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Extending the limits of cardiovascular disease risk estimation:

The roles of HDL cholesterol, resting heart rate and advancing years

Marie Therese Cooney

PhD Thesis

2010

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DECLARATION

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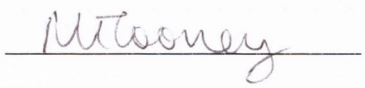

Marie Therese Cooney

ACKNOWLEDGEMENTS

Special thanks are due to three groups whose generous collaboration of their data allowed these analyses to be undertaken:

- The SCORE investigators: R.M. Conroy, K. Pyorala, A.P. Fitzgerald, S. Sans, A. Menotti, G. De Backer, D. De Bacquer, P. Ducimetiere, P. Jousilahti, U. Keil, I. Njølstad, R.G. Oganov, T. Thomsen, A. Tverdal, H. Wedel, P. Whincup, L. Wilhelmsen, I.M. Graham, contributed the data used in the HDL analyses and additionally in the SCORE O.P. section
- The National FINRISK study investigators, in particular Prof E. Vartiainen, Dr. A Joulevi and Dr. T. Laatikainen, provided the data used for the resting heart rate analyses
- The Cohort of Norway investigators, especially Dr. A. Lindman and Prof A. Tverdal, provided additional data used in the SCORE O.P. section.

I want to thank Prof Graham, my supervisor and principal investigator on the SCORE project, who taught me a great deal about epidemiology and prevention and provided constant advice and support. We have spent many enjoyable hours discussing the various findings, their interpretations and how to proceed with future analyses.

I also want to thank Dr. Alexandra Dudina, my colleague, for her help during the three very enjoyable years we spent working together.

I am very grateful for the help of both Dr. Ronan Conroy and Dr. Anthony Fitzgerald, the biostatisticians who derived the initial SCORE function. They provided extremely useful statistical advice, particularly regarding the methods for the derivation of the risk functions

Lastly, I want to thank my family and especially my husband, Dr. David Bradley, for their help and support during my research and the preparation of this thesis.

SUMMARY

Background: Atherosclerotic cardiovascular diseases (CVD) including coronary heart disease, stroke and peripheral vascular disease are the commonest causes of death worldwide. The underlying atherosclerosis starts early in life, progresses slowly and is often extensive by the time of clinical presentation. While recent advances in medical treatment of these diseases have resulted in reductions in age-specific mortality rates, myocardial infarction, stroke or aortic aneurysms can kill quickly, often before medical care is available. This makes treatments either not applicable or palliative if irreversible damage has already occurred. For these reasons prevention of CVD should be considered a key element of health policy.

The occurrence of atherosclerosis is known to relate to a number of risk factors, modification of which has been shown to prevent or delay their onset. The population strategy aims to reduce the incidence of disease by shifting the population risk factor distribution in a favourable direction. The high risk strategy is complementary and identifies those at highest risk and directs the most intensive risk factor modifications towards those who benefit most.

Multiple risk factors may combine to produce a level of risk that may be more than additive. For this reason guidelines on CVD prevention stress the importance of total CVD assessment. Those with established CVD have already declared themselves to be at highest risk. However, in asymptomatic individuals a system for estimating total CVD risk is required. SCORE (Systematic COronary Risk Evaluation) is the system recommended by the European guidelines on CVD prevention. It estimates 10 year risk of CVD death based on age, gender, systolic blood pressure(SBP), total cholesterol (TC) and smoking status.

In common with other systems, SCORE has a number of limitations that prompt research questions. Does the complexity of incorporating additional risk markers result in a measurable improvement in performance? Alternatively, can a simpler risk estimation system -which can be used outside the clinic perform usefully well? Can risk estimation in older persons be improved? This is particularly important in the presence of an aging population.

This thesis focuses on addressing some of these issues by (1) examining more sophisticated risk estimation by exploring the role of high density lipoprotein (HDL) cholesterol in depth; (2) exploring the relationship between resting heart rate(RHR) and CVD risk; (3) simplifying risk estimation by looking at the performance of a risk estimation system based on age, gender, smoking, body mass index (BMI) and RHR alone, and (4) by re-examining the issue of risk estimation in older persons by challenging the concept that risk coefficients apply uniformly regardless of age.

Study Populations: The SCORE dataset, which contains pooled data from 12 European cohort studies (>2.1 million person years of observation) was used for the HDL cholesterol analyses. The National FINRISK study, a representative cohort study of the general population with up to 27 years follow-up, was used for the RHR

and simple risk estimation analyses. Three of the original SCORE cohorts contained individuals aged over 65 years, these and the representative Norwegian cohort study, CONOR, were used for the analyses of risk estimation in older people.

Methods and Results: The Cox proportional hazards model was used for the multivariable analyses and for derivation of the risk functions. The major findings were:

1. HDL cholesterol was shown to be an independent protective factor in both genders, all age groups and at all levels of total CVD risk. Importantly, this finding was found to apply to older women.

2. SCORE HDL, a risk estimation system incorporating HDL cholesterol, was derived and internally validated. This incorporation resulted in a significant but modest improvement in discrimination (AUROC: 0.808 to 0.814}. Changes in risk classification are particularly important since treatment decisions are based on risk categorisation. Incorporation of HDL cholesterol resulted in 2.2% of the total population and 11.5% of women from high risk countries correctly reclassified as high (≥5% 10 year risk of CVD death)) or low risk(<5%).

3. Resting Heart Rate (RHR) was shown to predict future CVD, CHD and total mortality in the general population. RHR >90bpm compared to <60bpm was associated with an independent 2 fold increased risk of CVD mortality, 3 fold in women –similar to that of current smoking. The effect was consistent across subgroups, independent and persisted after exclusion of events occurring within the first 2 years, suggesting that elevated RHR is not merely functioning as a marker of sub-clinical disease. Incorporation of RHR into a risk estimation system containing the current SCORE variables did not result in a meaningful improvement.

4. A function containing only easily measured variables: age, gender, body mass index, RHR and smoking status resulted in good discrimination. The incorporation of RHR improved both discrimination (AUROC: 0.81 to 0.82 men, 0.85 to 0.87 women) and classification, with 14% of the group correctly reclassified.

5. Risk factors including SBP, total and HDL cholesterol, current smoking, and diabetes continued to function in the elderly, with lower relative risks compared to younger individuals. A risk estimation system was derived using data restricted to the older people, SCORE O.P. with age-specific weightings for the risk factors. This resulted in improved discrimination- AUROC: 0.68 to 0.70 in men and 0.74 to 0.79 in women.

Conclusions: This thesis adds to current knowledge in the area of CV epidemiology and prevention by:

- Demonstrating the independent protective effect of HDL cholesterol in the largest analysis of its kind and extending this to older women. Previous studies showing this were small and included high numbers of women with pre-existing CVD.

- Clarifying a number of issues relating to the role of elevated heart rate as a risk factor in the healthy population, specifically, demonstrating the independent effect in women, independence from physical activity and co-morbidities and a temporal sequence which would be consistent with a causal relationship.

- Developing an accurate risk estimation system including only easily measured variables and demonstrating that this system is improved through the incorporation of RHR.

- Demonstrating the effect of several conventional risk factors in the elderly in the largest multivariable analysis of this age group to date.

- Providing the first risk estimation system specifically derived from older men and women, SCORE O.P. which resulted in better discrimination than that demonstrated in any previously published analysis.

Future work will focus on external validation of these newly derived risk functions. Once this is completed they can be incorporated into HeartScore, which will enhance both accessibility and clinical application.

PUBLICATIONS

The work in this thesis has led to the following peer-reviewed publications:

- Cooney MT, Dudina AL, Graham IM. Value and limitations of existing scores for the assessment of cardiovascular risk: a review for clinicians. *J Am Coll Cardiol*. Sep 29 2009;54(14):1209-1227.
- Cooney MT, Dudina A, De Bacquer D, et al. HDL cholesterol protects against cardiovascular disease in both genders, at all ages and at all levels of risk. *Atherosclerosis*. 2009;206(2):611-616.
- Cooney MT, Dudina A, De Bacquer D, et al. How much does HDL cholesterol add to risk estimation? - A report from the SCORE investigators. *Eur J Cardiovasc Prev Rehabil*. 2009;16:304-314.
- Cooney MT, Vartiainen E, Laatikainen T, Juolevi A, Dudina A, Graham I. Is elevated heart rate a predictor of Cardiovascular Disease? A report of the National FINRISK study. *Am Heart J*. 2009; 159: 612-619.

The following publications have been accepted for publication:

- Cooney MT, Vartiainen E, Laatikainen T, Juolevi A, Dudina A, Graham I. Simplifying Risk Estimation using Resting Heart Rate. *Eur Heart J*. 2010.
- Cooney MT, Dudina A, D'Agostino R, Graham IM. Cardiovascular risk estimation systems in primary prevention: Do they differ? Do they make a difference? Can we see the future? *Circulation*. 2010.

The following manuscript is in preparation for submission for publication:

- Cooney MT, Dudina A, De Bacquer D, Lindman A, Tverdal A, Thomsen T, De Backer G, Graham IM. SCORE O.P. - Extending risk estimation to older people.

Sections of this work which were completed before end 2007 were included in the following publication:

- Graham I, Atar D, Borch-Johnsen K, Boysen G, Burell G, Cifkova R, Dallongeville J, De Backer G, Ebrahim S, Gjelsvik B, Herrmann-Lingen C, Hoes A, Humphries S, Knapton M, Perk J, Priori SG, Pyorala K, Reiner Z, Ruilope L, Sans-Menendez S, Op Reimer WS, Weissberg P, Wood D, Yarnell J, Zamorano JL, Walma E, Fitzgerald T, Cooney MT, Dudina A. European guidelines on cardiovascular disease prevention in clinical practice: full text. Fourth Joint Task Force of the European Society of Cardiology and other societies on cardiovascular disease prevention in clinical practice. *Eur J Cardiovasc Prev Rehabil. Sep* 2007;14 Suppl 2:S1-113.

PREAMBLE

Atherosclerotic cardiovascular disease (CVD) is the leading cause of death worldwide. In most people it is the product of several risk factors operating over many years. All current guidelines on the management of CVD stress the need for both a preventive strategy and for reliable methods of assessing risk of future CVD in apparently healthy persons to permit a logical approach to management.

Several CVD risk estimation systems are available. The best known are those based on the American Framingham project[1-3] and the European SCORE (Systematic COronary Risk Estimation project[4]. Both are relatively crude tools yet can assign persons to risk categories with reasonable certainty. Both share similar dilemmas. Does the expense and complexity of adding newer risk markers result in useful improvements in performance? Conversely, can risk estimation by simplified by using only simple variables not requiring laboratory input, such as age, gender, smoking, body mass index and resting heart rate. Of these, least is known about the role of resting heart rate as a risk factor. Would such a system perform well enough to be practically useful for developing societies and save money in developed ones? Finally, all developed societies are ageing. As far as we know, measures to prevent CVD mostly transfer it to older age groups, and to different CVD categories such as stroke, heart failure and perhaps dementia rather than actually preventing atherosclerosis. In general, the performance of most risk estimation systems deteriorates with increasing age, these reasons are explored and some solutions presented.

In this thesis, I use large cohort study datasets to address some of these issues by: examining more sophisticated risk estimation by exploring the role of **high density lipoprotein (HDL) cholesterol** in depth; exploring the relationship between **resting heart rate** and CVD risk in depth; **simplifying risk estimation** by looking at the performance of a risk estimation system based on age, gender, smoking, body mass index and resting heart rate alone, and by re-examining the issue of **risk estimation in older persons** by challenging the concept that risk coefficients apply uniformly regardless of age. Arising out of these considerations, several specific research questions arise:

RESEARCH QUESTIONS

HDL CHOLESTEROL (CHAPTER 2)

1. What is the effect of HDL level on cardiovascular risk? – including cardiovascular mortality, coronary heart disease mortality and stroke mortality
2. Does the effect apply equally in subgroups based on:
    a. Age

   b. Gender
   c. Country of origin
   d. Level of total risk calculated by SCORE
3. Is this effect independent of the effect of other CV risk factors?
4. Can we identify subgroups where HDL-C is particularly important or groups in whom HDL is not related to CV risk?
5. What other factors are associated with level of HDL?
6. Are any of these factors potentially involved in determining the level of HDL of an individual and are these factors modifiable; what are the public health implications of these findings?
7. Are there interactions between HDL and other cardiovascular risk modifiers?
8. Are the criteria for causality met?
9. Will development of a SCORE risk estimation system with HDL-C as an additional variable result in a significant improvement in our ability to estimate risk of CVD?
10. In a new risk estimation function containing HDL should the coefficients for the risk factors be calculated separately in men and women?
11. Does inclusion of HDL cholesterol improve risk estimation in the intermediate risk group – those on the borderline of high / low risk?
12. Can a simple paper-based system be developed whereby the HDL cholesterol value can be included only for the individuals for whom it makes the greatest difference to their risk estimate?

RESTING HEART RATE & SIMPLIFYING RISK ESTIMATION (CHAPTER 3)

1. What is the effect of resting heart rate level on risk? – including total mortality, cardiovascular mortality, coronary heart disease mortality and stroke mortality
2. What is the relationship between resting heart rate and non-fatal events?
3. Does the effect apply equally in men and women?
4. Does the effect apply equally in those with and without pre-existing hypertension?
5. Is this effect independent of the effect of other CV risk factors, particularly other conventional cardiovascular risk factors, measures of physical fitness and measures of general fitness and co-morbidities?
6. What other factors are associated with elevated resting heart rate?
7. Are the criteria for causality met?
8. Will development of a SCORE risk estimation system with resting heart rate as an additional variable result in a significant improvement in our ability to estimate risk of CVD? Improvement would be assessed based on measures of calibration, discrimination and the numbers corrected classified into high and low risk groups.

9. What level of risk estimation can be provided by a risk estimation system containing only easily measured variables such as heart rate, BMI, age, sex and smoking status?

SCORE O.P. EXTENDING RISK ESTIMATION TO OLDER PERSONS (CHAPTER 4)

1. Are there differences in the effects of risk factors on both CHD and non-CHD CVD mortality in the older and younger age groups?
2. Are there differences in the proportions of CVD mortality caused by CHD and stroke in different age groups?
3. Can a function for the estimation of CVD risk in the older age group, called SCORE O.P., result in improved risk estimation in older people?

   This risk estimation system would be derived specifically and exclusively from longitudinal data from the over 65 year old age group. The hypothesis is that by using the specific risk factors which are most important in the older age group and age-specific beta coefficients accuracy can be improved.

   The derivation dataset will include the original SCORE dataset over 65 year olds and additionally a large Norwegian dataset will be added. To facilitate this the baseline survival (both CHD and nCHD) of the Norwegian cohort will need to be examined in order to assess the most appropriate method for incorporating this newer data.
4. How does this risk function perform on internal validation?
5. How does this performance compare to that of the original SCORE function in the older age group?
6. What is the most appropriate method for dealing with blood pressure lowering treatment in risk estimation systems in the older age group?

ABBREVIATIONS

ACAT	Acyl-CoA:cholesterol acyltransferase
AUROC	Area under receiver operating characteristic curve
BMI	Body mass index
Bpm	beats per minute
CEPT	cholesterol ester transfer protein
CHD	Coronary heart disease
CI	confidence interval
CRP	C reactive protein
CV	Cardiovascular
CVD	Cardiovascular disease
DM	Diabetes mellitus
HbA1c	Haemoglobin A1c
HDL	High density lipoprotein
HRT	Hormone replacement therapy
ICAM-1	Intercellular adhesion molecule 1
IL	Interleukin
ISH	International Society of Hypertension
LCAT	Lecithin: cholesterolacyl transferase
LDL	Low density lipoprotein
LRCPS	lipid research clinics prevalence study
MCP-1	Monocyte chemotactic protein-1
mg/dl	milligrams per decilitre
MI	Myocardial infarction
mmHg	millimeters of Mercury
mmol/l	millimols per litre
MMP	matrix metalloproteinase
MONICA	Multinational MONItoring of trends and determinants in CArdiovascular disease
nCHD	non-coronary cardiovascular disease
NHANES	National Health and Nutrition Examination Survey
NRI	net reclassification index

O.P.	older persons
PAR	Population attributable risk
PDGF	Platelet derived growth factor
RCT	randomised controlled trial
RHR	resting heart rate
SBP	Systolic blood pressure
SCORE	Systematic COronary Risk Evaluation
std dev	standard deviation
TC	Total cholesterol
TGFB	Transforming growth factor beta
TNFa	Tumour necrosis factor alpha
UK	United Kingdom
USA	United States of America
VA-HIT	Veterans Affairs High density Lipoprotein Intervention Trial
VCAM-1	Vascular cell adhesion molecule 1
VEGF	Vascular endothelial growth factor
VO2 max	Maximal oxygen consumption
WHAS-1	Women's Health and Aging Study 1
WHO	World Health Organisation

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CHAPTER 1 BACKGROUND

CARDIOVASCULAR DISEASE – THE GLOBAL BURDEN OF THE PROBLEM

Globally, cardiovascular diseases are now the most common cause of death[5]. This is the case for both men and women with 32% of deaths in women and 27% of death in men in 2004 throughout the world being caused by cardiovascular diseases[5].

These proportions are currently considerably higher when looking specifically at developed countries. For example, in Europe as a whole in the time period 2002-2006, 43% of deaths in women and 38% of deaths in men were caused by CVD[6].

As countries go through epidemiological transitions the main causes of death change from communicable, maternal, prenatal and nutritional causes of death which affect younger individuals to non-communicable diseases which tend to cause death at older ages. Most of the world's countries have now made this transition, as shown in Figure 1.1, from [5]. Africa is now the only World Health Organisation(WHO) region in which communicable diseases continue to outnumber non-communicable diseases as a cause of death.

Overall, over the next 20 years, it is projected that the leading cause of death will be ischemic heart disease followed by cerebrovascular disease[5]. It seems counter-intuitive that the numbers of deaths due to these diseases will occur in the presence of decreasing age specific rates of CVD disease. However, this occurs because the epidemiological change (decrease in age specific rates of CVD) which will occur worldwide will be offset by increases in CVD deaths due to global population aging and, in low and middle income countries, to population increase (see Figure 1.2).

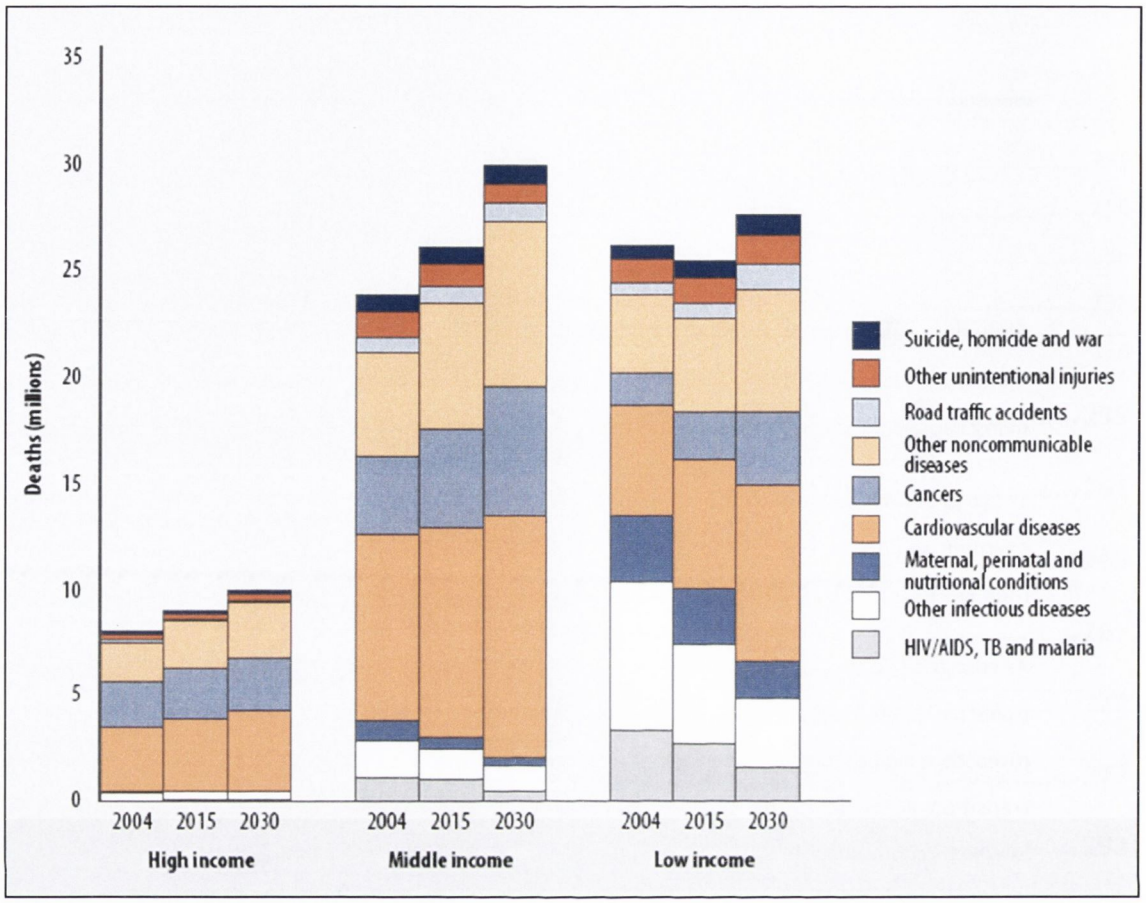


Figure 1.1: Projected deaths by cause for high, middle and low income countries (from [5] with permission)

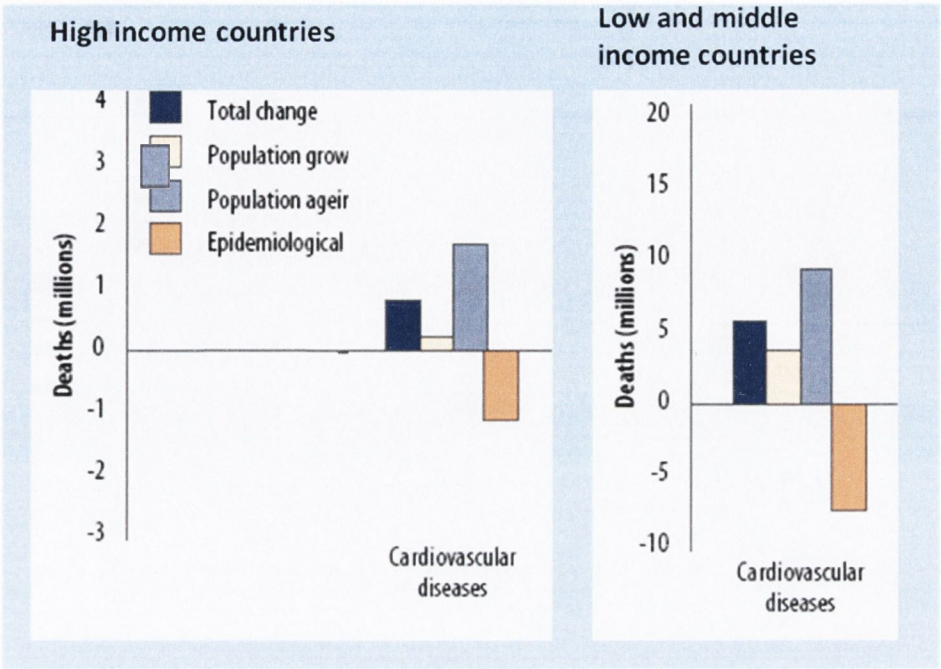


Figure 1.2: Decomposition of projected changes in annual numbers of deaths from CVD by income group, 2004-2030 (adapted from [5] with permission)

Across Europe there are marked gradients in the rates of CVD. Previously this high to low risk gradient went from North to South. Now though the gradient is steeper from West to East. Figure 1.3 shows the CHD mortality rates in men aged under 65 in each European country. I have used raw data from the WHO database and age standardized the rates using the European standard population. As can be seen in the figure, the rates of CHD are over 6 fold higher in Russian than Irish men.

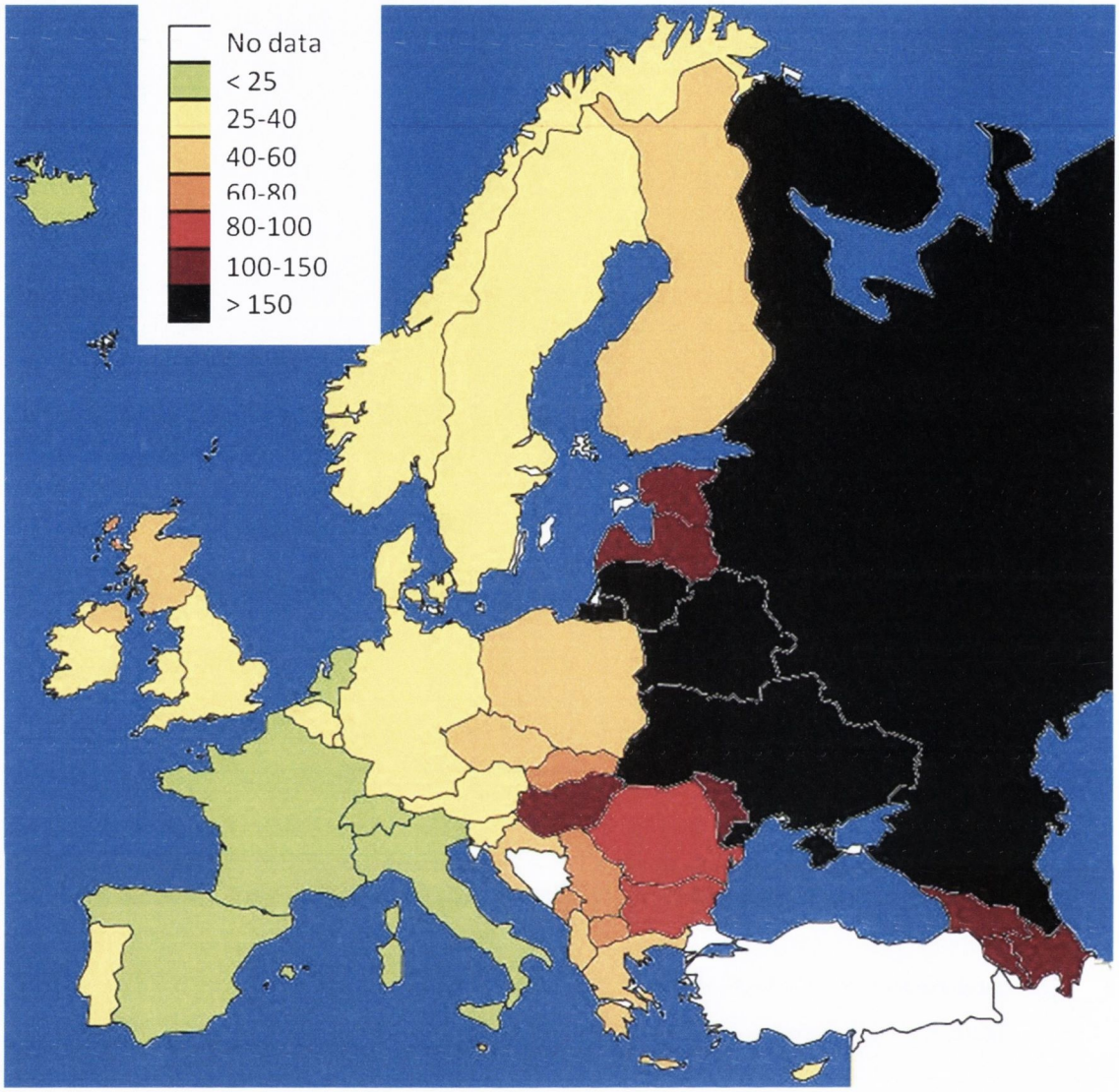


Figure 1.3: Age-standardised coronary heart disease mortality rates (per 100,000 population) in European men aged under 65 years

Over the last 30 years mortality rates from CVD have been falling rapidly in high Western and Northern European countries, as well as in the United States. However, in Eastern Europe and the states of the former United Soviet Socialist Republic the rates have risen dramatically over the last 10-15 years. In some of these countries the rates are now starting to decrease, although in others they continue to rise. These trends are illustrated in **Figure 1.10** on page 51. It has been estimated that approximately 50% of the reduction in CVD mortality is due to risk factor reduction at a population level, with the other 50% due to improvements in medical care for those who suffer coronary or cerebrovascular syndromes[7].

