HDL-cholesterol, Exercise Blood Pressure and Triglycerides as Predictors of Cardiovascular Disease or Diabetes

Influence of Physical Fitness and Re-examination

Thesis

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List of papers

1. Skretteberg PT, Grundvold I, Kjeldsen SE, Erikssen JE, Sandvik L, Liestøl K, Erikssen G, Pedersen TR, Bodegard J.
   HDL-cholesterol and prediction of coronary heart disease: Modified by physical fitness? A 28-year follow-up of apparently healthy men
   Atherosclerosis 2012; 220:250-6.

2. Skretteberg PT, Grundvold I, Kjeldsen SE, Engeseth K, Liestøl K Erikssen G, Erikssen J, Gjesdal K, Bodegard J.
   Seven-year increase in exercise systolic blood pressure at moderate workload predicts long-term risk of coronary heart disease and mortality in healthy middle-aged men.
   Hypertension 2013; 61:1134-40.

3. Skretteberg PT, Grytten AN, Gjertsen K, Grundvold I, Kjeldsen SE, Erikssen J, Mellbin L, Liestøl K, Fraser DA, Erikssen G, Pedersen TR, Bodegard J.
   HDL-cholesterol and prediction of coronary heart disease: Modified by physical fitness? A long-term follow-up of 1962 Norwegian men in the Oslo Ischemia Study
   Diabetes Res Clin Pract 2013;101:201-9.

Abbreviations

BP = Blood pressure

BMI = Body mass index (kg/m^2)

CHD = Coronary Heart Disease

CI = Confidence interval (95%)

CVD = Cardiovascular disease

DAGs = Directed Acyclic Graph approach

FTG = fasting serum triglycerides

HDL = High-density lipoprotein cholesterol

LDL = Low-density lipoprotein cholesterol

SBP = systolic blood pressure

SBP100W = peak systolic blood pressure at 100W workload

Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide and it causes 47% of all deaths in Europe (1). Most CVD deaths are caused by coronary heart disease (CHD), and CHD is the single most common cause of death in the in European Union (2). CVD consists of stroke, peripheral artery disease and aortic disease besides CHD. Great effort has been made to reduce the burden of CVD through improvements in lifestyle and medical intervention, and CVD mortality has fallen in many European countries during the last decades (2-4). CVD is, however, still among the leading causes of premature death in Europe accounting for about 30% of deaths before the age of 65 years (2). There is therefore still a strong need for more research on CVD to allow for more effective preventive measures.

Atherosclerosis is the predominant cause of CVD, especially CHD (5). Atherosclerosis represents a continuum, multi-factorial in aetiology beginning with injury to the endothelium covering the inside of the arterial wall. Lipid accumulation in macrophages leads to formation of foam cells which in turn degenerates. This subsequently leads extracellular deposition of lipid-rich plaques in the artery wall (6). The lipid deposition is accompanied by pathological proliferation of smooth muscle cells and infiltration of macrophages and other inflammatory cells in the arterial wall (7). The atherosclerosis often leads to narrowing of the arterial lumen and eventually limitation of blood flow. In advanced stages of atherosclerosis, formation of a fibrous cap covering the atherosclerotic plaque forms a vulnerable barrier between the plaque and the blood. According to current knowledge, most acute CHD events are caused by rupture of plaques with relatively high lipid content and thin fibrous cap, called unstable plagues (8).

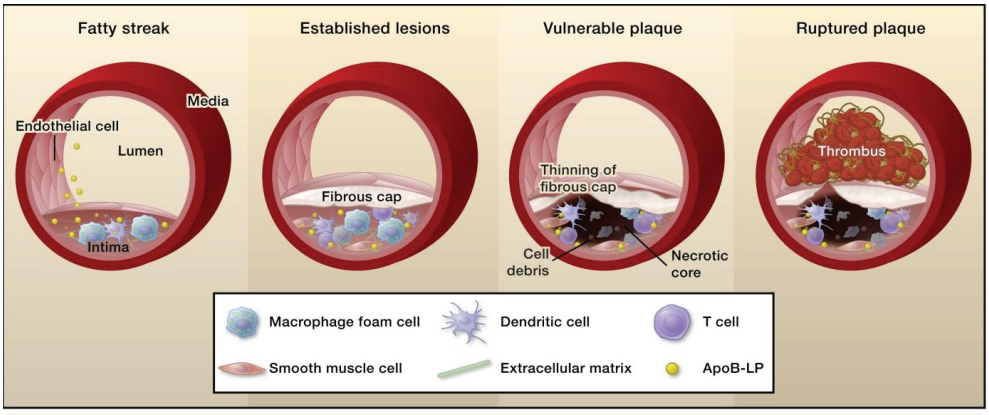


Figure 1 Stages of atherosclerosis from endothelial damage and fatty streaks (left side) to advanced atherosclerotic plaque with a ruptured fibrous cap and thrombus formation (right side).
(Modified from the internet, free download Google images)

The burden of atherosclerotic disease was recognized to reach epidemic proportions in the USA and Europe after Second World war, and the search for explanations and possible preventive measures was initiated (9). Several risk factors have been identified, and risk modification has proven successful with smoking cessation and reduction of blood pressure and cholesterol levels. Lifestyle interventions on physical activity and diet have also proven effective (3).

Risk factors and predictors

The term risk factor was introduced by Dr. William Kannel in his groundbreaking publication from the Framingham Heart Study in 1961(10). The underlying concept of risk factors had, however, been discussed long before the introduction of the term in medical literature. The derivation of the word "factor" from Latin implies causality, but the term risk factor was initially used both for causal and non-causal risk markers preceding development of diseases or disease events (11). During recent decades, most scientists have reserved the term risk factor for risk markers that have been shown to be causal in nature. Causality can in turn be difficult to evaluate and firmly establish in diseases of multi-factorial aetiology such as CVD. CVD risk factors may act by a known biological mechanism to induce a disease, but also be a surrogate for under underlying and often unknown factors or mechanisms. The term "predictor" is used when referring to a risk marker that is associated with subsequent development of disease, but where causality has not been found or potential causality has not been elucidated.

In this thesis, the term "risk factor" will be confined to risk markers generally accepted to be causal on the background of solid scientific evidence. The term "predictor" is used for markers that show association with subsequent disease but where causality has not been established. Causality cannot be elucidated solely from epidemiological studies. One may, however argue that a change in risk that parallels a change in the level of a predictor indicates a higher probability of a causal link than point measure-associations do.

Smoking

Smoking is associated with increased risk of all types of CVD, and the association show a clear dose-response relationship with the amount of tobacco smoked without a lower limit for harmful effects (12-14). Smoking has been firmly established as one of the most important modifiable CVD risk factors. Smoking cessation is therefore undoubtedly one of the most important measures in prevention of CVD, but may be challenging (15, 16).

Blood pressure

In the Global Burden of Disease Study in 2010, elevated resting blood pressure (BP) was estimated to be the leading single risk factor for death and disability adjusted life years worldwide (17). Overwhelming evidence exists for a firm, graded and consistent association between resting blood pressure levels and CVD risk (10, 18). Hypertension is defined as resting blood pressure ≥140/90 mmHg, and is probably the single most important modifiable CVD risk factor. There is incontrovertible evidence of beneficial effects on CVD risk when treating hypertensive individuals at high risk of CVD with blood pressure lowering drugs and lifestyle interventions (19, 20). There is also some evidence that blood pressure lowering treatment is beneficial for individuals with mild hypertension, and even in systolic blood pressure (SBP) in the high normal range (130-139/85-89 mmHg) if they are otherwise at high risk of CVD (20).

Systolic blood pressure measured during physical exercise tests, hereafter referred to as exercise SBP, has been shown to be an independent predictor of CHD and CVD mortality among apparently healthy individuals (21-23). The potential predictive impact of temporal changes in exercise SBP on CHD and CVD has not been investigated before we started the present study.

Cholesterol

An association between serum cholesterol levels and future development of CHD was first demonstrated in the Framingham Heart Study (10, 24), although a link between cholesterol and atherosclerotic disease had been proposed by Anitschkow in 1908 (25). The three major classes of lipoproteins, very low density (VLDL), low density lipoprotein (LDL) and high density (HDL) were first identified by John W. Gofman (26). He also laid the foundation for understanding the link between the lipoprotein classes and the risk of atherosclerosis and heart disease (27). There is now unequivocal evidence that LDL cholesterol levels are closely associated with CVD risk, and reduction of LDL cholesterol levels has become a cornerstone in CVD prevention due to overwhelming evidence of beneficial effects from lipid lowering trials (28-31).

