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Heart Rate Response to Exercise in the Prediction of Mortality and Myocardial Infarction

A Prospective Population Study in Men

Doctoral dissertation

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ABSTRACT

Heart rate (HR) is one of most easily measured exercise test variables. Although easily measured, the HR reflects a complex, integrated physiologic response: autonomic tone, central and peripheral reflexes, hormonal influences, and factors intrinsic to the heart are all important. During the last two decades exercise test derived HR variables have raised wide interest as prognostic markers of mortality and cardiac events both in asymptomatic persons and in patients with cardiovascular disease (CVD). The results of properly controlled studies suggest a possible bimodal relationship of HR to prognosis, in which both high HR at low workload and inappropriately low HR at maximal or near maximal workload are associated with adverse prognosis.

Workload achieved at HR of 100 beats/min (WL_{100}) and HR increase from 40% to 100% of maximal work capacity (HR40-100) were assessed using a maximal, symptom-limited exercise test on an electrically braked cycle ergometer. The complete data on exercise test variables was available for 1679 men in a population based sample of men. During the follow-up of eleven years, deaths were ascertained by linkage to the National Death Registry, and the classification of acute myocardial infarctions was carried out according to the multinational MONICA project protocol.

A new variable, HR40-100, quantifying an inappropriately low HR at maximal or near maximal workload was at least as strong a predictor of outcome as previously established related variables. A one standard deviation (SD) decrement in HR40-100 (13 beats/min) was related to an increased risk of all-cause death (relative risk, RR 1.3, 95% confidence interval, CI 1.1 to 1.6) and acute myocardial infarction (RR 1.3, 95% CI 1.1-1.6) in men without coronary heart disease (CHD) at baseline after adjustment for age, cardiovascular risk factors and exercise test variables. After exclusion of men whose test was possibly terminated prematurely because of submaximal effort, symptoms, or findings which could be interpreted to indicate latent CHD, a low HR40-100 still was an independent predictor of outcome. A one SD decrement in WL_{100} was related to an increased risk of CVD death (RR 1.7, 95% CI 1.3-2.4) and all-cause death (RR 1.7, 95% CI 1.3-2.2) in men without CHD at baseline and in men with known or suspected CHD at baseline after adjusting for risk factors, respectively. The exclusion of men who had an outcome event during the first two years of follow-up did not affect the results. A low WL_{100} was associated with a high resting HR and a low maximal oxygen uptake, but in survival analyses a low WL_{100} still provided additional prognostic information beyond these variables.

A blunted HR increase during the maximal exercise test is associated with an increased risk of death and adverse cardiac events in men without CHD at baseline. The association is particularly strong when the HR increase during the latter half of the test is considered. On the other hand, a low workload achieved at a submaximal HR of 100 beats/min predicts CVD and CHD mortality in men without CHD and all-cause death in men with known or suspected CHD. Contrary to previous interpretations, an exaggerated HR response at a low workload seems to indicate an increased risk by itself instead of being only a surrogate marker of a low cardiorespiratory fitness. The findings of the current thesis support the hypothesis that a bimodal relationship exists between HR and prognosis in which both an exaggerated HR response at submaximal workload and a blunted HR response at maximal or near maximal workload are associated with an adverse prognosis. In the current study sample several exercise test variables predict outcomes independent of each other and conventional risk factors. This emphasizes the importance of measuring several variables at submaximal and maximal workload and during recovery phase to maximize the prognostic yield obtained from the exercise test.

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Medical Subject Headings: Cardiovascular Diseases/epidemiology; Cardiovascular Diseases/mortality; Coronary Artery Disease; Exercise Test; Finland; Follow-Up Studies; Heart rate; Men; Myocardial Infarction; Risk Factor

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TIIVISTELMÄ

Sydämen sykintätaajuus, syke, on eräs helpoimmin mitattavista kuormituskoemuuttujista. Näennäisestä yksinkertaisuudestaan huolimatta mitattu syke on monimutkaisen säätelyn lopputulos, jossa autonomisen hermoston reflekseillä, hormonaalisilla tekijöillä ja sydämessä itsessään vaikuttavilla tekijöillä on kullakin oma osuutensa. Edeltäneiden kahden vuosikymmenen aikana kuormituskokeenaikaisen sykkeen on lukuisissa tutkimuksissa havaittu olevan yhteydessä kuolleisuuteen ja sydän- ja verisuonitautitapahtumiin sekä oireettomilla tutkittavilla että sydän- ja verisuonitautipotilailla. Tutkimusten perusteella sykkeen ja ennusteen yhteys on mahdollisesti kaksijakoinen niin, että sekä korostunut sykkeen nousu kevyessä kuormituksessa että toisaalta heikentynyt sykkeen nousu maksimikuormituksessa ovat yhteydessä huonoon ennusteeseen.

Tässä itä-suomalaisessa väestöpohjaisessa seurantatutkimuksessa tehtiin maksimaalinen kuormituskoe 1679 miehelle. Kuormituskokeessa määritettiin työmäärä, jonka tutkittava saavutti ennen sykkeen kohoamista yli 100 lyöntiä/min, ja sykkeen nousu välillä 40-100% tutkittavan maksimaalisesta suorituskyvystä. Tutkimuksessa selvitettiin näiden kahden sykemuuttujan ennusteellista merkitystä. Seurattavat päätetapahtumat 11 vuoden seuranta-aikana olivat kokonaiskuolleisuus, kuolleisuus sepelvaltimotautiin ja muihin sydän- ja verisuonisairauksiin, sekä akuutti sydäninfarkti. Nämä päätetapahtumat luokiteltiin hyödyntäen sairaalapoistotietoja ja valtakunnallisen kuolinsyyrekisterin tietoja.

Tutkimuksen keskeisinä löydöksinä todettiin, että heikentynyt sykkeen nousu välillä 40-100% tutkittavan maksimaalisesta suorituskyvystä ennustaa ennenaikaisen kuoleman ja akuutin sydäninfarktin riskiä miehillä, joilla ei ollut sepelvaltimotautia seurannan alkaessa. Tämä uusi heikentynyttä sykkeen nousua maksimikuormituksessa kuvaava muuttuja oli vähintään yhtä voimakas ennustaja kuin aikaisemmissa tutkimuksissa käytetyt vastaavaa ilmiötä kuvaavat muuttujat. Tämä tutkimus osoitti toisaalta sen, että matala saavutettu työmäärä ennen sykkeen kohoamista yli 100 lyöntiä/min ennustaa sydän- ja verisuonitautikuoleman riskiä miehillä, joilla ei ollut sepelvaltimotautia seurannan alkaessa. Sama muuttuja ennusti ennenaikaisen kuoleman riskiä myös miehillä, joilla oli todettu tai epäilty sepelvaltimotauti seurannan alkaessa. Matala työmäärä ennen sykkeen kohoamista yli 100 lyöntiä/min oli yhteydessä korkeaan leposykkeeseen ja huonoon kardiorespiratoriseen kuntoon, mutta elinaika-analyyseissä sen havaittiin kuitenkin tuovan ylimääräistä ennustearvoa näiden kahden ennestään tunnetun vaaratekijän lisänä. Kokonaisuutena tulokset tukevat hypoteesia, että sekä korostunut sykkeen nousu kevyessä kuormituksessa että heikentynyt sykkeen nousu maksimikuormituksessa ovat yhteydessä huonoon ennusteeseen.

Tämä väestötutkimus vahvistaa kliinisen kuormituskokeen merkitystä arvioitaessa myöhempien sydäntapahtumien ja enneaikaisen kuoleman vaaraa, sillä usealla kuormituskoemuuttujalla havaittiin olevan ennusteellista lisäarvoa perinteisiin vaaratekijöihin nähden. Tutkitut sykemuuttujat ennustivat päätetapahtumia sekä sepelvaltimotautia sairastamattomilla miehillä että sitä sairastavaksi todetuilla tai epäillyillä miehillä. Koska syke on eräs helpoimmin mitattavista kuormituskoemuuttujista, sykevasteen arviointi sekä kevyessä että maksimaalisessa kuormituksessa kannattaa ottaa huomioon arvioitaessa tutkittavan ennustetta kuormituskoetulosten perusteella.

Yleinen suomalainen asiasanasto: sydän- ja verisuonitaudit, epidemiologia; sydän- ja verisuonitaudit, riskitekijät; syke; sydäninfarkti, miehet, Suomi

“A man’s nature and way of life are his fate,
and that which he calls his fate is but his disposition.”

Menander (342-291 B.C.)

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Kuopio, April 2008

Kai Savonen

ABBREVIATIONS

CHD	Coronary heart disease
CVD	Cardiovascular disease
ECG	Electrocardiogram
HR	Heart rate
BP	Blood pressure
SA	Sinoatrial
AV	Atrioventricular
mV	Millivolt
rVLM	Rostral ventrolateral medulla
cVLM	Caudal ventrolateral medulla
VO_{2max}	Maximal oxygen consumption
VO_2	Oxygen consumption
BMI	Body mass index
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
W	Watts
WL_{100}	Workload achieved at a submaximal heart rate of 100 beats/minute
HR40-100	Heart rate increase from 40% to 100% of maximal work capacity in exercise test
KIHD	Kuopio Ischaemic Heart Disease Risk Factor Study
submaxCRI	Chronotropic response index at submaximal work
SBP	Systolic blood pressure
ICD	International Classification of Diseases
MONICA	MONItoring of Trends and Determinants in CArdiovascular Disease
AMI	Acute myocardial infarction
SD	Standard deviation
RR	Relative risk
CI	Confidence interval
ANOVA	Analysis of variance
maxCRI	Chronotropic response index at maximal work

LIST OF ORIGINAL PUBLICATIONS

This dissertation is based on the following original publications referred to in the text by their Roman numerals I – IV:

I Savonen K, Lakka T, Laukkanen J, Halonen P, Rauramaa T, Salonen JT, Rauramaa R. Heart rate response during exercise test and cardiovascular mortality in middle-aged men. *Eur Heart J* 2006;27:582-588.

II Savonen K, Lakka T, Laukkanen J, Rauramaa T, Salonen JT, Rauramaa R. Effectiveness of workload at the heart rate of 100 beats/min in predicting cardiovascular mortality in men aged 42, 48, 54, or 60 years at baseline. *Am J Cardiol* 2007;100:563-568.

III Savonen K, Lakka T, Laukkanen J, Rauramaa T, Salonen JT, Rauramaa R. Workload at the heart rate of 100 beats/minute and mortality in middle-aged men with known or suspected coronary heart disease. *Heart* 2008;94:e14.

IV Savonen K, Lakka T, Laukkanen J, Rauramaa T, Salonen JT, Rauramaa R. Usefulness of chronotropic incompetence in response to exercise as a predictor of myocardial infarction in middle-aged men without cardiovascular disease. *Am J Cardiol* 2008;101:992-998.

CONTENTS

1. INTRODUCTION

Coronary heart disease (CHD) is the leading cause of death in the developed world (1) and may become the leading cause of death in the entire world (2). Although the incidence of CHD has been decreasing over the last two decades, the prevalence is expected to increase given the growing elderly population (3-5). It is important to implement cost-effective strategies that direct the appropriate individuals to the optimal risk reduction procedures through risk prediction (4-6). There is a growing awareness of the need to apply statistical techniques to develop evidence-based scores for better decision making (7). The goal of risk prediction through statistical methods is to provide a logical estimate as to the likelihood of the occurrence of important deleterious clinical events (3-5). The most important outcome is death, but the future risk of nonfatal clinical outcomes is also an important element of risk evaluation (3-5). Based on the worldwide epidemiological experience, the evaluation of cardiovascular risk is based on four time-honoured classical cardiovascular disease (CVD) risk factors: age, serum cholesterol, resting systolic blood pressure and smoking status (8).

Exercise testing is not recommended in low-risk asymptomatic subjects due to both lacking evidence of its value and because false positive exercise electrocardiograms (ECGs) are common (9). However, the real issue is not to identify CHD, but to predict outcome (10). Because of this, increasing attention has been focused on the exercise test as a prognostic, as opposed to diagnostic, modality (11,12). It is well known that several exercise test indices in addition to ECG findings, such as exercise capacity (9,13), heart rate (HR) (14,15) and blood pressure (BP) responses (16) to exercise, are strong predictors of CVD events.

Exercise is the body's most common physiologic stress, and it places major demands on the cardiovascular system (17). The exercise test provides a precise and powerful noninvasive tool that permits the study of the regulation of the cardiovascular system under rigorously controlled and highly reproducible conditions, which include the full range of its functional capacity (18). The adaptations that occur during an exercise test allow the body to increase its resting metabolic rate up to 20 times, during which time cardiac output may increase as much as six times (17,19). The obvious advantage to the

researcher is that more is learned about how a system operates when it is forced to perform than when it is idle (18). Acute exercise can elicit cardiovascular abnormalities that are not evident at rest, and it can be used to determine the adequacy of cardiovascular function (19).

Heart rate is one of the most easily measured exercise test variables. Although easily measured, the HR reflects a complex, integrated physiologic response: autonomic tone, central and peripheral reflexes, hormonal influences, and factors intrinsic to the heart are all important (20-22). Recently, the changes in HR during and after exercise have emerged as powerful measures of risk for future CVD event in their own right (23). An interesting fact from the viewpoint of basic exercise physiology is that the mechanisms mediating the association of exercise HR variables and an increased risk of outcomes are largely unknown (24-26).

Office-based assessment of conventional risk factor burden is necessary, but may not accurately estimate risk of future CVD events (27). There is a need for easily available non-invasive methods to detect individuals with an increased risk of CVD events who would probably benefit most from preventive measures (28). The main aim of the current study is identify variables derived from exercise test HR which might serve as useful predictors for future CVD events and possibly provide additional prognostic information to conventional risk factors in a population-based sample of middle-aged men.

2. REVIEW OF LITERATURE

2.1 Sinoatrial node

The normal heart beat starts in the sinoatrial (SA) node (20,21,29). The normal HR is determined by the firing frequency of SA node (20,21,29). The SA node is a small, flattened, ellipsoid strip of specialized cardiac muscle and associated fibroelastic connective tissue about 3 mm wide, 15 mm long, and 1 mm thick (29-31). It contains clusters of cells, poor in contractile filaments, where the automatic activity resides mostly in the pacemaker or P cells (32). Near the periphery of the SA node lie also another group of cells which are called as transitional cells (33). The SA node is located in the superior lateral wall of the right atrium immediately below and slightly lateral to the opening of the superior vena cava, near the superior end of the sulcus terminalis (20,29,34). Its primary source of blood is from the SA nodal artery, which originates from the right coronary artery in about 60% of humans (29,35,36).

The normal cardiac impulses starts at the SA node, passes through the atrial tissue through preferential internodal tracts to the atrioventricular (AV) node where it slows, and then continues down the His-Purkinje system to the ventricular myocardium, where the wave of depolarization terminates when there is no further tissue to depolarize (20,31,37). Further conduction occurs only after a new impulse is formed in the SA node (38).

Many cardiac cells, especially the cells of the heart's specialized conducting system, have the capability of self excitation, a process that can cause automatic rhythmical discharge and conduction (39). The resting potential of a typical cardiac cell is -80 to -90 millivolts (mV) (38,39). When it is depolarized to a certain threshold level (threshold potential), an action potential is produced as a result of a complex series of ionic shifts (37-39). The appearance of the action potential of SA nodal cells is different from that of the typical myocyte (38,39). The normal resting potential of these cells is higher (-55 to -60 mV), and the spontaneous diastolic depolarization is much more pronounced (38-40). The slope of the diastolic depolarization determines the rate at which a cell will

spontaneously depolarize (automaticy) until it reaches the threshold potential, thus generating an action potential that is then propagated to surrounding cells (38,39,41).

The spontaneous cyclic depolarization of primary pacemaker cells in the SA node that establish intrinsic HR arises from the unique time-dependent characteristics of a variety of depolarizing and hyperpolarizing currents (37,39,42). The ionic basis of SA node pacemaker activity is such that action potential configuration is determined mainly by outward hyperpolarizing K^+ current, I_K, and two depolarizing inward currents, I_{Ca} and I_f, that are carried primarily by Ca^{2+} and Na^+, respectively (37,40,43). At the termination of one SA node action potential, the membrane voltage does not stabilize to a negative level but slowly creeps up with an approximately constant slope, until it reaches the threshold for a new SA node action potential (39,44). The gradual membrane depolarization has been attributed to an overall diminution in the net conductance of hyperpolarizing K^+ currents and a constant background inward current caused by the spontaneous inward movement along the concentration gradient for Na^+ ions (37,45,46). A major role, however, in the generation and control of the diastolic depolarization is played by a prominent increase in the inward depolarizing current I_f (37,39,46). An initial membrane depolarization leads to the activation of a transient (T-type) Ca^{2+} current ($I_{Ca,T}$) which results in Ca^{2+} influx into a confined subsarcolemmal space between the sarcolemma and sarcoplasmic reticulum, and [Ca^{2+}] in the subsarcolemmal space begins to increase (37,42). This triggers the focal release of Ca^{2+} (sparks) from sarcoplasmic reticulum Ca^{2+} release channels, further increasing [Ca^{2+}] in the space (37). This in turn leads to the activation of forward Na^+-Ca^{2+} exchange (I_{NCX}) and further membrane depolarization toward a threshold potential (37). Since the stoichiometry of the exchange is three Na^+ for one Ca^{2+}, the current is electrogenic and mediates a net inward current, I_{NaCa} (37,42). The interrelated actions of $I_{Ca,T}$, sarcoplasmic reticulum Ca^{2+} initiates, and I_{NaCa} creates a positive feedback loop that culminates in a progressive membrane depolarization to the threshold potential (37). Once threshold is achieved, an L-type inward Ca^{2+} current ($I_{Ca,L}$) rapidly activates, and an action potential is triggered (42,43,47).

The SA node usually has the fastest diastolic depolarization and thus functions as the normal pacemaker of the heart (20,21,29). If the SA node fails, the AV node has the next fastest pacemaker rate (approximately 40-60 beats/min) (21,38,39).

2.2 Cardiac innervation

The normal myocardium is richly innervated by the autonomic nervous system (31,48). The heart is supplied by autonomic nerve fibers from superficial and deep cardiac plexuses from which three major cardiac nerves project into the heart (35,49). These nerve networks lie between the bifurcation of the trachea and the ascending aorta, and superior to the bifurcation of the pulmonary artery (35). The parasympathetic supply is from preganglionic cardiac branches of the vagus nerves (21,50,51). The cell bodies of the parasympathetic postganglionic fibers constitute intrinsic ganglia in the vicinity of SA and AV nodes (20,48,50). The postganglionic parasympathetic fibers innervate primarily the atria. There are a few projections to the ventricles, however, and there is increasing evidence to show that the vagal nerves innervate the ventricular myocardium as well (48,52,53). The cell bodies of cardiac afferent vagal neurons are contained within the nodose ganglia inferior to the jugular foramen (35,50,54). The central fibers of these bipolar neurons continue to ascend in the vagus to enter the brain stem (35,50,54).

Both pre- and postganglionic cardiac parasympathetic fibers release acetylcholine as neurotransmitter (48,50,51). The effects of acetylcholine on the heart are mediated by muscarinic M_2-receptors, but the neural transmission between pre- and postganglionic fibers of both the sympathetic and parasympathetic systems is mediated by nicotinic N_N-receptors (35,50,51). Once acetylcholine has been secreted, it persists in the tissue for a few seconds. Thereafter, most is split into acetate and choline by the enzyme acetylcholinesterase (48,55).

The sympathetic supply is from postganglionic cardiac sympathetic fibers (20,21,51). The cell bodies of pre- and postganglionic fibers are located in the intermediolateral cell columns of the lateral horns of the superior five or six thoracic segments of the spinal cord, and in the cervical and superior thoracic paravertebral ganglia of the sympathetic

trunks, respectively (20,21,51). Sympathetic nerve terminals are located throughout the atria and ventricles (20,21,56). The cardiac afferent sympathetic neurons have their cell bodies in the C_6-T_6 dorsal root ganglia, and they enter the dorsal horn of the spinal cord (35,50,57). They synapse on cells in the outer part of the dorsal horn, the axons of the second-order neurons immediately decussate and ascend through the spinothalamic tract to reach the thalamus (50,57).

Pre- and postganglionic cardiac sympathetic fibers release acetylcholine and noradrenaline as neurotransmitters, respectively (20,50,51). In addition to innervation directly from sympathetic nerve endings, the sympathetic nervous system may have an effect indirectly by stimulating the adrenal medulla to secrete adrenaline and noradrenaline into the circulating blood (50,51,58). These two hormones can further bind to adrenergic receptors in the heart (50,51,58). The effects of noradrenaline and adrenaline on the heart are mediated by α_1-, β_1- and β_2-adrenergic receptors (50,51,58). β_1 is the most abundant subtype of adrenergic receptor in the heart, representing approximately 75% of total (20,51,58). Noradrenaline excites mainly α-receptors, but also excites the β-receptors to a lesser extent (39,59). Adrenaline excites both types of receptors approximately eqally (39,59). Noradrenaline is removed from the secretory site in three ways: (i) reuptake into the adrenergic nerve endings (accounting for removal of 50%-80% of the secreted noradrenaline); (ii) diffusion away into the surrounding body fluids; and (iii) destruction by enzymes (32,48,55).

The latency of the response of the SA node to vagal stimulation is very short (18,21,48). After a single stimulus, the maximum response has been reported to occur within only 400 milliseconds (18,48,60). Thus, vagal stimulation results in a peak response either in the first or in the second beat after its onset (21,48). On the other hand, following the onset of sympathetic stimulation, there is a latent period of up to 5 seconds. This is followed by a progressive increase in HR, which reaches a steady level in 20 to 30 seconds (18,21,48). Both parasympathetic and sympathetic preganglionic fibers are myelinated, whereas postganglionic fibers do not have a myelin sheath (50,61). The fact that parasympathetic postganglionic fibers are clearly shorter than sympathetic postganglionic fibers partly explains the slower cardiac responses to

sympathetic stimuli than to parasympathetic stimuli, because the neural transmission is faster in myelinated fibers (39).

The HR is determined from the ECG as the reciprocal of the time interval between two successive R peaks (which reflect depolarization of ventricles) and expressed as beats/minute (17,20). The intrinsic HR, in the absence of any neurohumoral influence, is about 100 to 120 beats/min and declines with age (21,62,63). Without neurohumoral influence maximal HR at peak exercise is 18-24% lower than with intact neurohumoral influence (63,64). In the intact, unblocked individual, the HR at any time represents the net effect of the vagal (parasympathetic) and the sympathetic nerves, which play a key role in the regulation of the HR by modulating the intrinsic pacemaker activity of the heart (19,36,65). In resting conditions, both autonomic divisions are thought to be tonically active with the vagal effects dominant (20,36,51). In normal adults at rest, the HR is about 70 to 85 beats/min and the normal range is 60 to 100 beats/min (29,65,66).

SA and AV nodes are the most densely innervated regions of the heart and are most affected by changes in autonomic tone, allowing for neural regulation of the HR (37,48). There is some asymmetry in the distribution of autonomic fibers to the heart, and the SA node is predominantly innervated by fibers from the right side (21,29,67). Weak to moderate vagal stimulation will slow the HR often to as little as one-half normal (39,48). Strong vagal stimulation of the heart can stop the heartbeat for a few seconds, but then the heart usually “escapes” and beats at a rate of 20 to 40 beats/min thereafter, paced by a pacemaker elsewhere than in the SA node (21,36,39). Increased vagal input into the SA node results in the release of acetylcholine from nerve endings in the SA node and the released acetylcholine binds to muscarinic receptors (37,39,48). Acetylcholine can directly activate a specific class of K^+ channels (K_{ACh}) in SA node cells, which produces a hyperpolarizing current that opposes the effects of depolarizing currents during diastole (37,42,48). Also, the release of acetylcholine slows HR by suppressing membrane-bound adenylate cyclase activity via a G-protein-coupled M_2-adenylate cyclase mechanism (see next paragraphs) (42,46,47). The former mechanism is more prominent at higher levels of vagal activation and explains acetylcholine-mediated action potential hyperpolarization (37,42). The latter mechanism occurs at lower levels of vagal activation and provides a conceptual explanation for a reduction in

the rate of diastolic depolarization without prominent membrane hyperpolarization (37,42). Besides these mechanisms vagal activation may affect diastolic depolarization by inducing an upward shift in action potential threshold (37). The net effect of these three mechanisms is to prolong the time required for diastolic depolarization to proceed to an action potential threshold (37).

An increase in sympathetic activity forms the principal method of increasing HR above the intrinsic level generated by the SA node to the maximal levels achieved (21,68,69). Strong sympathetic stimulation can increase the HR in adult humans to 180 to 200 beats/min (39,70). Sympathetic stimulation, either directly from sympathetic nerve endings in the heart (noradrenaline) or indirectly by means of circulating adrenaline, accelerates the pacemaker activity of SA node cells (46,47,56). This manifests itself as a marked increase in the rate of diastolic depolarization and an increase in the amplitude of the pacemaker action potential (37,43).

The increase in the rate of diastolic depolarization results from cyclic adenosine monophosphate-mediated enhanced intracellular Ca^{2+} handling and from an increase in the magnitude of I_f (42,46,47). The binding of sympathetic neurotransmitter to β-adrenergic receptor leads to the G-protein-mediated activation of a membrane-bound adenylate cyclase, the formation of cyclic adenosine monophosphate, and subsequent activation of a cyclic adenosine monophosphate-dependent protein kinase A (42,46,47). The active catalytic subunits of protein kinase A phosphorylates sarcoplasmic reticulum Ca^{2+} pumps and sarcoplasmic reticulum Ca^{2+} release channels leading to enhanced sarcoplasmic reticulum Ca^{2+} loading as well as increased Ca^{2+} sparks frequency (37). These events culminate in a faster increase in $[Ca^{2+}]$ in the subsarcolemmal space and a more robust activation of a depolarizing I_{NCX} (37). On the other hand, the channels mediating I_f are activated by cyclic adenosine monophosphate through the direct binding, and not by protein kinase A-mediated phosphorylation (46,47). The net effect of these cyclic adenosine monophosphate-mediated processes is a shortening of diastolic depolarization and an increase in HR (42,46,47). Still one possible β-adrenergic-mediated mechanism involves stimulation of Na^+-K^+-pump activity (32). The consequent hyperpolarization changes the pacemaker potential in early diastole into

the zone required for activity of the I_f current, so that less time is required to activate this current to initiate the following diastolic depolarization (32).