ATHEROSCLEROSIS

ATHEROSCLEROSIS – CLINICAL MANIFESTATIONS

Atherosclerosis, the process responsible for most cardiovascular disease, is a complex process which starts early in life. Atheroma or atherosclerotic plaques develop in the arteries of coronary, carotid, peripheral and other systems and result in progressive narrowing of the lumen of the vessel. As the process progresses the risk of clinical manifestations of the presence of atherosclerosis increases.

The manifestations of the atherosclerosis depend on both the site and type of atheromatous plaque. Angina implies a distal narrowing of a coronary artery, whereas an acute coronary syndrome, such as a myocardial infarction (MI) is usually the result of a plaque rupture with superimposed thrombosis leading to occlusion of the vessel [8]. Stroke may result from plaque rupture, thrombosis or embolization of plaque components. Gangrene of a lower limb may be the result of progression of atheroma or cholesterol emboli[8].

ATHEROSCLEROSIS - PATHOGENESIS

There are several stages in the development of atheroma. The process is considered to start with the formation of fatty streak. This progresses to the fibrous plaque which then progresses to the unstable plaque which is prone to rupture[9]. In recent years it has become increasingly clear that inflammation is an integral part of the process and that the progression of these stages may not occur as smoothly and continuously as previously thought[8, 9]. Described below is the complex process involved in the evolution of the atherosclerotic plaque.

Histologically, the plaque is composed of cells, including endothelial, smooth muscle and immune cells, lipids, debris and connective tissue elements[8]. Lipid droplets, foam cells and necrotic debris are contained in the core of the plaque, which is surrounded by smooth muscle cells and a collagen rich matrix. Immune cells are also abundant in the developing atheroma.

The first step in the formation of atheroma is the fatty streak. In the presence of increased levels of circulating cholesterol, lipid is laid down in the subendothelial space. These fatty streaks are common and can be found in young people. They may either regress spontaneously or progress to form atheroma[9].

The atherogenic process is initiated by the combination of haemodynamic strain and lipid deposition in the endothelium[8]. The importance of haemodynamic strain in the initiation of the process explains why certain vascular sites including branch points and arterial flow dividers are particularly prone to atherogeneisis[9]. The oscillating shear stress as opposed to laminar blood flow predisposes to the formation of atheroma at these sites.

Normal circulating low density lipoprotein cholesterol (LDL) particles contain small amounts of lipid hydroperoxides derived from the lipoxygenase pathway. LDL particles trapped within the endothelial cells accumulate additional lipid hydroperoxides produced by the lipoxygenase and myeloperoxidase pathways. It is hypothesised that once the concentration of LDL cholesterol within an endothelial cell crosses a certain threshold the LDL phospholipids become oxidised and pro-inflammatory[10].

Once LDL cholesterol becomes oxidised it leads to activation of the endothelial cells, through the release of phospholipids[8]. The activation / dysfunction of the endothelium is more likely in the presence of other risk factors associated with endothelial dysfunction including low HDL cholesterol, hypertension, smoking, oestrogen deficiency, advancing age, hyperhomocysteinaemia and diabetes[9]. This activation of the endothelium leads to the production of adhesion molecules and to the expression of inflammatory genes. Endothelial dysfunction also causes disruption of the normal endothelial cell secretion of the vasodilator nitric oxide (NO), which contributes further to the haemodynamic strain.

Adhesion molecules, including vascular cell adhesion molecule 1 (VCAM-1), intracellular adhesion molecule 1 (ICAM-1) and E-selectin, cause immune cells to adhere to the endothelium. E-selectin is expressed on the surface of endothelial cells; it mediates the rolling and loose tethering of leucocytes before the firmer binding and arrest of the leucocytes which are mediated by VCAM and ICAM[10]. ICAM is constitutively expressed on endothelial cells and leucocykes, whereas the expression of VCAM is induced by pro-inflammatory cytokines. Both VCAM and ICAM interact with integrins on the surface of leucocytes in order to cause their adhesion to the luminal surface of the endothelial cell. Monocytes and lymphocytes adhere preferentially, since they express receptors for VCAM1 on their surfaces[10].

Once the immune cells (mainly monocytes and T cells) are adherent to the endothelial surface they are drawn into the subendothelial space through the inter-endothelial junctions by the action of chemokines, including monocyte chemotactic factor 1 (MCP-1)[8]. Interleukin 8 (IL-8) may also have a role as a chemokine[11].

Once monocytes have entered the subendothelial space they are transformed into macrophages by the action of macrophage colony stimulating factor. This is a critical step because it causes the up regulation of scavenger and toll-like receptors.

Scavenger and toll-like receptors are both components of the innate immune system but act in opposing ways. Scavenger receptors internalise various elements including oxidised LDL, bacterial endotoxin and apoptotic cell fragments and dispose of them[8]. However, when ability of the macrophage to remove enough of the lipid derived from the uptake of oxidised LDL cholesterol is exceeded lipid droplets form inside the macrophage leading the hallmark cell of the atherosclerotic plaque – the foam cell[8].

Toll like receptors also recognise pathogen like particles, including oxidised LDL and other components of the plaque. However, when they intake particles as well as causing the accumulation of lipid inside the macrophage, it results activation of the macrophage[8]. This causes the secretion of inflammatory cytokines – tumour necrosis factor alpha (TNFa) and interleukin 1 (IL-1) along with proteases and cytotoxic oxygen and nitrogen radial molecules, leading to tissue damage[8, 11]. These inflammatory cytokines allow augmentation of the inflammatory cycle. In ApoE knockout mice, genetic modification to effect removal of toll like receptors results in reduced atherosclerosis[8].

The activated macrophages also secrete growth factors which lead to the intimal migration and proliferation of medial and adventitial smooth muscle cells and myofibroblasts. The latter results in production of the collagen rich matrix, which transforms the fatty lesion into a fibrous lesion and allows the formation of the fibrous cap of the lesion[11]. The death of foam cells by apoptosis leads to formation of the necrotic lipid-rich core of the lesion.

Angiogenic factors, including vascular endothelial growth factor (VEGF) and IL-8 are also secreted by activated macrophages. These lead to neoangiogeneisis within the plaque[11].

As well as monocytes, T cells are also attracted into the developing plaque, including natural killer cells, CD4 and CD8 T cells. These T cells can be activated by antigens (which include components of the atherosclerotic plaque e.g. oxidised LDL cholesterol and heat shock proteins) presented to them by macrophages and dendritic cells. These T cells produce two different responses. The Th 1 response signals the production of inflammatory cytokines including interferon gamma and the Th 2 response modulates the pro-inflammatory reaction and signals anti-inflammatory cytokines including transforming growth factor beta and interleukin 10. In the atherosclerotic plaque the Th1 response predominates. Interferon gamma further potentiates the activation of macrophages[8].

While the Th2 response is anti-inflammatory, it can also promote vascular disease due to the production of elastolytic enzymes which can contribute to the formation of aneurysms. B cells also contribute to modulating the atherosclerotic process and have anti-inflammatory effects[8].

Platelets are also attracted to the activated endothelium. Once they adhere to the endothelium and become activated they release the contents of these granules, including tissue factor and cytokines which amplify the process of monocytes and vascular smooth muscle cell migration and proliferation. Activation of platelets also leads to the release of arachadonic acid with can be converted to thomboxane A2 – known to cause vasoconstriction and platelet aggregation[9].

The overall inflammatory cascade described above leads to the release of interleukin 6, which causes the release of a variety of acute phase reactants from the liver[8]. These include CRP, which can be detected in

the blood and can be utilised clinically as a marker of the process[12]. Whether CRP actually contributes as a mediator of the process is still under considerable debate.

THROMBOSIS AND CONSEQUENCES OF ATHEROMA

The immune/inflammatory process is also involved in the transformation of a stable plaque to an activated plaque, which is prone to rupture. Activated T cells, mast cells and macrophages produce a number of inflammatory cytokines, proteases, coagulation factors, radicals, and vasoactive molecules. These prevent the formation of a stable fibrous cap on the atherosclerotic plaque and cause de-stablisation of the cap when present. Two proteases in particular are thought to be implicated in degradation of the collagen matrix - matrix metalloproteinases (MMPs) and cysteine proteases[8].

The shoulder regions of the plaque are particularly prone to rupture, because these areas have high concentrations of macrophages and T cells and because apoptosis occurs here[9].

Usually the fibrous cap prevents contact of material, including pro-coagulant factors, in the core of the lesion with the blood stream. Fissure or rupture of the fibrous cap exposes these elements to the bloodstream and causes thrombosis. The core also contains pro-thrombotic elements including phospholipids, tissue factor, and platelet-adhesive matrix molecules[8, 9]. It is this superimposed thrombus which causes acute occlusion of the coronary artery leading to myocardial ischemia and infarction unless the prompt re-vascularisation is instituted.

Endothelial dysfunction, resulting in reduced potential for vasodilatation also results in increased risk of luminal occlusion at the time of an acute thrombosis[9].

Another potential mechanism for sudden plaque progression is the rupture and micro-haemorrhage of the fragile new blood vessels produced in the atheroma as a result of neoangiogenesis[11]. The microhaemorrhage may lead to in situ thrombosis which can simulate the proliferation of smooth muscle cells within the atheroma and consequent growth of the plaque. The formation of thrombin leads to the activation of platelets which release platelet derived growth factor (PDGF) and transforming growth factor beta (TGFB), which are known to further increase smooth muscle cell proliferation (PDGF) and stimulate the deposition of collagen (TGFB)[11].

The main elements involved in the atherosclerotic process are summarised graphically in **Figure 1.4**.

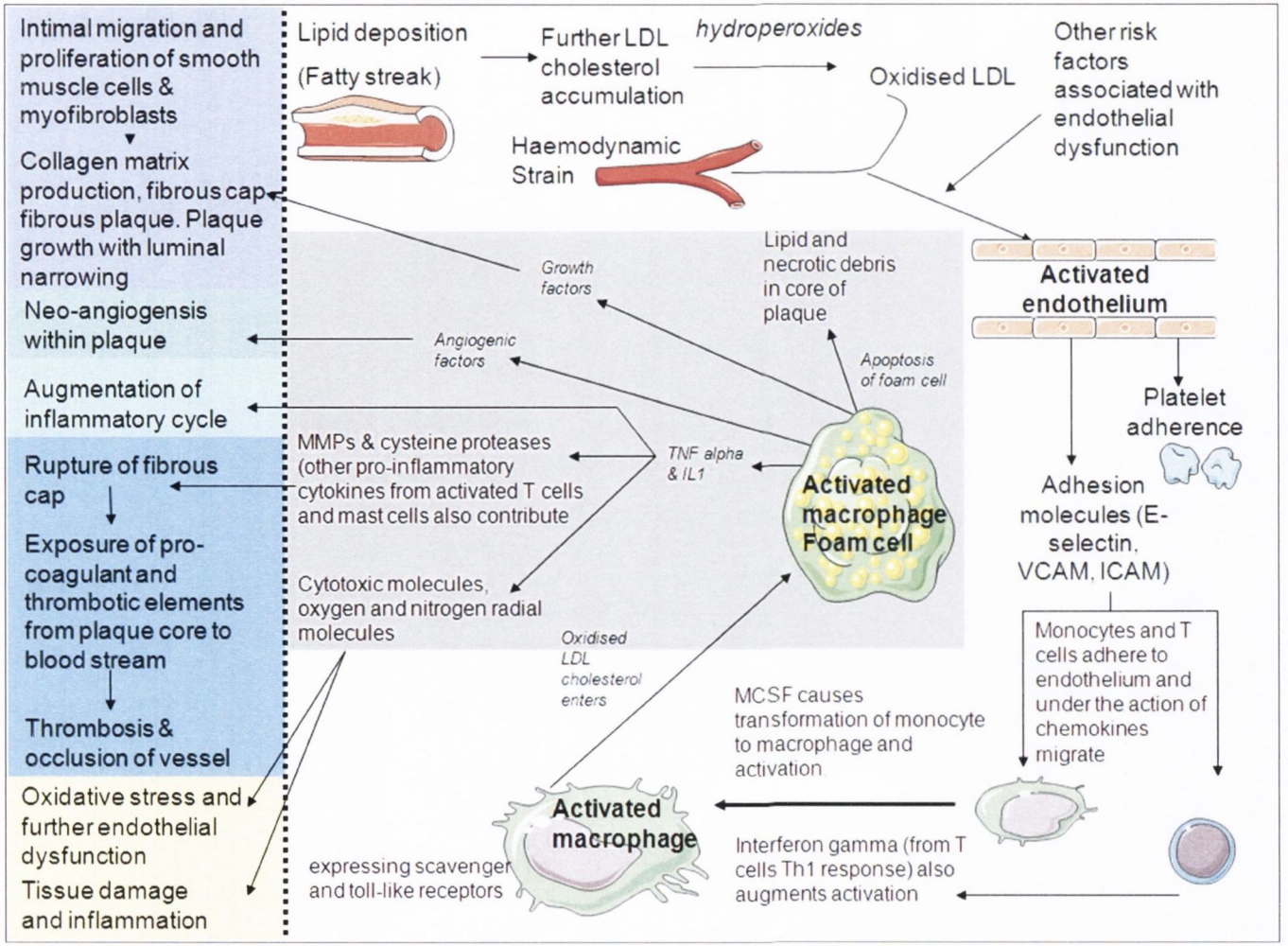


Figure 1.4: The atherosclerotic process

CAUSATION

A risk factor may be defined as a characteristic of an individual that is associated with an increased risk of developing a specific disease such as atherosclerotic CVD. However, because a certain characteristic is associated with the presence of a disease does not imply that the factor actually caused the disease. Potentially, several other situations could be confounding the association. For example, the characteristic could be a surrogate marker of another risk factor or the characteristic could be occurring due to the existence of undetected disease (reverse causality).

Austin Bradford Hill was a statistician who contributed very substantially to his field. In a seminal paper in 1965 he defined criteria for causality[13]. Fulfilment of these has since become accepted as evidence of a causal relationship. The criteria for causality are detailed in Table 1-1. Hill's other highly significant contributions to the area include his demonstration, with his protégé Richard Doll, of the association between cigarette smoking and lung cancer and his pioneering of the randomised controlled trial (RCT).

Strength	A large increase in risk when exposed to a factor is easier to detect and supports causation. However, it does not follow that a modest association excludes causality
Consistency	Findings which are repeatedly observed by different persons, in different places, circumstances and times make the association less likely due to chance and strengthen the likelihood of an effect
Specificity	Causation is more likely if only a certain population exposed to the risk factor develops the disease, assuming that that population does not have any other reason for developing the disease
Temporality	The effect has to occur after the cause, and this is particularly important if there are several factors involved
Biological gradient	The association is more likely to be causal if there is a dose-dependent response of the disease to dose of the risk factor exposed. Lack of this relationship may weaken but does not exclude causality
Plausibility	A biological plausible mechanism between cause and effect is helpful but this could be limited by present knowledge
Coherence	New findings on the association of the risk factor should not seriously conflict known facts about the disease
Experiment	If an intervention to reduce the risk factor in question prevents the disease, then this may be the strongest argument in favour of causality
Analogy	In some circumstances, it may be possible to judge through previous similar experiences

Table 1-1: Criteria for causality, from Hill [13]

These criteria have been adapted for CVD by Jeremiah Stamler and others. For example, independence from other risk factors is also considered important and the original criterion of specificity applies less to CVD, because it has a multifactorial aetiology.

RISK FACTORS FOR ATHEROSCLEROSIS

Numerous markers have been shown to be associated with atherosclerotic CVD. These include lifestyle characteristics, metabolic and biochemical abnormalities and genetic traits. However, as yet only a few of these can be considered to be truly causal. Uppermost of these are cigarette smoking, elevated blood pressure, elevated total cholesterol and diabetes. Several more factors fulfill most of the criteria and are generally accepted as risk factors, these include: elevated body weight, reduced physical activity, low HDL cholesterol and family history of CHD. The Framingham study, a cohort study which commenced in the 1950s was particularly influential in elucidating these relationships[14].

Age and gender are somewhat different. Male gender clearly increases risk of CVD. However, CVD is not more common in men than women. Eventually, more women will die from CVD than men. CVD occurs with the same frequency in men and women, just approximately 10 years later in women. Age may be considered to be exposure time to the other risk factor and not a risk factor in its own right.

In the INTERHEART study[15], nine key risk factors were identified as contributing 90% of the population attributable risk for myocardial infarction. These are illustrated in the pie chart in Figure 1.5 below. However, as INTERHEART is a case control study PAR cannot be calculated accurately and therefore caution is required in interpreting these results. As pointed out by others[16], the 90% PAR does not exclude the fact that other, including as yet undiscovered, risk factors could be potentially important, as individual PARs can sum to greater than 100%. Some novel risk factors with greatest potential as risk factors at present include highly sensitive CRP, homocysteine, lipoprotein (a) and fibrinogen[17].

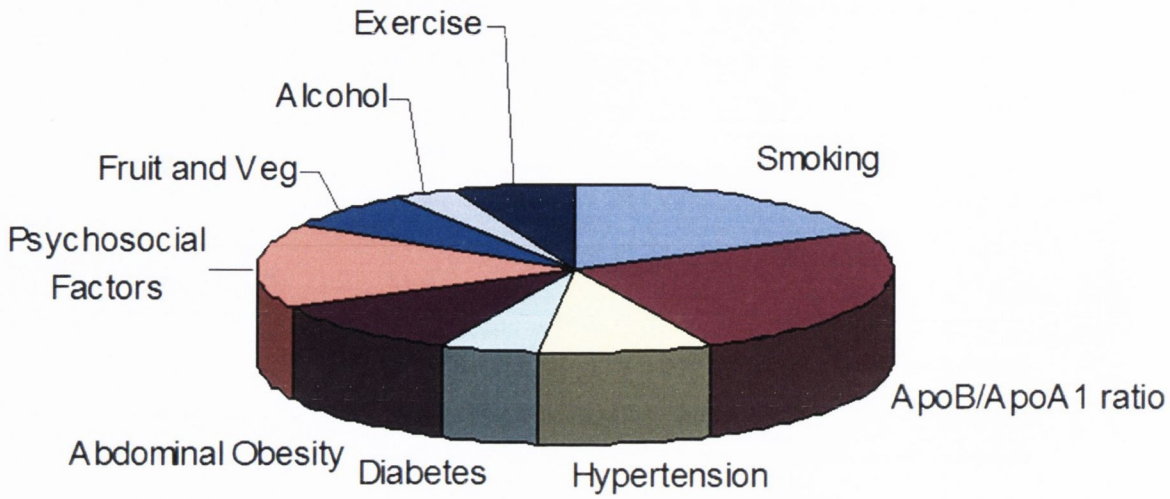


Figure 1.5: Relative contribution of the nine risk factors to the 90% PAR in INTERHEART, from [15]

It should be remembered that lifestyle characteristics are in general much less amendable to investigation in RCTs and therefore the weight of evidence may become balanced towards pharmacological interventions. This should not detract emphasis from these important factors in clinical practice.

Why adopt a preventive strategy?

The reasons why a health service should adopt a preventive strategy for CVD are based on the information above. Firstly, CVD is an important cause both of mortality and morbidity worldwide and as such causes a substantial economic burden, both in terms of lives lost prematurely and in terms of the healthcare of those who survive the initial event but with lasting serious consequences including congestive heart failure and disability due to non-fatal stroke.

Secondly, the atherosclerotic process which leads eventually to most CVD is known to start early in life and progress slowly. By the time individuals present with the symptoms of the disease atherosclerotic burden is already substantial. Therefore, prevention of the process should be initiated early, well before the onset of symptoms.

Over the last two decades, there have been substantial advances in high technology treatments, including percutaneous coronary interventions, thrombolysis which is becoming increasingly available for stroke as well as myocardial infarction and medical treatments which have been proven to provide long term benefit in these patients[7]. The substantial impact of these advances is reflected in the sharp fall in in-hospital mortality associated with acute coronary syndromes over this time period[7], although changes in policies relating to admission to coronary care units may also have contributed.

However, these treatments are not applicable to all those who suffer acute coronary or acute cerebrovascular syndromes. Many die within the first two hours of symptom onset, often before they reach medical attention. For others, these therapeutic interventions are palliative since irreversible damage has already occurred.

As detailed above, the occurrence of atherosclerosis, the pathophysiological process underlying most CVD, is known to relate to the presence of several lifestyle, metabolic and biochemical risk factors[15]. The favourable modification of these risk factors has been shown to prevent (or, when considering lifetime risk, at least to substantially delay) the onset of CVD[18]. Therefore, an approach which considers prevention to be an important aspect of CVD is clearly superior to an approach which focuses on therapeutics alone.

This rationale for the necessity for a preventive approach to CVD is clearly outlined in the guidelines on CVD prevention[17] and forms an important aspect of many policy documents on CVD prevention[19].

OBSERVATIONS OF GEOFFREY ROSE

Geoffrey Rose, a world renowned epidemiologist, drew attention to the fact that a large number of individuals exposed to a small risk may give rise to a much greater number of cases of disease than a small number of individuals exposed to a high risk[20]. Therefore, reducing the determinants of disease in the entire *population* is the most important means for reducing the incidence of disease. This is despite the fact that high risk *individuals* gain most from preventive measures. This theory applies not only to CVD but to a number of diseases, for example, the occurrence of fractures in the elderly; the majority of which occur in those in the intermediate categories of bone density and not in those with severe osteoporosis.

One of the most famous examples was the demonstration that most cases of Down's syndrome occur in babies with younger mothers. This was in spite of the proven sharp increase in risk of the disease with increasing maternal age and occurred because of the much higher numbers of infants born to mothers in the younger age group[20].

Figure 1.6 demonstrates graphically the higher absolute numbers of deaths from CVD occurring in those with low or medium cholesterol levels compared to those with high cholesterol levels, even though the **individual** risk in hypercholesterolaemic patients is much higher. Again this occurs because, on a **population** level, there are many more individuals in these low and medium cholesterol categories. This phenomenon is also demonstrated in **Figure 1.7**, which considers total CVD risk (as calculated using SCORE) and shows that the greatest number of deaths from CVD occurs in individuals in the lower risk categories, because most of the population falls into these categories.

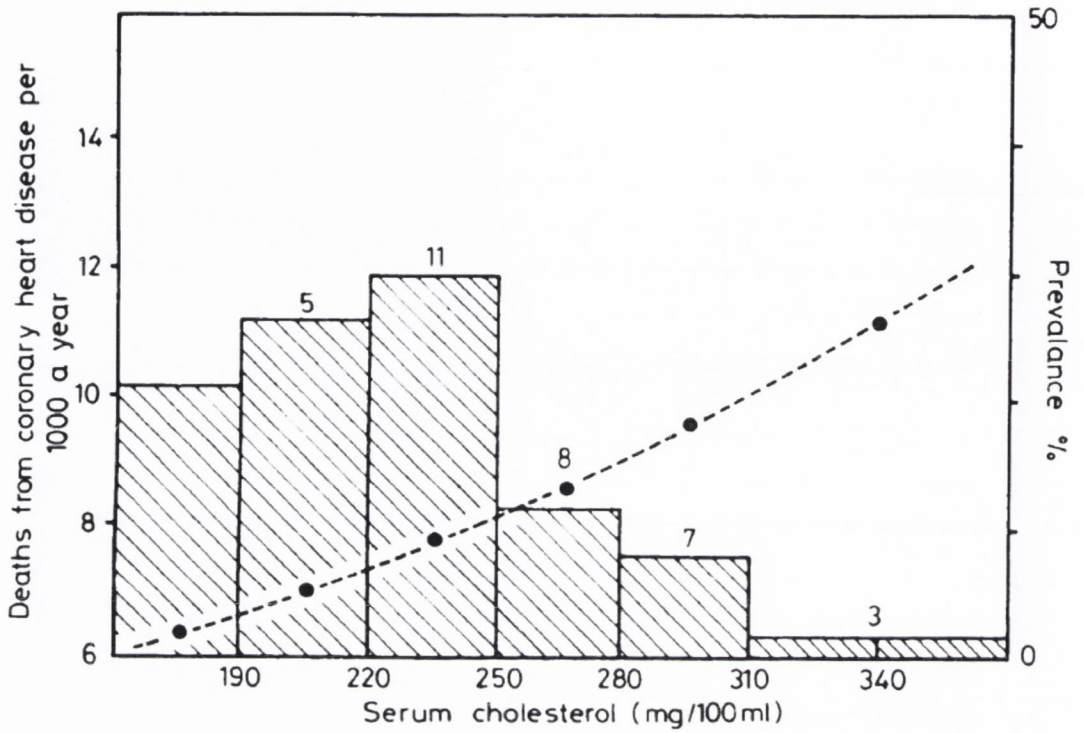


Figure 1.6: Prevalence distribution of serum cholesterol concentration related to coronary heart disease mortality (broken line) in men aged 55-64 years. Number above each bar represents estimate of attributable deaths per 1000 population per 10 years (based on Framingham), from Rose (with permission) [21]

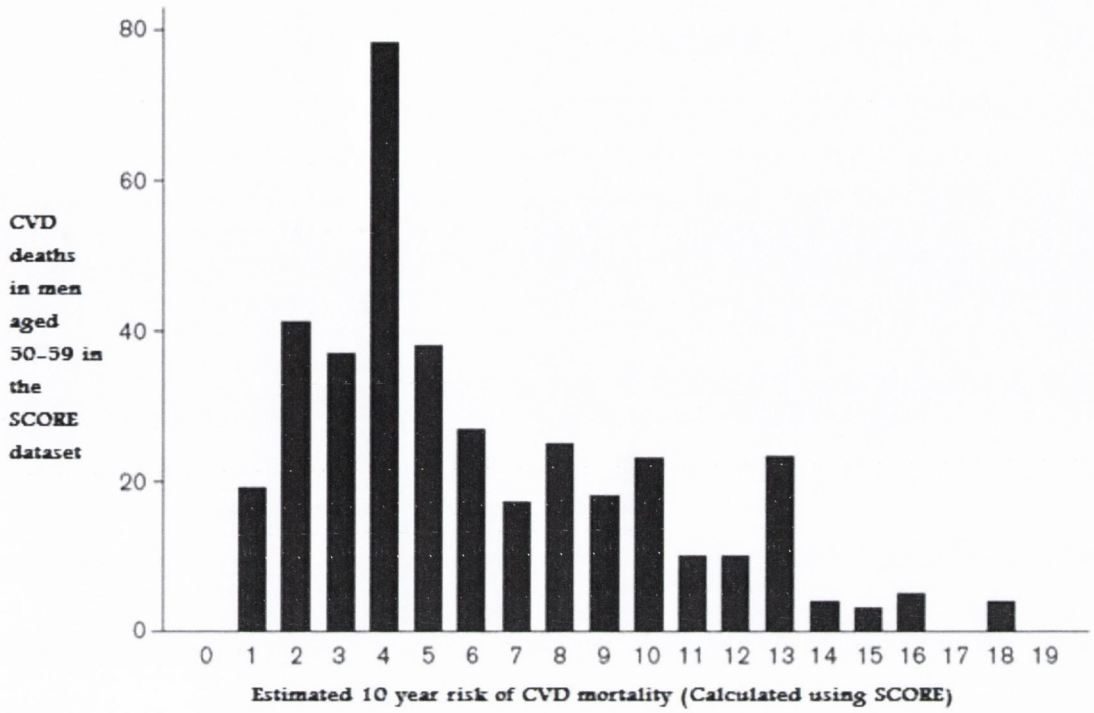


Figure 1.7: The observed number of deaths at each level of predicted risk in men aged 50-59 in the SCORE dataset (risk is % over 10 years, calculated using SCORE, from [17] with permission

Rose also emphasized the prevention paradox – preventive measures applied to an entire population may have a substantial impact on disease in the entire **population** but will offer little benefit to the **individual**.