HDL cholesterol levels have shown a consistent and graded inverse association with CHD and CVD risk (32, 33). HDL is thought to mediate its protective effects against CHD and CVD by improving cholesterol transport from peripheral tissues to the liver, called reverse cholesterol transport (34). Attempts to raise HDL cholesterol levels have so far given conflicting evidence with regards to net clinical benefits. The reason for this is debated, and

may be caused by negative side effects of drugs rather than lack of effect from increasing HDL levels per se (35). Increasing HDL levels or improving its function therefore continues to be appealing methods of potential risk modification. Physical exercise and increased physical fitness have been shown to be associated with a small but clinically significant increase in HDL cholesterol (36, 37). HDL cholesterol consists of relatively large heterogeneous particles, and subclasses with different anti-atherogenic properties have been identified. Some research on physical exercise has indicated that the relative contents of HDL or relative proportions of subclasses may be changed by exercise, thereby improving anti-atherogenic properties (38-40).

Physical fitness

Physical fitness, often also referred to as cardiorespiratory fitness has no universally accepted definition, but is predominantly considered to be a measure of aerobic exercise capacity. Maximal oxygen uptake (VO_2 max = maximum volume [V] of oxygen [O_2] in mL per kg body weight) is considered to be the gold standard of measuring physical fitness. High levels of physical fitness are associated with a significant and clinically important reduction in risk of CHD and CVD mortality as well as all-cause mortality among apparently healthy individuals of both genders (41-46). High levels of physical activity and physical fitness also show a clear inverse association with risk of developing type 2 diabetes (47-50).
Physical fitness has been measured among all men in the Oslo Ischemia Study using a standardized bicycle exercise-ECG protocol described in the methods section and in former papers (31, 51, 52). This method of measuring physical fitness has shown a high correlation with VO_2 max (53).

Type 2 Diabetes

Patients with type 2 diabetes mellitus have about four times higher incidence of CHD than the general population, and a two- to four-fold increased risk of CVD events (54, 55). CVD is the leading cause of mortality and morbidity among patients with diabetes mellitus, and it has been estimated that as many as 80% of patients with type 2 diabetes will develop CVD (56, 57). Patients with type 2 diabetes have been suggested to be at the same CVD risk as patients with established CVD, making these patients a target for secondary prevention. However, while this is debated (58, 59), evidence still points toward type 2 diabetes as a strong risk factor for CVD. Patients with type 2 diabetes seems to have accelerated stages of atherogenesis on several levels, from initial endothelial injury to advanced atherosclerotic lesions and thrombo-embolic events (60). The link between diabetes and atherosclerosis has,

however, not been fully elucidated. Diabetes is a gradually developing chronic disease of increasing incidence and prevalence worldwide, and will have major impact on the total burden of CVD (61). Identifying people at increased risk of developing diabetes mellitus early seems essential to implement effective preventive measures.

Prevention of CVD

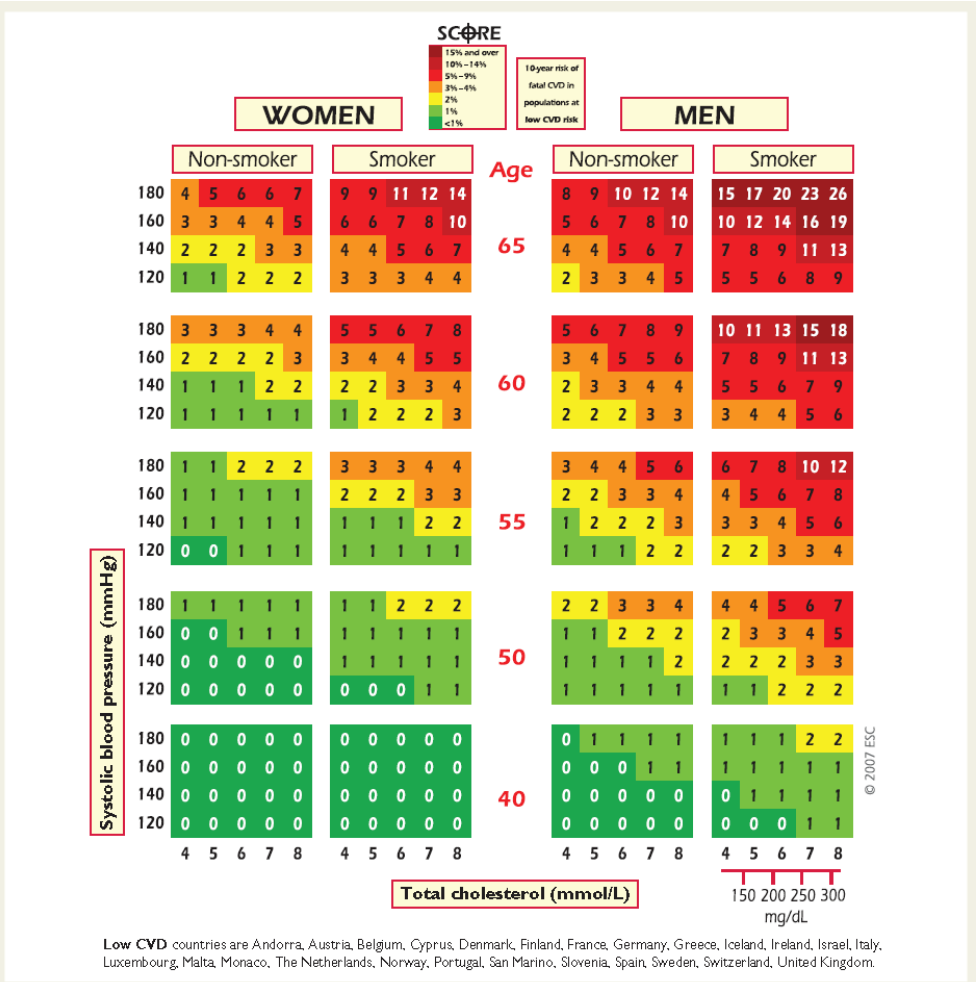
Prevention has traditionally been divided into *primary* and *secondary* prevention making a distinction between individuals who are asymptomatic, and those who have experienced one or more disease events, respectively. The usefulness of this distinction in CVD risk estimation has been questioned since current knowledge indicates that atherosclerosis evolves as an almost continuous process from asymptomatic damage of the arterial endothelium into symptomatic disease of various severity (3). Nevertheless, several interventional trials have used the distinction, and it is often easier to implement intensive preventive measures in secondary prevention since it may be easier to acknowledge the need for risk reduction after having experienced a CVD event (3). Despite major advances in secondary prevention, individuals who have best secondary prophylaxis available today still have a high residual risk of new CVD events (11, 28, 62). Identifying new predictors and risk factors seems necessary in order to further improve preventive measures.

It is estimated that 25 to 50 % of all serious CHD events occur among individuals with no prior symptoms (63, 64). Primary prevention seems uncontroversial in individuals at high risk of future CVD. Most CVD events in a general population occur, however, among people who are not categorized as being in a high risk group. Identifying persons that are likely to benefit from preventive measures therefore continues to be a great challenge and precise risk estimations tools are necessary (3).

Risk estimation

Smoking, hypertension and total cholesterol besides age and gender are often referred to as *classical risk factors,* and are used in most risk prediction models. The most widely used are the Framingham and the SCORE risk charts (3, 65). The SCORE system is designed to be used in primary prevention, estimating the 10-year risk of fatal CVD, and has made two different models; one for countries with high CVD risk and one for low-risk countries (3).

Figure 2 SCORE risk chart for low-risk countries



10-year risk of fatal cardiovascular disease (CVD) in countries at low CVD risk based on the following factors: age, sex, smoking, systolic blood pressure and total cholesterol. The risk of total (fatal + non-fatal) CVD events will be approximately three times higher than the figures given. (Adapted from the European Guidelines on cardiovascular disease prevention in clinical practice version 2012)

Risk charts have been widely applied in risk estimation, but their positive- and negative predictive value has been questioned. The positive predictive value of risk scoring systems is highly dependent of the local CVD prevalence and population characteristics, e.g the higher prevalence the better positive predictive value (66). Beside local adaptation of risk stratification models, attempts have been made to add other risk markers to improve the predictive value. Significant improvements have proven more difficult to achieve than expected (3). HDL cholesterol has been one of few predictors that has proven to improve risk estimation in all risk groups, and has therefore now been implemented in the electronic version (http://heartscore.org) of the SCORE risk system (3, 67). Besides HDL, physical fitness and other exercise predictors including exercise SBP have also shown to be possible candidates for improving risk estimation (42, 68, 69).

