RESTING HEART RATE IN CARDIOVASCULAR AGEING: FROM RISK MARKER TO RISK FACTOR

by

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ABSTRACT

An accumulation of epidemiological studies along with several lines of experimental research highlight sustained elevated resting heart rate as a significant predictor of cardiovascular morbidity and mortality. However, resting heart rate as a simple and inexpensive clinical parameter often remains overlooked by physicians. We therefore sought to raise awareness concerning the detrimental role of elevated resting heart rate in cardiovascular disease. Using three separate large prospective cohort studies, we examined the clinical importance of accelerated resting heart rate as a robust predictor of adverse cardiovascular prognosis. The current data supports the contention that a raised resting heart rate amplifies the risk of having several cardio-metabolic risk factors including type 2 diabetes mellitus, the metabolic syndrome, and increased pulse wave velocity. Resting tachycardia also appeared to increase the risk of cardiovascular mortality in otherwise healthy individuals, as well as negatively predicting outcome in patients already at-risk for the condition. Notably, we observed a strong synergistic effect between inflammatory activity and concurrent elevated resting heart rate among those who experienced a cardiovascular event. Overall, these findings underline the relevance of a high resting heart rate in the pathogenesis of atherosclerosis and in the clinical manifestations of cardiovascular mortality.

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LIST OF PUBLICATIONS

The following thesis includes five manuscripts which were recently published in peer-reviewed journals:

1) Ó Hartaigh B, Bosch JA, Thomas GN, Carroll D, Hemming K, Pilz S, Loerbroks A, Kleber ME, Grammer TB, Fischer JE, Böhm BO, März W. Evidence of a synergistic association between heart rate, inflammation, and cardiovascular mortality in patients undergoing coronary angiography. **Eur Heart J** 2012 (*in press*).

2) Ó Hartaigh B, Thomas GN, Jiang CQ, Bosch JA, Zhang WS, Cheng KK, Lam TH. Influence of heart rate at rest for predicting the metabolic syndrome in older Chinese adults. **Acta Diabetol** 2012 (*in press*).

3) Ó Hartaigh B, Thomas GN, Bosch JA, Pilz S, Loerbroks A, Kleber ME, Grammer TB, Fischer JE, Böhm BO, März W. Influence of resting heart rate on mortality in patients undergoing coronary angiography (from the Ludwigshafen Risk and Cardiovascular Health [LURIC] Study). **Am J Cardiol** 2012; 110: 515-20.

4) Ó Hartaigh B, Jiang CQ, Thomas GN, Tsvetanov KA, Bosch JA, Cheng KK, Lam TH. Usefulness of physical fitness and the metabolic syndrome to predict vascular disease risk in older Chinese (from the Guangzhou Biobank Cohort Study-Cardiovascular Disease Subcohort [GBCS-CVD]). **Am J Cardiol** 2011; 6: 845-50.

5) Ó Hartaigh B, Thomas GN, Zhang WS, Bosch JA, Cheng KK, Jiang CQ, Lam TH. Independent and combined associations of abdominal obesity and seated resting heart

rate with type 2 diabetes among older Chinese: the Guangzhou Biobank Cohort Study (GBCS). **Diabetes Metab Res Rev** 2011; 27: 298-306.

The following papers were also published in peer-reviewed journals during the period of postgraduate study at the University of Birmingham:

- Ó Hartaigh B, Thomas GN, Silbernagel G, Bosch JA, Pilz S, Loerbroks A, Kleber ME, Grammer TB, Fischer JE, Böhm BO, März W. Association of 25-hydroxyvitamin D with type 2 diabetes among patients undergoing coronary angiography: Cross-sectional findings from the LUdwigshafen RIsk and Cardiovascular Health (LURIC) Study. **Clinical Endocrinology** 2012 (*in press*).

- Ó Hartaigh B, Bosch JA, Thomas GN, Lord JM, Pilz S, Loerbroks A, Kleber ME, Grammer TB, Fischer JE, Böhm BO, März W. Which leukocyte subsets predict cardiovascular mortality? from the Ludwigshafen Risk and Cardiovascular Health (LURIC) Study. **Atherosclerosis** 2012; 224: 161-9.

- Silbernagel G, Sourij H, Grammer TB, Kleber ME, Ó Hartaigh B, Winkelmann BR, Boehm BO, März W. Isolated Post-Challenge Hyperglycaemia Predicts Increased Cardiovascular Mortality. **Atherosclerosis** 2012; 225: 194-9.

- Thomas GN, Ó Hartaigh B, Bosch JA, Pilz S, Loerbroks A, Kleber ME, Grammer TB, Böhm BO, März W. Vitamin D levels predict all-cause and cardiovascular disease mortality in patients with the metabolic syndrome: the LUdwigshafen RIsk and Cardiovascular Health (LURIC) Study. **Diabetes Care** 2012; 5: 1158-64.

- Ó Hartaigh B, Loerbroks A, Thomas GN, Fischer JE, Bosch JA. Age-dependent and -independent associations between depression, anxiety, DHEAS, and cortisol: from the MIPH Industrial Cohort Studies (MICS). **Psychoneuroendocrinology** 2011; 37: 929-36.

In addition, author contributions were provided for the following books during the period of postgraduate study at the University of Birmingham:

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AUTHOR ROLE IN STUDIES

The authors all contributed to the manuscripts consistent with the authorship criteria set by each of the above journals, including significant input in conception, design, and acquisition of data belonging to the GBCS (THL, CQJ, KKC, GNT), and LURIC (WM, BB) cohorts, analyses and interpretation of GBCS (BóH, GNT) and LURIC (BóH, GNT, JB, AL, SP, WM) data, initial hypotheses (BóH, GNT) and drafting of each manuscript (BóH), as well as subsequent drafting and critical revision of the manuscripts (all authors).

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ABBREVIATIONS AND ACRONYMS

CVD = cardiovascular disease

RHR = resting heart rate

ANS = autonomic nervous system

HRV = heart rate variability

HRR = heart rate recovery

BMI = body mass index

MetS = metabolic syndrome

CAD = coronary artery disease

CRP = C-reactive protein

MI = myocardial infarction

CHD = coronary heart disease

IHD = ischaemic heart disease

GBCS = Guangzhou Biobank Cohort Study

EPIC = European Prospective Investigation into Cancer and Nutrition

LURIC = LUdwigshafen RIsk and Cardiovascular Health

GHHARE = Guangzhou Health and Happiness Association for Respectable Elders

PWV = pulse wave velocity

ACS = acute coronary syndrome

SCD = sudden cardiac death

IL-6 = interleukin-6

OR/HR = odds/hazard ratio

IDI = integrated discrimination improvement

NRI = net reclassification improvement

AUC = area under the receiver operating characteristic curve

CHAPTER ONE

1.0. INTRODUCTION

1.1. CARDIOVASCULAR AGEING

".... A light heart lives long." *Love's Labour's Lost, V.ii.1895* William Shakespeare (1564-1616)

Undoubtedly, over the past few decades the world population has been progressively ageing, a trend which is likely to persist into the foreseeable future [1]. Despite this global increase in life expectancy, there has been a comparable growth in adverse health outcomes. Notably, with ageing of the population, cardiovascular disease (CVD) presents a significant health threat, and is considered the most common cause of morbidity and mortality among persons aged 65 and older. CVD accounts for 40% of the overall mortality rate, with 80% of deaths due to CVD occurring in those over 65 years [2]. Thus, given the substantial burden of CVD among the aged, ensuring identification of modifiable risk factors for attenuating the risk of CVD is an important dimension of preventive medicine. With increased longevity however, established risk factors for CVD such as smoking, obesity, and total cholesterol concentrations tend to lose their prognostic ability with advancing age [3-5], which in turn, has led researchers and clinicians to explore more potent constituents. To this end, an accumulation of epidemiological data supports the notion that sustained elevated resting heart rate (RHR) increases the risk of CVD morbidity and mortality. On the other hand, the predictive utility of this simple, easily-measured clinical parameter is often neglected by physicians. The following represents an effort to address the misconceptions which many clinicians seem to have regarding the detrimental role of high RHR in promoting CVD.

1.2. RESTING HEART RATE

It is well recognized that the autonomic nervous system (ANS) predominantly controls RHR as well as other essential life functions [6, 7]. The ANS consists of two key divisions, the sympathetic and parasympathetic limbs, both of which act in synergy or in opposition of each other to mediate essential physiological responses in real time [7]. Typically, accelerated sympathetic tone results in a faster RHR via stimulation of circulating epinephrine as well as neural release of norepinephrine [8, 9]. Conversely, a raised parasympathetic tone slows RHR as a consequence of acetylcholine release from efferent vagus nerve discharge [6, 9]. Indeed, the interplay between sympathetic and parasympathetic control of the heart creates a complex variability in heart rhythm that characterizes a healthy system [10]. For this reason, researchers and physicians have focussed on the application of readily available tools based on RHR for determining the link between dysfunctional ANS and subsequent adverse prognosis [6]. Moreover, epidemiologists have exploited such markers as RHR, heart rate variability (HRV), and heart rate recovery (HRR) as a means of exploring the relation between perturbed ANS function with morbidity and mortality at a population and environmental level (Figure 1.1). Evidently, many determinants of ANS function have been described, though as a non-invasive and inexpensive parameter, RHR is undoubtedly one of the most straightforward to obtain in the clinic.

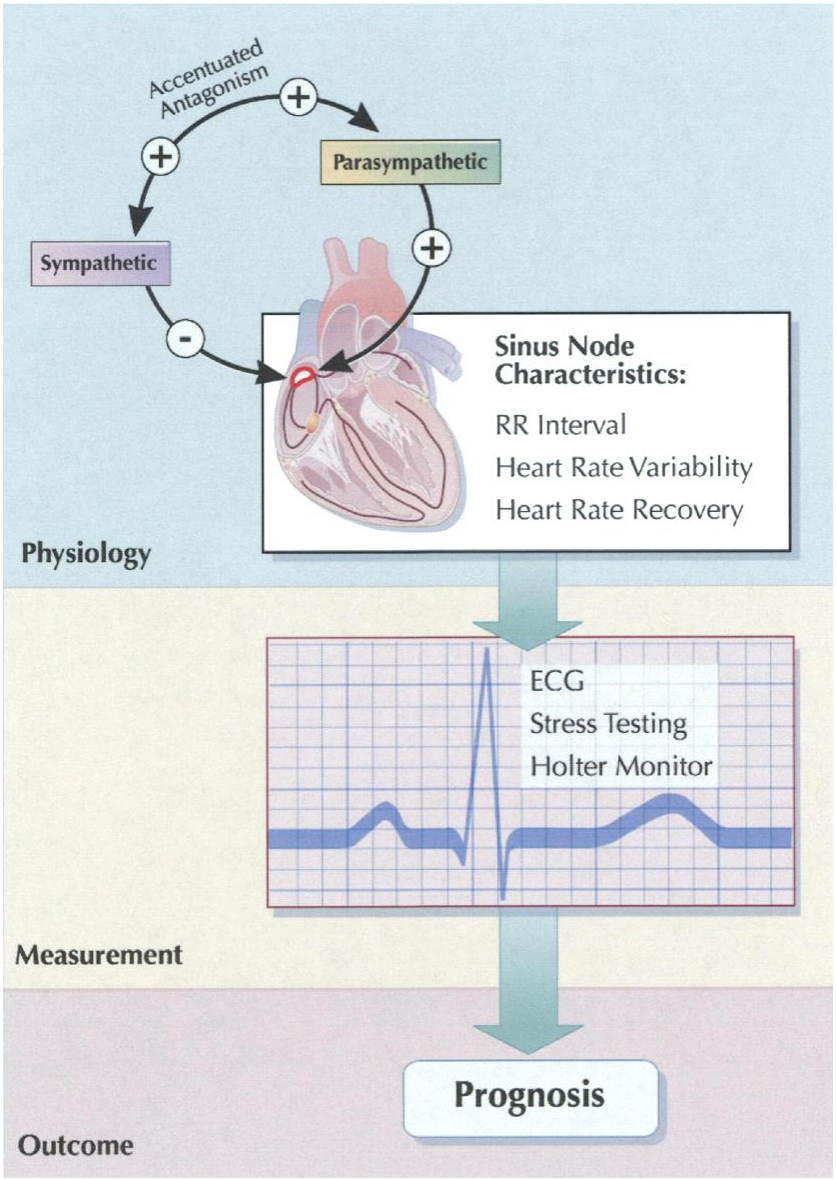

Figure 1.1. Schematic diagram showing the physiological effects of the sympathetic and parasympathetic pathways. Illustration highlights the accentuated antagonistic effects of the sympathetic and parasympathetic limbs on the sinus node to increase or decrease, respectively, the RR-interval (the distance between two successive R waves, representing the time between successive heart beats), heart rate variability, and heart rate recovery. These characteristics of autonomic nervous activity can be quantified via short-term electrocardiogram, Holter monitoring, or stress testing and are used to provide prognostic information in patients at risk for cardiovascular disease. Adapted from Lahiri et al. [6] with permission.

1.3. RESTING HEART RATE AND LIFE EXPECTANCY

From the smallest shrew or bumble-bee bat, to the largest blue whale [11], there is an extraordinary amount of variation in heart size and physiology. Indeed, the size of a mammalian heart can differ by more than seven orders of magnitude, ranging from approximately 12 mg to 600 kg [11]. In small animals such as a shrew, the heart can beat as high as 800-1,200 beats/minute (bpm), pumping 1-5 millilitres/minute, while in larger animals like the blue whale, RHR can be as low as 2-6 bpm while producing a cardiac output of 2,000-5,000 litres/minute [11]. Likewise, there is considerable disparity in maximum life expectancy among mammals (Figure 1.2). Those with a slower RHR tend to have a longer lifespan in contrast to those with a higher RHR who correspondingly have a shorter life expectancy [9, 12, 13]. Despite some inevitable variations among mammals, the total number of heart beats in a lifetime seems remarkably constant at approximately 1.1 x 10^9 (~1.1 billion) [11, 12]. In this regard, it appears mammals have a finite number of heart beats, whereby those who expend their overall "quota" at a faster rate ultimately have a shorter life [12]. However, one may speculate that given humans, on average, have a RHR of 60-100 bpm, our lifespan should be similar to that of a giraffe or tiger [13], or close to 28 years [11]. One noticeable exception is that the human heart can produce almost 3.03 x 10^9 (~3 billion) beats in a lifetime [11]. Evidently, it would appear modern humans have broken the trend by extending the biological boundaries, thus achieving life expectancies of around 80 years [13, 14]. A question which has therefore sparked much public interest is whether humans differ physiologically or biologically compared with other mammals in some way that enhances longevity? In short, the most plausible answer is probably not. Perhaps the most reasonable explanation how humans have extended their lifespan potential is largely attributable to recent cultural advancements including scientific, technological and evidence-based medical developments [15]. Indeed, throughout the majority of human history, average life expectancy

was rarely seen to go beyond 40 years [12, 15]. Albeit, acquired knowledge in science, technology, and medicine in the last 1-2 centuries has significantly improved disease control and prevention, raising the average life expectancy. Nonetheless, as the risk of death due to conventional diseases such as infection and malnutrition becomes increasingly smaller, it seems reasonable to speculate that the likelihood of succumbing to other noxious influences (i.e., CVD) will be greatly augmented with advancing age. Clearly, as the rise in life expectancy persists, it appears humans are entering an era where cardiovascular health represents a critical determinant of optimal longevity [12].

1.4. RESTING HEART RATE AND PHYSICAL FITNESS

The association between poor physical fitness and greater risk of adverse health outcomes is well described in the literature [16-19]. Notably, RHR and physical fitness appear to be inversely related. Individuals with poor cardio-respiratory fitness tend to have a faster RHR, and reciprocally, those who appear physically fit are more likely to possess a lower overall RHR [20]. However, the matter of whether RHR, and likewise, physical fitness, are strong predictors of poor health outcomes independent of one another has remained controversial. In particular, a limitation of most studies is the use of self-reported physical activity as a determinant of fitness. Indeed, self-reported measures of physical activity along with objectively measured physical fitness demonstrate only a poor-to-moderate correlation [21]. Nonetheless, two separate studies derived from a cohort of 1,960 healthy men residing in Oslo [22, 23] demonstrated both RHR and physical fitness appeared to be strong, graded, predictors of mortality from cardiovascular causes, independent of one another, suggesting both variables may contain important information towards risk of adverse health outcomes. Though clearly, further studies are required to test this notion.

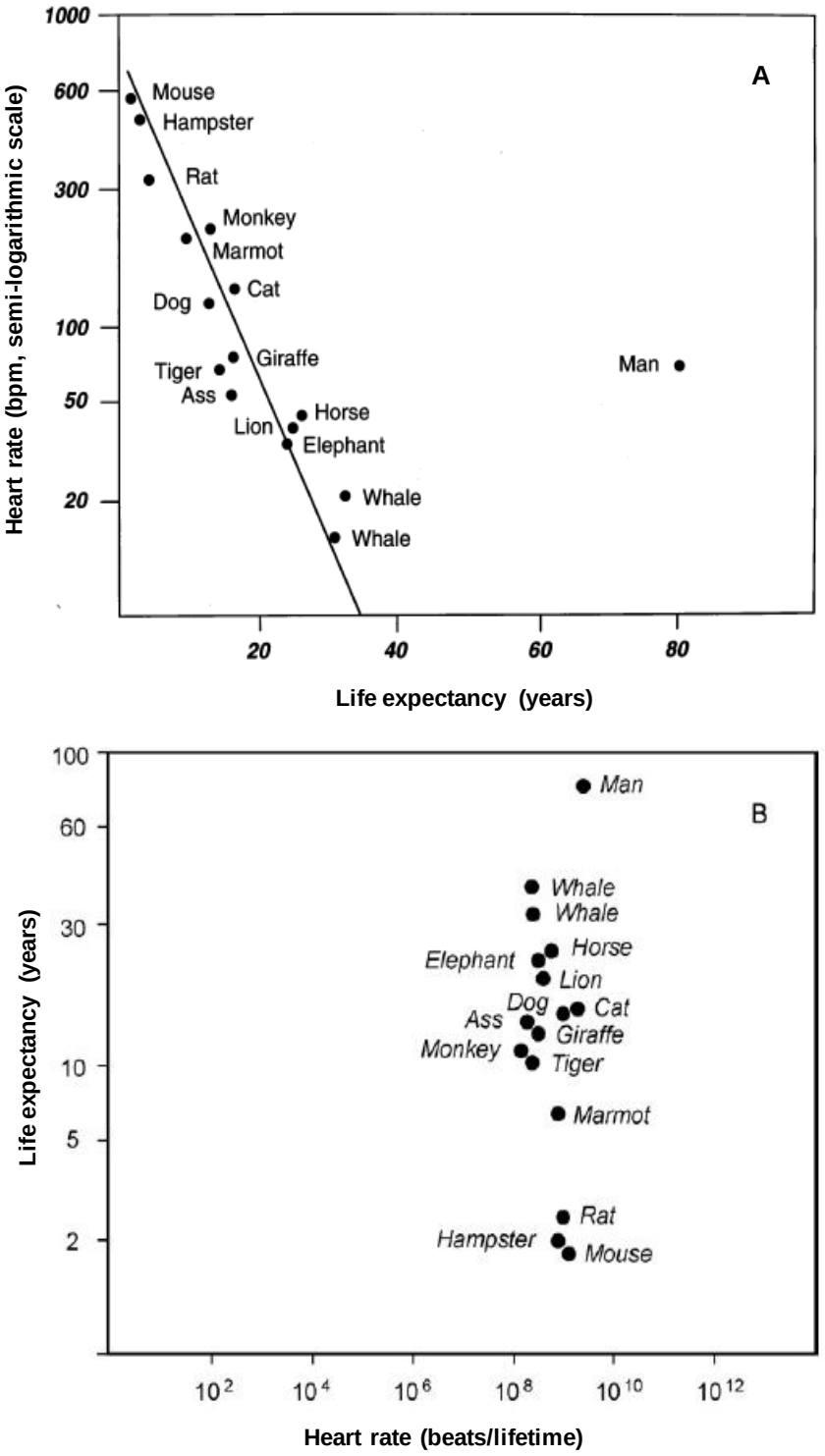

Figure 1.2. Relation between resting heart rate (beats/minute [A] and total beats/lifetime [B]) and life expectancy in 15 mammal species. Adapted from Levine [13], with permission.

1.5. ASSOCIATION OF RESTING HEART RATE WITH CARDIOVASCULAR RISK FACTORS

1.5.1. Obesity

Several large epidemiological studies have consistently shown that a number of cardio-metabolic risk factors are associated with increased morbidity; a relationship which may be explained, in part, by the deleterious effect of increased RHR. The predictive utility of RHR for the development of adverse glucose and lipid metabolism was assessed after 20 years follow-up in a general population, located in south-western Japan. Shigetoh and colleagues [24] reported that baseline RHR (≥80 bpm) independently predicted the development of obesity even after adjusting for a range of potential confounders. From the Hypertension and Ambulatory Recording Venetia Study (HARVEST) [25], both baseline clinic RHR and change in clinic RHR during follow-up were recorded among 701 persons screened for stage 1 hypertension who were subsequently followed for 7 years. In that study, both baseline clinic RHR (hazard ratio [HR], 95% confidence interval [95% CI] = 1.30, 1.10-1.50) and clinic RHR change during follow-up (HR, 95% CI = 1.17, 1.06-1.28) were found to be independent predictors of weight gain at study end.

1.5.2. Type 2 diabetes mellitus

In the Shanghai Women's Health Study [26], the relation between RHR and type 2 diabetes mellitus was examined. Around 47,571 Chinese women with no prior history of diabetes or other non-communicable diseases were included in this population-based cohort investigation. The incidence of diabetes was obtained via biennial in-person interviews [26]. During an average follow-up of 4.9 years, the incidence rates of diabetes per 1,000 person

years were 2.91, 3.31, 3.71, 4.16 and 5.34 according to RHR categories of ≤68, 69-72, 73-76, 77-80 and ≥80 bpm, respectively [26]. Shigetoh et al. [24] also demonstrated that a higher RHR may predispose to the development of type 2 diabetes mellitus. In fact, those with a RHR ≥80 bpm were considered to be over five-fold at greater odds of developing diabetes [24]. Likewise, in the San Antonio Heart Study [27], a population-based cohort of 3,301 Mexican Americans and 1,857 non-Hispanic whites, volunteers who were defined as hyperdynamic (i.e., RHR and pulse pressure in the upper-most quartile of their respective distributions) had an odds ratio (OR) of 3.97 for the prediction of future type 2 diabetes mellitus. In the Chicago Heart Association Detection Project in Industry [28], a 12 bpm increment in RHR was associated with a 10% increase in the odds of having diabetes, though following full adjustment, this relationship attenuated to non-significance. In the same study however, a raised RHR was a robust predictor (OR 1.21, 95% CI = 1.03-1.41) of diabetes mortality among adults aged between 35-49 years even after correcting for body mass index (BMI) and post-load glucose at baseline [28].

1.5.3. Hypertension

It has been shown that hypertensive patients, in general, tend to have a faster RHR compared to their normotensive counterparts [29, 30]. Data from the HARVEST study group indicated that baseline clinic RHR as well as altered RHR within the first 6 months of follow-up were important predictors for the development of sustained hypertension [31]. Indeed, the adjusted risk doubled (95% CI = 1.4-2.9) among patients whose RHR remained persistently elevated throughout the investigation [31]. Earlier studies have also noted the relation between sustained tachycardia and hypertension. Notably, findings from the four Chicago epidemiologic surveys [32] are of particular relevance. Across all four cohorts encompassing an overall sample size of greater than 35,000 men and women between the ages of 25-64 years, RHR emerged

as one of the strongest correlates of blood pressure [32]. Similar findings were reported in the Gubbio Study which consisted of 2,809 men and women from Italy. The findings in that investigation showed pulse rate to be significantly, albeit, modestly correlated with systolic (r = 0.20 [men], r = 0.19 [women]) and diastolic (r = 0.15 [men], r = 0.14 [women]) blood pressure following adjustment [33]. However, a number of prospective investigations have failed to find consistent results on the association between RHR and blood pressure [27, 34-37]. In this respect, some lost statistical significance after correcting for baseline blood pressure, while others had not considered adjusting for important confounders such as physical activity, or fitness. On the other hand, in a cohort of more than 8,000 Japanese men residing in Hawaii, bivariate and multivariate analyses of more than 50 variables revealed RHR was independently associated with changes in blood pressure [38]. In that study, multiple regression analyses of the first examination indicated that RHR was related cross-sectionally to levels of systolic (cumulative r^2 = 0.13) and diastolic (cumulative r^2 = 0.15) blood pressure, respectively [38]. Albeit, longitudinal changes in blood pressure between the first and third examinations found RHR to be associated with diastolic (cumulative r^2 = 0.28) blood pressure only [38]. In line with this data, the multi-centre longitudinal Coronary Artery Risk Development in Young Adults (CARDIA) study [39] found RHR to be an independent predictor of subsequent diastolic blood pressure regardless of initial blood pressure and other possible confounders among white and black men, as well as white women (0.7 mm Hg increase per 10 bpm increments). Thus, on the background of this information, it seems a raised RHR may be considered a risk factor for sustained elevated diastolic blood pressure [39].

1.5.4. The metabolic syndrome

Some epidemiological evidence supports the notion that elevated RHR is associated with several conventional risk factors known to amplify the CVD profile including obesity, diabetes, and hypertension [40]. To this end, we may speculate that a raised RHR is consequently related with the clustering of these risk factors, better known as the metabolic syndrome (MetS). A handful of studies have recently described the association between a faster RHR and the MetS. One such study was the Pressioni Arteriosi Monitorate E Loro Associazioni (PAMELA) project [41], a cross-sectional and longitudinal survey designed to examine variations in blood pressure at home, and in the office among more than 3,000 individuals representative of the general population living in Italy. Compared to healthy controls in that study, participants identified as having the MetS displayed significantly greater office, home, and 24-h ambulatory RHR values. Inuoe and colleagues [42] also examined the association of RHR and risk of developing the MetS in a large cohort of 6,281 Japanese individuals who participated in a health evaluation program. Over the 5 year follow-up period, men with a higher baseline RHR were more likely to experience the MetS. The OR for developing the MetS for men in the upper-most quartile for RHR was 1.73 (95% CI = 1.28-2.32) [42]. In another large sample consisting of 7,706 apparently healthy men and women who attended a general health screening program, a raised RHR was significantly associated with the presence of the MetS [43]. In that study, the adjusted OR increased progressively from 1.0 (reference figure) in the lowest RHR quintile (<60 bpm in men and <64 bpm in women) to 4.2 (95% CI = 3.0-5.9) and 3.6 (95% CI = 2.2-6.1), for men and women in the highest quintile (≥80 bpm in men and ≥82 bpm in women), respectively [43]. Moreover, in the Bypass Angioplasty Revascularization Investigation 2 Diabetes (BARI 2D) randomized clinical trial [44], regression analyses revealed that RHR was significantly higher in patients with the MetS compared to those without (regression coefficient; 2.9, P =0.002). From the same study, in

patients with type 2 diabetes or coronary artery disease (CAD), sustained elevated RHR was associated with an increasing number of components for the MetS, proposing that the risk of an elevated RHR for the development of the MetS extends beyond only those who seem apparently healthy.

1.5.5. Inflammation

Moving forward, inflammation is proposed to play an important role in the patho-physiological progression of atherosclerosis [45-49]. Yet, despite this bulk of evidence, we still lack an understanding with regards to the mechanisms responsible for modulating the inflammatory response during atheroprogression. With respect to RHR, only two investigations have shown an association with increased inflammation [50, 51]. In the Copenhagen Holter Study [50] the relation between RHR and HRV with subclinical inflammation was examined among 775 apparently healthy men and women. In that study, both RHR and HRV were significantly associated with increased inflammation. In the second, Rogowski et al. [51] explored the possibility that a raised RHR was related to an elevated microinflammatory response. In this investigation, concentrations of absolute leukocytes, C-reactive protein (CRP) as well as fibrinogen were analysed in a cohort of 4,553 subjects belonging to the Tel Aviv Medical Center Inflammation Survey (TAMCIS). In this relatively large survey, following adjustment, those in the fifth quintile of RHR (≥79 bpm) had significantly greater concentrations for each of the previously mentioned inflammatory markers compared to those in the first quintile (≤58 bpm); 4.74 and 4.23 x 10^9/L (leukocytes), 1.61 and 1.12 mg/L (CRP) and 8.11 versus 7.38 µmol/L, respectively [51]. Although the number of investigations evaluating the relationship between RHR and inflammation are few and far between, the findings from these two studies represent an attractive avenue for which

targeting RHR and inflammation via therapeutic manipulation may ultimately reduce the burden of CVD.

1.6. HIGH RESTING HEART RATE AS A PREDICTOR OF CARDIOVASCULAR MORTALITY

The integral relation between RHR and CVD has been reported as far back as the 1940s [52]. Since then, a number of studies have found an association between a raised RHR and CVD mortality including the Chicago People Gas Company Study [53], and the Framingham Heart Study [54]. These investigations are mentioned in passing because they are some of the earliest to document an association between rapid RHR and CVD death. Thereafter, it is not surprising that several other large epidemiological studies have shown sustained elevated RHR to be an important predictor of CVD mortality. These include the first National Health and Nutrition Examination Survey (NHANES 1) Epidemiological Follow-up Study (NNHEFS) [55], the Framingham Study [56], the British Regional Heart Study [57], the Secondary Prevention Reinfarction Israeli Nifedipine Trial (SPRINT) [58], the Cardiovascular Study in the Elderly [3], as well as a few other prospective cohort studies [59-61].