Rose's observations are the basis for the population strategy and have informed much of public health planning and policy over the last 25 years.

POPULATION AND HIGH RISK PREVENTIVE STRATEGIES FOR CVD

POPULATION STRATEGY

The population strategy for prevention of CVD aims to control the determinants of the disease by shifting the entire population's risk factor distribution in a favourable direction. This approach is necessary in order to reduce the incidence of disease.

Community interventions can be used to effect this reduction in risk factor levels in the population. These interventions are directed towards the entire population in one region. Many have compared the results in

terms of risk factor reduction with the changes observed over the same time period in a control region, which was not exposed to the intervention, in order to assess the benefit if the intervention.

Examples of interventions include government legislation to reduce smoking by banning smoking in public areas, limiting advertising for tobacco products or increasing taxation on cigarettes and improving food labelling. Other approaches which have been used in different community interventions include promoting healthy eating through free cookery demonstrations and classes, the provision of walking and cycle paths to promote physical activity and inclusion of physical activity as part of school curricula.

Several community intervention programmes have been established to reduce population risk factor levels, several of which have been highly successful [22-27]. Many have shown significant net changes including up to 28% reduction in daily smoking, [28] 10% reductions in total cholesterol, [24] 6% reductions in systolic blood pressure [22] and 6% reductions in diastolic blood pressure ; additionally Increases in physical activity and increases in HDL cholesterol of up to 5% have been observed. [26] However, these net figures often underestimate the effect of the intervention because the total change in the risk factors (net difference and the secular change) in the intervention area from the beginning to the end of the programme is often greater still. For example, in North Karelia cholesterol levels fell by over 11% during the first 10 years of the North Karelia project but because extensive public health programmes were ongoing throughout Finland the net difference was only 4%. [28] In Mauritius, a ban on imported saturated fats used as cooking oil reduced population cholesterol by 0.7mmol/l within five years [29]. Population strategies have also been shown to be more cost effective than high risk strategies in previous analyses, in both less and more developed regions. [30, 31]

Some community intervention studies have not shown significant net improvements in population risk factor levels. [32-35] As mentioned above this may be due to strong secular trends which were occurring in the control community at the same time or improvement in risk factors in the control community may have occurred due to the Hawthorn effect, migration between the communities or contamination of the control community by the health promotion activities occurring in the intervention community.

Reductions in population risk factors are not impossible in current developed societies. For example, the Hartslag Limberg (Netherlands) programme began in 1998 and resulted in a 6% net reduction in both SBP and DBP. [22] Another obvious benefit of the population strategy is the reduction in mortality and morbidities due to other pathologies that share common risk factors with CVD. For example, population wide reductions in smoking and obesity prevalence will generate substantial reductions in neoplastic and degenerative diseases, with consequent reductions in health care costs.

HIGH RISK STRATEGY

The high risk strategy aims to identify those at highest risk and to reduce susceptibility in these individuals, through the direction of the most intensive preventive measures, including pharmacotherapy when needed, towards these individuals.

The two approaches should be considered complementary. The success of either depends critically on the extent of population risk factor level reduction and the uptake of the high risk strategy both in terms of screening for high risk status and compliance with prescribed preventive efforts in those who are high risk. This has been demonstrated in two recent analyses[36, 37]. Moreover, high risk interventions will often result in greater success when introduced on a background of a population strategy. For example, advice to a high risk smoker may be more effective when a community wide smoking cessation programme is ongoing.

This thesis will focus more on the high risk strategy, since CVD risk estimation systems are particularly relevant to this strategy. Guidelines on CVD prevention that are intended for use in clinical practice are generally based on the high risk approach because they are directed towards physicians who are dealing with individual patients. Of course, these guidelines always acknowledge the importance of the population strategy, which is complementary, but applies less to physicians and more to health policy makers and those involved in public health decisions.

TOTAL CVD RISK & THE IMPORTANCE OF TOTAL RISK ESTIMATION IN PREVENTION OF CVD

Guidelines on the prevention of CVD stress the importance of assessment of total risk of future development of CVD so that the most intensive preventive measures can be directed towards those who will benefit most. These are the individuals with the highest absolute risk of CVD. Therefore, in the European CVD prevention guidelines, those with the highest risk of future CVD events receive the highest priority for preventive efforts.

The logic of this approach is supported by the results of trials of pharmacotherapy for the prevention of CVD. **Table 1-2**, adapted from Jackson and colleagues' review[38], demonstrates the greater absolute risk reduction for stroke and CHD observed in RCTs of blood pressure and lipid lowering treatment in those at higher baseline risk (due to pre-existing disease in this case) than lower risk individuals, despite an equivalent or even higher relative risk reduction. An interesting analysis by the same group calculated that the number needed to treat to prevent one CVD event in 5 years when using 3 preventive interventions (aspirin, lipid lowering and BP lowering) was only 6 in the very high risk group (30% 5 year risk of CHD) compared to 36 in the low risk group (5% 5 year risk of CHD)[39].

Lipid lowering trials meta-analysis		
Baseline risk of trial participants	Absolute risk reduction (CHD)	Relative risk reduction (CHD)
Few or no participants had a history of vascular disease	2.0% (1.7% to 2.3%)	24% (17% to 30%)
Most or all of participants had a history of vascular disease	3.4% (3.1% to 3.6%)	24% (18% to 21%)
Blood-pressure lowering trials meta-analysis		
	Absolute risk reduction (Stroke)	Relative risk reduction (Stroke)
Few or no participants had a history of stroke	1.4% (1.2% to 1.5%)	35% (29% to 41%)
Most or all of participants had a history of stroke	4.4% (3.4% to 5.4%)	24% (14% to 34%)

Table 1-2: Absolute and relative risk reductions demonstrated in meta-analyses of blood pressure and lipid lowering treatment in higher and lower risk groups, adapted from [38].

As mentioned, according to the European guidelines on CVD prevention, those with established CVD are considered to be at highest risk[17]. Additionally, those with type 2 diabetes or type 1 diabetes with microalbuminuria are automatically considered high risk. Those with markedly elevated levels of single risk factors also require intensive risk factor modification, irrespective of their other risk factor levels. This applies particularly to those with familial hypercholesterolaemia in whom atherosclerosis develops particularly early and quickly.

Other individuals are also at high risk due to the combination of their risk factors. Guidelines on the prevention of CVD stress the importance of total CVD risk because the even moderately elevated levels of risk factors can interact with each other, sometimes multiplicatively, to substantially increase the overall risk. **Figure 1.8** illustrates the importance of considering all of the risk factors and not taking a uni-risk factor approach. For example, from the figure it can be seen that the woman with a TC of 8 mmol/l and ideal other risk factor levels is at much lower total risk (2%) than the man with TC of 5mmol/l if he is also a smoker with moderate hypertension (14%).

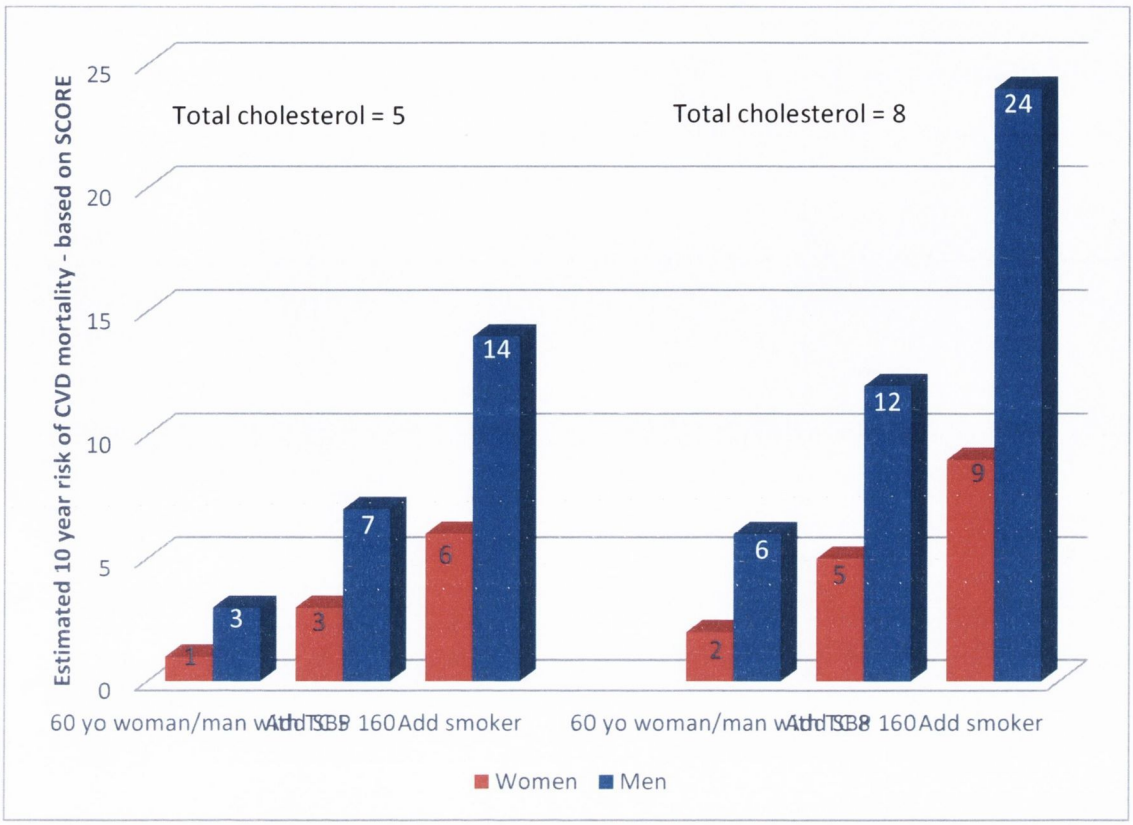


Figure 1.8: Illustration of the increase in total CVD risk which occurs with the addition of extra risk factors in men and women aged 60 years

In asymptomatic individuals a system for estimating the total CVD risk due to these risk factors is required. Of course, *total* CVD risk can never be assessed because the measurement of all known risk factor levels would not be possible and additionally, there may be many other risk factors for CVD which are as yet unrecognized. Therefore, for the purposes of guidelines on CVD prevention we consider total CVD risk to be the risk due to the combination of the main risk factors, which are commonly measured in clinical practice. In the European guidelines this includes the variables in the SCORE function – smoking, age, total cholesterol, SBP. Other risk factors which elevate total CVD risk include low HDL cholesterol, obesity (especially abdominal obesity), family history of CHD, social deprivation, high triglycerides, sedentary lifestyle and the presence of subclinical atherosclerosis.

CVD RISK ESTIMATION SYSTEMS

Calculation of the total risk caused by a combination of different risk factors is complicated and when physicians assess this risk using an "eyeball" method the results have been very inaccurate[40]. Risk estimation systems provide a convenient method for physicians and healthcare workers involved in CVD prevention to estimate risk quickly and easily. The first, and possibly most commonly used, risk estimation

system was developed by the Framingham group. More recently, other systems including SCORE have been developed. These systems calculate the risk of a certain outcome (usually risk of CVD events) occurring within a certain time frame (usually 5 or 10 years but lifetime risk can also be assessed) based on the individual's measured risk factor levels.

The basic principle of developing a risk estimation system is to use statistical methods (mainly Cox or Weibull) to combine the baseline risk of CVD in that population (the risk in an individual with the baseline level of risk factors) with the risk associated with that individual's combination of risk factor levels. Ideally, longitudinal cohort data from a sample representative of the population to which the function is to be applied should be used for the calculation of both the baseline risk and the risk factor weightings. The exact statistical formulae used for developing risk estimation functions are detailed in the methods sections below.

Table 1-3 below describes some of the characteristics of risk estimation systems and the terminology used for assessing their performance and usefulness.

Appropriate statistical methods for derivation of the function Representative sample from the population from which the system is to be applied Sufficient power (large enough sample size) Accepted statistical methods The endpoint predicted by the function should be defined in such a way that it is easily standardized across populations and relevant to the outcomes of RCTs of preventive measures
Performance of the function – internal and external validity Discrimination –the ability of the function to separate those who will develop the endpoint from those who will not Often assessed using: Area under receiver operating characteristic curve (AUROC) – a means for expressing the maximum achievable sensitivity and specificity. An AUROC of 1 indicates perfect discrimination. 0.5 equates to chance. Values in the region of 0.9 are often achieved for diagnostic tests. Values rarely exceed 0.8 for risk estimation. Harrell's C statistic gives the same information but can be used with variable follow-up. Sensitivity / Specificity / Positive predictive value / Negative predictive value Calibration – a measure of how closely predicted outcomes agree with actual outcomes Often assessed using either: Hosmer-Lemeshow goodness of fit testing – lower values indicate better fit, values less than 20 generally considered good fit. Significant p values indicate lack of fit. Predicted to observed ratios – the closer the value to 1, the better the fit. Values greater than 1 indicate overestimation and vice versa Reclassification Net reclassification index – a measure of the net percentage of those who do and who not develop the endpoint within the time period that are correctly reclassified to a different risk category when a new risk factor is added to the risk estimation system.
Usability of the system The format affects the ease of use of the system. This will also impact on the uptake of the system by users.
Inclusion of appropriate risk factors Most risk estimation systems include age, gender and conventional risk factors including lipid levels, smoking and blood pressure. Inclusion of other factors may be important, especially if they have been shown to be powerful risk determinations and prevalent in the population to which the system is to be applied (e.g. social deprivation) Some advocate the use of only risk factors which are potentially modifiable, although most agree that risk factors to be included should be chosen based on whether they improve risk estimation because those identified as high risk can still modify their risk by favourably altering their other risk factors. Systems using only easily measured non-laboratory measures have been developed recently
Has use of the system been shown to result in measurable health gains?

Table 1-3: Criteria for a clinically useful risk estimation system

SYSTEMATIC CORONARY RISK EVALUATION (SCORE)

SCORE (Systematic Coronary Risk Evaluation) was developed in 2003 at the request of the European Society of Cardiology (ESC). Several studies had shown that the Framingham function, which had been used as the system for risk estimation in the European guidelines on CVD prevention until that time[41, 42], overestimated risk in European populations[43].

SCORE was derived from a pooled dataset containing prospective data on over 205,000 people, with a combined observation time of 2.1 million person years. Twelve cohort studies from 11 European countries were included in the pooled dataset. The variables included in the function and used for the estimation of risk include: age, gender, systolic blood pressure, smoking status and either total cholesterol or TC / HDL cholesterol. Two functions were developed with different baseline survivals – one for use in lower risk European regions including Mediterranean countries and the other for use in higher risk regions including western and central Europe.

SCORE is available in two formats, a two dimensional coloured chart and an interactive electronic version, HeartScore. HeartScore was developed by combining the Danish computerized system PRECARD, with the SCORE function. HeartScore is now available on the internet and also as a standalone version on USB or CD-ROM.

SCORE estimates 10 year risk of fatal CVD, which included coronary heart disease, hypertensive heart disease, cerebrovascular disease, both haemorrhagic and ischemic, and peripheral vascular disease. ICD codes included were: 401 to 414, 426 to 443 and 798.1 – 798.2, with exclusion of the following definitely non-atherosclerotic causes of death: 426.7, 429.0, 430.0, 432.1, 437.3, 437.4 and 737.5. Fatal as opposed to total events were chosen as the endpoint for the function because standardization of this endpoint across the different studies and different countries was more accurate.

SCORE has been the system recommended by the European guidelines on CVD prevention since its release in 2003, in both the third and the fourth revisions[17, 44]. The SCORE chart for use in high risk European regions and using total cholesterol as the lipid measures is shown in **Figure 1.9**.

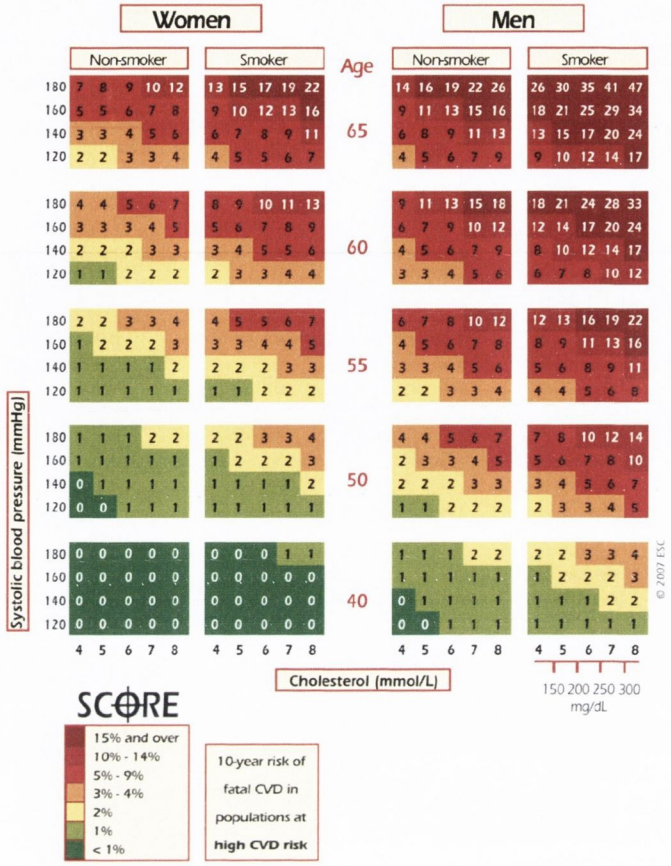


Figure 1.9: SCORE chart for use in high risk European regions

OTHER RISK ESTIMATION SYSTEMS

Many other risk estimations systems are also in existence[4, 45-50]. We recently reviewed current risk estimation systems in two articles[51, 52]. The Framingham function is widely used and the Framingham group also pioneered many of the methods commonly used in risk estimation[1, 2]. Several modified versions of the Framingham function have also been developed and presented as either charts or tables and have been included in national and international guidelines[39, 41].

Table 1-4 uses the characteristics outlined in **Table 1-3** compare to Framingham, SCORE and some of the other commonly used systems. Recently, several other systems have been introduced offering advantages in terms of inclusion of extra risk factors etc. In the table I have concentrated mainly on those systems which are recommended by guidelines on CVD prevention[4, 45-50].

	Framingham[45]	SCORE[4]	ASSIGN – SCORE[46]	QRISK1[53] & QRISK2[47]	PROCAM[48]	WHO / ISH[50]
Data:	Prospective Studies: Framingham Heart Study and Framingham offspring study. Latest version includes both.	Pooled prospective studies	SHHEC Prospective study	QRESEARCH database	Prospective study	Methods differ to other risk estimation functions – not based on prospective data.
Population:	General population Framingham Mass USA. Baselines: 1968-1971, 1971-1975, 1984-1987	12 prospective studies from 11 European countries Baselines: 1972 to 1991	Random sample from general population in Scotland, baseline 1984-1987.	Data collected from 1993 to 2008.	Healthy employees. Baseline: 1978 to 1995	Not applicable
Sample type:	Volunteer	Mostly random samples from general population, some occupational cohorts	Random	Health records of general practice attendees. Not random	Industrial employee volunteers. Not random	Not applicable
Sample size:	3,969 men and 4.522 women	117,098 men and 88,080 women	6540 men and 6757 women	1.28 million (QRISK1) 2.29 million (QRISK2)	18,460 men and 8,515 women	Not applicable
Statistical methods:	Cox (Weibull – earlier versions[1])	Cox & Weibull	Cox	Imputation of substantial missing data Cox	Cox & Weibull. Exploratory analyses with neural networks also[54]	Relative risks associated with risk factors were taken from the comparative risk assessment project. These were combined with the estimated absolute risks for each WHO sub-region based on the global burden of disease study.
Calculates:	10 year risk of CHD events originally. Latest version: 10 year risk of CVD events	10 year risk of CVD mortality	10 year risk of CVD events	10 year risk of CVD events	Two separate scores calculate 10 year risks of major coronary events	10 year risk of CVD events

	Framingham[45]	SCORE[4]	ASSIGN – SCORE[46]	QRISK1[53] & QRISK2[47]	PROCAM[48]	WHO / ISH[50]
					and cerebral ischemic events	
Age range:	30-75	40-65	30-74	35-74	20-75	40-79
Variables:	Gender, Age, total cholesterol, HDL cholesterol, SBP, Smoking status, Diabetes, Hypertensive treatment	Gender, Age, Total cholesterol or total cholesterol / HDL cholesterol ratio, SBP, Smoking status Versions for use in high and low risk countries	Gender, Age, Total cholesterol, HDL cholesterol, SBP, Smoking – no. cigs, Diabetes, Area based index of deprivation, Family history	QRISK1 - Gender, Age, Total cholesterol to HDL cholesterol ratio, SBP, Smoking status, Diabetes, Area based index of deprivation, Family history, BMI, Antihypertensive treatment. QRISK2 also includes ethnicity and chronic diseases	Age, gender, LDL cholesterol, HDL cholesterol, diabetes, smoking, SBP	Gender, Age, SBP, Smoking status, Diabetes +/- Total Cholesterol Different charts available for worldwide regions
Formats:	Simplified scoring sheets. Colour charts have been generated for some guidelines, eg. JBS and New Zealand guidelines. Online calculators. Portable calculators.	Colour coded charts, HeartScore – online and CD-based standalone electronic versions	Online calculator	Online calculator	Simple scoring sheet & Online calculators	Colour coded charts
Developments:	Latest version includes version based on non-laboratory values only, substituting BMI from lipid measurements	National, updated recalibrations		QRISK2 includes interaction terms to adjust for the interactions between age and some of the variables	Recent change in the methods (Weibull) allows extension of risk estimation to women and broader age range	
Recommended by	NCEP guidelines[55], Other national guidelines recommend adapted versions including New	European guidelines on CVD prevention[17]	Recommended by SIGN (Scottish Intercollegiate Guidelines Network)[56]	NICE guidelines on lipid modification[38]	International Task Force for Prevention of Coronary Disease	WHO guidelines on CVD prevention[50]

	Framingham[45]	SCORE[4]	ASSIGN – SCORE[46]	QRISK1[53] & QRISK2[47]	PROCAM[48]	WHO / ISH[50]
guidelines	Zealand[39]				guidelines	
Website	Online and downloadable risk calculator available at: www.nhlbl.nih.gov/guidelines/cholesterol/index/htm	Online and downloadable risk calculators available at: www.HeartScore.org	Online risk calculator available at: www.assign-score.com	Online risk calculator available at: www.qrisk.co.uk	Online calculator available at: www.chd-taskforce.com/calculator	Charts downloadable at: www.who.int/cardiovascular_diseases/guidelines/Pocket_GL_information/en/index.html
Internal validation - Discrimination	AUROC Men: 0.76 (0.75 to 0.78) AUROC Women: 0.79 (0.77 to 0.81)	AUROC high risk: 0.80 (0.80 to 0.82) AUROC low risk: 0.75 (0.73 to 0.77)	AUROC: Men: 0.73 AUROC: Women: 0.77	QRISK2: AUROC Men: 0.79 (0.79 to 0.79) AUROC Women: 0.82 (0.81 to 0.82)	AUROC 0.82 for coronary events AUROC 0.78 for cerebral ischaemic events	Not specified
Internal Validation - Calibration	HL Men: 13.48 HL Women: 7.79	Not specified	Observed 10 year CVD incidence rates: Men: 11.7%, Women 6.4% Median ASSIGN Men: 11.7%, Women: 6.2%	Good correlation between observed and predicted risks in both men and women – presented graphically only – in each decile of risk	Not specified	Not specified
External Validation - Discrimination	PRIME Study: Belfast: 0.68[57] PRIME Study France: 0.66[57] Dutch study:0.86 (0.84 to 0.88)[58] Cleveland Study:0.57[59] China: Men: 0.75 (0.72 to 0.78)[60] China: Women: 0.79 (0.74 to 0.85)[60] THIN (UK): Men: 0.74 (0.73 to 0.74)[61] Women: 0.76 (0.76 to 0.76)[61] EPIC Norfolk: 0.71[62]	Dutch study:0.85 (0.83 to 0.87)[58] Cleveland Study:0.73[59] Norwegian Study: Range for different age groups: Men: 0.65 to 0.68[64] Women: 0.66 to 0.72[64] Austrian Study: Men: 0.76 (0.74 to 0.79)[65] Women: 0.78 (0.74 to 0.82)[65] Icelandic Study: 0.80 (0.78 to 0.82) – SCORE	Not assessed	THIN database (UK): QRISK1: AUROC Men: 0.76 (0.76 to 0.77)[61] AUROC Women:0.79 (0.79 to 0.79)[61]	PRIME Study: Belfast: 0.61[57] PRIME Study France: 0.64[57]	Not assessed

	Framingham[45]	SCORE[4]	ASSIGN – SCORE[46]	QRISK1[53] & QRISK2[47]	PROCAM[48]	WHO / ISH[50]
	UK Women (BHHS): 0.66 (0.62 to 0.69)[63]	high[66] 0.80 (0.77 to 0.82) - SCORE low[66]				

Table 1-4: Characteristics of current risk estimation systems (WHO/ISH – World Health Organisation/ International Society of Hypertension)

COMPARING RISK ESTIMATION SYSTEMS - METHODS

Framingham and ASSIGN are based on intermediate sized samples which are representative of the general population[45, 46]. PROCAM is based on a sample of industrial employees[48]. It may be considered somewhat underpowered for risk estimation women, as only 49 events were recorded in women in the derivation dataset. SCORE is based on a substantially larger dataset which contains over 205,000 individuals, representing 2.1 million person years of observation[4]. Because it is a pooled dataset of 12 European prospective studies it has the potential to accommodate more of the heterogeneity across Europe in terms of baseline CVD risk. The majority of the included studies are representative of the general population, although in the lower risk European countries some occupational cohorts were also included[4].

