl K, Kjeldsen SE, Bodegard J, Erikssen J, Gjesdal K, Skretteberg PT.
Physical fitness is a modifiable predictor of early cardiovascular death: A 35-year follow-up study of 2014 healthy middle-aged men
European J Prev Cardiol. 2018;10.1177/2047487318793459

These papers are referred by their Roman numerals throughout the thesis.

Abbreviations

BMI = Body mass index (kg/m²)

BPM = Beats per minute

CHD = Coronary heart disease

CI = Confidence interval (95%)

CV = Cardiovascular

CVD = Cardiovascular disease

HDL = High-density lipoprotein cholesterol

HR = Heart rate

LDL = Low-density lipoprotein cholesterol

PF = Physical fitness

SBP = Systolic blood pressure

VO^2 = Oxygen consumption

VO^2-max = Maximal oxygen consumption

Introduction

Pathophysiology of cardiovascular disease

The term cardiovascular disease (CVD) refers to all diseases of the cardiovascular (CV) system, but the main contributors to mortality are coronary heart disease (CHD) (53%) and stroke (32%) (1). Atherosclerosis is the predominant cause of CVD and knowledge about this pathophysiological pathway prefacing a symptomatic atherosclerotic lesion forms the basis of CVD prevention and treatment.

The atherosclerotic continuum mainly affects large or medium-sized arteries such as the aorta, the carotid, iliac coronary and popliteal arteries. The key processes involved are thickening of the intima layer of the vessel wall, lipid accumulation, and inflammation. At lesion-prone sites, and accelerated by risk-factors, damage to the endothelium covering the inside of the artery wall will lead to dysfunction with increased permeability to lipoproteins, monocyte adhesion and migration, platelet adhesion and activation and release of growth factors that lead to smooth muscle cell proliferation and extracellular matrix synthesis in the intima layer of the artery wall. Within the intima, migrated monocytes will transform into macrophages that engulf oxidized lipoproteins and develop into foam cells that degenerate and deposit lipid-rich substance that calcifies in the intima layer of the artery wall. These processes induce a chronic and self-reinforcing inflammatory response that gradually leads to the development of atherosclerotic plaques. As the atherosclerotic changes advance, a vulnerable fibrous cap will be the only barrier between the plaque and the blood creating a lesion prone to rupture and activation of a thrombotic cascade. The three principal pathophysiological manifestations of atherosclerosis are; critical stenosis due to gradual plaque growth, acute occlusion by thrombosis due to plaque rupture, and formation of aneurysms and rupture due to vessel wall weakening. The atherosclerotic process is slow and often requires many decades to evolve into a clinically significant lesion. However, sudden changes in unstable plaque, such as rupture and thrombosis, can acutely induce symptomatic or even fatal atherosclerotic disease (2) (Figure 1).

Figure 1

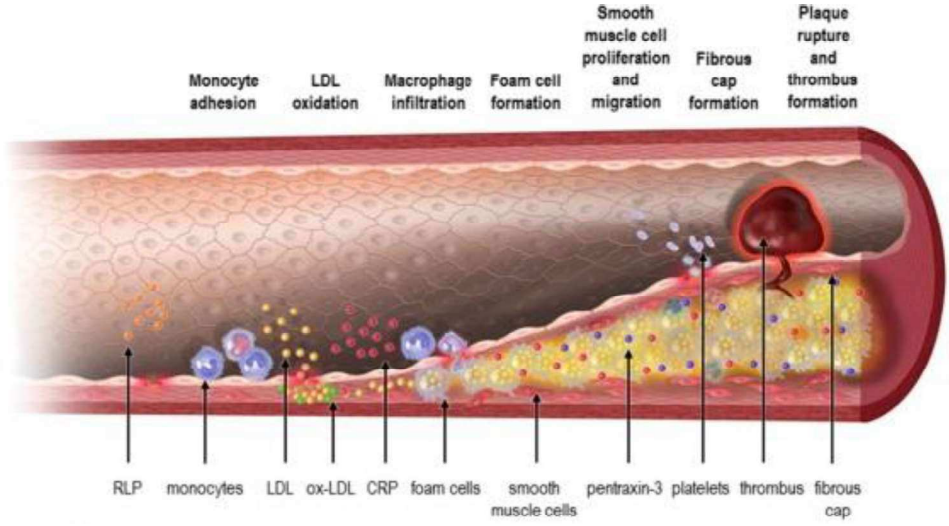

Figure 1: Schematic overview of the stages of atherosclerosis from endothelial damage (left side) to an advanced atherosclerotic plaque with a ruptured fibrous cap and thrombosis (right side). (Google images)

Although atherosclerosis and complications thereof, account for the majority of CVD, other pathophysiological pathways also contribute. Hypertension accelerates hyaline thickening of arteriole walls and laminated, concentric thickening of vessel walls due to hyperplasia of smooth muscle cells and duplicated basement membranes with increased vascular stiffness, loss of structural detail and narrowing of the lumen as consequences. These phenomena are often referred to as early vascular aging and cause increased total peripheral resistance, increased blood pressure and, hence, induce a self-reinforcing vicious circle (2). Another example is atrial fibrillation, the most common clinically significant arrhythmia, which affects millions of people globally (3, 4). This condition is associated with increased risks of thromboembolic events and heart failure (5, 6). The pathogenesis and risk factors associations for atrial fibrillation are, however, often related to hypertension and atherosclerotic disease and involve interlinked conditions such as previous myocardial infarction, hypertension, and obesity (7). Still, other and independent mechanisms such as autonomic nervous system activation, and structural remodelling of the myocardium due to genetic dispositions or without any identifiable cause, probably account for a substantial amount of arrhythmias, including atrial fibrillation, as well as less common types and even unexplained sudden cardiac death (8, 9).

Epidemiology of cardiovascular disease

The burden of CVD reached epidemic proportions during the mid-20th century. Vast, and successful, efforts were initiated to understand the nature of the disorders better and identify preventive strategies and treatment options. However, despite advances in modern treatment and prevention, CVD is still the leading cause of mortality in the world. According to the 2018 WHO health statistics, non-communicable diseases caused 41 million (71%) of the overall total of 57 million global deaths in 2016, and CVD caused 17.9 million (44%) of non-communicable diseases-related global deaths (10). In Europe, CVD causes 3.9 million (45%) of all deaths each year and, even as CV death is falling in most European countries, CVD is still the leading cause of both total and premature death among both men and women in most European countries. Due to better diagnostics and treatment options, more patients are diagnosed with CVD and patients with CVD live longer. As a result of this, the prevalence of CVD has increased in most European countries since the beginning of the 21st century. More than 85 million people in Europe now live with CVD, and this accounted for the loss of more than 64 million disability-adjusted life years in 2016 (23% of total disability-adjusted life years lost) and the current estimate suggests a yearly cost of CVD in the European Union of 210 billion Euros from health-care and loss of productivity (11).

Prevention of cardiovascular disease

CVD prevention is defined as a coordinated set of actions, individually targeted or at the population level, aimed at eliminating or minimizing the impact of CVDs and their related disabilities. Recommended preventive measures currently include lifestyle changes such as smoking cessation, nutritional guidance, weight-loss, and physical exercise; and pharmacological treatment with blood pressure-lowering, antiplatelet, and lipid-lowering drugs (12).

As mentioned above, the atherosclerotic continuum develops over decades from endothelium damage to clinically significant disease. The nature of this pathophysiological pathway indicates that small endothelial changes may occur early in life, even during childhood and adolescent years, and this underlines the usefulness of advocating CVD prevention at the population level. This strategy is in line with Geoffrey Rose's prevention theory; "*small shifts in the risk of disease across a whole population consistently lead to*

greater reductions in disease burden than a large shift in high-risk individuals only" (13). Individual behavior is carried out in an environment with multiple influences from family, school, advertisement, state regulations, and healthcare systems. Thus, preventive research should aim at influencing decision-makers with evidence-based suggestions on how to improve CVD risk at a group, community, regional, national or global level ensuring that even non-risk parts of the population such as children, adolescents, and young adults benefit from preventive measures *before* irreversible pathophysiological processes start (14).

Clinicians separate *primary* and *secondary* CVD prevention in daily practice. *Primary* prevention aims to avoid or postpone the development of disease among asymptomatic individuals at risk, whereas *secondary* prevention seeks to prevent new events among patients with established CVD. Literature shows that, even with optimal secondary preventive measures, patients with previous CVD events are at high risk of experiencing new events (12, 15, 16). The risk of recurrence is highest during the first 12 months. After this time, the risk is reduced by approximately 50%, but the risk of recurrence and CVD related mortality remains substantial when compared with individuals from the general population matched for age, sex, and area of residence (17-19). Still, even when aiming secondary preventive measures at patients who are likely to be motivated by the negative experience of a recent CVD event, adherence to secondary preventive medication and lifestyle interventions is low and improving this adherence is a persistent challenge (20).

In primary prevention, the aim is to identify healthy individuals with increased risk of developing CVD, *and who* are likely to benefit from prevention, and then educate and motivate these to comply with advice and prescriptions. Convincing an apparently healthy population to change lifestyle and take medication is demanding. Not surprisingly, adherence to primary prevention is even lower than is the case for secondary prevention (12, 21). Finding the parts of a population that will benefit from primary prevention is also hard. Both opportunistic and systematic CVD risk screening strategies have been investigated in several studies. A large (n=59616) Danish trial from 2014 showed that although screening and repeated life-style counseling of high-risk individuals over five years may lead to overall improvements in risk factor-levels, there was no impact on CVD

outcomes after ten years (22). Similarly, a recent Cochrane review concluded that general health checks with CVD screening did not reduce all-cause or CV death (23). Other recent Cochrane reviews demonstrated that only high-risk individuals with diabetes or hypertension decreased their CV death risk through modification of risk factor levels by counseling or education (24, 25). However, most studies that form the bases of these reviews were performed several decades ago and did not include optimal medical treatment in addition to contemporary lifestyle intervention. For this reason, current European guidelines advocate a systematic approach to CVD risk assessment while emphasizing the need to target populations likely to be at higher CVD risk and the usefulness of repeated risk assessments (12).

The risk factor-approach

> *"Since 1949, 5127 citizens from the Boston suburb of 60,000 have reported faithfully every two years for physical exams which are conducted at a rate of 60 a week. Out of this mountain of data have come revelations on heart attack risk factors that have helped American physicians to devise preventive measures for high-risk patients. These risk factors - high blood pressure, cigarettes smoking, obesity, high blood cholesterol levels, and others - were either unknown or guessed prior to the Framingham study".*
>
> Boston Globe *March 7th, 1971 (26).*

The above newspaper quote from the Boston Globe, 1971, summarizes a paradigm shift that occurred in medicine during the mid-20th century-years. The term *cardiovascular risk factor* emerged in literature from the early 1970s but was unheard of only a decade or two before. In particular, there had been a radical change in the understanding of CVD during the decades after World War II. The common belief was no longer that random, unpreventable degenerative changes caused this cluster of diseases. Follow-up studies, such as the Framingham Heart Study, had provided new, convincing and scientifically valid epidemiological data that uncovered undisputable associations between lifestyle, comorbidity, and CVD. This new insight gradually convinced the scientific community throughout the 1950s and 60s, and by the 1970s this understanding was about to become established among the general public (27).

Risk factors and causality

The term “risk factor” implies causality, and causality can be difficult to validate when discussing a cluster of diseases related to slowly developing changes with multi-factorial etiology such as CVD. Some risk markers are plausibly causal by acting through known biological mechanisms whereas other risk markers are surrogates for underlying and even unknown mechanisms. For this reason, the term “risk factor” will be used when discussing risk markers shown to be causal on through multidisciplinary medical and statistical evidence whereas the term “predictor” will be used for markers associated with death or disease in the following sections. The present work is based on data from long-time follow up of a healthy cohort and hence, not designed to investigate causality. Our unique study design with systematically performed repeated measurements of the same physiological markers accompanied with very long-term follow-up does, however, allow investigation of temporal co-variation of predictors and endpoints that might offer a higher level of evidence than point-measurements of the same variables would do.

Risk estimation

In apparently healthy persons, CVD risk is, in general, the result of multiple*, interacting* risk factors and this is the basis for the total CVD risk approach to prevention that has led to the development of several CVD risk estimation systems (12). The most widely used systems are the Framingham risk score (US), and the SCORE system (Europe) with locally adjusted versions, such as NORRISK 2 in Norway. The SCORE system estimates the 10-year risk of a *first fatal* atherosclerotic event, including all International Classification of Diseases (ICD) codes that could reasonably be assumed to be of atherosclerotic origin, including coronary artery disease, stroke, and aneurysm of the aorta (28). There is an ongoing debate on the choice to calculate the risk of CV death rather than the total risk of a fatal or non-fatal CVD event. Naturally, the 10-year risk of any CVD event is higher than that of the risk of death from a first CVD-event. However, local conditions will influence the mortality rate at the first CVD event, availability of reliable risk factor prevalence data, the accuracy of diagnoses at hospital discharge, and quality of data on causes of death. In Norway, the mortality from a first CVD-event has fallen dramatically over the last decades, and the availability and reliability of data allow estimation of total 10-year CVD-event risk rather than fatal events only in the NORRISK-2 system (29) (Figure 2). Common to all the above-mentioned risk

estimation tools is that they are based on levels of the so-called *"classical CV risk factors"*; age, blood pressure, smoking, cholesterol, and gender. In addition, most will offer risk multiplication factors for family history of ischemic heart disease, treated hypertension, diabetes mellitus, low HDL cholesterol, South Asian ethnicity, systemic rheumatoid arthritis and, in some cases, other factors such as obesity and psychiatric conditions (Figure 2.1).

Figure 2.