2.3 Regulation of heart rate by central nervous system

All levels of the central nervous system contribute to the regulation of cardiovascular activities, but the main cardiovascular regulating centers are located in the brain stem (20,29,48). Located bilaterally mainly in the reticular substance of the medulla and lower third of the pons is an area called the vasomotor center (35,39). The center transmits parasympathetic impulses through the vagus nerves to the heart and sympathetic impulses through the spinal cord and peripheral sympathetic nerves to the heart and blood vessels of the body (39,48). From the viewpoint of cardiovascular control the most important parts of the vasomotor center are the nucleus tractus solitarius, the ventrolateral medulla, the dorsal motor nucleus and the nucleus ambiguus (71). The nucleus tractus solitarius, which lies in the posterolateral portions of the medulla and lower pons, receives sensory nerve signals from thoracic and abdominal organs mostly via vagus nerves and from the carotid sinuses via the glossopharyngeal nerves (56,72,73). The output signals from the nucleus tractus solitarius controls the activities of those areas in the vasomotor center, which in turn regulate the descending parasympathetic and sympathetic output (72-74). Widespread areas of the higher nervous centers can either excite or inhibit the vasomotor center (56,67,75). The more lateral and superior portions of the reticular substance cause excitation, whereas the more medial and inferior portions cause inhibition (39). The hypothalamus can exert either powerful excitatory or inhibitory effects on the vasomotor center: the posterolateral portions cause mainly excitation, whereas the anterior part can cause mild excitation or inhibition, depending on the precise part of the anterior hypothalamus stimulated (39,75,76). Of various parts of the cerebral cortex, anterior temporal lobe, the orbital areas of the frontal cortex, the anterior part of the cingulate gyrus, the amygdala, the septum and the hippocampus can all either excite or inhibit the vasomotor center, depending on the precise portion of these areas that is stimulated and on the intensity of the stimulus (39,76).

The parasympathetic efferent preganglionic neurons are located for the most part in the nucleus ambiguus of the medulla. Lesser numbers are located in the dorsal motor nucleus and the regions in between these two medullary nuclei (20,77,78). Parasympathetic activity to the SA node originates from the central nervous system rather than from peripheral ganglia (76), and severing of the preganglionic fibers, leaving only postganglionic innervation intact, releases the heart from parasympathetic inhibition (79). Preganglionic cardiac vagal fibers are tonically active, with a firing pattern that is pulse synchronous and most active during expiration and reduced during inspiration (respiratory sinus arrhythmia) (71,79). The cardiac vagal neurons in the nucleus ambiguus, however, do not display any pacemaker-like activity such as repetitive or phasic depolarizations or action potentials, but in the absence of synaptic activity those neurons are normally silent (71,80). Synaptic input to cardiac vagal neurons are therefore important in maintaining normal heart rate and cardiac function (79). A major pathway to the nucleus ambiguus originates from the nucleus tractus solitarius, and electrophysiological experiments demonstrate that the pathway is glutamergic (52,81,82). It is still unknown whether the nucleus tractus solitarius neurons relays sensory information project directly to cardiac vagal neurons, or whether there are synapses within the nucleus tractus solitarius before the sensory information is ultimately communicated to cardiac vagal neurons (71,79). Cardiac vagal neurons also have excitatory input from cholinergic nicotinic neurons, which are possibly involved in the respiratory sinus arrhythmia (79), and from dopaminergic neurons, which induce bradycardia via activation of D_2-receptors (83).

The ventrolateral medulla consists of rostral (rVLM) and caudal (cVLM) parts. A population of rVLM neurons (premotor neurons) project onto the interomediolateral cell column of the spine and constitute the main and final integration center in the brainstem for generating the sympathetic outflow to cardiovascular effector organs (71,72,84). Under normal conditions rVLM transmits signals continuously to the sympathetic preganglionic fibers in interomediolateral cell column via glutamergic transmission (39,71,84). This tonic sympatho-excitatory activity of rVLM is, however, continuously inhibited by gamma-aminobutyric acid-mediated transmission from cVML (71,72,84). cVML in turn receives tonic glutamergic excitatory input from the nucleus tractus

solitarius (71,72). Hence the tonic sympatho-excitatory transmission from rVLM to sympathetic preganglionic fibers is modulated by the degree of inhibitory drive from cVLM neurons, which in turn are under the control of the nucleus tractus solitarius (71,72,84).

2.4 Factors modulating heart rate response to neural stimulation

A complex interaction between sympathetic and vagal activity may be more important in the modulation of the HR than either branch alone (29). Postganglionic sympathetic and vagal fibers often lie side by side in the walls of the heart (48,85). Therefore, the neurotransmitters and neuromodulators released from nerve fibers of one autonomic division can influence the release of transmitters from the nerve endings of the other division (48,86). When both divisions of the autonomic nervous system are stimulated simultaneously, the resultant cardiac effect is often different from the algebraic sum of the individual responses obtained by stimulating the nerves from the two divisions separately (48,87). A prominent feature of such cardiac autonomic interactions is that the vagal effects tend to predominate over the sympathetic effects with respect to the control of HR (accentuated antagonism) (48,79,87). Two major mechanisms have been suggested to explain the antagonist effects of vagal stimulation on sympathetically induced responses (48,79,87). The first is a presynaptic mechanism, in which acetylcholine reduces the amount of noradrenaline released from sympathetic nerve terminals (48,79,88). The second is a postsynaptic mechanism, in which acetylcholine reduces the magnitude of the response to a given adrenergic stimulus (48,79,87). This second mechanism presumably involves inhibitory G_i-protein-dependent inhibition of cyclic adenosine monophosphate synthesis (79,89).

On the other hand, intense sympathetic stimulation attenuates the chronotropic responses to vagal stimulation in the case when sympathetic stimulation antecedes vagal stimulation (90). This profound inhibition of vagal efficacy by antecedent sympathetic activity is believed to be mediated by the release of a specific neuromodulator, neuropeptide Y, from the sympathetic nerve endings (91). Neuropeptide Y inhibits acetylcholine release from vagal nerve endings (90). Additionally, catecholamines can

reduce the release acetylcholine by binding to α-adrenergic receptors in the presynaptic region of the parasympathetic nerve fibers (69). Finally, a high concentration of noradrenaline released into the synaptic cleft by a sympathetic postganglionic fiber may limit the subsequent release of further noradrenaline through binding to α_2-receptors on the presynaptic nerve terminal (a negative feedback mechanism) (20,69).

Opioids modulate parasympathetic control of HR via receptors in the SA node (92), prejunctionally on vagal nerve terminals (93), or within nearby parasympathetic ganglia (93) resulting in attenuation of vagally mediated bradycardia. Angiotensin II exerts inhibitory effect upon the cardiac vagal nerves (94) while having a facilitatory effect on the sympathetic ganglia (18,95,96). Nitric oxide facilitates parasympathetic control of HR by increasing central (97) and peripheral (98-100) vagal neuronal activity. Additionally, nitric oxide might have an inhibitory effect on sympathetic control of HR (101). Vasopressin (or antidiuretic hormone), although having the vasonstrictor action in vascular system, can increase cardiac vagal activity to some extent (102).

Besides the pre- and postsynaptic interactions described above, processing probably occurs within the intrinsic cardiac nervous system, which involves afferent neurones, local interconnecting neurones as well as both parasympathetic and sympathetic efferent postganglionic neurones (78). Intrinsic cardiac ganglionic interactions represent the organ component of the hierarchy of intrathoracic nested feedback control loops, which provide rapid and appropriate reflex coordination of efferent autonomic outflow to the heart (78).

The chronotropic response of the SA node to a fixed neuronal stimulus can vary as a result of the change in receptors number and activity (32,58). The number of receptors per unit area of the SA node sarcolemma (the receptor density) is not fixed, but can rise or fall in response to certain physiological or pathophysiological circumstances, processes called up- and downregulation (32,58). For example, in congestive heart failure there is a chronic high-level exposure to catecholamines, which causes a reduction in the number of β_1-receptors, whereas β_2-receptor density remains constant (32,103,104). The second form of the changed response is uncoupling, which refers to a state of the receptor where there is no loss in density, but functional activity is diminished (58,103). The underlying molecular mechanisms of uncoupling are the

increased levels of inhibitory G_i-proteins, leading to a reduced ratio of G_s/G_i and an impaired G_s-mediated coupling between the β-receptor and adenylate cyclase (103,104). The third form of the changed response is a change in receptor affinity (32). For example, β-agonist catecholamines induce or stabilize a high affinity form of the β-adrenergic receptor, which is specific for agonists and binds antagonists rather weakly (32).

The chronotropic response of the SA node to neural stimulation also involves genetic variation, which is still poorly understoood (105-107). The Arg389Gly (108,109) and Ser49Gly (58) polymorphisms of the β_1-adrenergic receptor are associated with difference in HR at rest, but this has not been a consistent finding, however (110). During exercise there is no difference between Arg389Gly genotypes (111-113). The gene GNAS1 encodes α-subunit of the stimulatory G-protein that couples β_1-adrenergic receptor with the adenylyl cyclase (106). The T393C polymorphism of GNAS1 modulates HR response when values at rest, maximal exercise and recovery are considered together (106). The Arg16Gly polymorphism of the β_2-adrenergic receptor is associated with difference in HR at rest (107,114), but during low or high intensity exercise the difference in HR does not persist between genotypes (107). The rs324640 polymorphism of the muscarinic M_2-receptor gene is associated with a difference in HR recovery after exercise, but maximal HR is not different between the genotypes (105).

2.5 Mechanisms controlling heart rate

Although easily measured, the HR reflects a complex, integrated physiologic response: autonomic tone, central and peripheral reflexes, hormonal influences, and factors intrinsic to the heart are all important (Figure 1) (20-22). Some reflexes may increase HR through a decrease in vagal tone, an increase in sympathetic activity, or both, whereas others exert the opposite effects (21,22). In the intact human cardiovascular system several reflexes and control mechanisms operate simultaneously, and the interactions are quite complex (21,22).

The relative importance of neural control mechanisms in determining the cardiovascular response to exercise is dependent upon the type of exercise (static or

dynamic), the intensity of the exercise, the time after the onset of exercise (immediate, steady state, exhaustion, etc.), and the effectiveness of blood flow to meet the increased metabolic needs of the contracting muscle (22,115). Control mechanisms for the cardiovascular response during exercise are somewhat redundant, rather than additive, and they impinge on the same regulatory neurons in the vasomotor center of medulla, and, possibly, other sites, where integration of afferent information occurs (115,116). Besides neurally mediated reflexes, some humoral factors, such as cortisol, glucagon, growth hormone and thyroxine may play a minor role in modifying the control of HR (20,29,55).

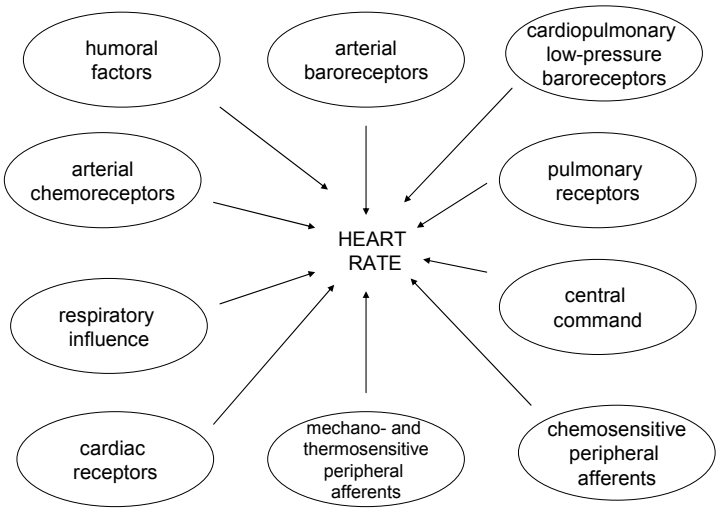

Figure 1. The overview of mechanisms influencing heart rate. See text for the more detailed discussion about the effects mediated by each individual mechanism.

2.5.1 Arterial baroreceptors and cardiopulmonary low-pressure baroreceptors

The function of the arterial baroreceptors is to maintain a normal BP (117-119). The arterial baroreceptors include carotid and aortic baroreceptors, which are spray-type nerve endings located in the walls of the large arteries (29,50,119). Carotid receptors lie in the wall of the internal carotid artery on either side (21,29,120). Each send impulses

centrally in the sinus nerve (of Hering), a branch of the glossopharyngeal nerve (50,120,121). Aortic arch receptors on the left lie within the aortic arch, but those on the right lie at the origin of the right subclavian artery and in the adjacent regions of the brachiocephalic artery (29,120,122). From the aortic receptors, activity travels centrally in small vagal branches (50,120,121). The nerve endings in the carotid sinus and aortic arch are activated by expansion of the arterial wall when BP is increased, and this stretching result in increases in discharge frequency in their afferent nerves (arterial baroreflex) (118,119,122).

Afferent nerves transmit the baroreceptor activity to the nucleus tractus solitarius, which modulates autonomic outflow to buffer the rise in pressure (21,29,119). Conversely, a fall in BP reduces baroreceptor discharge and trigger adjustments that oppose the hypotension (21,118,119). The baroreceptors respond much more to a rapidly changing pulsatile pressure than to a stationary pressure (39,69). In dogs, arterial rheoreceptors that respond to increased blood flow by sensitizing the function of baroreceptors have been identified in the carotid sinus (123). The existence of rheoreceptors in humans is not known.

Because mean arterial BP equals cardiac output times total peripheral resistance, the nucleus tractus solitarius can induce changes in BP by affecting either cardiac output, total peripheral resistance, or both via modulation of neural output (18,118,124). In response to a hypertensive stimulus, there is a rapid decrease in HR due to increased vagal discharge to the heart (18,121,125). In hypotensive stimulus there is an initial rapid increase in HR with withdrawal of vagal tone, followed by a slower rise in HR due to increased sympathetic discharge (18,121,125). The increase in total peripheral resistance (vasoconstriction) plays a major role in response to a hypotensive stimulus. In contrast, a decrease in HR is more important in response to a hypertensive stimulus (18,125). At rest roughly one third of the changes in arterial BP during carotid baroreceptor stimulation are due to changes in HR and two-thirds are dependent on alterations in total peripheral resistance, but the corresponding estimate for the overall baroreflex (carotid and aortic parts combined) is not known (126). Previously it was assumed that aortic baroreceptors operates over a higher range of arterial pressures than carotid receptors (21,39,127), but more recent studies have shown that both

baroreceptor populations operate over the same range of pressures (128). The estimated contribution of carotid baroreflex to overall baroreflex control is from 30% to 50 % (128,129). Aortic and carotid baroreceptors summate in their reflex effects (21,125). Because the relationship between total baroreceptor input and response is sigmoid, the type of summation (linear, inhibitory or facilitatory) depends critically on the size of the stimulus and the magnitude of the step in pressure to the baroreceptors (21,125,130).

The fact that during exercise BP and HR rise linearly with increases in work rate, whereas at rest the corresponding increases would induce powerful opposing reflexes through the baroreflex has puzzled physiologists for over a century (131,132). The current view is that the arterial baroreflex is reset in direct relation to the intensity of dynamic exercise without a change in sensitivity of the reflex (133-135). The resetting moves the baroreflex set point to the higher BP level so that baroreflex does not oppose the rising arterial BP but even actively tries to elevate BP (via further vagal withdrawal and/or sympathetic excitation to the heart and/or sympathetically mediated vasoconstriction) until the new set point is achieved (136-138). During exercise changes in arterial BP during carotid baroreceptor stimulation are mostly due to changes in total peripheral resistance so that only at light workload HR contributes to changes in BP (138,139). The central command is probably the primary regulator of baroreflex resetting during exercise (138,140,141). The muscle chemoreflex is also able to reset baroreflex, but in normal dynamic exercise it acts more as a modulator of central command-induced resetting (138,141,142).

Cardiopulmonary low-pressure baroreceptors monitor blood volume by sensing changes in the filling pressure of the chambers of the heart and pulmonary arteries and veins, as well as changes in cardiac contractility and afterload (18,143,144). Increases in these stimuli activate mechanically sensitive receptors in these structures, stimulating vagal afferent fibers that signal the nucleus tractus solitarius to inhibit sympathetic nervous system activity (18,124,144). The latter results in systemic vasodilatation and a reduction in total peripheral resistance, but physiological changes in cardiopulmonary low-pressure receptor activity cause little if any change in HR (18,124,144). When central venous pressure is normal, cardiopulmonary low-pressure baroreceptors tonically inhibit vasoconstriction induced by arterial baroreflex (18,144,145).

The cardiopulmonary low-pressure baroreceptors are not reset by the central nervous system during exercise, but they continuously inhibit arterial baroreceptor-induced vasoconstriction (18,118,144). If this tonic inhibition is eliminated by lower body negative pressure during exercise at a fixed workload, mean arterial BP and HR do not change, but total peripheral resistance increases and stroke volume decreases (146). Likewise, cardiopulmonary low-pressure baroreceptors inhibit the muscle metaboreflex-mediated vasoconstriction during dynamic exercise (147).

2.5.2 Central command and peripheral afferents

Central command is the term for motor command signals originating from subthalamic neurons involved in locomotion which is believed to be a primary stimulus mediating the autonomic nervous system adjustments to exercise (118,148,149). These signals activate separately both somatomotor and cardiovascular control systems at the onset of exercise (115,149,150). Activation is in direct proportion to the number of motor units required to maintain a given force of contraction (18,143,150). The magnitude of a central command- mediated cardiovascular response during exercise can be independent of force production (e.g. imagined exercise) and dictated more by an individual's perception of effort (18,116,151). Central command increases HR, cardiac output, and also BP immediately at the onset of exercise by rapid vagal withdrawal, but command signals have a minimal effect on the sympathetic nervous system-mediated vasoconstriction (116,150,152). Because both locomotor and cardiovascular responses, much like the responses to exercise, can be induced by either electrical or chemical stimulation of cells in hypothalamic locomotor region and in mesencephalic locomotor region, the current view is that these two neuroanatomical areas are strongly involved in the central command (115,116,152). Both these areas have connections with the vasomotor center in medulla, which enables them to influence cardiovascular control (18,116,152).

Originally it was thought that the central command acts as a pure feed-forward control mechanism (18,143). The close relationship of the central command with the number of motor units recruited and with the perception of effort suggests an important role for feedback from exercising muscles (18,124,153). Brain mapping studies have

showed that two regions of the cerebral cortex, the insular cortex and the medial prefrontal cortex, may function to interpret feedback signals from active muscles and elicit appropriate autonomic adjustments via connections to the vasomotor center (153).

The muscle chemoreflex is elicited from chemosensitive group III and IV afferent fibers in the muscle whenever muscle blood flow falls below the critical level needed to maintain adequate oxygen transport to the muscle (148,150,154). Release of hydrogen from the working muscles might signal the onset of the reflex, it may actually stimulate not chemically sensitive afferents but rather the conversion of monoprotonated phosphate to its diprotonated form (143,155). Excitatory action potentials from muscle sensory afferents project to the brain via synaptic transmissions in the dorsal root of the spinal cord (115,124,154). This exerts effects on the vasomotor center in medulla (primarily cVLM and rVLM), which in turn inhibit vagal and/or stimulate sympathetic preganglionic neurons, thus producing efferent autonomic nervous system responses to the heart and arterial vasculature (115,124,154). The muscle chemoreflex has a distinct threshold, and in mild exercise this reflex is not tonically active (18,143,150). As the severity of exercise increases to moderate, the margin for any blood flow error decreases and the reflex is tonically active (18,143). The baroreflex normally buffers the muscle chemoreflex by limiting chemoreflex-induced peripheral vasoconstriction so that the rise in arterial BP by the muscle chemoreflex occurs almost solely via an increase in cardiac output (i.e. rise in HR) (156).

Besides chemosensitive fibers, group III and IV afferent fibers in muscle also contain mechanosensitive fibers (115,148,154). These fibers are excited by mechanical stimuli (stretch and compression) in the active muscle (115,124,154). Their role in control of HR is not well understood (121,157), but it has been shown that a passive cycling of the legs significantly increased HR above baseline within one second of the onset of limb movement (158). In studies utilizing static muscle contraction this fast tachycardic response is vagally mediated (159), but evidence also exists for a slower sympathetically mediated tachycardiac response induced by mechanosensitive fibers (160). Since mechanoreceptor discharge quickly returns toward control levels during sustained static contractions, it is unlikely that these afferents contribute importantly to the maintained tachycardia in static exercise (157). It is not known, however, whether

this waning occurs to the same extent during dynamic contractions as well (18). Nevertheless, the muscle-heart reflex is not necessary for the vagally mediated increase in HR that occurs at the onset of exercise. This increase has been shown to be evoked by attempted exercise in subjects paralyzed with curare-like drugs (i.e. an absent afferent input from muscles) (161).

In addition to responding to chemical and mechanical stimuli, many group III and IV sensory afferents are thermosensitive (18,143,155). Muscle temperature, which is normally well below 37°C in the limbs, can increase above 40°C during severe exercise (18,143). Thus, it is possible that these afferents could be stimulated and provide feedback to the central nervous system regarding the thermal status of the active muscle fibers (18,143). While this remains a possibility, experimental findings are insufficient to determine the role of this mechanism in autonomic nervous system-mediated cardiovascular control during exercise (18,124).

2.5.3 Respiratory sinus arrhythmia, arterial chemoreceptors, and pulmonary and cardiac receptors

Respiratory sinus arrhythmia refers to the rhythmic variations HR, occurring at the frequency of respiration (21,55,79). Typically HR accelerates during inspiration and decelerates during expiration (21,55,79) as a result of complex central and reflex intearactions involving both vagal and sympathetic branches of autonomic nervous system (162). During exercise respiratory sinus arrhythmia gradually disappears despite gradually increasing respiration (163,164).

Peripheral arterial chemoreceptors are situated in the aortic and carotid bodies (21,29,50). They excite nerve fibers that pass through carotid nerves and the vagus nerves into the vasomotor center (39,50,74). Activity in their afferent nerves is increased by arterial hypoxia, hypercapnia, or acidemia (29,50,165). The primary effect of aortic body chemoreceptor stimulation on HR is excitatory (166). Conversely, carotid chemoreceptor stimulation causes a pronounced and consistent bradycardia, but with intact respiratory control it is normally counterbalanced by tachycardia accompanying the respiratory response induced by the same stimulation (29,55,167). Because exercise normally is associated with maintenance of PaO_2, normal or reduced arterial carbon

dioxide, and maintenance of blood pH within acceptable limits, the arterial chemoreceptors are not normally activated and therefore likely do not play an important role in HR control during conventional dynamic exercise at sea level (20,124).

The lungs are richly innervated and lung inflation, with moderate pressures, stimulates airways stretch receptors which results in a reflex increase in HR (168,169). The reflex response during hyperinflation of the lung and also during pulmonary congestion is to cause bradycardia (21). Because breathing frequency, tidal volume and minute ventilation all increase during exercise, it is possible that reflexes activated by lung inflation participate in HR control during exercise. At present, however, there is no compelling evidence for it (124).

Atrial receptors are concentrated near the junctions of the superior and inferior venae cavae and the pulmonary veins with the atria (20,21,29). The afferent fibers are contained in the vagus and the efferent pathway within the sympathetic nerves (21,29,170). Atrial receptors are stimulated mainly by stretching due to increases in atrial volume, which results in a reflex increase in HR (20,21,29). Because of the relatively slow time-course of sympathetic responses, the tachycardia following to stimulation of atrial receptors requires up to 30 seconds to reach a stable level (21).

Some authors suggest that a larger stimulation of atrial receptors via the increased venous return during exercise may be an important mechanism mediating a normal exercise-induced tachycardia (69,170,171). On the contrary, other authors conclude that the tachycardic reflex mediated by the atrial receptors is weak or nonexistent in humans and thus it does not play any role in exercise-induced tachycardia (18). A direct stretch of SA node can also increase HR to some extent, and this mechanism may be operative in exercise when venous return is increased (39,172). The mechanism might be augmented after heart transplantation (29,172), but in the normal heart this mechanism is largely masked or overridden by other reflex mechanisms (173). A rise in the temperature of the blood reaching SA node as the consequence of muscular work may also increase HR via a direct effect on SA nodal tissue (39,172).

Ventricular mechanoreceptors are situated mainly in the left ventricle. Afferent nerves travel either in the vagus or in the sympathetic nerves (170,174). Both populations of receptors can be divided into mechanosensitive and chemosensitive

endings (170,174). Normally their activity does not modulate HR either at rest or during exercise, but in the ischemic myocardium their activation results in powerful reflex responses (21,78,174). The vagal afferents mediate reflex cardio-inhibitory, sympatho-inhibitory and vasodepressor responses, while activation of sympathetic afferents results in cardio-accelerator, sympatho-excitatory, vasodepressor responses (170,174). Both mechanical and chemical stimuli may be involved in activation of receptors, but chemical stimuli are more important for triggering of reflexes (21,170,174). The anginal pain is mediated by sympathetic afferent fibers and causes tachycardia (21,170,174).

The vagal afferents are located nearer to the endocardial than to the epicardial surface, while the reverse is true for the sympathetic afferents (174). Thus subendocardial ischemia stimulates vagal afferents, resulting in bradycardia, whereas transmural ischemia more likely induces tachycardia mediated by sympathetic afferents (174). Left ventricular vagal afferents are preferentially distributed in the inferoposterior wall. In contrast, sympathetic afferents appear to be more uniformly distributed throughout the wall of the left ventricle (174). This probably explains why bradycardia usually occurs when the circumflex branch is occluded or when ischemia involves the inferior and lateral wall of the left ventricle (170,174). Occlusion of the anterior descending branch or ischemia involving the anterior wall is likely to result in an increase in HR (170,174). The chemical stimuli that activate receptors involve substances resulting from myocardial ischemia, including bradykinin, prostaglandins and adenosine (170,174). The bulging or dyskinesis of the ischemic zone may stimulate mechanosensitive vagal afferents, but the increase in discharge lasts only approximately two minutes. In contrast, the increased discharge of chemosensitive vagal afferent persists for the duration of the ischemic event (174).

2.6. Heart rate response to exercise: a synthesis

In 1990 Rowell & O'Leary introduced an overall model as to how adjustments of the autonomic nervous system to large-muscle dynamic exercise may be mediated (136). This hypothesis represents the most compelling integrative scheme attempting to explain the primary signals involved and how those signals may interact to produce

autonomic nervous system adjustments to exercise (124). The key concept in the model is that BP is the primary variable controlled during exercise and control of HR serves as an adjunct in this scheme (136,150,175).

From a resting value up to a rate of 100 beats/min during dynamic upright exercise HR increases rapidly primarily due to the activation of vagal withdrawal (51,65,73). The activation of vagal withdrawal is mainly due to augmented central command (136,150,157). The central command also resets the arterial baroreflex immediately to a higher operating point. Normally the baroreflex does not elicit an increase in sympathetic nervous system activity, however, because the rise in cardiac output (due to fast HR increase) is rapid enough to raise arterial BP to its new operating point (136,157,175). If any difference between the prevailing level of BP and a new, higher baroreflex operating point is detected by baroreceptors they can raise HR via further inhibition of parasympathetic tone (136,150,157). In this setting the muscle chemoreflex is not activated. The exact role of the afferents from muscle mechanreceptors is unknown, but in all probability their effect is negligible (136,150,157).

