

SUBMAXIMAL HEART RATE IN EXERCISE TEST AND
MORTALITY IN MIDDLE-AGED MEN

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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 the exercise test derived HR variables have excited widely as prognostic markers of mortality and cardiac events both in asymptomatic persons as well as in cardiovascular disease (CVD) patients.

Workload achieved at HR of 100 beats/min (WL_{100}) was 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, the deaths were ascertained by linkage to the National Death Registry.

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 low workload achieved at a submaximal HR of 100 beats/min predicts CVD and CHD mortality in men without CHD as well as 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. 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 as well as during recovery phase in order to maximize the prognostic yield obtained from the exercise test.

PREFACE

This M.Sc.-thesis is a part 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. The supervisors of this M.Sc.-thesis are professors Rainer Rauramaa, M.D., Ph.D., M.Sc., and Timo Lakka, M.D., Ph.D. The reviewers are professor Rauramaa and docent Hanna-Maaria Lakka, M.D., Ph.D. The study was performed at Kuopio Research Institute of Exercise Medicine

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1 INTRODUCTION

Coronary heart disease (CHD) is the leading cause of death in the developed world (Pasternak et al. 2003) and may become the leading cause of death in the entire world (Murray and Lopez 1997). Although the incidence of CHD has been decreasing over the last two decades, the prevalence is expected to increase given the increasing elderly population (Shetler et al. 2001, Froelicher et al. 2002, Froelicher et al. 2003). It is important to implement cost-effective strategies that direct the appropriate individuals to the optimal risk reduction procedures through risk prediction (Ashley et al. 2002, Froelicher et al. 2002, Froelicher et al. 2003). There is a growing awareness of the need to apply statistical techniques to develop evidence-based scores for better decision-making (Swets et al. 2000). 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 (Shetler et al. 2001, Froelicher et al. 2002, Froelicher et al. 2003). The most important outcome is death but the future risk of nonfatal clinical outcomes is an important element of risk evaluation as well (Shetler et al. 2001, Froelicher et al. 2002, Froelicher et al. 2003). 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 (De Backer et al. 2003).

Exercise testing is not recommended in asymptomatic subjects due to both lacking evidence of its value and because false positive exercise electrocardiograms (ECGs) are common (Erikssen et al. 2004). However, the real issue is not to identify CHD but to predict outcome (Gibbons et al. 2002a). Because of this, increasing attention has been focused on the exercise test as a prognostic, as opposed to diagnostic, modality (Ashley et al. 2000a; Lauer 2001b). It is well known that several exercise test indices, besides ECG findings, such as exercise capacity (Erikssen et al. 2004, Laukkanen et al. 2007), heart rate (HR) (Ekelund et al. 1988, Slattery and Jacobs 1988) and blood pressure (BP) responses (Mundal et al. 1994) 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 (Froelicher and Myers 2006). 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 (Rowell 1993). 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 (Navare and Thompson 2003, Froelicher and Myers 2006). 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 (Rowell 1993). Acute exercise can elicit cardiovascular abnormalities that are not present at rest, and it can be used to determine the adequacy of cardiovascular function (Navare and Thompson 2003).

Heart rate 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 (Hammond and Froelicher 1985, Hainsworth 1995, Iellamo 2001). Recently, the changes in HR during and after exercise have emerged as powerful measures of risk for future CVD event in their own right (Lauer 2001c). An interesting fact from 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 (Ellestad 1996, Chaitman 2003, Routledge and Townend 2006).

Office-based assessment of conventional risk factor burden is necessary but may not accurately estimate risk of future CVD events (Brindle et al. 2006). 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 (Laukkanen 2005). 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 (Hammond and Froelicher 1985, Hainsworth 1995, Camm and Fei 1996a). The normal HR is determined by the firing frequency of SA node (Hammond and Froelicher 1985, Hainsworth 1995, Camm and Fei 1996a). 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 (Hariman et al. 1980, Camm and Fei 1996a, Awtry and Loscalzo 2001b). It contains clusters of cells, poor in contractile filaments, where the automatic activity resides mostly in the pacemaker or P cells (Opie 1991). In contrast are transitional cells, which lie near the periphery of the SA node (Brown 1982). 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 (Hammond and Froelicher 1985, Bouman and Jongasma 1986, Camm and Fei 1996a). Its primary source of blood is from SA nodal artery which originates from the right coronary artery in about 60% of humans (Camm and Fei 1996a, Moore and Dalley 1999, Mangrum and DiMarco 2000).

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 because there is no further tissue to depolarize (Hammond and Froelicher 1985, Awtry and Loscalzo 2001b, Moore 2006). Further conduction occurs only after a new impulse is formed in the SA node (Awtry and Loscalzo 2001a).

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 (Guyton and Hall 1996). The resting potential of a typical cardiac cell is -80 to -90 millivolts (mV) (Guyton and Hall 1996, Awtry and Loscalzo 2001a). 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 (Guyton and Hall 1996, Awtry and Loscalzo 2001a, Moore 2006). The appearance of the action potential of SA nodal cells is

different from that of the typical myocyte (Guyton and Hall 1996, Awtry and Loscalzo 2001a). The normal resting potential of these cells is higher (-55 to -60 mV), and the spontaneous diastolic depolarization is much more pronounced (Binah and Rosen 1992, Guyton and Hall 1996, Awtry and Loscalzo 2001a). The slope of the diastolic depolarization determines the rate at which a cell will spontaneously depolarize (automaticity) until it reaches threshold potential, thus generating an action potential that is then propagated to surrounding cells (Braunwald and Ross 1979, Guyton and Hall 1996, Awtry and Loscalzo 2001a).

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 (Irisawa et al. 1993, Guyton and Hall 1996, Moore 2006). The ionic basis of SA node pacemaker activity is 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 (Podrid et al. 1990, Binah and Rosen 1992, Moore 2006). 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 (DiFrancesco 1993, Guyton and Hall 1996). 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 (Maylie and Morad 1984, Baruscotti et al. 2005, Moore 2006). A major role, however, in the generation and control of the diastolic depolarization is played by a prominent increase in an inward depolarizing current I_f (Guyton and Hall 1996, Baruscotti et al. 2005, Moore 2006). 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 (Irisawa et al. 1993, Moore 2006). This triggers the focal release of Ca^{2+} (sparks) from sarcoplasmic reticulum Ca^{2+} release channels, further increasing $[Ca^{2+}]$ in the space (Moore 2006). This in turn leads to the activation of forward Na^+ - Ca^{2+} exchange (I_{NCX}) and further membrane depolarization toward a threshold potential (Moore 2006). 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} (Irisawa et al. 1993, Moore 2006).

The interrelated actions of $I_{Ca,T}$, sarcoplasmic reticulum Ca^{2+} sparks, and I_{NaCa} creates a positive feedback loop that culminates in a progressive membrane depolarization to a threshold potential (Moore 2006). Once threshold is achieved, an L-type inward Ca^{2+} current (I_{Ca}) rapidly activates, and an action potential is triggered (Podrid et al. 1990, Irisawa et al. 1993, DiFrancesco 1995).

The SA node usually has the fastest diastolic depolarization and thus functions as the normal pacemaker of the heart (Hammond and Froelicher 1985, Hainsworth 1995, Camm and Fei 1996a). If the SA node fails, the AV node has the next fastest pacemaker rate (approximately 40-60 beats/min) (Hainsworth 1995, Guyton and Hall 1996, Awtry and Loscalzo 2001a).

2.2 CARDIAC INNERVATION

The normal myocardium is richly innervated by the autonomic nervous system (Levy and Martin 1979, Awtry and Loscalzo 2001b). The heart is supplied by autonomic nerve fibers from superficial and deep cardiac plexuses from which three major cardiac nerves project into the heart (Janes et al. 1986, Moore and Dalley 1999). These nerve networks lie between the bifurcation of the trachea and the ascending aorta, and superior to the bifurcation of the pulmonary artery (Moore and Dalley 1999). The parasympathetic supply is from preganglionic cardiac branches of the vagus nerves (Shields 1993, Hainsworth 1995, Freeman et al. 2006,). The cell bodies of the parasympathetic postganglionic fibers constitute intrinsic ganglia in the vicinity of SA and AV nodes (Levy and Martin 1979, Hammond and Froelicher 1985, Shields 1993). The postganglionic parasympathetic fibers innervates the atria but has few projections to the ventricles although there is increasing evidence to show that the vagal nerves innervate the ventricular myocardium as well (Levy and Martin 1979, Standish et al. 1994, Johnson et al. 2004). The cell bodies of cardiac afferent vagal neurons are contained within the nodose ganglia inferior to the jugular foramen (Shields 1993, Moore and Dalley 1999, Browning and Mendelowitz 2003). The central fibers of these bipolar neurons continue to ascend in the vagus to enter the brain stem (Shields 1993, Moore and Dalley 1999, Browning and Mendelowitz 2003).

Both pre- and postganglionic cardiac parasympathetic fibers release acetylcholine as neurotransmitter (Levy and Martin 1979, Shields 1993, Freeman et al. 2006). 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 (Shields 1993, Moore and Dalley 1999, Freeman et al. 2006). Once the acetylcholine has been secreted, it persists in the tissue for a few seconds; then most of it is split into an acetate ion and choline by the enzyme acetylcholinesterase (Levy and Martin 1979, Berne and Levy 1988).

The sympathetic supply is from postganglionic cardiac sympathetic fibers (Hammond and Froelicher 1985, Hainsworth 1995, Freeman et al. 2006). The cell bodies of pre- and postganglionic fibers are 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 (Hammond and Froelicher 1985, Hainsworth 1995, Freeman et al. 2006). Sympathetic nerve terminals are located throughout the atria and ventricles (Hammond and Froelicher 1985, Shepherd 1987, Hainsworth 1995). The cardiac afferent sympathetic neurons have their cell bodies in C_6 - T_6 dorsal root ganglia, and they enter the dorsal horn of the spinal cord (Shields 1993, Moore and Dalley 1999, Bear et al. 2001). 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 (Shields 1993, Bear et al. 2001).

Pre- and postganglionic cardiac sympathetic fibers release acetylcholine and noradrenaline as neurotransmitters, respectively (Hammond and Froelicher 1985, Shields 1993, Freeman et al. 2006). In addition to sympathetic innervation directly from sympathetic nerve endings, sympathetic nervous system may have an effect indirectly by stimulating adrenal medulla to secrete adrenaline and noradrenaline into the circulating blood and these two hormones can further bind to the adrenergic receptors of the heart (Shields 1993, Guyton and Hall 1996, Freeman et al. 2006,). The effects of noradrenaline and adrenaline on the heart are mediated by α_1 -, β_1 - and β_2 -adrenergic receptors of which β_1 is the most abundant subtype in the heart representing approximately 75% of total (Hammond and Froelicher 1985, Bristow et al. 1990, Freeman et al. 2006). Noradrenaline excites mainly α -receptors but excites the β -receptors to a less extent as well whereas adrenaline excites both types of receptors approximately eqally (Parkinson 1990, Guyton and Hall 1996). Noradrenaline is removed from the secretory site in three ways: (i) reuptake into the adrenergic nerve ending (accounting for removal of 50%-80% of the secreted noradrenaline); (ii) diffusion

away into the surrounding body fluids; and (iii) destruction by enzymes (Levy and Martin 1979, Berne and Levy 1988, Opie 1991).

The latency of the response of the SA node to vagal stimulation is very short (Levy and Martin 1979, Rowell 1993, Hainsworth 1995). After a single stimulus, the maximum response has been reported to occur within only 400 milliseconds (Levy et al. 1970, Levy and Martin 1979, Rowell 1993). Thus, vagal stimulation results in a peak response either in the first or in the second beat after its onset (Levy and Martin 1979, Hainsworth 1995). On the other hand, following the onset of sympathetic stimulation, there is a latent period of up to 5 seconds followed by a progressive increase in HR which reaches a steady level in 20 to 30 seconds (Levy and Martin 1979, Rowell 1993, Hainsworth 1995). Both parasympathetic and sympathetic preganglionic fibers are myelinated whereas postganglionic fibers do not have myelin sheath (Barron and Chokroverty 1993, Shields 1993). The fact that parasympathetic postganglionic fibers are clearly shorter than sympathetic postganglionic fibers partly explains the slower cardiac responses to sympathetic stimuli compared to parasympathetic ones because the neural transmission is faster in myelinated fibers (Guyton and Hall 1996).

HR is determined from ECG as the reciprocal of the time interval between two successive R peaks (which reflect depolarization of ventricles) and expressed as beats/minute (Hammond and Froelicher 1985, Froelicher and Myers 2006). The intrinsic HR, in absence of any neurohumoral influence, is about 100 to 120 beats/min and declines with age (Jose and Taylor 1969, Lewis et al. 1980, Hainsworth 1995). Without neurohumoral influence maximal HR at peak exercise is 18-24% lower than with intact neurohumoral influence (Ekblom et al. 1972, Lewis et al. 1980). 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 (Ekblom et al. 1973, Mangrum and DiMarco 2000, Navare and Thompson 2003). In resting conditions, both autonomic divisions are thought to be tonically active with the vagal effects dominant (Hammond and Froelicher 1985, Mangrum and DiMarco 2000, Freeman et al. 2006). In normal adults at rest, the HR is about 70 to 85 beats/min and the normal range is 60 to 100 beats/min (Ekblom et al. 1973, Spodick et al. 1992, Camm and Fei 1996a).

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 (Levy and Martin 1979, Moore 2006). 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 (Stone and Liang 1984, Hainsworth 1995, Camm and Fei 1996a). Weak to moderate vagal stimulation will slow the HR often to as little as one-half normal (Levy and Martin 1979, Guyton and Hall 1996). 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 SA node (Hainsworth 1995, Guyton and Hall 1996, Mangrum and DiMarco 2000). Increased vagal input into the SA node results in the release of acetylcholine from nerve endings at the SA node and released acetylcholine binds to muscarinic receptors (Levy and Martin 1979, Guyton and Hall 1996, Moore 2006). 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 (Levy and Martin 1979, Irisawa et al. 1993, Moore 2006). Second, 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 paragraph) (Irisawa et al. 1993, DiFrancesco 1995, Baruscotti et al. 2005). The former mechanism is more prominent at higher levels of vagal activation and explains acetylcholine-mediated action potential hyperpolarization (Irisawa et al. 1993, Moore 2006). 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 (Irisawa et al. 1993, Moore 2006). Besides these mechanisms vagal activation may affect diastolic depolarization by inducing an upward shift in action potential threshold (Moore 2006). The net effect of these three mechanisms is to prolong the time required for diastolic depolarization to proceed to an action potential threshold (Moore 2006).