QRISK[53] and QRISK2[47] are different because they are based on databases of general practice attendees and are therefore not random representative samples of the population; additionally the baseline risk factor measurements would have been obtained at varying times during the observation period, methods were not standardized and there are substantial amounts of missing data, which were imputed as part of the analysis. However, the advantage of use of these data is the substantially larger numbers which can be included. For example, the derivation dataset for QRISK2 included over 1.5 million people[47]. Additionally, these systems, based on GP registers, have the potential for ongoing revisions utilizing newer data[67, 68].

The choice of endpoint predicted by the function is also a consideration. Early systems usually estimated CHD risk[2]. Since atherosclerosis may manifest elsewhere, for example as stroke or peripheral vascular disease, more recent systems have tended to use total CVD as the primary endpoint[4, 45]. It is however, helpful to retain the capacity to estimate risk of cause-specific events, since stroke, for example, may be proportionately more common in certain populations such as low risk countries and in older persons[6].

The endpoint should be as clearly defined as possible to prevent coding difficulties when the function is applied to external populations. This was a problem with initial versions of the Framingham function, which included "softer" endpoints including onset of angina of effort and silent myocardial infarctions based on ECG re-examinations[1]. Additionally, this endpoint did not correspond to the endpoints used in clinical trials. More recent versions have been based solely on "harder" endpoints[45] or have allowed an option for calculation of risk of harder endpoints[2]. SCORE estimates risk of fatal CVD events only, whereas the other systems in table 1 estimate risk of CHD/CVD events[4]. Some have considered this a disadvantage of the SCORE because clearly the goal is to prevent all vascular events, not just deaths. The rationale behind the choice of this endpoint was that, in general, the risk of CVD death will also signal risk of nonfatal events and the use of this very clear endpoint definition was subject to much less variation in terms of coding and endpoint ascertainment when being applied across 12 different cohort studies[4]. The ease of application of this definition also aids the recalibration process, as will be discussed below.

STATISTICAL CONSIDERATIONS

All risk functions have the same basic structure. They take the baseline survival for the disease in question and adjust it to the level of the individual's risk factors. This is done by multiplying the amount the risk factor is above the baseline level by the relative risk weighting (or beta coefficient) for that risk factor.

Most of the current risk estimation systems are based on proportional hazards models – either Cox (semi-parametric)[45-47] or Weibull (parametric)[4, 48]. Earlier functions utilized logistic regression, which had the disadvantage of requiring complete follow-up in all participants and not accounting for those who withdrew before the end of the follow-up period due to either loss to follow-up or death from a non-CVD cause. The Cox method has the advantage of not making any assumptions regarding the shape of the underlying survival, in contrast to Weibull method which imposes a parametric function on the baseline survival. Weibull was chosen for the original SCORE function which included age as part of the time variable, as opposed to as a risk factor. This method has the advantage of allowing the effect of age to vary at different ages[4]. For example, it removes the assumption that an increase in the risk associated with increasing age is constant across all baseline ages. This method also makes more efficient use of the data by allowing risk to be estimated for follow-up times greater than the length of the study's follow-up period[4]. This advantage of the Weibull model was also utilized in the most recent version of PROCAM to allow a function to be derived in women – for whom limited data were available[48]. However, as demonstrated by the both the PROCAM and SCORE groups, the choice of Cox or Weibull makes little practical difference to risk estimation.

Other more complicated methods also exist, including cluster analysis, tree-structured analysis and neural networks[69]. These methods are particularly useful for selecting the most appropriate variables when a large number of potential predictors of risk are available. Neural networks are complicated classification functions which utilise a large number of predictor variables, sometimes multiple times within the same function. They do not assume that risk factors function in a constant and continuous fashion and can account for complex non-linear relationships and interactions between risk factors[54]. Cluster analysis focuses on the identification of groups of individuals with similar risk factor characteristics who have similar levels of risk. This system has the advantage of taking the interactions between risk factors and age into account. However, there is difficulty in obtaining large epidemiological datasets with extensive numbers of predictor variables available. Additionally, the necessity for measurement of multiple factors in clinical practice adds to complexity and is, therefore, likely to limit clinical usage of these systems. Tree-structured systems attempt to progressively split the population into smaller subgroups, through sequential introduction of the risk factors, starting with the simplest. The advantage is that some individuals can be

classified as high or low risk based on very few risk factors. This reduces unnecessary laboratory testing in these individuals. The main problem with all of these methods is model shrinkage – their predictive ability declines sharply once the model is applied to an external dataset, which limits their utility in clinical practice[69].

The WHO/ISH(International Society of Hypertension) risk prediction charts offer an advantage in that they have been developed for each specific WHO subregion[50]. The disadvantage is the methodology. Only a limited description of the methods has been provided[50]. This specifies that the charts were developed by creating a hypothetical dataset for each region – based on the risk factor prevalence in that area, using the mean and standard deviation of risk factor levels measured as part of the collaborative risk assessment study[70]. Each individual was then assigned a relative risk, based on the combination of their risk factor level and the relative risk associated with each risk factor, as estimated from mainly prospective studies. The relative risk for each individual was then scaled according to the baseline risk in that region, as estimated from the global burden of disease study, in order to estimate the absolute risk. These methods require substantial further investigation to determine accuracy and validity, as acknowledged by the authors[50].

COMPARING PERFORMANCE OF RISK ESTIMATION SYSTEMS – INTERNAL AND EXTERNAL VALIDATION

The main ways to describe the performance of a risk estimation system are discrimination, calibration and re-classification. These are explained in **Table 1-3**.

Internal validation, the assessment of model performance in the dataset from which it was derived, is important in checking the mathematical performance of the model used and appropriate fit of the model. As shown in **Table 1-4**, risk estimations systems generally perform well, when assessed in this way[4, 45-48]. However, using the derivation dataset (or a proportion of the same dataset from which the derivation dataset was drawn) is distinctly limited in terms of comparing one function with another. These methods will be inherently biased towards the new function not least because the exact baseline survival of the population is included in the new function, but also because the new function has been derived for prediction of the exact endpoint in the test dataset and the risk factors have been identically defined for the newly-derived function and the validation dataset. Therefore, assertions of superiority of new functions when assessed using the derivation dataset of the new function should be viewed with caution[46, 47]. Comparing the performance of functions in an external dataset is more appropriate.

External validation of the Framingham function has been assessed in numerous studies[57-63]. Most external validation exercises were based on either the Anderson et al 1991 function[1] or the Wilson et al 1998 function[71], which assessed risk of CHD events as opposed to the 2008 function by D'Agostino et al[45]

which estimates risk of CVD incidence. In general, external validations of Framingham functions have demonstrated good discrimination, with AUROCs or C statistics ranging from 0.66 to 0.88, with some exceptions[59, 72]. These have generally been higher in women. The discrimination in the elderly has been poorer, will be as discussed below[72].

The **discrimination** of various risk functions as calculated in some external studies is shown in **Table 1-4**. Some external validation studies have shown poor discrimination with the Framingham function. This may be due to a narrow age range[72] (which does not allow for the predictive ability of age as a risk factor) or differences in endpoint definition. For example, Aktas et al[59] showed SCORE to be a stronger predictor of CVD mortality than Framingham; however, the Framingham function used was intended to estimate risk of CHD events not mortality[59]. Likewise, the low AUROCs of Framingham in the PRIME study[57] may have been related to differences in ascertainment of endpoints in the two studies because earlier versions of the Framingham function included angina and silent MIs[73]. SCORE has been externally validated in a number of studies, yielding similar results to Framingham, as shown in **Table 1-4**. QRISK performed well when externally validated in the UK THIN GP register[61]. Experience of the external validity of PROCAM is more limited[48]. ASSIGN-SCORE, QRISK2 and the WHO/ISH investigators have not yet reported any studies external validation.

Jackson has drawn attention to the fact that, because in clinical practice a threshold is used for defining high / low risk and treatment decisions are based around this, it is important, as well as reporting these measures of summary discrimination (AUROC and C statistic) to consider the discrimination at the threshold of high /low risk[74]. For example, in SCORE the authors report the sensitivity and specificity of the function at a variety of cut-points for the threshold for high or low risk[4].

Calibration is a different issue. Differences in the baseline rates of CVD in different geographic regions mean that risk estimation systems which are well-calibrated in one region will lead to over or under estimation of risk in another[75]. Likewise, secular changes in the incidence of CVD over time mean that risk estimation systems become out-dated. For example, in most of the developed world CVD incidence is now decreasing[49, 76]. This means that over time risk estimation systems will start to overestimate risk. Conversely, in areas where CVD rates are still increasing, current risk estimation systems will under-estimate risk. CHD mortality trends in men across time and place are illustrated in **Figure 1.10**.

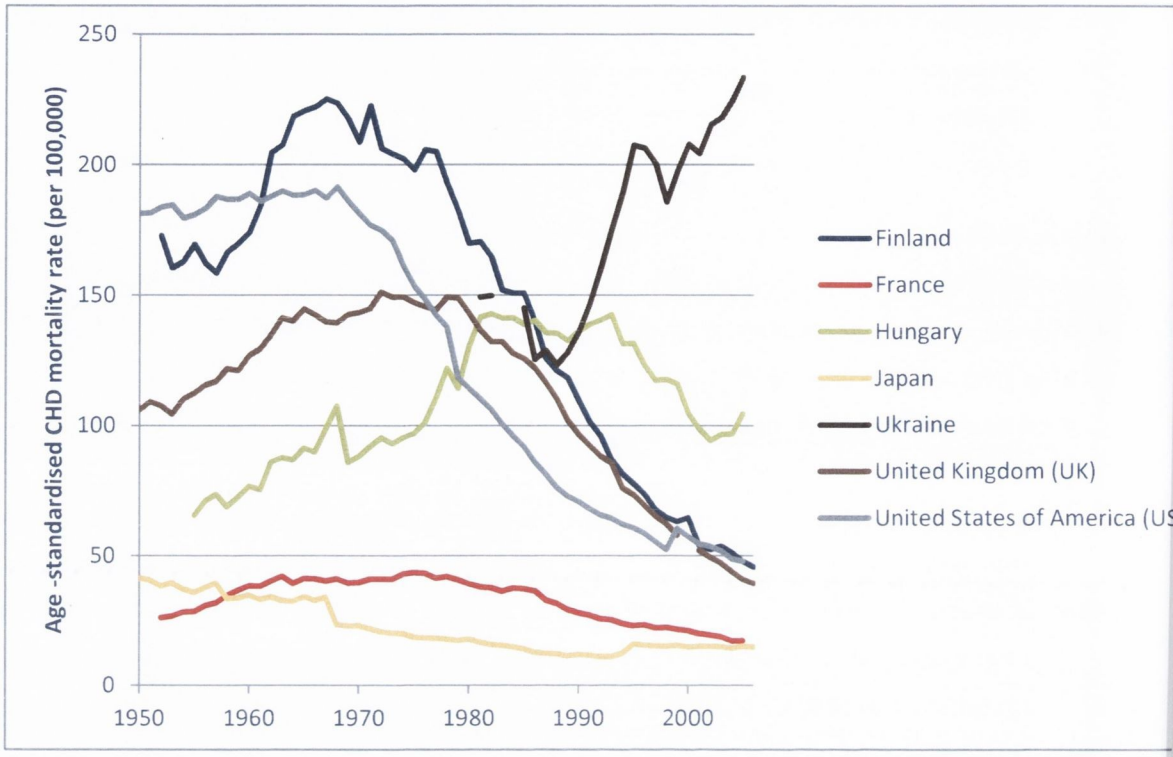


Figure 1.10: Global age-standardised CHD mortality rates in men aged under 65 (1950 to 2006), graphs drawn using data from WHO statistics[49]

This was well illustrated in a systematic review by Brindle et al which calculated the calibration of the Framingham function in several different cohorts[75]. The function overestimated risk in those cohorts where the baseline risk was lower than that of the Framingham cohort, for example cohorts in France and Germany. The risk was underestimated in cohorts with a worse baseline survival, for example cohorts of diabetic patients or patients with a family history of CHD. The Framingham function was well calibrated in most studies in the UK, Australia and the USA, because their baseline survival was similar to that of the Framingham cohort, at that time.

Risk functions can, however, be recalibrated to overcome this problem. This recalibration process will be discussed in the section on advances in risk estimation.

The net reclassification index is a novel method for assessing re-classification into more appropriate risk categories and is generally used for assessing the benefits of risk functions where a new risk factor has been incorporated. This measure is discussed below.

COMPARING RISK ESTIMATION SYSTEMS – USABILITY AND FORMATS

Risk estimation systems are of little value unless clinicians actually use them in day to day practice. Previous studies have shown that the format of the system affects the usage of risk estimation systems and the accuracy with which clinicians use them[77]. For example, simpler colour charts were shown to be preferred to more complicated numerical tables[77]. The SCORE chart[4], shown in **Figure 1.9** (pg 42), is a good example of a simple, easy to use risk chart. The SCORE investigators acknowledge that the format of the SCORE charts is based on that of the original New Zealand risk charts used in their guidelines on hypertension[39].

In general, electronic systems should be user-friendly, especially because previous studies have shown that healthcare providers are less likely to use computerised systems, compared with simpler paper charts, even after training[78]. An innovative solution to improving usability is the integration of the risk estimation system with the GP database. In this way the risk estimate is automatically calculated. PREDICT-CVD, an integrated system developed to aid implementation of the New Zealand guidelines, resulted in a fourfold increase in the rate of documentation of risk estimates in the medical notes[79].

The risk chart format has several advantages in that it is easy to use and inexpensive to produce. The SCORE chart which provides not only the colour-coded risk category but also the integer value for the 10 year risk has the advantage of combining ease of use and accuracy. Previous studies have shown risk estimates calculated using colour coded chart versions of Framingham can vary substantially from the Framingham risk estimate when calculated using the original mathematical function[38]. The weakness of the paper chart is that only limited number of variables can be incorporated.

Most of the current risk estimation systems include the conventional risk factors age, gender, smoking, blood pressure and lipid levels. Recently, there has also been increasing interest in the inclusion of family history of CHD[46, 53], social deprivation measures[46, 53], ethnicity[47], biomarkers[12, 80] and interactions variables which adjust for the use of anti-hypertensive medication[2, 46, 53]. Inclusions such as social deprivation may be considered particularly important in certain regions, for example where social gradients in health outcomes exist[46, 53]. However, increasing the number of variables has advantages and disadvantages. For example, introducing a postal code related measure of social deprivation will limit the use of the function in regions outside this geographical region. One assumes that the more independent CVD risk factors included, the better the risk estimate. However, the law of diminishing returns applies; once the basic risk factors are included most of the predictive ability has been realized and addition of extra factors results in only minor improvements[81], as will be discussed below.

As more factors are included the system becomes more complex, time-consuming and costly because a greater number of risk factors have to be measured in order to estimate the risk. This increase in complexity can impact on the usage of the system. Some have suggested the use of additional factors to refine the risk estimate only in those at intermediate risk[81]; this approach will be discussed below.

Recently, there has been increasing interest in reducing the number of measurements (particularly laboratory measurements) required for risk estimation in order to increase ease of use and cost-effectiveness. For example, the use of body mass index in place of lipid measurements has been shown to result in only minor reductions in discrimination of the function[45, 82]. The WHO/ISH risk charts are available in formats excluding lipid measurement[50]; these are particularly suited to areas in the developing world where access to medical facilities is limited.

UNIVERSAL LIMITATIONS OF RISK ESTIMATION SYSTEMS AND RECENT ADVANCES IN OVERCOMING THESE

One limitation of all risk estimation systems is that they assume constant effects of the risk factors at differing ages and levels of the other risk factors. QRISK2 has attempted to overcome the problem of differing effects of the risk factors with increasing age by including interaction variables between age and several of the other risk factors[47]. However, this method still assumes that the interaction effect with age remains constant at all ages. Certain combinations of risk factors may act synergistically to increase risk in a manner that is more than additive. Cluster analysis and neural networks attempt to account for this, but introduce other problems as discussed above. The ideal situation would be to have an extremely large dataset (a whole country or even continent) in which there were numerous individuals with each combination of risk factors and to examine the actual (not calculated) risk within each combination. In this way, particularly dangerous combinations of risk factors could be identified. However, development of such a dataset would be practically impossible, especially in the modern era when many of the identified risk factors have already been treated

RECALIBRATION OF RISK ESTIMATION SYSTEMS

As mentioned above, risk functions developed in one region will tend to over or under estimate risk in other populations with different baseline risks[75, 83], either due to secular changes over time or regional differences. The ideal solution to this problem would be the continual generation of updated risk functions based on recent prospective cohort studies. While this is possible in some countries, for example Finland[84] and Italy[85], this is not feasible in most areas. Recalibration of risk estimation systems represents a viable alternative. The fundamental principle of recalibration is adjustment of the baseline survival of the function to the current level in that particular geographic region[86].

Two recent, region-specific pieces of information are required: the current national CVD mortality rates (or CVD event rates) and representative surveys of risk factor levels in the population. The current survival

curve is taken to equate to the baseline survival at the population mean level of risk factors. This new baseline is then adjusted to the individual's level of risk factors, using the beta coefficients for each risk factor from the original risk function[86]. This represents a feasible option in many countries where current mortality statistics are easily accessible and cross-sectional surveys of risk factor distributions have been conducted. The assumption here is that while the baseline survival changes from place to place and over time, the relative risks or beta coefficients associated with each risk factor remain the same.

Both Framingham[60, 86-89] and SCORE[58, 90-92] [93] have been recalibrated for several different regions. One advantage of the SCORE system is that the use of CVD mortality as the endpoint, as opposed to CVD events, facilitates the recalibration process[4], because reliable and recent CVD mortality statistics are readily available in many regions. This information is much less easily obtained and standardized when CVD event rates are required, and regional differences in coding represent a difficulty in systems which include less hard endpoints[1, 2]. This advantage strongly influenced the choice of CVD mortality as the endpoint in the SCORE project. [4]

ASSESSING THE VALUE OF INCORPORATING NEW RISK FACTORS INTO RISK ESTIMATION SYSTEMS

Recently, much attention has focused on trying to improve risk estimation through the incorporation of new risk factors. Traditionally, the improvement in discrimination of risk estimation systems has been assessed by area under receiver operating characteristic curve (AUROC) or Harrell's C statistic. There have been several demonstrations of the lack of improvement in discrimination, as measured by AUROC, with the addition of these risk factors, despite the fact that many of these risk factors had strong and independent effects on the further occurrence of CVD[21, 47, 81, 94]. **Table 1-5** shows some examples of the effect that inclusion of extra risk factors had on discrimination, as measured by AUROC.

Risk Factor	Study	Odds ratio comparing	Endpoint	Improvement in AUROC
Multiple biomarker score – brain natriuretic peptide, CRP, homocysteine, renin, urinary albumin-to-creatinine ratio[94]	Framingham Offspring Study	1.84 (1.11–3.05) comparing the high quintile to the lowest two quintiles of multimarker score	Cardiovascular disease incidence – MI, coronary insufficiency, stroke & heart Failure	0.76 to 0.77
HDL Cholesterol[81]	Framingham	0.65 (0.53 to 0.80) for each 1 std dev increase in HDL	Coronary heart disease incidence - MI, angina pectoris, coronary insufficiency or CHD death	0.762 to 0.774
hsCRP - women	Women's Health	hsCRP: 1.22 per 1	CVD incidence: MI,	0.813 to 0.815

only[21]	Study	unit increase in log(hsCRP)	ischemic stroke, coronary revascularization, And CVD deaths	
hsCRP[81]	Framingham Offspring Study	1.34 (1.14 –1.58) for each 1 unit increase in log(hsCRP)	MI and CHD death	0.863 to 0.865
Ethnicity & Chronic diseases and Interactions between age and several other risk factors[47]	QRISK2	Ethnicity: 8 ethnicities – Ranging from 1.97 (Pakistani women) to 0.51 (Chinese women) compared to White women Atrial fibrillation: 3.06 in men Renal disease: 1.70 in men	Cardiovascular disease incidence – CHD, Stroke, TIA	Women: 0.814 to 0.817 Men: 0.788 to 0.792 - For all additions combined
Haemoglobin A1c (HbA1c)	EPIC Norfolk		CHD incidence	Men:0.72 to 0.73 Women: 0.80 to 0.80

Table 1-5: Adjusted odds ratios for risk factors newly incorporated in risk function and the improvement in AUROC afforded by their incorporation.

However, AUROC was a technique developed for assessing the performance of a diagnostic test which has a straightforward yes/no answer, against that of a gold standard. A perfectly sensitive and specific test will result in an AUROC of 1. However, because risk estimation is just that, an estimate, a perfect AUROC will never be achieved. The highest AUROCs for risk estimation achieved to date have been in the region of 0.88, when tested on the same data from which they were derived[80]. The usual AUROCs of risk estimation systems are in the region of 0.75 to 0.80. When one considers that age and gender alone can result in AUROC of up to 0.70, clearly there is little room for improvement in AUROC with addition of risk factors beyond the conventional risk factors.

For this reason, there has been increasing interest in the development of more appropriate methods for assessing the improvement in performance afforded by incorporation of new risk factors[95]. The method with most potential for clinical utility is reclassification. Clinically, the most important feature of a risk estimation system lies in its ability to classify individuals to appropriate risk categories, since treatment decisions are based on these classifications[74]. Therefore, improvement of discrimination of a function in those at intermediate risk, who are close to the thresholds of the risk categories, is particularly important[21].

A new method for assessing for superior classification has been developed by Pencina et al[81]. This measure, the net reclassification index (NRI), determines the net percentage of those who do and do not develop an event over the observation time who are correctly reclassified using the new function. For example, in an individual who develops the endpoint upward movement to a higher risk category, when using the new risk function, would be considered correct reclassification[81].

This system has the advantage over previous reclassification measures of quantifying the reclassification in the net correct direction, as opposed to reporting the overall reclassification which occurs on addition of the new factor[21, 81]. **Table 1-6** shows the NRIs associated with incorporation of various risk factors in risk estimation functions.

Risk Factor	Study	Improvement in AUROC	NRI
HDL[81]	Framingham	0.762 to 0.774	12.1%, p<0.001
CRP[81]	Framingham	0.863 to 0.865	11.8% , p<0.009
hsCRP – women only[21, 96]	Women's Health Study	0.813 to 0.815	5.7%, p<0.0001
HbA1c[62]	EPIC Norfolk	0.72 to 0.73 in men 0.80 to 0.80 in women	3.4% (p=0.06 in men) 2.2% (p=0.27)

Table 1-6: Improvement in AUROC and NRI associated with addition of various risk factors to risk estimation functions

Table 1-7 shows an exploratory analysis by the Framingham group which shows the change in AUROC when risk factors are sequentially added to a model initially containing only age and gender[81]. The NRI associated with each additional risk factor is also shown. Most of the risk factors result in considerable improvement in correct risk classification, as indicated by NRI, despite the fact that incorporation of some of the additional risk factors results in only very minor changes in AUROC. The authors emphasize the fact that the value of incorporating risk factors is dependent on the order in which the risk factors were added to the model[81].

	AUROC	NRI
Gender and age alone		
Systolic blood pressure	0.740	10.8%
Lipids	0.767	7.0%
Smoking	0.787	7.7%
Diabetes	0.795	- 0.5%
CRP	0.799	5.6%

Table 1-7: Change in AUROC and NRI associated with incorporation of various risk factors into the Framingham function. (Adapted from reference[81])

The exact utility of this system has yet to be determined. Some have suggested that those risk factors which provide superior classification could be measured in those at intermediate risk. High sensitivity CRP has been suggested as a potential extra risk factor to measure in those at intermediate risk to further define risk in this group[21, 81]. It has been pointed out that the NRI depends strongly on the thresholds chosen for the risk categories[81]. Therefore, it has been suggested that risk categories routinely used in clinical practice for decision making should be used in assessing reclassification[81].

LIMITATIONS OF SCORE

ESTIMATION OF RISK OF FATAL CVD EVENTS ALONE

Some consider the use of the CVD mortality, as opposed to CVD events as the endpoint to be a disadvantage of the SCORE system. However, as mentioned above the use of this endpoint was specifically chosen because it could be easily standardized across the different European cohort studies and additionally, because it simplifies the process of recalibration because mortality statistics are easily available from the WHO database.

However, a version of SCORE which estimates 10 year total events risk has recently been developed by Dr. Catherine McGorrian, called SCORE Plus. This version uses data from FINRISK and is due to be published in the near future.

DIABETES

At present the SCORE system does not include diabetes as a risk factor in the function. This is because the guidelines on CVD prevention consider that individuals with diabetes are automatically considered high risk and therefore risk does not need to be estimated for the purposes of risk stratification in those with diabetes. However, physicians are interested in having a system for estimating risk due to combinations of risk factors in individuals with diabetes as a means for monitoring their total CVD risk. For this reason, based on a re-examination of the SCORE dataset[97], the 4th version of the guidelines included the following advice about adjusting the SCORE risk for individuals with diabetes – the risk in those with self reported diabetes is 3 fold higher in men and 5 fold higher in women. However, it should be remembered that HDL cholesterol was not taken into account in these analyses and that some of the risk in the diabetic patients would be due to the low levels of HDL cholesterol which are often associated with diabetes.