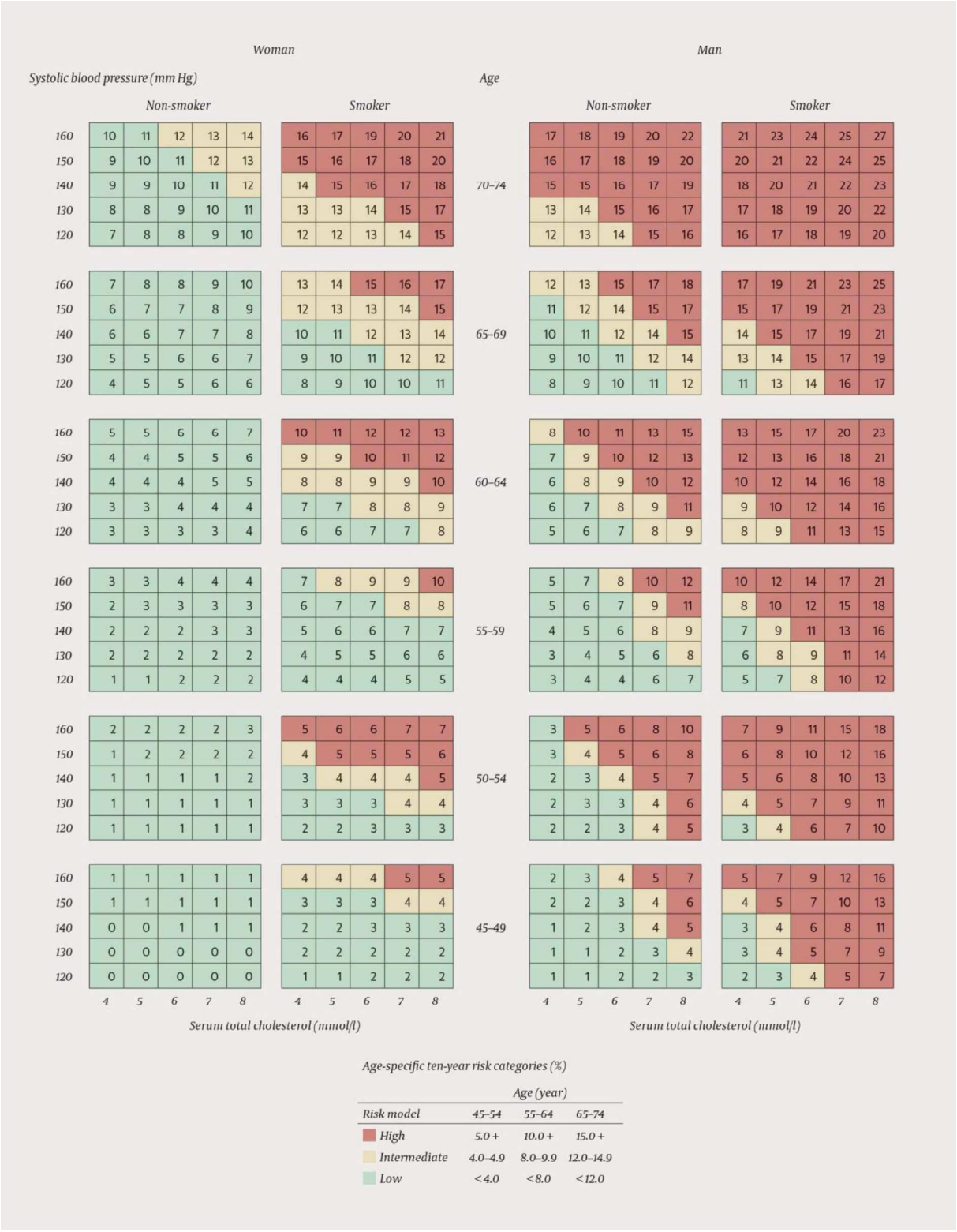

Figure 2. NORRISK-2 risk table: Ten-year risk of non-fatal or fatal myocardial infarction or stroke or death from coronary artery disease, given the absence of familial risk, low HDL cholesterol and medically treated hypertension (1). Risk estimates are based on the average age within each age group.

Figure 2.1.

Additional factor	Recommended multiplication factor
Included in risk calculation:	
Additional factor	Recommended multiplication factor
Myocardial infarction under the age of 60 in a first-degree relative	1.3
Myocardial infarction under the age of 60 in two or more first-degree relatives	1.7
Medically treated hypertension	1.3
Low HDL-cholesterol (i.e. < 1.0 mmol/l for men, < 1.3 mmol/l for women)	1.4
Further additional factors:	
South Asian ethnicity	1.5
Rheumatoid arthritis	1.4
Abdominal obesity (i.e. > 88 cm for women, > 100 cm for men)	Discretionary
Psychosocial load and stress	Discretionary
Depression, medication-controlled psychotic illness	Discretionary

(1) **Figure 2.1.** Key factors that should be studied for comprehensive risk assessment in the NORRISK-2 risk model

Established cardiovascular risk factors

Age

Increasing age is indisputably the most powerful predictor of CVD and many other diseases, and in the present work, statistical calculations have age included in all the adjustment models. However, a young person with many risk factors may have the same level of CVD risk as an older person with no risk factors. Hence, a 50-year-old may have the risk age of a 70-year-old and vice versa. The term *risk age* has therefore been implemented as an intuitive and easily understood method for communicating about risk and equations have been derived based on the ideal level of risk factors, such as blood pressure, smoking, and cholesterol in the SCORE system, corresponding to a certain age (30). This tool is designed to motivate lifestyle changes, particularly among younger patients, and is incorporated in other risk estimation tools, such as the physical fitness-based CV death risk calculator developed by The Cardiac Exercise Research Group in Trondheim, Norway (31). However, current prevention guidelines stress that absolute risk estimation rather than risk age estimation should form the basis of treatment decisions. Still, the need to identify younger persons (<50

years of age) at risk is encouraged (12). Life expectancy is expanding in most parts of the world, particularly in Europe and the US. CVD risk assessment among the elderly is controversial. One could argue against treating age-driven risk, while at the same time; preventive treatments might postpone morbidity, sustain a high quality of life and hence, prolong a good life. Along these lines, there is evidence that biological, rather than chronological age, should be applied when considering preventive treatment among the elderly (32). To ensure finding young individuals at high risk and elderly with a potential to benefit from life-prolonging preventive measures, one might argue that this principle is universal when considering risk assessment of individuals outside the traditional "preventive window" of 45 to 75 years of age.

Blood pressure

Worldwide prevalence of high blood pressure is increasing, and high blood pressure has been the most significant risk factor for premature death globally since 2003 according to the World Health Organization. In 2010, high blood pressure accounted for 9.4 million deaths and 7% of global disability-adjusted life years, and without further action, the World Health Organization has predicted that this number will increase. Systolic blood pressure (SBP) above 140 mmHg accounts for most of the mortality and disability burden, and the majority of SBP-related deaths per year are due to CHD (4.9 million) and stroke (3.5 million) (28, 33). In the present work, all statistical calculations have SBP included in the adjustment models. There is extensive evidence for a firm, graded and consistent association between blood pressure levels and CVD risk at all ages and in all ethnic groups, and extends from high levels to relatively low values (34, 35). High blood pressure induces a wide range of pathological processes in multiple organs such as the vasculature, heart, brain, and kidneys and often clusters and interacts with other CVD risk factors. Hence, current guidelines recommend that an estimate of total CVD risk and an evaluation of the presence of hypertension mediated organ damage should form the decision base when initiating antihypertensive treatment. Both lifestyle and pharmacological interventions will effectively lower blood pressure and hence reduce CVD risk (12, 28, 33).

Smoking

Smoking is an established cause of many diseases and among smokers, smoking is responsible for 50% of avoidable deaths, half of which due to CVD. The 10-year risk of fatal

CVD is approximately doubled in smokers and among individuals younger than 50 years, the relative risk is five-fold higher than in non-smokers (36). In the present work, all statistical calculations have smoking, and in some cases, smoking cessation, included in the adjustment models. Smoking enhances the development of atherosclerosis and thrombotic events, among others through affecting endothelial damage, oxidative processes, platelet function, fibrinolysis and vasomotor function (2). The risks associated with smoking show a dose-response relationship with no lower limit for harmful effects. The benefits of smoking cessation are well documented, and smoking cessation is possibly the most effective of all CVD preventive measures (37). Smoking is, however, an addictive disorder and cessation can be very difficult. Although the rate of smoking is declining I Europe, it is still widespread and increasing among women, adolescents and the socially disadvantaged (38).

Cholesterol

The role of dyslipidemia, especially hypercholesterolemia, in the atherosclerotic process is well documented (2). In the present work, all statistical calculations have total serum cholesterol included in the adjustment models. Lipids such as cholesterol and triglycerides circulate in the blood plasma bound to apolipoproteins, and the primary carrier of cholesterol in plasma, low-density-lipoprotein cholesterol (LDL) is atherogenic. There is a strong and graded positive association between total cholesterol as well as LDL and CVD risk. This association applies to men and women, and healthy individuals as well as individuals with established CVD (39). Therefore, both primary and secondary CVD prevention guidelines recommend LDL-lowering treatment through lifestyle intervention and medication (12). A low level of High-density lipoprotein cholesterol (HDL) is independently associated with high CVD risk as are high levels of fasting triglycerides. The combination of low HDL and moderately elevated triglycerides is very common in patients with abdominal obesity and insulin resistance, and among the physically inactive (40, 41). Attempts have been made to increase HDL levels pharmacologically, but partially due to adverse effects, and probably also due to other mechanisms related to reverse cholesterol transport, no benefits in CVD protection were seen. Hence, physical activity and other lifestyle factors, rather than drug treatment, remain important means of increasing HDL and lowering triglyceride levels.

Family history

Examining a patient's family history of premature CVD is a crude but straightforward assessment tool and reflects genetic dispositions as well as lifestyle habits within a household. Premature CVD is defined as the diagnosis of CVD among first degree male relatives before the age of 55 or female relatives before 65 years of age. A positive family history of premature CVD death is associated with an increased risk of early and lifetime CVD (42). Even so, family history fails to improve CVD risk prediction significantly beyond conventional risk factors alone. Some reasons might be that the definition of significant family history can vary and that other CVD risk factors can partly explain the impact of family history (43, 44). Current guidelines still recommend family history as part of the CVD risk screening routine. In particular, it can prove very useful in identifying young individuals with high CVD risk due to genetic disorders such as familial hypercholesterolemia (12). In the present work baseline examination of family history was uniform and performed by the same physician in all the participants and hence, we included family history of CHD in statistical adjustment models.

Obesity and type 2 diabetes

Body mass index (BMI) has increased substantially in all countries over the recent decades. If this trend continues, it will offset the favorable trends in key CVD risk factors such as blood cholesterol, blood pressure and smoking by 2025 (45). The main clinical complications of increasing BMI are elevated blood pressure, dyslipidemia, insulin resistance, systemic inflammation, a pro-thrombotic state, albuminuria, and the development of diabetes mellitus and CVD events. Both overweight and obesity are associated with increased risks of CV death and all-cause mortality (46). Physical fitness (PF) seems to influence the effect of obesity on CVD risk. Several findings indicate that normal weight unfit individuals have a higher risk of CVD than unfit individuals regardless of BMI whereas no CVD risk differences are seen between overweight and normal weight fit individuals. This phenomenon is often referred to as the obesity paradox. Results of the 2015 EPIC study, and other studies, suggest that the effect of inactivity is greater than the impact of BMI on cardiovascular status and all-cause mortality risk prediction (47, 48). In the present study, the study population was non-diabetic and, in line with previous findings, we found no strong impacts of BMI or fasting blood glucose on CVD or all-cause mortality prediction, especially not when including PF in

the prediction models. Hence, we did not include BMI and fasting blood glucose in the main statistical prediction models, but we included these variables in additional sensitivity analyses where appropriate.

Background of the present study

The primary aim of the Oslo Ischemia Study was to determine the prevalence of silent CHD in a population of apparently healthy middle-aged men. After completing the primary study, a long-term follow up was initiated, and since the start in 1972, the Oslo Ischemia Study has added valuable knowledge about PF, exercise test findings, and CVD epidemiology. In 1993, a paper from the study group, published in the New England Journal of Medicine, presented the novel finding that PF was an independent long-term predictor of CV and all-cause mortality among healthy middle-aged men (49). This finding was supplemented by another study group-paper, published in The Lancet in 1998, that showed how temporal changes in PF influenced mortality risk (50).

Both European and Norwegian guidelines on prevention of CVD stress that the use of additional "novel" risk markers is of limited value and rarely leads to re-classification after regular assessment of the individual 10-year risk of CVD or CV death (12, 29). Still, as mentioned previously, current guidelines for CV prevention emphasize that identifying characteristics of groups and individuals at high risk should be a cornerstone of the CV screening routine (12). According to the WHO, physical inactivity has recently been identified as the fourth leading risk factor for global mortality, causing an estimated 3.2 million deaths each year and most adults fail to meet the recommended level of physical activity (1, 11). A sedentary lifestyle is also one of the key risk factors for CVD independent of participation in physical exercise (51). In line with our previous findings, there is also growing evidence that individuals with abnormal heart rate response to exercise or low age-adjusted PF have increased long-term risks of CVD and CV death (52-56). In a large 2017 study (n=38,480), Nauman and Wisløff et al. concluded that low PF estimated by a non-exercise algorithm (including age, height, weight, resting and maximal heart rate, exercise habits, and waistline measurement) was independently associated with CVD and all-cause mortality. The inclusion of traditional CVD risk factors added little to risk discrimination and did not improve the classification of risk beyond estimated PF (57). This conclusion opposes to statements in current guidelines and hence, further epidemiological investigation of heart rate response to exercise and PF as possible predictors of CV death attained our interest.

In recent prospective cohort studies of a healthy population, a novel activity metric based on activity time and time in heart rate-zones during exercise called Personal Activity Intelligence

score was estimated by using data from the HUNT-study. The results showed that low single point scores assessed at inclusion were associated with a higher risk of premature CV death during 26 years of follow-up (58). When second scores were measured, ten years after the initial measurements, increased or sustained Personal Activity Intelligence scores were associated with lower risks of CVD and all-cause mortality during follow-up, and an improved score over ten years was associated with 6.6 years gained lifespan (59). The uncommon availability of repeated surveys with clinical examinations, blood tests ECG and exercise-ECG tests in the Oslo Ischemia Study gives the opportunity to study the impact of both point measures of-, and temporal changes in possible predictors of CV death and other outcomes. The very long and complete follow-up the Oslo Ischemia Study also allows investigating possible associations between predictors and risks of outcomes during different parts of the 35-year follow-up period. As a result of this, we studied how point measures of- and temporal changes in heart rate variables and PF associated with CVD outcomes in different parts of the observation period in the present study.

When investigating prediction of clusters of outcomes with complex and multifactorial genesis, such as CVD, it can be difficult to assess the independent effect of each factor as the effect of one factor may be dependent on the level of another factor and the effect of the two on the outcome might not be additive. This phenomenon is called statistical interaction and has been addressed since the early years of risk factor research, but not studied very widely, and our study contains data suitable for studying such interactions. We have previously shown that PF and heart rate increase during exercise are correlated (54). We then found it plausible that the predictive abilities of HRR and PF on CV disease and death may not be independent of each other, and this inspired the study of statistical interaction between heart rate variables and PF in the prediction of CV death in the present work.

Aims of the thesis

I. There is evidence that chronotropic incompetence and heart rate reserve (maximal heart rate minus resting heart rate) predicts CVD and CV death. It is physiologically plausible that physical fitness influences this relationship and this has, to our knowledge, not been studied in detail before.

Thus, the aim of paper I was: To test the hypothesis that physical fitness influences the predictive impact of heart rate reserve on the long-term risk of CV death in apparently healthy middle-aged men.

II. The chronotropic index ([achieved maximal heart rate – resting heart rate]/[estimated maximal heart rate – resting heart rate]) has previously been shown to predict CVD and mortality. Our data include repeated measurements of resting and maximal heart rates and allow investigating the effect of temporal changes in this index on CVD and mortality risk prediction. This impact has not been studied in detail before.

Thus, the aim of paper II was: To test the hypothesis that temporal changes in chronotropic index influence the long-term risks of CV and all-cause death in apparently healthy middle-aged men.

III. There is growing evidence that physical fitness and temporal changes in physical fitness influence risk of future mortality and CVD. However, research investigating how mid-life physical fitness and change in physical fitness during mid-life years affect risks of CVD and mortality in different parts of long-term follow-up is sparse.

Thus, the aim of paper III was: To test the hypothesis that physical fitness and temporal change in physical fitness influence risks of CV and all-cause death differently during the early, intermediate and late parts of a long-term follow-up of apparently healthy middle-aged men.