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 (Blomqvist and Saltin 1983, Hainsworth 1995, Åstrand et al. 2003). Strong sympathetic stimulation can increase the HR in adult humans to 180 to 200 beats/min (Guyton and Hall 1996, Wilmore and Costill 2004). Sympathetic stimulation, either directly from sympathetic nerve endings in the heart (noradrenaline) or indirectly by means of circulating adrenaline, accelerates

pacemaker activity of SA node cells (Shepherd 1987, DiFrancesco 1995, Baruscotti et al. 2005). This manifests itself in a marked increase in the rate of diastolic depolarization and an increase in the amplitude of the pacemaker action potential (Podrid et al. 1990, Moore 2006). The increase in the rate of diastolic depolarization results from cyclic adenosine monophosphate-mediated enhanced intracellular Ca^{2+} handling and from increase in the magnitude of I_f (Irisawa et al. 1993, DiFrancesco 1995, Baruscotti et al. 2005). 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 (Irisawa et al. 1993, DiFrancesco 1995, Baruscotti et al. 2005). 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 (Moore 2006). These events culminate in a faster increase in $[\text{Ca}^{2+}]$ in the subsarcolemmal space and a more robust activation of a depolarizing I_{NCX} (Moore 2006). 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 (DiFrancesco 1995, Baruscotti et al. 2005). The net effect of these cyclic adenosine monophosphate-mediated processes is a shortening of diastolic depolarization and an increase in HR (Irisawa et al. 1993, DiFrancesco 1995, Baruscotti et al. 2005). Still one possible β -adrenergic-mediated mechanism involves stimulation of Na^+ - K^+ -pump activity (Opie 1991). 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 (Opie 1991).

2.3 THE 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 (Levy and Martin 1979, Hammond and Froelicher 1985, Camm and Fei 1996a). Located bilaterally mainly in the reticular substance of the medulla and lower third of the pons is an area called the vasomotor center (Guyton and Hall 1996, Moore and Dalley 1999). The center transmits parasympathetic impulses through the vagus nerves to the heart and

transmits sympathetic impulses through the spinal cord and peripheral sympathetic nerves to the heart as well as to blood vessels of the body (Levy and Martin 1979, Guyton and Hall 1996). From the cardiovascular control viewpoint the most important parts of the vasomotor center are the nucleus tractus solitarius, the ventrolateral medulla, the dorsal motor nucleus, and the nucleus ambiguus (Seller 1991). 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 as well as from carotid sinuses via glossopharyngeal nerves (Shepherd 1987, Potts 2006, Chaitman 2007). The output signals from the nucleus tractus solitarius controls the activities of those areas in the vasomotor centre which in turn regulate the descending parasympathetic and sympathetic output (Spyer 1990, Potts 2006, Chaitman 2007). Widespread areas of the higher nervous centers can either excite or inhibit the vasomotor center (Stone and Liang 1984, Shepherd 1987, Mifflin et al. 1988). The more lateral and superior portions of the reticular substance cause excitation, whereas the more medial and inferior portions cause inhibition (Guyton and Hall 1996). The hypothalamus can exert either powerful excitatory or inhibitory effects on the vasomotor centre: 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 (Mifflin et al. 1988, Loewy 1990, Guyton and Hall 1996). 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 (Loewy 1990, Guyton and Hall 1996).

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 (Hammond and Froelicher 1985, Plecha et al. 1988, Armour 1999). Parasympathetic activity to the SA node originates from the central nervous system rather than from peripheral ganglia (Loewy 1990) and section of the preganglionic fibers, leaving only postganglionic innervation intact, releases the heart from parasympathetic inhibition (Mendelowitz 1999). Preganglionic cardiac vagal fibers are tonically active, with a firing pattern that is pulse synchronous and most active during postinspiration and reduced during inspiration (a respiratory sinus arrhythmia) (Seller 1991, Mendelowitz 1999). 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 from synaptic activity those neurons are normally silent (Seller 1991, Mendelowitz 1999). The synaptic input to cardiac vagal neurons are therefore important in maintaining normal heart rate and cardiac function (Mendelowitz 1999). A major pathway to the nucleus ambiguus originates from the nucleus tractus solitarius, and electrophysiological experiments demonstrate that the pathway is glutamergic (Andresen and Kunze 1994, Standish et al. 1994, Neff et al. 1998). It is still unknown whether the nucleus tractus solitarius neurons receiving 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 (Seller 1991, Mendelowitz 1999). Cardiac vagal neurons have also excitatory input from cholinergic nicotinic neurons which are possibly involved in the respiratory sinus arrhythmia (Mendelowitz 1999) and from dopaminergic neurons which induce bradycardia via activation of D₂-receptors (Chitravanshi and Calaresu 1992).

The ventrolateral medulla consists of rostral (rVLM) and caudal (cVLM) parts and population of rVLM neurons (premotor neurons) with their spinal projection to interomediolateral cell column constitutes the main and final integration center in the brainstem for generating the sympathetic outflow to cardiovascular effector organs (Reis et al. 1988, Seller 1991, Potts 2006). Under normal conditions rVLM transmits signals continuously to the sympathetic preganglionic fibers in interomediolateral cell column via glutamergic transmission (Reis et al. 1988, Seller 1991, Guyton and Hall 1996). This tonic sympatho-excitatory activity of rVLM is, however, continuously inhibited by gamma-aminobutyric acid-mediated transmission from cVLM (Reis et al. 1988, Seller 1991, Potts 2006). cVLM in turn receives tonic glutamergic excitatory input from the nucleus tractus solitarius (Seller 1991, Potts 2006). 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 (Reis et al. 1988, Seller 1991, Potts 2006).

2.4 THE 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 (Camm and Fei 1996a). Postganglionic sympathetic and vagal fibers often lie side by side in the walls of the heart (Ehinger et al. 1970, Levy and Martin 1979). 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 (Levy and Martin 1979, Levy et al. 1981). 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 (Levy 1971, Levy and Martin 1979). 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) (Levy 1971, Levy and Martin 1979, Mendelowitz 1999). Two major mechanisms have been suggested for the explanations for the antagonist effects of vagal stimulation on sympathetically induced responses (Levy 1971, Levy and Martin 1979, Mendelowitz 1999). The first is a presynaptic mechanism, in which acetylcholine reduces the amount of noradrenaline released from sympathetic nerve terminals (Starke 1977, Levy and Martin 1979, Mendelowitz 1999). The second is a postsynaptic mechanism, in which acetylcholine reduces the magnitude of the response to a given adrenergic stimulus (Levy 1971, Levy and Martin 1979, Mendelowitz 1999). This second mechanism presumably involves inhibitory G_i -protein-dependent inhibition of cyclic adenosine monophosphate synthesis (DiFrancesco and Tromba 1988, Mendelowitz 1999).

On the other hand, intense sympathetic stimulation attenuates the chronotropic responses to vagal stimulation in that case when sympathetic stimulation antecedes vagal stimulation (Yang and Levy 1992). 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 (Warner and Levy 1989). Neuropeptide Y inhibits acetylcholine release from vagal nerve endings (Yang and Levy 1992). Additionally, catecholamines can reduce the release acetylcholine by binding to α -adrenergic receptors in the presynaptic region of the parasympathetic nerve fibers (Åstrand

et al. 2003). 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) (Hammond and Froelicher 1985, Åstrand et al. 2003).

Opioids modulate parasympathetic control of HR via receptors at the SA node (Farias et al. 2001), or prejunctionally on vagal nerve terminals (Caffrey 1999) or within nearby parasympathetic ganglia (Caffrey 1999) resulting in attenuation of vagally mediated bradycardia.

Besides pre- and postsynaptic interactions described above, processing probably occurs within intrinsic cardiac nervous system which involves afferent neurones, local interconnecting neurones as well as both parasympathetic and sympathetic efferent postganglionic neurones (Armour 1999). 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 (Armour 1999).

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 (Opie 1991). 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, the processes called up- and downregulation (Opie 1991). For example, in the congestive heart failure there is a chronic high-level exposure to catecholamines which incurs reduction in the number of β_1 -receptors, while β_2 -receptor density remains constant (Bristow et al. 1990, Horn and Bilezikian 1990, Opie 1991). 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 (Bristow et al. 1990). The underlying molecular mechanisms of uncoupling are the increased level of inhibitory G_i -protein leading to a reduced ratio G_s/G_i , as well as an impaired G_s -mediated coupling between β -receptor and adenylate cyclase (Bristow et al. 1990, Horn and Bilezikian 1990). The third form of the changed response is a change in receptor affinity (Opie 1991). 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 (Opie 1991).

The chronotropic response of SA node to a neural stimulation also involves genetic variation which is yet poorly known (Hautala et al. 2006, Nieminen et al. 2006, Snyder et al. 2006). Arg389Gly polymorphism of the β_1 -adrenergic receptor is associated with difference in HR at rest (Bengtsson et al. 2001, Humma et al. 2001) but not during exercise (Buscher et al. 2001, Xie et al. 2001, Leineweber et al. 2004). Gene GNAS1 encodes α -subunit of the stimulatory G-protein that couples β_1 -adrenergic receptor with the adenylyl cyclase (Nieminen et al. 2006). T393C polymorphism of GNAS1 modulates HR response when values at rest, maximal exercise and recovery are considered together (Nieminen et al. 2006). Arg16Gly polymorphism of the β_2 -adrenergic receptor is associated with difference in HR at rest but during low or high intensity exercise the difference in HR does not persist between genotypes (Snyder et al. 2006). The rs324640 polymorphism of the muscarinic M₂-receptor gene is associated with difference in HR recovery after exercise but maximal HR is not different between the genotypes (Hautala et al. 2006).

2.5 THE 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) (Hammond and Froelicher 1985, Hainsworth 1995, Iellamo 2001). Some reflexes may increase HR through a decrease in vagal tone, an increase in sympathetic activity, or both, whereas others exert the opposite effects (Hainsworth 1995, Iellamo 2001). In the intact human cardiovascular system several reflexes and control mechanisms operate simultaneously, and the interactions are quite complex (Hainsworth 1995, Iellamo 2001). 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 (Mitchell 1990, Iellamo 2001). Different control mechanisms for 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 (Mitchell 1990, Waldrop et al. 1996). Besides neurally mediated reflexes, some humoral factors, such as cortisol, glucagon, growth hormone and

thyroxine may play a minor modifying at the control of HR (Hammond and Froelicher 1985, Berne and Levy 1988, Camm and Fei 1996a).

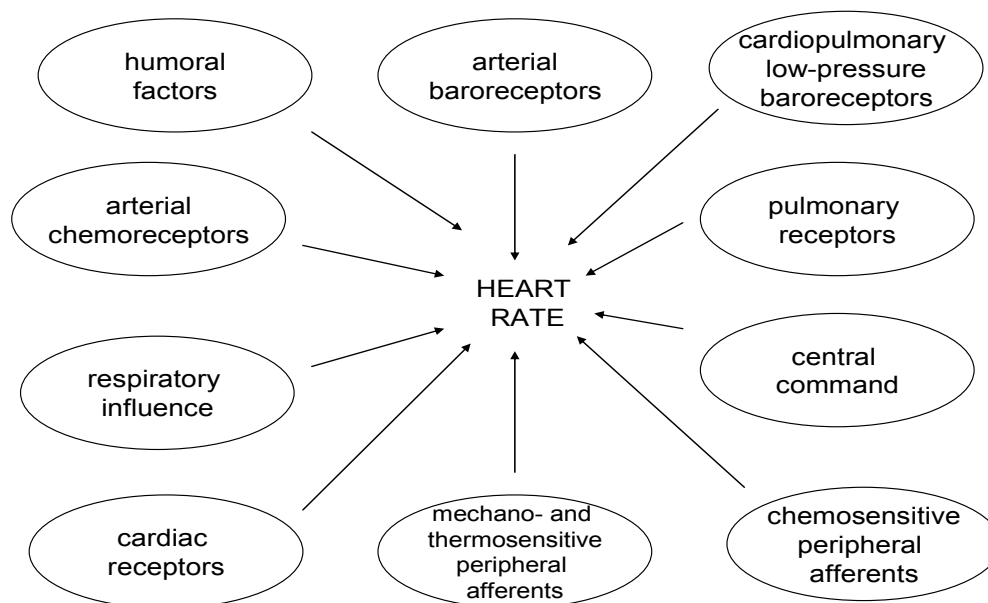


Figure 1. The overview of mechanisms influencing heart rate. See text for the more detailed discussion about the effects mediated by each individual mechanism.

2.5.1 Arterial baroreceptors and cardiopulmonary low-pressure baroreceptors

The function of the arterial baroreceptors is to maintain a normal BP (Kirchheim 1976, Raven et al. 1997, Raven et al. 2002). The arterial baroreceptors include carotid and aortic baroreceptors which are spray-type nerve endings located in the walls of several of the large arteries (Shields 1993, Camm and Fei 1996a, Raven et al. 2002). Carotid receptors lie in the wall of the internal carotid artery on either side (Joyner and Shepherd 1991, Hainsworth 1995, Camm and Fei 1996a). Each send impulses centrally in the sinus nerve (of Hering), a branch of the glossopharyngeal nerve (Joyner and Shepherd 1991, Shields 1993, O'Leary and Potts 2006). Aortic arch receptors on the left are close to the aortic arch, but those to the right lie at the origin of the right subclavian artery and in the adjacent regions of the brachiocephalic artery (Joyner and Shepherd 1991, Camm and Fei 1996a, De Sutter et al. 2006). From the aortic receptors, activity travels centrally in small vagal branches (Joyner and Shepherd 1991, Shields 1993, O'Leary and Potts 2006). 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) (Raven et al. 1997, Raven et al. 2002, De Sutter et al. 2006). Afferent nerves transmit the baroreceptor activity to the nucleus tractus solitarius which modulates autonomic outflow to buffer the rise in pressure (Hainsworth 1995, Camm and Fei 1996a, Raven et al. 2002). Conversely, a fall in BP reduces baroreceptor discharge and trigger adjustments that oppose the hypotension (Hainsworth 1995, Raven et al. 1997, Raven et al. 2002). The baroreceptors respond much more to a rapidly changing pulsatile pressure than to a stationary pressure (Guyton and Hall 1996, Åstrand et al. 2003). In dogs, arterial rheoreceptors has been identified in the carotid sinus that respond to increased blood flow by sensitizing the function of baroreceptors (Hajduczuk et al. 1988). The existence of rheoreceptors in humans, not to speak of their role in regulation of HR, 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 of them via modulation of neural output (Rowell 1993, Raven et al. 1997, Seals 2006). In hypertensive stimulus there is a rapid decrease in HR due to increased vagal discharge to the heart (Scher et al. 1991, Rowell 1993, O'Leary and Potts 2006). In hypotensive stimulus there is an initial rapid increase in HR with withdrawal of vagal tone but now accompanied by a slower rise in HR due to increased sympathetic discharge (Scher et al. 1991, Rowell 1993, O'Leary and Potts 2006). The increase in total peripheral resistance (vasoconstriction) plays a major role in hypotensive stimulus whereas in hypertensive stimulus a decrease in HR is more important (Scher et al. 1991, Rowell 1993). 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 (Ogoh et al. 2003). Previously it was assumed that aortic baroreceptors operates over a higher range of arterial pressures than carotid receptors (Sagawa 1983, Hainsworth 1995, Guyton and Hall 1996) but more recent studies have shown that both baroreceptor populations operate over the same range of pressures (Smith et al. 2001). The estimated contribution of carotid baroreflex to overall baroreflex control is from 30% to 50 % (Smith et al. 2001, Fadel et al. 2003). Aortic and carotid baroreceptors summate in their reflex effects (Scher et al. 1991, Hainsworth 1995). 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 as well as the magnitude of the step in pressure to the baroreceptors (Yamazaki and Sagawa 1989, Scher et al. 1991, Hainsworth 1995).