More recently, there has been renewed interest in the awareness of resting tachycardia and its implications towards CVD mortality. During an 8 year follow-up of 3,527 Israeli industrial employees, the adjusted relative risk (RR) for death due to CVD was 2.02 (95% CI = 1.1-4.0) among workers with a RHR >90 bpm compared to those with a RHR <70 bpm [62]. In the Malattie Cardiovascolari Aterosclerotiche, Istituto Superiore di Sanità (MATISS) Project, Seccareccia et al. [63] sought to verify the independent role of elevated RHR for predicting CVD mortality. Among 2,533 low-risk men, the adjusted HR for CVD mortality increased 1.63 (95% CI = 1.26-2.10) for each RHR category [63]. Likewise, in two separate

Japanese cohorts, RHR was found to be an important predictor of mortality. Over a 16.5 year follow-up period involving 8,800 subjects who participated in the National Survey on Circulatory Disorders [64], the RR in the upper-most quartile of RHR for CVD death in middle-aged men was 2.55 (95% CI = 1.22-5.31). Further, the role of RHR on CVD mortality did not materially change even after removing those who died within 5 years of study entry, thereby dismissing the hypothesis that RHR is simply an indicator of underlying chronic disease. Second, in the Ohasama Study, Hozawa and co-workers [65] examined the prognostic value of self-measured RHR at home for the risk of 10 year CVD mortality in 1,780 Japanese individuals. In that study, a 5 bpm increment in self-measured RHR during the morning was associated with a 17% increase in the risk of CVD death, and remained significant following adjustment for blood pressure. In the Paris Prospective Study I [66], a total of 5,713 asymptomatic middle-aged working men were followed prospectively for a total of 23 years. During this period, a RHR of more than 75 bpm was related to an increased risk (RR 3.92, 95% CI = 1.91-8.00) of sudden death due to myocardial infarction (MI). In the Coronary Artery Surgery Study (CASS), 24,913 patients with suspected or proven CAD were studied for a median follow-up duration of 14.7 years [67]. Patients whose RHR was ≥83 bpm at baseline presented with a greater risk (HR 1.31, 95% CI = 1.15-1.48) of CVD mortality, even after correcting for multiple clinical parameters. In line with this finding, the morBidity-mortality EvAlUaTion of the I_f inhibitor ivabradine in patients with coronary artery disease and left ventricULar dysfunction (BEAUTIFUL) Study [68] evaluated RHR as a prognostic risk factor of cardiovascular outcomes. After adjusting for baseline characteristics, those with a RHR ≥70 bpm had a greater risk of death due to CVD by around 34%. Accordingly, for every increase of 5 bpm in RHR, the risk of CVD mortality increased by 8% [68]. In the National FINRISK Study [69], the effect of RHR towards CVD mortality was determined among 10,519 men and 11,334 women. In men, each 15 bpm increase in RHR was associated

with an adjusted HR of 1.24 (95% CI = 1.11-1.40). For the women, a similar association was observed on the background of every 15 bpm increment in RHR (HR 1.32, 95% CI = 1.08-1.60). This relationship persisted even after removal of those with comorbidities and events occurring within the first 2 years of observation [69]. Two separate investigations from the Copenhagen City Heart Study (CCHS) examined the usefulness of RHR as a predictor of CVD mortality [70, 71]. In the first study, heavy, moderate, and former smokers had significantly greater risk (RR [95% CI] 1.13 [1.08-1.18], 1.15 [1.09-1.21] and 1.11 [1.05-1.17] respectively, per 10 bpm increase in RHR) of CVD death compared to never-smokers [70]. Second, Jensen and colleagues [71] assessed whether a raised RHR was a robust predictor of CVD mortality independent of inflammation. In that study, after adjusting for both CRP and fibrinogen, as well as other conventional risk factors, a 10 bpm increase in RHR was associated with increased (HR 1.14, 95% CI = 1.07-1.22) risk of CVD mortality.

Despite these observations, not all studies examining the relationship between RHR and CVD mortality achieved significance. In an earlier examination of the Paris Prospective Study I, adjusting for multiple confounders, Filipovsky et al. [72] failed to find an association between RHR and all-CVD mortality in middle-aged employees after 17 years of follow-up. In a large Finnish prospective study comprising 10,717 men and women between the ages of 30-59 years, elevated RHR was not shown to be related (RR 1.00, 95% CI = 0.79-1.26 in men with RHR ≥84 bpm and RR 1.05, 95% CI = 0.75-1.49 in women with RHR ≥94 bpm) to the risk of mortality from CVD [73]. In particular, the initial association between higher RHR and CVD risk was subsequently explained by high blood pressure [73]. In the Three-City (3C) Study [74], a French multi-centre investigation involving 7,147 community-dwelling older volunteers followed longitudinally for 6 years, RHR was significantly associated with overall and non-CVD mortality, though conversely, not with risk of death from CVD causes. In that study, however, it should be noted there was an 87% (95% CI = 0.98-3.6) increase in the risk

of CVD mortality in those with a RHR >79 bpm, while the non-significance observed was marginal (P = 0.06). More recently, the incremental prognostic value of RHR was assessed in a subgroup of 5,320 subjects from the Diabetes Cardiovascular Risk Evaluation Targets and Essential Data for Commitment of Treatment (DETECT) cohort study [75]. Here, it was concluded that a raised RHR provided minimal and non-significant prognostic value when added to a number of conventional risk factors as determined by the C-statistic (0.001, P =0.979). However, due to the known limitations of the C-statistic to discriminate between the additions of prognostic variables which usually result in small improvements, researchers and clinicians should continue to seek more appropriate methods for evaluating the prognostic utility of RHR in risk stratification.

Table 1.1. Studies of the association between resting heart rate and cardiovascular mortality

Reference	Study population	Country	Follow-up	Heart rate	Impact on cardiovascular mortality[¶]
Dyer et al. [53]	1,233 men aged 40-59 years	USA	15 years	RHR quintiles	Independent risk factor for sudden death due to CHD.
Kannel et al. [54]	5,070 subjects between 35-64 years free of CVD	USA	>30 years	Antecedent RHR measured biennially	CVD mortality rates increased progressively in relation to antecedent RHR in both sexes at all ages.
Gillum et al. [55]	14,407 individuals 25-74 years of age	USA	6-13 years	RHR >84 bpm	Risk of death from CVD was elevated in white men; incident CHD increased in white women, risk of death from CVD was also increased in black men and women.
Filipovsky et al. [72]	4,907 working men between the ages of 42-53 years	France	17 years	Baseline RHR	NS.
Gillman et al. [56]	4,530 hypertensive subjects aged 35-74	USA	36 years	40 bpm increments in RHR	1.68 times higher risk of CVD mortality in men and 1.70 for women.
Shaper et al. [57]	7,735 men aged 40-59 years without pre-existing IHD	UK	8 years	RHR ≥90 bpm versus <60 bpm	3.3 times greater risk for IHD mortality and 5.2 times increased risk of sudden cardiac death.
Disegni et al. [58]	1,044 patients (aged 50-79) hospitalized for acute MI	Israel	1 year	RHR ≥90 bpm on admission	Independent predictor of in-hospital and 1 year post-discharge mortality.
Aronow et al. [59]	1,311 men and women (60-100 years) with known heart disease and sinus rhythm	USA	4 years	Increment of 5 bpm measured from 24 h ambulatory electrocardiograms	1.14 higher chance of developing a new coronary event.
Palatini et al. [3]	1,938 white men and women 65 years or older	Italy	12 years	RHR quintiles (>80 bpm versus <64 bpm)	1.38 times greater risk of CVD death for elderly men in the top quintile of RHR.
Benetos et al. [60]	19,386 subjects aged 40-69 years	France	21 years	RHR categories (<60, 60-80, 81-100, and >100 bpm)	Relative risk of 2.18 for men in the highest RHR category according to CVD mortality.

Table 1.1. *Continued*

Reference	Study population	Country	Follow-up	Heart rate	Impact on cardiovascular mortality[¶]
Greenland et al. [61]	33,781 black and white men and women aged between 18-74 years	USA	22 years	Increment of 12 bpm in RHR	RHR independently predicted risk of fatal coronary disease as well as all-CVD mortality in younger men and middle-aged men and women.
Reunanen et al. [73]	10,717 men and women 30-59 years of age	Finland	23 years	RHR deciles	NS.
Kristal-Boneh et al. [62]	3,527 male employees mean age 43 years at study entry	Israel	8 years	RHR >90 bpm versus <70 bpm	Relative risk 2.02 for death due to CVD among workers with a RHR >90 bpm.
Seccareccia et al. [63]	2,533 men aged 40-69 years	Italy	4-13 years	Five RHR categories (<60, 60-69, 70-79, 80-89, and ≥90 bpm)	1.63 increased risk of CVD mortality per increment in RHR category.
Okamura et al. [64]	8,800 men and women ≥30 years	Japan	16.5 years	RHR quartiles	2.55 greater risk of CVD death for middle-aged men in the highest quartile.
Hozawa et al. [65]	1,780 individuals ≥40 years of age	Japan	10 years	Increment of 5 bpm in RHR	17% increase in the risk of CVD mortality.
Jouven et al. [66]	5,713 working men (between the ages of 42 and 53 years)	France	23 years	RHR >75 bpm	3.92 increase in relative risk of sudden death from MI.
Diaz et al. [67]	24,913 patients, mean age 53.2 years with suspected or proven CAD	Canada	14.7 years	RHR ≥83 bpm	1.31 increased risk for CVD mortality.
Fox et al. [68]	5,438 men and women aged 55 years or older with stable CAD and left ventricular dysfunction	International multi-centre trial	2 years	RHR >70 bpm versus <70 bpm and increments of 5 bpm	34% greater risk of CVD death for RHR >70 bpm and 8% increase in risk per 5 bpm increments.

Table 1.1. *Continued*

Reference	Study population	Country	Follow-up	Heart rate	Impact on cardiovascular mortality[¶]
Cooney et al. [69]	21,853 men and women, mean age of 43.2 years	Finland	6-27 years	RHR as 15 bpm increments	1.24 and 1.32 greater risk of CVD mortality in men and women, respectively.
Legeai et al. [74]	7,147 men and women ≥65 years	France	6 years	RHR quintiles	NS.
Jensen et al. [70]	43,293 men and women aged 20 years or older at study entry	Denmark	33 years	10 bpm increments in RHR	1.13, 1.15, and 1.11 increased risk of CVD mortality for heavy, moderate, and former smokers, respectively.
Leistner et al. [75]	5,320 men and women, mean age 55.9 years free of CAD at study entry	Germany	5 years	RHR quartiles	NS.
Jensen et al. [71]	6,518 men and women aged 20 years or older at baseline	Denmark	18 years	10 bpm increments in RHR	1.14 times greater risk of CVD mortality

[¶]Data reported from fully adjusted analyses.
RHR = resting heart rate, CHD = coronary heart disease, CVD = cardiovascular disease, bpm = beats per minute, NS = non-significant, IHD = ischaemic heart disease, MI = myocardial infarction, CAD = coronary artery disease.

1.7. SUMMARY AND AIMS OF THESIS

In light of the preceding evidence, however, RHR as an easily measured and inexpensive parameter, as well as potential risk factor for CVD appears to be overlooked in the clinical setting. Moreover, information regarding the association between RHR and adverse CVD prognosis beyond western populations or apparently healthy individuals is still lacking. Thus, the following studies aimed to explore the association between sustained elevated RHR with CVD morbidity and mortality. Specifically, we attempted to examine these associations via several large epidemiological cohorts including the Guangzhou Biobank Cohort Study (GBCS), consisting of an older Asian population; the European Prospective Investigation into Cancer and Nutrition (EPIC) Norfolk Study, where the relation between RHR and mortality was assessed in apparently healthy middle-aged British men and women; the LUdwigshafen RIsk and Cardiovascular Health (LURIC) Study, which included a population of German patients already at-risk for CVD.

The principle objectives of the current thesis therefore were to:

1) Evaluate the association of a raised RHR with a number of cardio-metabolic risk factors in older Chinese participants.

2) Assess whether a high RHR independently increases the risk of death due to CVD among generally healthy men and women, and separately, in those who are already at intermediate-to-high-risk for CVD.

3) Explore whether elevated RHR appears to increase the risk of CVD by amplifying the inflammatory process.

CHAPTER TWO

2.0. INDEPENDENT AND COMBINED ASSOCIATIONS OF ABDOMINAL OBESITY AND SEATED RESTING HEART RATE WITH TYPE 2 DIABETES MELLITUS AMONG OLDER CHINESE: THE GUANGZHOU BIOBANK COHORT STUDY

2.1. ABSTRACT

Central obesity and poor physical fitness predict the development of type 2 diabetes mellitus and cardiovascular mortality among Caucasian populations. We studied the independent and combined impact of abdominal obesity and seated resting heart rate used as an indicator of physical fitness, on the presence of type 2 diabetes mellitus among 30,519 older residents of Guangzhou, Southern China. Participants were stratified into four groups, based on the Asian criteria for abdominal obesity (≥90/≥80 cm in men/women) and the 75% cut-off point for seated resting heart rate (≥83 bpm). The association with type 2 diabetes mellitus was assessed using multivariable logistic regression. A total of 3,777 (12.7%) volunteers were diagnosed with type 2 diabetes mellitus, which was independently associated with seated resting heart rate and in particular, increasing levels of abdominal obesity ($P < 0.001$). An odds ratio of 3.93 (95% CI = 3.48, 4.43) was identified for type 2 diabetes mellitus in participants who were obese with a seated resting heart rate ≥83 bpm after adjusting for potential confounders. Higher seated resting heart rate, a marker of poor physical fitness, independently doubles the risk of type 2 diabetes mellitus. The strength of this association is further increased when abdominal obesity is considered.

2.2. INTRODUCTION

Studies have identified diabetes as a major determinant of all-cause and cardiovascular mortality [3]. Substantial evidence supports the notion that too much abdominal fat promotes the development of diabetes by increasing insulin resistance and hyperglycaemia [76-78]. In addition, abdominal adiposity appears to amplify the impact of other cardiovascular risk factors, including high blood pressure and dyslipidaemia [79]. According to the National Cholesterol Education Programme-Adult Treatment Panel III (NCEP-ATP III) [80] abdominal obesity, as measured by waist circumference, and not general obesity, determined by BMI, is recognised as the most prevalent form within the constellation of risk factors that constitute the metabolic syndrome.

Abdominal obesity is generally considered to result from caloric imbalance and reduced physical activity, which in the Global Burden of Disease study was among the top ten risk factors for health [81]. Although physical activity may ameliorate physical fitness, the strength of association between both is limited, and is likely the result of several factors including difficulties in quantifying activity, or low-intensity exercise being ineffective toward improving fitness [82, 83]. However, physical fitness has previously been identified as an important predictor of cardiovascular and non-cardiovascular mortality [17, 84]. This effect was independent of physical activity in the few studies that have addressed the association between cardio-respiratory fitness and mortality [17, 85].

Although some studies have examined the impact of abdominal obesity and physical fitness with poor health outcomes, certain aspects of this literature remain less developed. For instance, abdominal adiposity, in particular visceral abdominal obesity, is age- and gender- as well as ethnicity-dependent. While some health organisations have developed ethnicity-specific cut-points for abdominal obesity [86], data that form the basis of current guidelines according to waist circumference are limited for non-Caucasian populations. Likewise, large-

scale studies examining physical fitness either directly or as a surrogate (e.g. RHR) is scarce and predominantly derived from Caucasian populations. As developing nations such as China begin to increase their rate of modernisation similar to post-industrial societies, the prevalence of associated poor health conditions, including type 2 diabetes mellitus and the MetS, are likely to become more apparent, suggesting a large proportion of its 1.2 billion population may be at risk for adverse health consequences due to these conditions. Data highlighting such associations can be used as the foundation of health promotion initiatives that may offset this burden.

Given the impact of abdominal obesity and physical fitness on long-term health and the dearth of this information in non-Caucasian populations outside the developed world, the current study aimed to examine the independent and combined associations of abdominal obesity and seated RHR, as a measure of physical fitness [24, 87], on the presence of diabetes among 30,519 older residents of China.

2.3. METHODS

2.3.1. Subjects

The GBCS is a prospective population-based study aiming to examine determinants of health in an older Chinese population, and has been described previously [88]. Briefly, participants were drawn from the membership of a city-wide community social and welfare association in Guangzhou city, 'the Guangzhou Health and Happiness Association for Respectable Elders' (GHHARE). Baseline examinations were performed between 2003-2006, which included a detailed structured interview on lifestyle habits and medical history, as well as measurements of anthropometric, metabolic and biological indices. The Medical Ethics Committee of the

Guangzhou Medical Association approved the study, and written, informed consent was obtained from all participants.

2.3.2. Demographic and lifestyle data

Demographic data (e.g., age and gender), details of health and lifestyle (e.g., smoking, alcohol consumption and physical activity) and socio-economic variables (e.g. longest held occupation, education level and personal income) were obtained by qualified staff belonging to the Guangzhou study team. Participants were defined as current smokers if they answered yes to the question: "Do you currently smoke?" Also, participants were defined as current drinkers by answering yes to the question: "Have you consumed any alcohol in the past 12 months?" Level of physical activity was quantified using the short version of the International Physical Activity Questionnaire (IPAQ), validated in the Chinese population [89]. The formula for the computation of metabolic equivalent (MET)-minutes/week was; MET level x minutes of physical activity x events per week. In the current study, MET-minutes/week was presented as a continuous trait. For the socio-economic measures, participants were categorized based on manual (indicative of a "blue collar" working environment) or non-manual employment according to longest held occupation. Education level consisted of three categories; attended primary school, secondary school or college. Personal income was defined as net monthly income (in Yuan) according to the following categories; <10,000, ≥10,000-<15,000 and ≥15,000.

2.3.3. Experimental procedures

Waist circumference was measured in cm, horizontally around the smallest circumference between the ribs and iliac crest, or at the navel if no natural waistline was observed. Using

Asian criteria, abdominal obesity was present if waist circumference was ≥90 cm in men or ≥80 cm in women [90]. Seated RHR was measured three times (Omron 705CP); one minute apart, following a three minute rest. Subjects were classified as having a high seated RHR if their rate was ≥83 bpm, determined by the 75th percentile cut point. Blood samples were drawn to measure fasting plasma glucose, which was determined by nurses belonging to the Guangzhou study team. Diabetes was defined within each group if the fasting glucose level was ≥7.0 mmol/L or if the participant was receiving hypoglycaemic medication and impaired fasting glucose was identified if fasting glucose levels were ≥5.6 and <7.0 mmol/L or receiving hypoglycaemic medication. All pre- and diabetic states were based solely in accordance with the International Diabetes Federation criteria [91].

2.3.4. Statistical methods

Participants were stratified into four groups based on the presence of abdominal obesity and the 75% cut-off point for seated RHR; 1) non-obese/low RHR; 2) non-obese/high RHR; 3) obese/low RHR and; 4) obese/high RHR. Overall and gender comparisons between groups were performed by analysis of variance (ANOVA), with a Bonferroni post-hoc test for continuous parameters and χ^2 test with *P* for linear-by-linear test for categorical variables. MET-minutes/week, glucose and triglyceride levels were non-normally distributed and therefore logarithmically transformed with their geometric means and 95% CIs presented. Otherwise data are shown as Mean±SD for continuous parameters or prevalence for categorical variables.

Multivariable logistic regression was built to assess the independent and combined effects of abdominal obesity and seated RHR in determining the presence of diabetes. The first model adjusted for age and sex. The second model additionally adjusted for lifestyle factors including; smoking, alcohol, physical activity, socio-economic measures including;

longest held occupation, education level and personal income, and finally, chronic diseases such as; arterial hypertension, cardiovascular disease history and dyslipidaemia.

Additionally, we restricted the analyses to those who reported good health as a means of addressing potential reverse causality. We also excluded volunteers with potential sinus bradycardia (<50 bpm) and those on medication for blood pressure and heart disease, given that such medication including β-blockers and calcium channel blockers artificially reduce pulse rate. All data analyses were performed using SPSS 18.0 statistical package (SPSS Inc., Chicago, IL).

2.4. RESULTS

Of the 30,519 men and women who volunteered for the study, 684 (2.2%) were excluded due to missing data on a number of the variables examined, leaving a total of 29,835 (97.8%) available for analysis. Clinical attributes of the subjects are presented in Tables 2.1 to 2.3.

Table 2.1. Baseline characteristics overall

	Non-obese/low heart rate	Non-obese/high heart rate	Obese/low heart rate	Obese/high heart rate	*P* value[#]
Number of participants (%)	14,791 (49.6)	4,748 (15.9)	7,363 (24.7)	2,933 (9.8)	
Age (years)	62.2±6.7	62.2±6.7	63.4±6.4[*†]	63.7±6.5[*†]	< 0.001
Heart rate (beats/minute)	70±7	91±7[*]	71±7[†]	91±7[*§]	< 0.001
Waist circumference (cm)	74.6±6.5	74.4±6.7	87.2±5.9[*†]	87.8±6.4[*†]	< 0.001
Body mass index (kg/m^2)	22.2±2.5	22.0±2.5	26.4±2.6[*†]	26.6±2.8[*†]	< 0.001
Current smoker (%)	14.8	12.2	7.2	7.1	< 0.001
Current drinker (%)	33.4	29.4	25.2	23.5	< 0.001
MET-score (minutes/week)	2,550 (2,492-2,608)	2,312[*] (2,224-2,403)	2,627[†] (2,545-2,712)	2,336[*§] (2,216-2,463)	< 0.001
Manual occupation (%)	57.9	59.4	66.2	67.7	< 0.001
Education (%)					
Primary	36.5	39.0	53.6	54.6	< 0.001
Secondary	53.3	51.7	39.3	39.1	
University	10.2	9.4	7.1	6.3	
Personal income - Yuan (%)					
<10,000	6.7	6.7	9.1	9.6	< 0.001
≥10,000-<15,000	40.9	41.1	45.1	45.6	
≥15,000	52.4	52.3	45.8	44.9	
Fasting glucose (mmol/L)	5.35 (5.33, 5.37)	5.65 (5.60, 5.70)[*]	5.79 (5.75, 5.82)[*†]	6.25 (6.17, 6.33)[*†§]	< 0.001
Impaired fasting glucose (%)	22.3	25.9	33.0	33.1	< 0.001
Type 2 diabetes mellitus (%)	8.4	14.8	16.6	26.8	< 0.001
IFG/Type 2 diabetes (%)	30.7	40.7	49.6	59.9	< 0.001
Systolic blood pressure (mm/Hg)	127±22	131±21[*]	135±22[*†]	139±22[*†§]	< 0.001
Diastolic blood pressure (mm/Hg)	72±11	75±11[*]	75±11[*]	78±11[*†§]	< 0.001
Arterial hypertension (%)	34.3	40.6	52.7	60.1	< 0.001
Cardiovascular disease history (%)	35.8	37.8	50.3	53.4	< 0.001
Triglyceride (mmol/L)	1.23 (1.22, 1.24)	1.36 (1.34, 1.39)[*]	1.60 (1.57, 1.62)[*†]	1.79 (1.75, 1.84)[*†§]	< 0.001
Total cholesterol (mmol/L)	5.85±1.13	6.02±1.19[*]	5.94±1.14[*†]	6.10±1.21[*§]	< 0.001
LDL-cholesterol (mmol/L)	3.13±0.68	3.25±0.71[*]	3.16±0.68[†]	3.27±0.72[*§]	< 0.001
HDL-cholesterol (mmol/L)	1.68±0.41	1.69±0.42	1.63±0.37[*†]	1.63±0.39[*†]	< 0.001
Dyslipidaemia (%)	46.1	52.7	56.1	65.0	< 0.001

Data are presented as Mean±SD, Geometric mean (95% confidence interval) or percentage values. [#]*P* value was obtained by ANOVA or χ^2 test. [*]*P* < 0.05 when compared to Non obese/Low heart rate group; [†]*P* < 0.05 when compared to Non obese/high heart rate group; [§]*P* < 0.05 when compared to obese/low heart rate group. Abbreviations: MET = metabolic equivalent, IFG = impaired fasting glucose, LDL = low density lipoprotein, HDL = High density lipoprotein.

Overall, those with a low RHR undertook the most physical activity according to total MET-minutes/week (Tables 2.1 to 2.3). Of the total population, the highest proportion of current smokers and drinkers tended to be non-obese with a low RHR (Table 2.1). These observations were similar in the gender-specific analyses (Tables 2.2 and 2.3). Subjects who were obese with a high RHR were more likely to be manual employees who had received less education and were earning a lower income (Table 2.1). In addition, female subjects who were obese with a high RHR also reported earning a lower income, probably as a result of being less educated and having worked more years in manual occupation (Table 2.3).

A total of 3,777 (12.7%) subjects were diagnosed as having type 2 diabetes mellitus, 7,850 (26.3%) as having impaired fasting glucose and 11,627 (39.0%) had either of these hyperglycaemic states. The prevalence of type 2 diabetes mellitus increased across the abdominal obesity/RHR groups. The obese/high RHR group had the greatest prevalence of type 2 diabetes mellitus (26.8% overall, 38.8% for males and 36.9% for females). Similarly, this group also contained more subjects with impaired fasting glucose (33.1% overall, 46.7 for males and 42.7% for females) and the combined hyperglycaemic state (59.9% overall, 60.2% for males and 57.1% for females [Tables 2.1 to 2.3]).

Of the metabolic parameters, between-group differences were observed across the overall and gender stratified abdominal obesity/RHR groups for blood pressure (systolic and diastolic), lipids (total, high-density lipoprotein [HDL]- and low-density lipoprotein [LDL]-cholesterol and triglyceride levels) and glucose levels ($P < 0.001$). That is, the obese/high RHR group had the highest values for each of these parameters, although HDL-cholesterol was inversely associated.

Table 2.2. Baseline characteristics according to men

	Non-obese/low heart rate	Non-obese/high heart rate	Obese/low heart rate	Obese/high heart rate	*P* value[#]
Number of participants (%)	5,080 (60.2)	1,591 (18.8)	1,074 (12.7)	495 (5.9%)	
Age (years)	63.8±6.7	63.4±6.8	64.6±6.8[*†]	64.3±6.7[†]	< 0.001
Heart rate (beats/minute)	70±7	91±7[*]	70±7[†]	92±7[*§]	< 0.001
Waist circumference (cm)	78.6±6.9	79.0±7.0	94.3±4.3[*†]	95.2±5.3[*†]	< 0.001
Body mass index (kg/m^2)	22.6±2.6	22.6±2.7	27.3±2.4[*†]	27.4±2.4[*†]	< 0.001
Current smoker (%)	37.9	33.3	28.8	27.9	< 0.001
Current drinker (%)	46.2	43.9	42.6	42.2	0.008
MET-score (minutes/week)	2,668 (2,609-2,729)	2,355[*] (2,258-2,455)	2,664[†] (2,539-2,796)	2,477 (2,308-2,658)	< 0.001
Manual occupation (%)	51.4	53.4	53.4	51.3	0.40
Education (%)					
Primary	30.1	29.6	31.6	28.9	0.52
Secondary	53.4	52.6	50.7	53.3	
University	16.5	17.8	17.8	17.8	
Personal income - Yuan (%)					
<10,000	6.6	5.4	4.9	4.4	0.40
≥10,000-<15,000	42.4	42.0	47.7	41.3	
≥15,000	51.0	52.6	47.3	54.3	
Fasting glucose (mmol/L)	5.43 (5.41, 5.47)	5.74 (5.67, 5.81)[*]	5.83 (5.77, 5.90)[*]	6.21 (6.07, 6.36)[*†§]	< 0.001
Impaired fasting glucose (%)	27.1	33.0	42.9	46.7	< 0.001
Type 2 diabetes mellitus (%)	11.8	21.8	23.4	38.8	< 0.001
IFG/Type 2 diabetes (%)	33.6	43.5	51.4	60.2	< 0.001
Systolic blood pressure (mm/Hg)	130±21	134±22[*]	139±21[*†]	143±21[*†]	< 0.001
Diastolic blood pressure (mm/Hg)	75±11	77±12[*]	80±11[*†]	82±12[*†§]	< 0.001
Arterial hypertension (%)	39.2	46.4	62.0	68.1	< 0.001
Cardiovascular disease history (%)	36.4	37.1	54.1	58.8	< 0.001
Triglyceride (mmol/L)	1.77 (1.72, 1.84)	2.14 (2.02, 2.29)[*]	3,42 (3.18, 3.69)[*†]	4.59 (4.10, 5.12)[*†§]	< 0.001
Total cholesterol (mmol/L)	5.54±1.05	5.68±1.11[*]	5.59±1.03	5.78±1.09[*§]	< 0.001
LDL-cholesterol (mmol/L)	3.03±0.64	3.12±0.67[*]	3.07±0.66	3.17±0.67[*§]	< 0.001
HDL-cholesterol (mmol/L)	1.54±0.38	1.52±0.39	1.42±0.34[*†]	1.41±0.34[*†]	< 0.001
Dyslipidaemia (%)	38.1	46.9	52.3	66.8	< 0.001

Data are presented as Mean±SD, Geometric mean (95% confidence interval) or percentage values. [#]*P* value was obtained by ANOVA or χ^2 test. [*]*P* < 0.05 compared to Non obese/Low heart rate group; [†]*P* < 0.05 compared to Non obese/high heart rate group; [§]*P* < 0.05 compared to obese/low heart rate group. Abbreviations as in Table 2.1.

Table 2.3. Baseline characteristics according to women

	Non-obese/low heart rate	Non-obese/high heart rate	Obese/low heart rate	Obese/high heart rate	*P* value[#]
Number of participants (%)	9,711 (44.0)	3,157 (14.3)	6,289 (28.5)	2,438 (11.0)	
Age (years)	59.6±6.9	59.9±7.1	62.0±6.9[*†]	62.3±7.0[*†]	< 0.001
Heart rate (beats/minute)	71±6	91±7[*]	71.0±7[†]	91±8[*§]	< 0.001
Waist circumference (cm)	72.0±5.1	71.8±5.2	86.0±5.4[*†]	86.0±5.5[*†]	< 0.001
Body mass index (kg/m^2)	22.1±2.4	22.0±2.5	26.4±2.7[*†]	26.5±2.5[*†]	< 0.001
Current smoker (%)	2.7	1.6	3.5	2.9	0.09
Current drinker (%)	26.3	21.7	22.1	19.6	< 0.001
MET-score (minutes/week)	2,934 (2,887-2984)	2,641[*] (2,565-2,720)	2,999[†] (2,942-3,059)	2,734[*§] (2,647-2,824)	< 0.001
Manual occupation (%)	61.4	62.4	68.3	71.0	< 0.001
Education (%)					
Primary	39.9	43.7	57.4	59.8	< 0.001
Secondary	53.3	51.2	37.3	36.2	
University	6.9	5.1	5.3	4.0	
Personal income - Yuan (%)					
<10,000	6.8	7.3	9.9	10.7	< 0.001
≥10,000-<15,000	40.0	40.6	44.6	46.5	
≥15,000	53.1	52.1	45.5	42.8	
Fasting glucose (mmol/L)	5.32 (5.30, 5.34)	5.66 (5.62, 5.71)[*]	5.76 (5.73, 5.79)[*†]	6.19 (6.13, 6.26)[*†§]	< 0.001
Impaired fasting glucose (%)	21.1	29.7	37.2	42.7	< 0.001
Type 2 diabetes mellitus (%)	8.9	18.1	22.5	36.9	< 0.001
IFG/Type 2 diabetes (%)	26.8	39.2	46.9	57.1	< 0.001
Systolic blood pressure (mm/Hg)	125±21	128±21[*]	134±22[*†]	138±22[*†§]	< 0.001
Diastolic blood pressure (mm/Hg)	70±10	73±11[*]	74±11[*†]	77±11[*†§]	< 0.001
Arterial hypertension (%)	31.8	37.6	51.1	58.5	< 0.001
Cardiovascular disease history (%)	35.5	38.1	49.7	52.3	< 0.001
Triglyceride (mmol/L)	1.75 (1.71, 1.79)	2.25 (2.16, 2.35)[*]	3.09 (3.01, 3.18)[*†]	3.91 (3.73, 4.11)[*†§]	< 0.001
Total cholesterol (mmol/L)	6.03±1.12	6.10±1.15[*]	6.01±1.13[†]	6.12±1.18[*§]	< 0.001
LDL-cholesterol (mmol/L)	3.32±0.70	3.37±0.73[*]	3.30±0.71[†]	3.39±0.73[*§]	< 0.001
HDL-cholesterol (mmol/L)	1.76±0.41	1.76±0.42	1.63±0.35[*†]	1.64±0.38[*†]	< 0.001
Dyslipidaemia (%)	50.2	55.6	56.8	64.7	< 0.001

Data are presented as Mean±SD, Geometric mean (95% confidence interval) or percentage values. [#]*P* value was obtained by ANOVA or χ^2 test. [*]*P* < 0.05 compared to Non obese/Low heart rate group; [†]*P* < 0.05 compared to Non obese/high heart rate group; [§]*P* < 0.05 compared to obese/low heart rate group. Abbreviations as in Table 2.1.