It is likely that future versions of HeartScore, including SCORE plus, will contain diabetes as an extra risk factor.

OTHER RISK FACTORS

Other limitations of the current SCORE system include the lack of inclusion of other important risk modifiers for example, HDL cholesterol, obesity (particularly abdominal obesity), social deprivation, family history of CHD and elevated heart rate.

RISK ESTIMATION AT THE EXTREMES OF AGE

At present the SCORE system can only be used from ages 40 to 65 years. Extending the system to include older and younger individuals would enhance the usefulness of the system. However, it is necessary to ensure that any new system developed which extends risk estimation to these age groups is tested and proven to be accurate. To date, few systems have been evaluated for accuracy in specific age groups.

AIM - BROAD OBJECTIVES

The main aims of this project are to add to currently available knowledge on the independent roles of both HDL cholesterol and resting heart rate as modifiers of risk of CVD. Building on this, I aim to develop risk estimation systems incorporating these as additional variables and to evaluate the improvement in risk estimation as a result of their addition.

The ability of a simple risk estimation function using only easily measured variables will also be investigated. The potential for improving this score through incorporation of RHR will also be examined.

An additional aim is to investigate the role of CVD risk factors in the elderly, assessing whether there are differences in their effects in older and younger individuals. Based on these analyses I aim to derive and internally validate a new risk estimation function specifically, for use in the older age group. This will be derived fully from data based on individuals aged over 65 years as opposed to extrapolating from data on middle-aged individuals, as in current risk estimation systems.

SPECIFIC OBJECTIVES

1. HDL cholesterol

1.1. To determine the effect that HDL cholesterol has on risk of CVD (cardiovascular disease) mortality, CHD (coronary heart disease) mortality, stroke mortality and total mortality in the SCORE dataset. To determine whether this effect is independent of other risk factors. To examine whether this effect is demonstrable and consistent in both genders, in all age groups and at all levels of total CVD risk.

1.2. To derive and internally validate a function which contains HDL cholesterol and total cholesterol (TC) as two separate variables in addition to the other risk factors currently included in SCORE.

1.3. To assess whether this new risk function performs superiorly to the original SCORE function (both TC and TC/HDL ratio versions). Specifically, to assess whether the new function results in an improvement in correct risk reclassification.

2. Heart rate and CVD risk

2.1. To determine the effect of resting heart rate on CVD endpoints including CVD mortality, CHD mortality, total mortality and fatal and nonfatal myocardial infarction. To assess whether this is independent of other risk factors, specifically focusing on physical activity and systolic blood pressure. To determine if the effect is seen when those with co-morbidities have been excluded. To determine if the effect consistent across both genders, those with and without baseline

hypertension and in all strata of physical activity. To assess if the effect is a result of reverse causality or whether the effect remains once early events have been removed.

2.2. To investigate whether discrimination of risk estimation systems improves through inclusion of resting heart rate (as a continuous variable) when other risk factors are already included.

3. Simplifying Risk Estimation

3.1. To derive and assess performance of a risk estimation system including only easily measured variables: age, gender, smoking status, BMI and resting heart rate, in terms of discrimination and calibration.

4. Risk estimation in older persons (SCORE O.P.)

4.1. To examine the effects of CV risk factors on CVD and CHD mortality in the older and younger age groups in the SCORE dataset.

4.2. To assess whether a risk estimation function (SCORE O.P.) can be developed which will reliably estimate risk in the older age group.

4.3. To assess whether a risk estimation function derived specifically from data in the older age group (over 65 years) would perform better in terms of discrimination and calibration than the original SCORE function in which the risk factor weightings were derived from the entire group (mainly younger and middle aged individuals) and applied to each age group including the elderly.

CHAPTER 2 HDL CHOLESTEROL

In this section, I review the relationship between HDL Cholesterol and risk.

This sets the scene for the specific research questions listed on page 108.

Historically the focus has been on the inverse relationship between HDL cholesterol concentration and CVD risk. More recently, increasing attention has been paid to the quality and functionality of the HDL particle, especially with regard to the effect on inflammation, oxidation, endothelial function and thrombosis. **The process of reverse cholesterol transport and other scientific mechanisms for the protective effect of HDL cholesterol as well as the metabolism and biochemistry of HDL cholesterol are reviewed in detail in the appendix of this thesis.** The proposed effects of HDL cholesterol on the atherosclerotic process are graphically summarized in Figure 2.1.

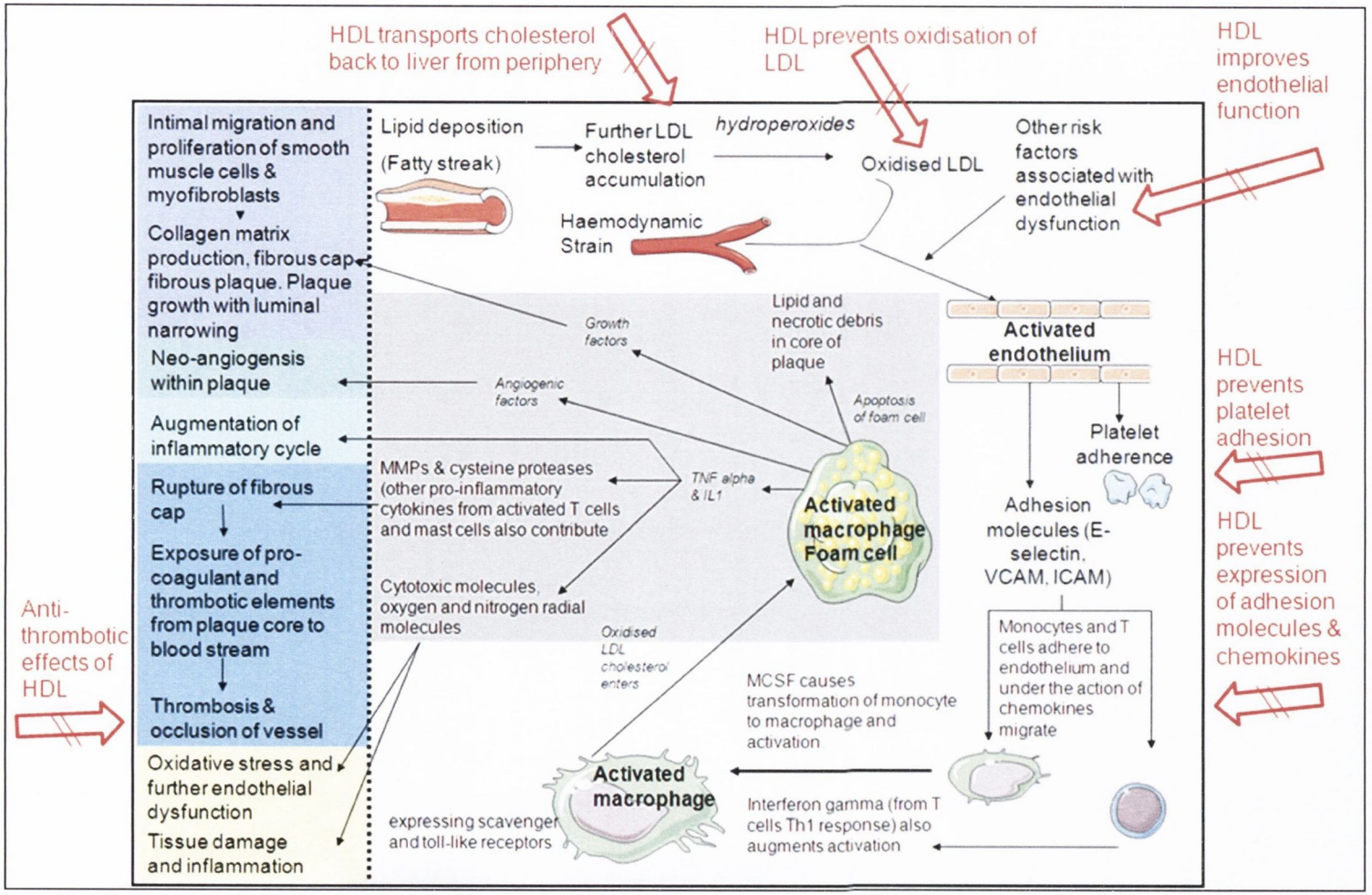


Figure 2.1: The atherosclerotic process - proposed protective effects of HDL cholesterol

HDL - EPIDEMIOLOGICAL EVIDENCE FOR THE EFFECT OF HDL ON CV RISK

HDL – DETERMINANTS AND ASSOCIATIONS WITH OTHER RISK FACTORS

BODY WEIGHT

Several epidemiological longitudinal studies (described in **Table 2-1** below) have also preformed cross-sectional analyses to assess the relationship between baseline HDL cholesterol level and other risk factors. The most consistent association seen both in men and women has been the inverse association with body weight.

A large cross-sectional analysis of the Women's Health Study included 27,158 apparently health women (mean age 54years) and showed significant associations between increasing body mass index and decreasing HDL cholesterol[98]. The risk of having low HDL cholesterol (<50mg/dl) was significantly reduced by 85% for those in the first quintile of BMI compared to those in the fifth, even after adjustment for other risk factors. The study of Japanese men also found BMI to be the strongest (inverse) correlate of HDL cholesterol levels. A relatively minor increase in BMI category was associated with a substantial and significant drop in mean HDL cholesterol levels – 57.8mg/dl with BMI ≤ 21.9 compared to 49.8mg/dl with BMI ≥ 24.2[99].

BMI was a strong inverse correlate of HDL level in the lipid research clinics prevalence study (LRCPS) also, even after adjustment for other risk factors known to be associated with HDL. Importantly, the effect of increasing BMI on HDL level was seen in children as well as adult men and women. Those in the 10^{th} percentile of BMI had 3mg/dl higher mean HDL than those in the 50^{th} percentile, whose HDL cholesterol levels were 3-4mg/dl higher than those in the 90^{th} percentile of BMI[100].

Lower concentrations of HDL cholesterol appear to be particularly related to abdominal obesity[101-103]. Indicators of body fat distribution appear to be inversely related to HDL cholesterol independently of the level of BMI and this is seen in elderly as well as younger individuals[104]. In the NHANES survey[105], the lower HDL cholesterol levels were seen in those with abdominal obesity across a range of ethnicities.

In a Canadian cross-sectional analysis, waist circumference was the best weight-related predictor of HDL cholesterol level, with superiority of waist circumference over BMI most marked in women[106, 107]. Both hip circumference and waist/hip ratio were also inferior to waist circumference. The relationships were demonstrated in each age group (18-34 years, 35-54 years and 55 -74 years).

In the Charleston health study, both BMI and waist circumference correlated negatively with HDL cholesterol in elderly (61 years to 106 years) white and black men and women[108].

PHYSICAL ACTIVITY

Higher levels of physical activity are associated with higher HDL cholesterol levels[109]. In the Women's Health Study, although body weight was a stronger predictor of low HDL levels the risk of having low HDL cholesterol was also significantly increased in inactive compared to active women, within each BMI category[98]. In Japanese men, a significant reduction in mean HDL cholesterol from 55.1mg/dl in the group exercising twice or more times weekly to 52.3mg/dl in the group who hardly ever exercised was demonstrated[99]. Interestingly in the LRCPS, the association appeared to be stronger for self report of exercise than for performance on exercise treadmill testing. Higher HDL levels were demonstrated in both active men and women than in their sedentary counterparts (self-report). These relationships remained significant after adjustment for age, BMI, smoking and alcohol[110]. The independent positive effect of physical activity on HDL cholesterol levels has also been demonstrated in the elderly[111]

In the recent analysis of the NHANES survey, higher levels of self reported physical activity were also significantly associated with higher HDL cholesterol in children and adolescents – after adjustment for other known cardiovascular risk factors[112].

SMOKING

Smoking (including passive smoking) has been associated with lower HDL cholesterol in several diverse populations[109, 113, 114]. In the study of Japanese middle aged men, mean HDL cholesterol decreased significantly from 55.3mg/dl in nonsmokers to 50.5mg/dl in those who smoked 30 or more daily[99]. This relationship was also demonstrated in the LRCPS, where an 11% difference between male smokers and non-smokers was demonstrated. In female smokers the HDL cholesterol level was 14% higher[115]. These figures were adjusted for other risk factors and were equal in women using and not using exogenous estrogens. There appears to be a dose response relationship, with lower HDL cholesterol levels in heavier than lighter smokers[115].

DIET

In LRCPS, there was a significant inverse association between sucrose and starch intake and HDL cholesterol levels[116].

A recent analysis of the NHANES survey showed that in children and adolescents there is an inverse relationship between sugar sweetened beverages and HDL cholesterol. A statistically significant 0.48mg/dl decrease in HDL cholesterol was associated with each one serving increase of these beverages[112]

The Framingham group have made use of cluster analysis to analyse the effect of different diet patterns on metabolic parameters including HDL cholesterol[117]. Individuals in the Framingham offspring study were divided into four food groups, depending on which foods or drinks contributed to the majority of the energy intake. The highest HDL cholesterol was in the "Beer" group and the lowest was in the "Soda" group – agreeing with the results of the above study in children. Intermediate levels of HDL cholesterol were seen in those in the "fruits, reduced fat dairy and whole grain" group and the "refined grains and sweets" group[117]. The expected reduction in HDL cholesterol in those taking higher quantities of refined carbohydrates is possibly offset by the higher saturated fat intake of these individuals and by the fact that those in the "fruits, reduced fat dairy and whole grain" group had lower saturated fat intakes.

ALCOHOL

Higher alcohol intake is associated with higher mean levels of HDL cholesterol, an association which has been demonstrated in several diverse cultures[109, 113, 114]. The mechanism for this is not fully understood. For example, in Japanese men the mean HDL cholesterol increased from 50.7mg/dl in non-drinkers to 55.3mg/dl in men drinking ≥ 2 units daily, $p<0.001$[99]. In the LRCPS lower HDL cholesterol levels were seen in those who reported not drinking[116].

ETHNICITY

Variations in HDL level based on race within countries have also been demonstrated, for example, in the LRCPS black children and adults of both genders had significantly higher HDL levels than those of white children and adults. This difference persisted after adjustment for potential confounders including age, BMI, alcohol, smoking, and educational status. The difference was attenuated but not eliminated once triglycerides, which were lower in the black population, were adjusted for[118].

GENETICS

Several genetic low HDL syndromes have been identified. However, the low HDL cholesterol associated with these syndromes does not always result in increased CVD risk. These are detailed in **Table 2-1** below.

Component of HDL metabolism affected	Specific Disease	Manifestations
Apo A1	Complete Apo A1 deficiency (deletion of APOA1 gene)	HDL is almost undetectable(<10mg/dl). Less severe reductions in HDL cholesterol in heterozygotes. Corneal opacities. Xanthomas. Increased risk of premature CHD.
	ApoA1 mutations (e.g. ApoA1 Milano)	Autosomal dominant. Low HDL levels (15-30mg/dl) not associated with increased risk of CHD. Corneal opacities.
Lecithin:	Complete LCAT deficiency	Very rare. Autosomal dominant.

cholesterolacyl transferase (LCAT)	Partial LCAT deficiency (fish eye disease) Results in decreased esterification of cholesterol to cholesterol esters in HDL particles, leading to accumulation of free cholesterol on lipoprotein particles and in peripheral tissues (see appendix illustrations).	Corneal opacities, normochromic anaemia, renal failure in young adults. Low HDL (<10mg/dl) High triglycerides. Not usually associated with increased risk of CHD. Partial deficiency is less severe
ABC1	Tangier disease – homozygous Tangier disease – heterozygous Familial hypoalphalipoproteinaemia (some families) Mutations in the ABC1 cause disruption of the passage of cholesterol from within to outside the cells and cholesterol esters become deposited in the reticuloendothelial system.	Autosomal codominant. HDL (<5mg/dl) and Apo A1 levels are extremely low. Orange tonsils. Peripheral neuropathy. Discoloration of rectal mucosa. Hepatomegaly. Splenomegaly. Opacities. Premature CHD.
Unknown genetic aetiology	Familial hypoalphalipoproteinaemia (most families) Familial combined hyperlipidaemia with low HDL-C	

Table 2-1: Genetic syndromes associated with variations in HDL cholesterol level

HDL cholesterol level appears to be related to both genetic and environmental factors.

High levels of HDL cholesterol can be considered a longevity factor. Substantially higher concentrations of HDL cholesterol have been demonstrated in healthy octogenarians in the Framingham study when compared with middle-aged subjects[119]. A higher level of HDL cholesterol has also been demonstrated in the offspring of centenarians[120]. This was associated with a higher prevalence of homozygosity for the VV variant of the I405 polymorphism in the cholesterol ester transfer protein (CEPT) gene, which leads to reduced activity of CEPT and increased levels of HDL[121].

The Taq1 polymorphism of the CEPT gene has a similar effect[122]. However, these CEPT polymorphisms and their associated elevation in HDL cholesterol have not been universally associated with consequent reductions in CHD incidence[123]. Clearly, the CEPT polymorphisms cause higher HDL levels but whether these higher HDL cholesterol levels due to CEPT dysregulation translate to reductions in CHD is currently unclear (see discussion on torcetrapib).

DRUGS

Specific pharmacotherapies for elevating HDL cholesterol will be discussed under the section HDL elevation. However, other commonly used drugs have been associated with reductions in HDL cholesterol and other metabolic changes, as adverse effects. These HDL lowering drugs include: non-selective beta blockers, phenytoin, anabolic steroids and to a lesser extent benzodiazepine derivatives[124].

EXOGENOUS HORMONES

Oestrogen is known to increase HDL cholesterol levels. This is reflected in higher level in those on the oral contraceptive pill, hormone replacement therapy (HRT) and additionally, in the decrease in HDL levels seen in women at the time of menopause.

In a meta-analysis of 107 trials examining the effect of HRT in postmenopausal women there was a 5.1% (95%CI: 3.6% – 6.7%) increase in HDL cholesterol in those randomized to HRT[125]. Since oestrogen also reduces LDL cholesterol the effect on LDL/HDL ratio is favourable in two ways. In subgroup analyses of the effect of HRT on LDL/HDL there was no difference in the effect of unopposed oestrogens compared to those combined with progestin. The effect of oral HRT was greater than that of transdermal preparations[125]. The HDL raising effect is thought to be mediated by an increase in HDL-apolipoprotein A-1 production and not by a decrease in the clearance rate[126]

Progestogens contained in the combined oral contraceptive medications antagonized the oestrogen-mediated increase in HDL cholesterol. However, this occurs less with the newer progestogens (desogestrel, gestodene) than the older versions (levonorgestrel, norethisterone[127]).

METABOLIC SYNDROME

The metabolic syndrome represents a clustering of cardiovascular risk factors, including, low HDL cholesterol, high triglycerides, abdominal obesity, glucose intolerance and mild hypertension. Some have suggested that the mechanism for the development of this cluster of risk factors is metabolically active visceral obesity. It has been suggested that this abdominal adipose tissue secrets cytokines (adipocytokines e.g. adiponectin) which in turn cause the development of these linked risk factors. However, this mechanism is still theoretical at present.

The metabolic syndrome can be considered a precursor to type 2 diabetes and a specific type of dyslipidaemia is common to both conditions. Known as atherogenic dyslipidaemia, it consists of low HDL cholesterol, moderately high triglycerides and relatively normal total levels of LDL cholesterol, but predominantly in the form of small dense LDL particles. The HDL particles are also in the small dense HDL3 form[103].

The association of low HDL cholesterol levels with both obesity and type 2 diabetes, which are currently reaching epidemic proportions in the developed world, suggests that the importance of low HDL cholesterol as a risk factor for CVD will continue to increase also.

HDL – EFFECT ON CHD

Several epidemiological studies have examined the relationship between HDL-C and CV risk; most have demonstrated a strong, inverse, independent relationship between HDL-C and CVD. [126, 128-151] The characteristics of these studies are detailed in Table 2-2.

In general, only those studies with a prospective design have been included in Table 2-2. This includes a limited number of prospective (nested) case control studies. Any studies identified from pubmed with a prospective design which included baseline measurement of HDL and long-term follow-up for the development of either mortality or CVD (or CHD) mortality +/- events were included if they reported on the effect of HDL on these outcomes (either as a categorical or continuous variable) were included. The reference lists for each of the publications were also searched in order to identify other investigations of the longitudinal effect of HDL on CVD outcomes. Two landmark case-control studies have also been included. Several studies have published on the effect of HDL in the same dataset, with increasing lengths of follow-up or addressing slightly different aspects. Where this occurred Table 2-2 focuses on the study with the longest follow-up but also mentions any different conclusions or extra analyses published on the same dataset. There are some additional smaller studies addressing HDL and risk listed in Table 2-3, which focuses on evidence for the effect of HDL in older individuals.

Author Study Year	Number Men Women Specific Group	Age range Follow-up	Overall effect	Hazard ratio	Endpoint	Hazard ratio (converted to per 0.5mmol/l increase)	Adjusted for	Associations with other risk factors	Other
Onat 2004[152] TEKHART Study	2,269 men and women	> 20 years FU: 4 year	Significant inverse (men and women are analysed together)	0.975 (0.959 to 0.99) Per 1mg/dl increase	CHD incidence	0.61 (0.45 to 0.82)	Age, TC, smoking, DM, SBP, WC, DBP, PA	Neg: smoking, WC, insulin, CRP Pos: Alcohol (men) PA (women)	
Gordon 1986 [153] LRCCPP – placebo group	1899 Men Free of CHD but high TC or TG	Aged: 35-59 FU: 7-10 years	Significant inverse (for the definite and suspect MI or CHD death endpoint)	0.972, p<0.01 Per 1mg/dl increase	Definite and suspect MI or CHD death	0.58	Age, cigs, SBP, LDL, TG, Exercise test		
Lehto 1997 [154] Finland	581 Men 478 Women NIDDM (previous MI not excluded)	Aged: 45-64 Mean FU: 7.2 years	Significant inverse (men and women analysed together)	1.9 (1.3 to 2.6) <1.0 mmol/l vs ≥ 1.0mmol/l	CHD mortality	NA	Age, sex, area, MI, TC, TG, glucose, diabetes duration		Similar results for men and women separately, figures not given
Laakso 1993[155] Same study	153 men 160 women	Mean age: men 56 women 58 FU: 7 years	Significant inverse	Beta – -1.6332 Per 1 mmol/l increase	CHD mortality	0.44	Age, duration DM, HbA1C, LDL-TG, VLDL-TG		Very similar hazard ratio in subgroup without previous MI

Author Study Year	Number Men Women Specific Group	Age range Follow-up	Overall effect	Hazard ratio	Endpoint	Hazard ratio (converted to per 0.5mmol/l increase)	Adjusted for	Associations with other risk factors	Other
Goldschmid 1994[156] Rancho Bernardo Study	327 Non-DM Men 496 Non-DM women 327 Non-DM men 76 DM Men 45 DM Women Oversampling of those with high TC/TG/ lipid lowering meds	Aged: >30 years FU: 16 year	Non-significant inverse in diabetic women. None in men or non-diabetic women	1.76 (0.91 to 3.43)- diabetic women only Per 10mg/dl decrease	CHD mortality	0.34 (0.09 to 1.20)	Age, HTN, obesity, smoking, exercise alcohol, estrogen use		Low HDL stronger risk factor in diabetic population
Woodward 2007[157] Asia Pacific Cohort Studies	36,659 women 43,035 men Baseline CHD not excluded	Mean age 49 FU: Median 6.8 years	Significant inverse	1.45 (1.26 to 1.66) – men and women together Per std dev decrease (0.4mmol/l)	CHD mortality	0.63 (0.53 to 0.75)	Age only		Additional adjustment for smoking and SBP decreased effect by 0.9% and for alcohol and BMI by 4%. Similar relationships in men and women (figures not given) Attenuation of relationship in older agegroups
Cremer 1997[158]	5,790 men Baseline CVD	Aged: 40-59.9	Significant inverse	2.2, p<0.0001	MI and CHD mortality	NA	LDL, fam hx, Lp(a), age, smoking, SBP,		

Author Study Year	Number Men Women Specific Group	Age range Follow-up	Overall effect	Hazard ratio	Endpoint	Hazard ratio (converted to per 0.5mmol/l increase)	Adjusted for	Associations with other risk factors	Other
GRIPS	excluded	FU: 10 years		< 0.9 mmol/l vs ≥ 0.9mmol/l			glucose		
Jeppesen 2003[159] Copenhagen Male Study	2,910 men Baseline CHD excluded	Aged: 53-74 FU: 8 years	Non-significant inverse	0.91 (0.85 – 1.03) Per 1 quintile increase	CHD incidence	NA	Age, alcohol, smoking, PA, BMI, HTN, SBP, DBP, NIDDM, social class, LDL, logTG		Effect is significant without adjustment for lipid measures
Reed 1985[160] Honolulu Heart Program	2,122 health men Baseline CVD, cancer, diabetes and lipid lowering med use excluded. Oversampling of high TC/TG	Mean age 58 FU: 10 years	Significant inverse	Beta - - 0.244 Per std dev increase	CHD incidence	0.70	Age, BP, BMI, glucose, alcohol, cigs, uric acid		No association with CHD mortality
Norrish 1995[161] Auckland RF Study	991 men No exclusions	Aged: 35-64 FU: 9 years	Significant inverse	0.48 (0.26 to 0.88) Comparing extreme tertiles	Total mortality	NA	Age, SBP, smoking, alcohol, social class, TC		
Turner 1998[162] UKPDS	3,055 men and women Recent MI,	Mean age: 52 FU: 7.9	Significant inverse	0.55 (0.41 to 0.73) Comparing	CHD incidence	0.44 (0.29 to 0.66)	Age, gender, LDL, HbA1C, DBP, smoking		

Author Study Year	Number Men Women Specific Group	Age range Follow-up	Overall effect	Hazard ratio	Endpoint	Hazard ratio (converted to per 0.5mmol/l increase)	Adjusted for	Associations with other risk factors	Other
	angina, heart failure and serious retinopathy or nephropathy were trial exclusion criteria	years		≥ 1.14 mmol/l to <0.95 mmol/l 0.85 (0.78 to 0.92) Per 0.1 mmol/l increase					
Salonen 1991[163] Kuopio IHD RF Study (KIHD)	1,799 men No exclusion of previous heart disease	Aged 42 – 60 FU: Up to 4.75 years	Non-significant inverse	1.82 (0.88 to 3.75) Comparing <1.07 mmol/l to > 1.47 mmol/l	MI	NA	Age, exam year, BMI, HxCHD, HxotherCVD, max O2 uptake, antihypertensive, SBP, LDL, TG, alcohol, cigs, years of smoking, leisure time activity		HDL2 more protective than HDL3 Significant relationship when adjusted for age and exam year only
Bainton 1992[164] Speedwell Caerphilly	2,348 men	Aged: 45 – 63 FU: Mean 3.2 years	Significant inverse	1.7 (1.0 to 2.8) Comparing extreme quintiles	CHD incidence		Age, TC, TG, DBP, BMI, pre-existing disease, smoking	Neg: BMI	
	2,512 men No exclusion of previous	Aged: 45-59 FU: Mean		1.22 (p<0.05) per 1 SD		0.75			