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 by baroreflex has puzzled physiologists for over a century (Zuntz and Geppert 1886, Krogh and Lindhard 1917). 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 (Potts et al. 1993, Papelier et al. 1994, Norton et al. 1999). 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 (Rowell and O'Leary 1990, Joyner 2006, Raven et al. 2006). 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 (Collins et al. 2001, Raven et al. 2006). The central command is probably the primary regulator of baroreflex resetting during exercise (Gallagher et al. 2001b, Gallagher et al. 2006, Raven et al. 2006). Muscle chemoreflex is capable to reset baroreflex as well but in normal dynamic exercise it acts more as a modulator of central command-induced resetting (Gallagher et al. 2001a, Gallagher et al. 2006, Raven et al. 2006).

Cardiopulmonary low-pressure baroreceptors monitor cardiopulmonary blood volume by sensing changes in the filling pressure of the chambers of the heart and pulmonary arteries and veins, as well as cardiac contractility and afterload (Rowell 1993, Rowell et al. 1996, Ray and Saito 2000). 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 (Rowell 1993, Ray and Saito 2000, Seals 2006). 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 (Rowell 1993, Ray and Saito 2000, Seals 2006). When central venous pressure is normal cardiopulmonary low-pressure baroreceptors tonically inhibit vasoconstriction induced by arterial baroreflex (Rowell 1993, Raven et al. 2000, Ray and Saito 2000).

The cardiopulmonary low-pressure baroreceptors are not reset by central nervous system during exercise, but they continuously inhibit arterial baroreceptor induced vasoconstriction (Rowell 1993, Raven et al. 1997, Ray and Saito 2000). 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 (Mack et al. 1988). Likewise, the cardiopulmonary low-pressure baroreceptors inhibit the muscle metaboreflex-mediated vasoconstriction during dynamic exercise (Collins and DiCarlo 1993).

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 (Rowell 1980, Raven et al. 1997, Delp and O'Leary 2004). These signals activate separately both somatomotor and cardiovascular control systems at the onset of exercise (Mitchell 1990, Rowell 1992, Delp and O'Leary 2004). Activation is in direct proportion to the number of motor units required to maintain a given force of contraction (Rowell 1992, Rowell 1993, Rowell et al. 1996). 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 (Rowell 1993, Waldrop et al. 1996, Nowak et al. 2005). Central command increases HR, cardiac output, and also BP immediately at the onset of exercise by rapid vagal withdrawal but command signals have minimal effect on the sympathetic nervous system mediated vasoconstriction (Rowell 1992, Waldrop et al. 1996, Waldrop and Kramer 2000). Because both locomotor and cardiovascular responses, much like those 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 (Mitchell 1990, Waldrop et al. 1996, Waldrop and Kramer 2000). Both these areas have connections with the vasomotor center in medulla which enables them to affect the cardiovascular control (Rowell 1993, Waldrop et al. 1996, Waldrop and Kramer 2000).

Originally it was thought that the central command acts as a pure feed-forward control mechanism (Rowell 1993, Rowell et al. 1996). The close relationship of the central command with the number of motor units recruited, or with the perception of effort

suggests an important role for feedback from exercising muscles (Rowell 1993, Seals 2006, Williamson et al. 2006). 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 (Williamson et al. 2006).

Muscle chemoreflex are 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 (Rowell 1980, Rowell 1992, Kaufman and Hayes 2002). 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 (Kaufman and Forster 1996, Rowell et al. 1996). Excitatory action potentials from muscle sensory afferents project to the brain via synaptic transmissions in the dorsal root of the spinal cord (Mitchell 1990, Kaufman and Hayes 2002, Seals 2006). 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 (Mitchell 1990, Kaufman and Hayes 2002, Seals 2006). The muscle chemoreflex has a distinct threshold, and in mild exercise this reflex is not tonically active (Rowell 1992, Rowell 1993, Rowell et al. 1996). As the severity of exercise increases to moderate, the margin for any blood flow error decreases, and the reflex is tonically active (Rowell 1993, Rowell et al. 1996). 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) (O'Leary 2006).

Besides chemosensitive fibers group III and IV afferent fibers in muscle contain mechanosensitive fibers as well (Rowell 1980, Mitchell 1990, Kaufman and Hayes 2002). These fibers are excited by mechanical stimuli (stretch and/or compression) in the active muscle (Mitchell 1990, Kaufman and Hayes 2002, Seals 2006). Their role in control of HR is not well understood (O'Leary 1996, O'Leary and Potts 2006) 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 (Nobrega and Araujo 1993). In studies utilizing static muscle contraction this fast tachycardic response is vagally mediated (McMahon and McWilliam

1992) but evidence also exists for a slower sympathetically mediated tachycardiac response induced by mechanosensitive fibers (Matsukawa et al. 1994). 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 (O'Leary 1996). It is not known, however, whether this waning occurs to a same extent during dynamic contractions as well (Rowell 1993). 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) (Leonard et al. 1985).

In addition to responding to chemical and/or mechanical stimuli, many group III and IV sensory afferents are thermosensitive (Rowell 1993, Kaufman and Forster 1996, Rowell et al. 1996). Because muscle temperature, which is normally well below 37°C in the limbs, can increase beyond 40°C during severe exercise (Rowell 1993, Rowell et al. 1996). 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 (Rowell 1993, Rowell et al. 1996). 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 (Rowell 1993, Seals 2006).

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 (Berne and Levy 1988, Hainsworth 1995, Mendelowitz 1999). Typically HR accelerates during inspiration and decelerates during expiration (Berne and Levy 1988, Hainsworth 1995, Mendelowitz 1999) as a result of complex central and reflex interactions involving both vagal and sympathetic branches of autonomic nervous system (Taylor et al. 2001). During exercise respiratory sinus arrhythmia gradually disappears despite gradually increasing respiration (Arai et al. 1989, Tulppo et al. 1996).

Peripheral arterial chemoreceptors are situated in the aortic and carotid bodies (Shields 1993, Hainsworth 1995, Camm and Fei 1996a). They excite nerve fibers that pass through carotid nerves and the vagus nerves into vasomotor center (Spyer 1990, Shields 1993,

Guyton and Hall 1996). Activity in their afferent nerves is increased by arterial hypoxia, hypercapnia, or acidemia (Sampson and Hainsworth 1972, Shields 1993, Camm and Fei 1996a). The primary effect of aortic body chemoreceptor stimulation on HR is excitatory (Karim et al. 1980). Conversely, carotid chemoreceptor stimulation causes a large and consistent bradycardia, but with intact respiratory control it is normally counterbalanced by tachycardia accompanying respiratory response induced by the same stimulation (Hainsworth et al. 1973, Berne and Levy 1988, Camm and Fei 1996a). Because exercise normally is associated with maintenance of PaO₂, 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 (Hammond and Froelicher 1985, Seals 2006).

The lungs are richly innervated and lung inflation, with moderate pressures, stimulates airways stretch receptors which results in a reflex increase in HR (Hainsworth 1974, Coleridge and Coleridge 1991). The reflex response during hyperinflation of the lung and also during pulmonary congestion is to cause bradycardia (Hainsworth 1995). 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 but at present there is no compelling evidence for it (Seals 2006).

Atrial receptors are concentrated near the junctions of the superior and inferior venae cavae and the pulmonary veins with the atria (Hammond and Froelicher 1985, Hainsworth 1995, Camm and Fei 1996a). The afferent fibers are contained in the vagus and the efferent pathway within the sympathetic nerves (Hainsworth 1991, Hainsworth 1995, Camm and Fei 1996a). Atrial receptors are stimulated mainly by stretching due to increases in atrial volume which results in a reflex increase in HR (Hammond and Froelicher 1985, Hainsworth 1995, Camm and Fei 1996a). Because of the relatively slow time-course of sympathetic responses, the tachycardia to stimulation of atrial receptors requires up to 30 seconds to reach a stable level (Hainsworth 1995).

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 (Hainsworth 1991, Wilkoff and Miller 1992, Åstrand et al. 2003). On

the contrary, the 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 (Rowell 1993). 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 (Shephard 1993, Guyton and Hall 1996). The mechanism might be augmented after heart transplantation (Shephard 1993, Camm and Fei 1996a) but in the normal heart this mechanism is largely masked or overridden by other reflex mechanisms (Blinks 1956). 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 (Shephard 1993, Guyton and Hall 1996).

Ventricular mechanoreceptors are situated mainly in the left ventricle and the afferent nerves travel either in the vagus or in the sympathetic nerves (Hainsworth 1991, Thames et al. 1993). Both populations of receptors can be divided into mechanosensitive and chemosensitive endings (Hainsworth 1991, Thames et al. 1993). 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 (Thames et al. 1993, Hainsworth 1995, Armour 1999). The vagal afferents mediate reflex cardio-inhibitory, sympatho-inhibitory, vasodepressor responses while activation of sympathetic afferents results in cardio-accelerator, sympatho-excitatory, vasodepressor responses (Hainsworth 1991, Thames et al. 1993). Both mechanical and chemical stimuli may be involved in activation of receptors, but chemical stimuli are more important for triggering of reflexes (Hainsworth 1991, Thames et al. 1993, Hainsworth 1995). The anginal pain is mediated by sympathetic afferent fibers and causes tachycardia (Hainsworth 1991, Thames et al. 1993, Hainsworth 1995). The vagal afferents are located nearer to the endocardial than to the epicardial surface while the reverse is true for the sympathetic afferents (Thames et al. 1993). Thus subendocardial ischemia stimulates vagal afferents resulting in bradycardia whereas transmural ischemia more likely induces tachycardia mediated by sympathetic afferents (Thames et al. 1993). Left ventricular vagal afferents are preferentially distributed to the inferoposterior wall whereas sympathetic afferents appear to be more uniformly distributed throughout the wall of the left ventricle (Thames et al. 1993). 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 (Hainsworth 1991, Thames et al. 1993). Occlusion of the anterior descending branch or ischemia involving anterior wall is

likely to result in increase in HR (Hainsworth 1991, Thames et al. 1993). The chemical stimuli which activate receptors involve substances resulting from myocardial ischemia including bradykinin, prostaglandins and adenosine (Hainsworth 1991, Thames et al. 1993). The bulging or dyskinesia of the ischemic zone may stimulate mechanosensitive vagal afferents but the increase in discharge lasts only approximately two minutes whereas the increased discharge of chemosensitive vagal afferent persists for the duration of the ischemic event (Thames et al. 1993).

2.6. HEART RATE RESPONSE TO EXERCISE: A SYNTHESIS

In 1990 Rowell & O'Leary introduced an overall model as to how the autonomic nervous system adjustments to large-muscle dynamic exercise may be mediated (Rowell and O'Leary 1990). 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 (Seals 2006). 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 (Rowell and O'Leary 1990, Rowell 1991, Rowell 1992).

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 (Ekblom et al. 1973, Freeman et al. 2006, Chaitman 2007). The activation of vagal withdrawal is mainly due to augmented central command (Rowell and O'Leary 1990, Rowell 1992, O'Leary 1996). Central command also resets the arterial baroreflex immediately to a higher operating point but normally the baroreflex does not elicit an increase in sympathetic nervous system activity because the rise in cardiac output (due to fast HR increase) is rapid enough to raise arterial BP to its new operating point (Rowell and O'Leary 1990, Rowell 1991, O'Leary 1996). 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 (Rowell and O'Leary 1990, Rowell 1992, O'Leary 1996). In this setting the muscle chemoreflex is not activated, the exact role of the afferents from muscle mechanoreceptors is unknown but in all probability their effect is negligible (Rowell and O'Leary 1990, Rowell 1992, O'Leary 1996).

During moderate exercise, when HR exceeds 100 beats/min, the activation of vagal withdrawal by central command still increases cardiac output, but not enough (Rowell and O'Leary 1990, Rowell 1991, Rowell 1992). 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 (Rowell and O'Leary 1990, Rowell 1991, Rowell 1992). Consequently, arterial BP cannot be increased immediately to its new, reset operating point so there is a pressure error detected by baroreceptors (Rowell and O'Leary 1990, Rowell 1991, Rowell 1992). As a consequence of this error, sympathetic nervous system activity to both the heart and to the resistance vessels increases in order to correct the pressure error (Rowell and O'Leary 1990, Rowell 1991, Rowell 1992). The sympathetically mediated increase in HR and cardiac output is much slower (by 15- to 20-fold) than the parasympathetically mediated rise (Rowell and O'Leary 1990, Rowell 1991, Rowell 1992). Thus vasoconstriction in resistance vessels all over the body (including active skeletal muscle) becomes a necessary adjunct to increased cardiac output in order to raise arterial BP as quickly as possible to minimize the pressor error (Rowell and O'Leary 1990, Rowell 1991, Rowell 1992). As workload increases, HR increases due to further sympathetic nervous system activation (Hammond and Froelicher 1985, Tulppo et al. 1996, Chaitman 2007). The increase in sympathetic nervous system activity can occur due to the arterial baroreflex (via further baroreflex resetting), the muscle chemoreflex (after a threshold has been passed after which this reflex becomes tonically active), or muscle mechanoreceptor activation (Hammond and Froelicher 1985, Rowell 1992, O'Leary 1996). Besides direct neural excitation SA node is also stimulated by increased level of circulating adrenaline which is secreted from the adrenal medulla (Kjaer 1989, Lauer 2001c, Chaitman 2007). 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 (Kjaer 1989, Mazzeo 1991, McArdle et al. 2001). 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 (Rowell and O'Leary 1990, O'Leary 1996, Tulppo et al. 1996). As 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 (Rowell and O'Leary 1990, O'Leary 1996, O'Leary and Potts 2006).

Pharmacological blockade studies have proved the differential contributions of the two autonomic branches during exercise (Rosenwinkel et al. 2001). 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 (Craig 1952, Tulppo et al. 1996, Rohrer et al. 1998). Withdrawal of vagal tone has been confirmed using time and frequency domain analyses of HR variability as well (Arai et al. 1989, Tulppo et al. 1996). Conversely, blockade of sympathetic control with β -adrenergic receptor antagonist reveals the importance of augmented sympathetic activity during moderate and heavy exercise (Epstein et al. 1965, Brown et al. 1986, Hespel et al. 1986). During light exercise, with workloads of 25% to 40% of $\text{VO}_{2\text{max}}$, 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 (Rowell and O'Leary 1990, Rowell 1991, Rowell 1992).

2.7 THE 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 (Bevegård et al. 1963, Wilkoff and Miller 1992, Navare and Thompson 2003). An appropriate increase of HR contributes significantly to attaining high levels of cardiac output (Stone and Liang 1984, Camm and Fei 1996a, Lauer 2001c). 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 (Poliner et al. 1980, Iskandrian et al. 1983, Froelicher and Myers 2006). When a normal human exercises maximally in upright position, the HR increase is 150-300% of resting while the stroke volume increase is about 10-100% (Mitchell et al. 1958, Plotnick et al. 1986, Camm and Fei 1996a). 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 (Saltin 1964, Fletcher et al. 2001, Navare and Thompson 2003). Thereafter stroke volume levels off (Stenberg et al. 1967, Gledhill et al. 1994, Seals et al. 1994) or there is a small decline (Higginbotham et al. 1986, Seals et al. 1994, Stratton et al. 1994) or increment (Åstrand et al. 1964, Zhou et al. 2001, Martino et al. 2002) at maximal work

intensity. An increase in HR accompanying dynamic exercise results in an increase in the force of myocardial contraction as well (i.e. frequency-force-relationship or the staircase phenomenon) (Hammond and Froelicher 1985, Rowell et al. 1996, Moore 2006). 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 (Rowell 1993, Rowell et al. 1996, Moore 2006). Although sufficient increase of HR is essential in order to raise cardiac output at heavier workloads, the absolute cardiac output which a person can attain is determined, however, by the magnitude of maximal stroke volume (Bevegård et al. 1963, Rowell 1974, Blomqvist and Saltin 1983).