Material and methods

The Oslo Ischemia Study

The Oslo Ischemia Study was initiated at Rikshospitalet, Oslo, Norway in 1971 by professor Ole Storstein and, at that time, Ph.D. candidate Jan Erikssen. The original name of the study was "Rikshospital-undersøkelsen." The primary aims of the study were to explore the extent of silent coronary heart disease in a population of apparently healthy men by anamnesis, clinical examination and exercise electrocardiography (ECG), identify possible risk factors among patients with coronary heart disease and explore the value of anamnesis and clinical examination in diagnosing silent ischemic heart disease. Secondary aims were to investigate the validity of different ECG-criteria at exercise testing by comparing the primary results with coronary angiography-findings. Tertiary objectives were to register the incidences of sudden cardiac death, myocardial infarction and angina pectoris during at least five years of follow-up and explore how initial findings relate to events of coronary heart disease later in life.

The study gradually developed into a long-term follow-up study in-line with the tertiary aims of the original protocol. One of the terms of the agreement at inclusion was that Jan Erikssen would continue as a health consultant for the test persons he recruited. During this work, he noticed that many of the men developed illnesses and characteristics that he had not suspected during his first examinations and interviews. Jan Erikssen's 1st opponent, Professor Kalevi Pyorala from Kuopio, Finland, had experience in leading population studies and he became a mentor in planning and organizing the developing follow-up project that is still on-going (Figure 3).

Figure 3.

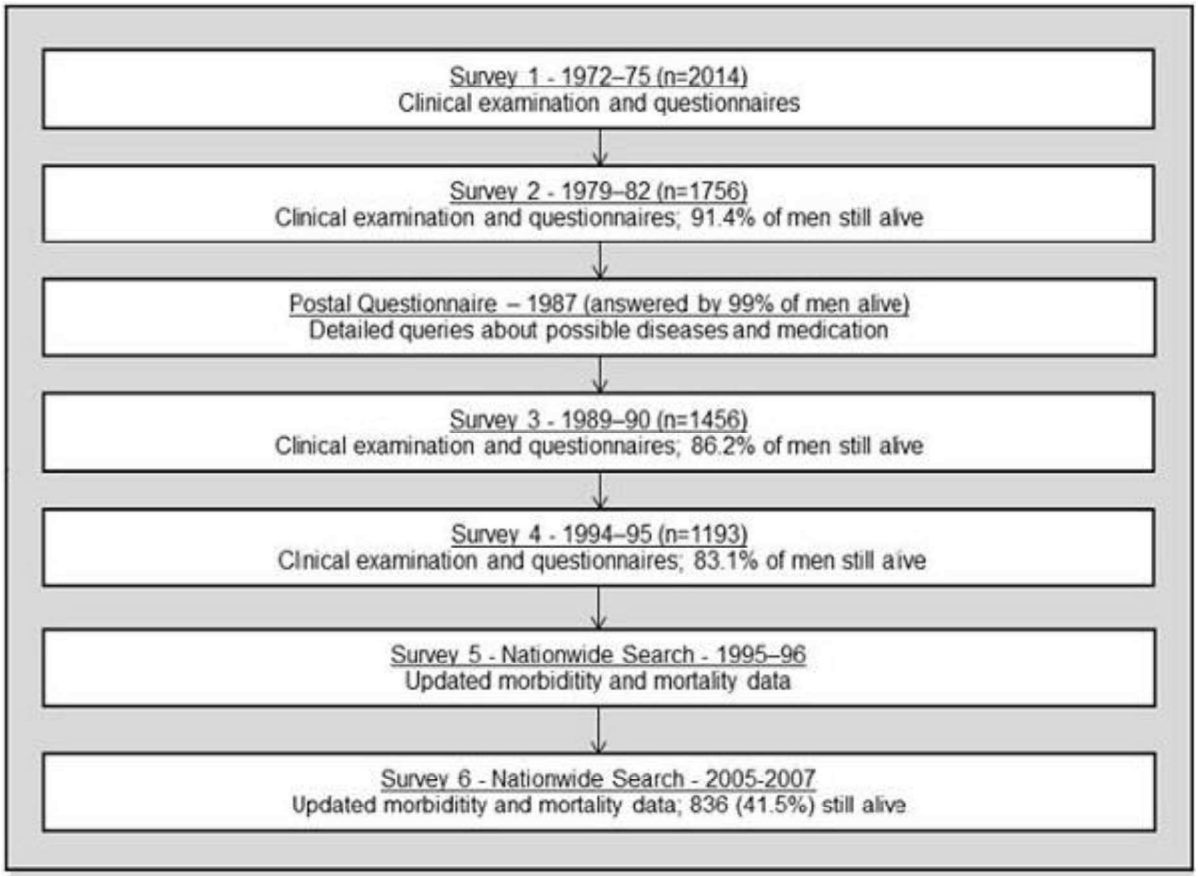

Figure 3. Oslo Ischemia Study flow-chart showing all surveys during 35 years of follow-up

Study population

All subjects in the studies of the present thesis are subsets of men included in the original survey. At baseline, Erikssen recruited a cohort of apparently healthy middle-aged men (40-59 years old) from five Companies based in the Oslo region (Custom Services, Norwegian Railways, Postal Services, Siemens Inc. and Telecommunication Services). These large institutions practiced annual or biennial health examinations of their employees allowing the Chief Medical Officer at each institution and the survey leader to decide the eligibility of each man after joint scrutiny of health records. To be considered eligible the men had to volunteer for examination, be physically capable of carrying out an exercise ECG-test (e.g., free of orthopedic, neurological or muscular conditions limiting the ability to conduct the test). The study subjects also had to be free of known or suspected coronary heart disease, diagnosed hypertension requiring drug treatment, diabetes mellitus, thyroid disorders, cancer, advanced pulmonary, renal or hepatic diseases, and other serious disorders. As a

courtesy to the volunteers, those who at arrival for the inclusion survey reported that any of the above-mentioned conditions had been diagnosed elsewhere after their last visit to the company health care office underwent a full study examination but were later excluded from our files. In total 2341 men fulfilled the criteria to be regarded as healthy and 2014 (86%) agreed to participate. As a result of this strict inclusion procedure, our study population was regarded to represent a group of apparently healthy and full-time employed middle-aged men.

Baseline Examinations

The men underwent an extensive CV health survey which took place between August 28, 1972, and March 21, 1975 (Survey 1). All participants were given a comprehensive health questionnaire one week before Survey 1 with queries on angina pectoris, family history, former diseases, use of drugs, lifestyle habits and working conditions, and included a Norwegian translation of the WHO angina, WHO claudication, and the MRC respiratory questionnaire. The questionnaires were reviewed together with all the participants by Jan Erikssen during the investigation day to ensure that all questions were understood and answered correctly. All participants underwent a thorough and meticulously detailed and standardized clinical examination, all conducted by Jan Erikssen, with measurements of height and weight, spirometry, 2-plane chest x-ray, fasting blood samples, intravenous glucose tolerance test, full-body clinical examination, resting ECG and blood pressure measurements and a symptom-limited bicycle exercise ECG test. The examinations started at 07.00 AM, and all men had been requested to abstain from smoking for at least 8 hours and from eating for 12 hours. Clinical parameters were examined under standardized conditions after 5 minutes rest in a supine position in a quiet room. Blood for cholesterol measurements and other blood tests was drawn before exercise testing and analyzed by standard methods. The study protocol generally followed the standardized principles described by Rose and Blackburn in their WHO-publication from 1968: CV Surveys and Methods. Clinical examinations and tests were performed in the order shown below (Figure 4).

Figure 4.

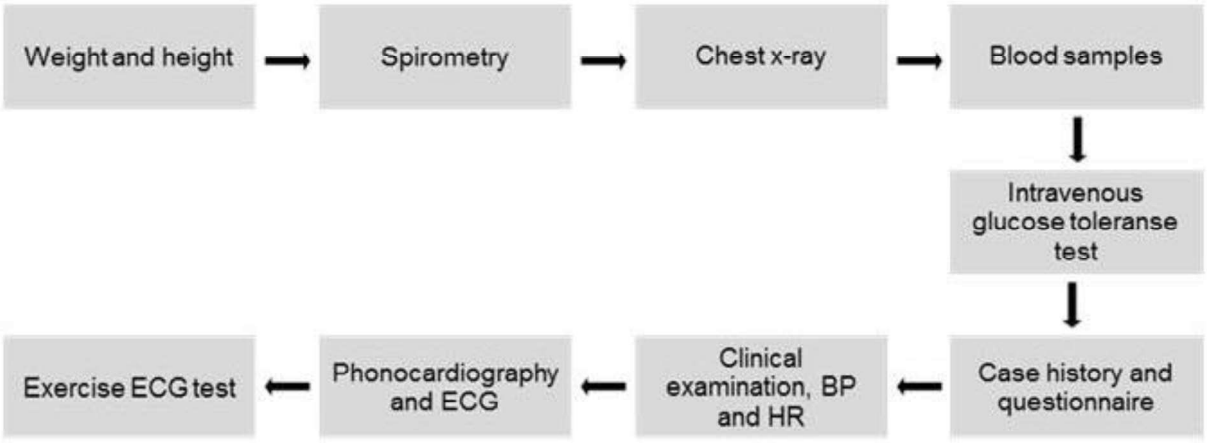

Figure 4. Order of examinations (from top left). BP: Blood pressure, HR: Heart rate

Bicycle exercise ECG- tests

All exercise ECG-tests were performed on an electrically braked Elema bicycle. The primary investigator carried out a pre-study with 80 representative men to determine the exercise protocol, and all the participants were able to cycle for 6 minutes at a workload of 100 Watts (W) indicating that most healthy middle-aged Norwegian men would be able to perform at least at this level. Hence, the initial workload was set at 100W and increased by 50W every 6^{th} minute for 98% of the men. The other 2% started with 50W at Survey 1. SBP was measured to the nearest 5 mmHg every second minute. All men were encouraged to continue until exhaustion. PF was defined as the total bicycle exercise work (Joule), calculated as the sum of work at all workloads, divided by body weight (kg) to correct measurements from a non-weight bearing activity. Tests were terminated for safety reasons if a subject stated that he felt unable to continue, even if no specific reason was communicated. The following pre-specified reasons validated premature termination by the investigator: Major cardiac arrhythmias such as atrioventricular block, ventricular tachycardia or supraventricular tachycardia, drop in systolic blood pressure of ≥ 10 mmHg on two successive measurements one minute apart, ST-depressions > 3 mm, severe dyspnoea or increasing chest pain. At Survey 1 in 1972-75, 1999 out of the 2014 men completed the 100W workload, while 1392 of the 1420 men who were still healthy at Survey 2 in 1979-82 completed the 100W workload. The reproducibility of exercise measurements was tested by repeating the test within two weeks in a random subset of 130 of the 2014 participants at

Survey 1. Blood pressures, heart rates and exercise capacity at the two tests were within ± 5% in 90% of the participants and within ± 10% in all participants. Men with suspected ischemia after the baseline examination underwent coronary angiography to determine the prevalence of true coronary artery disease in the study population. Although coronary angiography had been performed in other countries since the 1960s, and experimentally even decades before, the participants in the Oslo Ischemia Study were among the first persons to undergo this procedure in Norway.

Follow-up

Between the years of 1979-82 1756 of the original 2014 men revisited for Survey 2, and again Jan Erikssen performed all examinations. The participants also filled in similar questionnaires as at Survey 1. Survey 2 and subsequent clinical examinations were, with few exceptions, identical to Survey 1. In 1987 participants completed a postal questionnaire with detailed queries about possible diseases and medication (Survey 3). Between the years of 1989 and 1990 1456 (86.2% of men still alive) came in for a 3rd visit with clinical examinations and questionnaires (Survey 4) and in 1994-94, 1193 (83.1% of men still alive) came in again (Survey 5).

Survey 6

The last follow-up of the cohort (Survey 6) was initiated in 2005 and completed by the 31st of December 2008 and conducted by MD Ph.D. Johan Bodegard and Jan Erikssen. With permissions from the Norwegian Data Inspectorate and the Norwegian Board of Health, all 2014 subjects in the cohort were linked with patient journals at all Norwegian hospitals using their Norwegian social security number. Hospital records were manually scrutinized on-site. Using all available sections in records, e.g., outpatient notes, general practitioners letters, autopsy reports, ECG-readings, surgery notes, admission and discharge letters, both in paper- and electronic versions, the following data were obtained:

1. Name and date of birth with social security number
2. Date of admission and discharge, number of days admitted, and name of the hospital
3. Up to three diagnoses for each admission (ICD-10)
4. Separate registration of the following diseases and the year they occurred;
    - Unstable angina pectoris

- Myocardial infarction with localization (anterior wall, inferior wall etc)
- Myocardial infarction without symptoms
- Percutaneous coronary intervention (PCI)
- Coronary artery bypass (CABG)
- Aortic valve replacement (AVR)
- Intermittent claudication
- Surgery for claudication
- Transitory ischemic attack (TIA)
- Stroke (ischemic or hemorrhagic)
- Hypertension
- Diabetes mellitus
- Atrial fibrillation
- Chronic obstructive lung disease (COPD)
- Cancer, categorized

5. Date of death with category of diagnosis (coronary heart disease, CV disease, infection, cancer, joint and connective tissue diseases, poisoning, trauma, and suicide)
6. Whether the patient died in hospital and if an autopsy was performed. Results of the autopsy was registered
7. Important remarks

We obtained a complete update of cause-specific deaths from Statistics Norway until 2008. Statistics Norway has a record of the cause of death in all Norwegians who have died with a delay in the completeness of 6 months. Morbidity and mortality data are complete up to December 31st, 2007, and none were lost to follow-up.

End points

All papers included in the present thesis involve combined mortality endpoints. We defined CV death as death from myocardial infarction, sudden cardiac death, stroke (ischemic or hemorrhagic), pulmonary embolism, or aortic disease. We defined all-cause death as death from any cause. We defined coronary heart disease (CHD) as angina pectoris, non-fatal myocardial infarction or death from CHD. Only the first event of a specific disease was counted.

Statistical analyses

We performed statistical analyses through the JMP® 9 statistical software (SAS Institute Inc., Cary, NC, USA) following a pre-specified data analysis plan for the main hypotheses and aims. Additional analyses specific for each study were performed to explore new hypotheses emerging during our work, or as requested by reviewers. We used Kendall`s rank tests to assess correlations (trends) between subgroups (i.e., tertiles, quartiles or other specified categories) of participants according to levels of baseline- or examination characteristics. We tested differences in data between groups by Student's t-test or Pearson's chi-square test in accordance with the type of data. We considered a two-tailed p-value < 0.05 as statistically significant. Survival was analyzed using Kaplan Meier plots and tested with log-rank tests. For all endpoints examined, we used Cox proportional hazard regression to calculate hazard ratios and test the statistical significance in prediction. The distribution of all variables used in survival analyses was checked, and no variables had skewed distribution. We entered significant variables in univariate analyses ($p<0.05$) into multivariate analyses, and our prediction models were mainly identified by stepwise backward elimination. We analyzed interactions between predictors by entering the cross-product of the two predictors studied for potential interaction into the Cox analyses in addition to the other variables in the final prediction model. Where interaction was found, we stratified men and made separate Cox analyses in for the different strata. Specific details about statistical analyses are given in the respective papers when relevant.