Author Study Year	Number Men Women Specific Group	Age range Follow-up	Overall effect	Hazard ratio	Endpoint	Hazard ratio (converted to per 0.5mmol/l increase)	Adjusted for	Associations with other risk factors	Other
	CHD	5.1 years		decrease (0.35mmol/l)					
Njolstad 1996[165] FINNMARK Study	6,142 men 5,701 women Previous CHD excluded	Aged: 35-52 years FU: 12 years	Significant inverse	0.74 (0.66 to 0.83) men 0.70 (0.56 to 0.89) women Per 0.4 mmol/l increase	MI	0.69 (0.59 to 0.79) 0.64 (0.48 to 0.86)	Age, ethnic group, antihypertensives, angina, diabetes, TC, TG, BMI, smoking, SBP		Trend was similar in men and women, smokers and non-smokers
Wang 2001[166] Chin-Shan Community CV Cohort (CCCC)	1,703 men 1,899 women Exclusion of TG too high for LDL calculation, lipid lowering meds and previous CHD	Aged ≥ 35 FU: 8 years	Significant inverse	0.97 (0.96 to 0.99) Per 1mg/dl increase	CHD incidence	0.56 (0.46 to 0.82)	Age, gender, BMI, smoking, HTN, DM		
Jousilati 1999[136] FINRISK 82&87	7090 men 7696 women Previous MI excluded	Aged: 25-64 years FU: Mean: 10.6 years	Significant inverse men and women	Men: 0.91 (0.87 to 0.95) Women: 0.90 (0.34 to 0.98)	CHD mortality	0.62 (0.50 to 0.77) 0.59 (0.00 to 0.90)	Age, smoking, TC, SBP, DM, BMI		Results for CHD incidence very similar

Author Study Year	Number Men Women Specific Group	Age range Follow-up	Overall effect	Hazard ratio	Endpoint	Hazard ratio (converted to per 0.5mmol/l increase)	Adjusted for	Associations with other risk factors	Other
				Per 0.1 mmol/l increase					
Ridker Women's Health Study 2005[147]	15,642 women Initially healthy	Aged: Over 40 FU: Mean 10 years	Significant inverse	Women: 0.43(0.30 to 0.61) Comparing 5th (>1.59) to 1st quintile (<1.02mmol/l)	CVD incidence: nonfatal MI, CHD related death, stroke, revascularisation	NA	Age, BP category, DM, BMI, smoking		
Koro Indiana Medical Group 2006[138]	2,468 men 4,460 women Urban primary care attendees No exclusions	Aged: Mean 55 years FU: Mean 5.1 years	Significant inverse	0.89 (0.86 to 0.93) men & women together Per 0.26 mmol/l increase	Major adverse coronary event (MI, ACS or revascularisation)	0.80 (0.75 to 0.87)	Age, prior MI, smoking, race, gender, DM, LDL, HTN, SBP, trigs		Per 0.26 mmol/l in HDL over the observation period 0.93 (0.90 to 0.97)
Pocock BRHS 1986[167]	7,415 men General population No exclusions	Aged: 40-59 years FU: 4.2 years	Non-significant inverse	0.90 p = 0.21 Per std dev increase (0.27	CHD incidence: Nonfatal MI and CHD death	0.82	Age, BP, BMI, smoking, non-HDL cholesterol	Re-examination by Gordon et al 19990 showed significant inverse when	Statistical significance lost on additional of other risk factors to the model

Author Study Year	Number Men Women Specific Group	Age range Follow-up	Overall effect	Hazard ratio	Endpoint	Hazard ratio (converted to per 0.5mmol/l increase)	Adjusted for	Associations with other risk factors	Other
				mmol/l)				adjusting for TC and other risk factors	
Jacobs LRCPS FU 1990[168]	4,381 men 3,756 women Initially free of CHD	Aged: Over 30 years FU: Mean 8.4 years	Significant inverse men and women	Men: 0.66 Women: 0.79 Per 10mg/dl increase	CVD death: (includes definite and suspected)	0.45 0.64	Age, LDL, log trigs, BMI, SBP, cigs	Oversampled those with high TC & on lipid lowering meds	Paper on same population Pekkanen et al (1990) examines relationship in those with and without pre-existing CHD
Assmann PROCAM 2002[48]	5,389 men Excluded if MI, stroke or angina at baseline	Aged: 35-65 years FU: Mean 10 years	Significant inverse	Men: 0.968 (0.957 to 0.980) Per 1mg/dl increase	CHD incidence: CHD death or nonfatal MI	0.54 (0.43 to 1.00)	Age, LDL, smoking, SBP, Fam hx MI, DM, trigs	Greater effect in lower TC distribution	Figures from risk function (2002)
Stampfer Physician's Health Study 1991[126]	246 new MI cases 246 age and smoking matched conrols	NA	Significant inverse	Men: 0.38 (0.21 to 0.69) Comparing 5th quintile (med 1.67)to 1st quintile (med:0.92	New MI	NA	Ahe and smoking only	Nested case-control study Also, Rahilly0Tierney 2008 reports inverse assoc with increase in HDL over 12 years observation – prospective design	HDL2 and HDL3 also examined – both protective – 2>3

Author Study Year	Number Men Women Specific Group	Age range Follow-up	Overall effect	Hazard ratio	Endpoint	Hazard ratio (converted to per 0.5mmol/l increase)	Adjusted for	Associations with other risk factors	Other
Luc PRIME 2002[140]	9,073 men Initially free of CHD	Aged: Median: 55 years FU: 5 years	Significant inverse	Men: 0.57 (0.38 to 0.86) Comparing 5th quintile (>1.53mmol/l) to 1st quintile (<1.01 mmol/l)	CHD incidence: nonfatal MI, CHD death, angina	NA	Age, centre, smoking, HTN, DM, LDL trigs	HDL very similar relatioship to separate endpoints angina, CHD death etc.	ApoA1 more powerful predictor than HDL-C. LpA-1 or LpA-1:A-11 didn't add to predictive ability
Keys Minnesota prospective study 1980[169]	284 men	FU: 25 years	Non-significant inverse	Univariable difference between CHD dead and others: -3.30mg/dl, p=0.04	CHD mortality	NA	-	Negative correlation with body weight	
Paunio ATBC trial – placebo arm 1994[144]	7,052 men Male smokers CHD not excluded	Aged: 50-69 years Mean: 4.7 years	Significant inverse	0.988 (0.975 to 1.00) 0.983 (0.964 to 1.00) when adjusted for regression dilution	CHD mortality	0.79 (0.61 to 1.00) 0.72 (0.49 to 1.00)	Age, cigs, alcohol, BMI, PA, SBP, TC, education	Alcohol (underreported) Heavy drinkers mean HDL 20.5% higher than non-drinkers	Similar effect in both age groups

Author Study Year	Number Men Women Specific Group	Age range Follow-up	Overall effect	Hazard ratio	Endpoint	Hazard ratio (converted to per 0.5mmol/l increase)	Adjusted for	Associations with other risk factors	Other
				Per 0.026 mmol/l increase					
Rywik Poland and US collaborative study on CV epidemiology 1999[170]	2,610 US men 2,336 US women 2,490 Polish men 2,708 Polish women No exclusions	Aged: 50-64 years FU: 13 years	Significant inverse in men only	US: 0.41, p<0.0001 Poland: 0.62, p<0.01 Men & women combined	CVD mortality	NA	Gender, age, smoking, education, martial status, BMI, alcohol, SBP	Pos: lower education (Poland) Alcohol: (except Polish women) Neg: BMI, smoking, lower education (US women)	Polish men – U-shaped relationship with total mortality. Increase mortality in higher HDL groups – not CVD mortality
				Comparing high category (>1.32, >1.76, >1.63, >1.76) to low category (<0.91, 1.19, 1.16, 1.30) for US men, US women, Polish men, Polish women					

Author Study Year	Number Men Women Specific Group	Age range Follow-up	Overall effect	Hazard ratio	Endpoint	Hazard ratio (converted to per 0.5mmol/l increase)	Adjusted for	Associations with other risk factors	Other
Enger Oslo Study 1979[133]	93 cases 186 controls Healthy initially	Aged 40-49 years FU: NA	Significantly lower HDL in definite MI cases	HDL 8.6% lower in the definite MI group ($p<0.05$)	CHD events: Nonfatal MI or sudden CHD death	NA	Matched for smoking, TC and trigs	Nested case control study	
Miller Tromso Heart Study 1977[142]	17 cases men 31 matched controls 6 of 17 cases had pre-existing CHD	Aged: 37-49 years FU: NA	Significantly lower HDL in cases	35% lower HDL in cases	CHD events: Nonfatal MI or CHD death	NA	None. But no difference between cases and controls in other risk factors except LDL cholesterol	Nested case control study	
Russian LRC, US LRC Perova 1995[146]	1,739 US men 7,958 Russian men Excluded previous CHD Oversampled hyperlipidaemic men	Aged: 40-59 years FU: 12 years	Significant inverse US Russia – non consistent relationship	0.55 0.88 – Russia – visit 1 only & only when adjusted Per 10mg/dl increase	CHD mortality	0.32 0.78	Age, city, SBP, education, alcohol, cigs, TC, TG, BMI, exercise, calories from fat	Pos: Alcohol, exercise, low education (Russia only) TC (US only) Neg: cigs (US only) trigs, BMI	HDL positively associated with total mortality in Russian men. ? due to physically demanding and dangerous work / poor medical care US: significant relationship with CVD mortality also – reduced
Kitamura Urban Japanese	6,408 men Initially free of CHD and	Aged: 40-59 years FU: 8 years	Significant inverse	4.17 (1.37 to 12.7), $p<0.003$ for	CHD incidence – definite or		Age, TC, SBP, BMI, cigs, alcohol	Similar results for definite MI	Similar results in groups with TC > and < 5.69 mmol/l

Author Study Year	Number Men Women Specific Group	Age range Follow-up	Overall effect	Hazard ratio	Endpoint	Hazard ratio (converted to per 0.5mmol/l increase)	Adjusted for	Associations with other risk factors	Other
Study 1994[137]	stroke			trend	suspect MI or angina				
				< 1.24 compared to >1.66mmmol/l					
				0.943		0.32			
				Per 0.026 mmol/l increase					
Watkins MRFIT UC Group 1986[171]	5,792 white men 465 black men Free of overt CHD at baseline High risk (higher 10-15%)	Aged: 35-57 years FU: 10 years	Significant inverse in white men Reduced power in black men	Beta: -0.198, P<0.01 -0.0022 (NS)	CHD incidence	0.02 0.96	None	Pos: alcohol SE status (white) Neg: BMI Smoking SE status (black)	No statistical difference in the HDL relationship between blacks and whites but ++ underpowered in blacks
				Per 1mg.dl increase					

Author Study Year	Number Men Women Specific Group	Age range Follow-up	Overall effect	Hazard ratio	Endpoint	Hazard ratio (converted to per 0.5mmol/l increase)	Adjusted for	Associations with other risk factors	Other
De Backer BIRNH 1998[151]	9,116 men and women Initially free of CHD	Aged: 25 to 74 years FU: 10 years	Significant inverse in men Underpowered in women	Betas: M: -0.0866 (p0.0066) W: -0.0207 (p0.81) M: -0.0276 (p0.013) W: -0.0409 (p=0.32) Per 1mg/dl increase	CHD mortality CVD mortality	0.19 0.68 0.59 0.46	Age, smoking, BMI, DM, alcohol, Diet P?S, SBP, TC, BPtx	Pos: alcohol, age, SBP men only Neg: BMI, smoking, diet P/S, BPtx – women only	Quadratic relationship – significant in men. Trend also in women
Ingelsson Framingham offspring study 2007[172]	1,562 men 1,760 women Initially free of CHD	Aged: 30 to 74 years FU: 14 to 18 years	Significant inverse both men and women	M: 0.71 (0.60 to 0.83) W: 0.72 (0.57 to 0.92) Per 1 std dev increase	CHD incidence: MI, angina, coronary insufficiency or CHD death	0.55 (0.41 to 0.73) 0.57 (0.38 to 0.87)	Age, SBP, DM, smoking, BPtx		
Keys 1956 Finnish Study	787 men Initially	Aged: 30-61 years FU: 24	Non-significant inverse	M: -0.519 T = -1.12	CHD mortality	0.77	Age, BMI, PA, SBP, smoking	Neg: relative weight	

Author Study Year	Number Men Women Specific Group	Age range Follow-up	Overall effect	Hazard ratio	Endpoint	Hazard ratio (converted to per 0.5mmol/l increase)	Adjusted for	Associations with other risk factors	Other
1984[173]	healthy	years		Per 1 mmol/l increase			Additional inclusion of beta cholesterol – no change in relationship	Pos: age	
Okamura NIPPON DATA 90 2005[143]	3,014 men 4,161 women Initially free of CVD	Aged: Over 30 years FU: Mean 9.6 years	Significant inverse both men and women	0.70 (0.53 to 0.93) men and women together Comparing > 1.82mmo/l to 1.04 to 1.55 mmol/l	All cause mortality	NA	Age, BMI, trigs, non-HDL-C, HTN, DM, smoking, alcohol	Neg: BMI, trigs, DM, smoking, HTN – women only, age – women only Pos: alcohol	Stronger relationship in women than men
Sharett ARIC Study 2001[148]	5,432 men 6,907 women Initially free of CHD	Aged: 45-64 years FU: 10 years	Significant inverse both men and women	0.72 men 0.68 women, both p<0.01 Per SD increase (0.40 mmol/l)	CHD incidence (nonfatal MI, CHD death revascularisation)	0.66 0.62	Age, race, SBP, smoking, DM, BPtx, Lp(a), ApoB, ApoA1, LDL, trigs		HDL2 and HDL3 also analysed – both protective HDL3 > HDL2
Goldbourt Israeli IHD Study 1990[174]	6,547 men All employees	Aged: 40-65 years FU: 23	Significant inverse	1.36 (1.25 to 1.49) 1.09 (1.04 to 1.14)	CHD mortality All cause	0.55 (0.46 to 0.65) 0.85 (0.78 to 0.93)	Age, TC, smoking, SBP, angina, hx of MI, dx cancer, diabetes		

Author Study Year	Number Men Women Specific Group	Age range Follow-up	Overall effect	Hazard ratio	Endpoint	Hazard ratio (converted to per 0.5mmol/l increase)	Adjusted for	Associations with other risk factors	Other
	No exclsuions	years		Per 10mg/dl decrease in HDL-C	mortality				
Lewington Propsective Studies Collaboratio n 2007[139]	153,798 men and women Initially apparently healthy	Aged: 40-89 years FU: Mean 9.7 years	Significant inverse in each age group: 40-59 60-69 70-89	1.63 (1.44 to 1.85) 1.83 (1.65 to 2.03) 1.35 (1.22 to 1.49) Per 0.33 mol/l decrease	CHD mortality	0.48 (0.39 to 0.58) 0.40 (0.34 to 0.47) 0.63 (0.55 to 0.74)	None		Very little difference between HDL relationship in men and women
Wallidius AMORIS Study 2001[175]	95,857 men 76,214 women Healthy individuals ongoing health screening or outpatients No exclusions	Aged: M: Mean 47 years W: Mean: 50 years FU: Mean 5.6 years	Significant inverse – men and women	Women: 0.36 Men: 0.38 Comparing the 4th to the 1st quartile of HDL	CHD mortality	NA	None – database has no personal information		HDL is calculated from ApoA1, TC and TG

Author Study Year	Number Men Women Specific Group	Age range Follow-up	Overall effect	Hazard ratio	Endpoint	Hazard ratio (converted to per 0.5mmol/l increase)	Adjusted for	Associations with other risk factors	Other
Stensvold Norwegian Study 1992[150]	23,690 men 23,425 women General population No exclusions	Aged: 40-54 years FU: Mean 6.8 years	Significant inverse – men Non-significant in women Subgroup with CHD at baseline – significant both genders	Betas: M: -0.439 (-0.851 to -0.046) W: -0.706 (-2.005 to 0.593) M: -0.572 (-1.060 to -0.084) W: -1.264 (-2.374 to -0.154) Per 0.5 mmol/l increase	CHD mortality	0.64 (0.43 to 0.96) 0.49 (0.13 to 1.81) 0.59 (0.35 to 0.92) 0.28 (0.09 to 0.86)	Age, cigs, SBP, TC		Upswing in mortality in men with very high HDL – related to alcohol? No effect in healthy men with TC < 6.5mmol/l
Wilson Framingham 1988[176]	1,007 men 1,418 women Initially free of CHD	Aged: 50-79 years FU: 12 years	Significant inverse – men and women	M: 0.81, p=0.027 W: 0.72 (p=0.006) Per 10mg/dl increase	CHD mortality	0.67 0.53	Age, TC, SBP, BMI, smoking	W: Effect seen in each TC quartile M: Effect seen in first TC quartile only	Also inverse relationship with all-cause mortality in men. Borderline significance of CVD mortality relationship in men
Despres Quebec CV	2,103 men	Aged: Mean 56	Significant inverse	0.87 (0.77 to 0.96)	CHD incidence	0.51 (0.28 to 0.82)	None for figures given. But state that		

Author Study Year	Number Men Women Specific Group	Age range Follow-up	Overall effect	Hazard ratio	Endpoint	Hazard ratio (converted to per 0.5mmol/l increase)	Adjusted for	Associations with other risk factors	Other
Study 2000[132]	Initially free of CVD	years FU: 5 years		Per 10% increase in HDL	(nonfatal MI, CHD death, typical angina, coronary insufficiency)		relationship did not change on multivariable adjustment		
Knuiman Busselton Health Survey 2009[177]	3,041 men & women Initially free of CVD or DM	Aged: 25-84 year FU: Between 8 and 29 years	Significant inverse	1.60 (1.12 to 2.28) Comparing low HDL (<1.0mmol/l men <1.3 mmol/l women) to normal HDL	CHD incidence	NA	Age and gender only		Non significant inverse relationship with CVD incidence

Table 2-2: Observational studies addressing the effect of HDL cholesterol on CHD / CVD or mortality outcomes

Study Year Author	No of men and women in older age groups	Follow-up time Exclusions	Hazard ratio for HDL cholesterol - Overall	Hazard ratio for HDL cholesterol - Men	Hazard ratio for HDL cholesterol - Women	Other	Overall conclusion
FINE Study 2002 Houterman[178]	2170 men aged 65-84 years	10 years No exclusions	Men only	0.69 (0.49 to 0.96) - adjusted - per 1 mmol/l	Men only		Independent in men

Study Year Author	No of men and women in older age groups	Follow-up time Exclusions	Hazard ratio for HDL cholesterol - Overall	Hazard ratio for HDL cholesterol - Men	Hazard ratio for HDL cholesterol - Women	Other	Overall conclusion
	No women			increase - CVD mortality			
Uppsala Longitudinal Study of Adult men 2008 Zethelius[179]	1135 men aged 71 years	Median: 10 years Excluded if previous CVD	Men only	0.75 (0.62 to 0.91) - adjusted - per std dev(0.35mmol/l) increase - CVD mortality	Men only		Independent in men
Framingham 1993 Kronmal[180]	747 men and women in 66-75 age group	30 years	0.977 (0.957 to 0.997) - adjusted - per 1mg/dl increase - CHD mortality	Only analysed men and women together	Only analysed men and women together	No significant relationship in those aged over 76 years	Independent in men and women together
1990 Nikkila[181]	137 men and 398 women aged 85 years	5 years In-patients and nursing home residents excluded	Men and women not analysed together	1.74 - unadjusted - comparing <0.80 mmol/l to 1.30 – 1.79 mmol/l - All-cause mortality	2.58 (p<0.01) - unadjusted - comparing <0.80 mmol/l to ≥1.80 mmol/l - All-cause mortality		No adjustment
Leiden 85 plus 2003 Weverling-Rijnsburger[182]	250 women and 123 men aged 85 years	4 years No exclusions 64% had pre-existing CVD	2.0 (1.2 to 3.2) - unadjusted - CVD mortality - low HDL (mean 36mg/dl) compared to high (mean 64mg/dl)	1.6 (0.8 to 3.4) - unadjusted - CVD mortality - low HDL (mean 36mg/dl) compared to high (mean 64mg/dl)	2.2 (1.1 to 4.4) - unadjusted - CVD mortality - low HDL (mean 36mg/dl) compared to high (mean 64mg/dl)	Adjustment for LDL cholesterol, beta-blockade, thyroid dysfunction, albumin, DM and BMI did not affect the estmates	Inadequate adjustment

Study Year Author	No of men and women in older age groups	Follow-up time Exclusions	Hazard ratio for HDL cholesterol - Overall	Hazard ratio for HDL cholesterol - Men	Hazard ratio for HDL cholesterol - Women	Other	Overall conclusion
Rancho-Bernardo 1992 Barrett-Connor[183]	761 men and 983 women aged 65 to 89 years	3 years No exclusions	No combined analysis	1.09 (NS) - adjusted - CHD mortality - per 0.26mmol/l decrease in HDL	1.24 (NS) - adjusted - CHD mortality - per 0.26mmol/l decrease in HDL		No significant relationship but inverse trend in both men and women.
Zutphen Elderly Study 1996 Weijenberg[184]	885 men aged 64 to 84 years	5 years No exclusions; 25% had pre-existing CHD	No women included	0.80 (0.60 to 1.8) - adjusted - CHD incidence - per 0.26 mmol/l increase HDL	No women included		No significant relationship in men. Inverse trend
Systolic hypertension in the elderly program 1996 Frost[185]	4,736 men and women aged 60 years or older	Mean 7.5 years Mean:4.5 years All hypertensive Excluded those with pre-existing CVD	0.95 (0.81 to 1.12) - adjusted - CHD incidence - per 0.39 increase HDL	No separate analysis	No separate analysis		No independent effect. Significant inverse relationship on univariable analysis
Dubbo Study of Australian Elderly 1995 Simons[186]	1236 men and 1569 women aged 60 years and older	Median: 5.2 years No exclusions; 22% had pre-existing CHD	No combined analysis	0.82 (0.68 to 0.98) - adjusted - CHD events - per std dev increase (0.39mmol/l)	0.89 (0.75 to 1.07) - adjusted - CHD events - per std dev increase (0.39mmol/l)		Significant independent effect in men only
Cardiovascular study in the elderly (CASTEL)[187] 2005 Mazza[188]	1275 men and 1982 women aged 65 years or older	12 years No exclusions; 15% men and 11% women had pre-existing	No combined analysis	No relationship in men – results not given	Approx 1.5 Adjusted CHD mortality Comparing first to fifth quintile of HDL		Significant independent effect in women, not in men

Study Year Author	No of men and women in older age groups	Follow-up time Exclusions	Hazard ratio for HDL cholesterol - Overall	Hazard ratio for HDL cholesterol - Men	Hazard ratio for HDL cholesterol - Women	Other	Overall conclusion
		CHD					
Whitehall 2007 Clarke[189]	5344 men aged 68 to 97 years	7 year Separate analyses in those with prior CVD or statin use or without	No women included	0.74 (0.54 to 1.01) – no previous CHD 0.69 (0.50 to 0.69) – previous CHD - adjusted - CHD mortality - per 30mg/dl increase in HDL	No women included		Independent relationship in men. Women not included.
Cardiovascular Health Study 1999 Psaty[190]	1967 men and 2979 women aged 65 years and older	Mean: 4.8 years Previous MI excluded	0.89 (0.64 to 1.22) - adjusted - Myocardial infarction - per 1mmol/l increase HDL	No separate analyses	No separate analyses		Non-significant inverse trend
Established populations for epidemiologic studies of the elderly 1995 Corti[191]	2527 women and 1377 men aged 70 and older	6 years Separate analyses in those with and without previous CHD	1.4 (1.1 to 2.0) – no previous CHD - adjusted - CHD events - comparing <0.90mmol/l to ≥ 1.55 mmol/l	1.3 (0.8 to 2.2) – no previous CHD - adjusted - CHD events - comparing <0.90mmol/l to ≥ 1.55 mmol/l	1.5 (0.99 to 2.3) – no previous CHD - adjusted - CHD events - comparing <0.90mmol/l to ≥ 1.55 mmol/l	Risks greater in the younger (71-80 years) than the older (>80 years) subgroups	Independent risk factor – in both men and women, significant when all included, non-significant separately in men and women when restricted to those without previous CHD

Study Year Author	No of men and women in older age groups	Follow-up time Exclusions	Hazard ratio for HDL cholesterol - Overall	Hazard ratio for HDL cholesterol - Men	Hazard ratio for HDL cholesterol - Women	Other	Overall conclusion
Bronx aging study 1992 Zimetbaum[192]	226 women and 124 men aged 75 to 85 years	Mean: 6.3 years Those with dementia and terminal illness excluded 14% previous MI	No combined analysis	6.5 (2.0 to 20.9) - adjusted - CVD events - consistently lowest HDL tertile compared to consistently highest tertile of HDL	No effect in women – figures not given		Independent effect in men, not demonstrated in women
Honolulu Heart Program 2004 Curb[123]	2,340 men aged between 71 and 93 years	7 years Excluded those with pre-existing CVD or cancer	No women included	0.56 ($p<0.05$) - adjusted - CHD events - comparing high ≥ 60 mg/dl to low HDL <40 mg.dl	No women included		Independent inverse effect in men, women not studied
PROSPER – placebo group 2005 Packard[193]	1396 women and 1495 men aged between 70 and 82 years	3.2 years All had vascular disease or high risk of CVD	Significant trend for decreasing risk in increasing quintiles of HDL men and women combined – p=0.0019 Adjusted CVD events	Significant inverse association – hazard ratios not given Adjusted CHD events	Non-significant inverse association in women	Effect seen in primary and secondary prevention groups	Significant independent effect in men but not significant in women
Nilsson 2009[194]	199 men and 195 women aged 75 years	10 years Those with pre-existing vascular disease were excluded in separate	No combined analysis	0.71 (0.55–0.90) - adjusted - major cardiovascular events - per 10mg/dl increase in HDL	1.01 (0.83–1.23) - adjusted - major cardiovascular events - per 10mg/dl increase in HDL	Mentions that associations with MCVE were unchanged after exclusion of those with pre-existing vascular	Significant independent effect in men but not in women