Background for the present study

The *Oslo Ischemia Study* was initiated in 1971 at Rikshospitalet with the primary aim to search for possible, significant coronary artery disease among apparently healthy, middle-aged men. Since the study start in 1972, the Oslo Ischemia Study has provided significant contributions to the knowledge of CHD epidemiology. Physical fitness was for the first time shown to be an independent, long-term predictor of CVD mortality and all-cause mortality among healthy middle-aged men in the Oslo Ischemia Study (44). Most studies conducted in the cohort have provided valuable knowledge about exercise-ECG test findings and CHD (22, 23, 42, 43, 51, 52, 70-77). Findings from the Oslo Ischemia Study cohort have also contributed to the knowledge about maternal heritage in diabetes and the association between fasting blood glucose and CVD mortality (78, 79).

In most epidemiological studies to date, point measures of potential predictors and risk factor have been performed and study participants have thereafter been followed for a certain time. The uncommon availability of repeated surveys with clinical examinations, blood tests, ECG and bicycle exercise-ECG tests in the Oslo Ischemia Study represents an opportunity to study the impact of both point measures of-, and temporal changes in predictors of CVD and diabetes.

When identifying several predictors or risk factors, it may be challenging to estimate the independent effect of each factor on the outcome. The effect of one factor may be

dependent upon the level of another factor, and the effect of the two on the outcome is not additive. This is called interaction, and has been considered since the "early era" of risk factor research although the effect of single risk factor or predictors has received most attention (10, 24, 80). The Oslo Ischemia Study also contains data suitable to study interactions between predictors. On this background, we aimed to study interactions between markers in prediction of CVD and diabetes in the present study. Furthermore, we aimed to utilize the opportunity to investigate effects of temporal changes in predictors. Predictors that may improve CVD and diabetes risk estimation attained our interest.

Aims

1. There is firm evidence that the HDL cholesterol is inversely associated with risk of future CHD, and also substantial evidence that the level of physical fitness and physical activity may influence the HDL level. Some evidence indicates that the structure and function of HDL may be more favourable with increased physical activity and fitness.

   We therefore aimed to study: If the predictive ability of HDL for CHD (including angina pectoris, non-fatal myocardial infarction and CHD death) is dependent upon physical fitness and changes in physical fitness. Further, if physical fitness influences the predictive ability of HDL for CHD death, CVD death and all-cause death.

2. Increasing evidence indicates that SBP measured at low- or moderate exercise intensity may be even more closely associated with future risk of CVD death than resting SBP. The prognostic impact of temporal changes in exercise SBP has, however, not been studied before.

   We therefore aimed to study if seven-year changes in SBP measured at 100W workload predict CHD and mortality, and if these possible associations are influenced by physical fitness and changes in physical fitness.

3. Elevated fasting serum triglycerides (FTG) have been associated with diabetes risk. Whether physical fitness modifies the FTG-diabetes association remains, however elusive. Physical fitness also reflects important elements of lifestyle and is inversely associated with diabetes risk.

   We aimed to study whether the possible association between FTG and long-term risk of diabetes is modified by the level of physical fitness and temporal changes in physical fitness. Additionally; we studied if temporal changes in FTG levels predict future diabetes risk.

Material and methods

Subjects

All subjects in the studies of the present thesis constitute sub sets of men included in *The Oslo Ischemia Study* in the years 1972-75. The Oslo Ischemia Study was initiated in 1971 by Jan Erikssen (JE) who worked at Rikshospitalet at the time. Jan Erikssen was the primary investigator, designed the study and conducted the inclusion and the first and second survey. In subsequent surveys, Erikssen has cooperated with other clinicians and PhD students, but he has kept the overview over all examinations, data gathering and taken an active part in all publications. Originally, the main aim of the Oslo Ischemia Study was to examine apparently healthy men with bicycle exercise test, uncover subclinical coronary artery disease and thereby be able to estimate the prevalence and incidence of coronary artery disease among apparently healthy middle-aged men. Time of follow-up was initially planned to be approximately 5 years, but the men have been followed up to 35 years. Four clinical surveys, one postal questionnaire-survey and two nationwide searches of hospital records have been performed during the 35 years (Figure 3, flow chart).

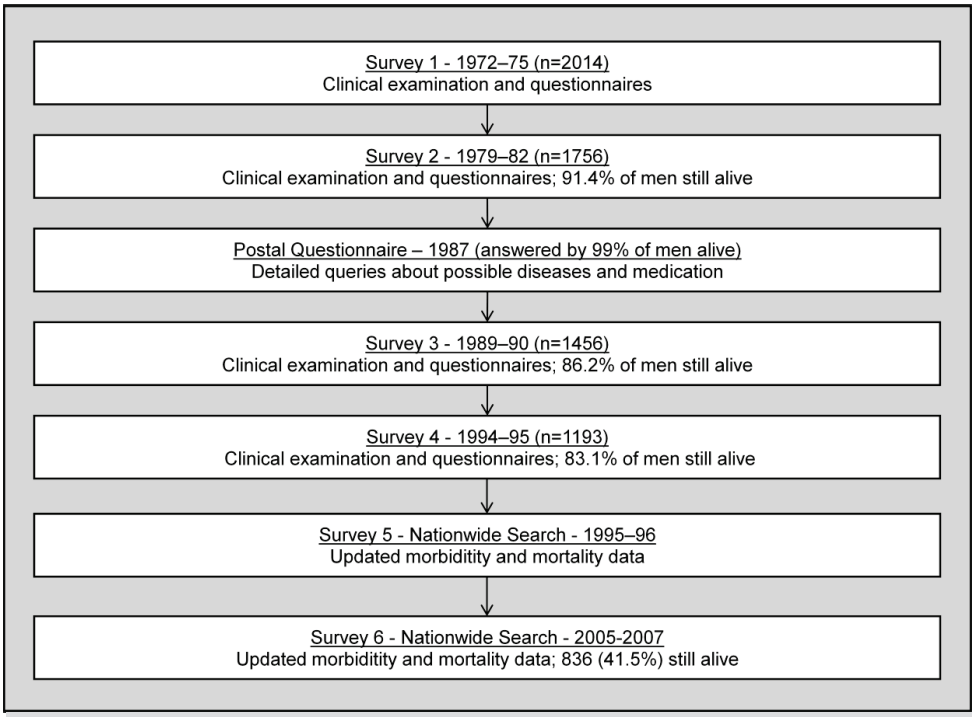


Figure 3 Oslo Ischemia Study flow-chart showing all surveys during up to 35 years follow-up

Selection and Inclusion

All men aged 40-60 years who were apparently healthy according to available health files and worked full time in five companies based in the Oslo region (Custom Services, Norwegian Railways, Postal Services, Siemens Inc. and Telecommunication Services) were invited to participate in the study. The above mentioned companies were selected because of their size in addition to having a company health department providing annual or biannual routine examination for the employees for at least the previous five years. All health records of the companies were scrutinized by Jan Erikssen in collaboration with the companies` chief physician during last half of 1971 and first half of 1972. Only men who were labelled "apparently healthy" according to predefined criteria were found eligible and invited to participate in the study. Criteria for being apparently healthy were the following: The men should be free from known or suspected heart disease, cancer, diabetes, severe lung, liver or renal disease, drug treated hypertension and any chronic drug regimen. Further, men who were judged unable to conduct a symptom-limited bicycle exercise test for muscular, orthopaedic or neurological reasons were not labelled apparently healthy. All 2341 men who fulfilled the inclusion criteria were invited to participate, and 2014 men (86%) gave their consent.

Clinical Surveys

All participants were given an extensive health questionnaire one week prior to Survey 1, administered through the respective health departments of their companies. The questionnaire was made in Norwegian specifically for the Oslo Ischemia Study, and included a Norwegian translation of the WHO angina-, the WHO claudicatio-, and the MRC respiratory questionnaire. A brief description of relevant questions is given in the publications when relevant. All questionnaires were reviewed together with all the participants by the primary investigator (JE) during the examination day to ensure that all questions were understood and answered correctly.

Survey 1 was conducted from August 1972 to March 1975 after having conducted a pilot study of 80 men from the community. The pilot study was performed partly in order to determine the level of the first workload on the exercise test. All men were asked to fast for at least 12 hours and abstain from smoking for at least 8 hours prior to examination starting at 07.00 AM. The study protocol generally followed the standardized principles described by

Rose and Blackburn in their WHO-publication of 1968: *Cardiovascular Surveys and Methods* (81). Clinical examinations and test were performed in the order as shown in Figure 2.