Permissions

The Norwegian Data Inspectorate has provided permission to store and analyze unidentifiable person data in the original study until 2042. The Norwegian Board of Health permitted to access the participants' health files until December 31st, 2008. Passive informed consent was obtained from all the participants alive in 2006.

Summary of the results

Paper I

Heart rate reserve predicts cardiovascular death among physically unfit but otherwise healthy middle-aged men; a 35-year follow-up study

In this paper, we tested the possible association between heart rate (HR) reserve and the risk of CV death among 2,014 healthy middle-aged men during 35 years of follow-up. Secondly, we tested a possible interaction between HR reserve and PF in CV risk estimation, and possible associations between HR reserve and CV death within subgroups of men according to their PF level. Finally, we tested possible associations between HR reserve, PF, and coronary heart disease (CHD). We measured HR reserve and PF at baseline and divided the men into tertiles by age-adjusted HR reserve before we analyzed interactions and risks of CV death and CHD in multivariable-adjusted Cox proportional hazard models.

Average age-adjusted HR reserve was 101 (±6) beats per minute (BPM), and high HR reserve was correlated with high PF and inversely correlated with smoking, time to CV death or CHD, and SBP. The crude incidence of CV death was 528 (26%) and CHD 765 (38%) during 60,420 person-years of follow-up. HR reserve was a significant predictor of CV death and CHD. PF was a significant predictor of CV death and CHD, but when we entered HR reserve into the same multivariate prediction model, PF was canceled out. We found significant statistical interactions between HR reserve and PF in prediction of CV death ($p = 0.0120$, $X^2 = 6.3$) and CHD ($p = 0.0054$, $X^2 = 7.8$). After stratifying the men by PF, HR reserve was a significant predictor of CV death (Table 1) and CHD in men with low PF, but not in men with intermediate or high PF.

Survival free from CV death and CHD decreased with decreasing HR reserve among the 2014 men and in the stratum of 677 men with lowest PF (Figure 5). There were no differences in survival between the HR reserve-groups in the strata of men with intermediate or high PF.

Figure 5.

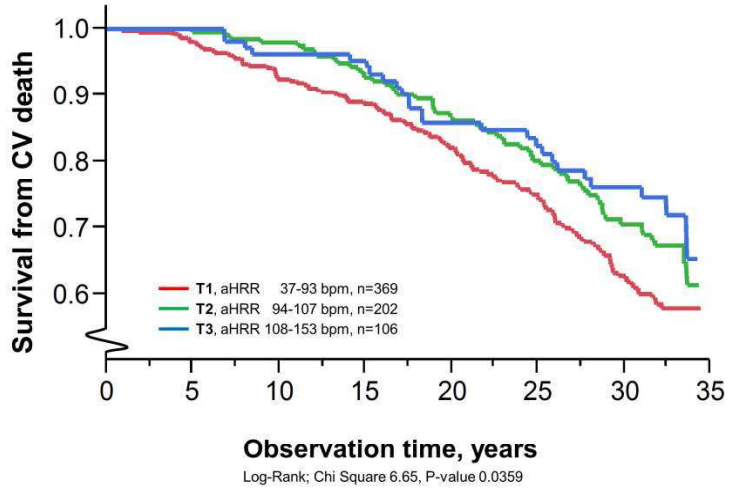

Figure 5. Survival free from cardiovascular death by HR reserve-groups among men with low PF. Kaplan-Meier curves showing survival (%) free from CV death in groups (T1-T3) of HRR among 677 healthy men with low age-adjusted PF.

Relative risks of CV death increased with decreasing HR reserve-tertiles among the 2014 men. In the lowest HR reserve-tertile, the risks were 41% (CV death) and 25% (CHD) higher than in the highest HR reserve-tertile after multivariable adjustment. Similar increases in risks of CV death and CHD, respectively, were shown among men with the lowest PF where risks in the low compared with high HR reserve-groups were 70% and 39% after multivariable adjustment. Among the men with intermediate or high PF, there were no trends towards differences in risks between the HR reserve-groups for CV death (Table 1) or CHD. We confirmed an independent association between low HR reserve and increased risks of CV death and CHD, but only among unfit men.

Table 1. Age-adjusted heart rate reserve as CV death predictor in men with a low, intermediate or high level of age-adjusted PF (n=2014)

Fitness level and adjustments	HR	95% CI	*P* Value	χ2
Low PF, range 0.19 to 1.11 kJ/kg, n = 677				
Age adjusted	0.77	0.68-0.89	0.0002	13.9
Multivariable	0.82	0.71-0.95	0.0006	11.7
Intermediate PF, range 1.12 to 1.37 kJ/kg, n = 672				
Age adjusted	0.85	0.72-1.01	0.0694	3.3
Multivariable	0.92	0.78-1.10	0.3427	0.9
High PF, range 1.68 to 5.71 kJ/kg, n = 665				
Age adjusted	0.96	0.78-1.18	0.6896	0.2
Multivariable	0.98	0.79-1.21	0.8585	0.0

Table 1. Hazard ratios (HR) per one standard deviation increase in each parameter. Possible predictors are measured at baseline. Heart rate reserve, maximal heart rate at exercise minus resting heart rate; physical fitness (PF), total work (kJ) divided by body weight (kg). CV Death, death from cardiovascular disease; multivariable analysis, adjusted for baseline age, smoking status, total cholesterol, family history of CHD, HR reserve, PF, and systolic blood pressure.

Paper II

Temporal Reduction in Chronotropic Index Predicts Risk of Cardiovascular death Among Healthy Middle-Aged Men: a 28-Year Follow-Up Study

In this study, we tested how temporal changes of the chronotropic index ([achieved maximal heart rate (HR) - resting HR]/[age-predicted maximal HR – resting HR]) after a symptom-limited bicycle ECG-test in 1420 healthy middle-aged men at two examinations seven years apart influenced the risk of cardiovascular (CV) death and all-cause death through up to 28 years of follow-up. Finally, we aimed to investigate whether there was an interaction between chronotropic index-changes and changes in PF. We measured PF, resting HR and maximal HR, and calculated age-predicted maximal HR according to Tanaka et al. [208-0.7 x age] (60), approximately seven years apart - at Survey 1 and Survey 2. From these variables, we calculated chronotropic indexes at both surveys, before we divided the men into quartiles by seven-year chronotropic index-changes and analyzed relative risks and interactions in multivariable Cox proportional hazard models.

Smoking cessation, Survey 1 and Survey 2 PF, seven-year change in PF, Survey 2 maximal HR and Survey 2 chronotropic index correlated with an increased chronotropic index over 7 years. Scatterplot analysis of the relationship between temporal chronotropic index-changes and PF-changes exhibited a correlation coefficient of 0.34 with outliers included (Figure 6).

We found 310 (21.8%) incidences of CV death and 740 (52.1%) incidences of all-cause death during follow-up, and incidences of death correlated inversely with chronotropic index-changes. Classical cardiovascular risk factors and chronotropic index-changes predicted CV and all-cause death in multivariable adjustment models, and chronotropic index-changes canceled out the effect of PF when entered into the same models. We found no statistical interactions between the chronotropic index and PF, or seven-year changes in these, in multivariable adjustment models, and hence, no stratification by PF was validated.

Figure 6.

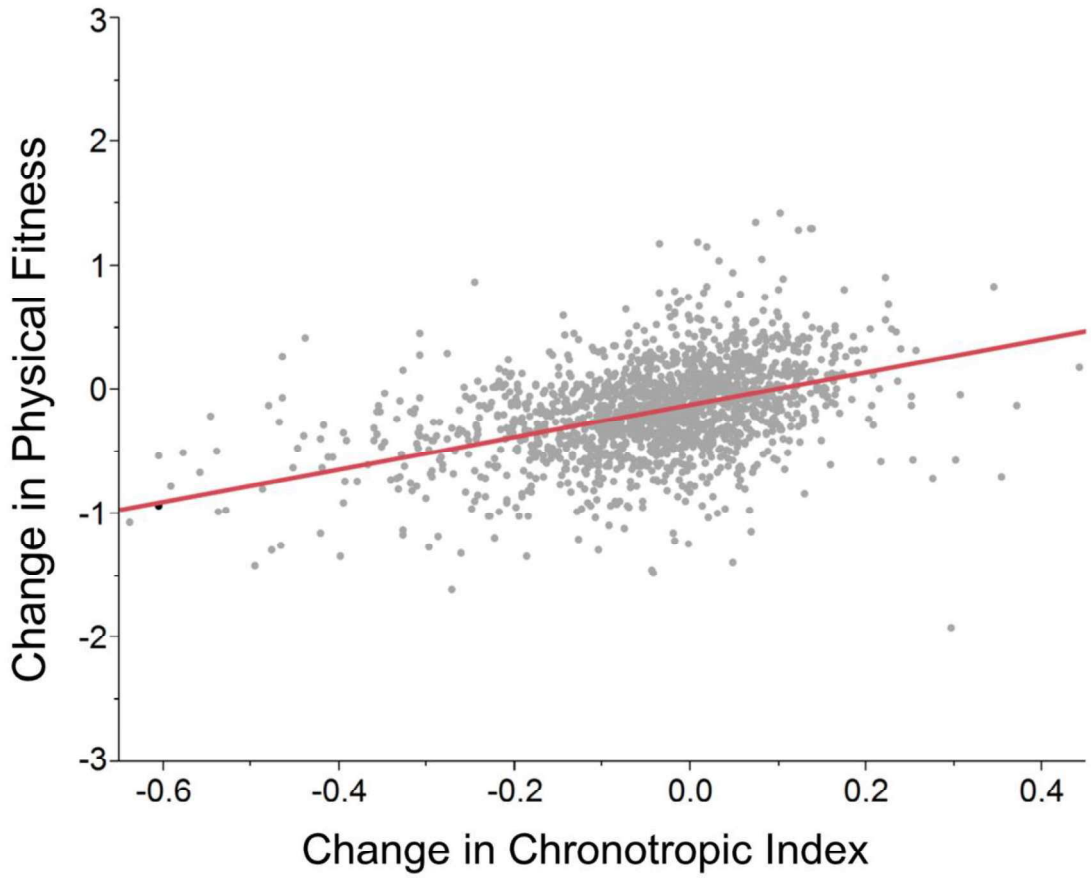

Figure 6. Scatterplot exhibiting the relationship between seven-year chronotropic index-changes (x-axis), and physical fitness-changes in kJ/kg (y-axis). Two outliers (X = 0.07, y = 4.16) and (x = -0.16, and y = 5.06) are not shown due to the scaling of the axes. Correlation coefficient, R = 0.34 (With outliers included)

When using the most negative change in chronotropic index-quartile, quartile one, as a reference, quartiles two, three and four were associated with lower risks of cardiovascular death (Table 2). When comparing the same quartiles, we found hazard ratios 0.78 (0.64-0.95), 0.67 (0.54-0.82 and 0.74 (0.61-0.91), respectively, for all-cause death. Hence, the results indicate a graded association between temporal chronotropic index-changes during mid-life and the risk of death during the next 28 years.

Table 2. Hazard ratios for CV death in quartiles according to seven-year change in chronotropic index, n = 1420

	Q1	**Q2**	**Q3**	**Q4**
N	355	355	355	355
Crude CV death, n (%)	102 (29)	73 (21)	65 (18)	70 (20)
Bivariable adjusted HR				
Q4 as reference	1.68 (1.24-2.30)	1.22 (0.88-1.71)	1.07 (0.76-1.50)	1
Q1 as reference	1	0.73 (0.54-0.98)	0.63 (0.46-0.87)	0.59 (0.44-0.81)
Multivariable adjusted HR				
Q4 as reference	1.50 (1.10-2.05)	1.10 (0.79-1.53)	1.04 (0.74-1.45)	1
Q1 as reference	1	0.73 (0.54-0.99)	0.69 (0.50-0.94)	0.67 (0.49-0.91)

Values are hazard ratios (HR) for CV death with 95 percent confidence intervals in parenthesis. Bivariable adjusted for baseline age and chronotropic index. Multivariable-adjusted for baseline values of the chronotropic index, physical fitness, age, systolic blood pressure, smoking status, total serum cholesterol, family history of coronary heart disease and smoking cessation between Survey 1 and Survey 2.

Paper III

Physical fitness is a modifiable predictor of early cardiovascular death: A 35-year follow-up study of 2014 healthy middle-aged men

In this study, we investigated the sustainability of middle-age physical fitness (PF) and other cardiovascular (CV) risk factor levels as predictors of cardiovascular (CV) death-risks during the early (0-11 years), intermediate (12-23 years) and late (24-35 years) parts of a 35-year observation period after Survey 1. Secondly, we investigated how temporal PF-changes during mid-life impacted risks of CV death during the early (0-9 years), intermediate (9-18 years) and late (18-28 years) parts of a 28-year observation period among 1420 men who remained apparently healthy at a second survey, approximately seven years after the first (Survey 2). Finally, we tested the sustainability of PF and classical CV risk factors as predictors of all-cause death risks during the early, intermediate and late parts of observation. We used multivariable-adjusted Cox proportional hazard models to calculate hazard ratios and stopped observation at 11, 23, and 35 years after Survey 1 and 9, 18 and 28 years after Survey 2, respectively, when investigating risks of early, intermediate and late risks. We excluded men with early events when investigating risks of intermediate events and excluded men with early and intermediate events when investigating risks of late events.

We found that PF at Survey 1 correlated inversely with cholesterol, systolic blood pressure (SBP), smoking, and body mass index (BMI). At Survey 2, we found that increased PF correlated with Survey 2 PF and Survey 1 SBP and correlated inversely with Survey 1 age, changes in age, Survey 1 PF, changes in cholesterol, changes in BMI, and both Survey 1 and Survey 2 percentages of smokers. There were 528 (26.2%) incidences of CV death and 1178 (58.4%) incidences of all-cause deaths during 35 years of follow up after Survey 1, and 311 (21.9%) incidences of CV death during 28 years of follow up after Survey 2. Time to CV death, the total incidence of CV death, incidences of early and intermediate CV death, time to all-cause death, the total incidence of all-cause death, and incidences of early, intermediate and late all-cause death, correlated inversely with increasing PF. Late CV death did not correlate with PF. Early, intermediate and late CV death did, however, correlate inversely with increased PF from Survey 1 to Survey 2.

We found that while PF predicted total CV death, PF was only a borderline significant predictor of early CV death, and did not predict intermediate or late CV death in multivariable analysis. Survey 1 to Survey 2 change in PF predicted CV death during all parts of observation, and PF predicted all-cause death during all periods of observation. Most classical CV risk factor remained strong throughout the whole observation period.

In multivariable models, relative risks of early and intermediate CV death increased with decreasing PF quartiles, but there were no late CV death-risk differences between the PF quartiles (Table 3). Relative risks of total, early, intermediate, and late all-cause death increased with decreasing PF-quartiles. Our findings suggest that PF is a modifiable CV death predictor with limited duration in contrast to the sustained impact of classical CV risk factors.