In multivariable analyses, an increased OR for type 2 diabetes mellitus was independently associated with the fourth (vs. first) quartile for abdominal obesity (OR [95% CI] = 3.51 [3.10, 3.98], $P < 0.001$) and RHR (OR [95% CI] = 2.59 [2.33, 2.90], $P < 0.001$), hereby indicating both contribute independently towards the prevalence of type 2 diabetes mellitus (Figure 2.1). Moreover, according to the obesity/RHR groups, the risk for type 2 diabetes mellitus doubled in those who were obese with a low RHR (OR [95% CI] = 2.13 [1.93, 2.35], $P < 0.001$) and in those who were non-obese with a high RHR (OR [95% CI] = 2.05 [1.83, 2.29], $P < 0.001$), highlighting the importance of both (Table 2.4). The OR for type 2 diabetes mellitus further increased peaking in those who were obese with a high RHR for overall (OR [95% CI] = 3.93 [3.48, 4.43], $P < 0.001$), male (OR [95% CI] = 2.73 [2.09, 3.57], $P < 0.001$) and female participants (OR [95% CI] = 4.31 [3.75, 4.94], $P < 0.001$), which remained robust after full adjustment (Table 2.4). Similar trends were present for impaired fasting glucose and the combined hyperglycaemic state with the adjusted risk increasing by 125% and 173% for overall, 85% and 112% for male and 136% and 189% for female subjects, respectively (Table 2.4).

Table 2.4. Odds ratios (with 95% confidence intervals) for the presence of impaired fasting glucose, type 2 diabetes mellitus and both combined among 29,835 older Chinese adults according to abdominal obesity and physical fitness parameters

	Odds ratios (95% confidence intervals)					
	Overall		Men		Women	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
	Impaired fasting glucose					
Non-obese/low heart rate	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Non-obese/high heart rate	1.48 (1.37, 1.61)	1.44 (1.33, 1.56)	1.32 (1.15, 1.51)	1.25 (1.09, 1.44)	1.58 (1.43, 1.74)	1.54 (1.39, 1.70)
Obese/low heart rate	2.04 (1.91, 2.19)	1.89 (1.76, 2.03)	1.99 (1.72, 2.31)	1.75 (1.51, 2.04)	2.07 (1.92, 2.24)	1.94 (1.79, 2.10)
Obese/high heart rate	2.52 (2.29, 2.90)	2.25 (2.04, 2.48)	2.21 (1.78, 2.75)	1.86 (1.49, 2.33)	2.61 (2.34, 2.91)	2.36 (2.11, 2.64)
P for trend	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Type 2 diabetes mellitus					
Non-obese/low heart rate	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Non-obese/high heart rate	2.24 (2.02, 2.50)	2.05 (1.83, 2.29)	2.14 (1.80, 2.56)	1.88 (1.56, 2.26)	2.30 (2.01, 2.63)	2.14 (1.86, 2.46)
Obese/low heart rate	2.59 (2.36, 2.85)	2.13 (1.93, 2.35)	2.28 (1.81, 2.74)	1.61 (1.29, 2.00)	2.70 (2.42, 3.02)	2.29 (2.04, 2.56)
Obese/high heart rate	5.27 (4.70, 5.91)	3.93 (3.48, 4.43)	4.47 (3.47, 5.74)	2.73 (2.09, 3.57)	5.52 (4.84, 6.29)	4.31 (3.75, 4.94)
P for trend	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Impaired fasting glucose/Type 2 diabetes mellitus combined					
Non-obese/low heart rate	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Non-obese/high heart rate	1.68 (1.57, 1.81)	1.60 (1.49, 1.72)	1.54 (1.36, 1.73)	1.42 (1.25, 1.60)	1.76 (1.62, 1.93)	1.69 (1.55, 1.85)
Obese/low heart rate	2.20 (2.07, 2.33)	1.96 (1.84, 2.10)	2.06 (1.80, 2.36)	1.72 (1.49, 1.98)	2.24 (2.09, 2.40)	2.03 (1.89, 2.18)
Obese/high heart rate	3.26 (3.00, 3.55)	2.73 (2.50, 2.98)	2.81 (2.31, 3.41)	2.12 (1.74, 2.58)	3.39 (3.08, 3.73)	2.89 (2.63, 3.20)
P for trend	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Model 1 adjusted for age and sex. Model 2 also adjusted for smoking, drinking, physical activity, longest held occupation, education, personal income, arterial hypertension, cardiovascular disease history and dyslipidaemia.

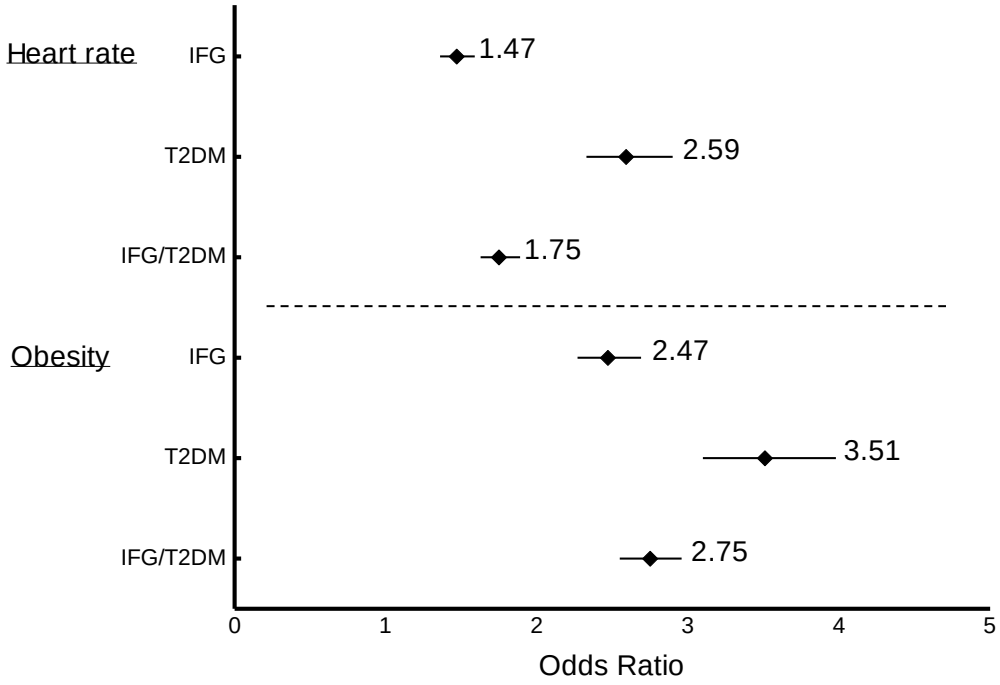

Figure 2.1. Odds Ratios (fourth vs. first quartile) for the independent associations of abdominal obesity and heart rate with impaired fasting glucose (IFG), type 2 diabetes mellitus (T2DM) and the combined hyperglycaemic state. Values are derived from multivariable analysis which adjusted for age, sex, smoking, drinking, physical activity, longest held occupation, education, personal income, arterial hypertension, cardiovascular disease history and dyslipidaemia.

In light of these findings, we tentatively explored whether the risk of type 2 diabetes mellitus in the presence of a high RHR differed among the non-obese and obese participants. A raised RHR appeared to double the risk of diabetes among the non-obese (Table 2.4). Likewise, in the obese subjects, the presence of a high RHR also almost doubled the risk for diabetes among the obese (OR [95% CI] = 1.89 [1.66, 2.12], $P < 0.001$).

We also addressed the potential issue of reverse causality by restricting to subjects who reported good or very good health (n = 24,346), and therefore were less likely to have poor physical fitness as a consequence of underlying chronic conditions. We found the risk of type 2 diabetes mellitus increased slightly from 3.93 (95% CI = 3.48, 4.43) to 4.10 (95% CI = 3.57, 4.71), which remained robust even after adjusting for several possible confounders

(fourth vs. first quartile). Further exclusion of those with potential sinus bradycardia (*n* = 115) or those receiving medication for blood pressure or heart disease (*n* = 815) did not affect these observations (data not shown). We additionally explored the data using the median heart rate of 75 bpm. The outcome risk of diabetes was similar whether we used the median (75 bpm) or 75% cut-off point (83 bpm) to stratify groups based on heart rate.

2.5. DISCUSSION

Our findings indicate, poor physical fitness determined by a raised RHR [24, 87] and abdominal obesity independently doubled the risk of type 2 diabetes mellitus. In addition, we observed those with a combination of both conditions may require more aggressive treatment, as the risk of diabetes was found to be two-fold higher. For instance, the strength of the association with type 2 diabetes mellitus was additionally increased on the background of abdominal obesity and a raised RHR by almost 300%, even after adjusting for potential confounding factors. Collectively, these data confirm earlier studies in Caucasians, showing that obese and physically less fit individuals are at a greater risk of metabolic complications such as type 2 diabetes mellitus [84].

We found a raised RHR may exacerbate the development of diabetes in addition to abdominal obesity, possibly contributing to the increased risk of mortality among those subjects [55]. However, the small differences in the anthropometric measures observed in the present study, although reaching significance in the latter groups, suggests these observations are further mediated through alternative mechanisms.

For example, other studies have reported an association between a raised RHR and increased sympathetic activity [3, 24], which has been implicated in the development of hyperglycaemia, including type 2 diabetes mellitus [3, 24]. Increased sympathetic tone both

amplifies insulin resistance and is further increased in response to insulin, potentially exacerbating hyperglycaemia [27, 92] and other metabolic abnormalities [93]. Laakso and colleagues [94] reported that insulin resistant subjects had an attenuated rise in the postprandial elevation in blood flow to skeletal muscles when compared to control subjects. Thus, sympathetic vasoconstriction may reduce blood flow leading to acute insulin resistance within peripheral tissue, further negatively impacting on the maintenance of glucose homeostasis [95].

There has also been renewed interest in other factors which mediate adipose tissue distribution and function, such as abnormal activity of the hypothalamic-pituitary-adrenal axis [96]. For instance, overweight populations may have a mild form of hypercortisolaemia [97, 98], and it is thought that cortisol counter-regulates the actions of insulin whereby the association between cortisol and type 2 diabetes mellitus is likely due to increased insulin resistance which is evident in the hypercortisolaemic condition [99]. Both clinical and experimental investigations show excess cortisol is related to insulin resistance [100] and may be a contributory cause of obesity [101]. However, studies representative of the general population, examining cortisol and its relative contribution to obesity, insulin resistance and type 2 diabetes mellitus are sparse and warrant further investigation.

As the rate of modernisation continues to rise in China, it seems unsurprising that the prevalence of type 2 diabetes mellitus continues to approach the figures observed in more developed societies. The changes that have occurred in the present study are likely to increase the burden of adverse health consequences associated with this condition. Thus, public health interventions are urgently required to delay or prevent the potential medical or societal ramifications that may occur in Chinas 1.2 billion population. In general, physical activity interventions have shown that exercise not only reduces basal sympathetic activity but also improves the metabolic profile overall. Improvements in physical fitness can also induce

these beneficial effects even in the absence of changes in body weight [102], supporting the impact of fitness independent of obesity levels as observed in the current study.

Our older Chinese sample were randomly drawn from the membership of a city-wide community social and welfare association in Guangzhou city which represents 7% of the city's older permanent population of that age strata. After limiting the analysis to those with good or very good self-rated health, the association between physical fitness and type 2 diabetes mellitus remained essentially unchanged, suggesting that our findings are unlikely to be affected by reverse causality. Likewise, further exclusion of those who may have artificially reduced RHR resulting from treatment or sinus bradycardia did not affect the observations. Sensitivity analyses also showed that the observations were not sensitive to the choice of cut-off for the classification of a high RHR with both the median (75 bpm) and 75% cut-off point (83 bpm) giving similar results. However, given the cross-sectional nature of the present study, confirmation using a longitudinal design to detect the causative role of physical fitness on the incidence of type 2 diabetes mellitus is required.

To our knowledge, the present study is the first to address the independent and combined effects of abdominal obesity and physical fitness as measured by RHR on the prevalence of type 2 diabetes mellitus in an older Chinese population. RHR is only a crude measure of physical fitness, but has shown to be an independent predictor of fitness as measured by maximal treadmill exercise test [103]. We demonstrate that a high RHR as mediated by poor physical fitness may lead to an increased prevalence of glucose intolerance in both non-obese and obese community dwelling older subjects. Although biologically plausible, further research is warranted as it remains equivocal whether elevated RHR in individuals with poor physical fitness is causal, consequential, or epiphenomenal. However, should these observations be confirmed, RHR may provide a cheap and simple means of risk

stratification in such populations for which targeted interventions, including lifestyle modification, should be implemented.

CHAPTER THREE

3.0. INFLUENCE OF HEART RATE AT REST FOR PREDICTING THE METABOLIC SYNDROME IN OLDER CHINESE ADULTS

3.1. ABSTRACT

The aim of this study was to examine the relationship between seated resting heart rate and the metabolic syndrome among older residents of Guangzhou, South China. 30,519 older participants (≥50 years) from the Guangzhou Biobank Cohort Study were stratified into quartiles based on seated resting heart rate. The associations between each quartile and the metabolic syndrome were assessed using multivariable logistic regression. 6,907 (22.8%) individuals were diagnosed as having the metabolic syndrome, which was significantly associated with increasing heart rate quartiles (P <0.001). Participants in the upper-most quartile (mean resting heart rate 91±8 bpm) of this cardiovascular proxy had an almost two-fold increased adjusted risk (odds ratio [95% CI] = 1.94 [1.79, 2.11], P <0.001) for the metabolic syndrome, as compared to those in the lowest quartile (mean resting heart rate 63±4 bpm). Heart rate, which is an inexpensive and simple clinical measure, was independently associated with the metabolic syndrome in older Chinese adults. We hope these observations will spur further studies to examine the usefulness of resting heart rate as a means of risk stratification in such populations, for which targeted interventions should be implemented.

3.2. INTRODUCTION

The MetS represents a constellation of closely associated cardiovascular risk factors including central adiposity, hyperglycaemia, hypertension, and hyperlipidaemia [90, 104]. The clustering of these factors has been known for over 80 years [105], and since that time, the concept of the MetS has evolved considerably [106]. Multiple lines of evidence suggest that RHR is associated with the presence of the MetS [107, 108], and in this regard, data highlighting such relationships may raise awareness for health promotion initiatives.

In three different populations, Palatini and co-workers [107] observed that men with elevated RHR also presented higher values of cholesterol, triglycerides, fasting insulin, and 2 h post-load glucose concentrations, all of which are indicative of the MetS. Results from the TAMCIS project reported a four-fold increase in the risk of the MetS among men and women with a RHR ≥80 bpm [43]. In a population-based study of healthy adults, Panzer and colleagues [109] found fasting plasma glucose was positively associated with RHR following an exercise test. In hypertensive patients, RHR was independently associated with obesity, waist girth, blood pressure and serum glucose [110]. Moreover, on several occasions RHR has been described as an independent predictor of cardiovascular morbidity and mortality [3l, 53-56, 59, 111, 112].

In light of this data however, these findings were mostly derived from Black and Caucasian populations, and large scale studies examining RHR with the MetS, particularly among Asian cohorts are largely unavailable. As developing nations such as China continue to increase their rapid rate of modernisation, the prevalence of cardiovascular-associated health conditions such as the MetS will likely become more apparent [113-115], possibly leading to overwhelming public health ramifications. Therefore, the purpose of our investigation was

to increase the recognition of RHR as an independent marker of the MetS, among older residents of Guangzhou, in South China.

3.3. METHODS

3.3.1. Participants and setting

The GBCS is a prospective population based study aiming to examine determinants of health in an older Chinese population, and has been described previously [88, 116]. Briefly, participants were drawn from the membership of a city-wide community social and welfare association (GHHARE) for older people in Guangzhou, China. Baseline assessment included a detailed structured interview on lifestyle habits and medical history, as well as measurements of anthropometric indices, blood pressure, and fasting plasma markers. The Medical Ethics Committee of the Guangzhou Medical Association approved the study, and written, informed consent was obtained from all participants. For the present study, data from 30,519 participants recruited from 2003-2006 were analysed.

3.3.2. Demographic and lifestyle data

Demographic data (e.g., age and gender), details of health and lifestyle (e.g., smoking, alcohol consumption and physical activity) and socio-economic variables (e.g. occupational status, personal income and education level) were obtained by qualified staff. Participants were defined as current smokers if they answered yes to the question: "Do you currently smoke?" Also, participants were defined as current alcohol drinkers by answering yes to the question: "Have you consumed any alcohol in the past 12 months?" Level of physical activity was quantified using the short version of IPAQ, validated in the Chinese population [89]. The

formula for the computation of MET-minutes/week was: MET level x minutes of physical activity x events per week. In the current study, MET-minutes/week was presented as a continuous variable. For socio-economic measures, participants were categorized based on manual (indicative of a "blue collar" working environment) or non-manual employment according to occupational status. Education level consisted of three categories; attended primary school, secondary school or college. Personal income was defined as net monthly income in Yuan (6.40 Yuan = 1.00 USD) according to the following categories; <10,000, ≥10,000-<15,000 and ≥15,000.

3.3.3. Experimental procedures

Seated RHR was measured three times (Omron 705CP), 1 minute apart, following a 3 minute rest, with the average calculated from the second and third measurements. For the purpose of this study, participants were stratified into quartiles based on seated RHR. The presence of three out of five factors from the following MetS guidelines were used to identify participants with the MetS: (a) waist circumference ≥90 cm in men and ≥80 cm in women (Asian central obesity criteria); (b) raised blood pressure (≥130/85 mm Hg) or receiving treatment for hypertension; (c) raised fasting plasma glucose (≥5.6 mmol/L) or previously diagnosed type 2 diabetes; (d) raised plasma triglycerides (≥1.7 mmol/L); and (e) reduced HDL-cholesterol (<1.00 mmol/L in men and <1.30 mmol/L in women) [90].

3.3.4. Statistical methods

MET-minutes/week, glucose and triglyceride concentrations were non-normally distributed and therefore logarithmically transformed with their geometric means and 95% CIs presented. Otherwise data are shown as Mean±SD for continuous parameters or prevalence for

categorical variables. Comparisons between groups were performed by ANOVA, with a Bonferroni post-hoc test for continuous parameters and χ^2 test with *P* for linear-by-linear test for categorical variables.

The associations between seated RHR and the MetS were determined using hierarchical multivariable logistic regression models. In these analyses, model 1 describes the unadjusted association; model 2 adjusted for age (continuous variable) and sex; and model 3 further adjusted for smoking, alcohol, physical activity, occupational status, personal income and education. To better understand the relationship between RHR and the MetS, the latter analyses were performed examining RHR as a categorical and continuous parameter. We conducted a sensitivity test on the above models including only those who reported good or very good health as a means of addressing underlying illnesses that may have otherwise influenced our analyses. We further removed participants with potential sinus bradycardia (<50 bpm) and those on medication for blood pressure and heart disease, given that such medication including β-blockers and calcium channel blockers artificially reduce pulse rate. All statistical tests were 2-sided, and statistical significance was defined as $P < 0.05$. All data were analyzed using SPSS (SPSS 18.0; SPSS Inc, Chicago, Illinois).

3.4. RESULTS

Of the 30,519 older Chinese subjects recruited, we obtained full information regarding heart rate and other relevant fasting biochemical, physiological, and metabolic parameters on 30,328, thus 99.4% of subjects recruited are included in this study. Logistic regression analysis revealed no significant interaction effect between gender and RHR with the MetS ($P = 0.14$), therefore the two sexes were combined.

As shown in Table 3.1, a total of 6,907 (22.8%) individuals were diagnosed with having the MetS. Subjects in the highest quartile for RHR had a higher BMI and waist circumference, but reported less physical activity, as compared to those in the lowest quartile. Likewise, these participants had higher concentrations of fasting glucose, total and LDL-cholesterol, triglycerides and higher systolic and diastolic blood pressures, reflecting the increased prevalence of type 2 diabetes, dyslipidaemia and arterial hypertension. Of the other parameters, those in the upper-most quartile for RHR were more likely to be manual employees, perhaps as a result of being less well educated. Further, these participants reported greater use of cardiovascular treatment including anti-hypertensives, lipid-lowering agents and anti-hyperglycaemic medication. In contrast, a greater proportion of participants in the lowest quartile tended to be current smokers and/or drinkers (Table 3.1).

Table 3.1. Participant characteristics according to quartiles of resting heart rate

	Resting heart rate quartiles				
	Q1	Q2	Q3	Q4	*P*-value*
No. (%)	7,628 (25.2)	7,709 (25.4)	7,427 (24.5)	7,564 (24.9)	
Age (years)	61.9±7.1	61.1±7.1	61.2±7.1	61.7±7.1	<0.001
Male (%)	32.2	25.4	25.2	27.8	<0.001
Heart rate (beats/minute)	63±4	72±1	78±2	91±8	<0.001
Body mass index (kg/m^2)	23.6±3.2	23.7±3.2	23.8±3.3	23.9±3.4	<0.001
Waist circumference (cm)	78.4±8.8	78.4±8.8	78.7±8.9	79.5±9.4	<0.001
Current smoker (%)	14.8	11.4	10.2	10.4	<0.001
Current alcohol drinker (%)	32.0	30.2	29.5	27.3	<0.001
Metabolic equivalent score (min/week)	2,690 (2,610-2,773)	2,509 (2,428-2,592)	2,529 (2,454-2,607)	2,307 (2,236-2,381)	0.46
Metabolic syndrome (%)	17.8	20.5	23.5	29.5	<0.001
Fasting glucose (mmol/L)	5.41 (5.39, 5.43)	5.49 (5.46, 5.51)	5.59 (5.57, 5.62)	5.88 (5.84, 5.91)	<0.001
Type 2 diabetes (%)	11.0	13.8	17.0	26.0	<0.001
Systolic blood pressure (mm/Hg)	130±23	129±22	129±21	134±22	<0.001
Diastolic blood pressure	72±10	73±11	74±11	76±11	<0.001
Arterial hypertension (%)	42.6	39.5	39.5	48.0	<0.001
Total cholesterol (mmol/L)	5.83±1.13	5.90±1.12	5.94±1.12	6.01±1.16	<0.001
HDL-cholesterol (mmol/L)‡	1.65±0.39	1.66±0.40	1.65±0.40	1.65±0.41	0.22
LDL-cholesterol (mmol/L)‡	3.20±0.70	3.24±0.69	3.28±0.71	3.31±0.71	<0.001
Triglyceride (mmol/L)	1.32 (1.30, 1.33)	1.39 (1.37, 1.40)	1.46 (1.44, 1.48)	1.56 (1.54, 1.58)	<0.001
Dyslipidaemia (%)	46.1	49.9	52.8	57.4	<0.001
Occupational status (% manual)	59.6	61.2	61.4	62.6	0.001
Personal income – Yuan (%)					
<10,000	7.9	7.1	7.6	7.6	0.26
≥10,000-<15,000	41.6	42.3	42.5	43.0	
≥15,000	50.6	50.6	49.9	49.4	

Table 3.1. *continued*

	Resting heart rate quartiles				
	Q1	Q2	Q3	Q4	*P*-value*
Education (%)					
Attended primary	42.4	42.4	42.1	45.0	<0.001
Secondary	47.4	49.0	49.5	46.8	
College	10.2	8.8	8.4	8.2	
Cardiovascular treatment (%)					
Anti-hypertensive medication	27.6	24.6	25.0	29.2	0.02
Lipid-lowering agents	6.2	6.8	6.5	7.6	0.003
Anti-hyperglycaemic medication	5.7	6.8	8.1	11.7	<0.001

Mean±SD, Geometric mean (95% confidence intervals) or percentage values are reported. *P-value was obtained by ANOVA or χ^2 test. HDL = high-density lipoprotein; LDL = low-density lipoprotein.

Figure 3.1 describes the relationship between RHR measured continuously with the MetS and its individual components. A 10 bpm increment in RHR was associated with a 16% increase in the risk of having the MetS (P <0.001). Likewise, a 10 bpm increase in RHR was most strongly associated with elevated fasting glucose as compared to the other individual components; surprisingly presenting a similar risk in comparison to the overall MetS score (see Figure 3.1). In Table 3.2, the relationship between each RHR quartile and the MetS is shown. In model 3, the adjusted risk associated with the MetS was almost two-fold greater for seated RHR among individuals in the highest quartile (OR [95% CI] = 1.94 [1.79-2.11], P <0.001), as compared to subjects in the lowest quartile.

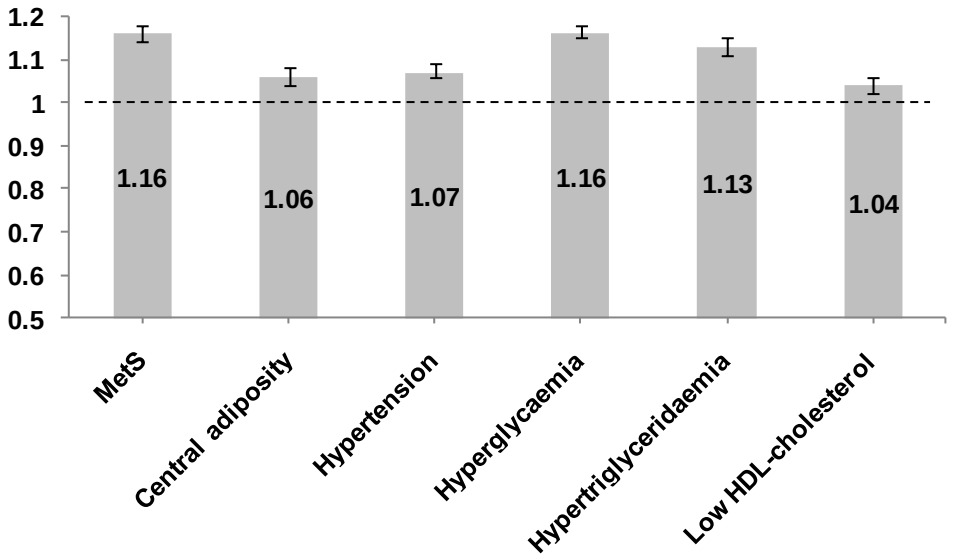

Figure 3.1. Fully adjusted odds ratios for the metabolic syndrome and the individual components according to seated resting heart rate (per 10 beats/minute increment). MetS = metabolic syndrome; HDL = high-density lipoprotein. Model adjusted as abbreviated in Table 3.2.

Table 3.2. Odds ratios for the metabolic syndrome according to quartiles of seated resting heart rate

	Resting heart rate quartiles				
	Q1	Q2	Q3	Q4	P_{trend}
No. MetS/without	1355/6273	1577/6132	1746/5681	2229/5335	
Unadjusted					
OR	1	1.22	1.41	1.97	<0.001
95% CI	⋯	1.12 – 1.33	1.30 – 1.54	1.82 – 2.14	
P value	⋯	<0.001	<0.001	<0.001	
Age and sex adjusted					
OR	1	1.22	1.41	1.96	<0.001
95% CI	⋯	1.11 – 1.32	1.29 – 1.54	1.80 – 2.13	
P value	⋯	<0.001	<0.001	<0.001	
Fully adjusted					
OR	1	1.21	1.41	1.94	<0.001
95% CI	⋯	1.11 – 1.32	1.29 – 1.53	1.79 – 2.11	
P value	⋯	<0.001	<0.001	<0.001	

Fully adjusted model included age, sex, smoking, alcohol, physical activity, occupational status, personal income and education. MetS = metabolic syndrome; OR = odds ratio; CI = confidence interval.

Lastly, we conducted a sensitivity test including only subjects who reported good or very good health (*n* = 24,286), and who were therefore less likely to have influenced the present analyses as a consequence of underlying chronic conditions. We found the risk for the MetS remained virtually unchanged (OR [95% CI] = 1.93 [1.76, 2.12], *P* <0.001) after adjusting for potential confounders. In a separate sensitivity check, exclusion of individuals with potential sinus bradycardia (<50 bpm) or those receiving medication for blood pressure or heart disease (*n* = 8,224) did not affect these results (OR [95% CI] = 2.12 [1.89, 2.38], *P* <0.001).

3.5. DISCUSSION

In this study, an elevated RHR was independently associated with the MetS in older Chinese adults. Participants in the upper-most quartile (≥91 bpm) of this cardiovascular proxy had an almost two-fold increased adjusted risk for the MetS, after correcting for a range of potential confounding factors. Our data confirm earlier investigations among non-Asian populations,

indicating that those with an elevated RHR were at greater risk of cardio-metabolic complications [43, 107, 110]. Though the prevalence of the MetS has become commonplace among affluent nations [117, 118], its implications within developing countries are of great concern [106]. As the rate of modernisation increases in China, the prevalence of the MetS will continue to rise, and may amplify the burden of adverse health outcomes associated with this condition. Thus, public health interventions are urgently needed. The results from this study suggest that RHR, which is an inexpensive and simple clinical measure, may be used as an indicator for the possible presence of the MetS in older Chinese adults.

Indeed, epidemiological studies have consistently shown that elevated RHR is a predictive factor for the MetS. For example, Singh et al. [30] observed that, apart from genetic factors, RHR was associated with several environmental causes including BMI, systolic, and diastolic blood pressure. Martin and co-workers [119] found individuals who presented with a higher RHR had substantially elevated levels of insulin and glucose, waist circumference, BMI, diastolic blood pressure and suggestively elevated triglycerides and systolic blood pressure. Given the potential clinical impact of RHR, the mechanisms on how it interacts with other cardiovascular risk factors are of significant biomedical interest. To this end, ANS activity is considered to play a central role in regulating pulse rate. Previous literature has implicated dysfunctional ANS activity with a number of deleterious health consequences pertaining to CVD [107, 120, 121]. This is often afforded to a sympatho-vagal imbalance, usually in favour of sympathetic over-activity [121]. Here, predominant sympathetic activity provokes numerous negative sequelae including abnormal heart-rate control, impaired central and peripheral vascular dynamics [122], as well as sustained insulin resistance and blood pressure elevation [123]. Moreover, due to the unwanted shift in ANS activity, lower parasympathetic outflow (i.e. reduced vagal tone) has been suggested to exacerbate a faster RHR and diastolic dysfunction [122, 124].

We may add that resting tachycardia is perhaps attributable to the consequent actions of leptin on cardiovascular control; a faster RHR may be influenced by a decline in vagal drive to the heart by leptin [125]. Indeed, high leptin has been shown to be a strong independent predictor of cerebrovascular disease [126] as well as acute MI [127]. Further, Narkiewicz et al. [125] observed that RHR in men with high leptin concentrations was faster in comparison to men with lower plasma leptin levels. Thus, if confirmed by additional studies, the link between leptin and RHR may well extend our understanding of the pathophysiological processes underlying cardiovascular function.