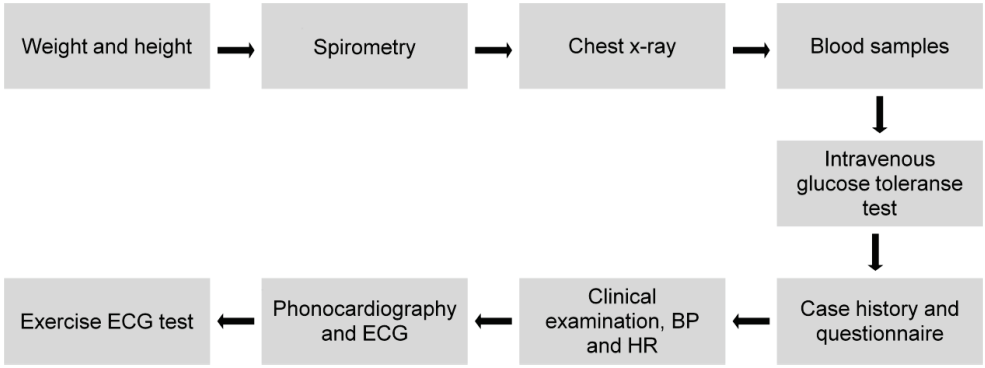


Figure 4 Order of examinations (from top left). BP: blood pressure, HR: heart rate

Details of the clinical examinations, blood tests and other test have been described in previous publications and in the publications in this thesis when relevant for the particular study. No independent method paper has been published from the Oslo Ischemia study.
Subsequent clinical surveys (as depicted in Figure 3) were virtually identical to Survey 1 with minor deviations. Deviations between surveys have been described in the publications when relevant.

Resting blood pressure measurements

Blood pressure measurements were performed under standardized conditions after the subjects had been familiarized with the laboratory and after 5 minutes supine rest in a quiet room. Measurements were repeated 3 times for all subjects using a mercury sphygmomanometer and auscultator method. Further details about blood pressure measurements have been described in previous publications (74, 82-84).

Blood tests

All blood samples were obtained in a fasting state, before exercise test was performed in all participants. Blood tests were analysed by standard methods of the time of analysis. Further details about tests and variation coefficients are provided in publications when relevant.

Bicycle exercise ECG test and physical fitness

All men performed a standardized bicycle exercise ECG-test at the clinical examinations. The initial workload was 6 minutes on 100W for 98 % of the men. The other 2% of the men started with 50W at Survey1. The workload was increased by 50W every 6 minutes and the men were encouraged to continue exercise until exhaustion or until exhibiting signs or symptoms causing an early test termination. SBP was measured to the nearest 5 mmHg every second minute throughout the test. Physical fitness was defined as the total bicycle exercise work (Joule), calculated as the sum of work at all workloads, divided by body weight (kg). Details about the exercise test have been described in present papers and previous publications (44, 51, 52, 70).

Endpoints and follow-up

Endpoints in the Oslo Ischemia Study have been identified by the postal questionnaire in 1987, the nationwide searches of hospital records in all Norwegian hospitals in 1995-96 and 2005-07 besides cases history and questionnaires at the clinical surveys, survey 1-4 (Figure 3). Only the last follow-up, survey 6, will be described in detail in this section.

Survey 6

Survey 6 was conducted during the years 2005-07 by MD Johan Bodegard with permission from *Norwegian Data Inspectorate* and the *Norwegian Board of Health.* The survey was performed as a search in all hospital records for all participants of the Oslo Ischemia Study in all Norwegian hospitals. Hospital records were manually assessed. All records found regarding admissions, outpatient notes and referral letters, both in paper- and electronic versions, were used and the following data were registered:

1. Name and date of birth with social security number
2. Date of admission and discharge, number of days they were 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 surgery (CABG)
- Aortic valve replacement (AVR)
- Intermittent claudication
- Surgery for claudication
- Transitory ischemic attack
- Stroke (ischemic and haemorrhagic)
- Hypertension
- Diabetes mellitus
- Atrial fibrillation
- Chronic obstructive lung disease (COPD)
- Cancer, categorised

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

Complete updates of cause-specific deaths were obtained from *Statistics Norway* until 2008. Statistics Norway has record of cause of death in all Norwegians who have died with a delay in completeness of 6 months.

Morbidity and mortality data are complete up to December 31st, 2007, and none was lost to follow-up.

Combined endpoints have been used in Paper 1 and 2. These endpoints are defined in the respective papers.

Statistical analyses

Statistical analyses were mainly performed using the JMP® 9 statistical software (SAS Institute Inc., Cary, NC, USA). Some additional analyses were performed using the SAS® version 9.2 (SAS Institute Inc., Cary, NC, USA). A data analysis plan was made for the main hypotheses and aims before statistical analyses were performed. Additional analyses specific for each study were performed to answer new hypotheses emerging during our work, or as requested by reviewers.

Kendall`s rank test was used to assess correlation (trend) between subgroups (i.e. tertiles, quartiles or other specified categories) of participants and baseline- or examination characteristics. Differences in data between groups were tested by Student's t-test or Pearson's chi-square test according to data type. A two-tailed p value < 0.05 was considered statistically significant.

Survival was analysed using Kaplan Meier plots and tested with log rank tests. Cox proportional hazard regression used for all endpoints examined to calculate hazard ratios and testing statistical significance in prediction.

The distribution of all variables used in survival analyses was checked, and variables with a skewed distribution were logarithmically transformed before entered into survival analyses. Significant variables in univariate analyses ($p<0.05$) were entered into multivariate analyses and prediction models were mainly indentified by stepwise backward elimination.

In paper 3, the Directed Acyclic Graph (DAG) approach was used (85). The DAG method is designed to provide suitable models for assessing the causal effect of one variable upon another. Given a list of potential causal relations between the total available set of variables, the DAG approach selects a set of variables to be included in the model that minimize bias by retaining in the model potentially confounders while eliminating intermediate variables.

Interactions between predictors were analysed 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. We also stratified men according to age-adjusted physical fitness and made separate Cox analyses in for the different strata.

Specific details about statistical analyses are given in the respective papers when relevant.

Summary of results

Paper 1 HDL, Physical fitness and coronary heart disease

In this paper, we tested the hypothesis that physical fitness influences the long-term prognostic impact of HDL for risk of CHD (main combined endpoint consisting of angina pectoris, non-fatal myocardial infarction and CHD death) and secondly death from CHD, CVD and all-cause when adjusting for classical CVD risk factors. Thirdly, we studied the possible interaction between physical fitness and HDL in CHD risk prediction and finally the possible influence of changes in physical fitness on the HDL-CHD risk association. This study included 1357 men who were apparently healthy and had HDL measured at Survey 2.

Mean HDL was 1.55 mmol/l (range 0.81-3.33 mmol/l) and follow-up from baseline until death or end of observation was 20.8 years (range 0.3-28.0 years). The highest (Q4) and the second highest (Q3) HDL quartiles were associated with lower risk of CHD, and adjustments for physical fitness had minor impact only on the results (Table 1).

Q4 was also associated with lower risk of CHD death (HR: 0.54, CI: 0.35 to 0.83), CVD death (HR: 0.63, CI: 0.45 to 0.87) and all-cause death (HR: 0.80, CI: 0.65 to 0.99) compared to Q1 when multiple adjusted (age, smoking status, SBP and total cholesterol) without physical fitness. Adjustments for physical fitness imposed minor changes only to the risk association between HDL and death from CHD and CVD, while all-cause death fell short of statistical significance. Q3 was associated with lower risk of death from CHD and CVD compared to Q1 when age-adjusted, but only with lower risk of CVD death when multiple adjusted (detailed data shown in paper, Table 1).

We found no interaction between HDL and physical fitness. The influence of changes in physical fitness over 8.6 years on the prognostic impact of HDL for CHD was studied in a sub population of 919 men free from CHD and cancer at Survey 3. Changes in physical fitness over 8.6 years had minor impact on the association.

Table 1 Risks of CHD among men in HDL cholesterol quartiles (Q1-Q4) compared to the lowest quartile (Q1)

	Q1 (0.81-1.30 mmol/l) n = 348	Q2 (1.31-1.51 mmol/l) n = 325	Q3 (1.52-1.71 mmol/l) n = 344	Q4 (1.72-3.30 mmol/l) n = 340
CHD, n (%)	**144 (41.4)**	**113 (34.8)**	**90 (26.1)**	**92 (26.1)**
Age-adjusted	1.00	0.78 (0.61-1.00)	0.55 (0.42-0.72)	0.57 (0.44-0.74)
Multiple adjusted* without PF	1.00	0.80 (0.62-1.02)	0.58 (0.44-0.75)	0.57 (0.44-0.74)
Multiple-adjusted* with PF	1.00	0.83 (0.64-1.06)	0.60 (0.46-0.79)	0.61 (0.47-0.80)

Risks are given in hazard ratios (95% CI), CI = 95% confidence interval, CHD = coronary heart disease including angina pectoris, non fatal myocardial infarction and CHD death. PF = physical fitness, n = number of men. *adjusted for age, smoking status, systolic blood pressure and total cholesterol.