Table 3. Hazard ratios for CV death in quartiles by baseline physical fitness

80 events of CV death before 12 yrs. observation (n = 2014)				
	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Univariate	1.92 (1.50-2.48)	1.37 (1.07-1.79)	1.34 (1.03-1.74)	1.00
Multivariate	1.60 (1.23-2.08)	1.22 (0.94-1.59)	1.27 (0.97-1.65)	1.00
207 events of CV death between 12 and 23 yrs. of observation (n = 1934)				
	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Univariate	1.91(1.46-2.52)	1.53 (1.17-2.01)	1.37 (1.04-1.80)	1.00
Multivariate	1.50 (1.13-1.99)	1.32 (1.00-1.74)	1.27 (0.96-1.68)	1.00
241 events of CV death between 23 and 35 yrs. of observation (n = 1727)				
	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Univariate	1.86 (1.29-2.68)	1.59 (1.12-2.27)	1.29 (0.89-1.85)	1.00
Multivariate	1.43 (0.97-2.90)	1.36 (0.95-1.96)	1.18 (0.82-1.71)	1.00

Values are hazard ratios for cardiovascular (CV) death with 95 percent confidence intervals in parenthesis. Univariate adjusted for Survey 1 age. Multivariate adjusted for Survey 1 age, systolic blood pressure, smoking status, total serum cholesterol and family history of coronary heart disease.

Discussion of the results

Paper I Heart rate reserve

We confirmed that HR reserve was a significant long-term predictor of CV death after adjustment for classical CV risk factors. The new finding was a significant statistical interaction between HR reserve and PF in our multivariable CV and CHD risk assessment models, and after stratifying the participants by low, intermediate and high PF, HR reserve remained predictive the group of men with low PF only.

Several previous studies have investigated associations between heart rate increment during exercise and CV endpoints. In the 1970s, Ellestad et al. showed that the risk of adverse cardiac events associated with an abnormal HR response during exercise was higher than that associated with ST-segment depression (61). Sandvik et al. published data from 16 years of follow up in the Oslo Ischemia Study in 1995, concluding that low HR reserve was better than maximal HR for recognizing individuals at risk of dying prematurely from CVD (54). In contrast to this, a 1992-study of 4,907 healthy men by Filipovski et al., concluded that HR reserve did not contribute to CV risk prediction (62). In a paper from 2006, Chen et al. criticized both these studies for having a limited number of participants, a narrow age-span, and inclusion of participants with manifest chronotropic incompetence (maximal HR < 85% of age-predicted) and presented a study of 27,459 healthy men free of chronotropic incompetence, aged from 20 to 59 years. This study concluded that HR reserve was inversely associated with CV death (RR = 0.6 [0.5-0.9]) in men younger than 40 years, even with PF included in a full multivariable prediction model, whereas in men older than 40 years, the association was borderline significant (RR = 0.9 [0.7-1.0]) in the fully adjusted model (63). In the present study, 147 (7.3%) of the participants had chronotropic incompetence according to the definition used by Chen et al. (Maximal HR < 85% of [220-age]). An ad-hoc re-analysis after excluding these 147 participants weakened the results significantly, but we still found the trend that HR reserve had the most reliable predictive impact among the physically unfit, as presented in the published paper (data not shown).

The concept of using heart rate-derived parameters to assess the risk of future CVD among *apparently* healthy individuals presupposes that neither the test subject nor the investigator has noted CVD-related signs or symptoms before the assessment. Still, the rationale for excluding men with chronotropic incompetence from long-term CVD-risk analyses is disputable. It is well established that patients with advanced CHD and heart failure often have a high resting HR and an attenuated ability to increase HR during exercise, and that reduced HR response to activity predicts myocardial perfusion defects and incident CHD (64-68). Subsequently, chronotropic incompetence can be a consequence of severe CVD, a clinical sign supporting the need for more diagnostic tests or a

predictor of increased risk of future CVD and CV death. In many cases, the presence of chronotropic incompetence will be unknown before testing, and the reasons for performing exercise tests are varied, including fitness-measurements, symptom-warranted investigations or clinical research. Investigators must, therefore, interpret measurements of chronotropic response according to the reason for testing. In our participants, chronotropic competence was unknown before the trial, and none had known CVD or other significant diseases, and after excluding 91 participants evaluated as likely to have silent CHD after Survey 1 investigations, our results remained robust. Hence, we ruled out that preexisting severe CVD influenced our results and we also find it doubtful that subclinical CHD should account for a substantial amount of incident chronotropic incompetence among our participants, and consequently, we included all the measured chronotropic responses in the primary analyses of the present work.

Heart rate-control - the effects of the autonomic nervous system and age

The sinoatrial node initiates the electrical impulses that lead to myocardial contraction, and the intrinsic rate of depolarization of the sinoatrial node is 100 BPM. The complex, interacting vagal and sympathetic mechanisms of the autonomic nervous system control HR-response to exercise, and these mechanisms are vital for tightly matching cardiac output to metabolic demands during exertion (69). In simplistic terms, vagal tone suppresses HR at rest, and sympathetic tone increases HR on demand. Vagal withdrawal causes the initial increase from resting HR to approximately 100 BPM during dynamic exercise, whereas from 100 BPM to maximal HR, sympathetic stimuli dominate (70), and the vagal tone determines the rate and magnitude of HR drop immediately after exercise is terminated (71).

Resting HR is modifiable by endurance training, and a low resting HR is associated with a high level of PF. There is no change in resting HR with aging. However, experimental studies using both sympathetic and parasympathetic blockade, show that the intrinsic depolarization rate of the sinoatrial node declines by 5 BPM for each decade, and that increase in HR after parasympathetic blockade is less than half in an older person than that in the young, indicating a low parasympathetic activity in older people (52).

On the other hand, there is a marked age-related decrease in maximum heart rate in healthy humans that starts from early adulthood, and this is the primary cause for the inevitable age-related decline in peak aerobic exercise capacity (72). This decline is not modifiable by aerobic exercise, and as the levels of sympathetic mediators such as epinephrine and

norepinephrine are higher in the old than they are in the young, it seems unlikely that this decline in HR is related to inadequate sympathetic stimulation (72). The causes for this decline remain somewhat unclear although it is plausible that the intrinsic response of the heart to sympathetic stimuli diminishes as the myocardium ages.

HR reserve and other HR variables reflect the complex interactions of the autonomic nervous system and the heart. As mentioned above, an inadequate HR response to a given metabolic state can, hence, arise as a result of contributions from a preexisting autonomic-imbalance and inability of the heart to respond aptly to autonomic stimuli.

Resting and maximal heart rate

Resting HR was not independently associated with CV death in our material (Hazard ratio 1.13 per one standard deviation increment, 95% confidence interval 0.79-1.60 in an ad-hoc multivariable Cox-model adjusted for classical CV risk factors), yet a number of studies find that high resting HR predicts CVD and CV death, and there is broad agreement that resting HR is associated with all-cause mortality (73-76). Furthermore, there is a direct association between high resting HR and hypertension, hypercholesterolemia, a high daily number of cigarettes smoked, hyperglycemia, and an inverse association with activity level, PF and pulmonary function (62, 77-81), and we found many of these associations in the present study. Taking all these associations into account, it is plausible that HR is not independent, and will interact with classical CV risk factors in multivariable prediction models. As an example, we found a significant interaction between resting HR and current smoking, $X^2 = 4.1$, p = 0.044, in an ad-hoc cross-product test in a multivariable CV death-prediction Cox model with HR, and classical CV risk factors included.

Age-adjusted maximal HR was, however, independently associated with CV death in our material (Hazard ratio 0.43 per one standard deviation increment, 95% confidence interval 0.29-0.64 in an ad-hoc multivariable Cox-model adjusted for classical CV risk factors), and this is in line with findings in similar studies (74, 82). A survey by Savonen et al. concluded that a blunted HR response after 100 BPM, corresponding to HR between 40% – 100% of the maximal workload (HR 40-100%) predicted CV death, whereas the increment from resting HR to 40% of the total workload did not (83). The results of the same study indicate that HR 40-100% is a better CV predictor than HR reserve, and a similar observation was also noted in a separate study by Leeper et al. in 2007 (84). The authors postulate that the role of vagal withdrawal might be less influential than that of sympathetic stimulus-response in CVD prediction.

Contrary to this, results from other studies indicate that an abnormal vagal tone is associated with a proneness to develop atrial fibrillation, and in-turn an increased stroke risk, and even lethal arrhythmias (85-87). From our study-population, Grundvold et al. showed that low HR at a moderate workload was a long-term predictor of incident atrial fibrillation, particularly among the fit but hypertensive, suggesting a relationship between high vagal tone, high stroke volumes, and atrial fibrillation (86). In a 23-year follow-up study of 5713 asymptomatic men, Jouven et al. found that subjects with increased resting HR, low HR reserve or slow post-exercise HR decrement, had increased risk of sudden CHD death but not non-sudden CHD death (87).

This summary of relevant findings in the present study and other recent literature illustrates that several observational studies find a broad, and to some extent diverging, spectrum of associations between HR-derived predictors and CVD.

Interaction of heart rate and physical fitness in cardiovascular prediction

In epidemiology, the terms interaction effect and effect modification, or effect-measure modification are often used interchangeably (88). Effect modification refers to a situation where the effect of one predictor variable is dependent on the value of one or several other covariates. In multivariable regression, effect modification is integrated by adding the cross-product of two or more prediction variables along with their corresponding individual variables in the regression models (88). In a paper published in 2015, Vachteva et al. demonstrate how failing to identify interactive effects in regression models could lead to significant bias, misinterpretation of results, and even incorrect public health interventions (89).

We have previously shown that both PF and HR reserve are predictive of CV death but physiologically, the two are not independent of each other. During maximal aerobic exercise, oxygen uptake (VO^2), increases approximately 4-fold, and this increase in VO^2 is due to a 2.2-fold increase in HR; a 0.3-fold increase in stroke volume, and a 1.5-fold increase in arteriovenous oxygen difference (90). Consequently, the ability to increase HR is the most substantial contributor to the ability to increase VO^2, and an individual's PF is largely influenced by HR reserve. The significant role of HR reserve in VO^2-increment indicates interacting roles between HR reserve and PF in CV prediction models. By adding the cross-

product of HR reserve and PF in the multivariable regression models presented in Paper I, we found significant interactions between HR reserve and PF in prediction of CV death (p = 0.0120, X^2 = 6.3) and CHD (p = 0.0054, X^2 = 7.8).

Results of most, but not all, other studies support that HR variables and PF have interacting and competing roles in CV prediction models. Savonen et al. found that maximal VO^2 (VO^2-max), the gold-standard in PF-measurement, attenuates the impact of HR reserve and HR 40-100 (83). This finding that the effect of exercise HR parameters alters the effect of PF, and vice versa, when entered into the same multivariable prediction models, are found in several other studies (83, 91, 92). Another study by Savonen et al. showed, however, that the workload at 100 BPM (WL100) predicted CV death, and that VO^2-max did not influence this predictive ability among men with known or suspected CVD (93).

The finding of a statistical interaction between two predictor variables in a multivariable regression model strengthens the rationale to investigate the effect of one predictor variable in strata by levels of the other predictor variable (88). In the present work, the predictive ability of age-adjusted HR reserve was investigated in strata by age-adjusted PF-level, and we unveiled that HR reserve only had a predictive impact in the stratum with the lowest PF.

Clinical relevance

We have previously shown that added use of exercise-derived variables such as SBP at 100W workload, maximal heart rate, heart rate at 100 W workload, and PF increases the precision of CV risk models (49, 50, 54, 86, 94). Heart rate response to exercise and approximates of VO^2-max are available for self-assessment with commonly available HR and exercise monitoring equipment such as watches and smartphone applications, and the same variables are available from non-invasive clinical testing. We suggest that the combination of HR reserve and PF can be used in CV risk assessment.

The results of the present work add to the increasing amount of evidence that autonomic imbalance, observable through HR at rest as well as HR responses during and after exercise, is strongly associated with the risk of future cardiovascular outcomes and all-cause death (95, 96). This aggregate of findings provides a rationale for assessing chronotropic response at exercise testing to assist CV risk assessment. However, as aptly put in an overview article on chronotropic incompetence by Brubaker and Kitzman from 2011, innovative approaches for

assessing the significance of chronotropic response to exercise will require further validation before clinical application (52).

Paper II Change in chronotropic index

The novel finding was that temporal change in the chronotropic index is an independent predictor of CV death after adjustment for PF and classical risk-factors among 1420 apparently healthy men followed for more than 29 000 person-years. We confirmed that chronotropic index measured at the start of follow-up was a significant independent predictor of CV death but a previous measurement of the chronotropic index, 7 years earlier, had no prognostic impact on CV death. For all-cause death, however, both measurements of the chronotropic index had a prognostic impact. We found no statistical interaction between the chronotropic index and PF or change in the chronotropic index and change in PF.

In Paper II we investigated another exercise HR-derived parameter and its predictive abilities in on CV mortality. The variable is based on measured and predicted values of maximal HR and measured resting HR. Many of the perspectives on HR and PF discussed in the above sections on Paper I are equally related to Paper II and will not be discussed further in the following sections.

Age-predicted maximal heart rate

Vigorous aerobic exercise or physical activity level does not modify the normal age-related decline in maximal HR, and the sympathetic tone and catecholamine response to stress is higher in the elderly than in younger persons (52). Hence, the age-related decline in maximal HR is non-reversible and cannot be explained by autonomic dysfunction. The traditional formula for age-predicted maximal HR (220-age) was derived from studies of middle-aged men, included men with known CHD and on beta-blocking medication, and is associated with high inter-subject variability with a standard deviation of ±11 BPM (60, 97, 98). As a result of this inaccuracy in traditional age-predicted maximal HR calculation, Tanaka et al. published the results of a meta-analysis including 351 studies with a total of 18,712 men and women (age range 18 to 81 years) who were non-medicated and healthy, and in whom maximal exertion was objectively documented with VO^2-max measurements. As a result, the regression equation for age-predicted maximal HR was defined as (208 - 0.7 x age), and stepwise regression revealed that age alone explained approximately 80% of the inter-individual variance in maximal HR (60). No significant differences were seen between men and women or between those with high or low PF in a healthy population.

An ad hoc analysis of the age-related decline in maximal HR among or baseline population of 2014 healthy middle-aged men gives the formula of (214 – 1,02 x age) for age-predicted maximal HR. Our age-related decline in HR is between the traditional 220-age formula and the formula suggested by Tanaka et al. Possible explanations for this difference could be that our population had an age-span of 39-59 years, which is more like the selection of men used to derive the traditional 200 – age-formula, but like Tanaka et al. we had no subjects with CHD or taking beta-blockers.

In-line with current recommendations (52, 60), we calculated age-predicted HR by the Tanaka-formula of (208 - 0.7 x age) before Paper II statistical work.

Chronotropic incompetence and the chronotropic index

The definition of chronotropic incompetence is inconsistent in literature, and chronotropic incompetence has most commonly been diagnosed when HR fails to reach an arbitrary percentage of age-predicted maximal HR, by the 200 – age-formula (varying from 70% to 85%), during an incremental dynamic exercise test (67, 99, 100). The chronotropic index, defined as the change in HR from rest to peak exercise divided by the difference of the resting HR and age-predicted maximal HR, is also commonly used when assessing chronotropic incompetence and an index of ≤ 0.8 defines the diagnosis (101).