During moderate exercise, when HR exceeds 100 beats/min, the activation of vagal withdrawal by central command still increases cardiac output, but not enough (136,150,175). The fast vagal component of the rise in cardiac output is not sufficient to raise cardiac output to a level that is needed to compensate fully the vasodilation in active muscle (136,150,175). Consequently, arterial BP cannot be increased immediately to its new, reset operating point so there is a pressure error detected by baroreceptors (136,150,175). As a consequence of this error, sympathetic nervous system activity to both the heart and to the resistance vessels increase in correct the pressure error (136,150,175).

The sympathetically mediated increase in HR and cardiac output is much slower (by 15- to 20-fold) than the parasympathetically mediated rise (136,150,175). Thus vasoconstriction in resistance vessels all over the body (including active skeletal muscle) becomes a necessary adjunct to increased cardiac output to raise arterial BP as quickly as possible to minimize the pressor error (136,150,175). As workload increases, HR increases due to further sympathetic nervous system activation (20,73,164). The increase in sympathetic nervous system activity can occur due to the arterial barofeflex

(via further baroreflex resetting), the muscle chemoreflex (after a threshold has been passed after which this reflex becomes tonically active), or muscle mechanoreceptor activation (20,150,157).

Besides direct neural excitation SA node is also stimulated by an increased level of circulating adrenaline, which is secreted from the adrenal medulla (23,73,176). Adrenaline secretion is increased only during moderate to heavy exercise (typically 50% of maximal oxygen consumption or above) and sympathoadrenal activation becomes progressively greater as exercise intensity increases up to maximum (58,176,177). As exercise approaches maximal levels, parasympathetic activity wanes and sympathetic nervous system activity increases such that at maximal oxygen consumption (VO_{2max}) little parasympathetic tone remains and sympathetic activity is greatly elevated (136,157,164). Because during severe exercise HR is at or near maximal level, any further pressor response (i.e. to a fall in a baroreceptor activity or further muscle afferent activation) can only occur via peripheral vasoconstriction in that cardiac output is already at maximal levels (121,136,157).

Pharmacological blockade studies have proved the differential contributions of the two autonomic branches during exercise (178). Blockade of vagal control with atropine (muscarinic receptor antagonist) reveals that most of the initial response to exercise, up to a HR of approximately 100 beats/min, is attributable to the withdrawal of tonic vagal activity (164,179,180). Withdrawal of vagal tone has been confirmed using time and frequency domain analyses of HR variability as well (163,164). Conversely, blockade of sympathetic control with β-adrenergic receptor antagonist reveals the importance of augmented sympathetic activity during moderate and heavy exercise (181-183). During light exercise, with workloads of 25% to 40% of VO_{2max}, plasma noradrenaline levels or directly measured muscle sympathetic nervous activity do not significantly increase, confirming the finding that the sympathetic nervous system is more important in the later stages of exercise (136,150,175).

2.7 Central circulatory response to exercise

During dynamic upright exercise cardiac output (i.e. stroke volume times HR) increases somewhat linearly in proportion to the oxygen consumption (VO_2), approximately 6 l/min of cardiac output per 1 l/min of VO_2 (19,171,184). An appropriate increase in HR contributes significantly to attaining high levels of cardiac output (23,29,67). Of the two major components of cardiac output, HR and stroke volume, HR is responsible for approximately two thirds of the total increase in cardiac output during dynamic upright exercise (17,185,186). When a normal human exercises maximally in the upright position, the HR increase is 150-300% of resting, while the stroke volume increase is about 10-100% (29,187,188).

The stroke volume normally reaches its maximum or almost maximum by the time the cardiac output has increased only halfway to its maximum, after which any further increase in cardiac output must occur by increasing the HR (19,189,190). Thereafter stroke volume levels off (191-193), or there is a small decline (193-195) or increment (196-198) at maximal work intensity.

An increase in HR accompanying dynamic exercise also results in an increase in the force of myocardial contraction (i.e. frequency-force relationship, or the staircase phenomenon) (20,37,143). The increase in force is secondary to a transient imbalance in cellular Ca^{2+} influx and efflux (favoring influx), an increase in sarcoplasmic reticulum Ca^{2+} content, and a larger sarcoplasmic reticulum Ca^{2+} release during each excitation-contraction coupling cycle (18,37,143). Although a sufficient increase of HR is essential to raise cardiac output at heavier workloads, the absolute cardiac output which a person can attain is determined by the magnitude of maximal stroke volume (68,184,199).

The relationship between HR and VO_2 or work intensity is approximately linear (19,200,201). It has been suggested that the HR might increase relatively less than VO_2 as the work rate becomes very heavy (171,202,203), but two studies have shown the opposite (204,205). In both studies HR rose slightly more steeply above anaerobic threshold (approximately 50-60% of VO_{2max}) than below anaerobic threshold, but the HR/work intensity -relationship was linear (205).

The linear relationship between HR and VO_2 or work intensity is widely employed in a number of submaximal exercise tests (200,206-216), in which VO_{2max} is estimated based on HR measured in a single or several submaximal workloads (69,217,218). By utilizing a linear HR-workload relationship a straight line is fitted to measured HR values (69,217,218). This line is extrapolated to the predicted maximal HR; the corresponding estimated maximal workload can then be approximated (69,217,218). VO_{2max} can then be estimated by using the relationship between work rate and VO_2 (69,217,218).

2.8. Factors modulating heart rate response to exercise

In the same person under standardized conditions, the variation from day to day in HR at a given VO_2 is 3-5 beats/min depending on the relative workload (%VO_{2max}), provided the state of training is the same (219-221). The HR at a given VO_2 is related to the maximal stroke volume (222,223), but it is not a measure of maximal cardiorespiratory fitness, unless the maximal HR is considered (69,224). Despite limitation some researchers have, however, considered the workload which a person can attain at some predetermined HR as a good estimate of cardiorespiratory fitness (225-227). There is a tendency for persons with a low resting HR to have a low HR at a fixed submaximal workload (14,228,229), but this has not been observed consistently (171,230). Concerning maximal HR, persons with a high maximal HR have a higher HR at the same relative workload compared with the persons with a low maximal HR (230,231). Furthermore, an inverse (229) or non-existent (230) relationship has been observed between maximal HR and HR at a fixed submaximal workload, which emphasizes a fundamental difference when expressing a submaximal workload in terms of either relative or absolute work.

The physiological limit on maximal HR in normal subject is determined by the steepness of the diastolic depolarization slope of SA nodal cells before they reach threshold potential, thus generating an action potential that is then propagated to surrounding cells (38,39). When HR reaches 195 beats/min in humans during severe exercise, ventricular diastolic filling time is only 0.12 seconds compared to 0.55

seconds at rest (HR 70 beats/min) (41,143,232). It seems logical that a limit would be approached where an increase in HR would not effectively increase cardiac output due to decreased diastolic filling; not only would the heart receive less blood to pump, but the degree of coronary artery perfusion would decrease (17,20). Although this theoretical limitation is reasonable, there is little experimental work to support it (17,20,196).

Age. HR at a given submaximal VO_2 (and workload) has been reported to be the same for individuals of the same gender and state of training regardless of age (14,229,233). On the other hand, HR at a fixed submaximal workload has also been suggested to be higher (15,226,234) or lower (228,235,236) in older subjects, but in these observations the state of training may differ between individuals.

The decline of maximal HR with age is a well-known phenomenon (217,237,238). A comprehensive review of the literature compiling over 23 000 subjects aged 5 to 81 years revealed that age alone accounted for 75% of the variability in maximal HR; other factors added only about 5% (239). Of mechanisms underlying age-related decrease in maximal HR, cardiac chronotropic responsiveness to β-adrenoceptor stimulation has been shown to be preserved (240) or reduced (241-243) in the elderly. Subclinical atherosclerosis accompanying aging has also been proposed as a mechanism (69). The slope of the decay of exercise maximal HR with age is very similar to the slope of the intrinsic HR with age, suggesting that the decline of maximal HR is independent of autonomic influence, but has more to do with the SA node and the myocardium (20,244,245). The age-related decline in maximal HR is steeper in men who have a low cardiorespiratory fitness (246) and are physically inactive (17).

Gender. HR at a given submaximal workload is higher in women (229,247,248). Maximal HR does not differ between genders (17,239,249) or is slightly lower (229,250,251) in women. **Menstrual cycle.** In women HR at a fixed submaximal workload has been reported to be higher during the mid-luteal phase (252), but most studies have reported no change in submaximal (253-255) or maximal HR (255-257) during the menstrual cycle.

Body height and weight. The persons with the heavier body weight have been reported to have a lower HR at a fixed submaximal workload (222,236), and the

association is more pronounced when lean body weight is considered instead of total body weight (258,259). Height and body weight (lean or total) do not affect on maximal HR (20,51,222). **Obesity.** The overweight persons have a higher HR at a fixed submaximal workload than normal weight persons. The relationship is valid especially when exercising on a treadmill, but also when on a cycle ergometer (251,260,261). In two studies, however, there was no relationship between body mass index (BMI) and HR at a given submaximal workload on a treadmill (14,262), and in one study (228) an inverse relationship was observed between BMI and HR at a given submaximal workload on a cycle ergometer. Overweight has not been reported to have an effect on maximal HR (20,51,261), except one study in which overweight was associated with lower maximal HR (263).

Mode of exercise. HR is slightly higher at a given submaximal VO_2 on a cycle ergometer than on a treadmill (264,265). Maximal HR probably does not differ between a cycle ergometer and treadmill (266-268), although it has been suggested to be slightly lower with cycle ergometer testing (29,239,269). Both HR at a given submaximal workload and maximal HR are lower on a cycle ergometer in the supine position (29,184,270). **Total mass of working muscles.** The HR at a given submaximal workload is higher when the dynamic exercise is performed with the arms than with the legs (171,271,272). The maximal HR with arm exercise is 88-100% of the maximal HR in leg exercise (171,273,274). **Exercise protocol.** Large increments in workload combined with a short duration of the step may result in HR not rising to a steady state level at that workload (275). Consequently, HR at that submaximal workload is lower than the actual HR at the identical work provided that steady state would have been achieved (275). The maximal HR is not markedly different between protocols as long as the same exercise mode is used (268,276). **Pedal frequency.** When exercising with cycle ergometer, HR at a fixed submaximal workload may be slightly lower with a pedal frequency of 40 to 50 revolutions/minute than at clearly higher pedal frequencies (277).

Habituation. Habituation to repeated exercise tests has been found to lead to a reduction in HR response to a fixed submaximal workload, but the habituation effect is difficult to separate from a training response (278,279). Other studies have, however,

shown no appreciable habituation effect (220). **True maximal effort.** Maximal effort should always be confirmed objectively before an attempt is made to measure a maximal HR (29). Objective measures of appropriate maximal effort include respiratory exchange ratio >1.10 and blood lactate level >7-8 mmol/l (17,20,29). Older individuals might be more afraid to achieve true maximal exertion but this effect may disappear on repeated testing (20). Even if the true VO_{2max} is achieved in exercise it is still possible that maximal HR is even slightly higher than HR measured at the workload corresponding to VO_{2max} (280). **Sampling interval of HR measurement.** The difference between measured HR and true HR (determined by the last 30 seconds of each minute during exercise) is inversely related to sampling interval (281). The 6-second rhythm strip at the end of each minute represents a reasonable balance between convenience and precision for measuring HR during exercise (281).

Environment. A hot environment causes a higher HR at a fixed submaximal workload than exercise at a low ambient temperature (171,282,283). Also a high relative humidity elicits a higher HR at a fixed submaximal workload (70). Maximal HR can even reach slightly higher values under hyperthermia than in normothermia (18). Again, HR at a given submaximal workload is lower in a cold environment (284) and also maximal HR is lower compared with neutral temperature (285). Emotional factors, nervousness, excitement and apprehension may raise the HR during exercise of light and moderate intensity (69,171,217). The heavier the workload, however, the less pronounced is this nervous effect on the HR such that it does not affect maximal HR (69). **Dehydration.** In dehydrated state HR at a given submaximal workload is higher than in euhydrated state (171,190,238), whereas acute expansion of blood volume decreases HR (286-288). Acute plasma volume expansion does not affect maximal HR (289), but in a dehydrated state maximal HR might be slightly higher than in the euhydrated state (18).

Level of fitness. HR at a fixed workload seems to be inversely related to a maximal cardiorespiratory performance of a subject (238,290,291), although maximal HR modulates this relationship (69). A high cardiorespiratory fitness accelerates the rate of attainment of the steady-state HR at submaximal work (275,292). Maximal HR has

been reported to be the same (293-295), higher (229,230,296) or lower (239,250,297) in subjects with a high cardiorespiratory fitness.

Training. Within the same subject endurance-typed exercise training reduces HR at a given submaximal workload, and reduced physical activity has the opposite effect (29,298,299). The rate of attainment of the steady-state HR at submaximal work occurs more rapidly after training as well (300). Endurance training does not change the maximal HR or slightly reduces it (301-303). Cessation of endurance-type exercise training may increase maximal HR (304). HR at a given submaximal workload has been reported to be lower (14,251,271) and maximal HR (230) higher, respectively, in subjects with a higher level of self-reported physical activity. **Bed rest.** After prolonged bed rest HR at a given submaximal workload is higher than before bed rest (190,280,305). The maximal HR is either the same (280) or increased after the bed rest (305,306).

Medications and alcohol. The effects of various medications on HR during exercise are summarized in Table 1. After acute ingestion of alcohol, HR at a fixed submaximal workload is increased (307) or unaltered (308) but maximal HR is not affected (307).

Arterial oxygen content. A reduced arterial oxygen content in anemia or after hemoglobin blocked by carbon monoxide (as acutely after smoking) results in a higher HR at a fixed submaximal workload (171,238,311). Parenthetically, also smokeless tobacco increases HR at submaximal work (70,312). The increased blood hemoglobin concentration lowers HR at a given submaximal workload (218,313), but does not affect on maximal HR (313,314). Long-term smokers seem to have a lower HR at a given submaximal workload (262,315,316), but unaltered (14,15,317) and higher (318) HR values have also been reported. Long-term smokers have been reported to have a lower maximal HR (230,263,315). **Thyroid gland function.** Hyperthyroid patients may have a high HR at a fixed submaximal workload (238,319), whereas hypothyroid patients may have the reverse (320).

Table 1. Medications affecting heart rate during exercise (20,308,309).

Medications	Effect on heart rate during exercise (↑ = increase, ↓ = decrease, ↔ = no effect)
atropine	↑
agents blocking β-adrenergic receptors	↓*†
nitrates	↑ or ↔
calcium channel blockers	
dihydropyridine agents	↑
diltiazem, verapamil	↓ or ↔
digitalis	↓‡
hydralazine, minoxidil	↑ or ↔
centrally acting antihypertensives (clonidine, methyldopa, moxonidine)	↔ or ↓
antiarrhytmic agents	
quinidine, disopyramide	↑ or ↔
propafenone	↔ or ↓
amiodarone	↓
bronchodilatators	
sympathomometic	↑ or ↔
anticholinergic	↑ or ↔
methylxanthines	↑ or ↔
psychotropic medications	
antidepressants	↑ or ↔
major tranquilizers	↑ or ↔
cold medications with sympathomimetic agents	↑ or ↔
thyroid medications	↑
anorexiants / diet pills	↑ or ↔

* β-blockers with intrinsic sympathomimetic activity have a reduced effect.
† The effect increases with a relative intensity of exercise (64,183,310).
‡ Heart rate decreases in patients with controlled atrial fibrillation and possibly congestive heart failure, but heart rate is not significantly altered in patients with sinus rhythm.

Circadian rhythm and seasons. HR display a circadian rhythm so that HR at a fixed submaximal workload is slightly higher at early afternoon (a peak around 13.30) compared with morning or late afternoon (321,322). On maximal HR the effect of circadian rhythm is negligible (322). HR at a fixed submaximal workload has been shown to be lower in the summer than in other seasons (251). **Eating and sleeping before the test.** HR at a fixed submaximal workload is increased for an hour or more after a heavy meal (70,217). An abnormally short sleep the night before exercise may raise HR at a fixed submaximal workload (70).

Genetics. The heritability estimates for HR at workload of 50 Watts (W) in cycle ergometer, and walking at submaximal speed on treadmill have been reported as 57% (323) and 32% (324), respectively. The workload that a person can attain at a

submaximal HR of 150 beats/min is characterized by a significant familial resemblance, but the heritability as a percent of the age and gender-adjusted phenotypic variance is only <10% (325-327). The genetic effect on maximal HR has been shown to be significant in two studies with brothers and twins (328,329), but this has not been a consistent finding, however (330). The current view is that the genetic effect for maximal HR is about 50% (331). Familial data has indicated that maximal HR may be characterized by a maternal effect (332).

2.9. Heart rate response to exercise and prognosis

During the last two decades, novel exercise test-derived HR variables like chronotropic incompetence and HR recovery have excited widely as prognostic markers of mortality and cardiac events both in asymptomatic persons and in patients with CVD (10-12,23-26,51,73,122,201,333-340). HR recovery is outside the scope of the present review, and therefore only HR variables measured during exercise test are discussed.

2.9.1 Submaximal heart rate and cardiovascular disease events in asymptomatic persons

Seven reports (14,15,227,341-344) from six separate follow-up studies have examined the relationship between submaximal HR and future CVD events in asymptomatic persons as summarized in Table 2. Six papers measured the HR at a fixed submaximal workload (14,15,341-344), and one paper (227) measured the workload which a person can attain at a fixed submaximal HR of 150 beats/min as the variable to quantify submaximal HR-work rate relationship. In two of the seven reports a high submaximal HR was found to be an independent predictor of a future CVD death (14,15). In one of the five papers that did not find an association survival analysis was not performed, the medications influencing HR were not explicitly reported, and the study sample included both healthy men and men with clinical evidence of definite or probable CHD (21.9% of the total sample) (341). Additionally, prevalent CHD was not controlled for in the analysis (341). Two separate reports from one study (342,343) did not find an association either, but in both reports neither survival analysis was performed nor the

Table 2. The summary of the follow-up studies examining the relationship between submaximal heart rate and future cardiovascular disease events in asymptomatic persons*

Study	Total number of subjects	Women (%)	Age at baseline (years, range or mean±SD)	Exclusion criteria	Length of the follow-up (years, average/median if reported)	Total number of events	Use of medications affecting HR	The variable to quantify submaximal HR-work rate relationship	Main result
Hinkle et al 1972 (341)	301 telephone company workers	0%	55-60	not reported	7	26 CHD related deaths	yes	HR at submaximal workload in step test	submaximal HR was not associated with the risk of CHD death†
Gothenburg Study 1976 (342)	730 men living in Gothenburg and born in 1913	0%	54	locomotor disturbances, recent MI, unwillingness to cooperate	8	30 nonfatal AMIs, 19 CHD related deaths	not reported	HR at 98 W in cycle ergometer test	127 bpm in men having a CHD event vs. 126 bpm in others (p=ns for difference)†
Gothenburg Study 1981 (343)	730 men living in Gothenburg and born in 1913	0%	54	locomotor disturbances, recent MI, unwillingness to cooperate	9	55 nonfatal or fatal CHD events	not reported	HR at 98 W in cycle ergometer test	"those who had a CHD event had the same submaximal HR as the others"†
Belgian Physical Fitness Study 1987 (227)	1476 factory workers	0%	40-55	self-reported angina pectoris, an abnormal resting ECG, use of β-blockers	5	19 CHD events (sudden death or fatal or nonfatal AMI)	β-blocker users excluded	the workload attained at a HR of 150 bpm (PWC_{150})	the relationship between quartiles of PWC_{150} and incidence of CHD events not significant†

Table 2. Continued

Lipid Research Clinics Mortality Follow-up Study 1988 (14)	3106 men, most of them having hyperlipidemia	0%	30-69	medication affecting HR, antihyper-tensive medication, signs of possible CVD	8.5 (average)	45 CVD related deaths	users excluded	HR at stage 2 in treadmill test (modified Bruce protocol)	RR of CVD and CHD related death 2.7 (95% CI 1.4-5.1) and 3.2 (1.5-6.7) with an increment of 35 bpm
US Railroad Study 1988 (15)	2431 railroad workers	0%	22-79	pre-existing CVD as defined by standardized assessment	20.0 (average)	258 CHD related deaths	not reported	HR at submaximal workload in treadmill test	RR of CHD related death 1.2 (95% CI 1.1-1.3) when HR >135 bpm vs. HR <115 bpm
Pardaens et al 1996 (344)	216 asymptomatic persons referred for the investigation of hypertension	33.8%	35±12 (men), 43±10 (women)	diabetes, evidence of ischemic or valvular heart disease, heart failure, claudication, renal insufficiency, pulmonary disease	16.5 (median)	53 nonfatal or fatal CVD events	antihyper-tensive medication stopped for at least 2 weeks	HR at 50 W in cycle ergometer test	submaximal HR was not associated with the risk of CVD event either in men or in women

* SD, standard deviation; HR, heart rate; CHD, coronary heart disease; MI, myocardial infarction; AMI, acute myocardial infarction; W, Watts; bpm, beats/minute; ns, not significant; ECG, electrocardiogram; CVD, cardiovascular disease; RR, relative risk, CI, confidence interval.

† The main result is from an unadjusted analysis. If not specified, the main result is from a multivariable analysis.

medications influencing HR were reported. In the fourth paper with a negative finding (227), the workload achieved at HR of 150 beats/min did not predict future CHD events as such, but after adjustment for body weight it was a strong independent predictor. In the fifth report with negative finding all subjects were hypertensive asymptomatic persons who were referred for the further investigation of hypertension (344). Additionally, from the results of the study by Lauer et al (345), it can be deduced that a high HR at a fixed submaximal workload did not predict CHD events, although the researchers did not directly examined that particular variable in their data.

Only one of the seven reports involved also women (344) and the negative finding was observed similarly in both genders. The length of the follow-up was addressed in one study (15) in which the exercise test was performed twice, enabling the researchers to calculate the results with two separate follow-up periods of about 15 and 20 years. They showed that the predictive value of HR at a fixed submaximal workload for future CHD death improved if a shorter follow-up after the second exercise test was used, even though the number of events decreased from 258 to 147 (15).

In two studies which found a high submaximal HR to be an independent predictor of future CVD death (14,15), a high submaximal HR was interpreted as a marker of low cardiorespiratory fitness, which was to considered explain the association with CVD death. The conjecture that the association is mediated by low cardiorespiratory fitness is supported by the facts that HR at a fixed workload is inversely related to the maximal cardiorespiratory performance of a subject (238,290,291), and low cardiorespiratory fitness is a major risk factor for future CVD event in asymptomatic persons (339,346).

Indirect support for the association between a high submaximal HR and increased risk of future CVD events is found from four follow-up studies (226,347-349). In these studies the actual submaximal HR was not reported, but either an age-adjusted value was used for analyses, or submaximal HR was utilized for indirect estimation of cardiorespiratory fitness (226,347-349). In all four studies (226,347-349), an estimated low cardiorespiratory fitness was associated with an increased risk of CVD events. Because a low cardiorespiratory fitness estimated from an indirect test is based on a high submaximal HR (69,217,218), it is presumable that persons with an increased risk of CVD death in all four studies also had a high HR at a fixed submaximal workload.

A high HR at a fixed submaximal workload, or a low workload attained at a fixed submaximal HR, has been shown to be associated with risk factors for CVD, such as a low serum high-density lipoprotein (HDL) cholesterol level (14,226); high serum total cholesterol (226,228), low-density lipoprotein (LDL) cholesterol (14) and triglyceride level (14); low HDL/total cholesterol ratio (226); low level of self reported physical activity (14,251,342); high resting systolic (15,228,236) and diastolic BP (14); diagnosed hypertension (350); and overweight (251,260,261) in asymptomatic persons. On the other hand, smokers seem to have a lower HR at a given submaximal workload than nonsmokers (262,315,316). Also left ventricular hypertrophy assessed from ECG has been reported to be related to a lower HR at a given submaximal workload (228).

Total cholesterol and BP level were controlled for, however, in two studies (14,15) which found the association between submaximal HR and the increased risk of CVD death. Slattery and Jacobs (15) performed stepwise analysis which revealed that the predictive value of the HR at a fixed submaximal workload was attenuated most by resting systolic BP, but the association remained statistically significant. Furthermore, the associations of submaximal HR with total (351,352) and HDL cholesterol (14,262,353) level; overweight (14,228,262); a low level of self reported physical activity (228); and smoking (14,15,317) have not been observed consistently.

2.9.2 Submaximal heart rate and cardiovascular disease events in patients with known or suspected coronary heart disease

The relationship between HR at a fixed submaximal workload and mortality as well as CVD events in patients with known or suspected CHD has not been investigated in previous studies. However, indirect evidence is available from two studies (354,355) in which the HR increment from rest to submaximal workload was measured. Falcone et al (354) followed 458 men with angiographically verified CHD for six years. They found that patients whose HR rose 12 beats/min or more from rest to one minute at a workload of 25 W at the beginning of exercise test had 5.8 and 13.5 times higher risk of adverse cardiac event and cardiac death, respectively, than patients with a milder HR increment (354). Leeper et al (355), however, did not find any association between HR increment

from rest to a fixed submaximal workload and all-cause or CVD mortality after following 1959 patients (5% women) referred for exercise testing for 5.4 years.

Falcone et al (354) reasoned the rapid HR increment resulting from a premature vagal withdrawal which in turn might be a marker of sympathetic overactivity or a reduced vagal activity, known risk factors for death or cardiac event in CHD patients especially after myocardial infarction (356-358). The explanation offered by Falcone et al (354) is not supported by the findings from a previous study in which HR increase from rest to one minute was lower in 12 CHD or cardiomyopathy patients with depressed baroreflex sensitivity (a marker of vagal activity) than in patients with a normal baroreflex sensitivity (359). Accordingly, Leeper et al (355) argued that the rapid HR increment at the beginning of exercise test reflects a high, rather than low, vagal activity, and they further suggested the early acceleration of HR in the study by Falcone et al (354) to be a marker of a low cardiorespiratory fitness, a known risk factor for death or cardiac event in persons with known or suspected CHD (17,360).

Some authors have suggested that a high HR at a fixed submaximal HR may result from an inadequate stroke volume increase accompanying an impaired left ventricular function originating from either myocardial ischemia (361-363) or from left ventricular dysfunction not directly related to ischemia (364). According to this view, a high submaximal HR is a baroreceptor-mediated compensatory mechanism as an attempt to preserve an adequate cardiac output rise during exercise in the face of an impaired left ventricular function (319,362). Hence, the association of a high submaximal HR with an increased risk of death could be explained by an impaired left ventricular function, a known risk factor for death in CHD patients (17). In accordance with this are the findings from two previous studies in dogs (365,366). The rise of HR at the early phase of an exercise stress was steeper in dogs with a healed myocardial infarction that were susceptible to ventricular fibrillation after experimentally induced coronary occlusion than in dogs that were resistant to ventricular fibrillation. The susceptibility to ventricular fibrillation was associated with a greater degree of left ventricular dysfunction which was possibly due to significantly higher proportion of transmural infarctions in susceptible dogs (365).