Study Year Author	No of men and women in older age groups	Follow-up time Exclusions	Hazard ratio for HDL cholesterol - Overall	Hazard ratio for HDL cholesterol - Men	Hazard ratio for HDL cholesterol - Women	Other	Overall conclusion
		analyses				disease	
2004 Li[195]	1109 men and 102 women aged between 60 and 102 years	Mean: 11.2 years No exclusions	Significant inverse effect Unadjusted CHD events and mortality Trend seen across low, medium, high categories – no hazard ratios given	No separate analyses	No separate analyses		No adjustment for other CV risk factors Significant inverse univariable effect – including men and women together
2007 Wang[196]	1025 men and women aged 65 to 74 years	13 years Those with diabetes excluded. Previous CHD or stroke not excluded	1.50 (1.12 to 2.01) - Adjusted - CVD mortality - comparing <1.0mmol/l to others	1.71 (1.17 to 2.49) - Adjusted - CVD mortality - comparing <1.0mmol/l to others	1.22 (0.74 to 2.01) - Adjusted - CVD mortality - comparing <1.0mmol/l to others		Significant independent inverse effect in men and women together and in men separately but not in women separately.
Rotterdam Study 1999 Houterman[197]	948 men and 1632 women aged 70 and older	Mean : approx 4 years No exclusions. Previous CHD: up to 9% previous MI in women and up to 19% in men	No combined analysis	0.96 (0.87 to 1.05) - adjusted - Fatal and nonfatal MI - per 0.1mmol/l increase HDL	0.90 (0.83 to 0.98) - adjusted - Fatal and nonfatal MI - per 0.1mmol/l increase HDL		Significant independent inverse effect of HDL in women, insignificant in men
Aronow 1996	664 white men and	Mean: 3.3 years (men)	No combined analysis	0.948 (0.935 to 0.962)	0.935 (0.926 to 0.945) - adjusted		Significant independent

Study Year Author	No of men and women in older age groups	Follow-up time Exclusions	Hazard ratio for HDL cholesterol - Overall	Hazard ratio for HDL cholesterol - Men	Hazard ratio for HDL cholesterol - Women	Other	Overall conclusion
Hebrew Hospital Home Study[198]	1488 women aged 60 - 100 years	4 years (women) No exclusions Care facility residents – 41% had previous MI		- adjusted - CHD events - per 1mg/dl increase HDL	- CHD events - per 1mg/dl increase HDL		inverse effect in older men and women.
Aronow 1998 Hebrew Hospital Home Study[199]	185 black men and 413 women aged 60 - 100 years	Mean: 3.5 years (men) 4 years (women) No exclusion Care facility residents – 52% of men and 24% of women had previous MI	No combined analysis	0.948 (0.925 to 0.971) - adjusted - CHD events - per 1mg/dl increase HDL	0.936 (0.920 to 0.952) - adjusted - CHD events - per 1mg/dl increase HDL		Significant independent inverse effect in both black men and women.
Prospective studies collaboration 2007 Lewington[139]	Exact no. not available older subgroup of total of 153,798 Aged between 70 and 89	Mean follow-up approx 9.7 years Initially apparently healthy	1.63 (0.44 to 1.85) - unadjusted - CHD mortality - per 0.33mmol/l decrease in HDL	1.37 (1.20 to 1.56) - unadjusted - CHD mortality - per 0.33mmol/l decrease in HDL	1.32 (0.22 to 1.53) - unadjusted - CHD mortality - per 0.33mmol/l decrease in HDL		Significant inverse effect in older men and women, but without adjustment for other risk factor

Study Year Author	No of men and women in older age groups Exclusions	Follow-up time	Hazard ratio for HDL cholesterol - Overall	Hazard ratio for HDL cholesterol - Men	Hazard ratio for HDL cholesterol - Women	Other	Overall conclusion
	years						
Asia Pacific Cohort Studies Collaboration 2007 Woodward[157]	Exact no. not available older subgroup of total of 79,694 Aged 75 years and older	Median follow-up approx 6.8 years Previous CHD not excluded	1.19 (NS) - unadjusted - CHD mortality - per std dev (0.40mmol/l) decrease in HDL	No separate analyses	No separate analyses		Non-significant inverse relationship in the older subgroup
ATBC Trial 1994 Paunio[144]	2,392 men aged between 60 and 69	Approx mean: 4.7 years Male smokers only	No women included	2.09 (0.87 to 5.04) - adjusted - CHD mortality - comparing first to fifth quintile of HDL	No women included		Non-significant inverse relationship in the older subgroup

"Adjusted" indicates adjustment for age and a minimum of smoking status, SBP and either TC or LDL cholesterol level. "Unadjusted" generally includes adjustment for age.

Table 2-3: Observational studies addressing the effect of HDL cholesterol on CHD / CVD or mortality outcomes in the older age group

Table 2-2 details for each study: the numbers of men and women included, the median follow-up time, the age range or mean age (as available), a summary of the overall effect of HDL, the adjusted hazard ratio, the endpoint studied and a description of the co-variables (other CV risk factors) which were adjusted for in the analysis. Where studies have reported the association between HDL cholesterol as a continuous variable, Table 2-2 also includes the hazard ratio for the effect converted to the unit of HDL reported in our study (per 0.5 increase in HDL). This was done to facilitate comparison between the previously published studies and our results. The methods used for converting the hazard ratios are described in Table 2-4.

Step	Equation used
Unit of HDL cholesterol converted from mg/dl to mmol/l:	$\alpha = \phi * 0.02586$
Convert original hazard ratio to beta coefficient:	$ß_{orig}$ = Natural logarithm(γ_{orig})
Convert to beta coefficient for 1mmol/l HDL increase:	$ß_{mmol/l} = ß_{orig/\phi}$
Convert to beta coefficient for 0.5mmol/l HDL increase:	$ß_{0.5mmol/l} = ß_{mmol/l}*0.5$
Convert to hazard ratio for 0.5mmol/l increase in HDL:	$\gamma_{0.5mmol/l}$ = Exponential ($ß_{0.5mmol/l}$)

Where,

Original unit used for hazard ratio of HDL as continuous variable = α

Original unit used for hazard ratio of HDL as a continuous variable converted to mmol/l= ϕ

Original hazard ratio (from study) for HDL as continuous variable = γ_{orig}

Beta coefficient for original unit of increase in HDL = $ß_{orig}$

Beta coefficient for 1 mmol/l increase in HDL = $ß_{mmol/l}$

Beta coefficient for 0.5 mmol/l increase in HDL = $ß_{0.5mmol/l}$

Hazard ratio for 0.5 mmol/l increase in HDL = $\gamma_{0.5mmol/l}$

Table 2-4: Steps and equations used for converting hazard ratios for effect of HDL as continuous variable in previous studies to hazard ratio per 0.5mmol/l increase in HDL

Also included in Table 2-2, where publications have made this information available, are the results of subgroup analyses, and cross-sectional analyses of the associations between HDL and other CV risk factors.

Those studies which reported only the risk associated with a defined HDL range compared to another range (e.g. quintiles or categories) we have not been able to standardize the hazard ratios. Where information on more than one endpoint is available we have focused on the CVD or CHD mortality (or event) endpoint as this is closest to what we intended studying in our analysis.

Studies including only those with established CHD are not included in Table 2-2, because our analysis focuses on the effect of HDL cholesterol in primary prevention. However, some studies have included those with and

without baseline CHD together. This has been stated in the table and we have reported on the effect in those without previous CHD where analysis of this subgroup has been made available.

To summarize the information in Table 2-2, there are 42 studies[48, 126, 132, 133, 136-140, 142-144, 146-148, 150-177] listed, of these 40 are prospective studies[48, 126, 132, 136-140, 143, 144, 146-148, 150-177]. Twenty of these included analyses of both men and women. 33 of a total of 41 studies included show a statistically significant protective effect of HDL on outcome[48, 126, 132, 133, 136-140, 142-144, 146-148, 150-177]. The remaining 8 show an inverse relationship which did not reach statistical significance[150, 151, 156, 159, 163, 167, 169, 173]. Examining the hazard ratios (converted to the standard unit (per 0.5mmol/l increase) the results are reasonably consistent. Twenty-four of the 28 studies on which this information is available have demonstrated hazard ratios between 0.40 and 0.80, for either CHD or CVD endpoints[48, 132, 136, 138, 139, 144, 146, 148, 150-154, 157, 160, 162, 164-166, 168, 172-174, 176]. Additionally, between subgroups, for example, individuals with diabetes[154, 155] or smokers[144], the relationship appears consistent. There is no consistent demonstration of a difference in the effect based on gender.

However, inconsistencies in the relationship still remain. Most studies have demonstrated that the relationship holds at each level of TC; [200, 201] some showed a stronger relationship at lower levels, [129, 134] but one of the largest prospective studies demonstrated that HDL-C was no longer related to CVD at TC levels lower than 6.5mmol/l. [150]. Additionally, only a limited number of studies have examined whether the relationship holds at all levels of either total or LDL cholesterol. The need for further investigation of this issue has been highlighted in a recent systematic review on the topic[202].

Other inconsistencies include the fact that several of the studies have been undertaken in populations which oversampled those with high total risk or high cholesterol levels. [135, 137, 138, 168, 171, 201] Others have shown that the relationship no longer holds in those without previous CHD. [150]

The effect of HDL cholesterol as a protective factor in the elderly deserves special consideration.

HDL – EFFECT ON CHD IN THE OLDER AGE GROUP

Table 2-3 shows the prospective studies which have examined the relationship between HDL cholesterol and CV risk specifically in the older age group. As can be seen in the table, the numbers included in many of the studies has been small. Where possible, we have focused on the subgroup which excluded those with a previous diagnosis of CHD, although this was not possible in most. Nineteen [123, 139, 178-182, 185, 186, 188, 189, 191-199] of 24 [123, 139, 144, 157, 178-186, 188-199] prospective studies of older individuals (or older subgroups of larger studies) demonstrated a significant inverse relationship. Six of the 24 studies shown in the table have only examined the relationship in men [123, 144, 178, 179, 184, 189]. Those that did include women, several only examined the effect in men and women together, adjusting for gender a as risk factor (5 of 18 studies which included women) [157, 180, 185, 190, 195]. Of 13 studies[139, 181-183, 186, 188, 191-194,

196-199] which have examined the effect specifically in older women 6 studies[139, 181, 182, 188, 197-199] have demonstrated a significant association – only 3[188, 197-199] of these were adjusted for other risk factors. However, of the 3 studies[188, 197-199] demonstrating an independent protective effect of HDL cholesterol in older women all have included those with previously diagnosed CHD and the prevalence of previous CHD in these populations was as high as 41%.

At present, therefore, research defining the role of HDL cholesterol in CVD in initially healthy older women from the general population is lacking. However, based on large pooled analyses of prospective studies in healthy older women which performed univariable analyses, it seems likely that the independent protective effect of HDL cholesterol on CVD risk would hold in older women.

HDL – EFFECT ON STROKE

Similar to coronary heart disease, atherosclerosis is also the underlying process in a substantial proportion of ischemic stroke events, as discussed above. While considerable and consistent epidemiological evidence supports this protective role of HDL cholesterol in the pathogenesis of coronary heart disease, as discussed above, evidence on the role of HDL cholesterol in the development of stroke is much more uncertain.

The latest version of the American Stroke Association[203] guidelines on primary prevention of stroke recommend that low HDL cholesterol should be considered a risk factor for stroke in men, but suggest that further research is required to clarify the role of HDL cholesterol in women. Several prospective studies[71, 123, 182, 204-214] and a meta-analysis[215], have demonstrated a protective effect of HDL cholesterol against stroke. However, other large studies[139, 157, 216-220] including the largest analysis to date, the prospective studies collaboration[139] as well as the Asia Pacific Cohort studies collaboration[46] have shown no significant relationship between HDL cholesterol and stroke endpoints. This has lead to considerable confusion around the issue.

While several studies have demonstrated the specific protective effect of HDL cholesterol on stroke risk in men[123, 210-214], analyses which looked at the effect in women specifically have not shown a significant independent relationship.[157, 210, 211, 216-219] Although, some of the combined analyses demonstrated no significant gender interaction. Additionally, some of the larger analyses which showed no relationship with stroke risk were not analysed separately in men and women[139]. The failure of larger pooled analyses to replicate the earlier demonstrations of the protective effect of HDL cholesterol on stroke outcomes may be caused by differences in the effect of HDL cholesterol in different geographical regions or genders. The prospective studies which examined the effect of HDL cholesterol on stroke are detailed in Table 2-5.

Author (Study)	Year	Number included	Age range	Follow-up	Endpoint	Significant relationship adjusted	Age-adjusted Hazard ratio	Multivariable Hazard ratio	Comparing	Hazard ratio (per 0.26 mmol/l ↑)	Gender Specific Analyses	Specific population	Sub-types
Wallidius (AMORIS)[175]	06	M: 98,722 W: 76,831	M: 47 mean W: 50 mean	Mean 10.3 years	Ischemic stroke mortality	Significant inverse	0.80 (0.72 to 0.89)	-	Per std dev (0.41) ↑	0.868 (0.812 to 0.929)	No	Participants in screening programs	Haem, all other stroke also available
Tanne (Israeli IHD Study)[213]	97	M: 8,586	Over 42	21 years	Stroke mortality (presumed ischemic)	Significant inverse	-	1.17(1.02 to 1.36)	Per 0.26mmol/l ↓	0.855 (0.735 to 0.98)	Men only	Civil servants	-
Weverling-Rijnsburger (Leiden 85 plus)[182]	03	M: 202 W:397	85 year olds	4 years Mean 2.6 years	Stroke mortality	Significant inverse	2.6 (1.0 to 6.6)	Not reported analyses adjusted for some other factors did not change relationship	Low to high tertile		No	85 year olds – includes previous CHD and stroke	All stroke only
Lindenstrom (Copenhagen City Heart Study)[204]	94	M&W: 12,630	Over 30	12 years	Stroke incidence (presumed ischemic)	Significant inverse	-	0.53 (0.34 to 0.83)	Per 1 mmol/l ↑	0.848 (0.755 to 0.95)	No (no interaction)	General	Definitely haem excluded
Lehto[209]	96	M: 581 W: 478	45-64	10 years	Stroke incidence	Significant inverse	1.9 (1.2 to 2.9)	-	Low (<0.9) to other		No	Type 2 diabetes	All
Wannamethee	00	M: 7735	40-59	Mean 16.8	Stroke incidence	Significant inverse	0.59 (0.41 to 0.83)	0.68 (0.46 to 0.99)	5th to 1st quintile		Men only	General	All; Fatal stroke no

Author (Study)	Year	Number included	Age range	Follow-up	Endpoint	Significant relationship adjusted	Age-adjusted Hazard ratio	Multivariable Hazard ratio	Comparing	Hazard ratio (per 0.26 mmol/l ↑)	Gender Specific Analyses	Specific population	Sub-types
(Brit Reg Heart Study)[214]				years	Stroke mortality	No relationship							assoc.
Soyama (Oyabe)[205]	03	M: 1,523 W: 3,466	35-79	~ 10 years	Stroke incidence Stroke mortality	Significant inverse Unclear	3.42 (1.45 to 8.08)	3.89 (1.35 to 6.20)	Low (<) to high (>1.56)		No (similar relationship)	Japanese general	All; ischemic stroke was similar
Psaty (CV Health Study)[210]	04	M: 1,954 W: 2,931	Over 65 72 mean	Mean 7.5 years	Ischemic stroke incidence	C: Non-significant inverse M: Significant inverse W: No relationship	C: 0.88 (0.78 to 0.98) M: 0.74 (0.58 to 0.94) W: 1.00 (0.87 to 1.16)	C: 0.92 (0.81 to 1.04)	Per std dev ↑ 15.7mg/dl	0.948 (0.874 to 1.03)	Yes – different	Over 65s	Ischemic stroke, haem available also
Iso[211]	07	M: 3,595 W: 5,492	40-69	Mean 18.4 years	Stroke incidence	M: Significant inverse W: No relationship	M: 1.9 (1.2 to 2.9) W: 1.1 (0.7 to 1.6)	M; 2.0 (1.3 to 2.3) W: 0.9 (0.6 to 1.3)	Low (M <1.03, W <1.29) to normal		Yes – different	Japanese rural general	Ischemic non-embolic also
Leppala (ATBC Trial)[212]	99	M: 28,519	50-69	Mean 6 years	Ischemic stroke incidence	Significant inverse	-	0.59 (0.46 to 0.74)	Low (<0.84) to > 1.14		Men only	Male smokers	Opposite relationship with haem stroke
Simons (Dubbo[206	98	M: 1,234	Over 60	Mean 8.2 years	Ischemic stroke	Significant inverse	-	0.64 (0.44 to 0.94)	Per 1 mmol/l ↑	0.89(0.808 to 0.984)	No	Australian general	Presumed ischemic

Author (Study)	Year	Number included	Age range	Follow-up	Endpoint	Significant relationship adjusted	Age-adjusted Hazard ratio	Multivariable Hazard ratio	Comparing	Hazard ratio (per 0.26 mmol/l ↑)	Gender Specific Analyses	Specific population	Sub-types
])		W: 1,571			incidence Ischemic Stroke mortality	Non-significant inverse		0.54 (0.27 to 1.07)					(436 inc)
Shahar (ARIC)[217]	03	M&W: 14,175	45-64	Mean 10 years	Ischemic stroke incidence	M: U-shaped non-significant W: Non-significant	-	- -	-		Yes	General – over-sampling of African Americans	Ischemic only
Kurth (Women's Health)[216]	07	W: 39,876	Over 45 55 mean	Mean 11 years	Ischemic stroke incidence	Non-significant inverse	-	0.91 (0.64 to 1.28)	Per 1 mmol/l ↑	0.976 (0.89 to 1.07)	Women only	Health workers Trial ASA vit E	Ischemic only, subtypes too small for analysis
Koren-Morag (BIP Registry)[208]	02	M&W: 11,177	40-74	6-8 years	Verified ischemic stroke incidence	Significant inverse	-	0.89 (0.81 to 0.98)	Per 0.26 mmol/l ↑	0.89 (0.81 to 0.98)	No	Documented CHD	Similar for non-haem and large vessel (NS).
Curb (Honolulu Heart)[123]	04	M; 2,444	71-93	Max 7 years	Ischemic stroke incidence	Significant inverse	-	2.7 (1.2 to 5.8)	<1.0 to >1.6		Men only	Hawaii Japanese descent	Thromboembolic only
Davis (SHEP trial)[71]	98	M: 2,036 W: 2,700	Over 60 72 mean	Mean 4.5 years	Ischemic stroke incidence	Significant inverse	†0.78 (0.67 to 0.91)	0.81 (0.69 to 0.95)	Per 0.39 mmol/l ↑	0.869 (0.781 to 0.966)	No	Elderly with isolated systolic HTN	Ischemic stroke subtype numbers too low
Gordon	81	M:	59-82	6 years	Stroke	Non-	*M: 0.93	M: 0.9 NS	Per std dev ↑	-	Yes	General	All;

Author (Study)	Year	Number included	Age range	Follow-up	Endpoint	Significant relationship adjusted	Age-adjusted Hazard ratio	Multivariable Hazard ratio	Comparing	Hazard ratio (per 0.26 mmol/l ↑)	Gender Specific Analyses	Specific population	Sub-types
(Framingham)[218]		2,036 W: 2,700			incidence Atherothrombotic stroke incidence	significant inverse Non-significant inverse	NS W: 0.96 NS M: 0.97 NS W: 1.16 NS	W: 0.93 NS M: 0.88 NS W: 1.13 NS					ischemic atherothrombotic
Njolstad (Tromso)[219]	96	M: 1,204 W: 1,584	35-52	~ 11 years	Stroke incidence	Non-significant inverse	-	M: 0.8 (0.6 to 1.0) W: 0.8 (0.6 to 1.1)	Per 0.4mmol/l ↑	M: 0.865 (0.781 to 1.0) W: 0.865 (0.781 to 1.064)	Yes	General	All
Woodward (Asia Pacific Collaboration)[157]	07	M: 44,629 W: 35,065	49 mean	Median 6.8 years	Ischemic stroke incidence	Non-significant inverse Categories show U shape	0.90 (0.75 to 1.07)	Reduction in hazard ratio of 4.8% on adjustment	Per 1 std dev ↓ HDL (0.4mmol/l)	0.934 (0.717 to 1.045)	Yes, HR 0.91 in men and 1.00 in women	Pooled dataset – mixed	Haem, all unclassified and CHD also available
Lewington (Prospective Studies Coll)[157]	07	M&W: 153,798	40-89	Mean 9.8 years	Stroke mortality	No relationship	-	-	-		No	Pooled dataset – mixed	All, definite subtype numbers were too low for analysis
Amarenco (Meta-analysis)[215]	07	M&W: 238,739	Mixed	-	Mixed – stroke incidence and mortality	Significant inverse 8 of 10 studies	Uncertain – check	0.85 to 0.89	Per 0.26 mmol/l ↑		No	Meta-analysis – mixed	No subtypes analysed

Author (Study)	Year	Number included	Age range	Follow-up	Endpoint	Significant relationship adjusted	Age-adjusted Hazard ratio	Multivariable Hazard ratio	Comparing	Hazard ratio (per 0.26 mmol/l ↑)	Gender Specific Analyses	Specific population	Sub-types
Knuiman (Busselton)[177]	09	M&W: 3,041	25 - 84	Between 29 and 8 years	Stroke incidence	Non-significant inverse	1.07 (0.64 to 1.80)	Not available	Comparing low HDL (<1.0 men <1.3 women) to normal	NA	No	General	Endpoint includes haemorrhagic and ischemic
Wang (Kuopio Study)[221]	08	M & W: 991	65to 74 years	Mean: 14 years	Incident stroke	Non-significant inverse	1.49 (1.00 to 2.22) 1.43 (0.93 to 2.20) – previous CHD excluded	Not given	Comparing low HDL (<0.9 mmol/l men <1.0 women) to normal HDL	NA	No	General	Haemorrhagic and ischemic stroke included in outcome

Table 2-5: Prospective studies examining the effect of HDL cholesterol on stroke endpoints.

EFFECT OF HDL ELEVATION

CONSERVATIVE MEASURES TO INCREASE HDL CHOLESTEROL

SMOKING CESSATION

As discussed above smoking has been shown to be associated with lower levels of HDL cholesterol. In prospective studies, smoking cessation has also been shown to associated with an increase in HDL cholesterol[222]. A meta-analysis of the effect of smoking cessation on HDL cholesterol levels in 27 intervention studies on smoking cessation and prospective studies on smoking status showed an pooled estimate of 0.100 (95% CI 0.074 to 0.127) mmol/l increase in HDL cholesterol in individuals who successfully quit smoking[223] This effect was reasonably consistent across the studies with 19 of 27 individual cohorts actually showing a significant elevation in HDL cholesterol. None of the other lipid subfractions changed significantly.

INCREASING PHYSICAL ACTIVITY

More physically active individuals are known to have higher HDL cholesterol levels. It seems reasonable that increasing physical activity, then, would be associated with elevation in HDL levels. However, meta-analyses of the effect of both exercise programmes in healthy individuals with risk factors and cardiac rehabilitation in those with established cardiovascular disease have yielded inconsistent results. A Cochrane review of the effect of rehabilitation showed no effect on HDL cholesterol[224]. Another systematic review demonstrated a similar lack of change in HDL cholesterol, despite favourable changes in total cholesterol, other risk factors and coronary events[225]. Of note, the lack of effect was for both comprehensive and exercise only cardiac rehabilitation[224, 225].

Two meta-analyses conducted separately in men[226] and women[227] which examined the RCTs investigating the effect of aerobic exercise on lipoproteins demonstrated overall significant but modest HDL elevations of 2% and 3% in men and women respectively. Another meta-analysis of RCTs of aerobic exercise and effects on HDL cholesterol demonstrated a similar modest but significant effect +2.53mg/dl (+0.065mmol/l)). The increase in HDL cholesterol was dependent on the exercise duration with an approximately 1.4mg/dl increase in HDL cholesterol for each 10 minute prolongation of exercise programme[228]. There was no significant effect of aerobic exercise on HDL cholesterol found in a meta-analysis of trials restricted to the examination of the effect in type 2 diabetic patients only[229]. However, they concluded that there may have been a lack of power for demonstrating the effect.

Aerobic exercise programs seem to be more efficacious for HDL elevation than weight training, for example, one meta-analysis of 29 RCTs examining the effect of progressive resistance training for periods of 4 weeks

or more on lipids showed an increase in HDL cholesterol of (0.7 mg/dl, −1.2 to 2.6) which equated to a 1.4% increase. However this was not statistically significant[230].

Some have suggested that in those with low baseline HDL cholesterol levels[231] or isolated low HDL cholesterol in the presence of normal triglyceride levels[232], exercise has the least effect. This is disputed, because the results of a meta-regression showed in men that those with lower initial HDL levels (as well as the subgroup of older men) tended to develop greater increases in HDL cholesterol[226]. Others have shown that the HDL elevating effects of exercise are stronger when low HDL cholesterol levels are combined with high triglycerides and abdominal obesity[232]. This study also showed that the only significant correlate of elevation in HDL cholesterol associated with exercise was the reduction in waist circumference[232]. This is backed up by the results of another meta-regression in men which showed greater reductions associated with those with higher baseline percent fat. In a meta-regression of trials restricted to women reductions in HDL cholesterol were predicted by the observed increase in VO2 max[227].

The non-significant or inconsistent results of RCTS of lifestyle interventions may be related to heterogeneity amongst the trials, for example, between the interventions used, methods and measurement of endpoints. Additionally, lifestyle interventions do not lend themselves to investigation in RCT settings as easily as trials of pharmacotherapy because compliance is difficult. Additionally, uptake of the intervention is difficult to ascertain and standardise. This has been acknowledged in the 4th version for the European guidelines on CVD prevention[17]. For this reason, the grading of the evidence for preventive measures has been omitted, to avoid the situation where drug therapies are given higher priority purely because more high level evidence supports their use.

FISH OIL / OILY FISH CONSUMPTION

Fish oil consumption has also been shown in a meta-analysis of 21randomised controlled trials to be associated with significant increases in HDL cholesterol; +1.6 (95% CI +0.8, +2.3) mg/dL, along with reductions in triglyceride levels – particularly in those with elevated baseline triglyceride levels[233].

WEIGHT REDUCTION

Weight reduction has been shown to be associated with favourable changes in lipids, including elevations in HDL cholesterol. For example, in an analysis comparing those who underwent bariatric surgery (mean baseline BMI 40) to BMI-matched controls subjects, there was a greater elevation in HDL cholesterol at 2 years follow-up – 22.0% increase , which was significantly different from the increase of 3.5% seen in the control group[234]. This group also lost 23.4% of their body weight, compared to an increase of 0.1% in the control group. The elevation at 10 years follow-up in the surgery group has 24%, although this was still

significantly different from the control group, during the ten years the control also had increased their HDL cholesterol levels by 10.8%[234].