The relationship between HR and VO_2 or work intensity is somewhat linear (Åstrand 1960, Lauer 2001a, Navare and Thompson 2003). It has been suggested that the HR might increase relatively less than VO_2 as the work rate becomes very heavy (Bates 1967, Brooke et al. 1969, Wilkoff and Miller 1992) but two studies have shown the opposite (Treese et al. 1993, Lewalter et al. 1995). In both studies HR rose slightly more steeply above anaerobic threshold (approximately 50-60% of $\text{VO}_{2\text{max}}$) than below anaerobic threshold but the HR/work intensity -relationship was linear (Lewalter et al. 1995).

The linear relationship between HR and VO_2 or work intensity is widely employed in a number of submaximal exercise tests (Åstrand and Ryhming 1954, Åstrand 1960, Maritz et al. 1961, Margaria et al. 1965, von Döbeln et al. 1967, Shephard et al. 1976, Siconolfi et al. 1982, Siconolfi et al. 1985, Kline et al. 1987, Golding et al. 1989, Ebbeling et al. 1991, Oja et al. 1991), in which $\text{VO}_{2\text{max}}$ is estimated based on HR measured in a single or several submaximal workloads (Lange Andersen et al. 1971, McArdle et al. 2001, Åstrand et al. 2003). By utilizing a linear HR-workload relationship a straight line is fitted to measured HR values (Lange Andersen et al. 1971, McArdle et al. 2001, Åstrand et al. 2003). This line is extrapolated to the predicted maximal HR; the corresponding estimated maximal workload can then be approximated (Lange Andersen et al. 1971, McArdle et al. 2001, Åstrand et al. 2003). $\text{VO}_{2\text{max}}$ can then be estimated by using the relationship between work rate and VO_2 (Lange Andersen et al. 1971, McArdle et al. 2001, Åstrand et al. 2003).

2.8. THE 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 ($\%\text{VO}_{2\text{max}}$), provided the state of training is the same (Davies 1968, Jones and Kane 1979, Greiwe et al. 1995). The HR at a given VO_2 is related to the maximal stroke volume (Jones and Campbell 1982, Ramamurthy et al. 1999) but it is no measure of maximal cardiorespiratory fitness unless the maximal HR is considered (Strandell 1964a, Åstrand et al. 2003). 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 (Wahlund 1948, Peters et al. 1983, Sobolski et al. 1987). There is a tendency for persons with a low resting HR to have a low HR at a fixed submaximal workload (Ekelund et al. 1988, Filipovsky et al. 1992, Blair et al. 1998) but this has not been observed consistently (Wilkoff and Miller 1992, Sandvik et al. 1995). 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 (Åstrand 1967, Sandvik et al. 1995). Furthermore, an inverse (Blair et al. 1998) or non-existent (Sandvik et al. 1995) relationship have 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 (Guyton and Hall 1996, Awtry and Loscalzo 2001a). 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) (Braunwald and Ross 1979, Oldershaw et al. 1983, Rowell et al. 1996). 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 (Hammond and Froelicher 1985, Froelicher and Myers 2006). Although this theoretical limitation is reasonable, there is little experimental work to support it (Åstrand et al. 1964, Hammond and Froelicher 1985, Froelicher and Myers 2006).

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 (Robinson 1939, Ekelund et al. 1988, Blair et al. 1998). On the other hand, HR at a fixed submaximal workload has also been suggested to be higher (Norris et al. 1953, Peters et al. 1983, Slattery and Jacobs 1988) or lower (Strandell 1964b, Jonsson and Astrand 1979, Filipovsky et al. 1992) 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 (Lange Andersen et al. 1971, Tanaka et al. 2001, Higgins and Higgins 2007). 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% (Londeree and Moeschberger 1982). Of mechanisms underlying age-related decrease in maximal HR, cardiac chronotropic responsiveness to β -adrenoceptor stimulation has been shown to be preserved (Poldermans et al. 1995) or reduced (Conway et al. 1971, White et al. 1994, Esler et al. 1995) in the elderly. Subclinical atherosclerosis accompanying aging has been proposed as a mechanism as well (Åstrand et al. 2003). 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 (Kostis et al. 1982, Bruce 1984, Hammond and Froelicher 1985). Age-related decline in maximal HR is steeper in men with a low cardiorespiratory fitness (Cooper et al. 1977) and physical inactivity (Froelicher and Myers 2006).

Gender. HR at a given submaximal workload is higher in women (Åstrand 1952, Blair et al. 1998, Wilmore et al. 2001). Maximal HR does not differ between genders (Åstrand and Christensen 1964, Londeree and Moeschberger 1982, Froelicher and Myers 2006) or is slightly lower (Hermansen and Andersen 1965, Sidney et al. 1992, Blair et al. 1998) in women. **Menstrual cycle.** In women HR at a fixed submaximal workload has been reported to be higher during the mid-luteal phase (Pivarnik et al. 1992), but most studies have reported no change in submaximal (Jurkowski et al. 1981, De Souza et al. 1990, Smekal et al. 2007) or maximal HR (Dombovy et al. 1987, Lebrun et al. 1995, Smekal et al. 2007) over 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 (Jonsson and Astrand 1979, Jones and

Campbell 1982), and the association is more pronounced when lean body weight is considered instead of total body weight (Cotes et al. 1973, Spiro et al. 1974). Height and body weight (lean or total) do not affect on maximal HR (Jones and Campbell 1982, Hammond and Froelicher 1985, Freeman et al. 2006). **Obesity.** The overweight persons have a higher HR at a fixed submaximal workload than normal weight persons. The relationship is valid especially when exercising in treadmill but in cycle ergometer as well (Buskirk et al. 1955, Dempsey et al. 1966, Sidney et al. 1992). In two studies, however, there was no relationship between body mass index (BMI) and HR at a given submaximal workload in treadmill (Gordon et al. 1987, Ekelund et al. 1988), and in one study (Filipovsky et al. 1992) an inverse relationship was observed between BMI and HR at a given submaximal workload in cycle ergometer. Overweight has not been reported to have an effect on maximal HR (Dempsey et al. 1966, Hammond and Froelicher 1985, Freeman et al. 2006) except one study in which overweight was associated with lower maximal HR (McHenry et al. 1977).

Mode of exercise. HR is slightly higher at a given submaximal VO_2 in cycle ergometer compared with treadmill (Hermansen et al. 1970, Niederberger et al. 1974). Maximal HR probably does not differ between cycle ergometer and treadmill (Åstrand and Saltin 1961, Hermansen and Saltin 1969, Myers et al. 1991) although it has been suggested to be slightly lower with cycle ergometer testing (Wicks et al. 1978, Londeree and Moeschberger 1982, Camm and Fei 1996a). Both HR at a given submaximal workload and maximal HR are lower in work on a cycle ergometer in the supine position (Bevegård et al. 1963, Hossack 1987, Camm and Fei 1996a). **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 (Christensen 1931a; Pendergast 1989, Wilkoff and Miller 1992). The maximal HR with arm exercise is 88-100% from the maximal HR in leg exercise (De Boer et al. 1982, Balady et al. 1986, Wilkoff and Miller 1992). **Exercise protocol.** Large increments in workload combined with a short duration of the step may result in HR not rising to steady state level at that workload (Sietsema et al. 1989). Consequently, HR at that submaximal workload is lower than the actual HR at the identical work provided that steady state would have been achieved (Sietsema et al. 1989). The maximal HR is not markedly different between protocols as long as the same exercise mode is used (Froelicher et al. 1974, Myers et al. 1991). **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 compared with clearly higher pedal frequencies (Eckermann and Millahn 1967).

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 (Davies et al. 1970, Wolthuis et al. 1977). Other studies have, however, shown no appreciable habituation effect (Jones and Kane 1979). **True maximal effort.** Maximal effort should always be confirmed objectively before an attempt is made to measure a maximal HR (Camm and Fei 1996a). Objective measures of appropriate maximal effort include respiratory exchange ratio >1.10 and blood lactate level $>7-8$ mmol/l (Hammond and Froelicher 1985, Camm and Fei 1996a, Froelicher and Myers 2006). Older individuals might be more afraid to achieve the true maximal exertion but this effect may disappear on repeated testing (Hammond and Froelicher 1985). 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} (Saltin et al. 1968). **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 (Atwood et al. 1989). The 6-second rhythm strip at the end of each minute represents a reasonable balance between convenience and precision for measuring HR during exercise (Atwood et al. 1989).

Environment. A hot environment causes a higher HR at a fixed submaximal workload than exercise at a low ambient temperature (Rowell et al. 1966, Ward et al. 1987, Wilkoff and Miller 1992). Also a high relative humidity elicits a higher HR at a fixed submaximal workload (Wilmore and Costill 2004). Maximal HR can even reach slightly higher values under hyperthermia than in normothermia (Rowell 1993). Again, HR at a given submaximal workload is lower in a cold environment (Stevens et al. 1987) and also maximal HR is lower compared with neutral temperature (Nielsen Johannsen 2000). Emotional factors, nervousness, excitement and apprehension may raise the HR during exercise of light and moderate intensity (Lange Andersen et al. 1971, Wilkoff and Miller 1992, Åstrand et al. 2003). The heavier the workload, however, the less pronounced is this nervous effect on the HR so that it does not affect maximal HR (Åstrand et al. 2003). **Dehydration.** In dehydrated state HR at a given submaximal workload is higher than in euhydrated state (Wilkoff and Miller 1992, Fletcher et al. 2001, Higgins and Higgins 2007)

whereas acute expansion of blood volume produces decreased HR (Fortney et al. 1981, Fortney et al. 1983, Hopper et al. 1988). Acute plasma volume expansion does not affect on maximal HR (Kanstrup and Ekblom 1982) but in dehydrated state maximal HR might be slightly higher than in euhydrated state (Rowell 1993).

Level of fitness. HR at a fixed workload seems to be inversely related to a maximal cardiorespiratory performance of a subject (Christensen 1931b, Tavel 2001, Higgins and Higgins 2007), although maximal HR modulates this relationship (Åstrand et al. 2003). A high cardiorespiratory fitness accelerates the rate of attainment of the steady-state HR at submaximal work (Jones et al. 1970, Sietsema et al. 1989). Maximal HR has been reported to be the same (Åstrand 1956, Pollock 1977, Balady and Weiner 1987), higher (Grimby and Saltin 1966, Sandvik et al. 1995, Blair et al. 1998) or lower (Hermansen and Andersen 1965, Lester et al. 1968, Londeree and Moeschberger 1982) in subjects with a high cardiorespiratory fitness.

Training. Within a same subject endurance-typed exercise training reduces HR at a given submaximal workload and a reduced activity has an opposite effect (Karvonen et al. 1957, Camm and Fei 1996a, Moore and Palmer 1999). The rate of attainment of the steady-state HR at submaximal work occurs more rapidly after training as well (Hagberg et al. 1980). Endurance training does not change the maximal HR or slightly reduces it (Benestad 1965, Rerych et al. 1980, Spina et al. 1992). Cessation of endurance-typed exercise training may increase maximal HR (Coyle et al. 1984). HR at a given submaximal workload has been reported to be lower (Christensen 1931a; Ekelund et al. 1988, Sidney et al. 1992) and maximal HR higher (Sandvik et al. 1995), 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 (Saltin et al. 1968, Myers 1995, Fletcher et al. 2001). The maximal HR is either the same (Saltin et al. 1968) or increased after the bed rest (Convertino et al. 1982, Myers 1995).

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 (Blomqvist et al. 1970) or unaltered (American College of Sports Medicine 2000) but maximal HR is not affected (Blomqvist et al. 1970).

Table 1. The medications affecting heart rate during exercise (Hammond and Froelicher 1985, Kendrick et al. 1987, American College of Sports Medicine 2000).

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 ↓
antiarrhythmic agents	
quinidine, disopyramide	↑ or ↔
propafenone	↔ or ↓
amiodarone	↓
bronchodilators	
sympathomimetic	↑ or ↔
anticholinergic	↑ or ↔
methylxanthines	↑ or ↔
psychotropic medications	
antidepressants	↑ or ↔
major tranquilizers	↑ or ↔
cold medications with sympathomimetic agents	↑ or ↔
thyroid medications	↑
anorexiant / diet pills	↑ or ↔

* β-blockers with intrinsic sympathomimetic activity have a reduced effect.
† The effect increases with a relative intensity of exercise (Robinson et al. 1966, Ekblom et al. 1972, Hespel et al. 1986).
‡ Heart rate decreases in patients with atrial fibrillation and possibly congestive heart failure but heart rate is not significantly altered in patients with sinus rhythm.

Arterial oxygen content. A reduced arterial oxygen content in anemia or after haemoglobin blocked by carbon monoxide (as acutely after smoking) results in a higher HR at a fixed submaximal workload (Ekblom and Huot 1972, Wilkoff and Miller 1992, Higgins and Higgins 2007). Parenthetically, also smokeless tobacco increases HR at submaximal work (Van Duser and Raven 1992, Wilmore and Costill 2004). The increased blood haemoglobin concentration lowers HR at a given submaximal workload (Robertson et al. 1982, McArdle et al. 2001) but does not affect on maximal HR (Ekblom et al. 1976, Robertson et al. 1982). Long-term smokers seem to have a lower HR at a given submaximal workload (Gordon et al. 1987, Lauer et al. 1997, Srivastava et al. 2000) but unaltered (Blackburn et al. 1960, Ekelund et al. 1988, Slattery and Jacobs 1988) and higher (Chevalier et al. 1963) HR values have been reported as well. Long-term smokers have been reported to have a lower maximal HR (McHenry et al. 1977, Sandvik et al. 1995,

Lauer et al. 1997). **Thyroid gland function.** Hyperthyroid patients may have a high HR at a fixed submaximal workload (Wasserman et al. 1999, Higgins and Higgins 2007), whereas hypothyroid patients may have an opposite finding (Gentlesk et al. 2004).

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 (Voigt et al. 1967, Reilly and Brooks 1990). On maximal HR the effect of circadian rhythm is negligible (Reilly and Brooks 1990). HR at a fixed submaximal workload has been shown to be lower in the summer than in other seasons (Sidney et al. 1992). **Eating and sleeping before the test.** HR at a fixed submaximal workload is increased for an hour or more after a heavy meal (Lange Andersen et al. 1971, Wilmore and Costill 2004). An abnormally short sleep the night before exercise may raise HR at a fixed submaximal workload (Wilmore and Costill 2004).