Paper 2 Changes in exercise blood pressure

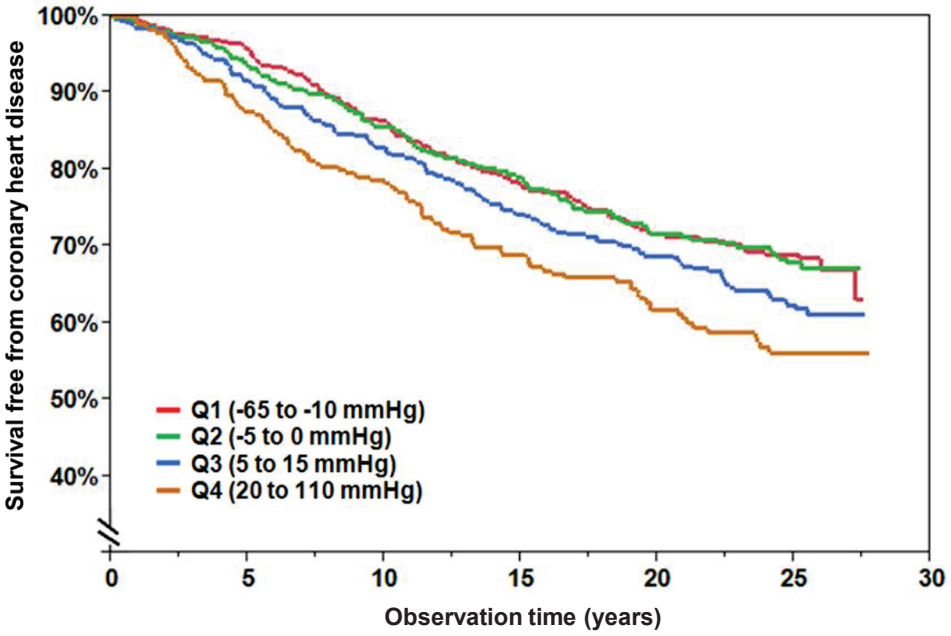
In this study, we tested the hypothesis that changes in exercise SBP during seven years predict CHD (including angina pectoris, non-fatal myocardial infarction and fatal coronary heart disease) and mortality over the following 28 years. Peak SBP at 100W workload (SBP100W) was measured among 1,392 men at Survey 1, and repeated at Survey 2. The men were divided into quartiles (Q1-Q4) of change in peak SBP100W.

Mean resting SBP was 127.3 mmHg and mean SBP100W was177.3 mmHg. Mean SBP increased by 3.4 mmHg and SBP100W with 3.0 mmHg from Survey 1 to Survey 2. Risk of CHD increased with increasing quartiles of seven year changes in SBP100W (Figure 5).

The highest quartile, Q4, was associated with 1.55-fold (95% CI; 1.17-2.03) risk of CHD and 1.93-fold (1.24-3.02) risk of CHD death compared to the lowest, Q1, when adjusted for family history of CHD, age, smoking status, resting SBP, SBP100W and total cholesterol at first examination (Model 1). Q4 had a 1.40-fold (1.06-1.85) risk of CHD and a 1.70-fold (1.08-2.68) risk of CHD death when further adjusted for physical fitness and change in physical fitness (Model 2). Q4 was associated with increased risk of death from CVD and all-cause compared to Q1 in Model 1, but not in Model 2.

Our results indicate that an increase in SBP100W over seven years is independently associated with increased long-term risk of CHD, CHD death and CVD death.

Figure 5 Survival free from coronary heart disease according to change in exercise blood pressure



Kaplan-Meier curves showing survival (%) free from coronary heart disease in quartiles (Q1-Q4) of seven-year change in peak systolic blood pressure at exercise load of 100W among 1392 initially healthy men.

Paper 3 Fasting serum triglycerides and diabetes

In this study, we tested the impact of physical fitness on the association between fasting serum triglycerides (FTG) and diabetes risk, and secondly if temporal changes in FTG predict diabetes risk.

A sub population of the Oslo Ischemia study cohort of 1,962 men who had fasting serum triglycerides (FTG) and fasting blood glucose measured at Survey 1 were included in this study. Out of the 1,962, 1; 387 were still healthy at Survey 2 and reached our criteria for studying temporal changes in FTG and diabetes risk.

During 35 years follow-up 202 out of the 1962 (10.3%) men developed diabetes. Compared with the lowest, the upper FTG tertile had 2.58-fold (95% CI; 1.81–3.74) diabetes risk adjusted for age, fasting blood glucose and maternal diabetes, and 2.29-fold (95%CI; 1.60–3.33) when also adjusting for physical fitness. Compared with unchanged (±25%) FTG levels (*n*=664), FTG reduction more than 25% (*n*=261) was associated with 56% lower (0.44; 95% CI; 0.24–0.75) diabetes risk, whilst FTG increase of more than 25% (*n*=462) was associated with similar risk. These associations were unchanged when adjusting for physical fitness and temporal changes in physical fitness.

When stratifying men into those with age-adjusted physical fitness below and above the median at inclusion, we found a possible interaction between FTG and physical fitness on diabetes risk. Our results indicated a stronger association between FTG levels and diabetes among unfit than fit men.

High FTG-levels predicted long-term diabetes risk in healthy middle-aged men, and the association was only modestly weakened when adjusted for physical fitness. A reduction in FTG was associated with decreased diabetes risk.

Discussion of results

HDL

The novel finding was that physical fitness and changes in physical fitness did not change the prognostic impact of HDL on CHD and death from CHD and CVD. In line with existing evidence, we found that HDL was a powerful long-term predictor for these endpoints (67, 86-89). HDL appeared to be the strongest predictor of CHD compared to other classical risk factors including physical fitness in this study. We also found that the largest difference in risk of future CHD in our study was between HDL levels of 1.31-1.51 mmol/l and 1.52-1.71 mmol/l. Men with HDL in the range 1.52-1.71 mmol/l and 1.72-3.30 mmol/l appeared to have similar risk, indicating that HDL increase above 1.50 mmol/l not necessarily is associated with further risk reduction which is in accordance with previous findings (90, 91).

Blood lipid response to exercise training and increases physical fitness is highly variable. Most studies on HDL and exercise have been small with less than 200 participants and of relatively short duration, although one study by Leon et al included 675 healthy subjects of both genders (92, 93). Results have been conflicting, but a meta-analysis by Leon et al. showed that the increase in HDL levels in the training groups of 20 studies without dietary intervention, including about 2200 subjects, was about 4.6% (range: decrease of 5.8% to increase if 25%) in average across studies (92). HDL cholesterol consist of complex particles with varying density and possibly also heterogeneous properties regarding reverse cholesterol transport and protection from CHD (94). HDL cholesterol consists mainly of two subclasses, the larger and less dense HDL_2 particles and the smaller and denser HDL_3 particles. HDL_2 particles have been considered to be most favourable in reverse cholesterol transport and to possess the most beneficial anti-atherogenic properties, although this has been questioned (93). Increase in HDL due to high physical activity or exercise intervention has mostly been ascribed to increase in HDL_2 or large HDL particles. The relative increase in HDL_2 and HDL_3 may, however, vary between individuals (40, 92, 94-97). On this background, it is reasonable to question if the predictive ability of HDL on CHD and CVD is equal along the whole spectrum of physical fitness in a population since HDL in fit and unfit people may have different composition. Our findings did, however, not support the hypothesis that the level of physical fitness modifies the predictive ability of HDL for CHD and CVD.

How do we explain our results, and should they be considered surprising? There are several reasons why we do not find our results surprising. First, the inverse association

between CHD and HDL levels has been consistent and of similar magnitude in several epidemiological studies likely to have included participants along the whole spectrum of physical fitness and physical activity (32, 33, 90, 98). Second, evidence of correlations between levels of specific HDL sub fractions and reverse cholesterol transport has been scarce and conflicting. Olchawa et al. compared measures of reverse cholesterol transport in 25 male athletes and 33 normally active men. They found increased cholesterol efflux from peripheral tissues to plasma among the athletes, but this could be fully accounted for by an increased number of HDL particles rather than an increased ability of HDL particles to promote cholesterol efflux (99). Third, exercise induced changes in HDL-composition and markers of reverse cholesterol transport have not been validated as surrogates for long-term protection against CHD. On the contrary, we cannot totally reject the possibility of enhancing beneficial properties of HDL in CHD protection from one single study.

The finding that physical fitness and temporal changes in physical fitness had minimal or no impact on the predictive ability of HDL should not be interpreted to question the association between increased physical fitness and increased HDL (36, 37). In line with existing evidence, we found a significant correlation between HDL levels and physical fitness. Since HDL measurements were not repeated in our study, we were not able to examine a possible association between temporal changes in physical fitness and HDL levels. Our findings, however, do not support earlier suggestions of clinically important differences in beneficial properties of HDL dependent upon physical fitness. We actually found the opposite because the predictive ability of HDL appears to be equal along the whole gradient of physical fitness in a population of apparently healthy men. If the structure and properties of HDL particles were highly dependent of the level of physical fitness, we would expect to find a significant interaction between HDL and physical fitness.