The level of effort at the point of test termination must be considered when evaluating maximal HR. Although subjective rating of perceived exertion is an acceptable method, a measurement of the respiratory exchange ratio (volume of carbon dioxide produced divided by the volume of oxygen consumed) in a direct VO^2-max-test is a more definite and objective was of assessing effort-level. The respiratory exertion ratio can be used in the Wilkoff equation to evaluate the metabolic-chronotropic relationship allowing a single HR achieved at any level of effort to be determined as consistent or inconsistent with a normal chronotropic function (102).

In the present work, the purpose of calculating the chronotropic index was not diagnostic, but the same considerations regarding effort-level at test termination must be taken into account, and it is plausible that a proportion of our maximal HR measurements are too low due to submaximal effort or non-cardiorespiratory limitations. From this viewpoint, exercise

testing with VO^2-max would be apter and, perhaps, add strength to the conclusions if the study had been repeated with modern technology not readily available in the 1970s.

Interestingly, the explicit *reason for test termination,* i.e., impaired breathing, lower limb fatigue, exhaustion, or safety reasons is found to be of prognostic value beyond that of suspected myocardial ischemia (103). In an Oslo Ischemia Study group-study by Bodegard et al., the authors found that apart from reaching a lower maximal HR, those who stopped the exercise test because of lower limb fatigue resembled the true exhaustion group in almost every respect, both regarding baseline and mortality findings (103). In contrast, those who terminated the test because of perceived breathing difficulties, when not part of exhaustion, had significantly higher mortality than those who terminated the test due to general exhaustion or leg fatigue (103). These findings could be considered confounding to our interpretation of maximal HR measurements. However, re-testing of 130 participants within two weeks of the initial examination showed high reproducibility for HR and working capacity, as described earlier. The findings in the present, and other studies relating to achieved maximal HR at test termination, are regardless of the above-mentioned concerns, highly significant, and, hence, of epidemiological interest, and could be of clinical value in preventive cardiology. After all, values of achieved maximal HR from simple testing or self-assessment are far more accessible than measurements from extensive cardiorespiratory laboratory testing.

Re-examination and temporal changes in predictor variables

Members of The Oslo Ischemia Study Group have been among the pioneers in examining the value of re-examination and temporal changes in predictor variables. In 1998, The Oslo Ischemia Study Group had a Lancet publication with the article "Changes in physical fitness and changes in mortality" (50), and this novel longitudinal approach has been applied to the present study as well as other studies from our study group during the last two decades (94, 104, 105). Although still not widely used, probably due to a relatively low number of studies with suitable data, the principle of re-examination and assessment of the prognostic value of the change in predictor variables has been used in other CV epidemiological studies recently (59, 106).

Our results find highly significant prognostic impact of temporal changes in some predictors of CHD, CV death and all-cause death. Hence, we argue that such analyses should be considered in epidemiological studies where data gives the opportunity. Co-variation of predictive variables might give a higher level of evidence than traditional point-measurements do. However, one must carefully consider inclusion of baseline levels, changes in confounders as well as the studied predictor and results of repeated measurements in the multivariable prediction models to avoid misadjusting the results. This is especially demanding where the co-variation of confounders might not be independent of each other. In the present work, the prediction models included both baseline level of the chronotropic index and change in the chronotropic index. Additionally, and as mentioned above, smoking interferes with HR, and thus smoking cessation between Survey 1 and Survey 2 was included in the final multivariable prediction models.

Clinical relevance

As well as the general addition to current evidence in support of adding HR-variables to the assessment of CV risk, the results of the present paper indicate that a temporal increase in the chronotropic index is associated with reduced long-term death risk and could encourage change to a healthier lifestyle with smoking cessation and increase in physical activity. These changes can influence the ability to reach maximal HR, decrease resting HR and modify classical risk factors such as BP and cholesterol (107, 108).

Paper III Physical fitness is a modifiable predictor of cardiovascular death

We have studied the predictive impacts of PF and change in physical fitness CV death risk before (49, 50) and PF and physical activity are well-established as CV and general risk predictors (47, 51, 53, 61, 82, 109-111). The novel findings were that PF measured at middle-age has a time-limited predictive power for CV death (approximately 20 years), but change in PF during middle age remains strong and significant as a predictor of CV death in a full lifespan perspective, and baseline PF was a significant predictor of all-cause death in a full lifetime perspective. The impact of temporal change in PF on CV death risk has also been investigated recently by the CERG study group in Trondheim, Norway, and the results are in-line findings in the present study (59).

Estimation of physical fitness

Measurement of VO^2-max would have been preferable when assessing PF and maximal HR in our participants. However, the equipment to perform such measurements was not easily obtainable at Survey 1 or Survey 2, and as discussed in detail in Paper III, we believe that our definition of PF as total work divided by body weight is the best possible measure given the data available in our study.

Physical fitness and classical cardiovascular risk factors

Aerobic exercise capacity, termed PF in the present thesis, reflects the accumulative ability of several coordinated organ-systems in the effort to sustain an increased output of power upon demand (112). PF is influenced by genetic factors (40%) and other factors (60%), and whereas the genetic causes of differences in PF are difficult to measure (113-115), effects of exercise on PF are more implicit (116). PF might be influenced by subclinical disease, lifestyle or both (117). In a New England Journal of Medicine-paper from 1995, Sandvik et al. demonstrated that smoking was associated with low PF and that smoking cessation was associated with increased PF during seven years of follow-up, among the men in our cohort (118). These findings are in line with numerous other studies (36, 45). Physical activity and high PF has beneficial effects on many factors that influence the atherosclerotic process and arterial stiffness, i.e., serum lipid levels, insulin sensitivity, glucose tolerance, platelet function, myocardial electrical stability, fibrinolysis, coronary artery dimensions and inflammation (2, 119).

The present study was commented in an editorial by Scherrenberg and Dale (120) where the authors highlight the novel finding of a time-limited protective impact of middle-age PF on

CV death risk and correlate the findings to a study that showed how physical activity level during adolescence did not influence cardiovascular risk factors at middle-age (121). Other studies, such as Lind et al. and the Honolulu Heart Program, showed that the impact of classical CV risk factors also generally decline with aging (122, 123). In the present study, impacts of baseline smoking status, SBP, and cholesterol on CV death risk weakened, but remained significant throughout 35 years of follow-up, whereas physical fitness and family history of CHD lost their predictive abilities as time progressed.

Clinical relevance

The results of the present study complement findings in other recent research and point out that repeated measurements of PF, and classical CV risk factors, are necessary to assess the lifelong cardiovascular risk in an individual fully.

Strengths and limitations

The present study is prospective in design, covers an extended follow-up, all data sets are complete with no participants lost to follow-up, and we have no work-up bias. All endpoint data are on the basis of complete cause-specific death records and standardized manual scrutiny of hospital records by physicians. The study investigators have not interfered with treatment in the case of subsequent disease, and all men were free of drug-treatment and healthy at inclusion. Changes in predictors studied are, hence, to a large degree likely to reflect lifestyle changes rather than interventions. Our exercise data were highly reproducible when verified by re-examination of approximately 5% of the participants within two weeks after Survey 1. PF, as defined in the present study, has been shown to be highly correlated with VO^2-max (124).

Our cohort was an all-male and middle-aged selection of healthy and full-time employed participants at inclusion, and hence, our results cannot be generalized to individuals of other ethnicities, ages or gender (125). Our findings cannot be applied in the setting of chronic medication or significant comorbidity as all our participants were healthy and non-medicated. Our results might be weakened by scatter in the data due to incident diseases and treatment activities outside our control during follow-up. When dividing the study population into groups and strata, and events into time-periods, some analyses may, despite up to 60.000 person-years of follow-up, suffer from low statistical power. Still, relative strengths between various risk factors should not be significantly affected by this. The men of the Oslo Ischemia were leaner and had a much larger percentage of smokers than a similar population would have today. There are also several other discrepancies in demographics and lifestyle in 2018 versus 1972 and one should consequently be careful projecting all associations into the current setting. All repeated measurements of biological variables might be influenced by regression to the mean, and this phenomenon cannot be ruled out in our data. Although hypotheses were formed before statistical work, the hypotheses were formed decades after the inclusion of the participants, and this might have influenced our findings.

Conclusions

Paper I

Low heart rate reserve was independently associated with increased risks of cardiovascular death and coronary heart disease in apparently healthy middle-aged men. The predictive impacts of heart rate reserve on cardiovascular death risk and risk coronary heart disease were, however, confined to unfit men. Our findings suggest that the use of heart rate reserve and physical fitness could improve cardiovascular risk prediction.

Paper II

A temporal reduction of the chronotropic index is independently associated with increased long-term risks of cardiovascular death and all-cause death in long-term follow up of apparently healthy middle-aged men. We also confirmed that point-measurement of the chronotropic index predicts cardiovascular death and all-cause death in our material. Our findings suggest that repeated measurements of the chronotropic index are of interest in cardiovascular death risk assessment.

Paper III

Physical fitness measured at a median age of 50 years was independently associated with risk of cardiovascular death before reaching the age of 73 years, but we found no association between physical fitness at middle-age and cardiovascular death among those who survived the first 23 years of observation. However, a change in physical fitness during middle-age was independently associated with the risk of cardiovascular death throughout life. Physical fitness and most classical cardiovascular risk factors were independently associated with risks of all-cause death during the whole observation period. Thus, our data suggest that physical fitness is a cardiovascular risk factor with limited duration in contrast to the sustained impact of blood pressure, smoking, and cholesterol on cardiovascular mortality, but a positive change in physical fitness during middle-age remains protective throughout life.

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I

II

ORIGINAL RESEARCH

Temporal Reduction in Chronotropic Index Predicts Risk of Cardiovascular Death Among Healthy Middle-Aged Men: a 28-Year Follow-Up Study

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Background- Chronotropic index is a standardized measure of heart rate (HR) increment during exercise that reflects the combined effects of age, resting HR, and physical fitness. Low chronotropic index has been reported to predict disease and death. We tested whether temporal change in chronotropic index over 7 years influenced risk of cardiovascular death through up to 28 years.

Methods and Results- Chronotropic index was calculated ([achieved maximal HR−resting HR]/[age-predicted maximal HR−resting HR]) after a symptom-limited bicycle ECG exercise test in 1420 healthy men at 2 examinations 7 years apart, in 1972 and 1979. Events of cardiovascular death were registered by manual scrutiny of all participants' hospital charts and the Norwegian Cause of Death Registry. The participants were divided into quartiles of temporal change in chronotropic index, with quartile one having the most negative value. Cox proportional hazard regression models were used to estimate risks and adjusted for classical cardiovascular risk factors. Incidence of cardiovascular death was 310 (22%) during median of 21 years of follow-up. After multivariable adjustment, and comparison with quartile four (mean +0.11), quartiles one (−0.16), two (−0.04), and three (+0.02) were associated with hazard ratios 1.50 (95% CI 1.10 2.05), 1.10 (0.79 1.53), and 1.04 (0.74 1.45) for cardiovascular death. Results remained robust also after exclusion of 31 participants with exercise ECG-induced signs of coronary ischemia.

Conclusions- Temporal reduction in chronotropic index was associated with increased long-term risk of cardiovascular death and might be a clinically important predictor when assessing risk in healthy individuals over a longer time. **(*J Am Heart Assoc*. 2016;5: e004555 doi: 10.1161/JAHA.116.004555)**

Key Words: all-cause death prediction • cardiovascular outcomes • chronotropic index • exercise testing • heart rate • physical exercise • risk prediction

Cardiovascular diseases remain leading causes of severe morbidity and death worldwide.[1] In preventive cardiology, improved cardiovascular prediction is important in order to choose appropriate risk-modifying strategies. Risk predictors derived from exercise testing have gained interest and are now important complements to classical risk factors, such as smoking, blood pressure, and cholesterol.[2–5] One established exercise-derived cardiovascular predictor is the chronotropic index ([achieved maximal heart rate−resting heart rate]/[age-predicted maximal heart rate−resting heart rate]), which is a standardized measure of heart rate (HR) change during exercise that reflects the combined effects of age, resting HR, and physical fitness (PF).[6] Measured HR increment during exercise, which is incorporated in the chronotropic index formula, is shown to be associated with cardiovascular death[6–8] and its predictive ability is influenced by PF.[9] Temporal changes in PF-related or exercise-derived parameters such as resting HR and exercise systolic blood pressure have been reported to predict cardiovascular disease and death.[5,10] Despite inevitable individual changes in resting HR, maximal HR, and PF over time,[11–13] the prognostic impact of temporal change in chronotropic index on cardiovascular death risk has not been studied before.

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Accompanying Tables S1 through S4 are available at http://jaha.ahajournals.org/content/5/12/e004555/DC1/embed/inline-supplementary-material-1.pdf

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Received August 23, 2016; accepted October 24, 2016.

The main aim of the present work was to study the possible association between temporal change in chronotropic index over 7 years and risk of future cardiovascular death among 1420 apparently healthy middle-aged men. Second, we tested the possible association between temporal change in chronotropic index and risk of all-cause death. Finally, we aimed to investigate whether there was an interaction between change in chronotropic index and PF that validated investigation of associations between chronotropic index and end points within subgroups of men according to their PF level.

Methods

Study Population

The present study included a population of 1420 men in the Oslo Ischemia Study who fulfilled our criteria for studying the possible prognostic impact of change in chronotropic index on cardiovascular death and all-cause death (Figure 1). The Oslo Ischemia Study consists of 2014 apparently healthy men aged 40 to 59 years recruited from 5 governmental institutions in Oslo during the years 1972 1975.[14] All men gave their informed consent before inclusion. Further details about selection procedures and exclusion criteria have been presented elsewhere.[14–16] All participants underwent standardized clinical examinations, blood tests, chest radiograph, resting ECG, and symptom-limited bicycle exercise ECG tests at inclusion (Survey 1, 1972 1975) and identical examination in the years 1979 1982 (Survey 2). Family history of coronary heart disease, including angina pectoris, nonfatal/fatal myocardial infarction among parents or siblings, was registered in questionnaires. To be included in the present study, the men had to be healthy at both Survey 1 and Survey 2. The study was approved by the regional committee for medical and health research ethics (REK).

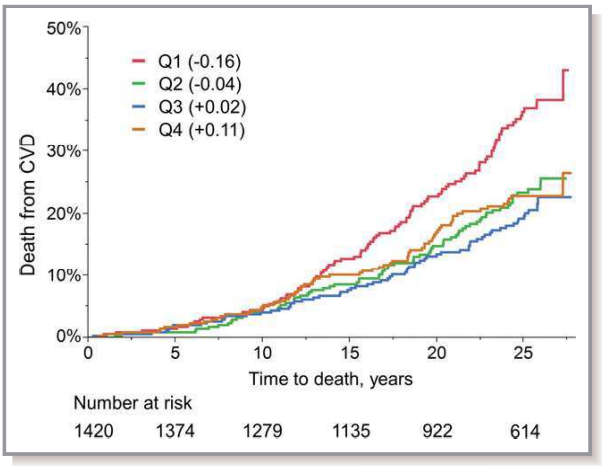

Figure 1. Cardiovascular death in quartiles of men according to 7-year change in chronotropic index. Kaplan Meier plot exhibiting death from cardiovascular disease in percent (*y*-axis) during 28 years of follow-up (*x*-axis) in quartiles (Q1 Q4) by 7-year change in chronotropic index among 1420 healthy middle-aged men. CVD, cardiovascular disease.