2.9.3 Chronotropic incompetence and cardiovascular disease events in asymptomatic persons

Chronotropic incompetence is a term that has been used to describe inadequate HR responses to metabolic demand (51,340,367). The simpliest measure of chronotropic incompetence is the peak HR attained in an exercise test (9,230,342-344,368-370), but maximal HR can be expressed as adjusted for age (315,316,345,368,371-376) or resting HR (9,230,345,370,377) or both (376), as summarized in Table 3. Additionally, chronotropic incompetence can imply the HR at submaximal workload which is unexpectedly low related to an age-adjusted expected maximal HR (315,316,345,373,376).

Seven reports (9,230,345,370,374,375,377) from five separate follow-up studies have found chronotropic incompetence to be an independent predictor of future CVD events in asymptomatic persons (Table 4). Additionally one report has found the same association after adjustment for age and gender (316), and four papers (342,343,368,371) from two follow-up studies have found the same association in unadjusted models without reporting results from multivariable models (Table 4). On the other hand, in one study maximal HR did not predict CVD deaths in multivariable models either in men or women (369). In another study maximal HR did not predict CVD events in hypertensive asymptomatic men and women (344).

Chronotropic incomptence did not predict an outcome either in men or in women in two (344,369) out of the five studies that included both genders, whereas in two studies (371,375) the association was observed in men, but not in women. In the only study including exclusively women (374), the risk of CVD death was increased in women who did not achieve an age-adjusted target maximal HR. Other subgroup analyses have shown that chronotropic incompetence is associated with an increased risk of CVD death in men with both low and intermediate or high level of cardiorespiratory fitness (230), and in both younger (20-39 yrs) and older (40-59 yrs) men (370). In the study by Balady et al (375) chronotropic incompetence predicted CHD events in 144 high risk men (according to the Framingham Risk Score), but not in 1276 men with intermediate or low risk in age-adjusted model.

Table 3. The definition of chronotropic incompetence in previous studies with asymptomatic subjects

Variable	Definition	Cut-off values used
Heart rate impairment (315,316,345,368,371-376)	Inability to achieve a fixed %-value of age-adjusted expected maximal heart rate	90% (316,368,371,374), 85% (315,345,372,373,375,376)
Heart rate reserve (9,230,345,370,377)	Maximal heart rate minus resting heart rate	
Maximal heart rate (maxHR) (9,230,342-344,368-370)	The highest heart rate recorded during the exercise test	
Chronotropic response index at submaximal work (submaxCRI) (315,316,345,373,376)	((Heart rate recorded at fixed submaximal work – resting heart rate) / (age-adjusted expected maximal heart rate – resting heart rate)) / ((METs at fixed submaximal work – METs at rest) / (METs at maximal work – METs at rest))*	0.80 (315,373), 0.87 (316)
Chronotropic response index at maximal work (maxCRI) (376)	(Heart rate recorded at maximal work – resting heart rate) / (age-adjusted expected maximal heart rate – resting heart rate)	0.80 (376)

* MET, metabolic equivalent.

Table 4. Summary of the follow-up studies examining the relationship between chronotropic incompetence and future cardiovascular disease events in asymptomatic persons*

Study	Total number of subjects	Women (%)	Age at baseline (years, range or mean±SD)	Exclusion criteria	Length of the follow-up (years, average/ median if reported)	Total number of events	Use of medications affecting HR	The variable(s) to quantify chronotropic incompetence	Main result
Gothenburg Study 1976 (342)	649 men living in Gothenburg and born in 1913	0%	54	locomotor disturbances, recent MI, unwillingness to cooperate	8	30 nonfatal AMIs, 19 CHD related deaths	not reported	maxHR	165 bpm in men having a CHD event vs. 172 bpm in others (p=0.05 for difference)†
Seattle Heart Watch 1980 (368)	2365 men	0%	44.5±7.7	clinical manifestation of CHD, cardiac or antihypertensive medication, diabetes, atypical chest pain syndrome	5.6 (average)	47 nonfatal or fatal CHD events	users excluded	inability to achieve 90% of age-adjusted expected maximal HR	5 year probability 0.066 in men not achieving the target HR vs. 0.015 in others (p<0.05 for difference)†
Gothenburg Study 1981 (343)	649 men living in Gothenburg and born in 1913	0%	54	locomotor disturbances, recent MI, unwillingness to cooperate	9	55 nonfatal or fatal CHD events	not reported	maxHR	"those who had a CHD event had considerably lower maxHR as the others"†

Table 4. Continued

Seattle Heart Watch 1983 (371)	4158 persons	13.2%	45.5±8.4 (men), 49.1±9.0 (women)	clinical manifestation of CHD	6.1 (average)	100 and 102 CHD events in men and women	not reported	inability to achieve 90% of age-adjusted expected maximal HR	incidence 1.84% in men not achieving the target HR vs. 0.59% in others ($p<0.001$ for difference), in women no difference†
Norwegian Study 1995 (230)	1960 men	0%	40-59	known or suspected heart disease, diabetes, malignancy, hypertension under drug treatment, medication affecting HR, inability to conduct cycle ergometer test	16	143 CVD related deaths	users excluded	maxHR, HR reserve	RR of CVD related death 1.9 (95% CI 1.2-3.0) when HR reserve <93 bpm vs. >103 bpm
Aerobic Center Longitudinal Study 1996 (369)	26621 persons	23.4%	42.2 (men), 41.9 (women)	history of hypertension, stroke, diabetes, or myocardial infarction, an abnormal resting or exercise ECG, inability to achieve 85% of age-adjusted expected maximal HR	8.1 (average)	92 and 13 CVD related deaths in men and women	not reported	maxHR	RR of CVD related death 1.6 (95% CI 0.9-2.9) and 1.1 (0.2-5.6) with a decrement of 35 bpm in men and women

Table 4. Continued

Framingham Offspring Study 1996 (345)	1575 men	0%	43	prevalent CHD, inability to reach stage 2 in a standard Bruce protocol, use of β-blockers	7.7 (average)	95 nonfatal or fatal CHD events	β-blocker users excluded	inability to achieve 85% of age-adjusted expected maximal HR, HR reserve, submaxCRI	RR of CHD event 1.3 (95% CI 1.1-1.5) with a decrement of 0.12 bpm in HR reserve
Pardaens et al 1996 (344)	216 asymptomatic persons referred for the investigation of hypertension	33.8%	35±12 (men), 43±10 (women)	diabetes, evidence of ischemic or valvular heart disease, heart failure, claudication, renal insufficiency, pulmonary disease	16.5 (median)	53 nonfatal or fatal CVD events	antihyper-tensive medication stopped for at least 2 weeks	maxHR	maxHR was not associated with the risk of CVD event either in men or women
Lipid Research Clinics Mortality Follow-up Study 2000 (316)	5354 persons, most of them having hyperlipidemia	39.0%	44±10	medication affecting HR, antihypertensive medication, signs of possible CVD	12.0 (average)	114 CVD related deaths	users excluded	inability to achieve 90% of age-adjusted expected maximal HR, submaxCRI	RR of CVD related death 1.8 (95% CI 1.2-2.6) in subjects not achieving the target HR vs. others

Table 4. Continued

Aerobic Center Longitudinal Study 2002 (370)	27459 men	0%	20-59	history of hypertension, heart disease, stroke, diabetes, cancer, or arthritis, an abnormal resting or exercise ECG, inability to achieve 85% of age-adjusted expected maximal HR	13.0 (average)	205 CVD related deaths	not reported	HR reserve	RR of CVD related death 1.7 (95% CI 1.1-2.0) and 1.1 (1.0-1.4) with a decrement of 10 bpm in younger (20-39 yr) and older (40-59 yr) men
Lipid Research Clinics Mortality Follow-up Study 2003 (374)	2994 women, most of them having hyperlipidemia	100%	30-80	medication affecting HR, antihypertensive medication, signs of possible CVD	20.3 (average)	147 CVD related deaths	users excluded	inability to achieve 90% of age-adjusted expected maximal HR	RR of CVD related death 1.5 (95% CI 1.0-2.1) in women not achieving the target HR vs. others
Framingham Offspring Study 2004 (375)	3043 persons	53.0%	45±9 (men), 45±9 (women)	prevalent CHD, inability to walk on the treadmill, BBB on resting ECG, use of β-blockers or digoxin, inability to achieve 70% of age-adjusted expected maximal HR	18.2 (average)	224 and 81 nonfatal or fatal CHD events in men and women (including 14 and 4 fatal)	β-blocker and digoxin users excluded	inability to achieve 85% of age-adjusted expected maximal HR	RR of CHD event 1.7 (95% CI 1.2-2.5) in men not achieving the target HR vs. others, no association in women

Table 4. Continued

Norwegian Study 2004 (9)	2014 men	0%	40-59	known or suspected heart disease, diabetes, malignancy, hypertension under drug treatment, medication affecting HR, inability to conduct cycle ergometer test	26	300 CHD related deaths	users excluded	maxHR	RR of CHD related death 1.3 (95% CI 1.2-1.5) with a decrement of 14 bpm
Paris Prospective Study 2005 (377)	5713 men	0%	42-53	known or suspected CVD, resting SBP >180 mmHg, an abnormal resting ECG, inability to achieve 80% of age-adjusted expected maximal HR	23 (average)	81 sudden deaths from MI	not reported	HR reserve	RR of sudden death from MI 4.0 (95% CI 1.5-10.6) when HR reserve <89 bpm vs. >113 bpm

* SD, standard deviation; HR, heart rate; MI, myocardial infarction; AMI, acute myocardial infarction; CHD, coronary heart disease; maxHR, the highest heart rate recorded during the exercise test; bpm, beats/minute; CVD, cardiovascular disease; HR reserve, maximal heart rate minus resting heart rate; RR, relative risk; CI, confidence interval; BP, blood pressure; ECG, electrocardiogram; submaxCRI, ((heart rate recorded at stage 2 of exercise test – resting heart rate) / (age-adjusted expected maximal heart rate – resting heart rate)) / ((METs at stage 2 of exercise test – METs at rest) / (METs at maximal work – METs at rest)); MET, metabolic equivalent; BBB, bundle-branch block; SBP, systolic blood pressure.

† The main result is from an unadjusted analysis. If not specified, the main result is from a multivariable analysis.

The length of the follow-up was addressed in two studies (345,375). In the study by Lauer et al (345) two of the three chronotropic incompetence variables used in the study remained independent predictors of combined fatal or nonfatal CHD event when analysis were restricted to men with at least two years of event-free follow-up, whereas all three chronotropic incompetence variables were independent predictors when the follow-up of 7.7 years was considered as a whole. In the study by Balady et al (375), the predictive value of chronotropic incompetence for combined fatal or nonfatal CHD event in men remained similar during both the first and the last 10 years of the total follow-up of 18.2 years.

Chronotropic incompetence has been associated with smoking in several studies (263,316,370), but smoking was controlled for in the studies which found the association between chronotropic incompetence and an increased risk of CVD event (9,230,345,370,375,377). Chronotropic incompetence has been shown to predict CVD events both in smokers and nonsmokers (230,315,316). In the study by Lauer et al (315) a synergistic effect between chronotropic incompetence and smoking was observed for the risk of CHD event, whereas in the study by Sandvik et al (230) a synergistic effect was not observed.

Chronotropic incompetence has not been shown to be associated with exercise induced ischemic changes in ECG (342,345). Bruce et al (368) observed, however, a synergistic effect between chronotropic incompetence and exercise-induced ischemic changes in ECG for the risk of combined fatal or nonfatal CHD event.

Besides smoking, chronotropic incompetence has been shown to be associated with risk factors for CVD, such as a low HDL/total cholesterol ratio (345); high total cholesterol (230,370) and triglyceride level (230,370); impaired glucose tolerance (230); low level of self reported physical activity (230); low cardiorespiratory fitness (229,296,370); high resting systolic (343,345,370) and diastolic BP (230,345); diagnosed hypertension (345,350,378); increased left ventricular mass (372) and left ventricular cavity dilatation (373); carotid atherosclerosis (376); and overweight (263,345,370) in asymptomatic persons. Most of the aforementioned CVD risk factors were controlled for, however, in the seven studies (9,230,345,370,374,375,377) that found an association between chronotropic incompetence and the increased risk of CVD

events. Pardaens et al (344) performed stepwise analysis which revealed that a low maximal HR predicted CVD events in hypertensive subjects after adjustment for age, gender and resting HR. Further adjustment for CVD risk factors did not substantially alter the finding, but the prognostic value of maximal HR disappeared entirely after additional inclusion of VO_{2max} in the model (344). Furthermore, the associations of chronotropic incompetence with total cholesterol levels (342), self-reported physical activity (342,345), and systolic BP (342) have not been observed consistently.

Several mechanisms have been suggested to mediate the association between chronotropic incompetence and increased risk of CVD event (Table 5) (23,24,26,51,122,171,230,320,338,345,369,377,379,380). A latent CHD has been proposed to be a mediating factor (26,171,338). According to this view, abnormal myocardial wall motion and accumulation of metabolic by-products caused by ischemia irritate ventricular mechano- and chemoreceptors (174) leading to vagal activation and consequently attenuation of normal HR increase during exercise (24,26). The imbalance between oxygen demand and delivery may worsen further because of increased wall stress induced by ventricular dilatation, a condition shown to accompany chronotropic incompetence (373). The view is supported by the findings that chronotropic incompetence is an independent predictor of the presence of CHD in patients referred for exercise testing to diagnose CHD (382-384) and that chronotropic incompetence is associated with an advanced carotid atherosclerosis in asymptomatic men (376).

Table 5. The summary of proposed mechanisms explaining the association of chronotropic incompetence with an increased risk of cardiovascular disease event.

latent coronary heart disease (26,171,338)
atherosclerosis of the artery supplying the sinoatrial node (338,369)
sinoatrial node dysfunction (171,320,379)
reduced bioavailability of nitric oxide within the sinoatrial node (26)
systemic low-grade inflammation (381)
sinoatrial node β-receptor downregulation due to chronic sympathetic activation (26,230)
reduced baroreflex sensitivity (359,377)
abnormal cardiovascular autonomic control (26,51,230)

The relationship between myocardial ischemia, chronotropic incompetence and mortality has been directly studied in patients referred for exercise testing, and in these studies chronotropic incompetence was associated with ischemic findings assessed by

angiography (extent (385)), exercise echocardiography (presence (386) and extent (387)) and thallium imaging (presence (388) and extent (389)). Importantly, in each study (385-389) chronotropic incompetence was an independent predictor of death in a multivariable model that also included myocardial ischemia, suggesting that myocardial ischemia does not solely explain the association between chronotropic incompetence and mortality in this patient group. More specifically, latent CHD involving an artery supplying SA node has been proposed to explain the association between chronotropic incompetence and an increased risk of CVD events (338,369). Against this notion is the finding that in a group of patients with chronotropic incompetence the artery to the SA node was free of significant stenosis in 90% of cases (390).

Other authors have suggested chronotropic incompetence to be a manifestation of SA node dysfunction, and consequently SA node dysfunction to be a link between chronotropic incompetence and an increased risk of CVD event (171,320,379). This view is supported by the observations that patients with SA node dysfunction, as evidenced by typical 12-lead and Holter ECG findings, often have an attenuated HR response to exercise (391-393), but this is not a consistent finding (171,379). Furthermore, Chin et al (390) did not find SA node dysfunction in 23 patients who had chronotropic incompetence in exercise.

Routledge and Townend (26) have proposed that chronotropic incompetence might be caused by reduced bioavailability of nitric oxide within the SA node. Animal experiment (394) and study in human heart transplant recipients (395) have shown that nitric oxide exerts a tonic chronotropic effect on the SA node probably by causing activation of the I_f current. In persons with CHD, endothelial nitric oxide bioactivity is impaired as a result of reduced synthesis and inactivation by reactive oxygen species. This may further manifest itself as endothelial dysfunction and perhaps systemic low-grade inflammation, known risk factors for future CVD events (26,396,397). In subjects undergoing investigation for angina, persons with chronotropic incompetence had raised markers of systemic low-grade inflammation compared with those having a normal HR response (381).

Alternatively, chronotropic incompetence has been suggested to be a manifestation of SA node β-receptor downregulation as a result of chronic sympathetic activation

(26,230). This has been shown to be the case in patients with congestive heart failure (398), but it is not known whether this mechanism is also operative in asymptomatic persons. Routledge and Townend (26) have proposed that a SA node β-receptor downregulation in consequence of a chronic sympathetic activation could result from metabolic syndrome which may induce chronic sympathetic overactivation, particularly if hypertension is present (399). Chronic sympathetic overactivity may induce numerous unfavorable changes from the viewpoint of cardiovascular health, including hypertension (400-402); insulin resistance (403); a tendency to develop obesity (404); development of left ventricular (405) and vascular hypertrophy (406); an increased electrical instability of the heart, favoring life-threatening arrhythmias (407); occurrence of coronary thrombosis through increased blood viscosity (408), platelet activation (409) and development of a procoagulant state (410); and increased mechanical stress on the arterial wall via tachycardia (411).

A reduced baroreflex sensitivity has also been mentioned as a link between between chronotropic incompetence and an increased risk of CVD events, and more specifically sudden cardiac death due to ventricular fibrillation (377). It has been shown that among patients who have had myocardial infarction and have similar left ventricular ejection fraction, the inability to sustain episodes of ventricular tachycardia was predicted by depressed baroreflex sensitivity (412,413). Impaired baroreflex sensitivity favors circulatory collapse during ventricular tachycardia, a condition that precipitates ventricular fibrillation and sudden death (377). Jouven et al (377) suggested that a reduced ability to increase HR during exercise to the maximum extent could be the clinical counterpart of an impaired baroreflex sensitivity. In cardiac patients the association between chronotropic incompetence and a reduced baroreflex sensitivity has been observed in CHD and cardiomyopathy patients (359) but not in patients with congestive heart failure (414).

Finally, many investigators have supposed chronotropic incompetence to be a marker of abnormal cardiovascular autonomic control (26,51,230). This view is supported by the observations that the majory of patients with primary autonomic failure have a clearly abnormal HR response to exercise (222,415,416) and that in patients with

congestive heart failure chronotropic incompetence is related to reduced heart rate variability (a marker of depressed vagal activity) (414,417).

Reduced heart rate variability is a risk factor for future CVD events in asymptomatic middle-aged (418) and elderly persons (419,420). The sympathetic nervous system and circulating catecholamimes can contribute to all three major mechanisms involved in the generation of cardiac arrhytmias (421), enhanced automaticy (40,422,423), triggered automaticy (early (424,425) and delayed (426,427) afterdepolarizations), and re-entry (428,429), in healthy or diseased myocardium. Quite the contrary, a high vagal activity protects from arrhytmias (430,431). Indeed, Freeman et al (51) have stated that HR response to exercise may help to identify persons with autonomic imbalance who are currently healthy and without CVD, but who are predisposed to to sudden cardiac death in the future. Likewise, among patients with CVD and concomitant autonomic imbalance, there might be a superimposed increase in the risk of CVD events (51).

2.10 Summary

The HR response to exercise, although simple to assess, reflects a complex, integrated physiologic response in which autonomic tone, central and peripheral reflexes, hormonal influences and factors intrinsic to the heart are all important (20-22). During the last two decades, the exercise test derived HR variables have generated wide interest as prognostic markers of mortality and cardiac events both in asymptomatic persons and in CVD patients (11,12,23-26,51,73,122,201,333-338,340). The results of the properly controlled studies (9,14,15,230,345,370,374,375,377) suggest a possible bimodal relationship of HR to prognosis in which both high HR at low workload and inappropriately low HR at maximal or near maximal workload (i.e. chronotropic incompetence) are associated with adverse prognosis (223). Indeed, in the Lipid Research Clinics Mortality Follow-up Study both a high HR at a fixed submaximal workload (14) and an inability to increase HR normally when approaching maximal wok capacity (316) were associated with an adverse prognosis in two papers from the same study sample.

A high HR at a fixed submaximal workload has consistently been interpreted as a marker of a low cardiorespiratory fitness, and therefore the association with CVD death has been explained by this mechanism (14,15,355). Interestingly, however, there are no published population-based follow-up studies exploring this hypothesis in which HR at a fixed submaximal workload and cardiorespiratory fitness have been entered into the same regression model predicting future CVD events. The mechanism mediating the association between chronotropic incompetence and an increased risk of CVD event is not known, although several possibilities has been presented (23,24,26,51,122,171,230,320,338,345,369,377,379,380).

The hypothetical graph based on the results from previous studies (9,14,15,230,345,354,355,365,366,370,374,375,377) is shown in Figure 2. In essence, it is the theory proposed by Ramamurthy et al (223) in a graphic form. The hypothesis has a physiologically relevant basis, because HR increase from rest to maximal exercise is known to consist of two consecutive phases: the early rise up to a rate of 100 beats/min is controlled mainly by the parasympathetic nervous system (164,179,180), whereas the increase from 100 beats/min to maximum is controlled mainly by the sympathetic nervous system (181-183). In the current study the hypothesis presented in Figure 2 is further extended by suggesting that the predictive value of a high HR at a submaximal workload can be optimized if a submaximal HR response is quantified as a workload achieved at HR of 100 beats/minute (WL_{100}). The HR of 100 beats/min is used, because it is the upper bound of the first, parasympathetically (164,179,180) controlled phase of the total HR rise during exercise and it has been proposed that an exaggerated HR response particularly during this phase indicates an adverse prognosis (354,365,366). Furthermore, the hypothetical graph in Figure 2 suggests that the predictive value of a blunted HR increase at maximal or near maximal workload is optimized if the HR increment particularly during the latter half of the exercise test is considered when quantifying chronotropic incompetence. The prognostic value of the two aforesaid new HR-derived variables, WL_{100} and the HR increment during the latter half of the exercise test, has not been studied formerly.

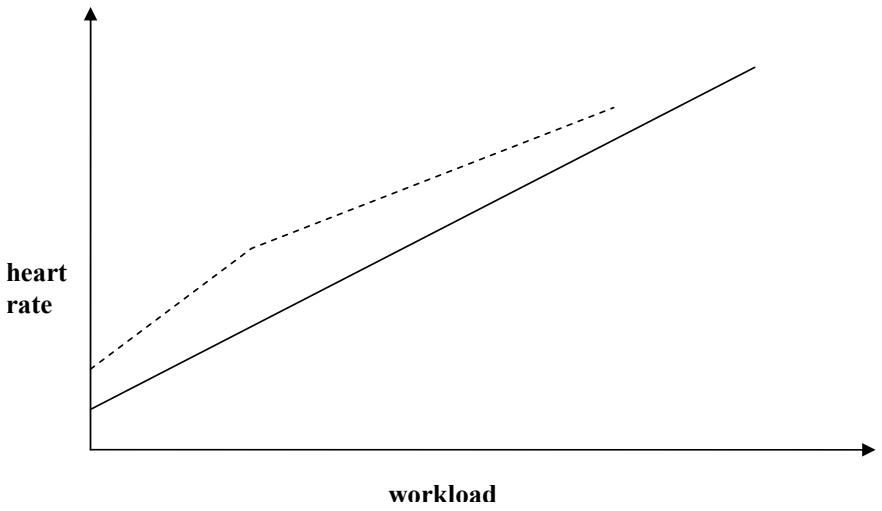

Figure 2. The hypothetical heart rate response to incremental exercise in subjects with an increased risk of future cardiovascular disease events (dashed line) and in normal subjects (continuous line) based on the data from previous studies (9,14,15,230,345,354,355,365,366,370,374,375,377).

3. MAIN HYPOTHESIS AND AIMS OF THE STUDY

The main hypothesis of the current thesis is that a bimodal relationship exists between HR and prognosis (Figure 2) in which both a low workload achieved at HR of 100 beats/min (WL_{100}) and a blunted HR increase particularly during the latter half of the maximal exercise test are associated with adverse prognosis. Accordingly, the aims of the study were:

a) to examine whether a blunted HR increase during the latter half of the maximal exercise test better predicts CVD and CHD mortality than resting HR, HR reserve, or maximal HR in middle-aged men free of CHD.

b) to investigate the association between WL_{100} and the risk of CVD death in middle-aged men free of CHD.

c) to study the association between WL_{100} and the risk of death in middle-aged men with known or suspected CHD and its prognostic value beyond other HR-derived and exercise test variables.

d) to explore the association between HR increase from 40% to 100% of maximal work capacity in an exercise test (HR40-100) and the risk of acute myocardial infarction in middle-aged men without CVD and to compare the predictive value of HR40-100 with other variables describing chronotropic incompetence.

4. METHODS

4.1 Study population

The subjects were participants of the Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD), a collaborative research project between the Kuopio Research Institute of Exercise Medicine and the Research Institute of Public Health, the University of Kuopio. The KIHD is an ongoing population study designed to investigate risk factors for CVD and related outcomes (432). The study involves men from East Finland, an area known for its high prevalence and incidence of CVD (432,433). The study protocol was approved by the Research Ethics Committee of the University of Kuopio and complies with the Declaration of Helsinki. Each participant gave written informed consent. The subjects are a representative age-stratified, age-balanced random population-based sample of men who lived in the city of Kuopio or neighbouring rural communities. Of the 3235 eligible men, 2682 (82.9% of those alive) were recruited in two cohorts. The first cohort consisted of 1166 54-year-old men (83.3% of those alive) enrolled in the study between March 1984 and August 1986; the second cohort was an age-stratified sample of 1516 42-, 48-, 54-, and 60-year-old men (82.6% of those alive) enrolled between August 1986 and December 1989. Table 6 describes the four follow-up studies of the thesis.

4.2 Examination protocol

Examinations at baseline were carried out over two days, one week apart, and consisted of a wide variety of biochemical, physiological, anthropometric and psychosocial measures (432). Invitations to attend the first study visit and written instructions to complete a detailed self-administered questionnaire were mailed four weeks in advance. At the first visit, a trained interviewer checked the completed questionnaire and a nurse measured body height and weight and blood pressure. The subjects underwent a medical examination, during which information about medical history and use of medications

obtained from the self-administered questionnaire was checked. At the first visit, a maximal, symptom limited cycle ergometer test was performed (434-439).

Table 6. The description of the study population and main variables*

Study	Total number of subjects	Population	Exercise HR-derived variable	Follow-up time	Main outcomes
I	1378	without CHD and use of β-blockers	heart rate increase at interval 40-100% of maximal workload	11.4 years	37 CHD deaths, 56 CVD deaths
II	1314	without CHD and use of HR-lowering medication	workload achieved at HR of 100 bpm	11.5 years	35 CHD deaths, 51 CVD deaths
III	365	known or suspected CHD	workload achieved at HR of 100 bpm	11.1 years	75 overall deaths
IV	1176	without CVD and use of HR-lowering medication	HR increase from 40% to 100% of maximal work capacity	11.0 years	106 acute myocardial infarctions

* HR, heart rate; CHD, coronary heart disease; CVD, cardiovascular disease; bpm, beats/minute.