Exercise blood pressure

Our study of changes in exercise blood pressure was the first to investigate the possible association between temporal changes in exercise blood pressure and long-term risk of CHD and death. We found that seven-year change in SBP at a moderate exercise load of 100 Watt (SBP100W) was a significant predictor of CHD and death. The prognostic impact remained significant when adjusted for classical cardiovascular risk factors. Our findings indicate that exercise SBP adds prognostic information beyond classical risk factors on risk of CHD death among healthy middle-aged men.

The association seem to be particularly strong for CHD death followed by death from CVD and all-cause (Paper 2, Table 3 and 4). Since no other study has examined the prognostic impact of temporal changes in exercise SBP, differences in significance levels and prognostic power between the various endpoints should be interpreted with caution.

Exercise blood pressure (BP) is a measure of several properties of the cardiovascular system, and it is therefore not surprising that exercise SBP shows a closer association with CVD death than all-cause death that also includes deaths from causes not related to cardiovascular function. Whether existing evidence supports differences in the associations between exercise SBP and various forms of CVD remain elusive. This is due to difficulties in comparing results obtained from studies with major differences in population selection, methodologies and test procedures (100).

Adjustments for physical fitness and seven-year change in physical fitness had a small but significant impact of the results, abolishing the association between all-cause death risk and seven-year change in SBP100W. We also found that the above mentioned associations concerned men with normal resting BP (<140/90 mmHg). Our results support and extend existing evidence of a consistent, graded and independent association between SBP measured at low- to moderate exercise intensity and CVD- risk.

The importance of exercise SBP and an exaggerated SBP response to exercise has been debated, and results have often been considered conflicting. One source of conflicting results has been lack of consensus of BP values or magnitude of BP increase that represents an exaggerated BP response (101). Whether BP measured at low to moderate exercise intensity or at maximal intensity, eventually peak SBP during exercise test is the best predictor of future CVD events has also been a matter of debate (101).

In both the present and previous publications from the Oslo Ischemia Study, SBP100W has been a significant predictor of CHD and death from CHD and CVD when adjusted for classical CVD risk factors. Maximal SBP (almost invariably SBP measured at maximum workload just prior to termination of exercise) has, however not been an independent predictor of future CVD events (22, 23, 73). Our findings are in line with results from a recent meta-analysis of 9 studies conducted by Schultz et al with data from 46,314 individuals followed 15 years in average (101). Schultz et al. found that an exaggerated SBP response at moderate-, but not at maximal workload predicted CVD events when adjusted for age and causal BP in addition to other classical CVD risk factors. The existing evidence for a significant association between exercise SBP at moderate workload and future CVD risk appears to be robust. The added evidence from the present study show that also temporal

changes in exercise SBP at moderate workload may be a powerful predictor of future CHD and CVD. Our findings underline the need for further studies to evaluate if an exaggerated SBP response to exercise may be involved in the pathophysiological pathways. Further, antihypertensive treatment to prevent CVD in individuals demonstrating exaggerated exercise SBP response seems even more relevant than before. Few studies have examined potential treatment so far, but exercise training, weight loss and spironolactone have shown promising results on exercise SBP (102, 103). These studies have, however, been small (n=115 in both studies) and not designed to assess effects on CVD risk. In order to conduct future studies of high quality with comparable results, the scientists should try to reach a consensus with regards to definitions of normal versus exaggerated BP responses to exercise.

Our findings may be important since they pave the way for further research using exercise BP e.g. for assessing risk modification over time.

Exercise BP at moderate versus maximal workload, mechanisms

One reason that exercise SBP at moderate workload seems to be closer associated with CVD risk than SBP at maximal workload may be that it is more difficult to obtain reliable measurements at high-intensity exercise because of noise and movement artefacts. In the meta-analysis by Schultz et al, it is also pointed out that the statistical power to detect an effect of maximal exercise BP was lower than for exercise BP at moderate workload due to small number of studies available for pooled analysis at maximal exercise workload (101). In the Oslo Ischemia Study cohort, there has been a consistent close association between exercise SBP at 100W and CHD, CHD death and CVD death in all studies and analyses. Further, the lack of association between maximal SBP and the same endpoints has also been consistent. All blood pressure measurements at Survey 1 and 2 both at rest and during exercise were performed by the same investigator (JE). Thus, the above mentioned limitations seem unlikely to explain why SBP100W is a better predictor of the two for CVD events.

In our study, the participants started directly at a workload of 100W. This workload represents a sudden and relatively demanding workload for most individuals. The sudden increase in cardiac output needed to meet metabolic demands causes an abrupt increase in blood pressure unless accompanied by reduction in peripheral resistance caused by vasodilatation. Reduction of peripheral resistance is in turn dependent upon arterial compliance, the autonomic nervous system and endothelial properties (104-106). A steep rise in exercise SBP, most often reflected in a high SBP at moderate workload may better reveal subtle impairments in vasodilatation than maximal SBP as the cardiovascular system has had

short time to adapt to the an increased cardiac output (21). At maximal workload, the cardiovascular system has had more time to adapt to the increased cardiac output, and the peak SBP reached may also be influenced by CHD or impairment in left ventricular function (107). Although the men in the Oslo Ischemia Study cohort were apparently healthy, subclinical disease cannot be excluded. Even so, it is important to make a distinction between healthy individuals and those with known CVD as blood pressure responses to exercise may be different (107).

Fasting serum triglycerides

The present study was the first to show that fasting serum triglyceride (FTG) levels remain significant predictors of diabetes risk also after adjustment for physical fitness, and that physical fitness modifies this association only modestly, however somewhat more among unfit than among fit subjects. As discussed in paper 3, there are strong indications that almost all cases of diabetes in this study were type 2 diabetes. For the first time, we have shown that a reduction in FTG levels over time predicts reduced risk of type 2 diabetes among healthy middle-aged men. Previous evidence for an association between longitudinal changes in triglycerides and type 2 diabetes-risk is scarce, albeit existing for younger men (108).

Direct comparisons of the association between FTG and type 2 diabetes between our study and studies of other cohorts are inferential because of differences in baseline characteristics (109, 110). Tirosh et al followed 13,953 men for 10.5 years (108) with similar FTG levels but lower mean age at inclusion (32.4 years), which may explain the lower incidence of type 2 diabetes as compared to our study. Their main finding was, however, similar to ours with a HR of 2.01 (1.20 –4.38) for risk of diabetes in the highest FTG tertile (108). Similar findings have been demonstrated among women (110). Consistent with findings from these studies, FBG and BMI were strong predictors of diabetes in the present study (109, 111, 112).

Whether triglycerides play a causal role in the development of diabetes, or if raised serum triglyceride levels is a consequence of underlying abnormalities in glucose metabolism elucidated remains unknown. There are, however, several supporting arguments for a potential causal role. Hypertriglyceridemia is associated with higher plasma concentrations of free fatty acids, reduced insulin sensitivity and increased hepatic glucose production (40, 113-116). Furthermore, increased free fatty acids and insulin levels stimulate hepatic esterification of free fatty acids to triglycerides that may in turn further increase serum triglyceride levels (117). Elevated serum triglyceride levels may therefore be part of a series of adverse

metabolic changes eventually leading to diabetes. This hypothesis is supported by studies reporting reductions in hepatic and pancreatic triglyceride following calorie restriction, also reflected in serum triglyceride levels. These findings have in turn been shown to be associated with improved insulin sensitivity in type 2 diabetes (118). Insulin sensitivity is associated with physical fitness, and may therefore explain some of the effect of physical fitness on the triglyceride-diabetes association (40).

Reduction in FTG levels of more than 25% over 7.3 years predicted significantly reduced risk of diabetes in the present study. Fasting serum triglyceride levels and changes in those over time are probably sensitive markers of lifestyle and alterations in lifestyle such as physical activity and eating habits (119). Our results indicate that changes in FTG are associated with changes in other parameters related to lifestyle such as cholesterol, BP, BMI and physical fitness. Changes in BMI were not associated with diabetes risk in our study, and did not change results. One should, however keep in mind that changes in BMI were numerically low among the 1392 men. The reduced risk associated with FTG reduction remained significant when adjusting for point measures and changes in other predictors of diabetes. This may indicate that factors other than lifestyle, e.g., genetics, also determine changes in FTG levels over time (120).

Even if serum triglycerides may be partly determined by genetics, our results indicate that is possible to influence triglyceride levels by improving physical fitness and lifestyle. Interventions to lower triglycerides might lower the risk of type 2 diabetes, but this has to be confirmed in randomised controlled trial.