Recent studies have also highlighted that a defect in nitric oxide (NO) bioavailability may contribute to the pathophysiology of the MetS [128]. In particular, a defect in the production of NO impairs oxidative metabolism by diminishing mitochondrial biogenesis, resulting in lipid accumulation in skeletal muscle, liver and pancreas, which may ultimately impede insulin signalling, glycaemic control, and NO-mediated vasodilation [120, 129, 130]. Interventions to improve mitochondrial function have been shown to correct insulin signalling as well as other metabolic and vascular abnormalities [130]. Interestingly, NO also modulates autonomic control of pulse rate by participating in parasympathetic vasodilation within the coronary arteries while inhibiting sympathetic vasoconstriction [111]. Further, experimental studies have shown NO augments cardiac vagal control in healthy adults, as well as patients suffering from heart failure [131, 132]. In summary, the relationship between RHR and the cluster of risk factors that form the MetS is well validated, and it seems plausible that RHR may well represent a member of this family, for which NO could be the missing link [111]; though clearly, further longitudinal and interventional studies are needed to help establish this notion.

To our knowledge, no other studies have examined the association between RHR and the MetS among older Chinese. The present large-scale sample was randomly drawn from the membership of a city-wide community social and welfare association in Guangzhou city,

representing 7% of the city's older permanent population of that age strata. In this regard, our sample may not be representative of all older Chinese in Guangzhou, although, we have previously shown that our sample has similar levels of metabolic disorders including type 2 diabetes and hypertension as compared to nationally representative samples of urban Chinese [133, 134], suggesting reasonable external validity. We cannot rule out the possibility of "healthy volunteer bias" among people who have chosen to join GHHARE, and by extension, in common with all cohorts in developing countries, our subjects are with increasing age selected survivors. In particular, our subjects survived childhood infections in a pre-antibiotic environment, and lived through significant periodic social turmoil up until the 1970s. However, if survivorship were an issue, we would expect different relationships in the older compared with the younger subjects, which we check for routinely.

After limiting the analysis to individuals with good or very good self-rated health, the association between RHR and the MetS remained essentially unchanged, suggesting that our findings are unlikely to be affected by other underlying illnesses. Further exclusion of participants who may have artificially lowered RHR resulting from treatment or sinus bradycardia did not affect these observations. However, given the cross-sectional nature of the present investigation, confirmation using a longitudinal design to study the prediction of RHR on the incidence of the MetS is necessary.

In conclusion, seated RHR was found to be significantly associated with the MetS in older Chinese adults. Our data highlights the importance of RHR as a distinct marker of health status, independent of other major risk factors. In light of these findings, RHR may provide a cheap and simple means of risk stratification in such populations for which targeted interventions should be implemented.

CHAPTER FOUR

4.0. USEFULNESS OF RESTING HEART RATE AND THE METABOLIC SYNDROME TO PREDICT VASCULAR DISEASE RISK IN OLDER CHINESE: FROM THE GUANGZHOU BIOBANK COHORT STUDY-CARDIOVASCULAR DISEASE SUB-COHORT

4.1. ABSTRACT

Physical fitness may independently lower the risk of cardiovascular disease. We explored the independent and combined associations of physical fitness measured by seated resting heart rate, and the metabolic syndrome, with cardiovascular disease risk, as described by elevated pulse wave velocity in older Chinese. Data from 1,996 participants were drawn from the Guangzhou Biobank Cohort Study-Cardiovascular Disease Sub-cohort. Analysis of Variance and logistic regression were employed to establish the independent and combined associations of resting heart rate and the metabolic syndrome with pulse wave velocity. Resting heart rate was independently associated with elevated pulse wave velocity; odds ratio (95% CI) = 1.63 (1.22, 2.18), as was the metabolic syndrome; odds ratio (95% CI) = 2.36 (1.76, 3.17). Participants with a high resting heart rate, but without the metabolic syndrome had an adjusted odds ratio (95% CI) of 1.63 (1.15, 2.30) for the presence of the cardiovascular proxy. Those with a low resting heart rate and the metabolic syndrome had an adjusted odds ratio (95% CI) of 2.35 (1.66, 3.33). The risk of an elevated pulse wave velocity increased almost four-fold on the background of both a high resting heart rate and diagnosis of the metabolic syndrome; odds ratio (95% CI) 3.87 (2.39, 6.28) (P = 0.52 for interaction). In conclusion, physical fitness, measured by seated resting heart rate and the metabolic syndrome are independently associated with elevated pulse wave velocity, a surrogate marker for cardiovascular disease. The strength of this association is further increased on the background of both. These findings confirm the beneficial effects of physical fitness on attenuating the risk of vascular disease among older Chinese.

4.2. INTRODUCTION

Risk factors for CVD attribute to a number of medical disorders and their clustering is known as the MetS. The MetS is largely the result of altered modifiable lifestyle factors, for instance physical fitness. Several studies have indicated poor physical fitness is associated with the MetS, exacerbating an unhealthy CVD risk profile and promoting all-cause mortality [16, 135]. However, the current contributions of RHR and the MetS toward the risk of CVD remain underexplored, particularly among Chinese populations. Therefore, in the present study we evaluated the independent and combined associations of physical fitness as measured by seated RHR, and the MetS, with CVD risk, as described by pulse wave velocity (PWV) in older Chinese residents of Guangzhou, China. We specifically hypothesized that poor physical fitness; 1) would be coupled with a higher prevalence of CVD risk and; 2) the risk of CVD would additionally increase on the background of the MetS, thus forewarning of a major developing health burden in a rapidly modernizing and burgeoning older Chinese population.

4.3. METHODS

4.3.1. Participants and setting

Data were drawn from 1,996 older Chinese volunteers (>50 years) as part of the Guangzhou Biobank Cohort Study-Cardiovascular Disease Sub-cohort (GBCS-CVD). This sub-cohort enables a thorough evaluation of volunteers' current CVD status through the measurement of a range of surrogate markers of vascular disease/risk, thus allowing for the development of testable hypotheses. Our older volunteers were permanent residents of Guangzhou, belonging to a community and welfare association (GHHARE), which represents a homogeneous

Cantonese group who has retained many traditional and cultural norms, despite previous turmoil and extensive economic transition. This population forms part of an ideal setting to assess, both cross-sectionally and longitudinally, the impact of rapid economic development on health outcomes. A more comprehensive description of this sub-cohort and its multidisciplinary measures is detailed by Jiang et al. [136]. The Medical Ethics Committee of the Guangzhou Medical Association approved the study, and written, informed consent was obtained from all volunteers.

4.3.2. Experimental procedures

Volunteers were classified according to physical fitness status as indicated by seated RHR and diagnosis of the MetS. Seated RHR was measured three times (Omron 705CP); 1 minute apart, following a 3 minute rest, with the average calculated from the second and third measurements. Volunteers were classified as having a high seated RHR, if their heart rate was ≥83 bpm, determined by the 75th percentile cut-point. The presence of three out of five factors from the following criteria were used to identify those with the MetS: (a) waist circumference ≥90 cm in men and ≥80 cm in women; (b) raised blood pressure (≥130/85 mm Hg) or receiving treatment for hypertension; (c) raised fasting plasma glucose (≥5.6 mmol/L or ≥100 mg/dL) or previously diagnosed type 2 diabetes; (d) raised plasma triglycerides (≥1.7 mmol/L or ≥150 mg/dL); and (e) reduced HDL-cholesterol (<1.00 mmol/L or <40 mg/dL in men and <1.30 mmol/L or <50 mg/dL in women) [90]. Volunteers with CRP levels ≥11mg/L (4.8%) were excluded due to a suspected inflammatory or infectious condition, leaving 1,902 participants available for analyses.

PWV was measured using a volume-plethysmographic apparatus with an automatic waveform analyzer (Colin VP1000; Colin Medical Technology, Komaki, Japan). Measurements were taken with volunteers lying in a supine position after 5 minutes of rest.

Occlusion and monitoring cuffs were placed around both sites of the lower legs and upper arms. Pressure waveforms of the brachial and tibial arteries were then recorded simultaneously using semiconductor plethysmographic and oscillometric pressure sensors. These waveforms allow the determination of the time interval between the initial rise in the brachial and tibial pressure waveforms (T). The path length from the suprasternal notch to the elbow (La) and also from the suprasternal notch to the ankle (Lb) were automatically statistically determined from the patient's height. PWV was calculated using the formula PWV ¼(Lb-La)/T. Measurements of the left and right brachial-to-ankle PWV were obtained for an average of 10 seconds. The method validity has been reported previously, with an inter-observer coefficient of variation of 8.4% and intra-observer coefficient of variation of 10.0% [137]. The average of the left and right brachial-to-ankle PWV was used for subsequent analyses.

4.3.3. Statistical methods

The independent effects of seated RHR and the MetS on CVD status were first examined. Subsequently these parameters were used to stratify the participants into four groups 1: Low RHR/no-MetS, 2: High RHR/no-MetS, 3: Low RHR/MetS and 4: High RHR/MetS. Comparisons between groups were performed by ANOVA with a Bonferroni post-hoc test for continuous parameters, and χ^2 test with *P* for linear-by-linear test for categorical variables. All variables following a non-normal distribution were logarithmically transformed and their geometric means and 95% CIs presented. Otherwise data are presented as Mean±SD for continuous parameters or prevalence for categorical variables.

Associations were tested using three hierarchical models derived from multivariate logistic regression. Model 1 included age (continuous) and sex as covariates. Model 2 further adjusted for lifestyle factors including; smoking (never, ever), alcohol (never, ever), physical

activity (MET-minutes/week), and personal history of CVD (yes, no), which volunteers reported if a physician had ever told them they had experienced; hypertension, dyslipidaemia, valvular heart disease, CHD, stroke, angina pectoris, MI, peripheral heart disease, rheumatic heart disease or any other CVD-related events. Finally, model 3 additionally adjusted for socio-economic measures including; education level (primary or below, middle, or college or above) and personal income (<10,000, ≥10,000-<15,000 and ≥15,000 Yuan). For the purpose of our analyses, PWV was dichotomized into low and high risk groups based on the 75% cut-point (16.98 m/s). For comparison, we additionally explored the relationship across each of the stratified groups using the median cut-point for PWV (14.80 m/s). All statistical tests were 2-tailed, and statistical significance was defined as P <0.05. Analyses were conducted using Statistical Software for Social Sciences, version 18 (SPSS, Chicago, Illinois, USA).

4.4. RESULTS

Demographic characteristics are presented in Table 4.1. The prevalence of those with a high RHR was 24.9% (n = 473), while 21.2% (n = 403) were classed as having the MetS. Of these MetS participants, 114 (28.3%) had a high RHR while the remaining 289 (71.2%) volunteers had a low RHR (Table 4.1). Physical activity levels were greatest in those who had a low RHR without the presence of the MetS (P = 0.006). Between-group differences were observed for waist circumference and BMI (P <0.001). Several parameters including PWV (Figure 4.1), blood pressure, fasting glucose, glycosylated haemoglobin, lipids and CRP were significantly higher in those with a high RHR and the MetS whilst HDL-cholesterol was significantly lower (Table 4.1). No interaction effect was observed between RHR and the MetS (P = 0.52).

Table 4.1. Baseline characteristics

	Low RHR/no-MetS (*n*=1,140)	High RHR/no-MetS (*n*=359)	Low RHR/MetS (*n*=289)	High RHR/MetS (*n*=114)	*P* value[#]
Age (Years)	59.0±6.8	58.5±6.9	60.8±6.9[*†]	60.6±7.0	<0.001
Male	48.8%	51.0%	53.6%	43.9%	0.689
Heart Rate (beats/minute)	71±7	91±7[*]	72±7[†]	92±7[*§]	<0.001
Pulse Wave Velocity (m/s)	14.8±2.8	15.6±2.7[*]	16.6±3.0[*†]	17.4±3.1[*†]	<0.001
Waist Circumference (cm)	76.2±7.9	76.8±7.8	86.0±8.0[*†]	86.2±9.0[*†]	<0.001
Body mass index (kg/m^2)	23.0±2.7	23.0±2.7	26.1±2.6[*†]	25.9±2.9[*†]	<0.001
Fasting glucose (mg/dL)	94.22 (93.67, 94.93)	97.82 (95.83, 99.62)[*]	108.44 (106.10, 110.97)[*†]	116.91 (110.79, 123.40)[*†§]	<0.001
Glycosylated haemoglobin (%)	5.9	6.0	6.3[*†]	6.7[*†§]	<0.001
Impaired fasting glucose	16.6%	27.3%	59.5%	61.4%	<0.001
Type 2 diabetes mellitus	2.1%	3.6%	12.5%	16.7%	<0.001
Total cholesterol (mg/dL)	223.89±40.60	224.28±41.37	231.24±43.31[*]	234.72±45.63[*]	0.006
LDL-cholesterol (mg/dL)	129.15±25.52	129.54±26.29	133.02±25.90	136.89±28.61[*]	0.003
HDL-cholesterol (mg/dL)	64.19 ± 14.69	62.64±15.08	51.43±12.37[*†]	50.65±12.37[*†]	<0.001
Triglyceride (mg/dL)	116.03 (113.37, 119.57)	129.88 (103.87, 130.20)	221.43 (208.14, 235.60)[*†]	237.37 (214.34, 263.06)[*†]	<0.001
Dyslipidaemia	41.8%	44.6%	70.2%	73.7%	<0.001
Systolic blood pressure (mm/Hg)	123±20	126±18	142±20[*†]	142±19[*†]	<0.001
Diastolic blood pressure (mm/Hg)	72±10	75±10[*]	81±10[*†]	81±10[*†]	<0.001
Arterial hypertension	27.0%	31.5%	74.7%	69.3%	<0.001
CVD history	35.2%	34.0%	58.2%	50.9%	<0.001
C-reactive protein (mg/L)	1.13 (1.06, 1.19)	1.34 (1.20, 1.50)[*]	1.92 (1.72, 2.15)[*†]	2.09 (1.75, 2.49)[*†]	<0.001
Current smoker	39.8%	42.3%	43.7%	38.1%	0.518
Current alcohol user	59.4%	52.9%	58.5%	60.2%	0.635
Physical activity (MET-minutes/week)	3,167 (2,973-3,373)	2,741 (2,224-2,886)[*]	2,784 (2,455-3,157)	3,128 (2,643-3,702)	0.006

Table 4.1. *Continued*

	Low RHR/no-MetS (*n*=1,140)	High RHR/no-MetS (*n*=359)	Low RHR/MetS (*n*=289)	High RHR/MetS (*n*=114)	*P* value[#]
Education					
College or above	12.9%	10.6%	14.9%	14.0%	0.059
Middle	61.4%	61.8%	54.9%	46.5%	
Primary or below	25.7%	27.6%	30.2%	39.5%	
Personal income (Yuan/USD)					
≥15,000/2,292	14.7%	16.6%	14.3%	14.7%	0.088
≥10,000-<15,000/≥1,528-<2,292	63.6%	62.7%	60.5%	51.4%	
<10,000/<1,528	21.7%	20.7%	25.2%	33.9%	

Data are presented as mean±SD, geometric mean (95% confidence interval) or percentage values. [#]ANOVA with Bonferroni for continuous data or χ^2 test for categorical data was used. [*]*P* <0.05 when compared to Low RHR/no-MetS; [†]*P* <0.05 when compared to High RHR/no-MetS; [§]*P* <0.05 when compared to Low RHR/MetS. Dyslipidaemia was defined as total cholesterol ≥200 mg/dL, triglycerides ≥150 mg/dL or lipid lowering agents. Abbreviations; LDL = Low-density lipoprotein, HDL = High-density lipoprotein, CVD = cardiovascular disease, MET = metabolic equivalent, USD = United States Dollar.

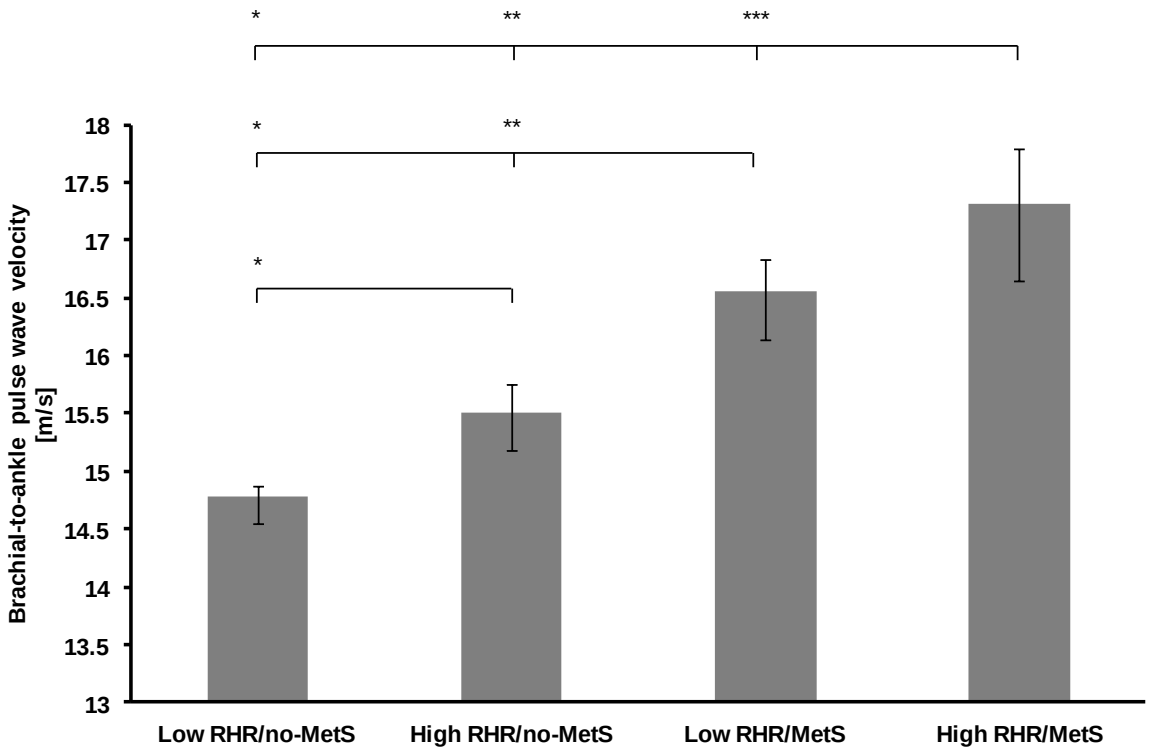

Figure 4.1. Brachial-to-ankle-pulse wave velocity according to seated resting heart rate (RHR) and the metabolic syndrome (MetS) groups. Values presented are geometric means with 95% CIs in meters per second (m/s). $^{*}P$ <0.05 when compared to the Low RHR/no-MetS group; $^{**}P$ <0.05 when compared to the High RHR/no-MetS group; $^{***}P$ <0.05 when compared to the Low RHR/MetS group.

In Figure 4.2, mutually adjusted logistic regression revealed a high RHR and the MetS were independently associated with PWV (P <0.001, respectively), indicating both contribute separately towards increasing the risk of this cardiovascular proxy. Further, in Table 4.2 we observed those with a high RHR, the MetS, or both, had a greater adjusted OR for increased PWV (P_{trend} <0.001 respectively). Through additional exploratory analysis using the median as the cut-point for PWV, we found a raised RHR, diagnosis of the MetS, and both combined, were similarly linked to an elevated PWV following adjustment; OR [95 % CI] = 2.18 [1.64, 2.89], 2.73 [1.97, 3.80] and 5.28 [3.10, 9.00], P_{trend} <0.001 respectively. To address the potential issue of reverse causality, we examined only those who had reported good or very good health (n = 1,594), and found the ORs were virtually the same. Further exclusion of

volunteers with potential bradycardia (RHR <50 bpm), or those on blood pressure-lowering medications did not affect these findings (data not shown).

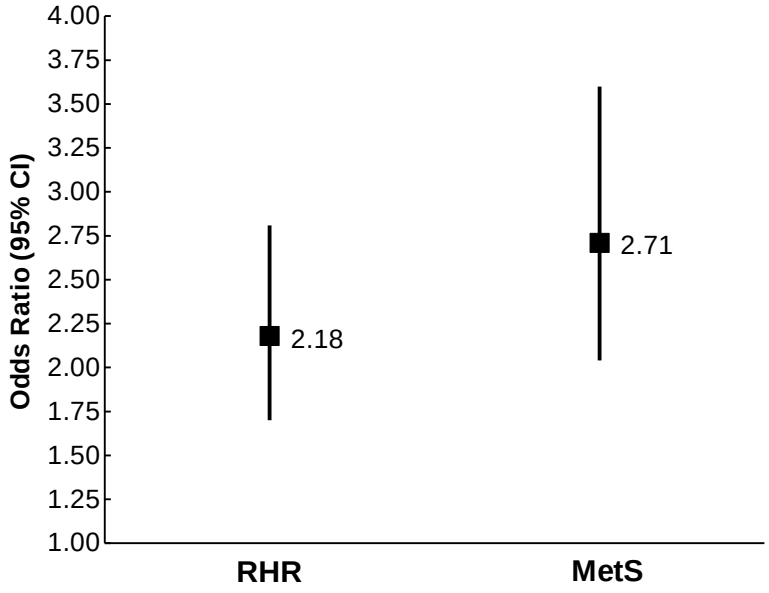

Figure 4.2 Independent associations of seated resting heart rate (RHR) and the metabolic syndrome (MetS) with pulse wave velocity. Values are dichotomous, and derived from mutually adjusted multivariate analyses which included age, sex, smoking, drinking, and physical activity, prior history of cardiovascular disease, education and personal income.

Table 4.2 Odds ratios (95% CI) for the risk of elevated pulse wave velocity among 1,902 older Chinese subjects according to seated resting heart rate and the metabolic syndrome

	Model[1]	Model[2]	Model[3]
Low RHR/no-MetS	1 (Reference)	1 (Reference)	1 (Reference)
High RHR/no-MetS	1.64 (1.17, 2.30)	1.61 (1.14, 2.27)	1.63 (1.15, 2.30)
Low RHR/MetS	2.66 (1.90, 3.73)	2.40 (1.70, 3.38)	2.35 (1.66, 3.33)
High RHR/MetS	4.26 (2.65, 6.84)	4.02 (2.49, 6.49)	3.87 (2.39, 6.28)
P_{trend}	<0.001	<0.001	<0.001

Model[1] adjusted for age and sex. Model[2] additionally adjusted for smoking, drinking, physical activity and prior history of cardiovascular disease. Model[3] further adjusted for education and personal income. Abbreviations; CI = confidence interval; RHR = resting heart rate; MetS = metabolic syndrome.

4.5. DISCUSSION

In the present study, we described the independent and combined effects of physical fitness as measured by RHR, and the MetS on a surrogate marker of CVD in older Chinese. We found the risk of a high PWV was independently associated with RHR and the MetS. These data confirm previous studies derived from Caucasians, whereby physical fitness was identified as an important predictor of cardiovascular mortality [17, 84], while the MetS was associated with an approximately two-fold increase in CVD risk [138, 139]. Further, participants who presented with both a raised RHR and the MetS had an increased risk for high PWV by almost 400%, which remained robust after controlling for a range of potential confounders.

Several epidemiological studies have addressed the importance of RHR as a predictor for CVD. Mounting evidence shows that RHR >80-85 bpm is directly associated with the risk of developing hypertension and atherosclerosis, and is a strong predictor of cardiovascular morbidity and mortality [140]. For instance, in the HARVEST study, RHR was found to be a significant marker for the development of hypertension, even after many clinical variables including physical activity were taken into consideration [29]. In the CASS investigation, RHR emerged as a significant predictor for CVD mortality independent of other risk factors [67]. Also, RHR was found to be strongly correlated with the severity of atherosclerosis among men who had developed MI at a young age [141]. Data from several other studies examined in a recent review provide additional evidence linking RHR to a greater risk of all-cause and CVD mortality, which pertains to both the general population and in those already at-risk for CVD [142]. In light of this evidence, recent reviews have recommended that improving physical fitness (i.e. reducing pulse rate) may have important benefits towards cardiovascular health [143, 144]. Several clinical and experimental studies have also suggested that a reduced RHR may increase coronary endothelial function while attenuating atheroprogression [112, 145]. These data along with our own support the relevance of examining the impact of reducing RHR, perhaps

lowering the risk associated with CVD morbidity and mortality. Subsequently, a potential role for RHR and its modulation should be included in prospective cardiovascular guidance documents [142].

Previous research has indicated physical fitness is influenced by dysfunctional ANS activity [146], suggesting that progressive autonomic damage may be involved in elevating RHR [147]. Autonomic dysfunction is characterized by a sympathetic-parasympathetic imbalance which may also, in part, be attributable to the individual components of the MetS [109, 148]. For instance, patients with a faster RHR tend to have an abnormal metabolic profile [147, 149]. This was shown in a population-based study of healthy adults [109], whereby fasting plasma glucose was associated with poor HRR following an exercise test. This association was particularly evident among volunteers whose RHR was ≥80 bpm [109]. Likewise, Shishehbor et al. [148] found individuals in the highest quartile of the triglyceride-to-HDL-cholesterol ratio, a strong correlate of insulin resistance [150], had a significantly poor HRR post-exercise. In the present study, we observed the risk of a high PWV doubled in those with a fast RHR even in the absence of the MetS, suggesting the risk of experiencing a cardiovascular event extends below those with a fast RHR and the MetS, and not just the MetS. The current findings may well reflect subtle changes of sympathetic and parasympathetic imbalance that occur as a consequence of minimal abnormalities to the metabolic profile. It seems those unfit individuals who might otherwise appear healthy may actually be at an increased risk for CVD.

Lack of consideration of fitness in studies examining CVD risk suggests their healthy control groups may have included participants with poorer physical health as determined by a high RHR, resulting in underestimation of CVD risk. We therefore recommend future studies addressing the risk of CVD in those with the MetS should consider physical fitness when determining a reference group, which is achievable in most studies if pulse rate is used as a

surrogate. The present study carries its inherent limitations. Given its cross-sectional nature, confirmation using a longitudinal design to detect the causative role of physical fitness on the incidence of CVD is required. Nonetheless, our study adds value to the existing literature highlighting RHR as a simple, easy-to-measure marker with implications towards the prediction of CVD risk. The study's large sample size allowed for us to establish the independent effects of physical fitness and the MetS in subgroups. Even after adjusting for several confounding factors, our findings remained robust. In addition, we controlled for acute inflammatory or infectious conditions and further controlled for reverse causality by excluding those with poor self-reported health, strengthening our findings.

CHAPTER FIVE

5.0. SUSTAINED ELEVATED HEART RATE PREDICTS INCIDENT CARDIOVASCULAR MORTALITY IN APPARENTLY HEALTHY MIDDLE-AGED BRITISH ADULTS: FROM THE EUROPEAN PROSPECTIVE INVESTIGATION INTO CANCER AND NUTRITION-NORFOLK STUDY

5.1. ABSTRACT

Despite increasing evidence underlining the relationship between a raised heart rate and cardiovascular mortality, this easily measurable, inexpensive, clinical parameter continues to be overlooked as a potential risk factor in the general population. Thus, we aimed to promote the clinical value of sustained tachycardia as a predictor of death due to cardiovascular disease in a cohort of generally healthy middle-aged British adults. A total of 25,639 participants (men *n* = 11,607 and women *n* = 14,032) aged between 39 and 79 years were followed prospectively. Risk estimates for cardiovascular mortality according to quartiles of resting heart rate were determined using hierarchical multivariate Cox proportional hazards regression. During a median follow-up time of 14.9 years, 1,734 incident cases of cardiovascular mortality were reported. In the upper-most quartile of resting heart rate, the overall adjusted risk for cardiovascular mortality was HR (95% CI) 1.19 (1.03-1.37). Sex-specific analyses revealed the strongest associations were found among the men HR (95% CI) 1.37 (1.14-1.64). No association was observed in the women. In conclusion, we recommend elevated heart rate should no longer be neglected as a predictor of cardiovascular mortality, at least in the general population.

5.2. INTRODUCTION

Over the past few decades, a number of epidemiological studies have been undertaken to examine the relationship of accelerating RHR with adverse cardiovascular outcome. One of the earliest in the series to describe this association was the Chicago Employee Study [53]. Across three groups of middle-aged Caucasian males, Dyer and colleagues [53] documented that a rapid RHR predicted both total and CVD mortality. Several other observational studies have indeed highlighted a similar association including, but not constrained to, the Framingham Heart Study [54], the British Regional Heart Study [57], the MATISS Project [63], and NHANES [55]. Despite literature emphasizing the independent contribution of sustained tachycardia for predicting poor cardiovascular prognosis, its current role as a modifiable determinant of health status remains suboptimal and lacks consideration, particularly in the general population. Therefore, the present investigation sought to further ascertain the importance of RHR as a predictor of CVD mortality in a large cohort of generally healthy middle-aged adults.

5.3. METHODS

5.3.1. Study design and population

The EPIC-Norfolk study is a prospective cohort study designed to investigate the relationship between diet, nutritional status, lifestyle and environmental factors for the risk of developing a range of chronic diseases [151]. A total of 25,639 participants (men n = 11,607 and women n = 14,032) aged between 39 and 79 years, were enrolled in the EPIC-Norfolk study. Thirty five general practices from the city of Norwich and surrounding rural towns were chosen as the study area, and all individuals within the age range in each general practise database were

invited to participate, with the exception of those deemed unsuitable by the general practitioner. Those who provided signed informed consent were invited to participate in a health examination. The EPIC-Norfolk study was approved by the Norfolk Local Research Ethics Committee.

5.3.2. Baseline examination

Participant examination began in 1993 and was completed at the end of 1997. An overall summary of the recruitment process, study design, and population characteristics have been described elsewhere [152]. In brief, participants completed a detailed health and lifestyle questionnaire including questions on smoking status (current, former, never), and a four-level (e.g., 1 = inactive, 2 = moderately inactive, 3 = moderately active, and 4 = active) physical activity index derived from the validated EPIC short physical activity questionnaire [153]. Information regarding prevalent hypertension, dyslipidaemia, and diabetes was based on self-reported physician diagnosis. From the baseline questionnaire, we further obtained information about self-reported use of anti-hypertensive and lipid-lowering therapy. At baseline, participants attended for a health check whereby a range of anthropometric indices were recorded by trained nurses using standardised procedures [154]. Waist was measured in cm, horizontally around the smallest circumference between the ribs and iliac crest. When the minimum circumference was not possible to identify, the minimum circumference at the level of the navel was used instead. BMI was estimated as weight (kg) divided by height (m^2). Blood pressure was determined using an Accutorr sphygmomanometer (Datascope, UK). Two measurements were taken 30 seconds apart after the participant had been seated for 3 min. RHR was also obtained using an Accutorr non-invasive blood pressure monitor. Likewise, 2 measures were taken 30 seconds apart following a 3 min rest in the seated

position. For the purpose of this study, we used the mean of the 2 measures for blood pressure and RHR in analyses.