At the second visit seven days later blood specimens were taken between 8 and 10 o'clock in the morning for laboratory determinations. For these blood samples, the subjects were instructed to fast and to abstain from smoking for 12 hours, to abstain from drinking alcohol for 3 days, and to abstain from using analgesics for 7 days. After the subjects had rested in a supine position for 30 minutes, blood was drawn with Terumo Venoject vacuum tubes (Tokyo, Japan). No tourniquet was used.

4.3 Exercise testing

HR response, cardiorespiratory fitness, exercise induced myocardial ischemia and BP response were assessed using a maximal, symptom limited cycle ergometer exercise test on an electrically braked cycle ergometer. For men examined before June 1986, the testing protocol comprised of a three-minute warm-up at 50 W followed by a step-by-step increase in the workload by 20 W per minute (early protocol) (Tunturi EL 400, Turku, Finland). The remaining men were tested with a linear increase in the workload

by 20 W per minute (later protocol) (Medical Fitness Equipment 400L, Mearn, Netherlands). For safety reasons and to obtain reliable information, the test was supervised by an experienced physician with the assistance of a trained nurse. Submaximal effort at peak exercise was defined as respiratory exchange ratio at peak exercise below 1.00. It is an objective marker of premature termination of exercise test because normally respiratory exchange ratio rises much above 1.00 at peak exercise as a consequence of ventilatory compensation for metabolic acidosis (10,17,319).

4.3.1 Assessment of heart rate response to exercise

HR was recorded from ECG at rest, at the end of each 60-second interval during the exercise test, and at peak exercise. HR represents the prevailing value at that time point obtained from sample interval of approximately 3 seconds and measured digitally by electrocardiograph. Resting HR was expressed as the lowest HR value, whether measured in lying position before the test or while sitting on bicycle before the test. WL_{100} was recorded directly at HR of 100 beats/min or interpolated linearly as a function of HR by using resting HR and the nearest HR value above 100 beats/min. Chronotropic index at HR of 100 beats/min was calculated as ((100 – resting HR) / (maximal HR – resting HR)) / (workload at HR 100 beats/min / maximal workload). Chronotropic index at HR of 100 beats/min quantitatively expresses how steep the early rise of HR from rest to 100 beats/min is in relation to the overall steepness of HR rise during the exercise test. A value of roughly 1 means that the steepness of the early HR rise from rest to 100 beats/min is about the same as the HR rise from that time point to maximum. Correspondingly, a value larger than 1 means that the early HR rise is steeper than the HR rise from that time point to maximum. HR at 40, 60, 80, and 100 % of maximal workload was interpolated linearly as a function of HR by using the nearest HR values below and above the time point respectively. HR increase from rest to 50 W as well as HR increase from rest to 33% of maximal workload were calculated as the difference between HR at corresponding time point and resting HR (354,355).

HR40-100 was calculated as maximal HR minus HR at the workload of 40% of maximal workload attained. All chronotropic incompetence variables listed in Table 3 were defined accordingly except chronotropic response index at submaximal work

(submaxCRI). When calculating submaxCRI the workload of 100 W as the submaximal workload was used. The workload was expressed in Watts instead of METs to simplify the equation of submaxCRI, because Watts at rest are explicitly zero. SubmaxCRI was calculated according to the hypothetical situation in which the exercise test would have been stopped at 85% of age-adjusted expected maximal HR although in reality our subjects continued until their symptom-limited maximum. Age-adjusted expected maximal HR was calculated as 220 – age.

4.3.2 Assessment of cardiorespiratory fitness, exercise electrocardiography and exercise blood pressure

VO_{2max} and exercise test duration were used as measures of cardiorespiratory fitness. Respiratory gas exchange was measured by the mixing chamber method with the use of a Mijnhardt Oxycon 4 analyzer (Gebr. Mijnhardt B.V., Netherlands) for men examined before June 1986 and by the breath-by-breath method with the use of a MGC 2001 analyzer (Medical Graphics Corp., St. Paul, Minnesota, USA) for the remaining men. VO_{2max} was defined as the highest value for VO_2 recorded during a 30-second interval.

ECG was recorded continuously with the Kone 620 electrocardiograph (Kone, Turku, Finland). The Mason-Likar lead system including V1, V5 and aVF lead connections was used (440). ECG was printed every 60 seconds intervals during exercise. Exercise ECGs were coded manually by one cardiologist. The criteria for ischemia in ECG during exercise was horizontal or downsloping ST depression with 0.5 or more mm at 80 milliseconds after J point in studies I, II and IV, and horizontal or downsloping ST depression with 1.0 or more mm in study III, and any ST depression with 1.0 or more mm at 80 milliseconds after J point in all studies.

Blood pressure was measured immediately before the test and every two minutes during and after the exercise test using cuff stethoscope method. The maximal systolic BP was the highest value achieved during the test. Systolic blood pressure (SBP) response was calculated as systolic BP at peak exercise minus systolic BP measured immediately before the test. BP was measured during recovery at regular 2 minutes intervals while subjects seated on the cycle without pedaling (437). Of these post-

exercise measurements, systolic BP at 2 minutes recovery was selected as the main variable because it was available for all men.

4.4 Biochemical analyses

Fasting blood glucose was measured using the glucose dehydrogenase method (Merck, Darmstadt, Germany) after proteins had been precipitated with trichloroacetic acid. The main lipoprotein fractions (LDL, HDL) were separated from fresh serum samples using precipitation and ultracentrifugation (441). The cholesterol contents of lipoprotein fractions were measured enzymatically (Boehringer Mannheim, Mannheim, Germany) on the day after the ultracentrifugal spin. Blood hemoglobin was determined photometrically (Gilford Stasar III, Gilford Instrument Laboratories Inc., Oberlin, Ohio, USA) using the cyanmethemoglobin method (442) within a few hours of blood sampling.

4.5 Resting blood pressure, body weight and body mass index

Resting BP was measured between 8 and 10 o'clock in the morning by two trained nurses, one during 1984 to 1985 and another during 1986 to 1989, with a random-zero mercury sphyghomanometer (Hawksley, Lancing, UK). The measurement protocol included, after supine rest of five minutes, three measurements in the supine, one in the standing and two in the sitting position with five minute intervals. Blood pressure was read with an accuracy of two mmHg. The disappearance of sounds (Korotkoff's fifth phase) was recorded as diastolic BP. In the present study the mean of all six measurements was used as systolic and diastolic BP.

Body weight was measured using a balance scale. The subject wore light clothing and no shoes. BMI was computed by dividing body weight in kilograms by the square of body height in meters.

4.6 Smoking and alcohol consumption

The current number of cigarette, cigars, and pipefuls of tobacco smoked daily and the duration of regular smoking in years were recorded using a self-administered questionnaire. Years smoked were defined as the sum of the years of smoking, regardless of when it had started, whether the subject had currently stopped smoking, or whether it had occurred continuously or during several periods. The lifelong exposure to smoking ("cigarette years") was estimated as the product of years smoked and the number of tobacco products smoked daily at the time of the examination, or for ex-smokers, at the time when they had smoked last time. Alcohol consumption was assessed with a structured quantity-frequency questionnaire using the Nordic Alcohol Consumption Inventory on drinking behavior over the previous 12 months. The average weekly consumption of alcohol in pure ethanol (g/week) was calculated based on the known alcoholic content of each beverage type and the reported doses and frequencies of drinking sessions (443).

4.7 Baseline diseases and medications

Medical history and the use of medications were assessed using a self-administered questionnaire. A physician reinterviewed the subjects regarding their medical history and the use of medications during a medical examination. Known or suspected CHD was defined as having either a history of myocardial infarction, angina pectoris on effort based on the London School of Hygiene Cardiovascular Questionnaire (444), or the use of nitroglycerin for chest pain once a week or more frequently. A prevalent CVD was defined as a history of CHD, cardiac insufficiency, cardiomyopathy, arrhytmias, stroke or claudication. Diabetes was defined as fasting blood glucose ≥6.7 mmol/l (445) or a clinical diagnosis of diabetes with either dietary, oral, or insulin treatment.

4.8 Collection and classification of follow-up events

4.8.1 Mortality

All deaths were ascertained by computer linkage to the Finnish National Death Registry using the Finnish social security number. There were no losses to follow-up. All deaths that occurred between study enrollment (from March 20, 1984 to December 5, 1989) and December 31, 1998 were included. Deaths from CVD and CHD were coded according to the Ninth International Classification of Diseases (ICD) codes (390-459 and 410-414, respectively) (446) or the Tenth ICD codes (I00-I99 and I20-I25, respectively) (447).

4.8.2 Acute coronary events

The collection of data on and the diagnostic classification of nonfatal and fatal coronary events by the end of 1992 were performed as part of the multinational WHO MONICA (MONItoring of Trends and Determinants in CArdiovascular Disease) project, in which detailed information on all coronary events was collected prospectively (448,449). All KIHD participants lived at baseline in the province of Kuopio, one of the monitoring areas of the Finnish part of the WHO MONICA project (FINMONICA) (450). In the FINMONICA study, regional coronary register teams collected data on coronary events from hospitals and wards of health centers and classified the events (450,451). The sources of information included interviews, hospital documents, death certificates, autopsy records, and medico-legal reports. The FINMONICA coronary register data were annually cross-checked with data obtained from the computerized national hospital discharge and death registers. Data on coronary events from the beginning of 1993 to the end of 1998 were obtained by computer linkage to the national hospital discharge and death certificate registers. A physician collected and classified the coronary events using the same procedures as in the FINMONICA study (450,451).

The diagnostic classification of coronary events was based on cardiac symptoms, ECG findings, cardiac enzyme elevations, autopsy findings, and history of CHD. Each suspected coronary event (ICD-9 codes 410-414 and ICD-10 codes I20-I25) was classified into 1) a definite acute myocardial infarction (AMI), 2) a probable AMI, 3) a

typical acute chest pain episode of more than 20 minutes indicating CHD, 4) an ischemic cardiac arrest with successful resuscitation, 5) no acute coronary event or 6) an unclassifiable fatal case. In the present study, definite AMIs, probable AMIs and prolonged chest pain episodes were used as outcomes. All chest pain episodes lead to hospitalization. If a subject had multiple nonfatal coronary events during the follow-up period, the first event after baseline was defined as the outcome.

4.9 Statistical methods

Statistical analyses were performed by using SPSS 11.5 for Windows (SPSS, Inc., Chicago, Illinois). Descriptive data are presented as mean and standard deviations (SDs), or medians and ranges, respectively, for continuous data and percentages for categorical data. Differences in baseline characteristics were examined using linear and logistic regression analyses after adjustment for age. Because of the skewed distribution, the exact Mann-Whitney U-test was used for age, smoking and alcohol consumption.

The association of HR-derived and other exercise test variables with the risk of outcomes were analyzed using Cox proportional hazards' models (452). In the Cox model, the hazard is assumed to equal the instantaneous death rate is given by the formula: $h_i(t)=h(t)C_i$, where $C_i=\exp(B_1X_{1i}+ B_2X_{2i}+\ldots+ BpXp_i)$ (3-5). The model assumes that the hazard (h) of death for patient I at time t ($h_1(t)$) equals the hazard of death for an "average patient" at the same time (h(t)) multiplied by the factor (C_i) that is the function of the prognostic profile of patient I; this is the proportional hazards assumption that gives the model its name (3-5). The proportional coefficient for patient i (C_i) is, in turn, a function of the values for that patient of a set of prognostic factors ($X_{1i},\ldots, Xp_i$), multiplied by a corresponding set of regression coefficients ($B_1,\ldots, Bp$) that measure the strength of the association between the prognostic factor and outcome of large number of subjects (3-5). Relative risks (RRs), adjusted for risk factors, were estimated as antilogarithms of coefficients from multivariable models. Their confidence intervals (CIs) were estimated under the assumption of asymptomatic normality of the estimates. The proportional-hazards assumption was verified by inspection of the plots

of Schoenfeld residuals for covariates (453). Linearity of associations was assessed with the Martingale residuals (454). No violations were observed. To detect the best cut-off point for a variable, the dichotomization cut-off point that maximized the log-rank test statistics was sought, and the predictive power of this categorized variable was tested by using Cox models. In further analyses, the sample was restricted to subjects who remained free of events during the first 2 years of follow-up. All tests for statistical significance were two-sided. A value of p less than 0.05 was considered statistically significant.

4.9.1 Study I

The analysis of variance (ANOVA) for repeated measures, adjusted for age and the length of follow-up, was used to detect whether the slopes of HR increase of men who died during follow-up and survivors differed from the beginning of the test or only later during the test. In order to eliminate dispersion from compound symmetry assumption (equal correlations between measurements) Greenhouse-Geisser corrected degrees of freedom were used when testing the effects in ANOVA. The Helmert contrasts, which compare HRs at each relative workload with the mean HRs of the next relative workloads, were used to locate the phase of the test (rest, 40, 60, 80, and 100 % of maximal workload) where the HR slopes of men who died during follow-up and survivors started to diverge. The statistically most significant contrast was used to construct a new variable. The correlations between the new HR variable and other HR-derived variables were analyzed using Pearson's correlation test. The new HR variable constructed according to ANOVA for repeated measures was entered into forced Cox proportional hazards' regression models. Two different sets of covariates were used: 1) age and examination year, 2) age, examination year, alcohol consumption, BMI, cigarette smoking, CVD history, diabetes, serum LDL cholesterol, systolic BP at rest and myocardial ischemia during exercise. To compare the additional predictive value of HR40-100 and other exercise test variables, a stepwise Cox regression analysis was used. After entering the conventional risk factors, the additional predictive value brought into the model by HR40-100 and each exercise test variable was compared in turns. In supplementary analyses, the sample was restricted to subjects who had none of

the following: history of cancer, submaximal effort observed at peak exercise, history of CVD, history of chronic obstructive pulmonary disease, bronchial asthma or pulmonary tuberculosis, or dizziness, dyspnea, chest pain, arrhythmia, ischemic ECG changes or change in BP as a cause of discontinuation of a test.

4.9.2 Study II

A multiple stepwise linear regression analysis including resting HR, chronotropic index at HR of 100 beats/min, maximal HR, and VO_{2max} was used to investigate the determinants of WL_{100}. Cox proportional hazards' regression models were fitted to compute the relative risk of death associated with a low WL_{100}, expressed as a continuous or dichotomous variable. Age, examination year, and exercise test protocol were forced into the Cox models, and rest of the variables were chosen by backward stepwise selection (p-value >0.1 for removal) from conventional risk factors, including alcohol consumption, BMI, cigarette smoking, CVD history, diabetes, myocardial ischemia during the exercise test, serum LDL and HDL cholesterol, and systolic and diastolic BP at rest. The additional predictive value brought by WL_{100} beyond other HR-derived and exercise test variables was explored by entering WL_{100} into a Cox model that included age, examination year, exercise test protocol, conventional risk factors chosen by stepwise selection, and the HR and exercise test variables in turns.

The difference in WL_{100} between two different testing protocols was tested using linear regression analysis after adjustment for age. To address specifically the effect of two different exercise test protocols the stepwise selection was performed separately in corresponding subgroups. In supplementary analyses, the sample was restricted to subjects who had none of the following: history of cancer, history of CVD, history of chronic obstructive pulmonary disease, bronchial asthma or pulmonary tuberculosis, or dizziness, dyspnea, chest pain, arrhythmia, ischemic ECG changes or change in BP as a cause of discontinuation of a test.

4.9.3 Study III

Difference in WL_{100} between men who used HR-lowering medication and those who did not was tested with independent samples t-test. A multiple stepwise linear

regression analysis was used to investigate determinants of WL_{100}. Cox proportional hazards' regression models were fitted to compute the relative risk of death associated with a low WL_{100}, expressed as a continuous or dichotomous variable. Age, examination year, exercise test protocol, and use of HR-lowering medication (beta-blockers, digoxin, clonidine) were forced into the Cox models, and rest of the variables were chosen by backward stepwise selection (p-value >0.1 for removal) from conventional risk factors, including alcohol consumption, BMI, cigarette smoking, cardiac insufficiency, history of myocardial infarction, diabetes, myocardial ischemia during the exercise test, serum LDL and HDL cholesterol, and systolic and diastolic BP at rest. The additional predictive value brought by WL_{100} beyond other HR-derived and exercise test variables was explored by entering WL_{100} into a Cox model that included age, examination year, testing protocol, use of HR-lowering medication, conventional risk factors chosen by stepwise selection, and the HR and exercise test variables in turns. Because the use of HR-lowering medication affects WL_{100}, the association of WL_{100} with mortality was examined separately in men who did not use HR-lowering medication and in men who used such medication.

Difference in WL_{100} between two different testing protocols was tested using linear regression analysis after adjustment for age and use of HR-lowering medication. To address specifically the effect of two different exercise test protocols the stepwise selection was performed separately in corresponding subgroups. In supplementary analyses, the sample was restricted to subjects who had none of the following: history of cancer, history of cardiomyopathy, stroke or claudication, history of chronic obstructive pulmonary disease, bronchial asthma or pulmonary tuberculosis, or dizziness, dyspnea, chest pain, symptoms of cardiac insufficiency, arrhythmia, ischemic ECG changes or change in BP as a cause of discontinuation of a test.

4.9.4 Study IV

The difference in HR40-100 between men whose test was terminated because of reason potentially caused by latent CVD (dizziness, dyspnea, chest pain, arrhythmia, ischemic ECG changes or change in BP) or because of submaximal effort and other men was tested with the independent samples t-test. The Pearson correlation coefficients between

chronotropic incompetence variables were calculated. Cox proportional hazards' regression models were fitted to compute the relative risk of AMI associated with a low HR40-100, expressed as a continuous or dichotomous variable. Two different sets of covariates were used: 1) age and examination year; and 2) age, examination year, alcohol consumption, BMI, cigarette smoking, diabetes, VO_{2max}, myocardial ischemia during exercise test, serum LDL and HDL cholesterol, systolic and diastolic BP at rest, SBP response, and SBP at 2 minutes after peak exercise. Age and examination year were forced into the model, and backward stepwise selection, with a p-value of >0.10 for removal, was used for the rest of the variables. In separate supplementary analyses, we excluded the subjects who died because of CHD within the next year after experiencing AMI, and men whose test was terminated because of reason potentially caused by a latent CVD or because of submaximal effort. Comparisons among chronotropic incompetence variables was addressed in a separate Cox model in which first stepwise selection was performed among the rest of the variables, and then forward stepwise selection was performed among chronotropic incompetence variables, with a p-value of <0.05 for entry. To study the joint effect of HR40-100 and SBP response to exercise, men with HR40-100 in the lowest quartile of HR40-100 and SBP response in the two highest quintiles of SBP response were compared with men who had a normal HR40-100 and SBP response. The cut-off point for SBP response was based on the results from a previous study in the same study population (439).

5. RESULTS

5.1 Baseline characteristics

The most important demographic and biochemical characteristics of all 1679 men according to baseline health status are presented in Table 7. The median age of the subjects was 52.4 (range 42.0-61.3) years. Among 1314 men with no CHD, 190 men with CVD were older, smoked less, had a higher diastolic BP, were more likely to have bronchial asthma, and were more likely to use antihypertensive medication (other than HR-lowering agents) or nitrates than 1124 men with no CVD after adjustment for age. Almost two thirds of self-reported CVDs were arrhytmias.

In 365 men with known or suspected CHD, 90 (24.7%) men reported a history of myocardial infarction, 226 (61.9%) men angina pectoris diagnosed by a doctor, 284 (77.8%) men angina pectoris on effort based on the London School of Hygiene Cardiovascular Questionnaire (444), and 61 (16.7%) men the use of nitroglycerine for chest pain at least once a week. Of these 365 men, 34.2% used HR-lowering medications and 14.0% reported symptoms of cardiac insufficiency. The men with known or suspected CHD were older, had a higher BMI, smoked more, had a lower HDL cholesterol concentration, and were more likely to have a history of stroke, cardiac insufficiency, cardiomyopathy, claudication, arrhytmias, or diabetes compared with 1314 men with no CHD after adjustment for age. Additionally, the 365 men were more likely to use antihypertensive medication, nitrates, or calcium channel blockers. The use of lipid lowering medication or ACE-inhibitors was not very common at the time of baseline examination.

5.2 Exercise test findings

Among 1314 men with no CHD history, 190 men with CVD were had a lower VO_{2max}, were more likely not to achieve 85% of age-adjusted expected chronotropic response at maximal workload, and were more likely to have the exercise test discontinued because of arrhytmias than 1124 men with no CVD after adjustment for age (Table 8). The 190

Table 7. Baseline characteristics of the men who had complete data on heart rate response to exercise test according to baseline health status

Characteristics	Mean (SD), median (range) or proportion				
	No CHD or CVD history (n=1124)‡	**No CHD history but CVD history positive (n=190)‡**	**p-value for difference¶**	**Known or suspected CHD (n=365)‡**	**p-value for the difference#**
Number of CVD/CHD/all-cause deaths‡	36/25/110	15/10/23	0.01/0.04/ 0.68	37/30/75	<0.001/<0.001/ <0.001
Number of AMIs*	102	22	0.51	85	<0.001
Age, years	52 (42-61)	53 (42-61)	0.001	54 (42-61)	<0.001
Body mass index, kg/m^2	26.5 (3.3)	26.7 (3.5)	0.38	27.3 (3.7)	<0.001
Cigarette smoking, cigarette-years‖	0 (0-2880)	0 (0-1400)	0.04	0 (0-2000)	0.008
Alcohol consumption, g/week	0 (0-889)	0 (0-728)	0.94	27 (0-2307)	0.44
Serum HDL cholesterol, mmol/l	1.33 (0.30)	1.30 (0.26)	0.17	1.26 (0.33)	<0.001
Serum LDL cholesterol, mmol/l	3.98 (0.96)	4.00 (1.03)	0.88	4.05 (1.05)	0.60
Diastolic blood pressure at rest, mmHg	88.0 (10.0)	89.9 (10.4)	0.02	87.5 (10.1)	0.34
Systolic blood pressure at rest, mmHg	132.2 (15.1)	135.0 (16.8)	0.06	132.9 (17.6)	0.76
Resting heart rate, beats/min	68.0 (10.0)	68.3 (10.8)	0.67	66.9 (10.6)	0.08
Blood hemoglobin, g/l	146.9 (8.8)	147.0 (9.8)	0.71	147.2 (9.5)	0.22
Self-reported diagnoses or symptoms					
History of myocardial infarction, %	0.0	0.0		24.7	
History of stroke, %	0.0	7.9		3.3	0.003
Cardiac insuffi-ciency, %	0.0	13.2		14.0	<0.001
Cardiomyopathy, %	0.0	7.5		3.6	0.004
Claudication, %	0.0	9.5		7.9	<0.001
Arrhytmias, %†	0.0	61.9		28.8	<0.001
Chronic obstructive pulmonary disease, %	5.7	7.9	0.34	8.8	0.13
Bronchial asthma, %	2.3	5.8	0.02	5.5	0.06
Pulmonary tuberculosis, %	3.3	2.1	0.29	5.2	0.23
Cancer, %	1.7	1.1	0.39	1.9	0.99
Diabetes, %§	3.5	4.7	0.49	7.1	0.02

Table 7. Continued

Regular use of medications					
Antihypertensive medication, %	2.4	7.9	0.001	38.1	<0.001
β-blockers, %	0.0	0.0		32.3	
Nitrates, %	0.4	2.1	0.03	21.4	<0.001
ACE-inhibitors, %	0.3	1.1	0.16	0.3	0.86
Calcium channel blockers, %	0.7	0.0		4.4	<0.001
Clonidine, %	0.0	0.0		0.3	
Digoxin, %	0.0	0.0		6.6	
Medication for hypercholestero-lemia, %				1.6	

* AMI, acute myocardial infarction.
† Arrhytmias included extrasystolia, regular or paroxysmalatrial fibrillation and supraventricular tachycardia.
‡ CVD, cardiovascular disease; CHD, coronary heart disease.
‖ Cigarette-years denotes the lifelong exposure to smoking which was estimated as the product of years smoked and the number of cigarettes smoked daily at the time of examination.
§ Diabetes was defined as fasting glucose ≥6.7 mmol/l or use of medication for diabetes.
¶ Difference between men with no coronary heart disease or cardiovascular disease history and men with no coronary heart disease history but positive cardiovascular disease history. Difference in age, smoking and alcohol consumption was tested with exact Mann-Whitney U-test. Differences in number of deaths and acute myocardial infarctions, self-reported diagnoses or symptoms, and regular use of medications were tested with logistic regression analysis and differences in rest of the variables with linear regression analysis after adjustment for age.
# Difference between men with no coronary heart disease history and men with known or suspected coronary heart disease. Differences between groups were tested as explained in previous footnote.

men were also more likely to have myocardial ischemia during exercise test, but their test was not discontinued more often because of ischemic ECG changes. Among 1314 men with no CHD, HR40-100 and WL_{100} were not different between men with CVD and those without it.

365 men with known or suspected CHD had a lower VO_{2max}, maximal workload, SBP response and SBP at 2 minutes after peak exercise, and were more likely to have submaximal effort at peak exercise than 1314 men with no CHD after adjustment for age and use of HR-lowering medication. Men with known or suspected CHD were more likely to have chronotropic incompetence and have the exercise test discontinued because of dyspnea, chest pain or ischemic ECG changes. WL_{100} was not different between men with CHD and those without it.