General discussion

We have investigated the predictive impact of HDL and exercise blood pressure on the risk of CHD and death, and the predictive impact of fasting serum triglycerides on diabetes. In all studies, we have studied the impact of physical fitness and changes in physical fitness in risk prediction. Relatively few epidemiological studies on healthy individuals have studied the impact of temporal changes in predictors and future CVD- or diabetes risk. The limited availability of repeated clinical examinations including exercise tests of all men in the Oslo Ischemia Study represents a rare opportunity to study the impact of repeated measures in risk prediction.

Physical fitness and physical activity

Physical fitness is usually defined as maximal aerobic capacity, and maximal oxygen uptake ($V0_{2max}$) is considered to be the gold standard for assessing physical fitness. Our method of measuring physical fitness has shown a high correlation with VO_{2max} (53). All methods of measuring physical fitness have, however, limitations because physical fitness is an integrated measure of aerobic performance dependent upon multi-organ function. Physical fitness also reflects psychological drive to exercise until exhaustion besides physiological ability. The mode of exercise may also play a role in the ability to reach the individuals maximal aerobic capacity (121).

HDL and Physical Fitness

One should expect that the association between baseline HDL and the outcome would be influenced if subsequent changes in physical fitness had the ability to improve HDL quality. The results must, however, be interpreted with caution since we did not have follow-up measures of HDL and therefore cannot study changes in HDL levels during the same time span. We have investigated the influence of the cross sectional gradient of physical fitness, and the natural course of changes in physical fitness on the predictive ability of HDL. This may influence HDL differently from exercise intervention as HDL response to exercise may vary with the kind of exercise, intensity, duration and acute effects of exercise may also differ from chronic effects of repeated exercise (40, 92, 94, 122). Our findings should therefore not be interpreted to mean that changes in HDL properties are impossible by physical exercise intervention which may affect lipid metabolism differently than spontaneous changes in physical fitness do. As previously mentioned, the response of blood lipids to physical exercise and increased physical fitness is complex and also vary with age, gender and several other

characteristics (94). In addition, it is estimated that as much as 25-40% of the level of physical fitness may be genetically determined. The ability to respond to exercise with increase in physical fitness also appears to vary significantly between individuals (123). We have shown that HDL seems to be independent of physical fitness when predicting CVD risk.

Exercise blood pressure and Physical Fitness

Adjustments for physical fitness and temporal changes in physical fitness weakened the association between changes in exercise blood pressure and disease risk in the present study. The changes in hazard ratios between the highest and the lowest quartile of exercise blood pressure imposed by adjusting for physical fitness and its changes were numerically in the similar range for all the endpoints, but there was no longer a significant association between exercise blood pressure and CVD- or all-cause death. Exercise blood pressure moderate workload and physical fitness are to some degree inversely correlated, and may represent different expressions of the same underlying physiological mechanisms. Adjusting for physical fitness and its changes might therefore be unnecessary, potentially underestimating the prognostic information of exercise blood pressure. On the contrary, it is possible that high levels of physical fitness and increase in physical fitness protect against the hazard associated with an increase in exercise blood pressure. This possible protective effect may be mediated through improving cardiovascular risk factors not accounted for in the present study, e.g. changes in endothelial properties (124, 125).

Physical fitness, triglycerides and diabetes

Adjustments for physical fitness had modest impact on the association between FTG and future diabetes risk. Directed acyclic graphs method (DAG) was used to determine the primary adjustment model for survival model, and body mass index (BMI) was not necessary to estimate the risk of type 2 diabetes. However, when adding BMI in a supplementary model, the adjustment for physical fitness had similar impact on the association compared with the primary adjustment model. Our results demonstrate an interaction between FTG and physical fitness in the type 2 diabetes risk prediction, being slightly stronger in unfit men compared to fit men. Changes in physical fitness over seven years did, however not modify the association between FTG changes type 2 diabetes risk.

Development of type 2 diabetes is caused by a complex interplay of genetic factors and lifestyle. Physical fitness could therefore modify the association between the risk of type 2 diabetes and FTG involving several possible mechanisms. To derive these mechanisms is challenging by the use of observational studies and we have attemted to suggest explanations

with existing data. First, FTG and physical fitness are both independent predictors of type 2 diabetes. Although inversely correlated, the two predictors convey somewhat different information about underlying genetic- and lifestyle factors. All these factors are not accounted for in our measurements and adjustments, and the modifying effect on the risk association might be explained by inclusion of more adjustment variables, weakening the independent impact of one predictor. This may also be the main reason why we found FTG to be a stronger predictor of diabetes in unfit than fit men.

Given the modifying effect of physical fitness on the association between point measures of FTG and diabetes risk, it was somewhat surprising that adjustments for physical fitness and seven-year changes in physical fitness had no impact on the association between seven-year changes in FTG levels and diabetes risk. Physical fitness had, however, a modest impact only on the FTG-diabetes association. Furthermore, physical fitness measured at survey 1 was not significantly different between categories of seven year change in FTG levels. Additionally, the change in physical fitness was numerically relatively small in all categories of FTG-change, -0.08 (kJ kg^{-1}) to -0.17 (kJ kg^{-1}) among men with a reduction in FTG of more than 25% and an increase of more than 25% respectively. Since we studied the FTG changes among a subset of 1387 men who were apparently healthy and free from diabetes at the second survey, it is unlikely that the levels of physical fitness and its changes are representative for men of the same age in the general population. This may have influenced the results.

Physical fitness or self reported physical activity?

Despite significant differences in physical fitness, we did not find any differences in self reported physical activity when comparing the baseline HDL quartiles in our study. Physical fitness was a significant, independent predictor of CHD and death whereas self-reported physical activity was not.

In line with previous reports, we found that physical fitness showed a stronger inverse association with diabetes risk than self reported physical activity did. Consequently, our findings are in line with previous knowledge that self reported physical activity and measured physical fitness are not necessarily highly correlated and that physical fitness should generally be preferred as a predictor of CVD and diabetes (48, 126). Self-reported physical activity is a subjective measure, and is burdened with recall bias and measurement errors. The level of physical fitness may also reveal physiological information not related to physical activity per se since an important proportion of physical fitness probably is genetically determined (127).

Exercise testing to determine physical fitness is, however costly and may not always be feasible for routine use. Estimates of physical fitness, not measured in exercise testing, might therefore seem attractive and may provide valuable prognostic information (128). However, further studies are needed to show the value of self-reported physical fitness when predicting CVD and type 2 diabetes.

The value of repeated testing

We found that both seven-years changes in exercise blood pressure was a significant predictor of CHD, CHD death and CVD death while a decrease in FTG was a significant predictor of reduced diabetes-risk. In both studies, the association was adjusted for the baseline level of the predictor studied. Given the significant prognostic impact of changes in some predictors of diabetes and CVD, repeated testing seems important in risk estimation, and should thus probably be considered in epidemiological studies when possible. Causality cannot be proven from epidemiological studies alone. One might, however, argue that associated changes in levels of a predictor and risk of outcome may indicate a higher likelihood of causality as compared to traditional point measure-outcome associations.

Repeated measurements in epidemiological studies to assess the predictive impact of spontaneous or life-style induced changes on outcome give important knowledge. This may be useful when designing intervention studies and interpreting results from them. This kind of epidemiological knowledge may be particularly important when an intervention fails to improve the disease risk despite improving a variable. An example of this was that the use of torcetrapib succeeded in raising HDL levels but failed to decrease CVD risk (129). In such cases, robust epidemiological evidence about associated temporal changes in risk marker and changes in outcome may help both in interpretation of results and also possibly in planning of further research on modifying the risk marker.

Is there a unifying link between HDL, exercise SBP and serum triglycerides?

Low HDL levels and high levels of serum triglycerides are associated with insulin resistance (130). Reduced insulin sensitivity has been shown to be associated with increased exercise SBP, poor physical fitness and impaired vasodilatation in apparently healthy young people (106, 131). As presented in this thesis, both low HDL levels and increased exercise SBP are associated with increased risk of CHD and death from CHD and CVD. Elevated FTG levels predict increased risk of diabetes, while temporal reductions in FTG levels predict reduced risk of diabetes. Fasting serum triglycerides also predict increased risk of CHD and CVD in

the Oslo Ischemia Study cohort, but the association did not reach statistical significance when adjusted for other CVD risk factors (unpublished data). The common denominator between the predictor presented in this thesis appears to be insulin sensitivity.

Strengths and limitations

Strengths

The cohort has been followed prospectively, all data sets are virtually complete and the findings refer to an apparently healthy population of middle-aged men followed for up to 35 years. All participants were examined under standardized conditions by the same physician (JE) at both Survey 1 and 2, and JE also closely supervised subsequent surveys. We have no work-up bias, and all event data are based on cause-specific death records and complete hospital records. Furthermore, all diagnoses of non-fatal events have been verified by physicians and cross-checked by two of the study investigators, and none are self-reported. Since the study group has not interfered with patient care, bias is minimized. None of the predictors investigated in our studies have been systematically or intentionally modified in any way neither before baseline, nor during the time span during which changes in predictors have been measured. Changes in predictors studied are therefore likely to reflect a natural course depending on lifestyle and age rather than treatment interventions.