Examinations

Resting HR was counted manually during 60 s measured with a stopwatch after a standardized period of supine rest. All participants performed a standardized bicycle exercise ECG test and were examined by the same physician (J.E.) at both surveys. The initial workload was 6 minutes at 100 W, increased by 50 W every 6 minutes. The exercise tests were continued until a HR of at least 90% of the maximal predicted HR was reached unless specific symptoms or signs necessitated a premature termination.[17] If an individual seemed physically fit despite his reaching 90% of maximal predicted HR +10 bpm at the end of 1 load, he was encouraged to continue as long as possible on the next load, ie, maximally for an additional 6 minutes on a higher load.[6] Exercise testing was repeated within 2 weeks in 130 of the participants and showed high reproducibility for HRs and working capacity between the 2 tests, within ±5% in 90% of the men, and within ±10% in all of them. HR was measured every second minute throughout the test. Peak exercise HR was recorded from ECG just before termination of the test. Chronotropic index was calculated ([achieved maximal HR−resting HR]/[age-predicted maximal HR−resting HR]). Age-predicted maximal HR was calculated according to Tanaka et al (208−0.7 age).[18] PF was defined as the total bicycle exercise work (Joules), calculated as the sum of work at all workloads divided by body weight (kg). Further details about HR and PF measurements have also been presented previously.[15,16]

Follow-Up

Morbidity and mortality data were consecutively obtained from 2 clinical surveys in 1989 1990 (Survey 3), and 1994 1995 (Survey 4), 1 questionnaire survey in 1987, and 2 nationwide searches of patient records from all Norwegian hospitals in 1995 1996 and in 2005 2008 with permission from relevant authorities. Mortality data were obtained from the Norwegian Cause of Death Registry and validated through scrutiny of medical records. All morbidity and mortality data are complete up to January 1, 2007, and none of the participants were lost to follow-up.

End Points

The main end point in the present study was cardiovascular death, consisting of fatal myocardial infarction, sudden

cardiac death, fatal stroke (cerebral infarction or hemorrhage), and death from pulmonary embolism or aortic disease. The secondary end point was death from any cause.

Statistical Methods

All statistical calculations were performed using SAS JMP 9 software. Kendall rank tests were used to assess correlation (trend) between 7-year change in chronotropic index quartiles and clinical characteristics of participants. The risks of end points in change in chronotropic index-quartiles were estimated by Kaplan Meier plots and tested with log-rank tests. Cox proportional-hazard modeling was used when calculating hazard ratios and observation time started at Survey 2. Significant variables in univariate analyses ($P<0.05$) were entered into multivariable analysis, and a final prediction model was reached by stepwise backward variable selection. We chose to keep chronotropic index at Survey 1 in all adjustment models because it forms the baseline level for change in chronotropic index. Hazard ratios for end points were then examined after bivariable adjustment for baseline chronotropic index and age; and then multivariable adjustment for baseline chronotropic index, age, smoking status, total cholesterol, resting systolic blood pressure and PF, as well as smoking cessation between Survey 1 and Survey 2 and family history of coronary heart disease. Statistical interactions between change in chronotropic index and change in PF were tested by adding the interaction term of these variables in the regression models. The same adjustment models and interaction analyses were used for both end points to obtain comparable results. Models were also tested for both endpoints after exclusion of 31 men who exhibited ischemia during the exercise test by developing ST-depressions of more than 2 mm or chest pain.[2,19] The same change in chronotropic index quartile-limits from the total material was also used for the group of 1389 men with no signs of ischemia at Survey 2 exercise test. Sensitivity analyses of other potential predictors of cardiovascular death and all-cause death were also performed (see Results section).

Results

Characteristics of Participants

At Survey 1, the 1420 men were on average 49.3 years, had a body mass index of 24.4, and 567 (40%) were current smokers. Mean resting HR was 61 beats per minute (BPM), and mean maximal HR was 165. Mean resting HR increased with 2 beats per minute, and mean maximal HR decreased with 7 beats per minute from Survey 1 to Survey 2. Smoking cessation, Survey 1 and 2 PF, 7-year change in PF, Survey 2 maximal HR, and Survey 2 chronotropic index were correlated with increasing chronotropic index. The relationship between temporal changes in chronotropic index and PF was further studied with a scatterplot analysis exhibiting a correlation coefficient of 0.34 (Figure 2). Survey 1 percentage of smokers, serum cholesterol, and chronotropic index were inversely correlated with increasing chronotropic index. There was no correlation between body mass index or change in body mass index and increasing chronotropic index (Table 1).

End Points

Crude incidence of cardiovascular death among the 1420 men was 310 (21.8%) during up to 27.7 years (median 20.8) comprising 29 536 person-years of follow-up after Survey 2. Crude incidence of all-cause death was 740 (52.1%). The incidences of cardiovascular death and all-cause death were inversely correlated with change in chronotropic index (Table 1).

Hazard Ratios for Death

Change in chronotropic index and classical cardiovascular risk factors were significant and independent predictors of cardiovascular death and all-cause death after multivariable adjustment (Tables 2 and S1). Crude risks of cardiovascular death increased with negative changes in chronotropic index-quartiles as shown in Figure 1. After multivariable adjustment and comparison with quartile four (mean +0.11), quartile one (−0.16) was associated with hazard ratio 1.50 (95% CI 1.10

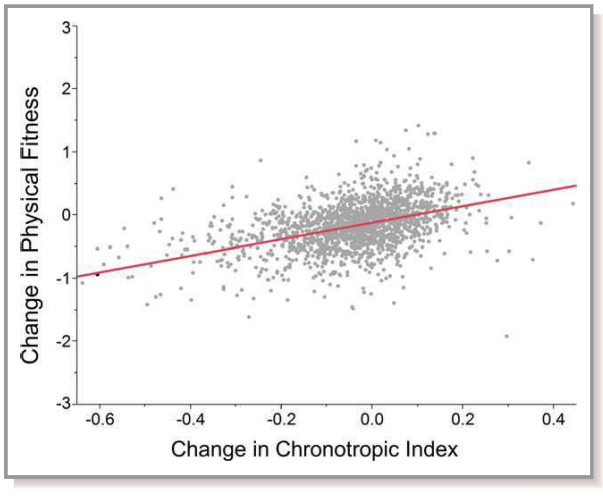

Figure 2. Relationship between temporal changes in chronotropic index and physical fitness. Scatterplot exhibiting the relationship between temporal change in chronotropic index (*x*-axis) and physical fitness in kJ/kg (*y*-axis). Two outliers ($x=0.07$, $y=4.16$) and ($x=-0.16$, $y=-5.06$) are not shown due to the scaling of the axes. Correlation coefficient, $R=0.34$.

Table 1. Characteristics of Participants in Quartiles According to 7-Year Change in Chronotropic Index

	Q1	Q2	Q3	Q4	*P* Value
N	355	355	355	355	Kendall
Age, y	50.2 (5.4)	48.6 (5.3)	48.6 (5.2)	49.7 (5.4)	0.3177
BMI, kg/cm^2	24.5 (2.4)	24.3 (2.7)	24.4 (2.7)	24.2 (5.4)	0.1834
ΔBMI, kg/cm^2	0.25 (1.49)	0.33 (1.23)	0.17 (1.23)	0.26 (1.21)	0.6838
Serum cholesterol, mmol/L	6.7 (1.3)	6.6 (1.2)	6.5 (1.1)	6.6 (1.2)	0.0254
Systolic blood pressure, mm Hg	128 (16)	128 (16)	126 (16)	128 (16)	0.2653
Resting heart rate 1, BPM	62 (11)	61 (9)	60 (9)	62 (9)	0.7157
Maximal heart rate 1, BPM	164 (13)	166 (13)	166 (13)	162 (14)	0.0314
Resting heart rate 2, BPM	63 (10)	63 (10)	61 (9)	65 (11)	0.0924
Maximal heart rate 2, BPM	144 (14)	157 (12)	162 (12)	168 (12)	<0.0001
Physical fitness 1, kJ/kg	1.45 (0.60)	1.60 (0.60)	1.61 (0.56)	1.46 (0.56)	0.0076
Physical fitness 2, kJ/kg	1.10 (0.55)	1.43 (0.60)	1.52 (0.57)	1.50 (0.65)	<0.0001
ΔPhysical fitness, kJ/kg	−0.35 (0.42)	−0.17 (0.33)	−0.09 (0.34)	0.04 (0.44)	<0.0001
Smoking, n (%)	171 (48)	133 (37)	135 (38)	128 (36)	0.0024
Smoking cessation, n (%)	38 (10)	33 (9)	49 (13)	51 (14)	0.0034
Family history CHD, n (%)	62 (18)	82 (24)	67 (19)	80 (23)	0.5033
Cardiovascular death, n (%)	102 (29)	73 (21)	65 (18)	70 (20)	<0.0001
All-cause death, n (%)	236 (66)	177 (50)	149 (42)	178 (50)	<0.0001
Chronotropic index 1	0.93 (0.10)	0.93 (0.10)	0.93 (0.10)	0.90 (0.11)	0.0067
Chronotropic index 2	0.77 (0.12)	0.89 (0.10)	0.94 (0.10)	1.00 (0.11)	<0.0001
ΔChronotropic index (mean)	−0.16	−0.04	0.02	0.11	<0.0001
ΔChronotropic index (range)	−0.60 to −0.08	−0.08 to −0.01	−0.01 to 0.05	0.05 to 0.44	

Values are mean with SD in parentheses or n, number, with percent in parentheses of characteristics of men in quartiles according to 7-year change in chronotropic index. Δ represents 7-year change of the denoted parameter. BMI indicates body mass index; BPM, beats per minute; CHD, coronary heart disease.

2.05) for cardiovascular death. When using the most negative change in chronotropic index-quartile, quartile one, as reference, and quartiles two, three, and four were all associated with hazard ratios 0.73 (0.54 0.99), 0.69 (0.50 0.94), and 0.67 (0.49 0.91) for cardiovascular death (Table 3).

Crude risks of all-cause death increased with more negative temporal changes in chronotropic index-quartiles. After multivariable adjustment and comparison with quartile four, quartile one was associated with hazard ratio 1.35 (1.10 1.64) for all-cause death. When using the most negative change in chronotropic index-quartile, quartile one, as reference, quartiles two, three, and four were associated with hazard ratios 0.78 (0.64 0.95), 0.67 (0.54 0.82), and 0.74 (0.61 0.91) for all-cause death (Table S2).

Sensitivity Analyses and Statistical Interaction

In the subgroup of 1389 men with no detectable ischemia at Survey 2 exercise ECG tests, the incidence of cardiovascular death and all-cause death were 295 (21.2%) and 715 (51.5%), respectively. The most negative change in chronotropic index-category (mean −0.16) was associated with 41% and 34% increased multivariable adjusted risk of cardiovascular death and all-cause death, respectively, compared with the largest change in chronotropic index-category (mean +0.11) (Table S3).

Several other possible predictors of cardiovascular death, including systolic blood pressure at 100 W workload, fasting blood glucose, triglycerides, radiograph-measured relative heart volume, forced expiratory volume at 1 s, hemoglobin level, resting HR, maximal HR, body mass index, and temporal change in body mass index, were introduced into a complete multivariable analysis model to evaluate potential impact on cardiovascular death prediction. However, none of these variables had significant impact on the prediction model (Table S4).

We found no statistical interaction between chronotropic index, temporal change in chronotropic index and PF, or temporal change in PF (data not shown) and hence, no stratification by PF level was validated.

Table 2. Impact of Predictors of Cardiovascular Death

	Univariable HR	Multivariable HR	χ^2	*P* Value
Age	2.09 (1.86 2.35)	2.37 (1.60 3.57)	19.9	<0.0001
Systolic blood pressure	1.35 (1.20 1.51)	1.31 (1.16 1.48)	18.7	<0.0001
Smoker, y/n	1.42 (1.13 1.77)	1.56 (1.20 2.03)	10.9	0.0010
Cholesterol	1.26 (1.13 1.39)	1.19 (1.07 1.33)	9.8	0.0018
Δ Chronotropic index	0.80 (0.70 0.91)	0.83 (0.72 0.95)	7.0	0.0080
Family history CHD, y/n	1.47 (1.13 1.89)	1.42 (1.09 1.83)	6.5	0.0108
Smoking cessation	0.62 (0.41 0.89)	0.70 (0.47 1.02)	3.4	0.0659
Body mass index	1.16 (1.03 1.30)	1.08 (0.96 1.23)	1.7	0.1928
Chronotropic index	0.90 (0.80 1.02)	0.47 (0.12 1.71)	1.3	0.2549
Maximal heart rate	0.90 (0.80 1.03)	2.08 (0.51 9.34)	1.0	0.3164
Resting heart rate	1.03 (0.91 1.15)	0.94 (0.81 1.09)	0.6	0.4412
Physical fitness	0.87 (0.76 0.99)	1.00 (0.86 1.16)	<0.1	0.9670

Values are hazard ratios (HR) of 1 SD increase in baseline value for continuous variables, and HR for yes vs no for baseline status of nominal variables, 95% CI in parentheses. Ranked by χ^2 in multivariate model with all possible predictors included. BMI indicates body mass index; CHD, coronary heart disease.

Discussion

We investigated a possible association between temporal changes in chronotropic index, measured by repeated symptom-limited bicycle exercise ECG tests, and long-term (up to 28 years) risks of cardiovascular death and all-cause death among 1420 apparently healthy middle-aged men followed for more than 29 000 person-years. We confirmed that chronotropic index measured immediately before the start of follow-up (at Survey 2) was a significant long-term predictor of cardiovascular death after adjustment for PF and classical cardiovascular risk factors, whereas baseline measurements of chronotropic index (at Survey 1) had no prognostic value for cardiovascular death. For all-cause death, however, both present and previous chronotropic index measurements are of prognostic value using the same adjustment models. The new finding was that temporal change in chronotropic index was an independent long-term predictor of cardiovascular death after adjustment for PF and classical cardiovascular risk factors. Similar findings were detected when separately assessing all-cause death risks. The results remain robust after exclusion of a limited number of participants with signs of myocardial ischemia on exercise ECG tests.

Potential Pathophysiological Mechanisms

Baseline values of chronotropic index and PF were correlated inversely with change in chronotropic index, and change in PF increased with increasing change in chronotropic index. Smoking elevates resting HR, slows HR increase during

Table 3. Hazard Ratios for Cardiovascular Death in Quartiles According to 7-Year Change in Chronotropic Index, n=1420

	Q1	Q2	Q3	Q4
N	355	355	355	355
Crude cardiovascular death, n (%)	102 (29)	73 (21)	65 (18)	70 (20)
Bivariable adjusted HR				
Q4 as reference	1.68 (1.24 2.30)	1.22 (0.88 1.71)	1.07 (0.76 1.50)	1
Q1 as reference	1	0.73 (0.54 0.98)	0.63 (0.46 0.87)	0.59 (0.44 0.81)
Multivariable adjusted HR				
Q4 as reference	1.50 (1.10 2.05)	1.10 (0.79 1.53)	1.04 (0.74 1.45)	1
Q1 as reference	1	0.73 (0.54 0.99)	0.69 (0.50 0.94)	0.67 (0.49 0.91)

Values are hazard ratios for cardiovascular death with 95% CI in parentheses. Bivariable adjusted for baseline age and chronotropic index. Multivariable adjusted for baseline values of chronotropic index, physical fitness, age, systolic blood pressure, smoking status, and total serum cholesterol as well as family history of coronary heart disease and smoking cessation between Survey 1 and Survey 2.

exercise, and impairs the ability to reach age-predicted maximal HR,[20,21] and physical exercise lowers resting HR.[11] However, resting HR at Survey 1 and 2 were not different among the change in chronotropic index-quartiles, whereas maximal HR at both Survey 1 and 2 were. Smoking cessation was most frequent in the highest change in chronotropic index-quartile. Adjustment for smoking cessation did not alter the results and other lifestyle changes, such as increased physical exercise, may also contribute. Maximal HR, which is not modifiable by endurance training, and is highly age dependent and genetically determined,[6] was inversely correlated with chronotropic index at Survey 1 and correlated with Survey 2 chronotropic index and change in chronotropic index. Subclinical cardiovascular disease can cause exercise intolerance and failure to reach maximal HR. Still, excluding 31 men who exhibited signs of coronary ischemia at the Survey 2 exercise test only weakened the results marginally.