Genetics. The workload which 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% (Bouchard et al. 1984, Perusse et al. 1987, Perusse et al. 1988). The genetic effect on maximal HR has been shown to be significant in one study with brothers and twins (Bouchard et al. 1986) but not in another study (Fagard et al. 1987). Familial data has indicated that maximal HR may be characterized by a maternal effect (Lesage et al. 1985).

2.9. HEART RATE RESPONSE TO SUBMAXIMAL EXERCISE AND PROGNOSIS

During the last two decades, the 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 (Ellestad 1996, Ashley et al. 2000a, Lauer 2001a, Lauer 2001b, Lauer 2001c, Gibbons et al. 2002a, Lauer 2002a, Lauer 2002b, Lauer and Froelicher 2002, Chaitman 2003, Lauer 2003, Lauer 2004, Kaplan et al. 2005, Lauer et al. 2005, De Sutter et al. 2006, Freeman et al. 2006, Kligfield and Lauer 2006, Routledge and Townend 2006, Chaitman 2007). 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 (Hinkle et al. 1972, Wilhelmsen et al. 1976, Wilhelmsen et al. 1981, Sobolski et al. 1987, Ekelund et al. 1988, Slattery and Jacobs 1988, Pardaens et al. 1996) 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 (Hinkle et al. 1972, Wilhelmsen et al. 1976, Wilhelmsen et al. 1981, Ekelund et al. 1988, Slattery and Jacobs 1988, Pardaens et al. 1996) and one paper (Sobolski et al. 1987) 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 (Ekelund et al. 1988, Slattery and Jacobs 1988). In one of the five papers which 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) (Hinkle et al. 1972). Additionally, a prevalent CHD was not controlled for in the analysis (Hinkle et al. 1972). Two separate reports from one study (Wilhelmsen et al. 1976, Wilhelmsen et al. 1981) did not find an association either, but in both reports neither survival analysis was performed nor were the medications influencing HR reported. In the fourth paper with negative finding (Sobolski et al. 1987), 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 (Pardaens et al. 1996). Additionally, from the results of the study by Lauer et al (Lauer et al. 1996), 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.

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 (Hinkle et al. 1972)	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 (Wilhelmsen et al. 1976)	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 (Wilhelmsen et al. 1981)	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 (Sobolski et al. 1987)	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

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
Lipid Research Clinics Mortality Follow-up Study 1988 (Ekelund et al. 1988)	3106 men, most of them having hyperlipidemia	0%	30-69	medication affecting HR, antihypertensive 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 (Slattery and Jacobs 1988)	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 (Pardaens et al. 1996)	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	antihypertensive 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

* For the first four studies the main result is from unadjusted analysis whereas for the next three studies the main results are from multivariable analyses. 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.

Only one of the seven reports involved also women (Pardaens et al. 1996) and the negative finding was observed similarly in both gender. The length of the follow-up was addressed in one study (Slattery and Jacobs 1988) 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 then a shorter follow-up after the second exercise test was used, even if the number of events decreased from 258 to 147 (Slattery and Jacobs 1988).

In two studies which found a high submaximal HR to be an independent predictor of future CVD death (Ekelund et al. 1988, Slattery and Jacobs 1988) a high submaximal HR was interpreted as a marker of a low cardiorespiratory fitness and therefore the association with CVD death was explained by this mechanism. The conjecture that the association is mediated by a low cardiorespiratory fitness is supported by the facts that HR at a fixed workload is inversely related to a maximal cardiorespiratory performance of a subject (Christensen 1931b, Tavel 2001, Higgins and Higgins 2007) and a low cardiorespiratory fitness is a major risk factor for future CVD event in asymptomatic persons (Mark and Lauer 2003, Lauer et al. 2005, Froelicher and Myers 2006).

Indirect support for the association between a high submaximal HR and increased risk of future CVD events is found from four follow-up studies (Peters et al. 1983, Lie et al. 1985, Arraiz et al. 1992, Hein et al. 1992). In these studies the actual submaximal HR was not reported but either age-adjusted value was used for analyses, or submaximal HR was utilized for indirect estimating of cardiorespiratory fitness (Peters et al. 1983, Lie et al. 1985, Arraiz et al. 1992, Hein et al. 1992). In all four studies (Peters et al. 1983, Lie et al. 1985, Arraiz et al. 1992, Hein et al. 1992) 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 (Lange Andersen et al. 1971, McArdle et al. 2001, Åstrand et al. 2003), it is presumable that persons with an increased risk of CVD death in all four studies had a high HR at a fixed submaximal workload as well.

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 (Peters et al. 1983, Ekelund et al. 1988); a high serum total cholesterol (Peters et al. 1983, Filipovsky et al. 1992), low-density lipoprotein (LDL) cholesterol (Ekelund et al. 1988) and triglyceride level (Ekelund et al. 1988); a low HDL/total cholesterol ratio (Peters et al. 1983); a low level of self reported physical activity (Wilhelmsen et al. 1976, Ekelund et al. 1988, Sidney et al. 1992); a high resting systolic (Jonsson and Astrand 1979, Slattery and Jacobs 1988, Filipovsky et al. 1992,) and diastolic BP (Ekelund et al. 1988); a diagnosed hypertension (Bruce et al. 1974); as well as overweight (Buskirk et al. 1955, Dempsey et al. 1966, Sidney et al. 1992) in asymptomatic persons. On the other hand, smokers seem to have a lower HR at a given submaximal workload than nonsmokers (Gordon et al. 1987, Lauer et al. 1997, Srivastava et al. 2000). Also left ventricular hypertrophy assessed from ECG has been reported to be related with a lower HR at a given submaximal workload (Filipovsky et al. 1992). Total cholesterol and BP level were controlled for, however, in two studies (Ekelund et al. 1988, Slattery and Jacobs 1988) which found the association between submaximal HR and the increased risk of CVD death. Slattery and Jacobs (Slattery and Jacobs 1988) 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 (Lopez et al. 1974, Montoye et al. 1976) and HDL cholesterol (Haskell et al. 1980, Gordon et al. 1987, Ekelund et al. 1988) level; overweight (Gordon et al. 1987, Ekelund et al. 1988, Filipovsky et al. 1992); a low level of self reported physical activity (Filipovsky et al. 1992); as well as smoking (Blackburn et al. 1960, Ekelund et al. 1988, Slattery and Jacobs 1988) 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 (Falcone et al. 2005, Leeper et al. 2007) which have measured the HR increment from rest to submaximal workload. Falcone et al (Falcone et al. 2005) 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 workload of 25 Watts (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 (Falcone et al. 2005). Leeper et al (Leeper et al. 2007), 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 (Falcone et al. 2005) 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, the known risk factors for death or cardiac event in CHD patients especially after myocardial infarction (Kleiger et al. 1987, Makikallio et al. 2005, Kiviniemi et al. 2007). The explanation offered by Falcone et al (Falcone et al. 2005) 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) compared with patients with a normal baroreflex sensitivity (Fukuma et al. 2004). Accordingly, Leeper et al (Leeper et al. 2007) argued that the rapid HR increment at the beginning of exercise test reflects a high, instead of low, vagal activity and they further suggested the early acceleration of HR in the study by Falcone et al (Falcone et al. 2005) 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 (Morris et al. 1991, Froelicher and Myers 2006).

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 (Hultgren et al. 1986, Wasserman 1997, Myers and Madhavan 2001) or from left ventricular dysfunction not directly related to ischemia (Weber et al. 1982). 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 (Wasserman 1997, Wasserman et al. 1999). 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 (Froelicher and Myers 2006). In accordance with this are the findings from two previous studies in dogs (Billman et al. 1985, Billman and Hoskins 1989). 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 as compared with 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 (Billman et al. 1985).

2.10 SUMMARY

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 (Hammond and Froelicher 1985, Hainsworth 1995, Iellamo 2001). During the last two decades, the exercise test derived HR variables have excited widely as prognostic markers of mortality and cardiac events both in asymptomatic persons and in CVD patients (Ellestad 1996, Ashley et al. 2000a, Lauer 2001a, Lauer 2001b, Lauer 2001c, Gibbons et al. 2002a, Lauer 2002a, Lauer 2002b, Lauer and Froelicher 2002, Chaitman 2003, Lauer 2003, Lauer 2004, Kaplan et al. 2005, Lauer et al. 2005, De Sutter et al. 2006, Freeman et al. 2006, Kligfield and Lauer 2006, Routledge and Townend 2006, Chaitman 2007). The results of the properly controlled studies (Ekelund et al. 1988, Slattery and Jacobs 1988, Sandvik et al. 1995, Lauer et al. 1996, Cheng et al. 2002, Mora et al. 2003, Balady et al. 2004, Erikssen et al. 2004, Jouven et al. 2005) 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 (Ramamurthy et al. 1999). Indeed, in the Lipid Research Clinics Mortality Follow-up Study both a high HR at a fixed submaximal workload (Ekelund et al. 1988) and an inability to increase HR normally when approaching maximal work capacity (chronotropic incompetence) (Srivastava et al. 2000) 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 (Ekelund et al. 1988, Slattery and Jacobs 1988, Leeper et al. 2007,). 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 would 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 (Wilkoff and Miller 1992, Sandvik et al. 1995, Camm and Fei 1996; Ellestad 1996, Kohl et al. 1996, Lauer et al. 1996, Lauer 2001; Curtis and O'Keefe 2002, Gentlesk et al. 2004, Jouven et al. 2005, Kaplan et al. 2005, De Sutter et al. 2006, Freeman et al. 2006, Routledge and Townend 2006).

The hypothetical graph based on the results from previous studies (Billman et al. 1985, Ekelund et al. 1988, Slattery and Jacobs 1988, Billman and Hoskins 1989, Sandvik et al. 1995, Lauer et al. 1996, Cheng et al. 2002, Mora et al. 2003, Balady et al. 2004, Erikssen et al. 2004, Falcone et al. 2005, Jouven et al. 2005, Leeper et al. 2007) is shown in Figure 2. In essence, it is the theory proposed by Ramamurthy et al (Ramamurthy et al. 1999) in a graph 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 parasympathetic nervous system (Craig 1952, Tulppo et al. 1996, Rohrer et al. 1998), whereas the increase from 100 beats/min to maximum is controlled mainly by sympathetic nervous system (Epstein et al. 1965, Brown et al. 1986, Hespel et al. 1986). 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 (Craig 1952, Tulppo et al. 1996, Rohrer et al. 1998) 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 (Billman et al. 1985, Billman and Hoskins 1989, Falcone et al. 2005). The prognostic value of the new HR-derived variable, WL_{100} , has not been studied formerly.

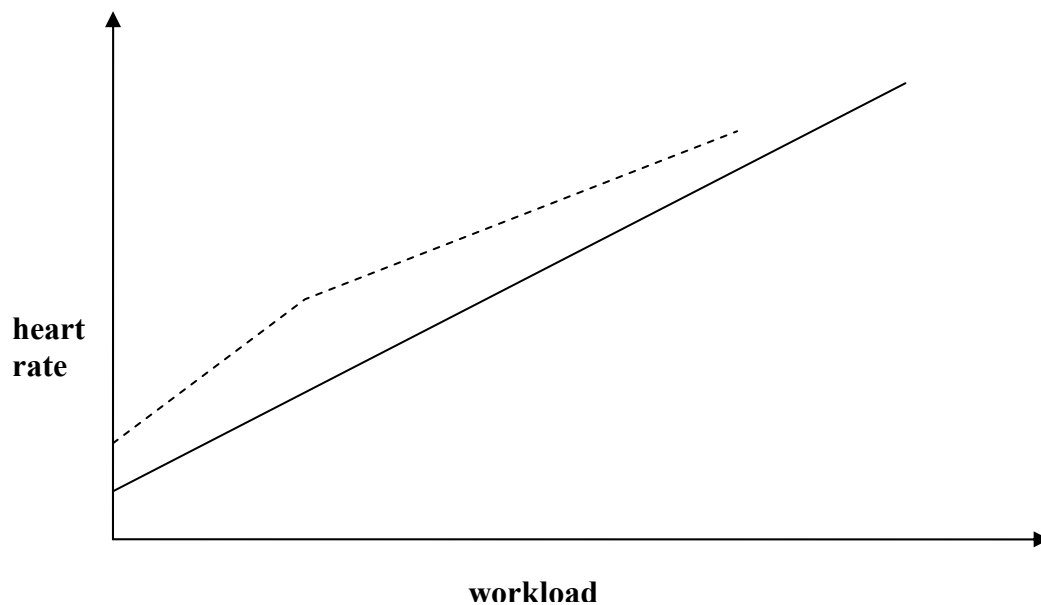


Figure 2. The hypothetical heart rate response to incremental exercise in subjects with an increased risk of future cardiovascular disease event (dashed line) and in normal subjects (continuous line) based on the data from the previous studies (Billman et al. 1985, Ekelund et al. 1988, Slattery and Jacobs 1988, Billman and Hoskins 1989, Sandvik et al. 1995, Lauer et al. 1996, Cheng et al. 2002, Mora et al. 2003, Balady et al. 2004, Erikssen et al. 2004, Falcone et al. 2005, Jouven et al. 2005, Leeper et al. 2007).

3 MAIN HYPOTHESIS AND AIMS OF THE STUDY

The main hypothesis of the current thesis is that a low workload achieved at HR of 100 beats/min (WL_{100}) is associated with adverse prognosis. Accordingly, the aims of the study were:

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

b) 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.

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 (Salonen 1988). The study involves men from East Finland, an area known for its high prevalence and incidence of CVD (Keys et al. 1984, Salonen 1988). 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 a written informed consent. The subjects are a representative age-stratified, age-balanced population-based random sample of men who lived in the town 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) and was 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) and was enrolled between August 1986 and December 1989.

Complete data on exercise test variables were available for 2240 men. WL_{100} could not be recorded or interpolated for 113 men because they had either a resting HR higher than 100 beats/min or no HR values above 100 beats/min during an exercise test. Data on the prevalence of CHD, ischemic changes in ECG during an exercise test, or the use HR-lowering medication (beta-blockers, digoxin, clonidin, methyldopa) were missing for 299 men. Of 1828 men with complete data, 149 men without CHD at baseline but using HR-lowering medication were excluded. After these exclusions, the final study sample included 1679 men who were divided into two groups: 1314 men without CHD at baseline, and 365 men with known or suspected CHD at baseline. Known or suspected CHD was defined as either a history of myocardial infarction (90 men in the final study sample) or angina pectoris diagnosed by a doctor (226 men), angina pectoris on effort based on the London School of Hygiene Cardiovascular Questionnaire (Rose et al. 1982) (284 men), or the use of nitroglycerine for chest pain at least once a week (61 men).

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 (Salonen 1988). 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 (Lakka et al. 1994, Lakka et al. 2001, Laukkanen et al. 2001, Laukkanen et al. 2004, Laukkanen et al. 2006a; Laukkanen et al. 2006b).

At the second visit after seven days the blood specimens were taken for laboratory determinations between 8 and 10 o'clock in the morning. 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, Mearns, Netherlands). For safety reasons and to obtain reliable information, the test was supervised by an experienced physician with the assistance of a trained nurse.

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 a 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 $((100 - \text{resting HR}) / (\text{maximal HR} - \text{resting HR})) / (\text{workload at HR 100 beats/min} / \text{maximal workload})$. 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 (Falcone et al. 2005, Leeper et al. 2007). Heart rate reserve was calculated as maximal HR minus resting HR.