5.3.3. Laboratory analyses

Samples of non-fasted venous blood were collected by venepuncture into plain and citrate monovettes, while participants were in the supine position. Blood samples for assay were immediately stored at 4^{o}C. Early the following morning, samples were transported to the department of clinical biochemistry, at the University of Cambridge for processing. All remaining samples were stored at -80 C for further analyses. Total-, and HDL-cholesterol, as well as triglycerides (mmol/L) were measured using the RA 1000 (Bayer Diagnostics, Basingstoke). LDL-cholesterol (mmol/L) was determined using Friedewald criteria [155]. CRP (mg/L) concentrations taken from serum were measured using a full-range, high-sensitivity assay on an Olympus AU640 clinical chemistry analyser (Olympus, UK, Ltd). Absolute white blood cell enumeration (10^3/μL) was obtained using a Coulter MD18 Haemolyser (Coulter Corporation, Miami, FL, USA). Fibrinogen (g/L) was measured using the Clauss method [156].

5.3.4. Study endpoint

Information on vital status was obtained at the UK Office of National Statistics. Follow-up time for mortality was calculated for each individual from time of entry to the study to time of death, or up until February 29, 2008. The primary endpoint in this study was death due to CVD. Qualified nosologists who were masked to any data of the study probands except for the information from the death certificates classified those who died from a CVD event. Death from CVD causes were defined using the International Classification of Diseases ninth

(ICD-9) and tenth (ICD-10) edition codes: CVD mortality (ICD-9 401–448, or ICD-10 I10–I79), CHD mortality (ICD-9 410–414 or ICD-10 I20–I25), and stroke mortality (ICD-9 430–438 or ICD-10 I60–I69).

5.3.5. Statistical methods

A total of 73 (0.3%) participants were excluded due to incomplete resting HR measurement at baseline. Hence the analytic sample consisted of 25,566 (99.7%) men and women. All continuous covariates were checked for normality and those which visually deviated on inspection of the frequency distribution were transformed onto the natural log (base *e*) scale. The study sample was summarised by comparing participants according to RHR quartiles, reporting the mean±SD (or median and inter quartile ranges [IQR] for those variables which were skewed) for continuous measures, and proportions for categorical variables. Comparisons between quartiles were performed by ANOVA with a Bonferroni post-hoc test for continuous parameters, and two-way tables of frequency counts with Pearson's χ^2 test for categorical variables. Kaplan-Meier survival function with Log rank test for equality was used to evaluate the predictive ability of RHR quartiles with CVD mortality. Due to funding, some covariates measured in the present analyses were restricted to approximately 71-86% of the entire cohort. Thus, we employed multiple imputation procedures permitting the substitution of each missing data point with an imputed value drawn from a distribution of the present study's complete data. Details of the variables which underwent multiple imputations are outlined in Table 5.1.

Table 5.1. Reporting multiple imputation according to missing data

	Observations per $m^{\dagger}$			
Variable	Complete	Incomplete	Imputed	Total[‡]
Glucose	18,639	7,000	6,949	25,639
C-reactive protein	18,586	7,053	7,002	25,639
Total white blood cells	18,195	7,444	7,396	25,639
Fibrinogen	22,171	3,468	3,425	25,639

[†]Reported imputed datasets created were *m*=5. [‡]Complete + Incomplete = Total number of observations.

Time-to-event analyses were then performed using stepwise multivariate Cox proportional hazards regression, reporting HRs with 95% CIs. Two models were fitted for analyses; Model 1 adjusted for age and sex, and Model 2 additionally corrected for smoking, physical activity, BMI, waist circumference, total-, LDL-, and HDL-cholesterol, triglycerides, dyslipidaemia, lipid-lowering therapy, glucose, type 2 diabetes, systolic and diastolic blood pressure, hypertension, anti-hypertensive treatment, CRP, fibrinogen, and total white blood count. To better understand the relationship between RHR and CVD mortality, the latter analyses were performed examining RHR as a categorical (quartiles) and continuous (10 bpm increment) parameter, respectively. As anti-hypertensive therapy may influence RHR, participants who reported the use of these agents were excluded from secondary analyses in order to explore whether the initial findings would be affected. Lastly, to minimise potential bias due to subclinical and undetected pre-existing disease at baseline, we also carried out the analyses removing those who died within 2 years of enrolment. The above analyses were considered significant at a *P*-value of <0.05 (two-tailed). All calculations were performed using STATA version 11.2 (StataCorp, Texas).

5.4. RESULTS

During a median follow-up time of 14.9 years, 1,734 (6.8%) deaths occurred due to CVD (incidence, 4.4 per 1,000 person-years). Overall, there was a greater incidence of CVD mortality in the highest quartile of RHR as compared to the others (Table 5.2 and Figure 5.1).

Table 5.2. Population characteristics at baseline according to quartiles of resting heart rate

	Resting heart rate quartiles				
	Q1	Q2	Q3	Q4	*P* value[#]
Patients (*n*)	6,659	6,451	6,300	6,156	
All-cardiovascular mortality (%)	7.2	6.1	6.0	7.8	<0.0001
Age (years)	59.6±9.3	58.6±9.3	58.9±9.3	59.7±9.4	<0.0001
Male sex (%)	59.1	44.9	39.3	36.9	<0.0001
Resting heart rate (beats/minute)	57±4	66±2	74±2	87±8	<0.0001
Waist circumference (cm)	89.3±11.9	87.6±12.3	87.6±12.4	88.9±13.0	<0.0001
Body mass index (kg/m^2)	26.1±3.5	26.2±3.8	26.3±4.0	26.8±4.4	<0.0001
Smoking status (%)					
Never	44.5	45.7	46.2	47.3	<0.0001
Former	47.0	43.5	40.5	38.0	
Current	8.5	10.8	13.3	14.7	
Physical activity status (%)					
Inactive	27.4	28.7	30.2	36.7	<0.0001
Moderately inactive	27.2	28.8	29.8	29.0	
Moderately active	24.8	23.1	22.0	20.1	
Active	20.6	19.4	18.0	14.2	
Glucose (mmol/L)	3.90 (3.40-4.50)	4.00 (3.40-4.60)	4.10 (3.50-4.80)	4.30 (3.60-5.20)	<0.0001
Type 2 diabetes (%)	2.0	1.8	2.3	3.1	<0.0001
Systolic blood pressure (mmHg)	133±18	133±18	135±18	140±18	<0.0001
Diastolic blood pressure (mmHg)	79±10	81±10	83±10	87±11	<0.0001
Hypertension (%)	62.3	60.1	65.0	75.4	<0.0001
Blood pressure lowering therapy (%)	25.4	15.4	15.1	18.7	<0.0001
Lipids (mmol/L)					
Total cholesterol	6.00 (5.30-6.80)	6.00 (5.40-6.80)	6.10 (5.40-6.90)	6.20 (5.50-7.00)	<0.0001

Table 5.2. *continued*

	Resting heart rate quartiles				
	Q1	Q2	Q3	Q4	*P* value[#]
LDL-cholesterol	3.87 (3.23-4.57)	3.85 (3.23-4.54)	3.90 (3.25-4.59)	3.91 (3.29-4.67)	0.004
HDL-cholesterol	1.29 (1.09-1.59)	1.40 (1.10-1.70)	1.40 (1.10-1.70)	1.41 (1.09-1.71)	<0.0001
Triglycerides	1.49 (1.00-2.09)	1.52 (1.10-2.20)	1.57 (1.18-2.20)	1.72 (1.20-2.50)	<0.0001
Dyslipidaemia (%)	67.8	69.0	71.0	76.3	<0.0001
Lipid lowering therapy (%)	1.8	1.4	1.3	1.4	0.10
Markers of inflammation					
C-reactive protein (mg/L)	1.30 (0.70-2.70)	1.50 (0.70-3.10)	1.60 (0.80-3.40)	1.90 (0.90-4.00)	<0.0001
Total white blood cells (10^3/μL)	6.09 (5.19-7.09)	6.19 (5.29-7.29)	6.39 (5.39-7.50)	6.60 (5.60-7.89)	<0.0001
Fibrinogen (g/L)	2.80 (2.37-3.27)	2.85 (2.40-3.35)	2.90 (2.46-3.40)	3.00 (2.50-3.50)	<0.0001

Categorical variables are shown as percentages, and continuous data are presented as mean±SD, or median (IQR) for skewed data. [#]One-way ANOVA was conducted for continuous parameters, and χ^2 test for categorical variables. LDL = low-density lipoprotein; HDL = high-density lipoprotein.

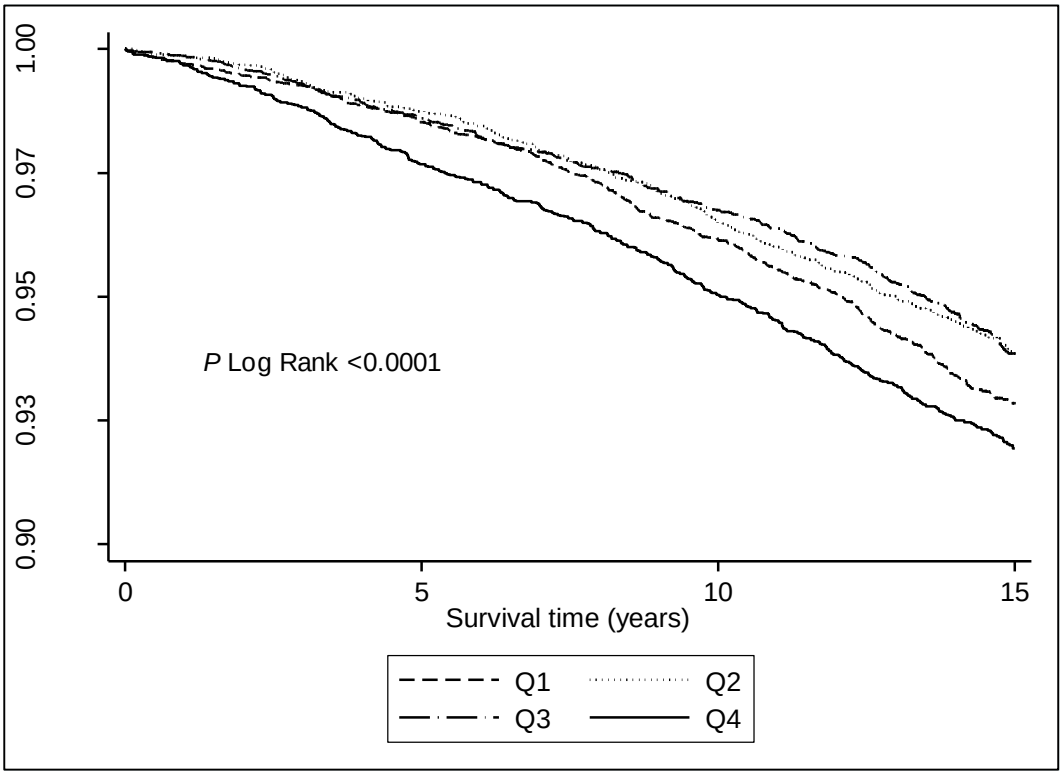

Figure 5.1. Kaplan-Meier curve for cardiovascular mortality according to resting heart rate quartiles. The log-rank test indicated significant differences between quartiles (*P* <0.0001).

Likewise, those in the highest quartile were older, had a higher BMI, consumed more tobacco and tended to be less physically active. Similar observations were observed in sex-specific analyses (Tables 5.3 and 5.4). Of the lipids, there were higher concentrations of total-, LDL-, and HDL-cholesterol, as well as triglycerides and glucose for participants in the upper-most quartile of RHR (Table 5.2). In men, no relationship was found for RHR with both LDL- and HDL-cholesterol (Table 5.3). Overall and sex-specific analyses also revealed both systolic and diastolic blood pressures were higher among participants in the fourth quartile of RHR as compared to those in the lower quartiles (Tables 5.2 to 5.4). As expected, the proportion of type 2 diabetes, arterial hypertension and dyslipidaemia were all significantly higher among those in the top quartile of RHR (Tables 5.2 to 5.4). Conversely, self-reported use of anti-hypertensive therapy was greater among participants in the lowest quartile of RHR. The inflammatory markers CRP, total white cell count, and fibrinogen were

all higher among individuals belonging to the fourth quartile of RHR (Table 5.2). Similar findings for the inflammatory markers based on gender-specific analyses are reported in Tables 5.3 and 5.4.

Table 5.3. Sex-specific characteristics at baseline according to quartiles of resting heart rate

	Resting heart rate quartiles (Men)				
	Q1	Q2	Q3	Q4	*P* value[#]
Patients (*n*)	3,936	2,898	2,473	2,269	
All-cardiovascular mortality (%)	8.7	8.0	8.1	11.2	<0.0001
Age (years)	60.1±9.3	59.0±9.3	59.0±9.3	60.1±9.2	<0.0001
Resting heart rate (beats/minute)	57±5	67±2	74±2	87±8	<0.0001
Waist circumference (cm)	95.1±9.2	95.3±9.6	96.0±9.8	98.0±10.7	<0.0001
Body mass index (kg/m^2)	26.3±3.1	26.4±3.2	26.5±3.4	27.0±3.7	<0.0001
Smoking status (%)					
Never	36.1	33.2	31.8	30.0	<0.0001
Former	55.6	55.0	53.3	53.4	
Current	8.3	11.8	14.9	16.6	
Physical activity status (%)					
Inactive	26.9	30.4	31.1	38.0	<0.0001
Moderately inactive	24.3	24.6	25.2	24.6	
Moderately active	25.7	22.4	22.0	19.9	
Active	23.1	22.6	21.7	17.5	
Glucose (mmol/L)	3.90 (3.40-4.50)	4.00 (3.50-4.70)	4.10 (3.50-4.90)	4.30 (3.70-5.30)	<0.0001
Type 2 diabetes (%)	2.5	2.8	3.4	4.4	<0.0001
Systolic blood pressure (mmHg)	135±17	136±17	138±17	142±18	<0.0001
Diastolic blood pressure (mmHg)	81±10	84±11	86±11	90±12	<0.0001
Hypertension (%)	67.2	67.3	72.3	80.8	<0.0001
Blood pressure lowering therapy (%)	23.8	14.8	15.0	18.8	<0.0001
Lipids (mmol/L)					
Total cholesterol	5.89 (5.19-6.59)	5.90 (5.30-6.60)	6.00 (5.29-6.70)	6.10 (5.30-6.90)	<0.0001

Table 5.3. *continued*

	Resting heart rate quartiles (Men)				
	Q1	Q2	Q3	Q4	*P* value[#]
LDL-cholesterol	3.88 (3.28-4.53)	3.84 (3.26-4.46)	3.90 (3.30-4.52)	3.86 (3.26-4.59)	0.46
HDL-cholesterol	1.20 (1.00-1.40)	1.20 (1.00-1.40)	1.20 (1.00-1.40)	1.20 (1.00-1.40)	0.47
Triglycerides	1.59 (1.20-2.29)	1.70 (1.20-2.50)	1.80 (1.30-2.60)	2.00 (1.40-2.90)	<0.0001
Dyslipidaemia (%)	69.5	72.4	75.1	79.4	<0.0001
Lipid lowering therapy (%)	2.0	1.2	1.3	1.3	0.06
Markers of inflammation					
C-reactive protein (mg/L)	1.30 (0.70-2.70)	1.50 (0.70-3.00)	1.60 (0.80-3.30)	1.90 (0.90-4.00)	<0.0001
Total white blood cells (10^3/μL)	6.19 (5.29-7.19)	6.29 (5.39-7.39)	6.39 (5.59-7.59)	6.79 (5.79-8.00)	<0.0001
Fibrinogen (g/L)	2.73 (2.30-3.20)	2.80 (2.34-3.30)	2.90 (2.40-3.40)	2.90 (2.50-3.50)	<0.0001

Categorical variables are shown as percentages, and continuous data are presented as mean±SD, or median (IQR) for skewed data. [#]One-way ANOVA was conducted for continuous parameters, and χ^2 test for categorical variables. LDL = low-density lipoprotein; HDL = high-density lipoprotein.

Table 5.4. Sex-specific characteristics at baseline according to quartiles of resting heart rate

	Resting heart rate quartiles (Women)				
	Q1	Q2	Q3	Q4	*P* value[#]
Patients (*n*)	2,723	3,553	3,827	3,887	
All-cardiovascular mortality (%)	4.9	4.5	4.7	5.8	0.06
Age (years)	59.0±9.2	58.3±9.2	58.8±9.2	60±9.5	<0.0001
Resting heart rate (beats/minute)	58±4	67±2	74±2	87±7	<0.0001
Waist circumference (cm)	81.0±10.2	81.3±10.5	82.1±10.7	83.8±11.5	<0.0001
Body mass index (kg/m^2)	25.9±4.1	26.0±4.2	26.2±4.3	26.7±4.7	<0.0001
Smoking status (%)					
Never	56.7	55.8	55.7	57.4	<0.0001
Former	34.4	34.2	32.1	29.0	
Current	8.9	10.0	12.2	13.6	
Physical activity status (%)					
Inactive	28.2	27.3	29.5	35.9	<0.0001
Moderately inactive	31.2	32.3	32.9	31.5	
Moderately active	23.6	23.7	22.0	20.1	
Active	17.0	16.7	15.6	12.5	
Glucose (mmol/L)	3.90 (3.40-4.40)	3.90 (3.40-4.50)	4.00 (3.40-4.70)	4.20 (3.60-5.10)	<0.0001
Type 2 diabetes (%)	1.3	1.0	1.5	2.3	<0.0001
Systolic blood pressure (mmHg)	130±19	131±18	133±18	139±19	<0.0001
Diastolic blood pressure (mmHg)	76±10	79±10	81±11	86±11	<0.0001
Hypertension (%)	55.1	54.2	60.2	72.3	<0.0001
Blood pressure lowering therapy (%)	27.7	15.9	15.2	16.6	<0.0001
Lipids (mmol/L)					
Total cholesterol	6.09 (5.39-6.89)	6.14 (5.39-7.00)	6.19 (5.39-7.00)	6.29 (5.59-7.19)	<0.0001

Table 5.4. *continued*

	Resting heart rate quartiles (Women)				
	Q1	Q2	Q3	Q4	*P* value[#]
LDL-cholesterol	3.87 (3.19-4.64)	3.88 (3.20-4.61)	3.90 (3.20-4.69)	3.98 (3.29-4.73)	0.0005
HDL-cholesterol	1.51 (1.29-1.79)	1.53 (1.31-1.78)	1.52 (1.29-1.81)	1.49 (1.28-1.77)	0.0009
Triglycerides	1.29 (0.89-1.79)	1.29 (0.89-1.90)	1.40 (1.00-2.00)	1.50 (1.09-2.19)	<0.0001
Dyslipidaemia (%)	65.4	66.5	68.3	74.5	<0.0001
Lipid lowering therapy (%)	1.6	1.6	1.3	1.5	0.70
Markers of inflammation					
C-reactive protein (mg/L)	1.40 (0.70-2.90)	1.50 (0.70-3.20)	1.60 (0.80-3.40)	1.80 (0.80-4.00)	<0.0001
Total white blood cells (10^3/μL)	6.00 (5.09-7.00)	6.09 (5.19-7.19)	6.29 (5.29-7.50)	6.50 (5.50-7.79)	<0.0001
Fibrinogen (g/L)	2.85 (2.40-3.30)	2.90 (2.41-3.40)	2.94 (2.50-3.48)	3.04 (2.60-3.57)	<0.0001

Categorical variables are shown as percentages, and continuous data are presented as mean±SD, or median (IQR) for skewed data. [#]One-way ANOVA was conducted for continuous parameters, and χ^2 test for categorical variables. LDL = low-density lipoprotein; HDL = high-density lipoprotein.

Cox proportional hazard regression models for the risk of CVD mortality according to RHR quartiles are shown in Figure 5.2. Overall, after correcting for a range of covariates, those in the highest quartile of RHR had an increased risk of CVD mortality by approximately 20% (P =0.02). Sex-stratified analyses indicated the adjusted risk for CVD mortality increased further by almost 20% (P =0.001) in men. However, no association was observed among women. In Figure 5.3, a 10 bpm increment in RHR was associated with an increased adjusted risk of death due to CVD in the overall population HR (95% CI) = 1.05 (1.01-1.10), P =0.02 and in males HR (95% CI) = 1.09 (1.03-1.15), P =0.002. Upon removal of those who reported the use of anti-hypertensive therapy (n = 4,408), we observed the initial findings did not differ materially for the entire cohort HR (95% CI) = 1.23 (1.01-1.48), P =0.03. On the other hand, the risk of CVD mortality slightly attenuated HR (95% CI) = 1.28 (1.04-1.62), P =0.04 for men in the fourth quartile of RHR as compared to the first. Lastly, the results remained similar after excluding participants who died within 2 years of follow-up (n = 307) for the overall cohort HR (95% CI) = 1.16 (1.01-1.34), P =0.04 as well as in men HR (95% CI) = 1.33 (1.10-1.62), P =0.004.

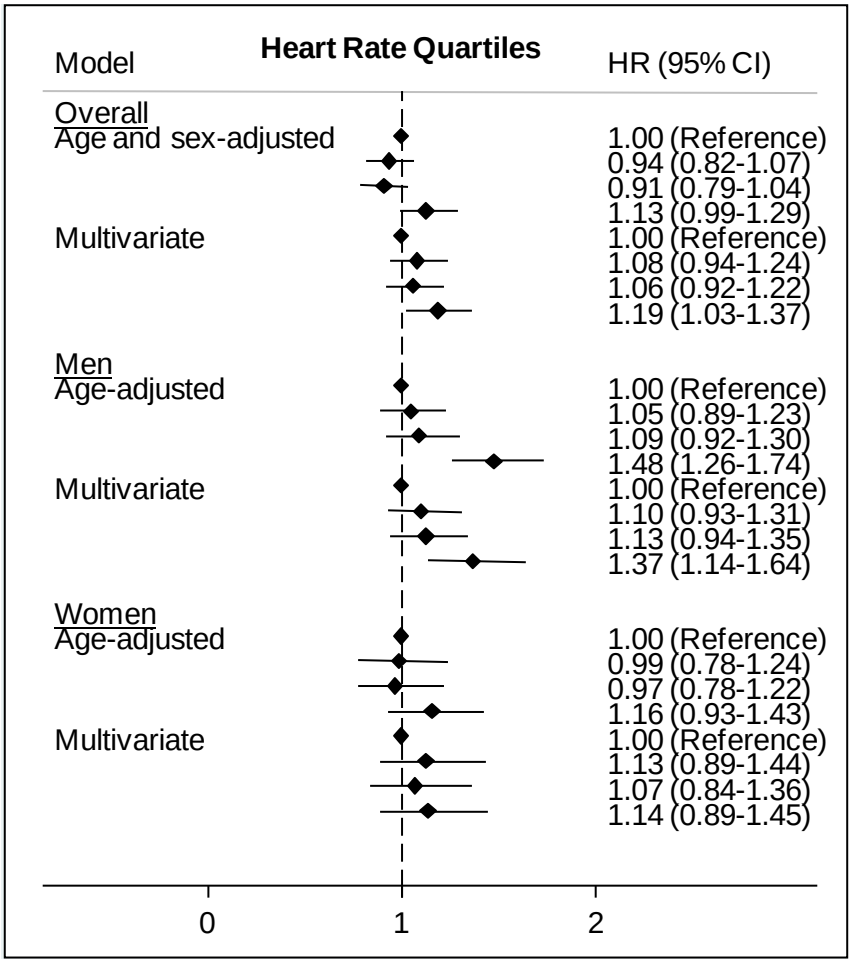

Figure 5.2. Hazard ratio estimates (with 95% CIs) using multiple imputation methods for incident cardiovascular mortality according to resting heart rate quartiles. Multivariate model included age, sex (Overall), smoking, physical activity, body mass index, waist circumference, total-, LDL-, and HDL-cholesterol, triglycerides, dyslipidaemia, lipid-lowering therapy, glucose, type 2 diabetes, systolic and diastolic blood pressure, hypertension, anti-hypertensive treatment, high sensitivity C-reactive protein, fibrinogen, and total white blood count.

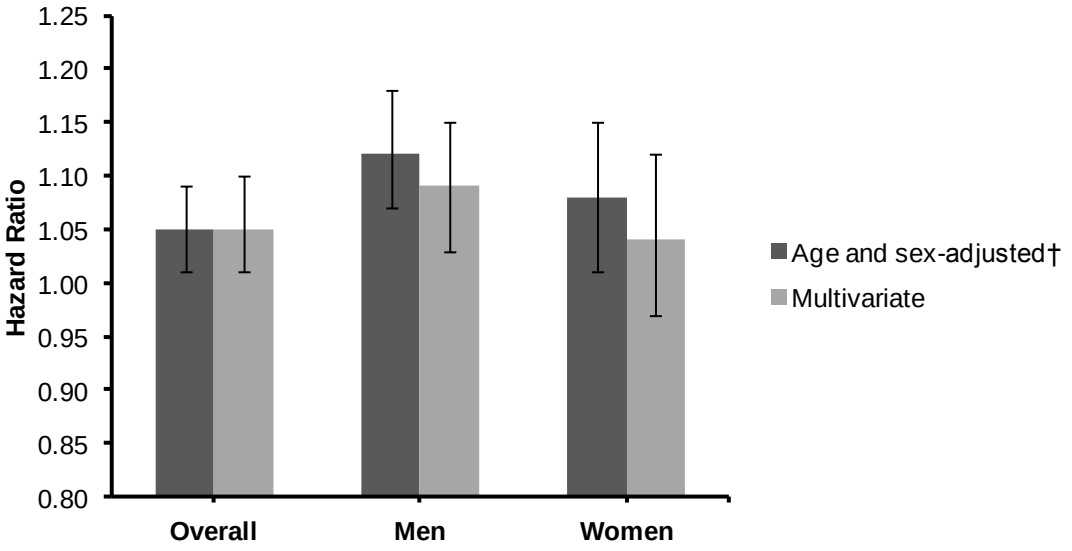

Figure 5.3. Hazard ratio estimates (with 95% CIs) using multiple imputation methods for incident cardiovascular mortality according to resting heart rate (per 10 beats/minute increments). Multivariate model was adjusted as abbreviated in Figure 5.2. †Sex was included as a covariate in Overall only.

5.5. DISCUSSION

In this study, the integral relationship between elevated RHR and CVD mortality was consistent with a number of published data in the general population [53, 54, 57, 60, 63]. Of particular note, a faster RHR was found to be a critical determinant of all-cardiovascular death in men, but not in women; though only a few investigations have reported a higher RHR as an independent predictor of CVD mortality in women [55, 69, 110, 157].

Consistently, several large population-based cohort studies with particularly lengthy follow-ups have emphasized the relevance of RHR as a significant predictor of cardiovascular risk. Across 30-years of follow-up, 894 incidents of CVD evolving from 5,070 participants were documented in the Framingham Heart Study, of which the number of case fatalities increased impressively with antecedent RHR, and was most striking among men aged 35 to 64 years [54]. In parallel, a study consisting of 5,713 asymptomatic working men

followed for a period of 23 years demonstrated the risk of sudden death due to MI increased in subjects with a RHR >75 bpm [66]. Likewise, in the MATISS Project including 2,533 Italian middle-aged males who were followed for a combined duration of 24,457 person-years, RHR was found to be a robust independent predictor of total, CVD and non-CVD mortality [63]. Collectively, these findings taken together with the present study observations underline the significance of RHR as an inexpensive, and easily measured clinical parameter for determining the risk of adverse CVD outcomes.

Moving forward, there appears little doubt that dysfunctional ANS activity plays a central role in the pathogenesis of a number of vascular anomalies. To this end, predominant sympathetic overactivity is a crucial feature in atherosclerotic plaque development via initiation of several haemodynamic (i.e., tachycardia, hypertension) and metabolic (i.e., hyperglycaemia, dyslipidaemia) alterations [158]. In light of this conjecture, it may seem that RHR is merely a marker of ANS dysfunction rather than a risk factor *per se*. Despite this, ample literature supports the notion that a rapid RHR may intervene along a chain of events, thereby promoting CVD [159, 160]. These mechanisms include, but are unlikely restricted to, disturbed haemodynamics, oxidative stress, vascular remodelling, endothelial dysfunction and inflammation [158, 159]. Moreover, elevated RHR may, in part, induce myocardial ischemia by amplifying myocardial oxygen demand as well as inhibiting coronary blood flow [159, 161]. We may add that these actions perhaps due to the involvement of RHR may subsequently enhance the progression of plaque vulnerability as well as erosion and rupture [159].

Accordingly, we speculate that slowing the RHR may prove a useful adjunct for interrupting the risk associated with CVD mortality. In the BEAUTIFUL study [162], the novel specific pulse rate-lowering agent ivabradine, afforded an opportunity to evaluate the efficacy of selectively slowing the RHR without altering other aspects of cardiac function. Here, the usefulness of ivabradine for lowering the primary endpoint was not achieved. However,

ivabradine did reduce a number of secondary outcomes including admission to hospital for fatal and non-fatal MI (36%), as well as coronary revascularization (30%). The Systolic Heart failure treatment with the *I*f inhibitor ivabradine Trial (SHIFT) [163] further confirmed the importance of RHR reduction by ivabradine. Here, patients who received ivabradine treatment had fewer serious adverse cardiovascular events (n = 3,388 events) as compared to patients who received placebo (n = 3,847 events). Significantly, in the latter trial, intervention to modulate RHR successfully resulted in parallel modulation of CVD risk. In this respect, it may well be postulated that a raised RHR should be considered a risk factor for CVD. Undeniably, these reports provide a compelling basis to develop similar, well-designed, randomized controlled trials aimed at exclusively slowing the RHR, as a means of addressing the risk associated with vascular disorders.

Though the present study was prospective in nature, RHR was only measured at a single momentary time point. Further, there is significant diurnal variation in RHR, and thus, may not reflect sustained chronic tachycardia. It therefore remains to be confirmed in our cohort whether rapid RHR is itself a cause, or is purely associated with mortality. Almost 7% less patients reported the use of anti-hypertensive treatment in the highest compared to the lowest quartile of RHR. Such agents are generally administered for the management of cardiac arrhythmias, which in part, slow the RHR. Therefore, this may have inflated the risk estimates observed in the present investigation. Nonetheless, we counter that after removing individuals who reported the use of anti-hypertensive medication, the results did not materially change.

Though the relationship between RHR and CVD is well documented, it has remained neglected as a potential risk factor for some time. Only recently have current guidelines begun to recognize RHR as an important parameter with prognostic implications for cardiovascular health. The European Society of Cardiology and the European Society of

Hypertension [164] along with the Global Registry of Acute Coronary Events score [165] have since included RHR as a risk factor because of the growing body of evidence describing its relationship with CVD morbidity and mortality. Our data support the contention that RHR as an inexpensive, easily measured and modifiable risk factor should no longer be overlooked, especially among the general population.