Limitations

Our cohort consists of middle-aged Caucasian men who were healthy and employed at baseline. Our findings therefore, cannot be generalized to individuals of differing ethnicity, age, gender or individuals with morbidity.

The men included in the Oslo Ischemia Study were relatively lean with a BMI of 24.6 $kg/m^{2,}$ and only 335 (16.6%) men out of the 2014 had a BMI of more than 27 kg/m^2 whereas it was estimated that 27% of Norwegian men 16 to 44 years and 38% of men 45 to 79 years of age had a BMI of more than 27 kg/m^2 in 2012. Further, 882 (43.8) men in the cohort were smokers at inclusion. The proportion of daily smokers in the Norwegian population has been more than halved since the study was initiated (132). These changes are only examples of several changes in lifestyle and demographics since initiation of the Oslo Ischemia Study which requires caution if projecting these associations into the future.

The original aim of the Oslo Ischemia Study was to assess the prevalence and incidence of significant coronary artery disease within a cohort of apparently healthy middle-aged men. Initially, the follow-up was planned to be 5 years. All studies presented in this

thesis are based on follow-up extended up to 35 years and hypotheses developed more than three decades after inclusion of participants in cohort. It is difficult to assess how this might have influenced our findings. This should be kept in mind when interpreting results since the likelihood of finding spurious associations might increase with this method (133). On the contrary, all our hypotheses were specified before analysing any data, and all were biologically plausible.

Men who got various diseases between the surveys used in our studies were excluded when analyzing the prognostic impact of changes between those surveys. This was done deliberately because we wanted to study the prognostic impact among apparently healthy men and not among a mixed group of healthy and not healthy men. By this method, one will end up with an increasingly selected group of healthy individuals; and thereby probably increase the likelihood of introducing type II statistical errors. This concern was investigated with additional analyses of triglyceride changes excluding only men who got diabetes between Survey 1 and 2 while those who got other disease e.g. CVD were included. We found no important impact on results by performing this sensitivity analysis. We therefore believe that the selection of men who stayed healthy until Survey 2 did not introduce important selection bias impacting into our study of the triglyceride changes and diabetes risk, but similar analyzes were not performed in the other studies presented in this thesis.

Regression to the mean

All repeated measurements of biological variables may be influenced by the "regression to the mean" phenomenon. This phenomenon occurs because of the variability of both the measurement method and inherent physiological variation. Values that are outliers, or extreme at its first measurement will have a greater likelihood of being closer to the mean on a repeated measurement (134). The magnitude of regression to the mean may be estimated by statistical methods, and may also be reduced by serial measurements and using the average of those (134). In clinical intervention studies, one of the important effects of randomization is to reduce the impact of regression to the mean. In our studies of spontaneous changes in predictors, none of the above methods have been applied in order to reduce regression to the mean. The numeric values of the changes are therefore likely to have been higher than the real biological change, but will thereby also probably underestimate the strength of the associations between true changes and outcome.

Furthermore, the levels of many biological variables show a considerably inherent seasonal-, or circadian variation (83). Some variables may also vary during the week,

probably as a cause of changes in mental and physiological stress, eating and alcohol intake. Measurements should therefore preferably be repeated the same time of the day, same weekday and same time of the year each time when studying changes. Our measurements were not standardized this way at repeated surveys, but all measurements were performed in the morning in otherwise standardized conditions. When assessing changes of fasting serum triglycerides in prediction of diabetes, we performed additional adjustments for the time at which measurements were performed, and this had no impact on the results.

Endpoints

The diagnosis of angina pectoris was not confined to men with objective evidence of myocardial ischemia, and misdiagnosis cannot be ruled out. This potential limitation has been thoroughly discussed in Paper 1. After assessment of later CHD events among men diagnosed with angina pectoris compared to men without the diagnosis, we found that our diagnostic criteria appear to be sufficiently precise.

As discussed in Paper 3, our diagnosis of diabetes was based on the WHO 85 criteria to enable us to keep uniform criteria throughout the whole observation period. Available diagnostic information was not nearly sufficient to be able to apply modern diagnostic criteria retrospectively. It is difficult to estimate how modern diagnostic criteria would have influenced our results with respect to the FTG-diabetes association.

We believe that all other endpoints have high diagnostic validity compared to other long-term cohort studies as discussed in *Strengths*. Although we always have used the same diagnostic criteria for e.g. myocardial infarction in Oslo Ischemia Study, diagnostic criteria for myocardial infarction have changed since the seventies. We can only speculate have modern criteria would have influenced our results (135). We are however, confident that our definitions were robust in diagnosing myocardial infarctions, and that our results are more likely to underestimate than to overestimate risks. Further, the incidence, prevalence and treatment of CHD have changed since the Oslo Ischemia Study was initiated (1-4). All these changes make generalization of our exact results into today's epidemiology somewhat difficult. Even though, we believe that the above mentioned limitations are unlikely to affect individuals with different levels of predictors in a much skewed manner. We therefore believe that our results to a large extent are still valid for apparently healthy middle-aged men of Caucasian origin.

Alcohol

The participants responded to a questionnaire at inclusion in the Oslo Ischemia Study about drinking habits the last days prior to examination. These answers were considered to reveal part of their propensity to use alcohol. We have, however no detailed data with respect to amounts of alcohol consumed and no systematic information on alcohol consumption during follow-up. Lack of detailed data on alcohol consumption must be considered as an important limitation in our studies as alcohol intake may have significant impact on the levels of HDL, TG and SBP besides several other effects on the cardiovascular system (136). On the contrary, all participants in Oslo Ischemia Study were fully employed at inclusion and none reported alcohol abuse. This should support the notion that the average use of alcohol was within normal limits.

Clinical perspective

Our results are in line with existing evidence that HDL levels and physical fitness are associated to some extent. We found, however that HDL`s predictive ability on CHD and CVD remains similar at all levels of physical fitness among healthy middle-aged men. This finding supports that HDL level may be used for risk estimation without considering the level of physical fitness. This is in accordance with what has been done in the electronic version of the SCORE system as mentioned in Introduction. Further, our results indicate that HDL levels should be taken into account when estimating risk of CHD and CVD regardless of the level of physical fitness. Further research is needed to assess if our findings are valid for women, all age groups and people of other ethnicity.

Based on a previous publication from the Oslo Ischemia Study and some other studies that have shown prognostic impact of exercise blood pressure on CVD, the 2013 ESH/ESC hypertension guidelines have been changed and recommend ambulatory blood pressure measurement in the setting of normal resting blood pressure and an exaggerated blood pressure response to exercise (20, 73, 100).

The association we found between temporal changes in exercise blood pressure and future risk of CHD and death support previous evidence of a strong association between blood pressure measured at low- to moderate exercise and CVD risk. If confirmed in other studies, exercise blood pressure should be further investigated as a potential target of risk modification.

Future research should also compare the cost-effectiveness and predictive ability of ambulatory blood pressure and exercise blood pressure at low- to moderate workload both in CVD risk estimation and to reveal masked hypertension.

We found that high levels of physical fitness reduce the increased risk of diabetes only modestly. High levels of FTG should therefore not be neglected as a risk marker of future diabetes in fit individuals although the absolute risk of diabetes is lower than in unfit individuals. Significant reductions in FTG levels appear to be closely associated with significant and clinically important reduction in diabetes risk among healthy middle-aged men. If our findings are supported by future studies, methods of reducing FTG levels and their impact on diabetes risk should be investigated.

Conclusions

1. High-density lipoprotein cholesterol is a strong predictor of long-term risk of coronary heart disease and death from coronary heart- and cardiovascular disease in apparently healthy middle aged men. Physical fitness at baseline and changes in physical fitness after 8.6 years did not reduce the ability of HDL to predict CHD.

2. An increase in exercise SBP at 100W over seven years is independently associated with significantly increased long-term risk of CHD, CHD death, CV death and all-cause death. These associations are, however to some degree attenuated by physical fitness and temporal change in physical fitness. Our findings also support that increased exercise SBP is a strong long term cardiovascular risk factor in healthy men.

3. Fasting serum triglyceride levels are significant long-term predictors of diabetes among healthy middle-aged men and adjustments for physical fitness attenuates this association only modestly. Fasting serum triglycerides appear to be a stronger predictor of diabetes in men with poor physical fitness than in fit men. A reduction in fasting triglycerides over time is associated with reduced long-term risk of diabetes.

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