4.3.2 Assessment of cardiorespiratory fitness and exercise electrocardiography

$VO_{2\max}$ 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. $VO_{2\max}$ was defined as the highest value for VO_2 recorded during the 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 (Mason and Likar 1966). 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 men without CHD at baseline, and horizontal or downsloping ST depression with 1.0 or more mm in men with known or suspected CHD at baseline.

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 (Salonen et al. 1991). The cholesterol contents of lipoprotein fractions were measured enzymatically (Boehringer Mannheim, Mannheim, Germany) on the day after the ultracentrifugal spin. Blood haemoglobin was determined photometrically (Gilford Stasar III, Gilford Instrument Laboratories Inc., Oberlin, Ohio, USA) using the cyanmethaemoglobin method (Fairbanks 1976) 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 sphygmomanometer (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 minutes' 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 behaviour 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 (Kauhanen et al. 1997).

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. A prevalent CVD was defined as a history of CHD, cardiac insufficiency, cardiomyopathy, arrhythmias, stroke or claudication. Diabetes was defined as fasting blood glucose ≥ 6.7 mmol/l (World Health Organization 1985) or a clinical diagnosis of diabetes with either dietary, oral, or insulin treatment.

4.8 COLLECTION AND CLASSIFICATION OF DEATHS DURING THE FOLLOW-UP

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) (World Health Organization 1977) or the Tenth ICD codes (I00-I99 and I20-I25, respectively) (World Health Organization 1992).

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 age-adjusted linear and logistic regression analyses, and exact Mann-Whitney U-test was used with age. 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} .

The association of HR-derived and other exercise test variables with the risk of outcomes were analyzed using Cox proportional hazards' models (Cox 1972). The Cox model assumes that the hazard which equals the instantaneous death rate is given by the formula: $h_i(t)=h(t)C_i$, where $C_i=\exp(B_1X_{1i}+ B_2X_{2i}+\dots+ B_pX_{pi})$ (Shetler et al. 2001, Froelicher et al. 2002, Froelicher et al. 2003). The model assumes that the hazard (h) of death for patient I at time t ($h_i(t)$) 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 a proportional hazards assumption that gives a model its name (Shetler et al. 2001, Froelicher et al. 2002, Froelicher et al. 2003). 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}, \dots, X_{pi}), multiplied by a corresponding set of regression coefficients (B_1, \dots, B_p) that measure the strength of the association between the prognostic factor and outcome of large number of subjects) (Shetler et al. 2001, Froelicher et al. 2002, Froelicher et al. 2003). 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 (Grambsch and Therneau 1994). Linearity of associations was assessed with the Martingale residuals (Therneau et al. 1990). No violations were observed.

Age, examination year, exercise test protocol (and the use of HR-lowering medication in men with known or suspected CHD at baseline) 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. In men without CHD at baseline these variables included alcohol consumption, BMI, cigarette smoking, CVD history, diabetes, myocardial ischemia during exercise test, serum LDL and HDL cholesterol, and systolic and diastolic BP at rest. In men with known or suspected CHD at baseline the corresponding covariates included alcohol consumption, BMI, cigarette smoking, cardiac insufficiency, history of myocardial infarction, diabetes, myocardial ischemia during exercise test, serum LDL and HDL cholesterol, and systolic and diastolic BP at rest.

In further analyses, the sample was restricted to subjects who remained free of events during the first 2 years of follow-up. 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. 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, the use of HR-lowering medication in men with known or suspected CHD at baseline, conventional risk factors chosen by stepwise selection, and the HR and exercise test variables in turns.

In men with known or suspected CHD at baseline, 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 the use of HR-lowering medication in men with known or suspected CHD at baseline). To address specifically the effect of two different exercise test protocols the stepwise selection was performed separately in corresponding subgroups. In supplementary analyses with men without CHD at baseline, 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. In the corresponding analysis with men with known or suspected CHD at baseline, 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.

All tests for statistical significance were two-sided. A value of p less than 0.05 was considered statistically significant.

5 RESULTS

5.1 WORKLOAD AT HEART RATE OF 100 BEATS/MIN DURING EXERCISE TEST AND MORTALITY IN MEN WITHOUT CORONARY HEART DISEASE

At baseline, the median (range) age of the men was 52 (42-61) years, and the mean (SD) WL_{100} was 63 (31) W (Table 3). Baseline characteristics according to halves of WL_{100} are shown in Table 1. 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 3.

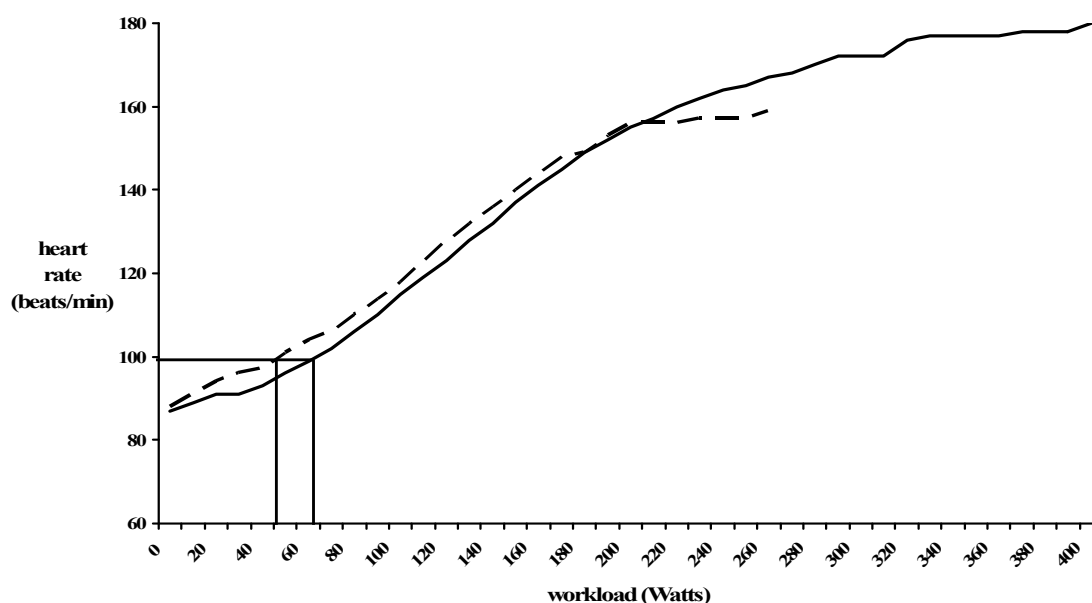


Figure 3. Heart rate (mean) as a function of workload in men who died during follow-up due to cardiovascular disease (n=51, dashed line) and survivors (n=1263, continuous line). Workload achieved at heart rate of 100 beats/min is indicated with vertical lines for each group. Standard deviations are not shown because the number of subjects in both groups gradually decreased along with increasing workload.

Men with a low WL_{100} were over 2-times more likely to die of CVD and about 3-times more likely to die of CHD than those with a high WL_{100} (Table 3). CVD mortality increased by 72% (95% CI 27%-138%, $p=0.001$), CHD mortality by 96% (32%-186%,

Table 3. Baseline Characteristics in 1314 Men with No History of Coronary Heart Disease and Not Using Heart Rate Lowering Medication at Baseline

Characteristics	Mean (SD), median (range) or proportion			p-value for difference between groups¶
	All men (n=1314)	WL ₁₀₀ below median value 64 Watts††	WL ₁₀₀ above median value 64 Watts††	
Number of CVD / CHD / all-cause deaths†	51 / 35 / 133	37 / 26 / 73	14 / 9 / 60	0.002 / 0.006 / 0.25
Age (years)	52 (42-61)	52 (42-61)	52 (42-61)	0.56
Body mass index (kg/m ²)	26.5 (3.5)	26.6 (3.5)	26.4 (3.2)	0.25
Cigarette smoking (cigarette-years)§	144 (299)	151 (319)	138 (277)	0.42
Alcohol consumption (g/week)	73 (110)	76 (119)	70 (101)	0.33
Cardiovascular disease history (%)*	14.5	13.8	15.1	0.50
Diabetes (%)	3.7	4.7	2.6	0.04
Serum HDL cholesterol (mmol/l)	1.32 (0.29)	1.30 (0.28)	1.35 (0.30)	0.003
Serum LDL cholesterol (mmol/l)	3.98 (0.97)	4.02 (0.99)	3.95 (0.95)	0.18
Diastolic blood pressure at rest (mmHg)	88 (10)	90 (10)	86 (9)	<0.001
Systolic blood pressure at rest (mmHg)	133 (15)	135 (16)	130 (14)	<0.001
Maximal oxygen uptake (ml/kg/min)	33.2 (7.3)	31.9 (6.9)	34.5 (7.5)	<0.001
Myocardial ischemia during exercise test (%)**	13.9	12.0	15.9	0.04
Resting heart rate (beats/min)	68 (10)	73 (10)	63 (8)	<0.001
Chronotropic index at heart rate of 100 beats/min‡	1.13 (0.28)	1.28 (0.29)	0.98 (0.17)	<0.001
Maximal heart rate (beats/min)	162 (17)	166 (16)	158 (17)	<0.001
Heart rate reserve (beats/min)#	94 (19)	93 (19)	96 (19)	0.03
Workload at heart rate of 100 beats/min (Watts)	63 (31)	38 (17)	88 (19)	<0.001

* Cardiovascular diseases included arrhythmias, cardiomyopathy, claudication, heart failure, and stroke.

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

‡ Chronotropic index at heart rate of 100 beats/min was calculated as ((100 – resting heart rate) / (maximal heart rate – resting heart rate)) / (workload at heart rate of 100 beats/min / maximal workload) (Salonen et al. 1991).

§ 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 (19).

|| Diabetes was defined as fasting glucose ≥ 6.7 mmol/l or use of medication for diabetes.

¶ Difference in age was tested with Mann-Whitney U-test. Differences in cardiovascular disease history, diabetes, myocardial ischemia during exercise test, and number of deaths were tested with logistic-regression analysis and in rest of the variables with linear regression analysis after adjustment

Table 3. Continued

	for age.
#	Heart rate reserve was calculated as maximal heart rate – resting heart rate.
**	Myocardial ischemia during exercise test was defined as a horizontal or downsloping ST depression of >1.0 mm at 80 msec after the J-point.
††	WL ₁₀₀ , workload at heart rate of 100 beats/min.

p=0.001), and all-cause mortality by 23% (2%-47%, p=0.03) with a decrement of 31 W (1 SD) in WL₁₀₀ adjusting 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 WL₁₀₀, but no association was found between WL₁₀₀ and all-cause mortality (Table 4). Entering the whole set of covariates into the model weakened the independent predictive value of WL₁₀₀ 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 2 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 WL₁₀₀, respectively.

The best WL₁₀₀ cut-off point for predicting CVD mortality was 50 W, and 497 men (37.8%) had WL₁₀₀ <50 W. Altogether 32 (63% of total) CVD deaths and 24 (69%) CHD deaths were observed among men with WL₁₀₀ <50 W. When WL₁₀₀ 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), WL₁₀₀ <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 predictors of CHD death were myocardial ischemia during exercise test (p<0.001), smoking (p<0.001), WL₁₀₀ <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 WL₁₀₀ <50 W and men with WL₁₀₀ ≥50 W continued to diverge with extended time of follow-up as shown in Figure 4.

Table 4. 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 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 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 \geq 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.

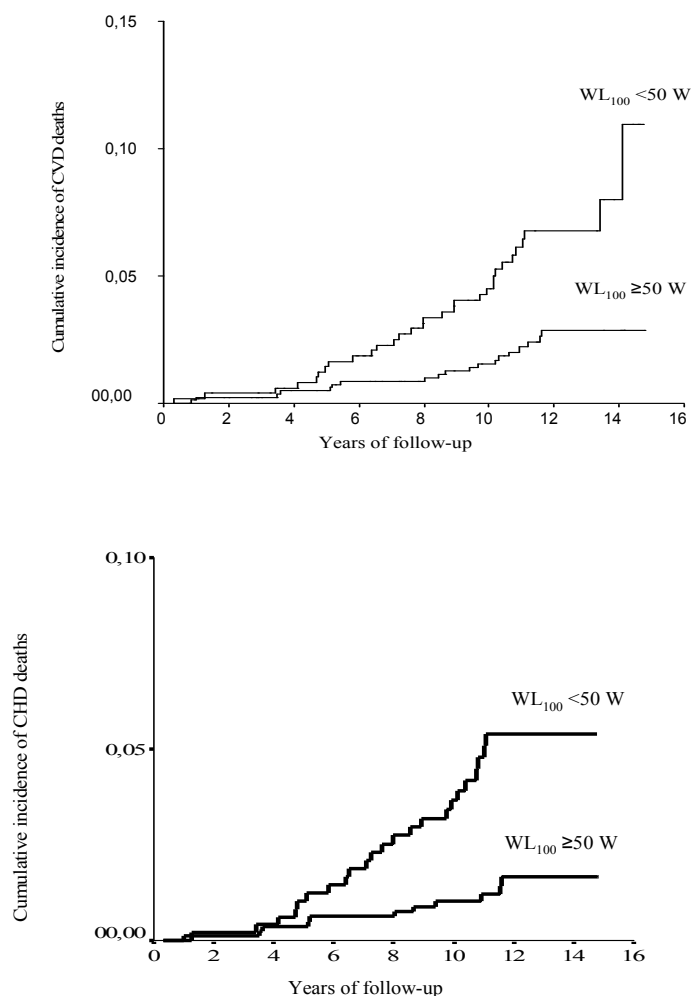


Figure 4. Kaplan-Meier curves for cumulative incidence of CVD (upper graph) and CHD (lower graph) deaths in men men with $WL_{100} < 50 W$ and men with $WL_{100} \geq 50 W$.

5.1.1 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 chronotropic index at HR of 100 beats/min 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} , that equals to about 2.1 metabolic equivalents, was associated with a 59% (95% CI 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 in Table 5, no other HR-derived or exercise test variable remained a significant predictor of death in models that included WL_{100} .

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

When a low blood haemoglobin 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 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} .