CHAPTER SIX

6.0. INFLUENCE OF RESTING HEART RATE ON MORTALITY IN PATIENTS UNDERGOING CORONARY ANGIOGRAPHY (FROM THE LUDWIGSHAFEN RISK AND CARDIOVASCULAR HEALTH STUDY)

6.1. ABSTRACT

Several epidemiological studies have reported an association between elevated resting heart rate and reduced survival. The usefulness of resting heart rate in predicting endpoints in high-risk patients is yet to be definitively established. The purpose of this study was to clarify the relation between resting heart rate with total and cardiovascular mortality in patients who had undergone coronary angiography. A total of 3,316 Caucasian individuals with availability of a coronary angiogram were prospectively followed from 2001-2011 (median 9.9 years). We explored the effect of resting heart rate on total and cardiovascular mortality, while correcting for a number of confounders. Patients in the highest quartile (resting heart rate ≥84 bpm) had a survival time reduced by 1.2 and 1.4 years for overall and cardiovascular mortality, respectively. Likewise, these patients had a significantly elevated adjusted risk for total (HR [95% CI] = 1.39 [1.17, 1.67], P_{trend} <0.001) and cardiovascular mortality (HR [95% CI] = 1.38 [1.08, 1.78], P_{trend} =0.004). In conclusion, resting heart rate is an inexpensive, easily measured and modifiable predictor of mortality.

6.2. INTRODUCTION

Numerous epidemiological studies have linked resting tachycardia to increased risk of morbidity and mortality [53-55, 111, 112]. One of the first to describe this association was the Chicago People's Gas Company Study [53]. Here, mortality from CVD and non-CVD causes generally increased with accelerating RHR. Despite these studies having identified an association between RHR and CVD outcome in a general population, few have explored this question in populations at intermediate-to-high risk. Examining the prognostic value of RHR in patients with stable CAD, Diaz and colleagues [67] found that high RHR independently predicted overall and CVD mortality. Likewise, admission RHR was considered to be the best predictor for in-hospital and 1 year post-discharge mortality in patients hospitalized for acute MI [58]. In light of these observations, however, resting tachycardia as a modifiable CVD risk factor is often overlooked [111, 112], especially among high-risk patients. Therefore, the present investigation sought to determine the role of RHR as an important predictor of mortality in patients who have undergone coronary angiography.

6.3. METHODS

6.3.1. Patients and setting

The LURIC study is an ongoing prospective cohort study of patients referred for coronary angiography, and is designed to evaluate determinants of cardiovascular health [166]. In total, 3,316 subjects (2,309 men and 1,007 women) between the ages of 18-95 years were recruited from July 1997 to January 2000, at the Herzzentrum (Cardiac Center) Ludwigshafen in southwest Germany. In order to reliably classify the cardiovascular disease phenotype at study entry and to minimise misclassification of silent CAD as non-CAD, inclusion criteria

for LURIC demanded the availability of a coronary angiogram in all participants. Other inclusion and exclusion criteria such as restriction to Caucasians of German ancestry and to stable clinical disease, with the exception of acute coronary syndromes (ACS) were established in order to limit the genetic and the clinical heterogeneity of the sample. An ACS was diagnosed if patients presented within 7 days of onset of symptoms of unstable angina pectoris or acute MI, comprising non-ST-elevation MI (troponin T > 0.1 µg/L) and ST-elevation MI (troponin T > 0.1 µg/L). Participants with a history of malignancy within the past five years, or any predominant non-cardiac disease were excluded from the study. Written, informed consent was obtained from each participant, and the study was approved by the institutional review board at the Ärztekammer Rheinland-Pfalz (Medical Association of Rheinland-Pfalz).

6.3.2. Baseline examination

Detailed descriptions of the LURIC baseline examination are described elsewhere [166]. Briefly, all variables included in the present investigation were selected on the background of previous literature, data availability, and the possibility of confounding. Between the hours of 06:00-09:00am, RHR was obtained by trained nurses using electrocardiography. Five measures were taken 30 seconds apart, following a 10 minute rest in the supine position away in a quiet room, with the average derived from the last 2 measures. Death certificates obtained from local person registries were reviewed to classify those who died from cardiovascular and non-cardiovascular events. Death from cardiovascular causes included sudden cardiac death (SCD), fatal MI, death due to heart failure, death after intervention to treat CAD, stroke and other deaths due to heart disease. Two experienced physicians masked to any data of the study probands except for the information from the death certificates independently classified the causes of death.

6.3.3. Statistical methods

Continuous parameters following a non-normal distribution underwent natural logarithmic transformation. Baseline characteristics according to quartiles of RHR are given as percentages for categorical data, and depending on their distribution, continuous data are presented as means±SD values (normal distribution) or as geometric means with 95% CIs (skewed distribution). Comparisons between groups were performed by ANOVA for continuous parameters, and by Pearson's χ^2 test for categorical variables. Kaplan-Meier survival function with Log rank test for equality was used to evaluate the predictive ability of RHR quartiles with overall and CVD mortality. We also fitted a fractional polynomial model in order to evaluate the non-linear pattern of baseline RHR as a continuous parameter with all-cause and CVD mortality.

Time-to-event analyses were performed using multivariable Cox proportional hazards regression. Three models were fitted for analyses; Model 1 was unadjusted, Model 2 adjusted for age and sex, and Model 3 also corrected for a range of conventional risk factors including smoking, alcohol, exercise, obesity, CRP, hypertension, diabetes mellitus, dyslipidaemia, and angina pectoris, symptoms of heart failure, CAD severity, family history and medical therapy. As anti-hypertensive therapy (i.e., ACE-inhibitors, β-blockers, and statins) may influence RHR, patients who reported the use of these agents were removed from secondary analyses in order to examine whether the initial findings would be affected. Further, to minimise potential bias due to subclinical and undetected pre-existing disease at baseline, we also carried out the analyses excluding those who died within 2 years since the time of enrolment. The above analyses were considered significant at a *P*-value of <0.05 (two-tailed). All calculations were performed using STATA version 11.2 (StataCorp, Texas).

6.4. RESULTS

The median duration of follow-up was 9.9 years. There were 995 (30%) deaths, of which 558 (56%) occurred due to CVD. A higher incidence of all-cause and CVD mortality was observed with increasing quartiles of RHR (Table 6.1). Patients in the upper quartile (RHR ≥84 bpm) had a higher waist circumference, BMI, systolic and diastolic blood pressure, total, LDL- and LDL-to-HDL-cholesterol ratio, glucose, glycosylated haemoglobin A_{1c}, interleukin-6 (IL-6), CRP, and symptoms of heart failure (Table 6.1). A greater proportion of MetS, hypertensive, diabetic, and less physically active patients were found among higher quartiles (Table 6.1). In contrast, there was a greater prevalence of angina pectoris in the lowest (RHR <64 bpm) compared to the highest quartile of RHR. Likewise, β-blockers and statins were more prevalent in the lowest quartile compared to the others (Table 6.1). Figure 6.1 presents Kaplan-Meier survival plots for total and CVD mortality according to quartiles of RHR. Patients in the highest quartile for RHR showed a greater risk for all-cause and CVD mortality. Log rank test for equality revealed a significant difference between quartiles (*P* <0.001).

Table 6.1. Baseline characteristics of the LURIC study population according to quartiles of resting heart rate

	Resting heart rate quartiles				
	Q1 (*n*=829)	Q2 (*n*=815)	Q3 (*n*=843)	Q4 (*n*=829)	*P* value
All-cause mortality	221 (26.6%)	225 (27.6%)	248 (29.4%)	301 (36.3%)	<0.001
Cardiovascular disease mortality	123 (14.8%)	126 (15.4%)	138 (16.3%)	171 (20.6%)	0.007
Age (years)	62.7±10.5	62.9±10.7	62.8±10.3	62.2±10.7	0.49
Men	616 (74.3%)	566 (69.4%)	558 (66.1%)	570 (68.7%)	0.003
Waist (cm)	98.4±10.4	98.2±11.7	99.5±12.4	99.5±12.6	0.04
Body mass index (kg/m^2)	27.2±3.6	27.3±3.8	27.6±4.2	27.8±4.5	0.03
Metabolic syndrome	429 (51.7%)	415 (50.9%)	495 (58.7%)	512 (61.7%)	<0.001
Heart rate (beats/minute)	55±4	64±2	71±2	84±8	<0.001
Systolic blood pressure (mm Hg)	140±24	139±23	140±23	144±23	<0.001
Diastolic blood pressure (mm Hg)	78±11	79±11	81±10	84±11	<0.001
Prevalence of hypertension	421 (50.7%)	393 (48.2%)	450 (53.3%)	500 (60.3%)	<0.001
Triglycerides (mg/dl)	147.92 (142.60, 152.34)	148.80 (143.49, 154.11)	152.34 (147.92, 157.66)	155.00 (149.69, 160.32)	0.16
Total cholesterol (mg/dl)	185.23 (182.91, 187.55)	186.77 (184.06, 189.01)	189.48 (187.16, 192.19)	191.80 (189.01, 194.51)	0.002
Low density lipoprotein cholesterol (mg/dl)	108.66 (106.34, 110.10)	110.21 (107.90, 112.53)	111.76 (109.44, 114.11)	112.92 (111.37, 116.41)	0.02
High density lipoprotein cholesterol (mg/dl)	37.51 (36.74, 38.28)	37.12 (36.35, 37.90)	36.74 (36.10, 37.51)	37.12 (36.35, 37.90)	0.59
Low/high density lipoprotein cholesterol ratio	2.89 (2.82, 2.96)	2.96 (2.88, 3.03)	3.02 (2.95, 3.10)	3.06 (2.97, 3.14)	0.008
Prevalence of dyslipidaemia	557 (67.1%)	557 (68.3%)	596 (70.7%)	577 (69.6%)	0.43
Glucose (mg/dl)	93.67 (91.33, 94.21)	94.39 (92.77, 96.01)	96.73 (94.93, 98.36)	103.40 (101.24, 105.74)	<0.001
Glycosylated haemoglobin A_{1c} (%)	6.1	6.2	6.3	6.5	<0.001
Diabetes mellitus	91 (10.9%)	134 (16.4%)	155 (18.3%)	210 (25.3%)	<0.001
Interleukin-6 (ng/L)	2.95 (2.79, 3.13)	3.22 (3.03, 3.42)	3.50 (3.29, 3.71)	4.03 (3.78, 4.30)	<0.001
C-reactive protein (mg/L)	2.59 (2.37, 2.82)	3.20 (2.93, 3.49)	3.85 (3.52, 4.21)	4.85 (4.41, 5.32)	<0.001
Smoking status					
Never	274 (33.0%)	303 (37.1%)	319 (37.8%)	298 (35.9%)	0.23
Former	396 (47.7%)	354 (43.3%)	348 (41.2%)	370 (44.6%)	
Current	159 (19.1%)	158 (19.3%)	176 (20.8%)	161 (19.4%)	
Current alcohol drinker	443 (53.4%)	386 (47.3%)	410 (48.6%)	414 (49.9%)	0.08
Exercise[†]					
Low	189 (22.8%)	199 (24.4%)	202 (23.9%)	261 (31.4%)	0.003
Average	448 (54.0%)	423 (51.9%)	460 (54.5%)	420 (50.6%)	
High	173 (20.8%)	175 (21.4%)	165 (19.5%)	133 (16.0%)	

Table 6.1. *Continued*

	Resting heart rate quartiles				
	Q1 (*n*=829)	Q2 (*n*=815)	Q3 (*n*=843)	Q4 (*n*=829)	*P* value
Family history of cardiovascular disease	442 (53.3%)	427 (52.3%)	440 (52.1%)	420 (50.6%)	0.75
Angina status					
Stable	257 (31.4%)	272 (34.2%)	269 (32.9%)	219 (27.0%)	0.04
Unstable	321 (39.2%)	292 (36.7%)	303 (37.0%)	316 (39.0%)	
New York Heart Association functional class					
1	471 (56.8%)	410 (50.3%)	460 (54.5%)	381 (45.9%)	<0.001
2	223 (26.9%)	248 (30.4%)	256 (30.3%)	240 (28.9%)	
3	113 (13.6%)	130 (15.9%)	110 (13.0%)	175 (21.1%)	
4	22 (2.6%)	27 (3.3%)	17 (2.0%)	33 (3.9%)	
Coronary Artery Disease severity					
0	241 (29.3%)	239 (29.6%)	269 (32.5%)	286 (35.1%)	0.01
1	154 (18.7%)	151 (18.7%)	146 (17.6%)	169 (20.7%)	
2	182 (22.1%)	158 (19.6%)	144 (17.4%)	139 (17.0%)	
3	244 (29.7%)	257 (31.9%)	268 (32.4%)	220 (27.0%)	
Cardiovascular medication use					
Angiotensin converting enzyme-inhibitors	444 (53.5%)	428 (52.5%)	438 (51.9%)	459 (55.3%)	0.52
β-blockers	600 (72.3%)	553 (67.8%)	519 (61.5%)	427 (51.5%)	<0.001
Statins	423 (51.0%)	397 (48.7%)	399 (47.3%)	336 (40.5%)	<0.001

Data are expressed as *n* (%), mean±SD, or geometric mean (95% confidence interval). [†]Exercise was recorded using an 11-point scale ranging from bedridden to extremely active, and categorised into “low” (not very active), “average” (usual office work) and “high” (heavy work or sports).

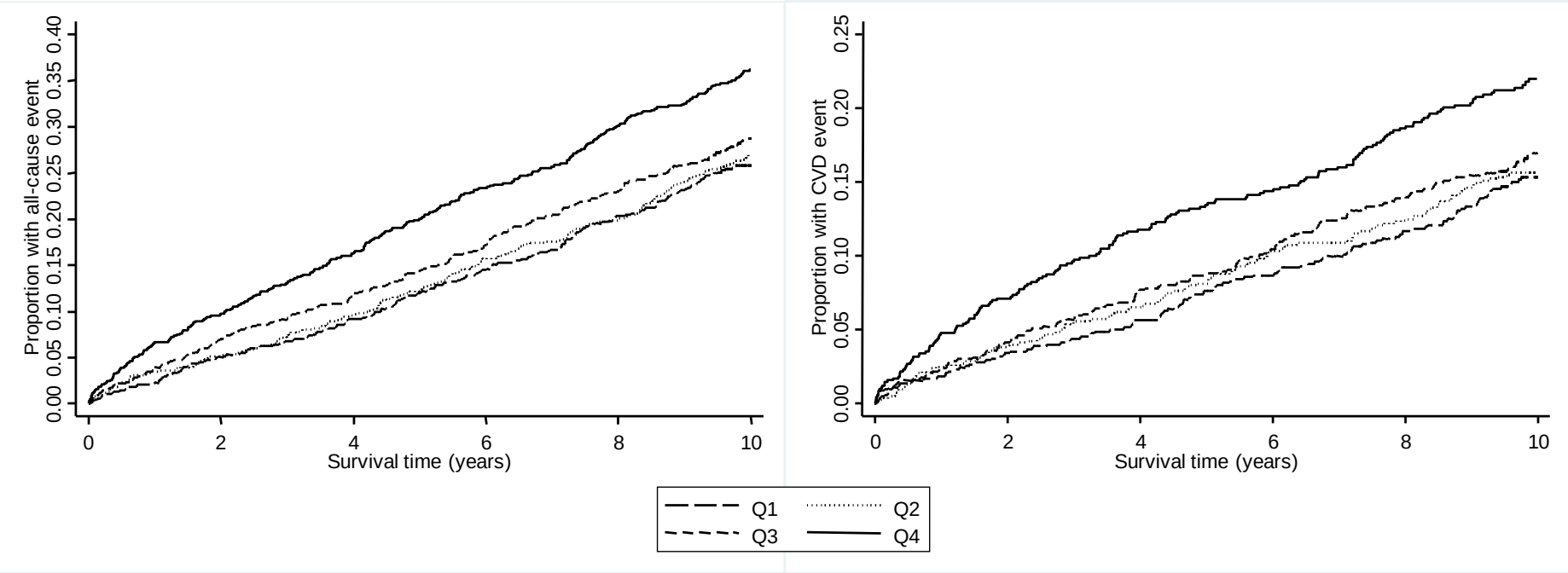

Figure 6.1. Kaplan-Meier plots for all-cause and cardiovascular mortality according to resting heart rate quartiles. Log-rank tests revealed significant differences between resting heart rate quartiles (*P* <0.001). CVD = cardiovascular disease.

Interestingly, after correction for covariates, fractional polynomial models revealed a J-shaped curve for RHR with overall and CVD mortality (Figure 6.2). That is, in addition to those with a high RHR, patients with a very low RHR also appear to be at risk of experiencing either endpoint. Cox proportional hazard regression models for overall and CVD mortality are presented in Table 6.2. There was a survival reduction of 1.2 and 1.4 years for those in the highest compared to those in the lowest quartile for overall and CVD mortality, respectively. Patients in the highest quartile had an increased risk for overall mortality by almost 40% after adjusting for a range of potential confounders (Table 6.2). Likewise, these patients had a significantly greater risk for CVD mortality by around 40% (Table 6.2). In Figure 6.3, a 10 bpm increase in RHR was significantly associated with overall (HR [95% CI] = 1.12 [1.06, 1.19], P <0.001) and CVD mortality (HR [95% CI] = 1.13 [1.05, 1.22], P =0.001) following adjustment.

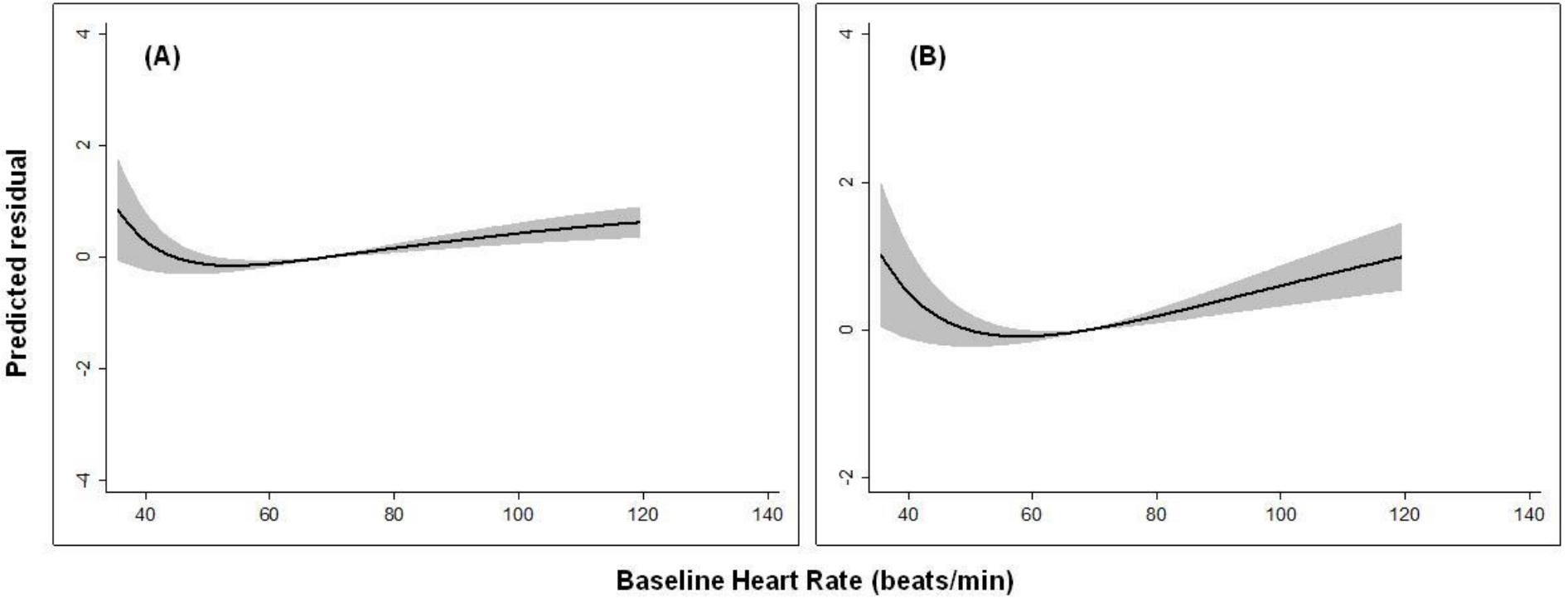

Figure 6.2. Fractional polynomial models present the non-linear pattern for baseline resting heart rate (continuous) according to overall (A) and cardiovascular mortality (B). Models adjusted for covariates as seen in Table 2. Grey regions are 95% confidence intervals.

Table 6.2. Crude and adjusted hazard ratios (with 95% CIs) for all-cause and cardiovascular mortality according to resting heart rate quartiles

	Resting heart rate quartiles				
	Q1	Q2	Q3	Q4	P_{trend}
All-cause mortality					
Median follow-up time (yrs)	5.5	5.4	5.0	4.3	
No. alive/deaths	608/221	590/225	595/248	528/301	
Model 1, HR (95% CI)	1 (reference)	1.06 (0.88, 1.28)	1.16 (0.96, 1.39)	1.54 (1.29, 1.84)	<0.001
Model 2, HR (95% CI)	1	1.05 (0.87, 1.27)	1.26 (1.05, 1.52)	1.70 (1.42, 2.03)	<0.001
Model 3, HR (95% CI)	1	0.94 (0.78, 1.14)	1.19 (0.98, 1.43)	1.39 (1.17, 1.67)	<0.001
Cardiovascular mortality					
Median follow-up time (yrs)	4.9	4.7	4.6	3.5	
No. alive/deaths	706/123	689/126	705/138	658/171	
Model 1, HR (95% CI)	1 (reference)	1.10 (0.86, 1.42)	1.14 (0.89, 1.47)	1.56 (1.24, 1.98)	<0.001
Model 2, HR (95% CI)	1	1.10 (0.85, 1.41)	1.25 (0.97, 1.60)	1.72 (1.36, 2.18)	<0.001
Model 3, HR (95% CI)	1	0.97 (0.75, 1.26)	1.15 (0.89, 1.48)	1.38 (1.08, 1.78)	0.004

Model 1 was unadjusted. Model 2 adjusted for age and sex. Model 3 also adjusted for smoking, alcohol, exercise, obesity, high sensitivity C-reactive protein, hypertension, diabetes mellitus, dyslipidaemia, un/stable angina, family history of stroke or myocardial infarction, New York Heart Association functional class, number of diseased vessels, and cardiovascular medication. HR = hazard ratio; CI = confidence interval.

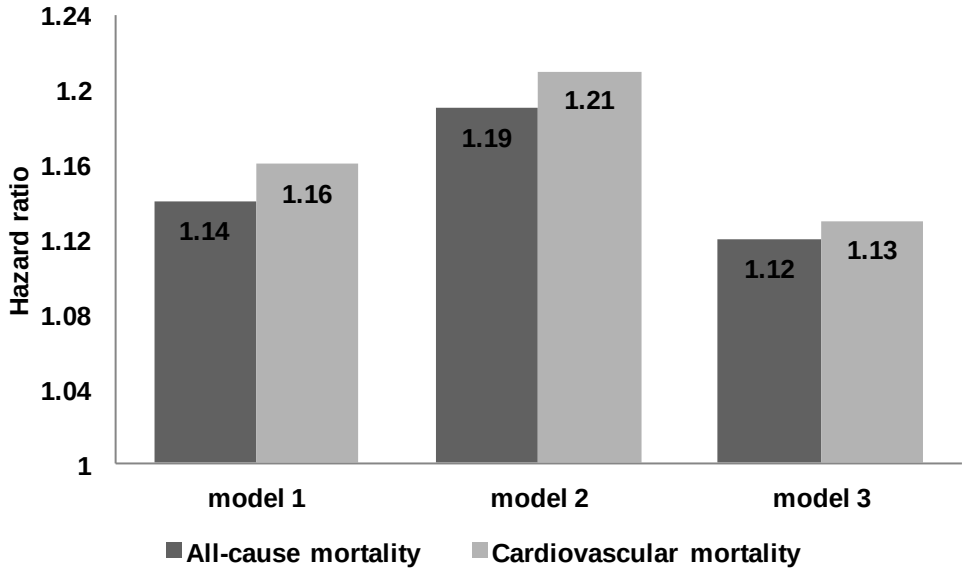

Figure 6.3 Hazard ratio estimates for resting heart rate (per 10 beats/minute increments) according to all-cause and cardiovascular mortality. Models were adjusted as abbreviated in Table 6.2.

The analyses were also carried out after excluding those who reported the use of ACE-inhibitors, β-blockers and/or statins (n = 758). For the highest quartile, the association between RHR with total (HR [95% CI] = 1.38 [1.12, 1.70], P_{trend} <0.001) and CVD mortality (HR [95% CI] = 1.30 [0.99, 1.73], P_{trend} =0.02) remained essentially unchanged. Lastly, we removed patients who died within 2 years (n = 224) since the time of enrolment, likely the result of a pre-existing chronic illness. We found RHR continued to remain a strong independent predictor of overall (HR [95% CI] = 1.32 [1.07, 1.62], P_{trend} =0.002) and CVD mortality (HR [95% CI] = 1.26 [0.94, 1.67], P_{trend} =0.04).

6.5. DISCUSSION

In the present study, RHR, a familiar and easily accessible clinical parameter was found to be an independent risk predictor for total and CVD mortality. Though these findings apply to

persons at intermediate-to-high cardiovascular risk, they are nonetheless consistent with published data from a number of epidemiological studies in general populations [53-55, 69, 70].

Among the few studies that examined these associations in high risk patients, one investigation retrospectively examined data from 106 patients who underwent coronary angiography, and observed a positive association between plaque rupture and RHR >80 bpm [167]. Similarly, in the oral glycoprotein IIb/IIIa inhibition with Orofiban in Patients with Unstable coronary Syndromes-Thrombolysis In Myocardial Infarction trial, Kovar and colleagues [168] showed mortality at 30 days and 10 months increased progressively according to higher RHR strata. Likewise, in the Asymptomatic Cardiac Ischemic Pilot Study, Pratt and co-workers [169] demonstrated that the incidence rate of ischemic events in patients with stable CAD was associated with their mean RHR, and those who had a RHR >80 bpm presented an almost two-fold increase in signs/symptoms of ischemia compared to those with a RHR <70 bpm.

Though the pathophysiologic mechanisms underlying the development of CAD are, in general, well recognized [170, 171], it is possible in theory that an elevated RHR is influential in identifying the risk of plaque instability. For example, sympathetic over-activity plays a vital role in the pathogenesis of atherosclerosis, most often through stimulation of several hemodynamic (i.e. tachycardia) and metabolic changes [158]. In this regard, resting tachycardia likely reflects an imbalance between sympathetic and parasympathetic tone [172]. A higher RHR may well be an indicator of dysregulated hemodynamic and ANS function, encouraging CVD morbidity and mortality by inducing atherosclerotic plaque disruption [173].

Indeed, resting tachycardia directly affects vascular risk by contributing to an imbalance between demand and supply of myocardial oxygen consumption [112]. An elevated RHR causes both an increase in myocardial oxygen demand, and a decline in coronary blood supply (the latter occurring via a shorter diastolic period) [67, 112], inducing fatigue and fracture

of elastic fibres within the arterial wall [172]. Any increase in RHR would therefore be deleterious, as it further decreases diastolic perfusion, augments stealing from the ischemic zone and impairs blood supply at the ischemic obstruction, further compromising coronary flow [174]. Seemingly, these functional alterations would likely provoke the onset of atherosclerotic plaque formation, and precipitate the later stages of atherosclerosis that lead to plaque rupture and coronary thrombosis [112].

There are several limitations to the present data that bear mention. Inclusion of only Caucasian subjects limits the generalisability of our findings which are therefore not applicable to other ethnic populations with CAD. That said it is unlikely that the pathophysiology differs significantly between ethnicities as supported by similar associations observed in other ethnic groups [55, 114, 175]. Although prospective in patient enrollment, RHR was only determined at a single time point, making it observational in nature. Thus, it cannot be confirmed from the present data whether rapid RHR is itself a cause, or is merely associated with mortality. Nevertheless, through multivariable analyses, we did however, take into account several risk factors that are most often associated with pulse rate, and which may have confounded the relationship between resting tachycardia and mortality. Moreover, two recent epidemiological studies with particularly lengthy follow-ups support a possible link. In the first study [66] RHR was determined in 5,713 middle-aged asymptomatic men, with a mean follow-up duration of 23 years. Here, the risk of death from total mortality and MI increased incrementally with RHR, which remained significant after correction for a number of known risk factors including age, diabetes, arterial hypertension, obesity and physical activity. Second, in the CASS investigation [67] involving 24,913 patients with suspected or proven CAD followed for a median of 14.9 years, RHR was also a robust independent predictor of overall and CVD mortality. On the background of this data, a faster RHR presents as a significant cardiovascular parameter, playing an integral role in predicting adverse

complications at different stages of the cardiovascular continuum. Interestingly, fractional polynomial models revealed that patients with a very low RHR also presented with greater risk in respect to both endpoints. To this end, our results may, in part, provide additional insight into the relative prognostic contribution of RHR as a risk factor for mortality. However, because the number of our patients with a very low RHR was minimal, and perhaps underpowered, we did not subsequently assess its significance with the risk of mortality. Nonetheless, we encourage forthcoming studies to consider the usefulness of a very low RHR as a potential hazard, rather than simply focussing on the effects of a high RHR, as here, its relationship with mortality seems non-linear.

CHAPTER SEVEN

7.0. EVIDENCE OF A SYNERGISTIC ASSOCIATION BETWEEN RESTING HEART RATE, INFLAMMATION, AND CARDIOVASCULAR MORTALITY IN PATIENTS UNDERGOING CORONARY ANGIOGRAPHY

7.1. ABSTRACT

Both elevated inflammatory activity and sustained tachycardia reflects unfavourable cardiovascular risk profiles, and there is evidence to suggest the deleterious effects of inflammation are amplified by increased heart rate. The purpose of this study was to assess the interaction between resting heart rate and inflammation in cardiovascular mortality. 3,267 patients (2,283 men), aged 18-95 years, scheduled for coronary angiography, were followed prospectively. By principle component analysis, we developed an overall multi-marker index of inflammation weighting the respective coefficients of five inflammatory markers including: interleukin-6, C-reactive protein, serum amyloid A, neutrophils, and fibrinogen. Cox proportional hazard models were employed to evaluate the relationship between inflammation and resting heart rate with cardiovascular mortality. Across 29,940 person years of follow-up, there were 546 (17%) deaths due to cardiovascular disease. Significantly, we observed a strong synergistic effect of inflammatory activity and concurrent elevated heart rate. For cardiovascular disease mortality, patients in the highest quartile of inflammation had an adjusted HR (95% CI) of 1.84 (1.31-2.57), P <0.0001 if their resting heart rate was <75 beats/minute. Substantially, patients had a greater adjusted HR of 7.50 (3.21-17.50), P <0.0001 if their resting heart rate was ≥75 beats/minute. The present analyses underline elevated inflammation as a risk factor for cardiovascular mortality. The effects of inflammation were strongly amplified by a faster resting heart rate. If confirmed by additional studies, this association may prove a useful adjunct for therapeutic approaches to alleviate symptoms and prolong survival.