Table 8. Characteristics of exercise testing in the men who had complete data on heart rate response to exercise test according to baseline health status

Characteristics	Mean (SD) or proportion				
	No CHD or CVD history (n=1124)‖	**No CHD history but CVD history positive (n=190)‖**	**p-value for difference§**	**Known or suspected CHD (n=365)‖**	**p-value for difference¶**
Workload at heart rate of 100 beats/min, Watts	63 (31)	64 (33)	0.85	69 (34)	0.12
Chronotropic index at heart rate of 100 beats/min*	1.13 (0.28)	1.12 (0.27)	0.59	1.12 (0.27)	0.98
Heart rate increase from rest to 50 Watts, beats/min	29 (9)	28 (10)	0.85	28 (10)	0.76
Maximal oxygen consumption, ml/kg/min [METs] ‡‡	33.5 (7.4) [9.6 (2.1)]	31.6 (6.7) [9.0 (1.9)]	0.03	26.8 (7.0) [7.7 (2.0)]	<0.001
Maximal workload, Watts	211 (45)	203 (47)	0.53	168 (43)	<0.001
HR increase from 40% to 100% of maximal work capacity, beats/min	55 (13)	53 (15)	0.23	43 (15)	<0.001
Maximal heart rate as a proportion of age-adjusted expected maximal heart rate	0.97 (0.09)	0.96 (0.11)	0.19	0.86 (0.13)	<0.001
Men unable to attain 90% of age-adjusted expected maximal heart rate, %	19.5	25.8	0.05	57.5	<0.001
Heart rate reserve, beats/min††	95 (19)	91 (21)	0.17	75 (22)	<0.001
Maximal heart rate, beats/min	163 (16)	159 (19)	0.20	142 (22)	<0.001
SubmaxCRI at workload of 100 Watts#	0.98 (0.13)	0.96 (0.14)	0.14	0.89 (18)	0.002
MaxCRI**	0.95 (0.15)	0.92 (0.18)	0.16	0.77 (0.21)	<0.001
Men unable to attain 85% of age-adjusted expected chronotropic response at maximal workload, %	24.1	29.5	0.11	61.5	<0.001
Systolic blood pressure response, mmHg ‖ ‖	64 (23)	61 (24)	0.15	47 (24)	<0.001
Systolic blood pressure at 2 minutes after peak exercise, mmHg	184 (27)	187 (29)	0.56	181 (27)	0.03
Myocardial ischemia during exercise test, 0.5 mm, %†	13.0	19.5	0.03	83.7	<0.001
Myocardial ischemia during exercise test, 1.0 mm, %‡	6.5	6.1	0.86	11.5	0.001
Respiratory exchange ratio at peak exercise below 1.00, %	4.1	6.3	0.27	12.3	<0.001

Table 8. Continued

Cause of discontinuation of exercise test					
Dizziness, %	0.6	0.0		1.1	0.67
Dyspnea, %	2.7	2.1	0.51	8.2	0.001
Chest pain, %	0.3	0.0		8.5	<0.001
Symptoms of cardiac insufficiency, %	0.0	0.0		0.3	
Arrhytmia, %	2.1	5.3	0.04	2.7	0.84
Ischemic changes in electrocardiogram, %	0.2	1.1	0.10	4.7	<0.001
Change in blood pressure, %	2.0	2.6	0.69	1.9	0.50

* ((100 – resting heart rate) / (maximal heart rate – resting heart rate)) / (workload at heart rate of 100 beats/min / maximal workload).

† The criteria for myocardial ischemia in electrogram during exercise was horizontal or downsloping ST depression with 0.5 or more mm at 80 milliseconds after J point or any ST depression of more than 1.0 mm at 80 milliseconds after J point.

‡ The criteria for myocardial ischemia in electrogram during exercise was any ST depression of more than 1.0 mm at 80 milliseconds after J point.

|| CVD, cardiovascular disease; CHD, coronary heart disease.

§ Difference between men with no coronary heart disease or cardiovascular disease history and men with no coronary heart disease history but positive cardiovascular disease history. Differences in continuous and dichotomized variables were tested with linear and logistic regression analysis, respectively, after adjustment for age.

¶ Difference between men with no coronary heart disease history and men with known or suspected coronary heart disease Differences in continuous and dichotomized variables were tested with linear and logistic regression analysis, respectively, after adjustment for age and use of heart rate-lowering medication.

((Heart rate recorded at workload of 100 Watts – resting heart rate) / (age-adjusted expected maximal heart rate – resting heart rate)) / ((100 Watts – 0 Watts) / (Watts at maximal work – 0 Watts)).

** (Heart rate recorded at maximal work – resting heart rate) / (age-adjusted expected maximal heart rate – resting heart rate).

†† Maximal heart rate minus resting heart rate.

‡‡ MET denotes metabolic equivalent (1 MET equals 3.5 ml/kg/min oxygen consumption).

|| || Systolic blood pressure at peak exercise minus systolic blood pressure measured immediately before the test.

5.3 Heart rate increase from 40% to 100% of maximal work capacity and mortality in men without coronary heart disease (Study I)

In ANOVA for repeated measures, the slope of HR increase was steeper in survivors as compared with those who died during follow-up due to CVD ($F=12.9$; $df=1.757$; $p<0.001$ for interaction effect adjusting for age and length of follow-up). By using Helmert contrasts, the difference in the steepness of HR slope between the groups was

the strongest at interval 40-100 % (F=19.6, p<0.001) as shown in Figure 3. Based on these results, a new variable called HR40-100 was constructed as maximal HR minus HR at the workload of 40% of maximal workload attained. The average (SD) HR40-100 was 54 (13) beats/min in the whole study sample. HR40-100 correlated negatively with resting HR (r=-.33, p<0.001) and positively with HR reserve (r=.79, p<0.001) and maximal HR (r=.66, p<0.001).

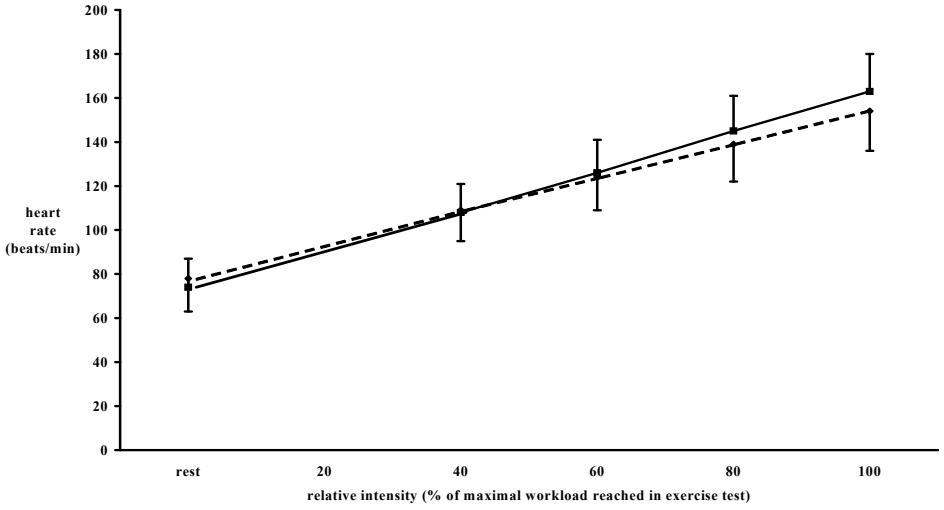

Figure 3. Heart rate (mean ± standard deviation) as a function of relative intensity (% of maximal workload reached in exercise test) in men who died during follow-up due to cardiovascular disease (n=56, dashed line) and survivors (n=1322, continuous line).

When adjusted for age and examination year, CVD mortality increased by 82% (95 % CI 39%-138%, p<0.001), CHD mortality by 127% (61%-213%, p<0.001), and all-cause mortality by 59% (35%-85%, p<0.001) for a 1-SD (13 beats/min) decrement in HR40-100. To investigate independent associations of HR40-100, it was entered simultaneously with age, examination year and known risk factors for CVD death into Cox models. CVD mortality increased by 35% (95% CI 1%-79%, p=0.04), CHD

mortality by 69% (19%-144%, p=0.004), and all-cause mortality by 33% (11%-59%, p=0.002) for a 1-SD (13 beats/min) decrement in HR40-100 (Table 9).

Table 9. Risk factors for cardiovascular disease, coronary heart disease, and all-cause death in 1378 men with no history of coronary heart disease at baseline*

Risk factor	Death due to cardiovascular disease		Death due to coronary heart disease		All-cause death	
	Relative risk (95 % CI)	p-value	Relative risk (95 % CI)	p-value	Relative risk (95 % CI)	p-value
Age, for each increment of 1 year	1.08 (1.02-1.16)	0.02	1.04 (0.97-1.12)	0.31	1.08 (1.04-1.13)	<0.001
Alcohol consumption ≥ 91 g/week, highest fourth vs. others	1.14 (0.62-2.09)	0.68	0.97 (0.45-2.09)	0.94	1.52 (1.06-2.19)	0.02
Body mass index, for each increment of 3.4 kg/m^2	1.20 (0.93-1.54)	0.16	1.19 (0.88-1.60)	0.26	1.07 (0.91-1.26)	0.42
Cardiovascular disease history, yes vs. no	1.81 (0.98-3.34)	0.06	1.66 (0.77-3.57)	0.20	1.02 (0.66-1.58)	0.93
Cigarette smoking, for each increment of 301 cigarette-years	1.43 (1.19-1.72)	<0.001	1.41 (1.11-1.78)	0.004	1.43 (1.29-1.60)	<0.001
Diabetes, yes vs. no	1.29 (0.48-3.47)	0.62	1.14 (0.33-4.00)	0.84	1.25 (0.64-2.45)	0.52
Myocardial ischemia during exercise, yes vs. no	2.35 (1.32-4.19)	0.004	3.32 (1.68-6.59)	0.001	1.25 (0.83-1.90)	0.29
Serum LDL cholesterol, for each increment of 0.97 mmol/l	1.16 (0.89-1.51)	0.28	1.26 (0.92-1.72)	0.15	1.01 (0.86-1.18)	0.92
Systolic blood pressure at rest, for each increment of 16 mmHg	1.36 (1.09-1.70)	0.008	1.19 (0.89-1.58)	0.24	1.28 (1.11-1.49)	0.001
HR increase from 40% to 100% of maximal work capacity, for each decrement of 13 bpm	1.35 (1.01-1.79)	0.04	1.69 (1.19-2.44)	0.004	1.33 (1.11-1.59)	0.002

* From Cox regression model adjusted for age, examination year and all variables shown in a table. Except for age, alcohol consumption, cardiovascular disease history, diabetes and myocardial ischemia, the relative risks were calculated for a change of 1 standard deviation, as shown. CI, confidence interval; LDL, low-density lipoprotein; HR, heart rate; bpm, beats/min.

To address specifically the late events, the analyses were restricted to 1367 subjects who had at least two years of event-free follow up. These analyses included 52 CVD deaths, 35 CHD deaths, and 135 all-cause deaths. The risk of CVD, CHD and all-cause

death increased by 33% (95% CI -1%-79%, p=0.06), 59% (10%-127%, p=0.01), and 28% (8%-54%, p=0.007) for a 1-SD (13 beats/min) decrement in HR40-100, respectively.

The best cut-off point of HR40-100 for predicting CVD mortality was 43 beats/min, and 272 (20%) subjects had low HR40-100 (<43 beats/min). Altogether 28 (50% of total) CVD deaths and 23 (62%) CHD deaths were observed among men with a low HR40-100. When HR40-100 was entered as a dichotomous variable into a Cox model, the strongest predictor of CVD death was smoking (p<0.001) followed by a low HR40-100 (RR 2.4, 95% CI 1.4-4.2, p=0.002), myocardial ischemia during exercise (p=0.007), high systolic BP at rest (p=0.007), high age (p=0.01), and CVD history (p=0.05). The strongest predictor of CHD death was a low HR40-100 (RR 4.3, 95% CI 2.1-8.7, p<0.001), followed by myocardial ischemia during exercise (p=0.001), and smoking (p=0.002).

The associations of HR40-100 with mortality were compared also with those of VO_{2max}, resting HR, maximal HR, HR reserve and systolic blood pressure response. All variables were considered as continuous variables, and relative risks were calculated for 1 SD increment. When HR40-100 and each of the other exercise test variables were entered into the fully adjusted model using the forward stepwise method, HR40-100 remained in the model for CVD and CHD mortality, whereas other exercise test variables did not. In the corresponding model for all-cause mortality, both VO_{2max} and HR40-100 were included in the model, but VO_{2max} was a stronger predictor (p=0.008) than HR40-100 (p=0.05).

Finally, 463 men who had cancer (n=29), submaximal effort at peak exercise (n=61), CVD (n=203), chronic obstructive pulmonary disease, bronchial asthma or pulmonary tuberculosis (n=157), or dizziness, dyspnea, chest pain, arrhythmia, ischemic ECG changes or change in BP as a cause for discontinuation of the test (n=118) were excluded. When adjusted for age, examination year, and known risk factors, CVD mortality increased by 33% (95% CI -7%-89%, p=0.11), CHD mortality by 61% (8%-144%, p=0.02), and all-cause mortality by 15% (-5%-39%, p=0.14) with a 10 beats/min decrement in HR40-100. After exclusions, the study sample included 137 (15%) men who had a low HR40-100 (<43 beats/min). When expressed as dichotomized variable, a

low HR40-100 was an independent predictor of CVD (RR 2.9, 95% CI 1.2-6.9, p=0.01), CHD (RR 4.4, 1.6-12.2, p=0.005) and all-cause death (RR 2.0, 1.2-3.2, p=0.007).

5.4 Workload at heart rate of 100 beats/min during exercise test and mortality in men without coronary heart disease (Study II)

The mean (SD) WL_{100} was 63 (31) W. Resting HR explained 39%, chronotropic index at HR of 100 beats/min 21%, VO_{2max} 5%, maximal HR 5%, and all these variables together 70% of the variance in WL_{100}. Heart rate vs. workload for those who died during follow-up due to CVD and survivors is shown in Figure 4. CVD mortality increased by 72% (95% CI 27%-138%, p=0.001), CHD mortality by 96% (32%-186%, p=0.001), and all-cause mortality by 23% (2%-47%, p=0.03) with a decrement of 31 W (1 SD) in WL_{100} after adjustment for age, examination year, and exercise test protocol. After further adjustment for conventional risk factors, CVD mortality increased by 72% (95% CI 27%-138%, p=0.001) and CHD mortality by 89% (28%-178%, p=0.001) with a decrement of 31 W in WL_{100}, but no association was found between WL_{100} and all-cause mortality (Table 10). Entering the whole set of covariates into the model weakened the independent predictive value of WL_{100} for CVD (p=0.006) and CHD death (p=0.001) marginally. To address specifically the late events, the analyses were restricted to 1303 subjects who had at least two years of event-free follow-up. The analyses included 47 CVD deaths and 33 CHD deaths. The risk of CVD and CHD death increased by 67% (95% CI 20%-133%, p=0.002) and 96% (32%-194%, p=0.001) for a 1-SD (31 W) decrement in WL_{100}, respectively.

The best WL_{100} cut-off point for predicting CVD mortality was 50 W, and 497 (37.8%) men had WL_{100} <50 W. Altogether 32 (63% of total) CVD deaths and 24 (69%) CHD deaths were observed among men with WL_{100} <50 W. When WL_{100} was entered as a dichotomous variable with conventional risk factors into a backward stepwise Cox model, the strongest predictors of CVD death were smoking (p<0.001), WL_{100} <50 W (RR 3.2, 95% CI 1.8-5.8, p<0.001), myocardial ischemia during exercise test (p<0.001), a high BMI (p=0.001), a high age (p=0.001), and CVD history (p=0.008). The strongest

Table 10. The relative risk for cardiovascular disease, coronary heart disease, and all-cause death in 1314 men with no history of coronary heart disease at baseline*

Risk factor	Death due to cardio-vascular disease		Death due to coronary heart disease		All-cause death	
	Relative risk (95% CI)	p-value	Relative risk (95% CI)	p-value	Relative risk (95% CI)	p-value
Age, for increment of 1 year	1.12 (1.05-1.20)	0.001	1.09 (1.01-1.19)	0.03	1.10 (1.06-1.15)	<0.001
Alcohol consumption ≥91 g/week, highest fourth vs. others					1.64 (1.13-2.39)	0.009
Body mass index, for increment of 3.5 kg/m^2	1.48 (1.18-1.87)	0.001	1.44 (1.10-1.90)	0.009	1.20 (1.01-1.43)	0.04
Cardiovascular disease history, yes vs. no	2.31 (1.24-4.28)	0.008	2.19 (1.03-4.68)	0.04		
Cigarette smoking, for increment of 299 cigarette-years	1.44 (1.22-1.70)	<0.001	1.43 (1.15-1.76)	0.001	1.45 (1.30-1.61)	<0.001
Myocardial ischemia during exercise test, yes vs. no	3.13 (1.75-5.59)	<0.001	4.29 (2.17-8.49)	<0.001		
Serum HDL cholesterol, for decrement of 0.29 mmol/l					1.22 (1.01-1.47)	0.04
Systolic blood pressure at rest, for increment of 15 mmHg					1.32 (1.14-1.53)	<0.001
Workload at heart rate of 100 beats/min, for decrement of 31 Watts	1.72 (1.27-2.38)	0.001	1.89 (1.28-2.78)	0.001		

* From Cox regression models adjusted for age, examination year, alcohol consumption, body mass index, cigarette smoking, cardiovascular disease history, diabetes, myocardial ischemia during exercise test, serum low-density and high-density lipoprotein cholesterol, systolic and diastolic blood pressure at rest, and exercise test protocol. The relative risks are shown only for variables included in the final model after a backward stepwise selection. Except for age, alcohol consumption, cardiovascular disease history, diabetes, and myocardial ischemia during exercise test, the relative risks were calculated for a change of 1 standard deviation, as shown. CI, confidence interval; HDL, high-density lipoprotein.

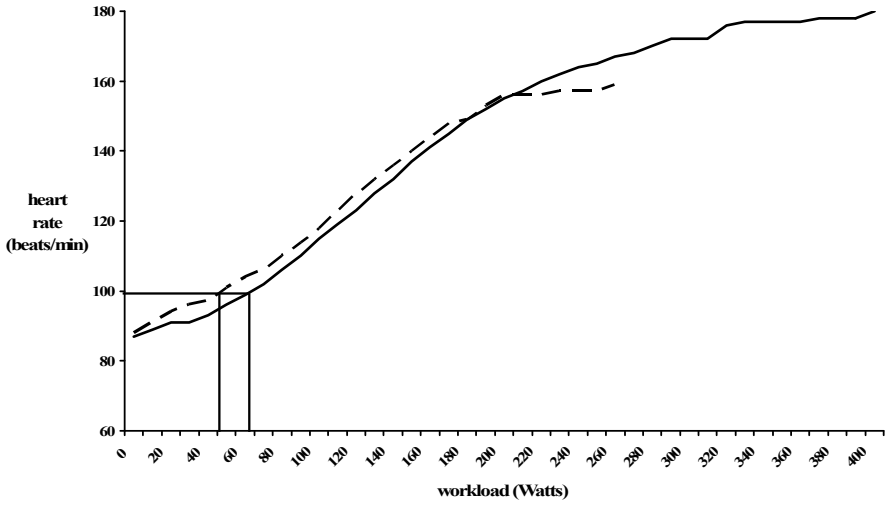

Figure 4. Heart rate (mean) as a function of workload in men who died during follow-up due to cardiovascular disease (n=51, dashed line) and survivors (n=1263, continuous line). Workload achieved at heart rate of 100 beats/min is indicated with vertical lines for each group. Standard deviations are not shown because the number of subjects in both groups gradually decreased along with increasing workload.

predictors of CHD death were myocardial ischemia during exercise test ($p<0.001$), smoking ($p<0.001$), WL_{100} <50 W (RR 3.9, 95% CI 1.9-8.2, $p<0.001$), a high BMI ($p=0.01$), a high age ($p=0.03$), and CVD history ($p=0.05$). The Kaplan-Meier curves for cumulative incidence of CVD and CHD deaths between men with WL_{100} <50 W and men with $WL_{100} \geq 50$ W continued to diverge with extended time of follow-up, as shown in Figure 5.

5.4.1 Workload at heart rate of 100 beats/min, other heart rate-derived and exercise test variables, and mortality

In a Cox model that included age, examination year, exercise test protocol, conventional risk factors chosen by stepwise selection, and the HR-derived and exercise test variables in turns, a 28-unit (1 SD) increment in the chronotropic index at HR of 100 beats/min

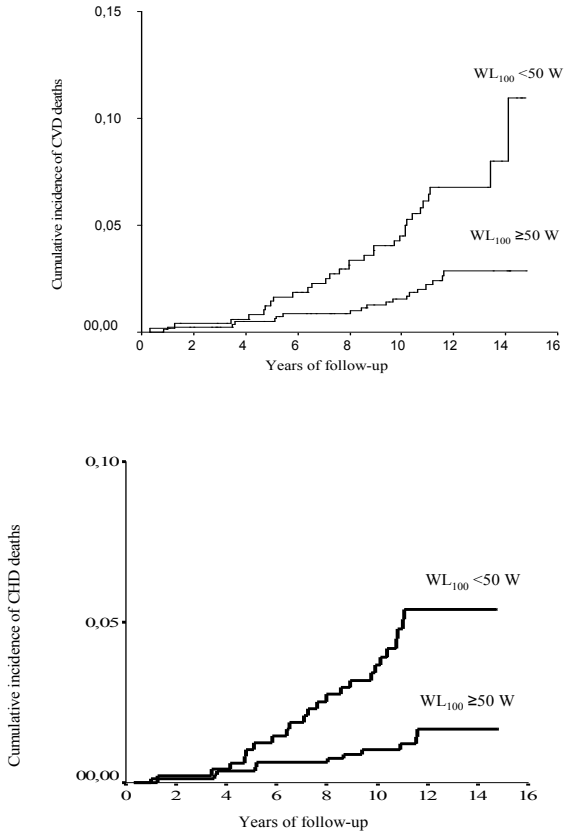

Figure 5. Kaplan-Meier curves for cumulative incidence of CVD (upper graph) and CHD (lower graph) deaths in men men with WL_{100} <50 W and men with WL_{100} ≥50 W.

was associated with a 43% (95% CI 9%-88%, p=0.01) increase in CVD mortality and a 65% (19%-128%, p=0.003) increase in CHD mortality; an increment of 10 beats/min (1 SD) in resting HR was associated with a 39% (0%-92%, p=0.05) increase in CHD mortality; a decrement of 7.3 ml/kg/min (1 SD) in VO_{2max} (about 2.1 metabolic equivalents) was associated with a 59% (0%-150%, p=0.05) increase in CHD mortality; and a decrement of 46 W (1 SD) in maximal workload was associated with a 52% (0%-133%, p=0.05) increase in CHD mortality. WL_{100} improved the predictive value of all models, except the model predicting CVD mortality that included chronotropic index at

HR of 100 beats/min. Whereas the predictive value of WL_{100} was consistent across the models shown, no other HR-derived or exercise test variable remained a significant predictor of CVD and CHD death in models that included WL_{100}.

5.4.2 Workload at heart rate of 100 beats/min and mortality: further adjustments

When a low blood hemoglobin concentration (<135 g/l) was entered into the model, the predictive value of WL_{100} for CVD and CHD death remained unchanged. The mean (SD) WL_{100} was 65 (29) W and 62 (32) W in men tested according to early and later testing protocol, respectively (p=0.30 for difference after adjustment for age). To address the effect of a testing protocol, the survival analysis was conducted separately for subjects performing the two different protocols. In 528 men tested according early protocol, WL_{100} as a continuous variable remained in the final model after stepwise selection for both CVD (p=0.04) and CHD (p=0.02) mortality. Among the 786 men tested according to the later protocol, WL_{100} also remained in the final model for both CVD (p=0.02) and CHD mortality (p=0.03). Finally, 400 men who had cancer (n=21), CVD (n=190), chronic obstructive pulmonary disease, bronchial asthma or pulmonary tuberculosis (n=149), or dizziness, dyspnea, chest pain, arrhythmia, ischemic ECG changes or change in BP as a cause of discontinuation of a test (n=109) were excluded. After stepwise selection CVD mortality increased by 59% (95% CI 4%-144%, p=0.03), and CHD mortality by 100% (25%-223%, p=0.004) with a 30 W decrement in WL_{100}.

5.5 Workload at heart rate of 100 beats/min during exercise test and mortality in men with coronary heart disease (Study III)

The mean (SD) WL_{100} was 69 W (34 W). Resting HR explained 38%, chronotropic index at a HR of 100 beats/min 18%, maximal HR 11%, VO_{2max} 5%, and all these variables together 72% of the variance in WL_{100} in a multiple stepwise regression analysis.

The risk of death increased by 56% (95% CI 20%-100%, p=0.001) for a 1-SD (34 W) decrement in WL_{100} when adjusted for age, examination year, testing protocol, and use of HR-lowering medication. After further adjustment for conventional risk factors, the

risk of death increased by 72% (95% CI 32%-122%, p<0.001) for a 1-SD (34 W) decrement in WL_{100} (Table 11). Finally, entering the whole set of covariates did not affect WL_{100} as an independent predictor of death (p<0.001). WL_{100} was also predictive of CVD and CHD death. After adjustment for conventional risk factors, the risk of CVD death increased by 59% (95% CI 11%-127%, p=0.01), and the risk of CHD death increased by 56% (4%-138%, p=0.03) for a 1-SD (34 W) decrement in WL_{100}. In men who had at least 2 years of event-free follow-up from baseline, the risk of death increased by 69% (95% CI 28%-127%, p<0.001) for a 1-SD (34 W) decrement in WL_{100} after adjustment for other risk factors.

Table 11. Risk factors for death in 365 men with known or suspected coronary heart disease at baseline*

Risk factor	Relative risk (95% CI)	p-value
Age, for increment of 1 year	1.08 (1.01-1.15)	0.02
Cardiac insufficiency, yes vs. no	2.42 (1.36-4.32)	0.003
History of myocardial infarction, yes vs. no	2.21 (1.32-3.71)	0.003
Workload at heart rate of 100 beats/min, for decrement of 34 Watts	1.72 (1.32-2.22)	<0.001

* From Cox regression adjusted for age, examination year, alcohol consumption, body mass index, cigarette smoking, cardiac insufficiency, diabetes, history of myocardial infarction, myocardial ischemia during exercise test, serum low-density and high-density lipoprotein cholesterol, systolic and diastolic blood pressure at rest, testing protocol, and use of heart rate-lowering medication. The relative risks are shown only to the variables which were included in the final model of a backward stepwise selection. Except for age, cardiac insufficiency, and history of myocardial infarction, the relative risks were calculated for a change of 1 standard deviation, as shown. CI, confidence interval.

The best cut-off point for predicting mortality was 55 W, and 130 (35.6%) of all 365 men had WL_{100} <55 W. Altogether 38 (51% of total) deaths were observed among men with WL_{100} <55 W. When WL_{100} was entered as a dichotomous variable into the backward stepwise Cox model including other risk factors, the strongest predictor of death was WL_{100} <55 W (RR 2.4, 95% CI 1.5-4.0, p<0.001), followed by a self-reported cardiac insufficiency (p=0.004), a history of myocardial infarction (p=0.006), a low diastolic BP at rest (p=0.03), and high age (p=0.04).

5.5.1 Workload at heart rate of 100 beats/min, other heart rate-derived and exercise test variables, and mortality

In a Cox model that included age, examination year, testing protocol, use of HR-lowering medication, conventional risk factors chosen by stepwise selection, and the HR-derived and exercise test variables in turns, the risk of death increased by 32% (95% CI 5%-66%, p=0.02) with 11 beats/min (1 SD) increment in resting HR; by 47% (10%-100%, p=0.01) with 7.0 ml/kg/min (1 SD) decrement in VO_{2max}; and by 35% (3%-75%, p=0.03) with 43 W (1 SD) decrement in maximal workload. Entering WL_{100} into these models improved the predictive value of the model statistically significantly in each case (Table 12). The predictive value of WL_{100} remained stable in various models. Of other HR-derived and exercise test variables, only VO_{2max} remained a statistically significant predictor of death in the models including WL_{100}. In that model, mortality increased by 35% (95% CI 1%-82%, p=0.05) with a 7.0 ml/kg/min decrement in VO_{2max}, and WL_{100} was also an independent predictor (p=0.002).