5.2 WORKLOAD AT HEART RATE OF 100 BEATS/MIN DURING EXERCISE TEST AND MORTALITY IN MEN WITH KNOWN OR SUSPECTED CORONARY HEART DISEASE

At the beginning of the follow-up, the median age of the subjects was 54 years (range 42-61 years). A total of 125 men (34.2%) used HR-lowering medication, including beta-blockers (118 men, 32.3%), digoxin (24 men, 6.6%), and clonidin (1 man, 0.3%). Of 118 beta-blocker users, 95 men (80.5%) used β_1 -selective agents and 23 men (19.5%) used non-selective agents. The use of HR-lowering medication was not suspended because of exercise test. The mean (SD) WL_{100} was 69 W (34 W). Baseline characteristics according to halves of WL_{100} are shown in Table 6. Resting HR explained 38%, chronotropic index at

Table 5. Workload at Heart Rate of 100 Beats/min and Cardiovascular Disease and Coronary Heart Disease Mortality After Adjustment for Heart Rate and Exercise Test Variables*

Heart rate –derived or exercise test variable entered into adjusted model before WL ₁₀₀	Cardiovascular disease mortality		Coronary heart disease mortality	
	P value for improvement of the model after entering WL ₁₀₀ into the model	Relative risk (95% CI) of CVD death per each increment of 1 SD in WL ₁₀₀	P value for improvement of the model after entering WL ₁₀₀ into the model	Relative risk (95% CI) of CHD death per each increment of 1 SD in WL ₁₀₀
Resting heart rate	0.002	1.89 (1.25-2.86)	0.005	2.04 (1.23-3.45)
Maximal heart rate	0.004	1.64 (1.16-2.33)	<0.001	2.13 (1.41-3.23)
Maximal oxygen uptake	0.006	1.56 (1.12-2.17)	0.002	1.79 (1.22-2.63)
Chronotropic index at heart rate of 100 bpm	0.09	1.39 (0.95-2.00)	0.03	1.67 (1.05-2.63)
HR reserve	0.008	1.54 (1.11-2.17)	0.001	1.89 (1.25-2.78)
Maximal workload	0.009	1.56 (1.11-2.22)	0.004	1.79 (1.19-2.70)

* CI, confidence interval; CVD, cardiovascular disease; CHD, coronary heart disease; WL₁₀₀, workload at heart rate of 100 beats/min; bpm, beats/min. 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.

HR of 100 beats/min 18%, maximal HR 11%, and VO_{2max} 5%, and all these variables together 72% of the variance in WL_{100} in multiple stepwise regression analysis.

Men with a low WL_{100} were 2 times more likely to die during follow-up than men with a high WL_{100} (Table 6). The risk of death increased by 56% (95% CI 20%-100%, $p=0.001$) with 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%, $p<0.001$) with 1 SD (34 W) decrement in WL_{100} (Table 7). Finally, entering the whole set of covariates did not affect on 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%, $p=0.03$) with 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%, $p<0.001$) with 1 SD (34 W) decrement in WL_{100} after adjustment for other risk factors.

Table 6. Baseline Characteristics in 374 Men with Known or Suspected Coronary Heart Disease at Baseline

Characteristics	Mean (SD), median (range) or proportion			p-value for difference between groups§
	All men (n=365)	WL_{100} below median value 69 Watts (n=182)#	WL_{100} above median value 69 Watts (n=183)#	
Number of deaths	75	48	27	0.004
Age (years)	54 (42-61)	54 (42-61)	55 (42-61)	0.65
Body mass index (kg/m ²)	27.3 (3.7)	27.4 (4.1)	27.2 (3.3)	0.63
Cigarette smoking (cigarette-years)†	195 (359)	226 (409)	165 (300)	0.11
Alcohol consumption (g/week)	81 (170)	94 (216)	69 (105)	0.18
Cardiac insufficiency (%)	14.0	11.5	16.4	0.22
Diabetes (%)‡	7.1	8.2	6.0	0.40
Use of heart rate-lowering medication (%)	34.2	19.8	48.6	<0.001
History of myocardial infarction (%)	24.7	18.7	30.6	0.01
Serum HDL cholesterol (mmol/l)	1.26 (0.33)	1.28 (0.32)	1.25 (0.33)	0.34
Serum LDL cholesterol (mmol/l)	4.05 (1.05)	4.10 (1.07)	4.01 (1.03)	0.42

Table 6. Continued

	All men (n=365)	WL₁₀₀ below median value 69 Watts (n=182)#	WL₁₀₀ above median value 69 Watts (n=183)#	
Diastolic blood pressure at rest (mmHg)	87 (10)	90 (10)	85 (10)	<0.001
Systolic blood pressure at rest (mmHg)	133 (18)	136 (17)	130 (17)	0.001
Maximal oxygen uptake (ml/kg/min)	26.8 (7.0)	27.1 (7.5)	26.6 (6.6)	0.68
Myocardial ischemia during exercise test (%)¶	11.5	11.0	12.0	0.78
Resting heart rate (beats/min)	67 (11)	73 (10)	61 (8)	<0.001
Chronotropic index at heart rate of 100 beats/min*	1.12 (0.27)	1.25 (0.29)	0.98 (0.15)	<0.001
Maximal heart rate (beats/min)	142 (22)	151 (20)	134 (21)	<0.001
Heart rate increase from rest to 50 Watts (beats/min)	28 (10)	32 (10)	24 (8)	<0.001
Heart rate increase from rest to 33% of maximal workload (beats/min)	27 (10)	30 (11)	24 (9)	<0.001
Heart rate reserve (beats/min)	75 (22)	78 (22)	73 (22)	0.03
Workload at heart rate of 100 beats/min (Watts)	69 (34)	42 (18)	97 (22)	<0.001

* Chronotropic index at heart rate of 100 beats/min was calculated as $((100 - \text{resting heart rate}) / (\text{maximal heart rate} - \text{resting heart rate})) / (\text{workload at heart rate of 100 beats/min} / \text{maximal workload})$ (16).

† 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 (Salonen et al. 1991).

‡ Diabetes was defined as fasting glucose ≥ 6.7 mmol/l or use of medication for diabetes.

§ Difference in age was tested with Mann-Whitney U-test. Differences in number of deaths, cardiac insufficiency, diabetes, history of myocardial infarction, heart rate-lowering medication, and myocardial ischemia during exercise test were tested with logistic-regression analysis and differences in rest of the variables with linear regression analysis after adjustment for age.

|| Heart rate reserve was calculated as maximal heart rate – resting heart rate.

¶ Myocardial ischemia during exercise test was defined as a horizontal or downsloping ST depression of ≥ 1.0 mm at 80 msec after the J-point or any ST depression of >1.0 mm at 80 msec after the J-point

WL₁₀₀, workload at heart rate of 100 beats/min.

The best cut-off point for predicting mortality was 55 W, and 130 (35.6%) of all 365 men had WL₁₀₀ <55 W. When WL₁₀₀ was entered as a dichotomous variable into the backward stepwise Cox model including other risk factors, the strongest predictor of death was WL₁₀₀ <55 W (RR 2.4, 95% CI 1.5-4.0, p<0.001), followed by a self-reported history of cardiac insufficiency (p=0.004), a history of myocardial infarction (p=0.006), diastolic blood pressure at rest (p=0.03), and age (p=0.04).

Table 7. 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.

5.2.1 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 increment in resting HR; by 47% (95% CI 10%-100%, $p=0.01$) with 7.0 ml/kg/min decrement in VO_{2max} ; and by 35% (95% CI 3%-75%, $p=0.03$) with 43 W decrement in maximal workload. Entering WL_{100} into these models improved the predictive value of the model statistically significantly in each case. The predictive value of WL_{100} remained stable in various models summarized in Table 8. 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 7.0 ml/kg/min decrement in VO_{2max} , while WL_{100} was an independent predictor ($p=0.002$) as well.

5.2.2 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 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 ($p=0.01$).

Table 8. 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. WL₁₀₀, workload at heart rate of 100 beats/min; CI, confidence interval; SD standard deviation.

The risk of death increased by 54% (95% CI 14%-108%, p=0.005) with 1 SD (29 W) decrement in WL₁₀₀. In men using HR-lowering medication, the strongest predictor of death was cardiac insufficiency (p=0.004), followed by WL₁₀₀ and diastolic blood pressure at rest (p=0.04). The risk of death increased by 72% (95% CI 14%-163%, p=0.01) with 1 SD (36 W) decrement in WL₁₀₀. There was no interaction between WL₁₀₀ and the use of HR-lowering medication (p=0.94). No interactions of WL₁₀₀ with clinical or exercise test variables shown in Tables 1 and 3 were found, either.

5.2.3 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 WL₁₀₀ for death remained unchanged. The mean (SD) WL₁₀₀ was 69 (31) W and 70 (36) W in men tested according to 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 WL₁₀₀ as a continuous variable remained in the final model after stepwise selection (p=0.04). Among the 196 men tested according to later protocol WL₁₀₀ 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 a 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 WL₁₀₀ among the remaining men.

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 (Brotman et al. 2005). Well-controlled experimentation 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 (Brotman et al. 2005). 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 (Froelicher and Myers 2006). 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 (Shetler et al. 2001, Brotman et al. 2005, Froelicher and Myers 2006). Such models help identify risk factors for an outcome, allow formulating risk stratification tools, and often may suggest pathophysiological mechanisms (Brotman et al. 2005). However, causation can never be definitively discerned from observational data sets, regardless of the statistical tools used, including testing for independence (Brotman et al. 2005). The most common technique for multivariable survival analysis is the Cox proportional hazards model (Shetler et al. 2001, Froelicher et al. 2002, Froelicher et al. 2003) which was used in the present study as well. Only three (Ekelund et al. 1988, Slattery and Jacobs 1988, Pardaens et al. 1996) of seven previous studies exploring the association between submaximal HR at exercise test and CVD events in asymptomatic subjects (Table 2) reported the result from multivariable model.

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 (Brotman et al. 2005). If, after adjustment, a risk factor maintains a statistically significant association with the outcome, it is called an independent risk factor for the outcome (Brotman et al. 2005). 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 (Brotman et al. 2005). Any claim that a given variable is an independent risk factor for a given CVD outcome (except in a particular study) ignores the likelihood of residual confounding, ie. that valuable predictors also associated with a variable of main interest have been excluded, poorly measured, or incorrectly modeled (Brotman et al. 2005). Hence, terms such as independent risk factor or independent predictor have meaning only in the context of a particular statistical model (Brotman et al. 2005). Importantly, in the current study a large number of established and potential risk factors were measured at baseline, and consequently it was possible to evaluate their impact on the association of WL₁₀₀ 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 weaken 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. The long follow-up may weaken the associations if the values of variables measured at baseline change considerably during a follow-up although the statistical power increases along with increasing number of outcome events (Peters et al. 1983). Because it is not known whether WL₁₀₀ changed during the follow-up of the present study, it is impossible to evaluate the relative effects of aforesaid mechanisms. Nonetheless, the question can be addressed indirectly by recalculating the results according to a notional situation where the follow-up would have been only eight years instead of true 11 years. The recalculation shows that the prognostic value of WL₁₀₀ impairs clearly with a shorter follow-up of eight years (data not shown). Assuming an unchanged trend, it can be speculated that the prognostic value of WL₁₀₀ would have been even larger if the follow-up time had been extended over 11 years.

Censoring refers to the removal of intervened subject from observation when the intervention occurs during follow-up because the intervention changes a natural course of a disease of a subject (Shetler et al. 2001, Froelicher et al. 2002, Froelicher et al. 2003). 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 (Shetler et al. 2001, Froelicher et al. 2002, Froelicher et al. 2003). 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 seven previous studies in asymptomatic subjects censoring was performed either.

Collinearity means that at least one of the covariates can be predicted well from the other covariates in the model (Harrell 2001). In the present study, collinearity was tried to minimize by utilizing a backward stepwise selection method in Cox models. 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 remained very much unchanged when all covariates were entered simultaneously into the model instead of stepwise selection method.

6.1.2 Study population

The 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 (Keys et al. 1984, Salonen 1988). 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 in male populations. 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 studies.

The baseline characteristics of the previous studies exploring the association between HR at exercise test and CVD events in asymptomatic subjects as well as those of the current study are gathered in Tables 2, 3 and 6. 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 length of the follow-up. According to this equation, in two 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 two previous studies might be due to a considerably longer follow-up in one study (Slattery and Jacobs 1988), as well as the inclusion of men with a clinical evidence of definite or probable CHD in another study (Hinkle et al. 1972).

Because of the limited number of CVD deaths during the follow-up, men with a history of CVD (involving cardiac insufficiency, cardiomyopathy, arrhythmias, stroke or claudication) were included in the current study. 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 (Ellenberg 1994). The potential selection bias was taken into account by including a history of CVD as a covariate in the survival analyses with men without CHD at baseline. Furthermore, in these men the main analyses were repeated after excluding men with CVD or other diseases or conditions potentially affecting the exercise test findings or the outcomes, but the results did not change considerably. Finally, the prevalence of CVD was not different between men with WL_{100} below or above the median value (Table 3). It is noteworthy, however, that by far the most common CVD were arrhythmias that are generally benign and 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 have not been investigated. Nonetheless, for descriptive purposes the present study can be compared with two recent studies (Falcone et al. 2005, Leeper et al. 2007) which 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 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. By 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 (Falcone et al. 2005), or consecutive patients referred for exercise testing (Leeper et al. 2007). The self-reporting unavoidably involves inaccuracy and a risk of misclassification (Froelicher and Myers

2006). 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 (Rose et al. 1982) is a widely used and well validated tool for a standard, unbiased assessment of chest pain in epidemiological studies (Ashley et al. 2000b). It is possible that the subjects in the present study represent the wide spectrum of severity of CHD, and consequently the results can be generalized to even larger group of patients with known or suspected CHD than the results from studies involving subjects referred for exercise testing (Ellenberg 1994). Moreover, men in the current study can be considered as patients who according to current guidelines (Gibbons et al. 2002b) 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 resemble those in the study by Leeper et al (Leeper et al. 2007). 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 as in two previous studies in patients with known or suspected CHD (Falcone et al. 2005, Leeper et al. 2007), 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. The same trend has been observed also in previous studies exploring the association between submaximal HR at exercise test and CVD events in asymptomatic subjects because in three (Sobolski et al. 1987, Ekelund et al. 1988, Pardaens et al. 1996) of seven studies the users have been excluded and in only one (Hinkle et al. 1972) study they have been included in the study sample (Table 2). In three (Wilhelmsen et al. 1976, Wilhelmsen et al. 1981, Slattery and Jacobs 1988) studies the presence of the users of HR-lowering medication was not reported. Definitely the future studies are needed to clarify the prognostic value of submaximal HR among the users of HR-lowering medication as well. In the current thesis a low WL_{100} predicted, however, all-cause deaths both in subjects who used and not used 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 (Wasserman et al.

1999, Fletcher et al. 2001, Gibbons et al. 2002a). 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 (Fletcher et al. 2001, Gibbons et al. 2002a, Froelicher and Myers 2006). 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. Furthermore, the potential effect of two different protocols was taken into account by including testing protocol as a forced covariate in all Cox models. 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. These findings suggest that the difference in the testing protocols had no effect on the results.

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 (Atwood et al. 1989). 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 strengthens the predictive value of HR variables observed in the present study.

In men without CHD at baseline myocardial ischemia during exercise test was defined as 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. A ST depression of 0.5 mm was used as a cut-off value for definition, as in the early reports from the KIH data (Salonen et al. 1991, Lakka et al. 1994, Salonen et al. 1995), because in univariate Cox model it was a stronger predictor of the outcome than 1.0 mm ST depression. In men with known or suspected CHD, 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%) if 0.5 mm ST depression had been used. More importantly, the main results were practically unchanged although ST-depression of 0.5 mm was replaced by 1.0 mm as a cut-

off value in men without CHD at baseline, and vice versa in men with known or suspected CHD.