7.2. INTRODUCTION

CAD represents an important public health burden. In particular, lifetime risk of CHD is around one in two for men and one in three for women over the age of 40 [176]. Aside from the more recognised parameters of CAD risk such as smoking, diabetes, hypertension, and dyslipidaemia, compelling evidence has now accumulated in support of chronic inflammation as another risk factor, contributing towards all stages of atherosclerosis from endothelial dysfunction and plaque formation to plaque disruption and thrombosis [48, 177].

Recent evidence indicates that inflammation and sustained tachycardia interact at several levels of the cardiovascular continuum [159], and may hereby exert a synergistic effect on cardiovascular morbidity and mortality. For example, elevated RHR increases tensile stress which apart from inducing endothelial injury also increases endothelial permeability to circulating inflammatory mediators [158]. In addition, dysfunctional ANS activity may underlie both progression of inflammation as well as elevated RHR [7]. Stimulation of efferent vagus nerve activity has been associated classically with normalizing tachycardia, whereas experimental evidence has shown that this process also inhibits inflammation via stimulation of the cholinergic anti-inflammatory pathway [178, 179]. Similarly there is ample evidence that elevated sympathetic activity modifies the inflammatory process and thereby promotes endothelial dysfunction and subsequent atheroprogression [160, 180]. Though, clearly, further studies are required to test the notion that a high RHR will exacerbate the effects of inflammation on CVD.

In light of the preceding discussion, this study was undertaken to evaluate the inter-relationship between RHR and inflammation with CVD mortality in a large cohort of German patients enrolled in the LURIC study.

7.3. METHODS

7.3.1. Study population

The LURIC study is a prospective cohort study designed to investigate environmental and genetic risk factors for CVD [166]. A total of 3,316 patients (2,309 men and 1,007 women), aged 18 to 95 years were enrolled in the LURIC study. A baseline examination was performed between July 1997 and January 2000 in a single tertiary care medical centre in South-West Germany (Herzzentrum, Ludwigshafen). Inclusion criteria were availability of a coronary angiogram, Caucasians of German ancestry to limit genetic heterogeneity, and clinical stability, with the exception of ACS. An ACS was diagnosed if patients presented within 7 days of onset of symptoms of unstable angina pectoris or acute MI, comprising non-ST-elevation MI (troponin T > 0.1 µg/L) and ST-elevation MI (troponin T > 0.1 µg/L). Participants with a history of malignancy within the past five years, or any predominant non-cardiac disease were excluded from the study. The LURIC study was approved by the institutional review board of the ethics committee at the "Landesärztekammer Rheinland-Pfalz" (Mainz, Germany) and written informed consent was obtained from all study participants.

7.3.2. Patient examination

An overall summary of the LURIC study objectives and baseline examination procedures have been published elsewhere [166]. Briefly, all body measures were recorded by trained study nurses on the same day, during early morning, between the hours of 06:00-09:00am. RHR was obtained by electrocardiography. Five measures were taken 30 seconds apart, following a 10 minute rest in the supine position in a quiet room, with the average derived from the last 2 measures. Other variables included in the study were selected on the basis of previous

literature, data availability, and the possibility of confounding. Details of these variables are provided in Table 7.1.

Table 7.1. Description of variables measured in the present study

Variable	Definition
Age	Taken at time of enrolment
Sex	Male/female
Waist	Measured in cm, horizontally around the smallest circumference between the ribs and iliac crest
Body mass index	weight (kg) divided by height (m^2)
Smoking	Never/ever smoker
Physical activity	Recorded using an 11-point scale ranging from bedridden to extremely active, and categorised into "below average" (not very active), "average" (usual office work) and "above average" (heavy work or sports)
Arterial hypertension	Systolic ≥140 and/or diastolic ≥90 mmHg, or anti-hypertensive medication – confirmed by a physician
Diabetes mellitus	Fasting glucose ≥7.0 mmol/L, or patients receiving anti-diabetic medication – confirmed by a physician
Dyslipidaemia	Total cholesterol ≥6.2 mmol/L, HDL-cholesterol <1.03/1.29 mmol/L (male/female), triglycerides ≥1.7 mmol/L, or use of lipid-lowering medication – confirmed by a physician
Angina pectoris	Stable (graded severity classes I to IV described by the Canadian Cardiovascular Society); Unstable (subdivided into different classes by subjective assessment of clinical symptoms using Braunwald's classification)
Family history	Family questionnaire regarding the history of cardiovascular disease in first and second degree relatives
Cardiovascular medication use	Use of ACE-inhibitors, β-blockers or Statins

HDL = high density lipoprotein; ACE = angiotensin converting enzyme.

7.3.3. Laboratory analyses

Samples of fasted venous blood were collected during baseline examination, with patients in the supine position. Routine laboratory parameters were immediately measured on a daily basis as previously mentioned [166]. Remaining blood samples were snap-frozen and stored in -80 C for further analyses. All markers were processed at the Institute of Haemostaseology and Transfusion Medicine of the Ludwigshafen General Hospital. IL-6 was measured using a high sensitivity enzyme immunoassay (R&D Systems, Wiesbaden, Germany). CRP concentrations were measured by high sensitivity immunoturbidimetric assay (Roche Mannheim, Germany). Serum amyloid A was determined by immunonephelometry (Dade Behring, Marburg, Germany). Fibrinogen was measured using the Clauss method (Roche Mannheim, Germany). Neutrophil count was determined using EDTA whole blood and was later quantified using an automated analyser (Technicon H-1, Bad Vilbel, Germany until Dec 1998; Advia 120, Siemens Healthcare Diagnostics, Eschborn, Germany since Jan 1999).

7.3.4. Outcome measure

The primary endpoint was death due to CVD. Information on vital status was obtained from local community registries. Death certificates were reviewed to classify the deceased into those who died from CVD and non-CVD causes. Death from CVD causes included SCD, fatal MI, and death due to heart failure, death after intervention to treat CAD, stroke and other deaths due to heart disease. Two experienced physicians who were masked to any of the study data independently classified the causes of death. In the case of a disagreement concerning the classification, it was discussed and the final decision was made by one of the principal investigators of LURIC (W.M.), who was also masked to the key study variables.

7.3.5. Statistical methods

For the purpose of this investigation RHR was dichotomised into low (<75 bpm, n = 2,391) and high (≥75 bpm, n = 876) according to the 75 percentile. The study sample was then summarised by comparing those with a low and a high RHR, reporting the mean±SD (or median and IQRs for those variables which were skewed), or by numbers and percentages for categorical variables. The two groups were compared using *t*-tests for continuous data, and the Mann-Whitney *U* test for those continuous variables which were skewed, as well as χ^2 tests for categorical variables. All inflammatory markers and continuous covariates were checked for normality and those which visually deviated on inspections of the frequency distribution were transformed onto the natural (base *e*) log scale. In this study, only the single markers of inflammation required logarithmic transformation. Inflammatory activity can be assessed by a number of correlated markers, such as IL-6, CRP, serum amyloid A, fibrinogen, and the neutrophil count (Table 7.2). We employed a principle component analysis to extract from the individual markers of inflammation a single weighted multi-marker index of inflammation. In this study, the first principle component accounted for 61.3% of the explained variance and no additional significant principal components were identified. Accordingly, we developed the overall multi-marker index of inflammation by weighting the respective coefficients of each of the five inflammatory markers that contributed towards the primary underlying factor (i.e. inflammation). Kaplan-Meier survival curves with the log rank test for equality were used to illustrate the predictive ability of inflammation (quartiles) with CVD mortality, stratified by low and high RHR.

Table 7.2. Pairwise Pearson correlation coefficients between the individual markers of inflammation

	Interleukin-6	C-reactive protein	Serum amyloid A	Fibrinogen	Neutrophils
Interleukin-6	1.00				
C-reactive protein	0.58***	1.00			
Serum amyloid A	0.52***	0.79***	1.00		
Fibrinogen	0.50***	0.73***	0.62***	1.00	
Neutrophils	0.33***	0.33***	0.30***	0.33***	1.00

For the single markers of inflammation, natural log transformations were applied to normalize distributions.
***P <0.0001.

The association between inflammation quartiles and CVD mortality for those with a low and a high RHR was further tested using Cox proportional hazard survival models, reporting HRs with 95% CIs and *P* for trend. Three models were employed to test these relationships. The first model was unadjusted; the second model corrected for age and sex; the final model additionally controlled for BMI, smoking, physical activity, arterial hypertension, type 2 diabetes, dyslipidaemia, angina pectoris, family history of CVD, and cardiovascular medications. To further understand the relationship between inflammation and CVD mortality, the latter analyses were also performed examining the single markers of inflammation in those with a low and a high RHR, respectively. Due to the large heterogeneity of LURIC patients, we re-performed the survival models according to a number of separate sensitivity checks. First, we examined the initial findings among those who were clinically stable at baseline. Second, patients with signs of heart failure were removed in order to examine whether the initial findings would be affected. In the current study, heart failure was determined according to a classification developed by the New York Heart Association (i.e., whereby/in whom slight physical activity caused symptoms; inability to carry on any physical activity without discomfort; symptoms of cardiac insufficiency present even at rest) [181]. Third, patients accompanied with an ongoing infection who may have spuriously contributed towards elevated inflammation were excluded. Fourth, to minimise potential bias due to subclinical and undetected pre-existing disease at baseline which is associated with increased mortality, we also carried out the analyses after removing

patients who died within 12 months from the time of enrolment. Fifth, we excluded those who reported the use of β-blockers, as these agents are known to artificially lower pulse rate, which may have biased our initial assignment of patients to the RHR categories. We then assessed the discriminatory value of the multi-marker index of inflammation by computing the c-statistic and integrated discrimination improvement (IDI), as well as the net reclassification improvement (NRI). For the latter, we chose *a priori* meaningful risk category of <6, 6–20, and >20 per cent 10-year risk of CHD based on the Third Adult Treatment Panel risk classification [80]. Model calibration was evaluated using the Hosmer-Lemeshow goodness-of-fit test according to 10 risk categories. All calculations were performed using STATA version 11.2 (StataCorp, Texas).

7.4. RESULTS

Of the 3,316 patients enrolled in the LURIC study, 49 (1.5%) were excluded due to missing data on a number of parameters examined. Hence, the analytic sample included a total of 3,267 patients (2,283 men and 984 women). Preliminary analyses revealed that there were no sex-specific differences present. Baseline characteristics of the overall population are reported in Table 7.3. The median duration of follow-up was 9.9 years. Across the 29,940 person years of follow-up, there were 546 (17%) deaths due to CVD. There was a trend towards higher BMI, lower physical activity, arterial hypertension, and type 2 diabetes mellitus in patients with a high RHR. In contrast, there was a higher incidence of unstable angina pectoris in patients with a low RHR. Similarly, use of β-blockers and statins was significantly higher among patients with a low RHR (Table 7.3). Of the individual inflammatory markers, IL-6, CRP, serum amyloid A, fibrinogen and neutrophils were all significantly higher in patients with a high RHR.

Table 7.3. Baseline characteristics of patients according to low and high resting heart rate

	Low RHR (<75 beats/minute)	High RHR (≥75 beats/minute)	*P* value
No.	(*n*=2,391)	(*n*=876)	
Cardiovascular mortality, n (%)	366 (15.3)	180 (21.0)	<0.0001
Age, mean±SD, y	62.7±10.5	62.3±10.7	0.32
Male sex, n (%)	1,682 (70.4)	601 (68.6)	0.34
Waist circumference, mean±SD, cm	98.8±11.6	99.5±12.6	0.11
Body mass index, mean±SD, kg/m^2	27.4±3.9	27.7±4.5	0.04
Resting heart rate, mean±SD	63±7	83±8	<0.0001
Smoking status, n (%)			
Never	858 (35.9)	312 (35.6)	0.89
Ever	1,533 (64.1)	564 (64.4)	
Physical activity, n (%)			
Low	564 (23.6)	278 (31.7)	<0.0001
Average	1,277 (53.4)	443 (50.6)	
High	550 (23.0)	155 (17.7)	
Arterial hypertension, n (%)	1,208 (50.5)	528 (60.3)	<0.0001
Type 2 diabetes mellitus, n (%)	363 (15.2)	217 (24.8)	<0.0001
Dyslipidaemia, n (%)	1,642 (68.7)	612 (69.9)	0.52
Angina pectoris, n (%)			
Stable	893 (37.7)	339 (38.9)	0.01
Unstable	776 (32.8)	241 (27.7)	
Family history of CVD, n (%)	1,266 (53.0)	440 (50.2)	0.17
Cardiovascular medication use, n (%)			
ACE-inhibitors	1,268 (53.0)	483 (55.0)	0.29
β-blockers	1,617 (67.6)	465 (53.1)	<0.0001
Statins	1,189 (49.7)	354 (40.4)	<0.0001
Inflammatory markers			
Interleukin-6, median (IQR), pg/mL‡	3.00 (1.74-5.69)	3.89 (2.11-7.29)	<0.0001
C-reactive protein, median (IQR), mg/L‡	3.02 (1.21-7.69)	4.69 (1.63-10.7)	<0.0001
Serum amyloid A, median (IQR), mg/L‡	4.80 (2.70-10.80)	6.15 (3.30-18.60)	<0.0001
Neutrophils, median (IQR), 10^3/μL‡	3.94 (3.11-4.88)	4.39 (3.52-5.76)	<0.0001
Fibrinogen, median (IQR), g/L‡	3.71 (3.16-4.43)	3.96 (3.34-4.76)	<0.0001

Categorical variables are shown as percentages, and continuous data are presented as mean±SD, or median (IQR). *t* test was computed for continuous parameters (with Mann-Whitney *U* test for skewed data)‡, and χ^2 test for categorical variables. Abbreviations; RHR = resting heart rate; CVD = cardiovascular disease; ACE = angiotensin converting enzyme; IQR = interquartile range.

Patients with a low RHR and who were in the highest quartile on the inflammation index had an increased risk of CVD mortality compared to those with lower levels of inflammation (Figure 7.1A). For those with a high RHR there was an even stronger increase in CVD mortality with increasing levels of inflammation. That is, patients in the highest quartile of inflammation had a greater risk of CVD mortality when RHR was ≥75 bpm (Figure 7.1B). Log rank test for equality revealed that there was a significant difference between quartiles (*P* <0.001).

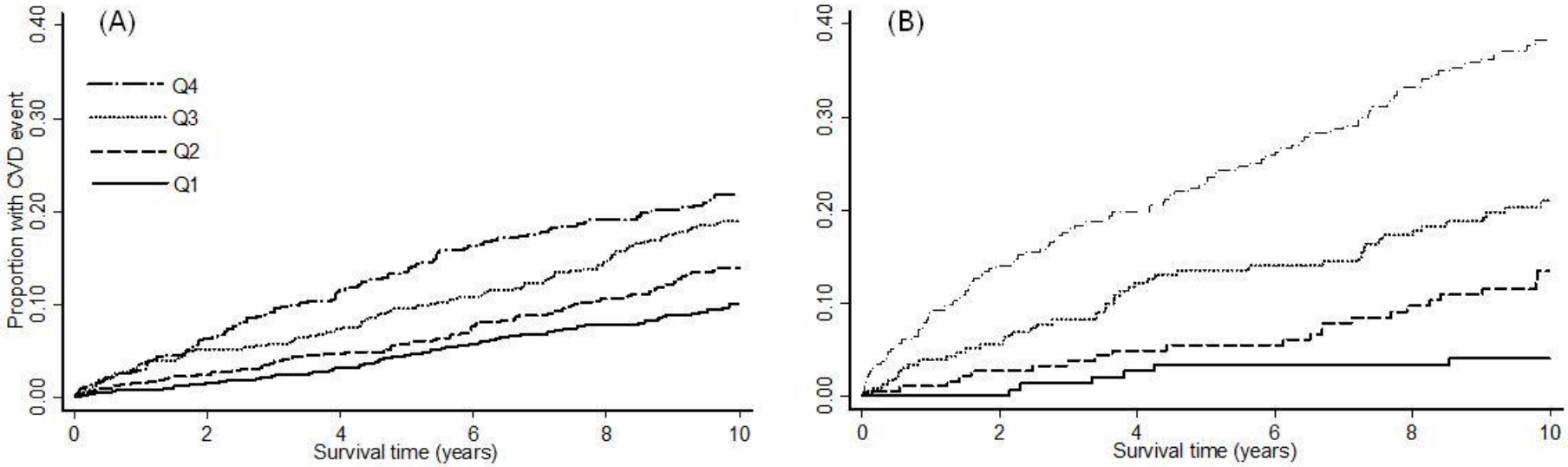

Figure 7.1. Kaplan Meier plots indicating the proportion of cardiovascular deaths according to quartiles of inflammation by (A) low (<75 beats/minute) and (B) high (≥75 beats/minute) resting heart rate. Log rank test for equality found quartiles of inflammation were significantly different in both models (*P* <0.0001, respectively).

Cox proportional hazard regression reported a significant interaction effect between heart rate and inflammatory activity for the primary endpoint (P = 0.04). Separate Cox proportional hazard regression models for those with a low and a high RHR are presented in Table 7.4. There was a survival reduction of 1.4 and 2.7 years due to the primary endpoint in those with a low and a high RHR, respectively, when the highest quartile of inflammation was compared with the lowest quartile. For those with a low resting RHR, the fully adjusted HR (95% CI) for CVD mortality among patients in the upper quartile of inflammation was 1.84 (1.31-2.57), P_{trend} <0.0001. However, for patients with a high RHR, the fully adjusted risk increased by more than seven-fold, HR (95% CI) = 7.50 (3.21-17.50), P_{trend} <0.0001, for those in the highest inflammatory quartile (Table 7.4).

Since patients involved in this study were scheduled for coronary angiography, we subsequently explored if the degree of CAD severity could explain, in part, the interaction between RHR and inflammatory status in predicting risk of CVD mortality. Interestingly, we observed that the adjusted risk of death due to CVD increased further depending on the magnitude of CAD severity (Figure 7.2). Patients with ≥20% stenosis of at least one main luminal vessel and who were in the upper-most quartile of inflammation with a high RHR had a comparable risk, [HR (95% CI) = 6.68 (2.66-16.78)], P_{trend} <0.0001 for CVD mortality compared to the overall cohort (*see* Table 7.4). On the other hand, the magnitude of cardiovascular risk increased further to approximately ten-fold HR [(95% CI) = 9.83 (3.05-31.65), P_{trend} <0.0001] among those with ≥50% narrowing of at least one main coronary artery, who were in the highest quartile of inflammation on the background of a high RHR.

Table 7.4. Relationship between inflammation and cardiovascular mortality by low and high resting heart rate

	Inflammation quartiles				
	Q1	Q2	Q3	Q4	P_{trend}
Resting heart rate (<75 bpm)					
No. alive/CVD event	603/65	546/89	474/111	411/110	
Median follow-up time, y	9.7	9.1	8.8	8.3	
Model 1, HR (95% CI)	1 (reference)	1.49 (1.07-2.06)	2.13 (1.56-2.90)	2.52 (1.85-3.44)	<0.0001
Model 2, HR (95% CI)	1 (reference)	1.39 (1.01-1.93)	1.95 (1.43-2.66)	2.09 (1.53-2.86)	<0.0001
Model 3, HR (95% CI)	1 (reference)	1.28 (0.91-1.79)	1.69 (1.22-2.34)	1.84 (1.31-2.57)	<0.0001
Resting heart rate (≥75 bpm)					
No. alive/CVD event	150/6	164/25	193/46	199/104	
Median follow-up time, y	9.7	9.0	8.5	7.0	
Model 1, HR (95% CI)	1 (reference)	3.49 (1.43-8.53)	5.61 (2.40-13.13)	11.72 (5.15-26.72)	<0.0001
Model 2, HR (95% CI)	1 (reference)	3.09 (1.26-7.57)	4.67 (1.99-10.96)	9.21 (4.03-21.03)	<0.0001
Model 3, HR (95% CI)	1 (reference)	2.40 (0.97-5.92)	4.08 (1.72-9.64)	7.50 (3.21-17.50)	<0.0001

Values were obtained from Cox proportional hazard models. Model 1 was unadjusted. Model 2 corrected for age and sex. Model 3 additionally corrected for body mass index, smoking, physical activity, arterial hypertension, type 2 diabetes, dyslipidaemia, angina pectoris, family history of cardiovascular disease and cardiovascular medication. Inflammation index included: interleukin-6, high sensitivity C-reactive protein, serum amyloid A, neutrophil numbers, and fibrinogen. Abbreviations; bpm = beats/minute; HR = hazard ratio; CI = confidence interval.

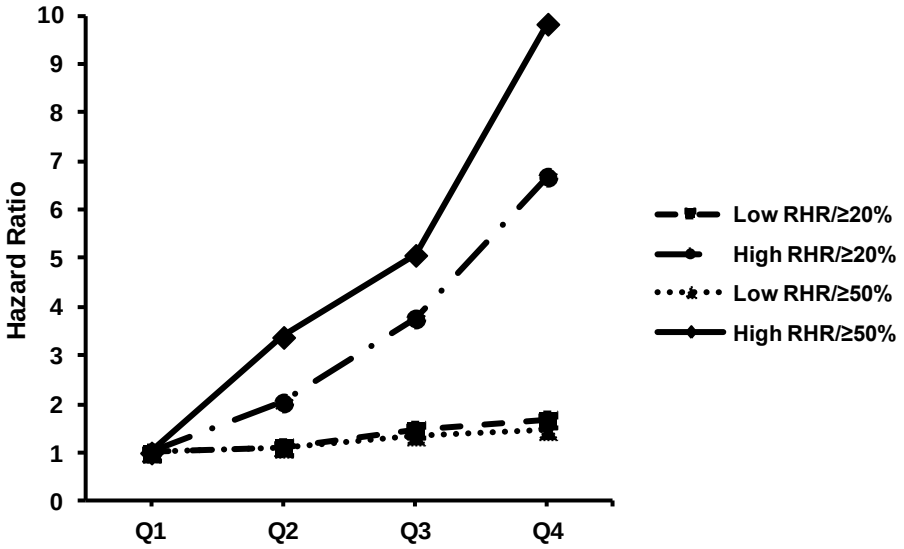

Figure 7.2. Hazard ratios for incident cardiovascular mortality according to quartiles (Q) of inflammation by low (<75 bpm) and high (≥75 bpm) resting heart rate (RHR) with either ≥20% or ≥50% stenosis of at least one main coronary artery. Model fully adjusted as outlined in Table 7.4.

In Figure 7.3, the fully adjusted HR estimates for the single markers of inflammation are presented. For each marker, we compared the upper-most quartile to the lowest and found the risk of CVD mortality was greater among patients who had a high RHR (Figure 7.3). Of all these markers, the greatest prediction was provided by fibrinogen, HR (95% CI) = 3.68 (2.02-6.70), *P* <0.0001). Though each individual marker was predictive of CVD mortality among those with a high RHR, the strongest prediction for CVD mortality was afforded by the overall marker of inflammation (Figure 7.3).

To better understand the relationship between inflammation and CVD mortality, we repeated the latter analyses including only patients with clinical stability (*n* = 2,234) at baseline. After comparing the upper-most quartile to the lowest, we found the relationship between inflammation and CVD mortality increased HR (95% CI) = 2.37 (1.56-3.60), *P* <0.0001 in patients with a low RHR, as well as for those HR (95% CI) = 9.18 (3.23-26.00), *P* <0.0001 with a high RHR. The analyses were then carried out after excluding patients with suspected heart failure (*n* = 619). The fully adjusted association between inflammation and CVD mortality remained unchanged, HR (95% CI) = 1.87 (1.26-2.78), *P* =0.001 for those

with a low RHR. However, there was a slight attenuation in the risk HR (95% CI) = 6.81 (2.63-17.63), *P* <0.0001 for those with a high RHR. We also removed patients accompanied by an ongoing infection (*n* = 315) which may have contributed towards a heightened inflammatory status at baseline. Likewise, we found the adjusted association between inflammation and CVD mortality for the highest compared to the lowest quartile did not materially change HR (95% CI) = 1.86 (1.31-2.64), *P* <0.0001 in patients with a low RHR, while again, there was a slight attenuation for individuals HR (95% CI) = 6.19 (2.63-14.60), *P* <0.0001 with a high RHR. To minimise potential bias due to subclinical and undetected pre-existing disease at baseline which is associated with increased mortality, we removed patients who died within 12 months (*n* = 130) of enrolment. The results were virtually identical, HR (95% CI) = 1.85 (1.29-2.65), *P* =0.001 for those with a low RHR, and HR (95% CI) = 6.23 (2.64-14.72), *P* <0.0001) for patients with a high RHR. Finally, after excluding those who reported the use of β-blockers, the adjusted association between inflammation and CVD death among those with a low RHR was somewhat attenuated HR (95% CI) = 1.78 (1.03-3.08), *P* =0.04, albeit the association remained significant. Conversely, the strong independent relationship between inflammation and CVD mortality HR (95% CI) = 5.35 (2.05-14.00), *P* =0.001 persisted among patients with a high RHR, even after removing those who reported the use of these agents. Further exploration of the data, using instead, the median cut-off value (median = 68 bpm) for RHR revealed similar findings (data not shown).

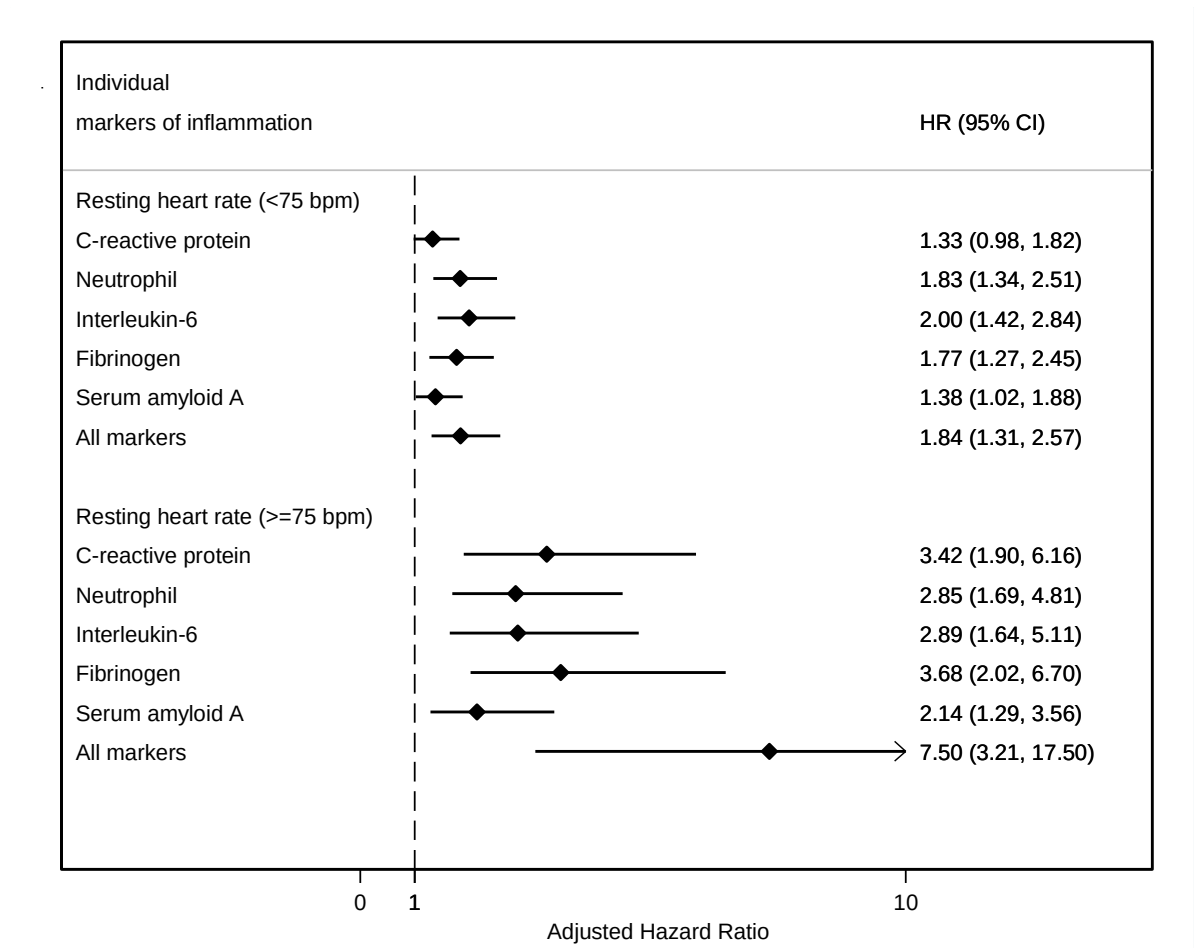

Figure 7.3. Hazard ratios (quartile 4 versus quartile 1) for incident cardiovascular mortality according to the individual markers of inflammation and the composite score, by low (<75 beats/minute) and high (≥75 beats/minute) resting heart rate. Model was fully adjusted as abbreviated in Table 7.4.

We assessed the discriminatory value of the multi-marker index of inflammation by computing the c-statistic and area under the receiver operating characteristic (AUC) curve; comparing a model including the conventional risk factors with a model based on a combination of these risk factors and the multi-marker index. Initially, the conventional risk factor model achieved reasonable discrimination, obtaining a c-statistic of 0.751 (Figure 7.4). After including the multi-marker index of inflammation, the discriminatory value marginally improved by 0.010 (0.761, P =0.015) (Figure 7.4). In addition, we employed the reclassification statistics of the IDI and NRI. According to the IDI, inclusion of the multi-marker index significantly improved model discrimination by 0.010 (P <0.0001). Moreover,

the addition of the multi-marker index to the conventional risk factors significantly improved reclassification of patients to a different risk category by 6% (P =0.002) (Table 7.5). Model calibration using the Hosmer-Lemeshow goodness-of-fit test also yielded a chi-square of 6.29 (P =0.61), indicating no significant deviation between observed and predicted risk (Figure 7.5).