We previously showed that HR reserve (the difference between maximal HR and resting HR) predicts cardiovascular death and showed that HR reserve and PF interact on cardiovascular risk prediction. After stratifying the same study population included in the present study by low, intermediate, or high PF, the predictive abilities of HR reserve were confined to the group of men with low physical fitness.[9] We found no statistical interaction between chronotropic index or temporal change in chronotropic index and PF and hence, no stratification by PF level was validated. Chronotropic index is, however, a standardized measure of HR increment during exercise that reflects the combined effects of age, resting HR, and physical fitness. Similar to HR reserve, the chronotropic index reflects the complex interaction between the autonomic nervous system and the cardiovascular system during exercise. We have discussed this relationship in more detail elsewhere.[9]

Clinical Relevance

Our past and previous results add to the growing amount of evidence that autonomic imbalance, as revealed by measurement of resting HR, maximal HR during exercise, HR reserve, or chronotropic index during exercise, is an important death risk predictor.[3,6,13,22–24] Resting and maximal HR are easily measured during exercise testing and allows self-assessment during rest and endurance training using commonly available HR monitoring equipment such as watches or smart-phone applications. Chronotropic index can then be calculated by a simple formula and results can be logged to assess temporal changes. Our results suggest that a reduction in chronotropic index as time progresses is associated with increased death risk.

Equally interesting, the results also indicate that a temporal increase in chronotropic index is associated with reduced long-term death risk. This finding encourages change to a healthier lifestyle with cessation of smoking and start of regular physical exercise. Such changes can, in the absence of relevant morbidity, influence the ability to reach maximal HR, lower resting HR, and modify classical cardiovascular risk factors such as blood pressure and cholesterol level.[20,21] It is possible that use of temporal change in chronotropic index and other markers of autonomic imbalance could improve the accuracy of cardiovascular death risk prediction, for example, by reclassifying individuals for medium to low or high cardiovascular death risk.[3,6,7,9,22]

Strengths of the Study

The present study is prospective in design, and all data sets are complete with none lost to follow-up. We have no work-up bias, and all event data are on the basis of complete hospital records and cause-specific death records. All men were healthy and free of drugs, and the study group has not interfered with treatment in case of disease. The reproducibility of our exercise data was verified by re-examination of 130 participants within 2 weeks. PF, as defined in the present study (total exercise capacity divided by body weight), has been shown to be highly correlated with maximum oxygen uptake, which is the most accepted measure of PF.[25,26]

Limitations

Predicted maximal HR may underestimate true maximal HR in middle-aged and older persons.[27] We cannot rule out that this and intravariability in exercise capacity and response in some individuals could have influenced the results. Our cohort consists of middle-aged white men who were healthy and employed full-time at inclusion, and our findings cannot necessarily be generalized to individuals of other ages, ethnicities, or sex. Only those who stayed healthy and free of medical treatment between Survey 1 and Survey 2 were included in our study. As a result of this, our findings cannot be applied in the setting of significant comorbidities and/or chronic drug use.

Conclusions

We have shown that a temporal reduction in chronotropic index is associated with increased long-term risk of cardiovascular death. Chronotropic index is derived from a normal exercise test, and our data suggest that repeated measurements might become a clinically important tool when assessing cardiovascular preventive strategies in healthy individuals followed in the routine clinical practice.

Sources of Funding

This work was supported by a 3-year PhD scholarship from the South Eastern Norway Health Authority.

Disclosures

Dr Kjeldsen received honoraria from Bayer, MSD, and Takeda. Dr Bodegard holds a full-time position as epidemiologist with AstraZeneca. The other authors have no conflicts of interest.

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SUPPLEMENTAL MATERIAL

Table S1. Impact of predictors of all-cause death

	Univariable HR	Multivariable HR	X2	P
Smoker, y/n	1.68 (1.47-1.94)	1.72 (1.46-2.03)	38.6	<0.0001
Age	2.03 (1.88-2.19)	1.89 (1.47-2.43)	27.6	<0.0001
Δ Chronotropic index	0.79 (0.72-0.86)	0.81 (0.75-0.89)	19.7	<0.0001
Systolic blood pressure	1.18 (1.09-1.27)	1.19 (1.09-1.29)	16.3	<0.0001
Smoking cessation	0.61 (0.48-0.77)	0.69 (0.54-0.87)	9.61	0.0019
Physical fitness	0.80 (0.73-0.87)	0.89 (0.81-0.99)	4.8	0.0287
Cholesterol	1.15 (1.07-1.23)	1.08 (1.01-1.17)	4.6	0.0314
Family history CHD, y/n	1.14 (0.96-1.36)	1.15 (0.96-1.37)	2.2	0.1392
Resting heart rate	0.98 (0.91-1.06)	0.95 (0.86-1.05)	0.9	0.3380
Chronotropic index	0.79 (0.72-0.86)	0.86 (0.37-1.95)	0.1	0.7263
Maximal heart rate	0.84 (0.77-0.91)	1.04 (0.43-2.62)	<0.1	0.9410
Body mass index	1.04 (1.95-1.12)	0.99 (0.92-1.07)	<0.1	0.8474

Values are hazard ratios (HR) for all-cause death of one standard deviation increase in baseline value for continuous variables, and HR for yes vs. no for baseline status of nominal variables, 95% confidence interval in parenthesis. Ranked by chi square in multivariate model with all listed possible predictors included. CHD: Coronary heart disease.

Table S2. Hazard ratios for all-cause death in quartiles according to 7-year change in chronotropic index, n = 1420

	Q1	Q2	Q3	Q4
N	355	355	355	355
All-cause death, n (%)	236 (66%)	177 (50%)	149 (42%)	178 (50%)
Bivariable adjusted HR				
Q4 as reference	1.59 (1.30-1.93)	1.19 (0.96-1.47)	0.97 (0.77-1.20)	1
Q1 as reference	1	0.75 (0.62-0.91)	0.61 (0.50.0.75)	0.63 (0.51-0.77)
Multivariable adjusted HR				
Q4 as reference	1.35 (1.10-1.64)	1.05 (0.85-1.30)	0.90 (0.72-1.12)	1
Q1 as reference	1	0.78 (0.64-0.95)	0.67 (0.54-0.82)	0.74 (0.61-0.91)

Values are hazard ratios for CV death with 95 per cent confidence intervals in parenthesis. Bivariable adjusted for baseline age and chronotropic index. Multivariable adjusted for baseline values of chronotropic index, physical fitness, age, systolic blood pressure, smoking status and total serum cholesterol as well as family history of coronary heart and smoking cessation between Survey 1 and Survey 2.

Table S3. Hazard ratios for death in quartiles according to 7-year change in chronotropic index in men with no detectable ischemia at Survey 2, n = 1389

End points	Q1	Q2	Q3	Q4
CV death	1.41 (1.02-1.93)	1.03 (0.74-1.45)	1.01 (0.72-1.43)	1
All-cause death	1.34 (1.09-1.64)	1.08 (0.87-1.34)	0.92 (0.74-1.15)	1

Values are hazard ratios for death with 95 per cent confidence intervals in parenthesis after multivariable adjustment for baseline values of chronotropic index, physical fitness, age, systolic blood pressure, smoking status and total serum cholesterol as well as family history of coronary heart and smoking cessation between Survey 1 and Survey 2. CV: Cardiovascular.

Table S4. Impact of predictors of CV death, full multivariable model

	Multivariable HR	X2	P
Age	2.21 (1.47-3.36)	15.3	<0.0001
Smoker, y/n	1.64 (1.26-2.13)	13.0	0.0003
Cholesterol	1.19 (1.06-1.34)	8.4	0.0038
Smoking cessation	0.58 (0.38-0.86)	7.2	0.0073
Systolic blood pressure	1.21 (1.03-1.41)	5.6	0.0178
Δ Chronotropic index	0.84 (0.73-0.97)	5.6	0.0178
Family history CHD, y/n	1.38 (1.05-1.79)	5.4	0.0196
Fasting blood glucose	1.11 (0.99-1.23)	3.3	0.0711
Δ Body mass index	1.12 (0.98-1.27)	2.7	0.0987
Systolic BP 100W	1.11 (0.94-1.31)	1.6	0.2044
Body mass index	1.07 (0.93-1.24)	1.0	0.3085
Resting heart rate	0.92 (0.79-1.08)	1.0	0.3093
Chronotropic index	0.54 (0.14-2.07)	0.8	0.3845
Maximal heart rate	1.73 (0.40-7.96)	0.6	0.4676
Relative heart volume	1.03 (0.90-1.16)	0.2	0.6808
Physical fitness	0.98 (0.82-1.15)	0.1	0.7817
Triglycerides	0.98 (0.87-1.09)	0.1	0.7405
FEV1	1.01 (0.88-1.16)	<0.1	0.8596
Haemoglobin level	1.01 (0.90-1.15)	<0.1	0.8269

Values are hazard ratios (HR) of one standard deviation increase in baseline value for continuous variables, and HR for yes vs. no for baseline status of nominal variables, 95% confidence interval in parenthesis. Δ is temporal change in the denoted variable, FEV1 is forced expiratory volume at one second, CHD is coronary heart disease, Systolic BP 100W is systolic blood pressure at 100 Watts workload. Variables are ranked by chi square in a multivariate model with all possible predictors included.

SUPPLEMENTAL MATERIAL

Table S1. Impact of predictors of all-cause death

	Univariable HR	Multivariable HR	X2	P
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Physical fitness	0.80 (0.73-0.87)	0.89 (0.81-0.99)	4.8	0.0287
Cholesterol	1.15 (1.07-1.23)	1.08 (1.01-1.17)	4.6	0.0314
Family history CHD, y/n	1.14 (0.96-1.36)	1.15 (0.96-1.37)	2.2	0.1392
Resting heart rate	0.98 (0.91-1.06)	0.95 (0.86-1.05)	0.9	0.3380
Chronotropic index	0.79 (0.72-0.86)	0.86 (0.37-1.95)	0.1	0.7263
Maximal heart rate	0.84 (0.77-0.91)	1.04 (0.43-2.62)	<0.1	0.9410
Body mass index	1.04 (1.95-1.12)	0.99 (0.92-1.07)	<0.1	0.8474

Values are hazard ratios (HR) for all-cause death of one standard deviation increase in baseline value for continuous variables, and HR for yes vs. no for baseline status of nominal variables, 95% confidence interval in parenthesis. Ranked by chi square in multivariate model with all listed possible predictors included. CHD: Coronary heart disease.

Table S2. Hazard ratios for all-cause death in quartiles according to 7-year change in chronotropic index, n = 1420

	Q1	Q2	Q3	Q4
N	355	355	355	355
All-cause death, n (%)	236 (66%)	177 (50%)	149 (42%)	178 (50%)
Bivariable adjusted HR				
Q4 as reference	1.59 (1.30-1.93)	1.19 (0.96-1.47)	0.97 (0.77-1.20)	1
Q1 as reference	1	0.75 (0.62-0.91)	0.61 (0.50.0.75)	0.63 (0.51-0.77)
Multivariable adjusted HR				
Q4 as reference	1.35 (1.10-1.64)	1.05 (0.85-1.30)	0.90 (0.72-1.12)	1
Q1 as reference	1	0.78 (0.64-0.95)	0.67 (0.54-0.82)	0.74 (0.61-0.91)

Values are hazard ratios for CV death with 95 per cent confidence intervals in parenthesis. Bivariable adjusted for baseline age and chronotropic index. Multivariable adjusted for baseline values of chronotropic index, physical fitness, age, systolic blood pressure, smoking status and total serum cholesterol as well as family history of coronary heart and smoking cessation between Survey 1 and Survey 2.

Table S3. Hazard ratios for death in quartiles according to 7-year change in chronotropic index in men with no detectable ischemia at Survey 2, n = 1389

End points	Q1	Q2	Q3	Q4
CV death	1.41 (1.02-1.93)	1.03 (0.74-1.45)	1.01 (0.72-1.43)	1
All-cause death	1.34 (1.09-1.64)	1.08 (0.87-1.34)	0.92 (0.74-1.15)	1

Values are hazard ratios for death with 95 per cent confidence intervals in parenthesis after multivariable adjustment for baseline values of chronotropic index, physical fitness, age, systolic blood pressure, smoking status and total serum cholesterol as well as family history of coronary heart and smoking cessation between Survey 1 and Survey 2. CV: Cardiovascular.

Table S4. Impact of predictors of CV death, full multivariable model

	Multivariable HR	X2	P
Age	2.21 (1.47-3.36)	15.3	<0.0001
Smoker, y/n	1.64 (1.26-2.13)	13.0	0.0003
Cholesterol	1.19 (1.06-1.34)	8.4	0.0038
Smoking cessation	0.58 (0.38-0.86)	7.2	0.0073
Systolic blood pressure	1.21 (1.03-1.41)	5.6	0.0178
Δ Chronotropic index	0.84 (0.73-0.97)	5.6	0.0178
Family history CHD, y/n	1.38 (1.05-1.79)	5.4	0.0196
Fasting blood glucose	1.11 (0.99-1.23)	3.3	0.0711
Δ Body mass index	1.12 (0.98-1.27)	2.7	0.0987
Systolic BP 100W	1.11 (0.94-1.31)	1.6	0.2044
Body mass index	1.07 (0.93-1.24)	1.0	0.3085
Resting heart rate	0.92 (0.79-1.08)	1.0	0.3093
Chronotropic index	0.54 (0.14-2.07)	0.8	0.3845
Maximal heart rate	1.73 (0.40-7.96)	0.6	0.4676
Relative heart volume	1.03 (0.90-1.16)	0.2	0.6808
Physical fitness	0.98 (0.82-1.15)	0.1	0.7817
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FEV1	1.01 (0.88-1.16)	<0.1	0.8596
Haemoglobin level	1.01 (0.90-1.15)	<0.1	0.8269

Values are hazard ratios (HR) of one standard deviation increase in baseline value for continuous variables, and HR for yes vs. no for baseline status of nominal variables, 95% confidence interval in parenthesis. Δ is temporal change in the denoted variable, FEV1 is forced expiratory volume at one second, CHD is coronary heart disease, Systolic BP 100W is systolic blood pressure at 100 Watts workload. Variables are ranked by chi square in a multivariate model with all possible predictors included.

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