Table 12. Workload at heart rate of 100 beats/min and mortality after adjustment for heart rate -derived and exercise test variables in 365 men with known or suspected coronary heart disease at baseline*

Heart rate –derived or exercise test variable entered into adjusted model before WL_{100}	Improvement of the model after entering WL_{100} into the model	Relative risk (95% CI) of death per each decrement of 1 SD in WL_{100}
Resting heart rate	0.006	1.56 (1.12-2.17)
Heart rate increase from rest to 50 Watts	0.001	1.59 (1.20-2.13)
Heart rate increase from rest to 33% of maximal workload	<0.001	1.61 (1.23-2.08)
Maximal heart rate	<0.001	1.72 (1.32-2.27)
Maximal oxygen consumption	0.001	1.52 (1.18-1.96)
Chronotropic index at heart rate of 100 beats/min	0.001	1.64 (1.20-2.17)
Heart rate reserve	<0.001	1.59 (1.23-2.04)
Maximal workload	0.002	1.52 (1.16-2.00)

* Adjusted for risk factors chosen after stepwise selection before the variables in the left column and workload at heart rate of 100 beats/min were entered into the model in turns. WL_{100}, workload at heart rate of 100 beats/min; CI, confidence interval; SD standard deviation.

5.5.2 Workload at heart rate of 100 beats/min, the use of heart rate-lowering medication, and mortality

The mean (SD) WL_{100} was 61 W (29 W) in 240 men not using HR-lowering medication and 86 Watts (36 W) in 125 men using the medication ($p<0.001$ for difference between groups). WL_{100} was chosen after stepwise selection among conventional risk factors to final models predicting mortality in both subgroups. In men not using HR-lowering medication, the strongest predictor of death was a history of myocardial infarction ($p=0.001$), followed by WL_{100} and age ($p=0.01$). The risk of death increased by 54% (95% CI 14%-108%, $p=0.005$) for a 1-SD (29 W) decrement in WL_{100}. In men using HR-lowering medication, the strongest predictor of death was self-reported cardiac insufficiency ($p=0.004$), followed by WL_{100} and diastolic BP at rest ($p=0.04$). The risk of death increased by 72% (95% CI 14%-163%, $p=0.01$) for a 1-SD (36 W) decrement in WL_{100}. There was no interaction between WL_{100} and the use of HR-lowering medication ($p=0.94$).

5.5.3 Workload at heart rate of 100 beats/min and mortality: further adjustments

When a low blood hemoglobin concentration (<135 g/l) was entered into the model the predictive value of WL_{100} for death remained unchanged. The mean (SD) WL_{100} was 69 (31) W and 70 (36) W in men tested according to the early and later testing protocol, respectively ($p=0.36$ for difference after adjustment for age and use of HR-lowering medication). To address the effect of a testing protocol the survival analysis was conducted separately for subjects performing the two different protocols. In 169 men tested according to early protocol WL_{100} as a continuous variable remained in the final model after stepwise selection ($p=0.04$). Among the 196 men tested according to later protocol WL_{100} also remained in the final model ($p=0.001$). Finally, 176 men who had cancer (n=7), cardiomyopathy, claudication, or a history of stroke (n=53), chronic obstructive pulmonary disease, bronchial asthma or pulmonary tuberculosis (n=61), or dizziness, dyspnea, chest pain, symptoms of cardiac insufficiency, arrhythmia, ischemic ECG changes or change in BP as a cause of discontinuation of the test (n=100) were excluded. After stepwise selection mortality increased by 89% (95% CI 28%-178%, $p=0.001$) with a 30 W decrement in WL_{100} among the remaining men.

5.6. Heart rate increase from 40% to 100% of maximal work capacity and the risk of acute myocardial infarction in men without cardiovascular disease (Study IV)

The mean (SD) HR40-100 was 55 (13) beats/min. The correlation coefficients between all continuous chronotropic incompetence variables varied between 0.60 and 0.99, but the correlation coefficients between submaxCRI and other variables describing chronotropic incompetence were between -0.05 and 0.30.

The risk of AMI increased by 45% (95% CI 20%-75%, p<0.001) for a 1-SD (13 beats/min) decrement in HR40-100 when adjusted for age and examination year. After further adjustment for conventional risk factors, the risk of AMI increased by 33% (95% CI 9%-64%, p=0.006) for a 1-SD (13 beats/min) decrement in HR40-100 (Table 13). Entering the whole set of covariates into the model weakened the independent predictive value of HR40-100 for AMI slightly (p=0.03). The increase in HR from rest to 40% of maximal work capacity did not remain in the final model after stepwise selection. To address specifically later events, the analyses were restricted to 1153 subjects who had at least 2 years of event-free follow up. These analyses included 91 AMIs, and the risk of AMI increased by 37% (95% CI 9%-72%, p=0.008) for a 1-SD (13 beats/min) decrement in HR40-100.

Twenty of the 106 men who had an AMI during the follow-up died because of CHD within the next year after experiencing AMI. After excluding these 20 men from analyses, HR40-100 was still an independent predictor of AMI after stepwise selection: the risk of AMI increased by 33% (95% CI 5%-67%, p=0.02) for a 1-SD (13 beats/min) decrement in HR40-100.

Altogether 140 men had either a respiratory exchange ratio at peak exercise <1.00 as an objective marker of premature termination of the test (n=50) or stopped the exercise test because of a reason (dizziness, dyspnea, chest pain, arrhythmia, ischemic ECG changes or change in BP) that could be interpreted to indicate latent CVD (n=99). HR40-100 was lower in these men compared with other men (45 beats/min vs. 56 beats/min, p<0.001). After exclusion of 99 men with termination of the test because of suspicious symptoms or findings, HR40-100 remained a statistically significant predictor of AMI after stepwise selection: the risk of AMI increased 23% (95% CI 3%-

49%, p=0.03) with a 10 beats/min decrement in HR40-100. Further exclusion of 41 men with a respiratory exchange ratio at peak exercise <1.00 weakened the predictive value of HR40-100 only marginally: the risk of AMI increased 22% (95% CI 1%-49%, p=0.04) with a 10 beats/min decrement in HR40-100.

Table 13. The risk of acute myocardial infarction in 1176 men without cardiovascular disease or the use of heart rate -lowering medication at baseline*

Risk factor	Relative risk (95 % CI)	p-value
Cigarette smoking, for increment of 306 cigarette-years	1.33 (1.15-1.54)	<0.001
Diabetes, yes vs. no	2.71 (1.42-5.18)	0.003
Myocardial ischemia during exercise, yes vs. no	1.76 (1.10-2.82)	0.02
Serum LDL cholesterol, for increment of 0.96 mmol/l	1.33 (1.10-1.60)	0.003
Systolic blood pressure response, for increment of 24 mmHg	1.25 (1.02-1.53)	0.03
Systolic blood pressure at 2 minutes after peak exercise, for increment of 27 mmHg	1.26 (1.03-1.54)	0.02
HR increase from 40% to 100% of maximal work capacity, for decrement of 13 bpm	1.33 (1.09-1.64)	0.006

* From Cox regression adjusted for age, examination year, alcohol consumption, body mass index, cigarette smoking,, diabetes, maximal oxygen consumption, myocardial ischemia during exercise test, serum low-density and high-density lipoprotein cholesterol, systolic and diastolic blood pressure at rest, systolic blood pressure reserve, and systolic blood pressure at 2 minutes after peak exercise. The relative risks are shown only to the variables which were statistically significant predictors of acute myocardial infarction in the final model after a backward stepwise selection. Except for age, alcohol consumption, diabetes, and myocardial ischemia during exercise test, the relative risks were calculated for a change of 1 standard deviation, as shown. CI, confidence interval; LDL, low-density lipoprotein; HR, heart rate; bpm, beats/minute.

5.6.1 Heart rate increase from 40% to 100% of maximal work capacity, other chronotropic incompetence variables, and the risk of acute myocardial infarction

When HR40-100 was replaced by other chronotropic incompetence variables in separate stepwise selections all other chronotropic incompetence variables were chosen into the final models among statistically significant predictors except HR reserve, chronotropic response index at maximal work (maxCRI) as a dichotomous variable, and submaxCRI both as a continuous and dichotomous variable. The risk of AMI increased by 25% (95% CI 5%-49%, p=0.01) with 1 SD (0.09) decrement in maximal HR as a proportion

of age-adjusted expected maximal HR; by 30% (6%-59%, p=0.01) with a 1-SD (17 beats/min) decrement in maximal HR; and by 27% (5%-52%, p=0.01) with a 1-SD (0.16) decrement in maxCRI. The risk of AMI was 1.8-fold (95% CI 1.2-2.8, p=0.005) in 228 (19.4%) men who were unable to reach 90% of age-adjusted expected maximal HR as compared with men who could reach the target value. The comparison among the variables describing chronotropic incompetence was performed by exploring how much additional information they bring to an optimal model constructed without them. After stepwise selection among the rest of the variables, only HR40-100 improved the constructed model both as a continuous and as a dichotomous (the lowest quartile vs. others) variable (p=0.05 and p=0.03 for improvement of the model, respectively).

5.6.2 Heart rate increase from 40% to 100% of maximal work capacity, systolic blood pressure response and the risk of acute myocardial infarction

Both HR40-100 and SBP response remaind in the model as independent predictors of AMI after stepwise selection (Table 13). The subjects were classified according to HR40-100 and SBP response so that 296 men with HR40-100 <46 beats/min (the lowest quartile) were categorized as having a low HR40-100, and 473 men with SBP response >67 mmHg (two highest quintiles) were categorized as having a heightened SBP response, respectively. After stepwise selection, men with a low HR40-100 had a 1.6-fold (95% CI 1.0-2.5, p=0.03) higher risk of AMI than other men. In a separate stepwise selection, men with a heightened SBP response had a 1.7-fold (95% CI 1.1-2.6, p=0.01) higher risk of AMI than other men. After adjusting for age, examination year, cigarette smoking, diabetes, myocardial ischemia during exercise test, serum LDL cholesterol, and SBP at 2 minutes after peak exercise, 89 men with a low HR40-100 and a heightened SBP response had a 3.1-fold (95% CI 1.7-5.7, p<0.001) higher risk of AMI than the reference group consisting of men with both variables normal (Table 14). The interaction between HR40-100 and SBP response was not statistically significant (p=0.55) in an unadjusted model.

Table 14. The risk for acute myocardial infarction in 1176 men without cardiovascular disease or the use of heart rate -lowering medication at baseline according to heart rate increase from 40% to 100% of maximal work capacity and systolic blood pressure response*

Group	Number of acute myocardial infarctions/subjects	Relative risk (95 % CI)
Normal HR40-100, normal SBP response	28/496	1.00 (reference group)
Normal HR40-100, heightened SBP response	30/384	1.56 (0.91-2.68)
Low HR40-100, normal SBP response	29/207	1.73 (1.01-2.96)
Low HR40-100, heightened SBP response	19/89	3.07 (1.66-5.66)

* From Cox regression adjusted for age, examination year, cigarette smoking,, diabetes, myocardial ischemia during exercise test, serum low-density lipoprotein cholesterol, and systolic blood pressure at 2 minutes after peak exercise. CI, confidence interval; HR40-100, heart rate increase from 40% to 100% of maximal work capacity; SBP, systolic blood pressure.

6. DISCUSSION

6.1 Methodological aspects

6.1.1 Study design

The prevention of diseases can only be achieved when factors that predict disease outcomes are identified and prevented or treated (455). Conduction of randomized controlled trials is the best way to determine if a variable causally contributes to an outcome, but for practical and ethical reasons, it is usually impossible to hold biological factors constant in human research (455). Additionally, several risk factors for a given outcome interact so that a particular variable may predict an outcome when considered univariately, but in reality the association may be through other variables (17).

Therefore, multivariable statistical models called survival analysis are used to assess possible causal factors for an outcome, permitting estimation of the unique effects of a particular variable on the outcome while statistically holding other variables constant (3,17,455). Such models help identify risk factors for an outcome, allow to formulate risk stratification tools, and often may suggest pathophysiological mechanisms (455). However, causation can never be definitively discerned from observational data sets, regardless of the statistical tools used, including testing for independence (455). The most common technique for multivariable survival analysis is the Cox proportional hazards model (3-5), which was also used in the present study. Seven (9,14,15,230,316,344,345,369,370,374,375,377) of 11 previous studies exploring the association between HR at exercise test and CVD events in asymptomatic subjects (Tables 2 and 4) reported the result from multivariable models.

The accepted way to analyze relationships between multiple risk factors and an outcome is, if possible, to include all relevant risk factors in the statistical model to determine the adjusted effect of each risk factor on the outcome (455). If, after adjustment, a risk factor maintains a statistically significant association with the outcome, it is called an independent risk factor for the outcome (455). However, no study will ever properly model all cardiovascular risk factors to assert that a particular variable is truly an independent risk factor for a given CVD outcome (455). For any

variable described as an independent risk factor for a given CVD outcome, residual counfounding may remain, ie. potential predictors also associated with a variable of main interest have been excluded, poorly measured or not measured at all, or incorrectly modeled (455). Hence, terms such as independent risk factor or independent predictor have meanings only in the context of a particular statistical model (455). Importantly, in the current study a large number of established and potential risk factors were measured at baseline. Consequently it was possible to evaluate their impact on the associations of WL_{100} and HR40-100 with outcomes thoroughly. However, the possibility of residual confounding due to some unmeasured factors can not be excluded.

The length of follow-up may influence the results via opposing mechanisms. The short follow-up may weaken the associations if only a relatively small number of outcome events occur during the follow-up. A limited number of outcome events inevitably weakens the power of a study to find out whether the given difference in outcome is statistically significant. On the other hand, false positive findings can be made due to small number of outcome events. A long follow-up may weaken the associations if the values of variables measured at baseline change considerably during the follow-up although the statistical power increases along with the increasing number of outcome events (226). Because it is not known whether WL_{100} or HR40-100 changed during the follow-up of the present study, it is impossible to evaluate the relative effects of the aforesaid mechanisms. Nonetheless, the question can be addressed indirectly by recalculating the results according to a hypothetical situation where the follow-up would have been only eight years instead of true 11 years. The recalculation shows that the prognostic values of HR40-100 and WL_{100} are clearly attenuated with a follow-up shorter than eight years (data not shown). It can therefore be speculated that the prognostic values of WL_{100} and HR40-100 would have been even larger if the follow-up time had been extended longer than 11 years.

Censoring refers to the removal of intervened subject from observation when the intervention occurs during follow-up because the intervention changes the natural course of a disease of a subject (3-5). In observational studies for cardiac events the interventions leading to censoring are coronary artery by-pass surgery and percutaneous transluminal coronary angioplasty with or without stenting (3-5). In the current study,

subjects who underwent coronary artery by-pass surgery or percutaneous transluminal coronary angioplasty during the follow-up were not censored at the time of intervention because we did not have a possibility to monitor those events during the long follow-up. However, in none of the 13 previous studies in asymptomatic subjects and patients with known or suspected CHD was such censoring performed either.

Collinearity means that at least one of the covariates can be predicted well from the other covariates in the model (456). In the present study, collinearity was tried to minimize by utilizing a backward stepwise selection method in Cox models (studies II-IV). This method effectively limits a number of variables so that only one of two highly correlated covariates is left in the final model, provided its predictive value is high enough. However, the main results of studies II-IV remained very much unchanged when all covariates were entered simultaneously into the model instead of stepwise selection method. In study I, the results were reported after entering all variables simultaneously into the model. Nonetheless, when the main analysis were repeated in study I with backward stepwise selection, HR40-100 remained as a statistically significant predictor in the final models predicting CVD, CHD and all-cause death (data not shown).

6.1.2 Study population

A strength of the current study is that subjects are a representative population-based sample of middle-aged men from eastern Finland, an area known for its high prevalence and incidence of CVD (432,433). Second, the participation rate was high, and there were no losses to follow-up. The representative sample of men makes it possible to generalize the observed results to male populations of European background. One limitation of the present study is that only men were enrolled. The extent to which age, gender, ethnic population, underlying diseases, and regular physical activity possibly modify the observed findings deserves further study.

The baseline characteristics of the previous studies exploring the association between HR during the exercise test and CVD events in asymptomatic subjects and in subjects of the current study are gathered in Tables 2, 4 and 7. The age of the subjects in the present study is on the upper end of the range reported in previous studies. It is impossible to

evaluate the exact CVD or CHD death rates in all previous studies, but crude estimates can be calculated in studies which reported CVD or CHD death as an outcome and the average or median length of the follow-up: the number of outcome events divided by the number of subjects divided by the the length of the follow-up. According to this equation, in all three previous studies which reported CHD death as an outcome, the event rate was higher than in the present study. The lower CHD mortality rate in the present study as compared with three previous studies might be due to a considerably longer follow-up in two studies (9,15), the inclusion of men with a clinical evidence of definite or probable CHD in one study (341), and the intitiation of earlier studies in the 1970s, when the incidence of CHD was higher.

Because of the limited number of CVD deaths during the follow-up, men with a history of CVD (involving cardiac insufficiency, cardiomyopathy, arrhytmias, stroke or claudication) were included in studies I and II. This may potentially lead to selection bias, which means that the study sample includes individuals with symptomatic or asymptomatic CVD who perform poorly in the exercise test and have an increased risk of future CVD events during follow-up (457). The potential selection bias was taken into account by including a history of CVD as a covariate in the Cox models (studies I and II). Furthermore, when the main analyses were repeated after excluding men with CVD or other diseases or conditions potentially affecting the exercise test findings or the outcomes, the results did not change considerably (studies I, II and IV). Finally, two exercise HR variables of main interest in the present study, HR40-100 and WL_{100}, were not different between men with CVD and those without it (Table 8). It is noteworthy, however, that by far the most common CVD was arrhythmias (Table 7), which are generally benign and usually have no prognostic value.

The relationship of HR at submaximal workload with mortality and the risk of CVD events in patients with known or suspected CHD has not been investigated. Nonetheless, for descriptive purposes the present study can be compared with two recent studies (354,355) that explored HR increment from rest to submaximal workload as a predictor of death in men with known or suspected CHD. The sampling of subjects in the present study (study III) differs markedly from that of the previous two studies. The men in the present study were from a large population-based sample and the

inclusion criteria involved a self-reported history of myocardial infarction or angina pectoris, or a regular use of antianginal medication. However, the study was not carried out in a clinical setting, and therefore the complete medical records of subjects were not available.

In contrast, in studies conducted in one or several academic clinics the detailed objective clinical history of participants can be gathered easily. The subjects in the two clinical studies were either patients with angiographically documented CHD (354), or consecutive patients referred for exercise testing (355). Self-reporting unavoidably involves inaccuracy and a risk of misclassification (17). However, in the present study the history of myocardial infarction or angina pectoris were based on a diagnosis made by a physician. Furthermore, the London School of Hygiene Cardiovascular Questionnaire (444) is a widely used and well validated tool for a standard, unbiased assessment of chest pain in epidemiological studies (458).

It is therefore possible that the subjects in the present study represent a wide spectrum of severity of CHD, and consequently the results can be generalized to an even larger group of patients with known or suspected CHD than the results from studies involving subjects referred for exercise testing (457). Moreover, men in the current study III can be considered as patients who according to current guidelines (459) should be referred for exercise testing either for diagnostic purposes or for evaluation of prognosis and treatment options. In this respect, the subjects of the current study III resemble those in the study by Leeper et al (355). The number of subjects was lower and the subjects were younger, but the length of follow-up was about twice as long in the current study III as in two previous studies in patients with known or suspected CHD (354,355), respectively. The estimated crude outcome rates for all-cause, CVD and CHD death were very similar in all these studies.

We deliberately excluded men who used HR-lowering medication in studies which involved men without CHD at baseline (studies I, II and IV). This exclusion has been used also in previous studies exploring the association between HR at exercise test and CVD events in asymptomatic subjects. In seven (9,14,227,230,316,344,345, 368,374,375) of 11 studies users of HR-lowering medication have been excluded, and in only one (341) study have they been included in the study sample (Tables 2 and 4). In

four (15,342,343,369,370,377) studies the presence of users of HR-lowering medication was not reported. Definitely future studies are needed to clarify the prognostic value of chronotropic incompetence also among the users of HR-lowering medication. In the current thesis, however, a low WL_{100} predicted all-cause deaths both in subjects who used and did not use HR-lowering medication among men with known or suspected CHD.

6.1.3 Exercise testing

A maximal, symptom limited exercise test was performed on an electrically braked cycle ergometer. The advantage of cycle ergometer is that an upper body motion is usually reduced, that makes it easier to measure BP and to record the ECG (10,190,319). It is argued that a major limitation to cycle ergometer testing is the fatigue of the quadriceps muscles in subjects who are not experienced cyclists, which may cause them to stop before reaching a true maximal HR (10,17,190).

Unfortunately the testing protocol was changed midway through the baseline data collection so that the increment of workloads during first minutes of a test was different between men tested earlier or later during the course of baseline data collection. Because WL_{100} characterizes the early rise of HR at the beginning of a test, two different protocols could affect WL_{100}. However, WL_{100} was not different between men tested according to different protocols (studies II and III). Furthermore, the potential effect of two different protocols was taken into account by including testing protocol as a forced covariate in all Cox models in studies II and III. Finally, WL_{100} was included as a statistically significant predictor in the final Cox models after stepwise selection performed separately in men tested with both earlier and later protocol (studies II and III). These findings suggest that the difference in the testing protocols had no effect on the results in studies II and III. The different testing protocols should have no effect on HR40-100 or other chronotropic incompetence variables because they are determined by a HR increase during the latter half of the test which was identical in both testing protocols. The main results were unchanged when the analyses were performed with the testing protocol as a forced covariate in Cox models (data not shown).

HR was recorded from ECG as the reciprocal of the time interval between successive R peaks obtained from sample interval of approximately three seconds and measured digitally by ECG. The difference between the measured HR and the true HR (determined by the last 30 seconds of each minute during exercise) is inversely related to the sampling interval (281). It is possible that the measurement of HR would have been more accurate if a longer measurement interval had been used. Hence, the possible inaccuracy associated with a short sampling interval rather weakens than falsely strengtens the predictive value of HR variables observed in the present study. On the other hand, HR can climb steeply even in the final seconds of exercise (17,460), in which case an inappropriately long sample interval would prevent the detection of the true maximal HR.

In studies I, II and IV myocardial ischemia during exercise test was defined as a horizontal or downsloping ST depression with 0.5 or more mm at 80 milliseconds after J point instead of conventionally used cut-off value of 1.0 mm. An ST depression of 0.5 mm was used as a cut-off value for definition, as in the early reports from the KIHD data (434,441,461), because in univariate Cox models (studies I, II, III, IV) it was a stronger predictor of the outcome than a 1.0-mm cut-off for ST depression. In study III, a 1.0 mm ST-depression was used as a cut-off value, however, because the prevalence of ischemic change in exercise ECG would have been quite large (84%) had a 0.5 mm cut-off been used. More importantly, the main results were practically unchanged even when an ST-depression of 0.5 mm was replaced by 1.0 mm as a cut-off value in studies I, II and IV, and vice versa in study III.

Another strength of the present study is that cardiorespiratory fitness was measured objectively by direct expiratory gas analysis instead of using predicted values. The use of direct expiratory gas analysis can greatly supplement exercise testing by adding precision and reproducibility as well as increasing the yield of information concerning cardiopulmonary function (319,363,460). Importantly, a submaximal effort at peak exercise can also be objectively evaluated based on respiratory exchange ratio (10,319,460). VO_{2max} is considered the best index of aerobic capacity and maximal cardiorespiratory function (10,190,460). As VO_2 is determined primarily by cardiac output in the absence of pulmonary or skeletal limitations, this allows for the use of

VO_{2max} as an estimate of cardiovascular function during physical stress (18,319). Predicting VO_2 from cycle ergometer workload is a common clinical practice, but such predictions can be misleading (319,363,460). In the current study, exercise testing with both the conventional indirect definition of exercise capacity and respiratory gas analysis was used, which is unique in a large cohort study. The accurate measurement of VO_{2max} assured the reliable estimation of the predictive value of cardiorespiratory fitness. The predictive value of other exercise test variables independent of cardiorespiratory fitness could also be assessed more reliably as well.

Maximal HR was relatively low in the current study compared with previous studies (9,230,342,344,368-370) exploring the association between HR at exercise test and CVD events in asymptomatic subjects. This might be explained by the higher age of our subjects, because the prevalence of chronotropic incompetence in the current study, as measured by variables that take age into account, was similar to those reported in previous studies (316,345,368,371,374,375). The exercise characteristics in the present study III were similar to those in two previous studies in patients with known or suspected CHD (354,355).

6.1.4 Collection and classification of outcome events

During the follow-up of the current study it was possible to to assess both cause-specific and overall mortality as hard end points. The present study is based on reliable data on outcome events because deaths were ascertained from the Finnish National Death Registry using personal identification codes. The coding of cause of death in the Finnish National Death Registry has been validated (462). Additionally, the validity of diagnoses of CHD deaths in the Finnish National Death Registry has been addressed and its use in endpoint assessment in epidemiological studies has been justified (463,464). Data on coronary events were obtained by computer linkage to the national hospital discharge and death registers. The source of this information was checked by interviews, hospital documents, death certificates, autopsy reports and medico-legal reports. The diagnosis of an acute coronary event was typically based on symptoms, ECG and cardiac enzymes or autopsy findings.

Five (227,342-345,368,371,375) of 11 previous studies exploring the association between HR at exercise test and CVD events in asymptomatic subjects (Tables 2 and 4) used composite end points (fatal or nonfatal CVD event) as an outcome. The several problems accompanying composite end point has been discussed thoroughly (465,466). One of the main problems is that variables predicting nonfatal CVD events can be different than those predicting CVD death, creating a situation where one variables's contrasting effects with respect to two end points can cancel each other out (3-5). Among fatal end points, all-cause mortality is suggested to be objective, unbiased, and clinically relevant (467,468). On the other hand, CVD mortality has been proposed to be more appropriate for evaluating the prognostic value of exercise test variables because exercise test is used to assess the response of a cardiovascular system to a standardized stress (17). In the present study, to avoid inherent problems associated with composite end points, incident AMI and mortality from CHD, CVD and all causes were used as the main outcomes. When mortality was used as an outcome (studies I-III), the results were reported separately for CHD, CVD and total mortality.

6.2. Results

The main finding of the current thesis is that both a low workload at HR of 100 beats/min and an inability to raise HR appropriately during the latter half of the maximal exercise test are prognostically adverse findings in the population-based sample of middle aged-men without CHD at baseline. These observations are from two separate studies (studies I and II) which involved virtually the same subjects. The results support the main hypothesis that a bimodal relationship exists between HR and prognosis as presented in Figure 2. The actual HR-workload-curve shown in Figure 4 resembles the hypothetical curve in Figure 2, although the distance between curves is exaggerated in the latter curve.Among asymptomatic subjects, only the Lipid Research Clinics Mortality Follow-up Study has shown that both a high HR at a fixed submaximal workload (14) and a low HR near maximal workload (316) are associated with an adverse prognosis. Although the two reports (14,316) are from a single study, it is impossible to derive the proportion of subjects that were included in both papers.