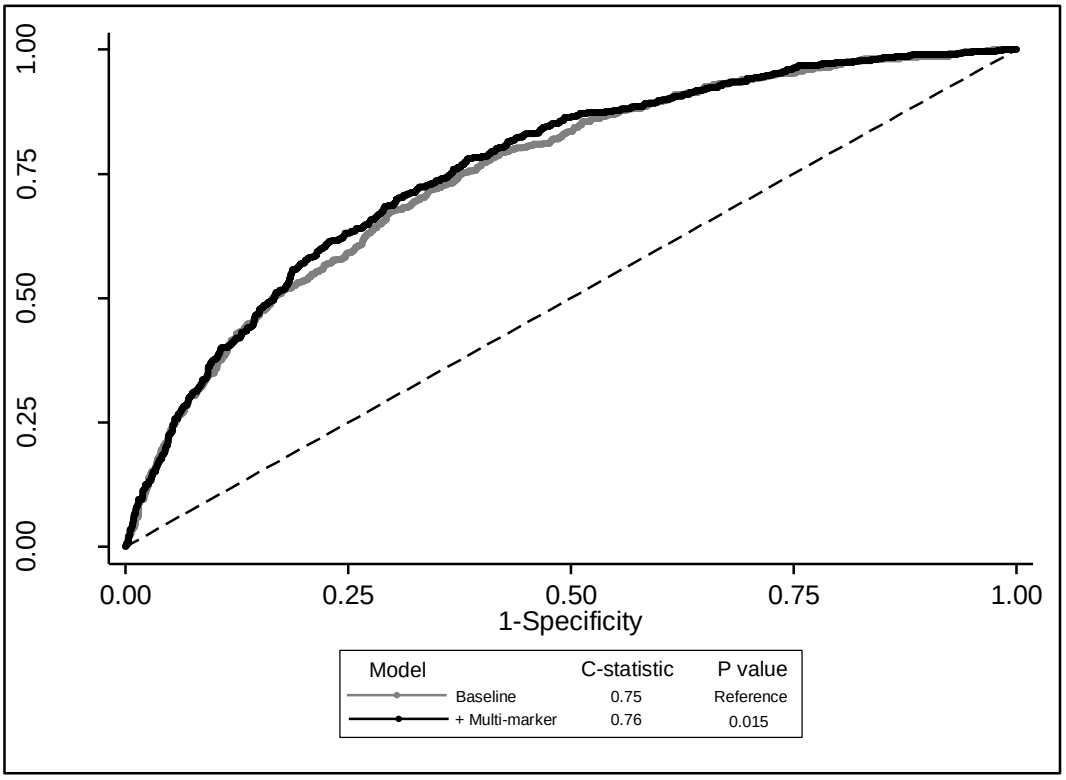

Figure 7.4. C-statistic estimates for the prediction of cardiovascular mortality according to the baseline conventional risk factors and multi-marker index of inflammation. Baseline model (grey) included age, sex, waist circumference, smoking, physical activity, arterial hypertension, Type 2 diabetes, dyslipidaemia, family history of cardiovascular disease, angina pectoris, and cardiovascular therapy.

Table 7.5. Reclassification of intermediate (defined as 6%-20%) risk patients with and without the cardiovascular endpoint

	Reclassification accounting for multi-marker			
Classification according to conventional risk factors	Low	Intermediate	High	Total no.
Patients with CVD endpoint				
Low <6% in 10 yrs	16	6	0	22
Intermediate 6%-20% in 10 yrs	5	157	34	196
High >20% in 10 yrs	0	17	301	318
Total no. with event	21	180	335	536
Patients without CVD endpoint				
Low <6% in 10 yrs	587	58	0	645
Intermediate 6%-20% in 10 yrs	95	1,165	79	1,339
High >20% in 10 yrs	0	88	571	659
Total no. without event	682	1,311	650	2,643
Net reclassification improvement		6.0% (*P* =0.002)		

Conventional risk factor model included age, sex, body mass index, smoking, physical activity, arterial hypertension, type 2 diabetes, dyslipidaemia, angina pectoris, family history of cardiovascular disease and cardiovascular medication.

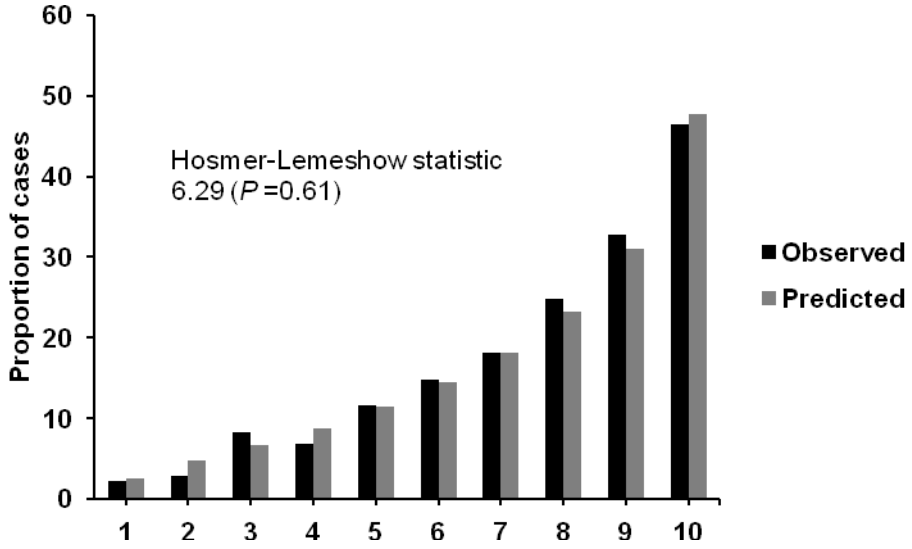

Figure 7.5. Proportion of observed and predicted cases according to the risk model that included the multi-marker index of inflammation. Hosmer-Lemeshow goodness of fit test yielded no significant deviation between observed and predicted risk (*P* =0.61).

7.5. DISCUSSION

The present study assessed the inter-relationship between inflammation and RHR with CVD mortality. We found the risk associated with elevated inflammation was amplified four-fold in patients with a high RHR (≥75 bpm), compared to those with a low RHR (HR 7.50 versus 1.84). This synergistic effect remained unaltered after adjusting for an extensive number of established risk factors. Further, these observations appeared somewhat dependent on the degree of CAD, since in the current investigation the risk of experiencing the study endpoint increased by almost ten-fold among those with severe CAD (≥50% luminal stenosis of at least one major coronary artery). Our study adds to the current literature because, it is, to our knowledge, the only investigation to address RHR as an effect-modifier of inflammation for predicting future CVD outcome.

The European Society of Hypertension/European Society of Cardiology guidelines recently proposed the inclusion of elevated RHR when evaluating the cardiovascular risk profile of an individual [164]. The present results indicate that the predictive utility of RHR may be substantially enhanced when considered alongside other risk factors, specifically inflammation. Consequently, we may speculate that slowing the RHR may prove a useful adjunct for interrupting the patho-physiological process initiated by inflammation within the local pro-atherosclerotic vascular environment. If this conjecture is correct, then it may be anticipated that the protective effects of selective heart rate-lowering agents (i.e., β-blockers, calcium antagonists, and the more novel specific heart rate-lowering agent ivabradine) may differ as a function of lower and higher inflammatory activity.

At this point the exact mechanisms by which resting tachycardia modulates the effects of inflammation remain to be elucidated, although the extant literature provides compelling evidence for several candidate processes. Among these is ANS damage. Sympathetic

overactivity plays a key role in atheroprogression by inducing tachycardia [158] as well as by causing immune dysregulation. For example, the sympathetic mediators adrenaline and noradrenaline have potent effects on cytokine release by activated T cells and macrophages and also regulate the migratory behaviour of these cells. Likewise, stimulation of parasympathetic outflow, which normalizes tachycardia, also short circuits the inflammatory cascade via the cholinergic anti-inflammatory pathway [7, 10, 178, 179], which involves down regulation of macrophage activity via direct neural stimulation of macrophage cholinergic receptors.

Following this above logic, a raised RHR could be considered a marker of ANS dysfunction, rather than a risk factor *per se* [112, 158, 182, 183]. Nonetheless, several lines of research provide evidence to suggest that RHR is also a primary risk factor for CVD [160, 163, 184], and therefore other interactive mechanisms could be considered as well. For instance, disturbed flow-generated shear stress as a consequence of increased pulse rate stimulates specific mechanosensors located on the surface of endothelial cells [158, 159], subsequently upregulating pro-atherogenic genes and downregulating athero-protective genes [158, 185, 186]. Many of these genes also control inflammatory processes. These include, but are unlikely limited to, reduced NO activity, which has potent immunomodulatory properties, increased vascular permeability, which is a hallmark of the inflammatory process, as well as increased expression of inflammatory components such as adhesion molecules (e.g., vascular cell adhesion molecule-1, intercellular adhesion molecule-1), chemoattractants (monocyte chemoattractant protein-1), and cytokines (IL-6, tumour necrosis factor-α) [158, 160, 187]. Thus, an elevated RHR may enhance the magnitude of an unfavourable cardiovascular risk profile by amplifying the effects of ongoing inflammatory processes.

Of additional importance, we derived a composite marker of inflammation from a principle component analysis. In doing so, we were able to combine each of the individual

markers into a single component, thereby reflecting a general marker of inflammation, permitting us to retain most of the information attributed to each marker. From a clinical perspective, the inclusion of a multi-marker strategy in this study based on CRP, neutrophils, IL-6, fibrinogen and serum amyloid A, was more informative for improving risk estimation, compared to single marker determination. Foremost, the clinical utility of individual biomarkers towards predicting subsequent cardiovascular events has recently been questioned. Indeed, the prognostic reliability of CRP, the most commonly measured inflammatory marker in cardiovascular epidemiology has been challenged by other authors [188-190]. Thus, we postulate that simultaneous assessment of multiple markers of inflammation may provide additional prognostic information beyond single marker determination, at least among secondary care patients.

The present study is not without limitations. Patients in the present investigation were Caucasian of German ancestry. Thus, caution is warranted in generalising our findings to the understanding of CVD in other populations. Though prospective in patient enrollment, RHR was only assessed at a single time point, and therefore may not reflect pulse rate over the longer term which would allow us to identify sustained tachycardia. Further, heart rate shows significant diurnal variation which presents additional problems for a one-off measure. Nevertheless, we would counter that RHR was measured in a sufficiently standardised manner to yield reliable data, permitting comparison among patients, while minimizing methodological bias as much as possible [191]. For the purpose of this investigation, resting heart rate was dichotomized according to the 75 percentile cut-off value. Thus, subsequent studies may wish to include other metrics of heart rate when evaluating its relationship with inflammation and adverse cardiovascular outcome. Similarly, the individual markers of inflammation were determined at a single time point. We also cannot exclude the possibility that other functionally distinct markers of inflammation may be implicated in CVD risks that

were not examined here. Although we performed multivariable adjustments, we cannot rule out that our results may be influenced by unmeasured or residual confounding. However, due to the large sample size and extensive range of measurements taken, we were able to adjust for a range of important covariates including arterial hypertension, type 2 diabetes, dyslipidaemia and family history of CVD. In this study, the observed association between resting heart rate and inflammation with CVD mortality appeared somewhat stronger with more severe CAD. However, due to the lack of non-fatal CVD endpoints in LURIC, we were unable to explore this relationship further. In light of this, we hope these findings spur future investigations to evaluate the putative link between resting heart rate and inflammatory activity with non-fatal CVD events.

In conclusion, the present data highlight a potential role for RHR as a risk factor for CVD mortality by amplifying the effect of inflammation, which may explain, in part, the poor cardiovascular prognosis in individuals with an elevated RHR. Should these observations be confirmed, slowing the heart rate may prove a useful adjunct in prolonging survival among CVD patients that exhibit an elevated inflammatory activity.

CHAPTER EIGHT

8.0. DISCUSSION

8.1. OVERVIEW

With advancing age, it has become more apparent that older individuals seem bedevilled by a number of adverse cardiovascular health conditions which account for considerable disability in later life [5]. Of all these complications, diseases of the coronary arteries are among the most common of vascular disorders attributable to poor health in the aged. Moreover, CAD accounts for over half of all mortality among individuals beyond 65 years [2], and overall CVD tends to outrank any other cause of death in persons belonging to this age strata. For this reason alone, it seems justified for researchers and clinicians to identify and forestall, as early as possible, the multitude of CVD risk factors in an effort to enhance the quality of health during later years of life [5].

8.2. SUMMARY AND INTEGRATION OF MAIN STUDY FINDINGS

8.2.1. Relation between resting heart rate and cardiovascular morbidity

Foremost, studies examining the interplay between sustained elevated RHR and cardiovascular risk factors beyond Caucasian populations and developed nations are, at present, sub-optimal. Notably, several investigations have described ethnic-specific variations in the number of cardio-metabolic indices including abdominal adiposity [192], phenotypes of body fat distributions [192, 193], ectopic fat depositions, namely, the accumulation of fat surrounding the liver [194], as well as biochemical parameters such as hyperinsulinaemia, hyperglycaemia, dyslipidaemia, hyperleptinaemia, low levels of adiponectin and higher levels of CRP [195]. In view of these ethnic disparities, it is of prime importance to better understand whether the role of RHR as a predictor of CVD health among Caucasian individuals is

comparable in other ethnic backgrounds. Collectively, the findings derived from studies one to three (chapters two to four) highlight the relevance of RHR and its negative implications towards heightened cardio-metabolic risk within a non-western cohort. In the first study (chapter two), we explored the independent associations of seated RHR as well as in combination with abdominal obesity for the presence of type 2 diabetes among older Chinese residents. A novel finding of the first investigation is resting tachycardia independently doubled the risk of type 2 diabetes mellitus in older Chinese adults, confirming previous results obtained in Caucasian populations [27, 28]. For instance, in the San Antonio Heart Study [27], volunteers defined as hyperdynamic (i.e., RHR and pulse pressure in the upper-most quartile of their respective distributions) had an almost four-fold adjusted risk for the prediction of future type 2 diabetes. Likewise, Carnethon et al. [28] reported a 12 bpm increment in RHR was associated with a 10% increase in the odds of having diabetes; albeit, this relation attenuated to non-significance following full adjustment. Nonetheless, the same study confirmed a raised RHR was a robust predictor (OR 1.21, 95% CI = 1.03-1.41) of death due to diabetes in those aged between 35-49 years, even after adjusting for BMI and post-load glucose at baseline [28]. To our knowledge, two other studies similarly examined the association between RHR and risk of diabetes in Asian-specific cohorts. In the Shanghai Women's Health Study [26], the incidence rates for diabetes were 2.91, 3.31, 3.71, 4.16 and 5.34 per 1,000 person years according to RHR categories of ≤68, 69-72, 73-76, 77-80 and ≥80 bpm, respectively. In spite of this, the observed associations reported in the Shanghai Women's Health Study [26] were limited to female participants only, and so those findings may not necessarily be replicated in older Chinese men. On the other hand, our investigation is indeed one of the first to address the independent effects of seated RHR towards the prevalence of type 2 diabetes mellitus in both community-dwelling older Chinese men and women. Second, Shigetoh and co-workers [24] demonstrated a high RHR predisposed older

Japanese subjects to the development of diabetes. We may add, however, due to the low incidence of obesity among Japanese individuals [24], the findings taken from that study are unlikely to be representative of other Asian cohorts.

Since the available literature regarding the relation between elevated RHR and the MetS were predominantly derived from non-Asian individuals, the purpose of the second study (chapter three), therefore, was to investigate the usefulness of a raised RHR as an independent marker of the MetS among older Chinese subjects. In this study, a rapid RHR was independently associated with the MetS in older Chinese men and women. Specifically, those in the highest quartile (≥91 bpm) of this cardiovascular proxy had an almost two-fold increased adjusted risk for the MetS. Related to this matter in hand, the resulting data from chapter three confirm earlier studies among non-Asian populations that indicated those with sustained elevated RHR were at greater risk for cardio-metabolic complications [41, 43, 44]. In one study consisting more than 3,000 Italian individuals, Mancia et al. [41] compared the values of RHR obtained from healthy controls with those whom were identified as having the MetS. In that investigation, participants diagnosed as having the MetS presented with significantly greater office, home, and 24-h ambulatory RHR values. Rogowski and colleagues [43] also reported the OR towards risk of having the MetS according to men and women in the highest quintile of RHR (≥80 bpm in men and ≥82 bpm in women) increased considerably by over four-fold (95% CI = 3.0-5.9) and three-fold (95% CI = 2.2-6.1), respectively. Moreover, in the BARI 2D trial [44], regression analyses indicated RHR was amplified in patients with the MetS compared to those without (regression coefficient; 2.9, P =0.002). Notably, in the same trial, a persistently high RHR was related to an increasing number of components belonging to the MetS in patients already at-risk for CVD, suggesting the risk of elevated RHR for development of the MetS likely extends beyond those who appear generally healthy [44].

Although there is little understanding of the specific mechanisms as to how elevated RHR detrimentally affects the risk of CVD, some evidence suggests that a perturbed ANS function may be responsible for the rise in RHR. More precisely, as with several other cardiovascular risk factors such as type 2 diabetes mellitus, abdominal obesity, and hypertension, resting tachycardia likely reflects the same common denominator: sympathetic overactivity [31, 123, 174]. Rapid RHR is perhaps a consequence of both enhanced sympathetic tone as well as a reduced parasympathetic outflow to the sinus node. To this end, one may assume a raised RHR is merely a marker of abnormal ANS activity. Though, conversely, RHR may likely possess some independent effects with regards to exacerbating the risk of CVD, since at present, the exact patho-physiological mechanisms that lead to impaired ANS activity are less clear, and therefore, require further investigation.

Moving forward, the third study (chapter four) reported in this thesis attempted to examine high RHR as an important indicator of adverse cardiovascular health by exploring the utility of this simple clinical parameter with an established surrogate measure of arterial stiffness, namely PWV. In the same study, we separately examined the relationship of a faster RHR in combination with the MetS for risk of CVD as described by increased PWV. After correcting for numerous potential confounders, the results of chapter four indicated that elevated RHR and the MetS independently amplified the risk of a high PWV. On the background of both high RHR and the MetS, the adjusted risk increased almost four-fold. Recent epidemiologic evidence supports the notion that higher PWV is independently associated with greater risk of CVD mortality, and is dominantly influenced by advancing age [196]. Thus, we may add, the results from the fourth chapter are of particular importance considering the significant relation between higher RHR and increased PWV among these older Chinese individuals. It is feasible to note that the increase in PWV observed in chapter four may have been mediated, in part, by a raised RHR. Indeed, the balance between

myocardial oxygen demand and supply is severely compromised under the presence of a high RHR due to its impact on shortening the fraction of cardiac cycle occupied by diastole, which in turn, lowers coronary blood supply, promoting greater myocardial oxygen consumption and oxidative stress [174, 197]. These functional alterations induce an increased inflammatory response by up-regulating various circulating pro-inflammatory cytokines such as IL-6 and tumour necrosis factor-alpha. The latter plays a critical role in endothelial dysfunction by restricting the bioavailability of nitric oxide which substantially impairs endothelial-dependent dilation [198, 199]. This type of behaviour ultimately exposes the endothelium to a myriad of adverse risk factors, provoking impaired vascular tone and structural wall dysregulation, which may result in an increased PWV [200].

In light of the findings reported in chapter's two to four, the data presented in this thesis adds to the paucity of literature among non-Caucasian subjects, confirming the strong independent contribution of RHR towards vascular anomalies. As developing nations such as China continue to increase their rapid rate of modernisation, cardio-metabolic complications such as type 2 diabetes mellitus as well as the MetS will likely become more apparent [113-115]; possibly forewarning of a major developing health burden in a rapidly modernizing and burgeoning older Chinese population.

8.2.2. Resting heart rate in cardiovascular mortality

Substantial epidemiological evidence indicates that a faster RHR may well be implicated in the progression of cardiovascular-related death [3, 54, 55, 60, 61, 64, 69, 71]. Yet, in the face of this literature, resting tachycardia as a modifiable cardiovascular risk factor continues to be overlooked [111, 112]. Moreover, data based on sex-specific stratification is limited, thus it remains unclear whether the threat of CVD mortality according to elevated RHR is similar between men and women, or whether the risk differs in magnitude. Of additional importance,

the usefulness of RHR in predicting cardiovascular endpoints among patients who are already at-risk for this condition is yet to be definitively established since the vast majority of the existing literature consists of a general population. In this thesis, study's four and five (chapters five and six) afforded an opportunity to explore these matters in hand. First, in chapter five, we sought to ascertain the importance of RHR as a marker of CVD mortality in a large cohort of apparently healthy middle-aged British men and women. In this chapter, the integral relation between a raised RHR and death due to CVD, according to the overall study sample, was fitting with a number of published data in the general population [61, 70, 71]. Of particular note, however, a faster RHR was found to be a critical determinant of all-cardiovascular death in men, but not in women. That the relationship between a high RHR and CVD mortality seems mainly driven by men in chapter five appears consistent with few other investigations that have explored this association according to sex-specific stratification [3, 54, 60, 64]. Though, paradoxically, a handful of studies also demonstrated a high RHR to be an independent predictor of CVD mortality in women [55, 69, 110, 157]. Findings from two separate studies reported by Gillum and colleagues [55, 110] show dissimilarities between elevated RHR and CVD mortality when age, sex, and ethnicity were accounted for. More specifically, the relation between high RHR and CVD death seemed particularly striking among black women (RR [95% CI] = 3.03 [1.46-6.28]). Further, in the Jerusalem 70-year-old Longitudinal Study, Perk et al. [157] demonstrated that the risk of CVD death increased in response to a raised RHR among older women only. Of particular note, however, is the latter study consisted of a relatively small sample size (n = 422, of which n = 193 (45%) women), and only included participants aged 70 years upon study entry. Unlike other ethnically homogenous cohorts, this study also consisted of participants born in more than 40 different countries [157]. Collectively, the heterogeneous findings ascribed to the Jerusalem 70-year-old Longitudinal Study are perhaps due, in part, to the important distinctions observed in cohort size, age of study

participants at entry, as well as the large number of differences in places of birth. Nevertheless, the findings from these studies taken together with the observations reported in chapter five, clearly suggest additional investigations are required to elucidate the complex interactions that exist between RHR, age, gender, ethnicity and CVD complications.

Second, in chapter six, the usefulness of elevated RHR in predicting death due to CVD is described among patients who underwent coronary angiography. In this study, patients in the highest quartile (≥84 bpm) had an increased adjusted risk for CVD mortality by approximately 40%. Although the results of the sixth chapter may only apply to persons at intermediate-to-high cardiovascular risk, they are nonetheless consistent with published findings from several epidemiological studies obtained in the general population [53-55, 69, 70]. Beyond this, the current data of chapter six may be of clinical importance because it provides specific information on patients undergoing coronary angiography, and who are otherwise of significant interest regarding risk assessment and therapeutic interventions; a limitation which currently exists in the available literature.

Moving forward, after correcting for a number of covariates in chapter six, fractional polynomial models revealed a J-shaped curve for RHR with CVD mortality. In particular, patients with a very low RHR also appeared to be at greater risk for experiencing the study endpoint; proposing the observed association between elevated RHR and CVD mortality seems non-linear. To this end, these findings may provide additional insight into the relative prognostic contributions of RHR as a risk factor of CVD death. Albeit, one limitation was the minimal number of patients (n = 98 for RHR <50 bpm) identified as actually having a very low RHR, which may have underpowered any subsequent evaluation with respect to the relative impact of a very low RHR towards the risk of CVD outcomes. Notwithstanding, we hope these findings shown in chapter six will spur forthcoming studies to consider the

implications of a very low RHR as a potential hazard, rather than simply dismissing it and focussing solely on the detrimental effects of a high RHR.

8.2.3. Resting heart rate: a potential modifier of inflammatory activity

Recent evidence indicate both inflammatory activity and sustained tachycardia interact at several levels of the cardiovascular continuum [159], and may hereby exert a synergistic effect reflecting an unfavourable cardiovascular risk profile. Preliminary evidence according to baseline characteristics reported in chapters five and six signify the deleterious effects of inflammation may be amplified by increasing RHR. Though, clearly, further studies are required to test this notion. Subsequently, in an attempt to address this question, the sixth study (chapter seven) was undertaken to investigate the inter-relationship between RHR and inflammation with CVD death in a large cohort of German patients undergoing coronary angiography.

In line with the demographics shown in chapters five and six, a number of individual markers of inflammation including IL-6, CRP, serum amyloid A, neutrophil count and fibrinogen, were significantly higher among patients with a raised RHR compared to those with a lower RHR. In addition, inflammatory activity was also assessed by combining each of the single markers into an overall weighted multi-marker index of inflammation. Foremost, the analyses reported in chapter seven underline the robust independent effects of a persistently high inflammatory activity as a risk factor for CVD mortality. Second, these detrimental effects were substantially amplified on the background of a faster RHR. In fact, patients in the upper-most quartile of inflammation who had a RHR $\geq$75 bpm appeared to have more than a seven-fold increased adjusted risk of CVD death.

Despite the observed associations in chapter seven, the exact mechanisms by which resting tachycardia modulates the effects of inflammation remain to be elucidated; although the extant literature provides compelling evidence for several candidate processes. In particular, local haemodynamic forces play a critical role in predisposing a number of arterial areas to atherosclerotic plaque development [158, 201-204]. These local forces generally comprise of flow-generated shear stress and blood pressure-derived tensile stress [158]. Conceptually, a faster RHR may, in part, amplify the burden of CVD by prolonging the exposure of disturbed flow-generated stress imposed on the wall of the artery (Figure 8.1). In light of these functional alterations, a high RHR may therefore critically promote the inflammatory process by provoking permanent alterations to endothelial cells, as well as inducing direct endothelial injury by initiating increased permeability to the endothelial cell wall, perhaps, via up-regulation of numerous inflammatory components (i.e., adhesion molecules, chemoattractants, and cytokines) [158, 203, 204]. In keeping with this, it would appear feasible to propose that an elevated RHR may therefore enhance the magnitude of an unfavourable cardiovascular risk profile by amplifying the effects of ongoing inflammatory processes. Consequently, we may speculate, that slowing the RHR may prove a useful adjunct for interrupting the patho-physiological processes initiated by inflammation within the local pro-atherosclerotic vascular environment. If this conjecture is correct, then it may be anticipated that the protective effects of selective heart rate-lowering agents (i.e., β-blockers, calcium antagonists, and the more novel specific heart rate-lowering agent ivabradine) may differ as a function of lower and higher inflammatory activity.

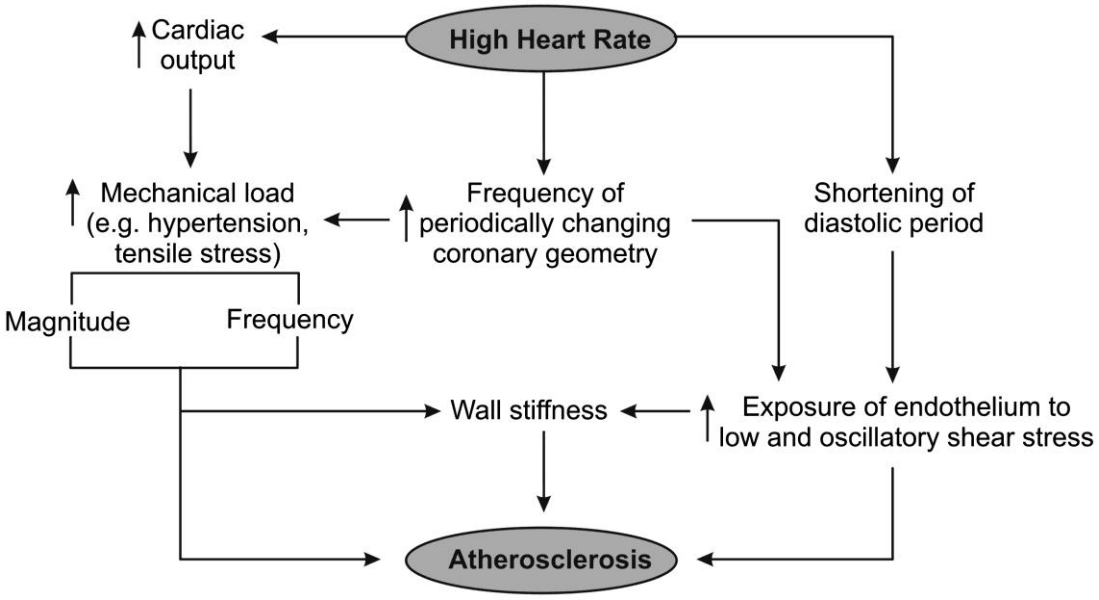

Figure 8.1. A conceptual framework of pathogenetic mechanisms influenced by resting heart rate in coronary atherosclerosis. Adapted from Giannoglou et al. [158] with permission.

8.3. LIMITATIONS

There are several limitations that bear mentioning in this thesis. Foremost, given the cross-sectional nature of the GBCS, we were unable to explore the causal relation between sustained elevated RHR and incident CVD risk. To this end, we would counter that our large-scale GBCS sample are currently being followed-up at 4 year intervals. Though biologically plausible, the continual monitoring of the GBCS participants for morbidity and mortality will eventually allow us to confirm whether a raised RHR does in fact amplify the risk of poor cardiovascular prognosis in older, community-dwelling Asians. Our GBCS sample is representative of approximately 7% of all individuals greater than 50 years of age in Guangzhou. Thus, caution is warranted when extrapolating our findings to other individuals belonging to this age strata, as our study participants do not resemble all older Chinese citizens residing in Guangzhou City. By extension, our sample was randomly selected from GHHARE,

thus we cannot rule out the possibility of "healthy volunteer bias". In spite of these limitations, however, our GBCS participants reflect similar levels of adverse chronic conditions observed in nationally representative samples of older urban Chinese adults [26, 133, 134], which in turn, enhance the external validity of the present findings.

In the LURIC investigation, inclusion of Caucasian patients only limits the generalisability of our results to other ethnic populations who are already intermediate-to-high risk for CAD. Although prospective in nature, the measurement of RHR was determined at a single momentary time-point in both LURIC and EPIC-Norfolk studies, therefore, it is difficult to establish whether elevated RHR is causally related, or simply an epiphenomenon of progressive CVD. Nevertheless, we attempted to record RHR in a standardized method, thereby yielding reliable data while permitting comparison between patients, as well as reducing the risk of methodological bias. Since the frequency of RHR illustrates significant diurnal variation and is easily influenced by several other confounders (i.e., psychic stimuli, postural position, environmental conditions etc.) one should employ strict standardized criteria as a means of evaluating the relationship between RHR and adverse CVD prognosis [191]. Under such conditions, a standardized measurement of RHR would limit the opportunity of methodological bias, while enhancing the scientific value of RHR itself, yielding more accurate and reliable data, allowing for the comparison of RHR between studies. Lastly, across all cohorts, we did however, correct for a large number of potential confounders that are most often considered to be associated with increased pulse rate.

8.4. CONCLUSION AND FUTURE DIRECTIONS

For some time, a raised RHR has remained a neglected risk factor of poor health in the clinical setting. Despite this, we propose that RHR should no longer be overlooked since our data along

with others indicate that sustained elevated RHR is strongly associated with increased cardiovascular risk as well as mortality. In the present thesis, an accelerated RHR tended to amplify the risk of CVD in generally healthy individuals, as well as among patients already at intermediate-to-high-risk for the condition. Though we were unable to determine the relation between RHR and mortality in Asian participants, nevertheless, we were able to identify a strong association between high RHR and a number of surrogate markers of vascular risk, suggesting the burden of increased RHR extends beyond Caucasian subjects. In light of the growing literature describing the relation between resting tachycardia and adverse CVD prognosis, it comes as no surprise that the European Society of Cardiology and the European Society of Hypertension [164] along with the Global Registry of Acute Coronary Events score [165] have recently included RHR as a risk factor for unfavourable CVD outcomes. These guidelines support the contention for slowing the pulse rate in an effort to attenuate the risk attributable to CVD. Indeed, few trials have already demonstrated the potential health benefits afforded by effective pharmacological therapy when slowing the RHR [162, 163]. Albeit, a practical limitation is these data are mainly confined to patients already at-risk for CVD. As a departing point for this conclusion, I would encourage forthcoming trials to consider the potential pharmacological implications of manipulating pulse rate among non-cardiac patients as well. Should a beneficial effect be confirmed, reducing the pulse rate pharmacologically may constitute a major public health benefit in one of the largest areas of human disease.

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