The 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 (Myers 1996, Wasserman et al. 1999, Myers and Madhavan 2001). Importantly, a submaximal effort at peak exercise can be objectively evaluated based on respiratory exchange ratio as well (Myers 1996, Wasserman et al. 1999, Gibbons et al. 2002a). VO_{2max} is considered the best index of aerobic capacity and maximal cardiorespiratory function (Myers 1996, Fletcher et al. 2001, Gibbons et al. 2002a). 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 (Rowell 1993, Wasserman et al. 1999). Predicting VO_2 from cycle ergometer workload is a common clinical practice, but such predictions can be very misleading (Myers 1996, Wasserman et al. 1999, Myers and Madhavan 2001). In the current study, exercise testing with both conventional indirect definition of exercise capacity and respiratory gas analysis was used which is unique in a large population study. The accurate measurement of VO_{2max} assured the reliable and unbiased estimation of the predictive value of cardiorespiratory fitness. The predictive value of other exercise test variables independent of cardiorespiratory fitness could be assessed more reliably as well.

6.1.4 Collection and classification of outcome events

During the follow-up of the current study it was possible 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 (Lahti and Penttila 2001). 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 (Rapola et al. 1997, Mahonen et al. 1999).

Four (Wilhelmsen et al. 1976, Wilhelmsen et al. 1981, Sobolski et al. 1987, Pardaens et al. 1996) of seven previous studies exploring the association between submaximal HR at exercise test and CVD events in asymptomatic subjects (Table 2) used composite end points (fatal or nonfatal CVD event) as an outcome. The several problems accompanying composite end point has been discussed thoroughly (Freemantle et al. 2003, Lauer and Topol 2003). 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 (Shetler et al. 2001, Froelicher et al. 2002, Froelicher et al. 2003). Among fatal end points, all-cause mortality is suggested to be objective, unbiased, and clinically relevant (Gottlieb 1997, Lauer et al. 1999). On the other hand, CVD mortality is 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 (Froelicher and Myers 2006). In the present study, to avoid inherent problems associated with composite end points, death from CVD or CHD, as well as total mortality was used as main outcome.

6.2. RESULTS

The results of the present study 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 3 resembles the hypothetical curve in Figure 2 although the distance between curves is exaggerated in the latter curve.

The workload achieved at 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 overall HR increase (Figure 5). Because both a high resting HR (Keys et al. 1966, Hesse et al. 2005, Jouven et al. 2005) and a low cardiorespiratory fitness (Morris et al. 1991, Mark and Lauer 2003, Lauer et al. 2005) 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 the study is that a low WL_{100} was associated with an increased CVD and CHD mortality in men who did not have a prior CHD at baseline, and with an increased mortality in men with a known a 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 (Ekelund et al. 1988, Slattery and Jacobs 1988), 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 5). 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 a low cardiorespiratory fitness did not explain the association of a low WL_{100} with outcomes.

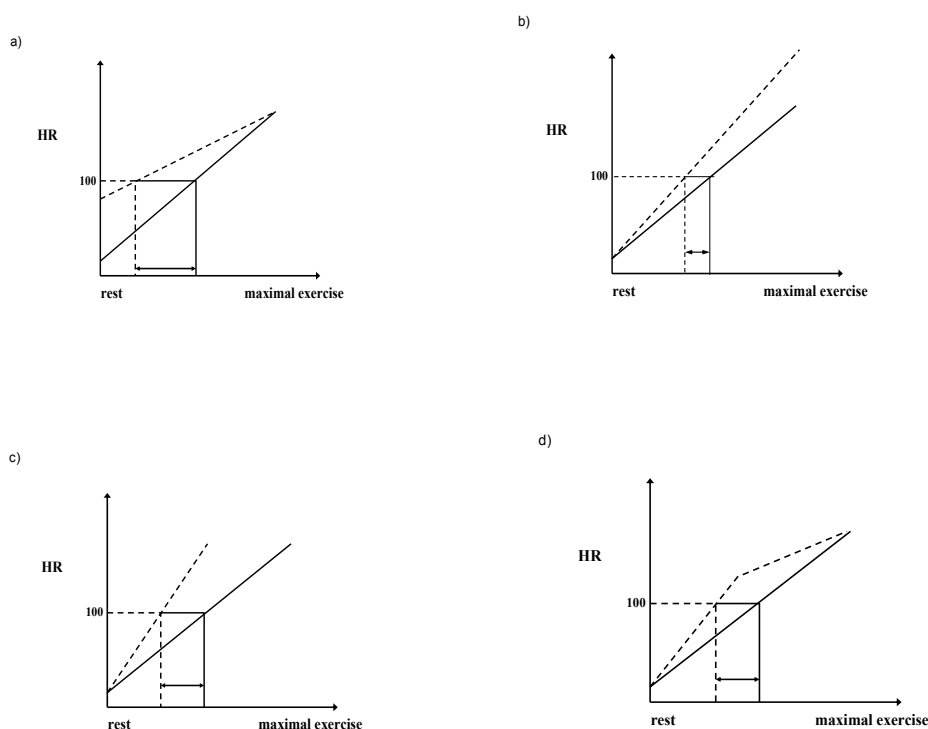


Figure 6. The graph illustrating how a high resting heart rate (a), a high maximal heart rate (b), a low cardiorespiratory fitness (c), and a disproportionately increasing heart rate at light workloads (d) each can have a lowering effect on workload achieved at heart rate of 100 beats/min as shown by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

This interesting finding suggests that an exaggerated HR response at low workload indicates an increased risk by itself instead of being only a surrogate marker of a low cardiorespiratory fitness.

Resting HR was the most significant individual determinant of WL_{100} and an independent predictor of outcomes as well. A high resting HR has been considered as a surrogate measure of a reduced parasympathetic tone (Palatini and Julius 1997b) which is a known risk factor for CVD events in asymptomatic subjects (Tsuji et al. 1996, Huikuri et al. 1998, Makikallio et al. 2001) and cardiac patients (Schwartz et al. 1992, Schwartz 1998, Freeman et al. 2006). 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 (Schwartz et al. 1992, Schwartz 1998). 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, however, that a 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 in the present study.

A continuously elevated resting HR and exaggerated HR responses to daily stressful situations may burden cardiovascular system and enhance progression of atherosclerosis (Perski et al. 1992, Huikuri et al. 1999) via several mechanisms (Palatini 1999a, Palatini and Julius 2004, Palatini et al. 2006). An elevated HR may be a sign of chronic sympathetic nervous system overactivity which may induce numerous unfavourable 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 (Julius et al. 1998). 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 (Gordon et al. 1981, Palatini 1999a, Palatini et al. 2006). 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 (Palatini and Julius 1997a, Palatini 1999a). The increase in arterial wall stress caused by a high HR can also be the result of the higher mean BP in individuals with tachycardia (Palatini 1994) which is due to the progressive shortening of the diastolic phase of the cardiac cycle (Palatini and Julius 1999, Palatini and Julius 2004). The

increased arterial wall stress may perturb intercellular junctions, increase permeability of the endothelial cells and favour the ingress of atherogenic particles leading to atherosclerotic plaques (Gordon et al. 1981, Bassiouny et al. 1994, Palatini 1999a). In rats, carotid artery compliance and distensibility have been shown to be markedly impaired by the progressive increase in HR caused by pacing (Mangoni et al. 1996). This might be due to the fact it takes a certain time for the arterial wall to distend fully in response to BP variations (Palatini and Julius 2004). On the other hand, pharmacologic HR reduction induced a significant decrease in thoracic aorta wall thickness in rats (Albaladejo et al. 2003). In CHD patients, hemodynamic forces resulting from increased HR may favour vulnerable coronary plaque disruption (Heidland and Strauer 2001). In patients with restricted coronary blood flow a high HR can further increase cardiac ischemia and precipitate the occurrence of arrhythmias as well as impair left ventricular performance by increasing myocardial oxygen demand, facilitating desynchronization of ventricular myocardial cells and worsening coronary perfusion (Palatini 1999b, Palatini and Julius 2004).

WL₁₀₀ 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 WL₁₀₀ 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 testing environment (Palatini and Julius 1997b). Because the data of 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 WL₁₀₀. 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 (Lange Andersen et al. 1971, Wilkoff and Miller 1992, Åstrand et al. 2003), indicated by a low WL₁₀₀. 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 (Kamarek et al. 2000), and a heightened BP response during mental stress has been linked to enhanced carotid

atherosclerosis (Kamarck et al. 1997, Jennings et al. 2004). Furthermore, a trend was observed between an exaggerated HR response during mental stress and enhanced carotid atherosclerosis in 4-year follow-up (Kamarck et al. 1997) but no association was observed to a progression of atherosclerosis from fourth to eleventh follow-up year (Jennings et al. 2004). Thus, a low WL_{100} may represent a trait characterized by an exaggerated cardiovascular responsiveness to stressful situations (corresponding to graph d in Figure 5). 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, and it 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, the careful inspection of Figure 3 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 which have used treadmill as a testing mode (Ekelund et al. 1988, Slattery and Jacobs 1988) and not in studies using cycle ergometer (Wilhelmsen et al. 1976, Wilhelmsen et al. 1981, Sobolski et al. 1987, Pardaens et al. 1996) whereas in the patients with known or suspected CHD the findings have been just opposite (Falcone et al. 2005, Leeper et al. 2007). 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 as compared with maximal tests. A low level of exercise enables testing of individuals with a limited exercise capacity, and cardiovascular risks associated with a high-intensity exertion can be largely avoided (Åstrand et al. 2003, Froelicher and Myers 2006). 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 a short recovery period needed.

6.2.1 Workload achieved at heart rate of 100 beats/min during an 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 (Ekelund et al. 1988, Slattery and Jacobs 1988) 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 (Ekelund et al. 1988, Slattery and Jacobs 1988). While the minimum HR required for a risk assessment was 116 (Slattery and Jacobs 1988) and 133 beats/min (Ekelund et al. 1988) in two previous studies, the mean HR to enable the risk assessment (ie. a first HR value above 100 beats/min) was only 104 beats/min in the present study.

Interestingly, three of the four studies (Hinkle et al. 1972, Ekelund et al. 1988, Slattery and Jacobs 1988), 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 four (Wilhelmsen et al. 1976, Wilhelmsen et al. 1981, Sobolski et al. 1987, Pardaens et al. 1996) 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 one variables' contrasting effects with respect to two end points can cancel each other out (Shetler et al. 2001, Froelicher et al. 2002, Froelicher et al. 2003). Because of a limited number of studies it is precipitated to state that a submaximal HR is a variable predicting 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 (Sobolski et al. 1987) 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. It is possible that the association was not 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 3 this could have been the case in the present study as well. We did not divide WL_{100} by body weight although there is theoretical basis for it (Åstrand et al. 2003). Furthermore, because a high BMI was a risk factor for death in men without CHD at baseline, it is possible that a prognostic value of WL_{100} would have even improved if had been expressed as indexed for 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 (Rowell 1993, Åstrand et al. 2003). 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 a latent CVD did not weaken the prognostic value of WL_{100} much. This suggests that a latent CVD most probably does not explain the increased risk associated with a low WL_{100} .

6.2.2 Workload achieved at heart rate of 100 beats/min during an 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 the 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 (Falcone et al. 2005). In the present study, HR increase from rest to the 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 and coworkers (Falcone et al. 2005), the 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 but unfortunately an 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 (Billman et al. 1985, Billman and Hoskins 1989). 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 as compared with dogs that were resistant to ventricular fibrillation. The susceptibility to ventricular fibrillation was associated with a greater degree of left ventricular dysfunction (Billman et al. 1985) which thus probably explains a higher HR at submaximal workloads as well.

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 (Leeper et al. 2007). Again, WL_{100} was not measured in the study by Leeper et al (Leeper et al. 2007) but the closer inspection shows that WL_{100} most 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 (Leeper et al. 2007) is that the latter used an individualized ramp treadmill protocol in which workload increments are tailored according to an estimated work capacity of a patient (Myers et al. 1991). 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 a total exercise capacity of a subject (Mundal et al. 1994). 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 (Leeper et al. 2007) could be to some extent attributed to different testing protocols used in these studies.

A low WL_{100} could result from left ventricular dysfunction in subjects with a more severe CHD (Weber et al. 1982, Wasserman 1997, Myers and Madhavan 2001). Although this possibility can not 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 (Hultgren et al. 1986, Wasserman 1997, Myers and Madhavan 2001). An early appearance of myocardial ischemia has been shown to associate with an adverse prognosis (McNeer et al. 1978, Weiner et al. 1980) and an angiographically more severe CHD (Weiner et al. 1980). Because the data on ST-segment depression at workloads below HR of 100 beats/min is not available, the role of an 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 and the American College of Cardiology (Gibbons et al. 2002a) as well as from the US Preventive Services Task Force (Fowler-Brown et al. 2004) have led to recommendation against the use of exercise testing as a screening tool for detecting a latent CHD in asymptomatic persons. 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 (Lauer et al. 2005). 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 (Ashley et al. 2000a, Lauer 2001b, Lauer et al. 2005). A latest version of the textbook written by major authorities in the field (Froelicher and Myers 2006) states, indeed, that exercise testing should be used for screening healthy, asymptomatic individuals along with risk factor assessment (Froelicher 2005). In this context assessment of exercise test result includes not only ST-segment diagnostics but other exercise test variables shown to have a prognostic value as well (Froelicher and Myers 2006). 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 on further risk stratification (Greenland et al. 2001, Kullo and Malik 2007). 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 (Aktas et al. 2004, Balady et al. 2004, Erikssen et al. 2004). 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 (Califf et al. 1996). 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 (Lauer et al. 2005). Additionally, these trials would provide much-needed evidence about the cost-effectiveness in exercise testing in asymptomatic persons (Lauer et al. 2005).

In patients with known or suspected CHD the value of exercise testing is more clearly established so that the post-exercise test risk assessment serves as a guide to a particular management strategy that is viewed as most appropriate, based on expected outcomes (Fletcher et al. 2001, Gibbons et al. 2002a). According to the guidelines of the American Heart Association and the American College of Cardiology (Gibbons et al. 2002a), 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 whereas 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 (Gibbons et al. 2002a).

An obvious question with regard to the clinical use of WL_{100} is whether it is a modifiable risk factor. The existing literature shows that physical activity, or training lowers HR at a fixed submaximal workload (Fletcher et al. 2001), and thus increases WL_{100} as well. This has been observed both in healthy subjects (Karvonen et al. 1957, Camm and Fei 1996a, Moore and Palmer 1999) and in patients with CHD (Detry and Bruce 1971, Ehsani et al. 1981, Myers et al. 1984). Weight loss in obese subjects is another physiological means to increase WL_{100} and this effect is especially pronounced in activities involving weight-bearing, such as walking in treadmill.

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 as well as during recovery phase. The underestimation of exercise testing as a

prognostic tool has been based on a constricted assessment of solely ST-segment response to exercise. Several studies conducted during the last 15 years in healthy individuals as well as in clinical populations have undoubtedly shown that exercise testing can offer additional prognostic information above conventional risk markers. Consequently, multivariable equations and scores derived from clinical variables and exercise test results are considered as a highly recommended method to evaluate an individual risk for adverse CVD events in the future (Lauer 2001b, Lauer et al. 2005, Kligfield and Lauer 2006).

7 SUMMARY AND CONCLUSIONS

1. 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, as well as 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.

2. Several exercise test variables predict premature death independent of each other and conventional risk factors. This emphasizes that the maximal prognostic yield from exercise test is obtained by measuring several variables at submaximal and maximal workloads as well as during recovery phase.

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