6.2.1 Heart rate increase from 40% to 100% of maximal work capacity, mortality and the risk of acute myocardial infarction in men without coronary heart disease (Studies I and IV)

According to the hypothesis shown in Figure 2 we expected that a blunted increase in HR particularly during the latter half of the maximal exercise test predicts CVD events in a population-based sample of middle-aged men free of CHD. We observed that the HR response to exercise between those who died of CVD during follow-up and survivors began to diverge only after 40% of maximal work capacity was achieved. The survival analysis show that a blunted HR increase between 40 and 100 % of maximal work capacity (HR40-100) during an exercise test was associated with increased CHD, CVD and all-cause mortality and an increased risk of AMI. The magnitude of the association was comparable with that of other major CVD risk factors and exercise test variables, including other variables quantifying chronotropic incompetence.

The present findings are in line with the results of previous studies in which chronotropic incompetence has predicted adverse cardiac events in asymptomatic persons (9,230,316,342,343,345,368,370,371,374,375,377) and confirm the hypothesis that chronotropic incompetence is an important risk factor for death and cardiac events regardless of the chronotropic incompetence variable used. A new chronotropic incompetence variable, HR40-100, was as strong predictor of mortality and AMI as previously established chronotropic incompetence variables. HR40-100 was formulated based on the finding that when compared with survivors, the blunted HR rise in subjects who died of CVD during follow-up was statistically significantly only when calculated during the latter half of the exercise test. This observation was confirmed in multivariable Cox models in which HR increase from rest to 40% of maximal work capacity was not associated with an increased risk of adverse outcome, whereas HR40-100 was a strong independent predictor. The findings furthermore support the hypothesis of a bimodal relationship between exercise HR and prognosis (223).

Symptoms originating from existing non-cardiovascular disease or latent CVD may interrupt the test prematurely, and in such case the underlying disease may explain the association between chronotropic incompetence and an outcome (230). In the present study, the exclusion of subjects who had an outcome event within two years after

baseline and those whose test was terminated because of symptoms or findings potentially due to existing disease or latent CVD did not weaken the prognostic value of HR40-100 dramatically. This suggests that neither existing baseline disease nor latent CVD explain the increased risk associated with a low HR40-100.

The mechanism mediating the association between chronotropic incompetence and an increased risk of CVD events is unknown. The proposed mechanisms include abnormal myocardial wall motion and accumulation of metabolic by-products caused by ischemia which irritate ventricular mechano- and/or chemoreceptors (174) leading to vagal activation and consequently attenuation of normal HR increase during exercise (24,26); abnormal cardiovascular autonomic control (26,51,230), and more specifically sinus node β receptor down regulation caused by chronic heightened sympathetic activation (26,230); SA node dysfunction (171,320,379); reduced bioavailability of nitric oxide in sinus node (26); latent ischemia of sinus node (338,369); reduced carotid baroreflex sensitivity (377), possibly due to carotid atherosclerosis (376); and systemic low-grade inflammation (381). Numerous proposed mechanisms may simply mean that the prognostic value of chronotropic incompetence is based on several overlapping mechanisms instead of a single one.

Three follow-up studies, the Norwegian Study (9), the Paris Prospective Study (228,377) and the KIHD Study, have shown that a heightened BP response and a blunted HR increase are independent predictors of CVD events in asymptomatic men (Table 13). The present findings add to current knowledge in that the heightened BP response and the blunted HR increase during the exercise test were independent risk factors for AMI and provided additional information for risk prediction beyond each of them alone (Table 14).

The finding that the heightened BP response and the blunted HR increase during exercise test both were independent risk factors for AMI is somewhat puzzling, because they simultaneously reflect exaggerated and blunted hemodynamic response. We hypothesize that there is a common physiological link between the heightened BP response and the blunted HR increase during the exercise test, such as arterial dysfunction due to subclinical adverse vascular changes, e.g. atherosclerosis and media thickening. Arterial dysfunction leads to reduced arterial compliance, which is known to

heighten the increase of SBP in response to exercise (469,470). It may also interfere with the normal exercise-induced HR rise. Because of a nonlinear pressure-volume-relationship in conduit arteries (471), a heightened SBP increase rapidly shifts the pressure-volume-curve towards an even more noncompliant region. With reduced arterial compliance, the pressure wave reflects back from peripheral circulation abnormally fast and results in an augmented central SBP in the latter half of systole (472). Both of these phenomena stretch aortic baroreceptors, the activation of which may lead to a vagally mediated inhibition of the normal increase in HR in response to exercise. These phenomena may be obscured at rest or during light dynamic work, but at higher workloads the inability to normally reduce total peripheral resistance may become evident. This could also explain the finding in the present study that a blunted HR rise during the latter, but not during the first half of the test predicted outcomes.

Several prospective studies have shown that measures of arterial function and structure provide prognostic information incremental to conventional risk factors for CVD (472,473). We suggest that arterial dysfunction, indicated by the heightened BP increase and the blunted HR increase during the exercise test, is a potential and currently overlooked mechanism explaining partly, although not completely, the association between chronotropic incompetence and an increased risk of CVD events. Supporting this hypothesis, flow mediated vasodilatation as a marker of endothelial function in conduit arteries has been shown to be markedly lower in patients with chronotropic incompetence compared with patients with normal HR response to exercise (381).

To address a potential overlapping between results of study I and study IV, the predictive value of HR40-100 for AMI was separately studied after excluding 20 men who died because of CHD within the next year after experiencing AMI. HR40-100 was still an independent predictor of AMI. This indicates that the main results of the studies I and IV do not overlap to a great extent, but instead represent two separate and clinically meaningful relationships.

6.2.2 Workload at heart rate of 100 beats/min during exercise test and mortality (Studies II and III)

The workload achieved at a HR of 100 beats/min during exercise test (WL_{100}) reflects physiological responses to early stages of the exercise test and is determined by resting HR, maximal HR, cardiorespiratory fitness and steepness of the early HR rise as related to the overall HR increase (Figure 6). Because both a high resting HR (377,474,475) and low cardiorespiratory fitness (339,346,360) are known risk factors for death and CVD events both in asymptomatic persons and in patients with known or suspected CHD, we hypothesized that a low WL_{100} is an independent predictor for adverse outcomes.

The principal findings of this part of the study is that a low WL_{100} was associated with an increased CVD and CHD mortality in men who did not have prior CHD at baseline, and with an increased mortality in men with known suspected CHD at baseline. The association was independent of other HR-derived or other exercise test variables, and the magnitude of the association was comparable with that of conventional risk factors. In two previous studies with a similar finding (14,15), a high HR at a fixed submaximal workload, which equals a low workload at a fixed HR, has been considered as a surrogate measure of a low cardiorespiratory fitness, and the prognostic value of a high submaximal HR has been explained by this assumption (corresponding to graph c in Figure 6). Moreover, in those studies the actual maximal cardiorespiratory fitness was not measured and thereby could not be included as a covariate in the analysis. In the present study, a low WL_{100} was a strong predictor of premature CVD, CHD and all-cause death even after adjustment for directly measured VO_{2max}, which means that low cardiorespiratory fitness did not explain the association of a low WL_{100} with outcomes. This interesting finding suggests that an exaggerated HR response at low workload indicates increased risk in and of itself, instead of being only a surrogate marker of low cardiorespiratory fitness.

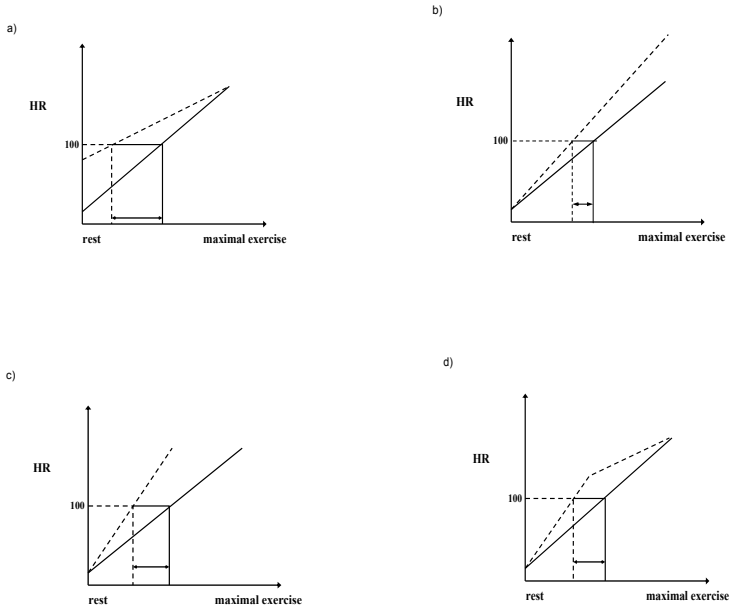

Figure 6. The graph illustrating how a high resting heart rate (a), a high maximal heart rate (b), a low cardiorespiratory fitness (c), and a disproportionately increasing heart rate at light workloads (d) each can have a lowering effect on workload achieved at heart rate of 100 beats/min as shown by broken lines.

Resting HR was the most significant individual determinant of WL_{100} and was also an independent predictor of outcomes. A high resting HR has been considered as a surrogate measure of reduced parasympathetic tone (476), which is a known risk factor for CVD events in asymptomatic subjects (418-420) and cardiac patients (51,430,431). Although the mechanism of the association is unknown, it has been suggested that a reduced parasympathetic tone makes an individual vulnerable to fatal ventricular arrhythmias in circumstances that may induce them, such as myocardial ischemia (430,431). It is possible that a high resting HR in men with a low WL_{100} reflects an impaired vagal control of the heart, but it is noteworthy that resting HR did not weaken the prognostic value of WL_{100} when entered in the same Cox model. Unfortunately, directly measured information on autonomic nervous system status (e.g. heart rate variability) is not available is the present study.

A continuosly elevated resting HR and exaggerated HR responses to daily stressful situations may burden cardiovascular system and enhance progression of athesclerosis (477,478) via several mechanisms (479-481). An elevated HR may be a sign of chronic sympathetic nervous system overactivity, which may induce numerous unfavorable changes from the viewpoint of cardiovascular health, as discussed previously. Because the cardiac work is a product of HR, stroke volume and BP, a consistently elevated HR will impose greater cardiac work (482).

The direct atherosclerotic effect of a high HR on the arterial wall can be explained by the intensification of the pulsatile nature of the blood flow and the associated changes in the shear stress (479,481,483). High HR is associated with a longer time spent in systole, during which changes in the rate of blood flow and departures from laminar flow are largest (479,484). The increase in arterial wall stress caused by a high HR can also be the result of a higher mean BP in individuals with tachycardia (485), which is due to the progressive shortening of the diastolic phase of the cardiac cycle (480,486). The increased arterial wall stress may perturb intercellular junctions, increase permeability of the endothelial cells and favor the ingress of atherogenic particles, leading to atherosclerotic plaques (479,483,487).

In rats, carotid artery compliance and distensibility have been shown to be markedly impaired by the progressive increase in HR caused by pacing (488). This might be due to the fact it takes a certain time for the arterial wall to distend fully in response to BP variations (480). On the other hand, pharmacologic HR reduction induced a significant decrease in thoracic aorta wall thickness in rats (489). In CHD patients, hemodynamic forces resulting from an increased HR may favor vulnerable coronary plaque disruption (490). In patients with restricted coronary blood flow a high HR can further increase cardiac ischemia and precipitate the occurrence of arrhythmias and impair left ventricular performance by increasing myocardial oxygen demand, facilitating desynchronization of ventricular myocardial cells and worsening coronary perfusion (480,491).

WL_{100} was recorded directly at HR 100 beats/min or interpolated linearly as a function of HR by using resting HR and the nearest HR value above 100 beats/min. The advantage of this method for defining WL_{100} is that the lowest HR values at the early

phase of the exercise test are not required to be below 100 beats/min. An exaggerated HR rise above the level of 100 beats/min at the first workload is an actual phenomenon in individuals with a limited exercise capacity, particularly if the first workload is not adjusted for the reduced performance. Resting HR, when measured before the exercise test, is higher than the true resting HR because of nervousness, excitement and apprehension related to the testing environment (476). Because data on the true resting HR measured at less stressful conditions was not available in the present study, it is impossible to evaluate to what extent interindividual differences in pre-test excitement affects resting HR values measured before test and consequently on WL_{100}. It is possible that a pronounced anticipatory HR response to test includes not only a high resting HR before the test, but also an exaggerated HR response at the first workloads (69,171,217), indicated by a low WL_{100}.

In previous reports from the KIHD Study, an exaggerated anticipatory BP response to exercise test has been linked to an increased left ventricular mass assessed by echocardiography (492), and a heightened BP response during mental stress has been linked to enhanced carotid atherosclerosis (493,494). Furthermore, a trend was observed between an exaggerated HR response during mental stress and enhanced carotid atherosclerosis in 4-year follow-up (493), but no association was observed to a progression of atherosclerosis from the fourth to eleventh follow-up year (494). Thus, a low WL_{100} may represent a trait characterized by an exaggerated cardiovascular responsiveness to stressful situations (corresponding to graph d in Figure 6).

A high chronotropic index at HR of 100 beats/min, reflecting a disproportionately steep HR rise at the early phase of a test, was an independent predictor of CVD and CHD death in men without CHD at baseline. This observation may further suggest that a trait characterized by an exaggerated cardiovascular responsiveness to stressful situations can to some extent explain the association of a low WL_{100} with an increased risk of outcomes in the current study.

To recapitulate, we suggest that a low WL_{100} concurrently reflects a high resting HR, an exaggerated HR increase at the beginning of an exercise test, and a low cardiorespiratory performance. While these variables separately had only a limited

predictive value in the present study, the combined variable WL_{100} provided valuable prognostic information beyond these variables.

We intentionally used the workload achieved at a fixed submaximal HR instead of using HR at a fixed submaximal workload. This approach has a solid physiological basis, as explained. Use of the workload achieved at a fixed submaximal HR enables the testing of subjects at approximately the same relative intensity independent of age, gender, size of body or fitness level, unlike when a fixed workload is used. However, careful inspection of Figure 4 suggest that the maximal predictive value might be found if a slightly lower HR value than 100 beats/min was used to quantify a submaximal HR response. Among asymptomatic subjects the association between a high HR at submaximal work and an increased risk of CVD death has been observed in studies that have used a treadmill as a testing mode (14,15), but not in studies using a cycle ergometer (227,342-344). In contrast, in patients with known or suspected CHD the findings have been just opposite (354,355). Therefore it is unclear whether the testing mode affects the prognostic value of a high HR at submaximal work or that of WL_{100}, and further studies are needed to address the issue.

Submaximal exercise tests have some benefits over maximal tests. A low level of exercise enables testing of individuals with a limited exercise capacity, and cardiovascular risks associated with high-intensity exertion can be largely avoided (17,69). This is an important aspect in subjects who have any contraindication for maximal test. If necessary, the exercise test can also be repeated frequently because of the short recovery period needed.

6.2.2.1 Workload at heart rate of 100 beats/min during exercise test and cardiovascular disease mortality in men without coronary heart disease at baseline

The results of the present study are in accordance with two previous population-based studies (14,15) which reported that a high HR at a fixed submaximal workload predicts CVD and CHD deaths. As explained, cardiorespiratory fitness did not explain the association of a low WL_{100} with outcomes. Furthermore, the level of exercise used to explore the association of a submaximal HR and workload with outcomes was lower in the present study compared with two previous studies (14,15). While the minimum HR

required for risk assessment was 116 (15) and 133 beats/min (14) in two previous studies, the mean HR to enable the risk assessment (ie. the first HR value above 100 beats/min) was only 104 beats/min in the present study.

Interestingly, three of the four studies (14,15,341), including the present study, investigating CVD and CHD death as an outcome have found a submaximal HR variable to be a statistically significant predictor. Conversely, all three (227,342-344) previous studies exploring a composite end point, including fatal and nonfatal CVD events, as an outcome have not found a submaximal HR variable to be a statistically significant predictor. It has been suggested that variables predicting nonfatal CVD events can be different than those predicting death, creating a situation where a variables's contrasting effects with respect to the two end points can cancel each other out (3-5). Because of a limited number of studies it is precipitated to state that a submaximal HR predicts fatal CVD events more potently than nonfatal CVD events. However, in the current study a low WL_{100} was not associated with an increased risk of AMI or nonfatal AMI, but it was an independent predictor of fatal AMI (15% of all AMIs).

One previous study (227) did not find the association between a low workload attained at HR of 150 beats/min and an increased risk of CHD event, but the association was observed when the workload attained was expressed as divided by body weight. The association may not have been observed because at the HR of 150 beats/min the difference in workload between groups with adverse and good prognosis had already narrowed considerably, although the difference might have been observed at lower HR levels. According to Figure 4 this could also have been the case in the study II of the current thesis. We did not divide WL_{100} by body weight, although there is a theroretical basis for it (69). Furthermore, because a high BMI was a risk factor for death in study II, it is possible that the prognostic value of WL_{100} would have improved if it had been expressed in relation to body weight.

A low stroke volume may be a common denominator for a high resting HR, an exaggerated HR increase at the beginning of an exercise test, and a low cardiorespiratory performance (18,69). We could not, however, directly assess stroke volume in the present study, and thereby its role in the association between WL_{100} and

mortality can not be considered any further. The exclusion of subjects who had an outcome event within two years after baseline and those whose test was terminated because of symptoms or findings potentially due to latent CVD did not weaken the prognostic value of WL_{100} much. This suggests that latent CVD probably does not explain the increased risk associated with a low WL_{100}.

6.2.2.2 Workload at heart rate of 100 beats/min during exercise test and mortality in men with known or suspected coronary heart disease at baseline

The results of the present study basically agree with the findings of a recent study in which a large HR increase from rest to a workload of 25 W at the onset of an exercise test was a strong predictor of adverse cardiac events and cardiac deaths in patients with CHD verified by angiography (354). In the present study, HR increase from rest to a workload of 50 W was associated with a nonsignificant trend ($p=0.11$) toward increased risk of death after stepwise selection (data not shown). Because of the testing protocol, the workload of 50 W was used instead of 25 W that hampers the comparison of the results of these two studies. Although WL_{100} was not measured in the study by Falcone et al (354), closer inspection indicates that men with an exaggerated HR response obviously had a low WL_{100}. The researchers suggested that a rapid HR increase was caused by a rapid vagal withdrawal reflecting an autonomic imbalance. Exercise capacity was lower in men with an exaggerated HR response. Unfortunately, exercise capacity was not included in the Cox model as a covariate, leaving open the possibility that an exaggerated HR response was caused by a low cardiorespiratory fitness.

The results of the present study also agree with the findings of two previous studies in dogs (365,366). The rise of HR at the early phase of an exercise stress was steeper in dogs with a healed myocardial infarction that were susceptible to ventricular fibrillation after experimentally induced coronary occlusion than in dogs that were resistant to ventricular fibrillation. The susceptibility to ventricular fibrillation was associated with a greater degree of left ventricular dysfunction (365), which probably also explains the higher HR at submaximal workloads.

On the contrary, in another recent study a HR increase from rest to 2 METs workload in treadmill was not associated with an increased risk of CVD death in patients referred

for exercise testing (355). Again, WL_{100} was not measured in the study by Leeper et al (355), but closer inspection shows that WL_{100} obviously was not different between subjects who died of CVD during the follow-up and survivors. One disparity between the present study and the study by Leeper et al (355) is that the latter used an individualized ramp treadmill protocol in which workload increments are tailored according to the estimated work capacity of a patient (268). It has been discussed that a prognostic value of an exaggerated BP response to submaximal exercise is directly related to the strain of the first workload(s) as related to the total exercise capacity of a subject (16). If a similar relationship exists for HR response too, then a discrepancy between the findings in the present study and in the study by Leeper et al (355) could to some extent be attributed to the different testing protocols used in these studies.

A low WL_{100} could result from left ventricular dysfunction in subjects with a more severe CHD (362-364). Although this possibility cannot be ruled out in the current study, an argument against this notion is that a low WL_{100} was not associated with a more prevalent history of myocardial infarction or self-reported cardiac insufficiency either in men using HR-lowering medication or in nonusers (data not shown). More specifically, a low WL_{100} could originate from myocardial ischemia beginning already at early exercise when HR is below 100 beats/min (361-363). An early appearance of myocardial ischemia has been shown to be associated with an adverse prognosis (495,496) and angiographically more severe CHD (496). Because the data on ST-segment depression at workloads below HR of 100 beats/min is not available, the role of early ischemia as a link between a low WL_{100} and an increased risk of death in the present study can not be excluded either.

6.3 Clinical implications

The recent consensus statements from the American Heart Association, the American College of Cardiology (10) and from the US Preventive Services Task Force (497) have led to recommendation against the use of exercise testing as a screening tool for detecting latent CHD in asymptomatic persons at low to intermediate risk. These recommendations have been largely based on an extensive body of literature

documenting the limitations of the ST-segment for diagnosing CHD in asymptomatic subjects (339). Still, reports on evaluation of the exercise test as a prognostic rather than a diagnostic test suggest that the prognostic value of the screening exercise test may have been underestimated (11,12,339). The latest version of the textbook written by major authorities in the field (17) states, indeed, that exercise testing should be used for screening healthy, asymptomatic individuals along with risk factor assessment (498). In this context assessment of exercise test result includes not only ST-segment diagnostics, but also other exercise test variables shown to have a prognostic value (17).

Nonetheless, no consensus exists whether this further risk stratification should be targeted to persons at an intermediate or high risk of events based on office tools such as the Framingham Risk Score or European Systematic Coronary Risk Evaluation (SCORE). Some authors have suggested that the current risk assessments based on conventional risk factors are especially ineffective among persons at an intermediate risk of events, and these individuals may benefit from further risk stratification (472,499). Recent prospective studies in asymptomatic persons have shown, however, that an additional prognostic value from exercise testing above conventional risk factors seems to be the largest in persons at a high risk of events (9,375,500). Whatever the target group, the primary aim of enhanced risk stratification is to detect those individuals who would benefit most from targeted aggressive treatment of risk factors (501). The recent expert statement concluded that the next major priority is the design and implementation of large-scale randomized trials to determine whether an exercise screening strategy leads to an improvement in outcomes (339). Additionally, these trials would provide much-needed evidence about the cost-effectiveness in execise testing in asymptomatic persons (339).

Because in patients with known or suspected CHD the value of exercise testing is more clearly established, the post-exercise test risk assessment serves as a guide to a particulatr management strategy that is viewed as most appropriate, based on expected outcomes (10,190). According to the guidelines of the American Heart Association and the American College of Cardiology (10), patients with a low-risk exercise test result can be treated medically without need for referral to cardiac catheterization. Patients with a high-risk exercise test result should usually be referred for cardiac

catheterization. Patients with an intermediate-risk exercise test result should be referred for additional testing, either cardiac catheterization or an exercise imaging study, depending on other clinical variables (10).

An obvious question with regard to the clinical use of WL_{100} or HR40-100 is whether it is a modifiable risk factor. For WL_{100}, the existing literature shows that physical activity or training lowers HR at a fixed submaximal workload (190), and thus also increases WL_{100}. This has been observed both in healthy subjects (29,298,299) and in patients with CHD (502-504). Weight loss in obese subjects is another physiological means to increase WL_{100}. This effect is especially pronounced in activities involving weight-bearing, such as walking on a treadmill.

As far as HR40-100 and other chronotropic incompetence variables are concerned, the effects induced by physical training are not as straightforward. In healthy persons, physical training lowers resting HR, but usually has no effect on maximal HR (17,190). Hence, chronotropic incompetence variables (Table 3) which are based solely on maximal HR (inability to achieve the fixed %-value of age-adjusted expected maximal HR or maximal HR) do not change as a consequence of training. On the other hand, chronotropic incompetence variables that do not depend exclusively on maximal HR (HR reserve, submaxCRI, maxCRI, HR40-100) may change towards values attributed to a lower risk after training, depending on whether resting HR decreases. Cessation of smoking has been speculated to improve chronotropic incompetence variables to a favorable direction, but direct evidence of this is lacking (315,316). In contrast to healthy persons, maximal HR may increase as a result of physical training in CHD patients (190). Changes in maximal HR may reflect a greater level of effort applied after training (190). Whether it is due to habituation to the testing situation, improved self-confidence to exercise until a true maximum is achieved, or physiological alterations induced by training, is not known.

In the current study, several exercise test variables predicted outcomes independent of each other or conventional risk factors. This highlights the fact the maximal prognostic yield from exercise test is obtained by measuring several variables at submaximal and maximal workload and during the recovery phase. The underestimation of exercise testing as a prognostic tool has been based on a constricted assessment of solely the ST-

segment response to exercise. Several studies conducted during the last 15 years in healthy individuals and patients have shown without doubt that exercise testing can offer additional prognostic information beyond conventional risk markers. Consequently, multivariable equations and scores derived from clinical variables and exercise test results are considered to be a highly recommended method to evaluate an individual's risk for adverse CVD events in the future (12,339,340).

7. SUMMARY AND CONCLUSIONS

1. Both a low workload achieved at HR of 100 beats/min and a blunted HR increase particularly in the latter half of the maximal exercise test are associated with an adverse prognosis in men without CHD at baseline. The findings support the main hypothesis that a bimodal relationship exists between HR and prognosis in which both an exaggerated HR response at submaximal workload and a blunted HR response at maximal or near maximal workload are associated with an unfavorable prognosis.

2. The heart rate increase from 40% to 100% of maximal work capacity (HR40-100) is a strong predictor of all-cause, CVD and CHD mortality in middle-aged men without CHD at baseline. Additionally, a low HR40-100 is associated with an increased risk of future myocardial infarction in the same population. The magnitude of the association is comparable with that of other major CVD risk factors and exercise test variables, including other variables quantifying chronotropic incompetence.

3. A low workload achieved at HR of 100 beats/min during an exercise test predicts CVD and CHD death in middle-aged men without CHD at baseline, and also all-cause death in men with known or suspected CHD at baseline. The association between workload achieved at HR of 100 beats/min and mortality is not explained solely by cardiorespiratory fitness, as previously assumed.

4. Several exercise test variables predict CVD events independently of each other and conventional risk factors. This emphasizes that the maximal prognostic yield from an exercise test is obtained by measuring several variables at submaximal and maximal workloads and during the recovery phase.

5. A complex interplay exists between an exaggerated BP response to exercise and a blunted HR increase during the latter half of maximal exercise test so that their simultaneous presence is related to a markedly increased risk of future myocardial infaction. Arterial dysfunction may be a link between an exaggerated blood pressure response, a blunted HR increase and an increased risk of future myocardial infarction.

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