An interesting trial by Wood et al[235] randomised 131 sedentary overweight men to one of three programs – usual care, weight reduction focusing on increasing exercise and weight reduction focusing on diet modification. All groups were discouraged from altering the composition of their diets – however, those in the diet group were instructed to consume less calories. Those in both weight loss programs experienced significantly greater weight loss than the control subjects; with greater total body weight loss in dieters than exercisers: -7.8kg in dieters and -4.6kg in exercisers[235]. However, there was no difference in fat weight loss between individuals in the two weight loss programs. HDL cholesterol increased significantly and by a very similar proportion In both dieters (0.13mmol/l) and exercisers (0.12mmol/l). Of note, the changes in HDL subfractions were also remarkably similar[235]. The authors concluded that weight reduction is associated with increases in HDL cholesterol regardless of the method through which it is achieved.

DIET MODIFICATION

It can be difficult to separate the effects of change in diet on lipid sub-fractions because many diet interventions will also lead to reduction in body weight, which is itself associated with favourable lipid effects. A meta-analysis by Nordmann et al[236] compared the effects of low fat and low carbohydrate diets on both lipids and weight reduction. The meta-analysis included five trials (447 individuals). Inclusion criteria specified that the participants were in the overweight or higher BMI category and they were randomised to low carbohydrate (<60g carbohydrate daily without energy restriction) or low fat (<30% of energy intake as fat) diet. Those in the low carbohydrate group initially lost more weight than the low fat diet group (-3.3kg after 6 months), however, at 1 year of follow-up there was no overall difference in weight reduction (-1.0kg (95%CI: -3.5kg to 1.5kg).

Those on the low carbohydrate diet achieved significantly greater increases in HDL cholesterol after 6 months of the intervention: 0.12 mmol/l (95%CI: 0.04 to 0.21 mmol/l). There were also significantly greater reductions in triglycerides. However, it is important to weigh these favourable effects against the more favourable effect of the low fat diet on LDL cholesterol (0.14mmol/l greater LDL reduction in the low fat diet group)[236].

PHARMACOLOGICAL ELEVATION OF HDL CHOLESTEROL

Decreasing HDL cholesterol concentrations continue to be associated with increased levels of risk even in those on high dose statin therapy and with particularly low levels of LDL cholesterol (<1.8mmol/l)[201]. For the purpose of effecting further risk reduction in secondary prevention, particularly, there is interest in the

addition of other lipid lowering agents in high risk individuals with lower levels of HDL cholesterol. However, HDL cholesterol elevation is not a target of therapy in guidelines on CVD prevention as yet[17, 237].

Several agents have been shown to be successful in elevation of HDL cholesterol in different populations. The mean increases (or changes) associated with different lipid-lowering modalities are shown in Table 2-6, as estimated from a recent meta-analysis[238]. Using the mean HDL cholesterol concentration at baseline in the placebo and active treatments groups it has also been possible to give an indication of the percentage change in HDL cholesterol which has been achieved with each drug class.

	No . RCTS	Change in HDL cholesterol (mg/dl)	% change in HDL c holesterol
Statins	62	1.6	4%
Fibrates	9	2.6	6%
Resins	3	2.7	6%
Combinations with niacin	6	12	30%
n3 fatty acids	9	-0.1	0%
Diet/surgery	5	-0.1	0%
ACAT inhibitors	2	-0.3	-1%
Probucol	2	-12.3	-26%
Glitazones	2	3.1	7%
Hormones	9	4.3	8%
Torcetrapib	2	27.7	57%
Niacin*	4		28%

Table 2-6: Mean change in HDL cholesterol seen in RCTs of different lipid lowering treatments from (1)*Estimate for change in HDL cholesterol with niacin taken from meta-analysis from Brown et al, because no niacin trials were included in the Briel et al(2)

As shown, the agents with the most specific action on increasing HDL cholesterol are niacin, either alone or in combination with other lipid lowering agents, and fibrates. In general, statins also have a moderate effect on HDL cholesterol. Estrogen also raises HDL cholesterol, but it no longer used for this purpose due to the harmful effects which became obvious during randomized controlled trials[239-242].

Torcetrapib is a cholesterol ester transport protein (CEPT) inhibitor whose use was associated with very substantial elevations in HDL cholesterol (up to 72%)[243]. The elevations in HDL cholesterol were mainly in the large HDL_2 form of HDL cholesterol, since blockage of CEPTs will lead to accumulation of this form (see appendix for illustration). Unfortunately, these elevations in HDL cholesterol did not lead to the expected reductions in angiographic or clinical outcomes. In fact, the Illuminate trial had to be terminated early due to a 66% increase in total mortality in the active treatment group[243]. Development of this agent has now ceased. Possible explanations for the harmful effect of torcetrapib include off target effects on blood pressure a (5.4mmHg increase – probably mediated through an increase in circulating aldosterone)[243] or

to the specific subtype of HDL cholesterol formed. Some have suggested that this large form may be dysfunctional[244]. Others have suggested that the blockage of the CEPT interrupts the normal reverse cholesterol transport and therefore the excess HDL cholesterol cannot return excess lipid to the liver in the usual way. At this point it is unclear whether the harmful effects of torcetrapib are specific to the molecule or could represent a class effect.

IS PHARMACOLOGICAL ELEVATION OF HDL CHOLESTEROL BENEFICIAL?

Similar to the situation, discussed above with estimating the benefit of HDL cholesterol when this has been achieved using lifestyle measures, it is also difficult to calculate the independent benefit of pharmacological HDL cholesterol elevation. This is because each of the treatments also causes considerable favourable effects on other lipid levels (including LDL cholesterol reduction and triglycerides reduction). The evidence which is available to answer the question of whether elevation of HDL cholesterol results in beneficial outcomes will be reviewed below.

RANDOMISED CONTROLLED TRIALS OF AGENTS WITH POTENTIAL FOR HDL ELEVATION

The Veterans Affairs High density Lipoprotein Intervention Trial (VA-HIT) was a randomised controlled trial designed to assess the effect of gemfibrozil (1,200mg daily) versus placebo in a group of 2,531 men with a history of CHD who had low HDL cholesterol combined with low LDL cholesterol levels[245]. After 5 years of follow-up there was a significant 22% reduction in coronary events associated with active treatment. There was also a significant elevation in HDL cholesterol (6%), reduction in triglycerides (33%) and a non-significant elevation in LDL cholesterol levels (3.6%)[245]. In an analysis designed to determine the relative contributions of changes in each lipid subfraction to the favourable effect on clinical outcomes of the treatment, Robins et al demonstrated that only the on-treatment increase in HDL cholesterol significantly predicted a lower level of coronary events[246]. Neither the change in LDL cholesterol nor triglycerides predicts the outcome. This suggests that the favourable effect of fibrates on clinical outcomes is partly mediated through elevations in HDL cholesterol[246].

Similar evidence of the contribution of HDL elevation to the effect of fibrates is available from an analysis of the Helsinki Heart Study (HHS) trial. This primary prevention trial of dyslipidemic men demonstrated a significant 34% reduction in the incidence of CHD after 5 years. This was associated with an 11% increase in HDL cholesterol, an 11% decrease in LDL cholesterol and a 35% decrease in triglycerides[247]. A Cox proportional hazards regression showed that changes in both HDL and LDL were predictive of the outcome[247].

The coronary drug project examined the effect of niacin versus placebo (and four other lipid lowering agents) in 8,341 men with a history of myocardial infarction. This showed a significant 27% relative risk reduction in recurrent nonfatal myocardial infarction events in those treated with niacin during the follow-up period of the trial. However, after 15 years there was also a 11% reduction in the risk of total mortality, (52.0 versus 58.2%; p = 0.0004). Unfortunately, on treatment LDL and HDL cholesterol levels were not measured in this trial and therefore, it has not been included in many of the meta-analyses and systematic reviews of the effect of HDL cholesterol elevation.

REVIEWS, META-ANALYSES AND META-REGRESSIONS OF THE EFFECT OF HDL ELEVATION

Several reviews have made use of different statistical approaches to attempt to summarise the benefit in terms of regression/delayed progression of coronary stenoses[248] or clinical endpoints[238, 248], adjusting for the effects on other lipid parameters. The conclusions of these regarding the benefits of HDL elevation have been inconsistent.

A recent meta-regression by Briel et al included 108 randomised controlled trials of different lipid lowering treatments. The majority of these (n= 62) were statin trials. Other modalities studies were: nicotinic acid combinations, fibrates, torcetrapib, diet/surgery, hormonal treatment, n3 fatty acids, resins, Acyl-CoA:cholesterol acyltransferase (ACAT) inhibitors, glitazones and probucol.

On univariable analysis they demonstrated a non-significant 8.2% (95%CI: −24.7% to 8.1%) reduction in risk of CHD events associated with each 10mg/dl increase in HDL cholesterol[238]. This effect was no longer demonstrated once LDL cholesterol was included in the model. They showed that 32% of the variability in terms of risk reduction in CHD events was explained by differences in effect on LDL cholesterol, but that inclusion of change in HDL cholesterol as well as change in LDL cholesterol resulted in no improvement in the explained proportion of variability (R2 for HDL = 0.01)[238].

The sensitivity analyses which excluded known harmful agents (torcetrapib and hormones – since their harmful effects are unlikely to be mediated by HDL cholesterol – as discussed above) showed a much greater beneficial effect of HDL cholesterol elevation on univariable analysis: 28.9% risk reduction (95%CI: −51.7 to −6.6) for the risk of CHD events per 10mg/dl increase in HDL cholesterol. However, again in the multivariable model the effect not only became statistically insignificant but the effect of increase in HDL cholesterol actually became hazardous – 15% increase in risk of CHD events per 10mg.dl increase in HDL cholesterol - although this was not statistically significant.

Possible explanations for the apparent lack of effect of HDL cholesterol include the fact that the vast majority of trials included were statin trials in which little change in HDL cholesterol occurred and the inclusion of drug class as a categorical variable in the model. The regression coefficients for each drug class

have not been given, however if agents such as fibrates and niacin were associated with a protective effect, this may have limited the ability to detect favourable effects of HDL cholesterol raising on outcome. Another possibility is that HDL elevation is more beneficial in those who have low HDL cholesterol levels at baseline. The majority of the trials included in this meta-regression did not have low HDL cholesterol as an inclusion criteria.

These results disagree with the results of previous systematic reviews on the subject including a review by Brown et al[248] which demonstrated the effects (both in terms of progression/regression of coronary stenoses and clinical outcomes) to be linearly related to the combined percentage changes in HDL and LDL cholesterol. As shown in Figure 2.2, greater reductions in LDL cholesterol and elevations in HDL cholesterol were associated with greater reductions in risk. The relationship was linear for both endpoints and a high degree of the variability across the trials was explained by the lipid changes; R2 = 0.96 for the angiographic outcome and R2 = 0.93 for the clinical outcomes[248].

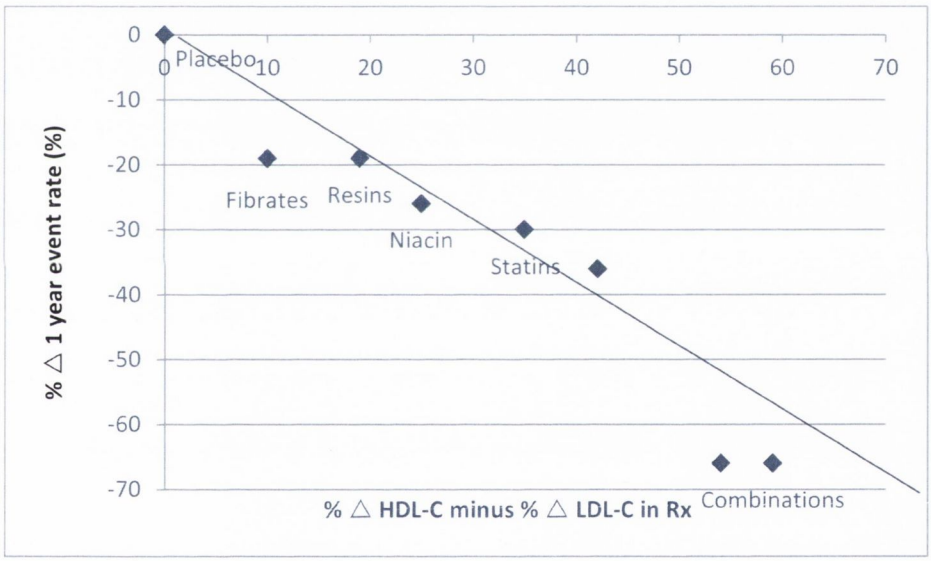


Figure 2.2: Correlation between change in both HDL cholesterol and LDL cholesterol and clinical outcomes in RCTs of lipid lowering agents (redrawn from Brown et al[248] with permission)

Clearly, Figure 2.2 does not definitely demonstrate that the change in HDL cholesterol is important, since all of the lipid change could be due to LDL decrease. However, the authors point out that if LDL cholesterol change alone were included on the X axis of the graph, those agents which cause considerable increases in HDL cholesterol (fibrates and nicotinic acid) would be moved off the line with greater reductions in endpoints than would be expected based on LDL cholesterol change alone. I have illustrated their point by drawing this graph, based on the figures given in the paper (see Figure 2.3). (Of note, the coronary drug project (niacin arm) is included here – the change in HDL and LDL cholesterol associated with niacin have been estimated from other trials in which the same dose was used.)

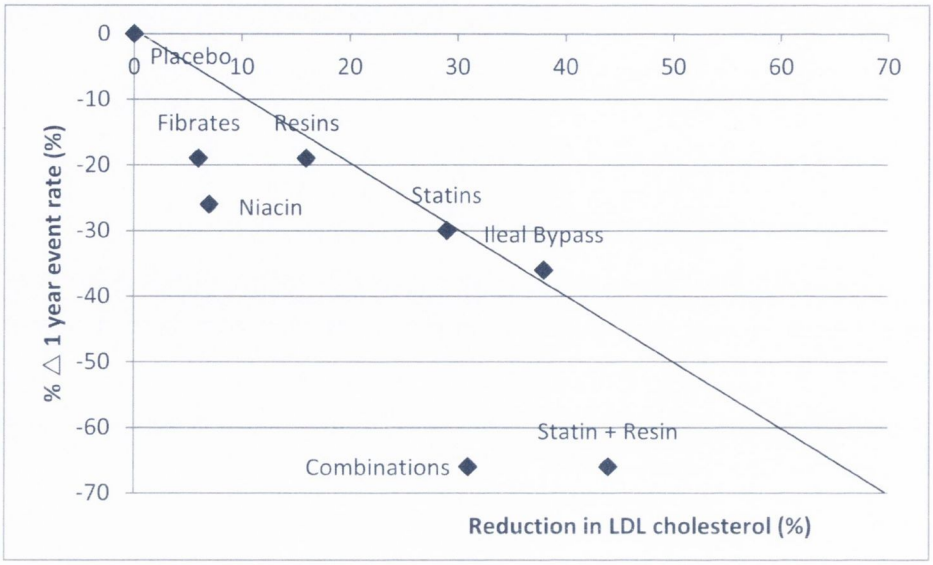


Figure 2.3: Correlation between change in LDL cholesterol and effect on clinical outcomes (drawn based on figures reported in Brown et al[248])

At present, it can be concluded that safe and effective methods for HDL cholesterol elevation are available, both through lifestyle changes and pharmacologically. However, the benefit of HDL cholesterol elevation has not been definitively proven. The current advice of the US and European guidelines on the topic seem reasonable – those with low HDL cholesterol should be considered to be at increased risk. They suggest that the reaction to this increased risk should be more intensive modification of other risk factors in order to lower total CV risk because HDL cholesterol is not considered a target of therapy at this point. Considering the inconsistent results discussed above this appears rational at present. As suggested by the authors of the large meta-regression[238], a large meta-regression with individual patient data from each of the trials, similar to the Cholesterol Trialists' collaboration, may shed further light on the issue and would also allow investigation of the effect of HDL elevation in subgroups based on baseline HDL levels and other factors (e.g. diabetes).

HDL – UNANSWERED QUESTIONS REGARDING RELATIONSHIP BETWEEN HDL AND CV RISK

Several questions regarding the effect of HDL cholesterol on CVD outcomes require clarification. These include the differences between the effect of HDL cholesterol on risk in men and women and particularly whether the association holds in all age groups. Specifically, as mentioned above there is insufficient evidence available at present regarding the effect of HDL cholesterol on risk in elderly women without a previous history of CHD. However, the finding of a continued protective effect of HDL cholesterol on CVD risk in older women would be expected based on the finding of a univariable association in large pooled cohort studies.

Whether HDL cholesterol functions at all levels of total CVD risk has not been investigated. This is important as this may give an idea of whether inclusion in risk estimation systems would result in superior risk estimation.

The effect of HDL cholesterol on stroke is unclear as yet. While many prospective studies, mainly in men, have demonstrated a significant inverse relationship large pooled analyses have failed to demonstrate an effect. This may be due to the use of a combined haemorrhagic and ischemic stroke endpoint. Since this meta-analysis pooled studies from many geographic regions it may be that there is a differential effect of HDL cholesterol on stroke individuals from different countries or of different races.

One of the main focuses of research regarding HDL cholesterol recently has been the investigation of the mechanisms through which HDL cholesterol exerts is protective effect. Related to this is the determination of which subtypes and sizes of HDL are particularly important in exerting the protective effect. Substantial research into the development of appropriate methods for the detection of dysfunctional HDL cholesterol is also underway. Further information on these questions may clarify why elevation of HDL cholesterol does not always result in a benefit. This may also aid the development of pharmacological approaches which improve not just the quantity by also the function of HDL cholesterol.

Epidemiological studies can also contribute to the understanding of the mechanism effect. The possibility remains that factors known to increase risk of CVD which are associated with low HDL cholesterol, for example diabetes, abdominal obesity, triglycerides, genetics, physical activity and smoking, are actually responsible for the association as opposed to HDL cholesterol being directly atheroprotective. Statistical adjustment for these potential confounders and subgroup analyses may help to clarify these issues.

HDL – UNANSWERED QUESTIONS REGARDING THE ROLE OF HDL IN RISK ESTIMATION

Many of the currently available risk estimation systems include HDL cholesterol and total cholesterol as two separate variables. In the original SCORE project, two versions of the SCORE charts were produced; one used total cholesterol (TC), the other used TC/HDL-C ratio as the lipid measure. However, the two charts had very similar properties and classified persons to virtually identical levels of risk. [4] This finding appears counterintuitive given that a strong and independent association between HDL-C and CV risk has been demonstrated in previous studies. The inclusion of extra variables as part of the two dimensional paper chart is not possible, however, the authors commented that entering TC and HDL-C separately into the model, as would be possible as part of HeartScore[249], may result in an improvement in risk estimation[4]. Whether the use of total cholesterol and HDL cholesterol separately improves risk estimation requires investigation.

HDL – SPECIFIC RESEARCH QUESTIONS

1. What is the effect of HDL level on cardiovascular risk? – including cardiovascular mortality, coronary heart disease mortality and stroke mortality
2. Does the effect apply equally in subgroups based on:
    a. Age
    b. Gender
    c. Country of origin
    d. Level of total risk calculated by SCORE
3. Is this effect independent of the effect of other CV risk factors?
4. Can we identify subgroups where HDL-C is particularly important or groups in whom HDL is not related to CV risk?
5. What other factors are associated with level of HDL?
6. Are any of these factors potentially involved in determining the level of HDL of an individual and are these factors modifiable; what are the public health implications of these findings?
7. Are there interactions between HDL and other cardiovascular risk modifiers?
8. Are the criteria for causality met?
9. Will development of a SCORE risk estimation system with HDL-C as an additional variable result in a significant improvement in our ability to estimate risk of CVD?
10. In a new risk estimation function containing HDL should the coefficients for the risk factors be calculated separately in men and women?
11. Does inclusion of HDL cholesterol improve risk estimation in the intermediate risk group – those on the borderline of high / low risk?
12. Can a simple paper-based system be developed whereby the HDL cholesterol value can be included only for the individuals for whom it makes the greatest difference to their risk estimate?

STUDY POPULATION – THE SCORE DATASET

SCORE DATASET - STUDY METHODS – SAMPLING & REPRESENTATIVENESS

The study population used was the SCORE dataset. [4] This comprises pooled data from 12 European cohort studies. Seven of the nine studies which had data available on HDL-C were included in this analysis[76, 84, 250-254]; the Russian cohort was not included as no information was available on glucose level or self-reported diabetes, meaning that this cohort could not be included in multivariable analyses which included this co-variable and the Scottish cohort was removed as the authors requested to withdraw their

participation from the collaboration. Those individuals with a definite previous diagnosis of CHD were excluded from the study, as in the original SCORE project[4].

The methods of the individual studies have been described in detail previously. [76, 84, 250-254] The sampling methods and representativeness of the individual studies are detailed below. As shown in the table, 96% of the data used for the HDL analysis were from studies which were representative of the general population.

Country	Recruitment	Population	% of the study population for HDL analyses
Belgium	Stratified random sample	General population	10%
Britain	Cluster sample	General population	7%
Denmark	Random sample	General population	10%
Finland	Random Sample	General population	13.5%
Germany	Stratified random sample	General population	4%
Italy	Random Sample (39 of 41 cohorts included– 93.2% of RIFLE pooling project) Occupational (2 of 52 cohorts included– 6.8% of RIFLE pooling project)	General population Occupational	54% (total) 50 % (general population) 4% (occupational)
Spain	Random Sample	General population	2.5%

Table 2-7: Population, methods of selection and contribution to the dataset of each of the European prospective studies.

HDL - METHODS

SCORE DATASET STUDY METHODS – LABORATORY MEASUREMENT OF HDL CHOLESTEROL

Laboratory methods for measurement of HDL cholesterol differed slightly between the studies. All used the precipitation method (with some variability in the reagent used) and all used enzymatic methods for determination of cholesterol content, apart from 13 of 24 towns in the British Regional Heart Study which used the Libermann Burchard method; a small correction factor was used to adjust for this. Studies which were part of Multinational MONItoring of trends and determinants in CArdiovascular disease (MONICA)[84, 251-253] underwent quality control according to MONICA methodology[255]. The methods of the individual studies are detailed in Table 2-8.

	Method	Precipitation reagent	Cholesterol determination	Other comments

			method	
Belgium	Precipitation	Heparin-manganese	Enzymatic	
Italy	Precipitation	Either Phosphotungstate Mg++, heparin or dextran sulphate Mg++	Enzymatic	Quality control centralised from WHO lipid reference laboratory in Praque
Britain	Precipitation	Phosphotungstate Mg++	Libermann Burchard (11 towns) enzymatic (13 towns).	Small correction factor included to adjust measurements done using LB method.
Finland	Precipitation	Dextran sulphate Mg++	Enzymatic	Part of MONICA – standardised quality control
Denmark	Precipitation	Phospotungstate Mg++,with updated method in later surveys	Enzymatic	Part of MONICA – standardised quality control
Spain	Precipitation	polyethylene glycol	Enzymatic	Part of MONICA – standardised quality control
Germany	Precipitation	Phosphotungstate Mg++	Enzymatic	Part of MONICA – standardised quality control

Table 2-8: Laboratory methods for HDL measurement in each of the cohorts.

ENDPOINT DEFINITIONS

The ICD 9 coding system was used. CHD mortality included ICD 9 codes 410-414. CVD mortality included in addition 401-409, 426 – 443, 798.1 and 798.2. The following definitely non atherosclerotic causes of death were excluded: 426.7, 429.0, 430.0, 432.1, 437.3, 437.4, 437.5, as in the SCORE project[4].

ASSESSING THE RELATIONSHIP BETWEEN HDL CHOLESTEROL AND OTHER CARDIOVASCULAR RISK FACTORS

Student's t test was used to assess for differences in the mean HDL-C in men and women.

The group was divided by quintile of HDL cholesterol. The mean of each other continuous risk factor (including total cholesterol, triglycerides, systolic blood pressure, body mass index and age) The proportion of individuals with diabetes and current smokers was also examined in each quintile of HDL. The p for trend across the quintiles was calculated.

The pair-wise correlation coefficients between HDL cholesterol and each of the continuous variables were calculated.

The mean levels of HDL cholesterol in current smokers versus non-current smokers and the mean HDL cholesterol level in those with and without diabetes was also calculated. These were compared using Student's t test. The mean level of HDL cholesterol in those from high and low risk European regions were also compared using Student's t test.

Each of these analyses was performed separately in men and women.

EFFECT OF HDL ON CVD ENDPOINTS

The cohort was divided into gender-specific quintiles of HDL-C. Crude CVD mortality rates per 1000 person years in each quintile were calculated. CVD mortality rates were assessed in gender-specific tertiles of HDL-C in each age group (under 45, 45-55, 55-65 and over 65 in men; the two younger age groups were combined in women due to low event numbers). The CVD mortality rate in each HDL-C quintile within gender specific total cholesterol (TC) quintile was also calculated.

Ten year risk of CVD mortality was calculated for each participant using the SCORE risk function. The cohort was then divided into categories of SCORE risk; ≤2%, 3-4%, 5-9% and ≥10%, separately in men and women. The CVD mortality rate in each HDL-C quintile within each of these categories was calculated. (The SCORE risk function used was the original total cholesterol version.)

The Cox proportional hazards model was used to assess the effect of HDL-C on CVD mortality, adjusting for age alone and additionally adjusting for other possible confounders of the relationship: systolic blood pressure (SBP), TC, current smoking status and diabetes and body mass index (BMI). Age was used as the time variable; participants entered the model at time equal to age at baseline and exited at time equal to age at study exit. Hazard ratios for HDL-C as a continuous variable were calculated, both per 0.5mmol/l increase in HDL-C and per 1 standard deviation (gender specific) increase in HDL-C.

The hazard ratios in each specific age group were also calculated. Hazard ratios for men and women were calculated separately throughout. These analyses were repeated for the CHD mortality endpoint. To examine the interaction between gender and HDL-C, men and women were analysed together, stratified by gender and an interaction term was added to the model. The interaction between age and HDL-C was similarly analysed.

Hazard ratios for HDL-C as a continuous variable (per 0.5mmol/l increase) were calculated separately in each country and a pooled estimate of the effect obtained. Using the meta command of Stata, a forest plot of the hazard ratio for HDL in each country was constructed, along with a pooled estimate.

The hazard ratios for HDL-C as a continuous variable within each SCORE category adjusted for age alone and adjusted for the other CV risk factors were also calculated. We tested for heterogeneity in the multivariable effect of HDL on CVD mortality in subgroups based on country, TC quintile, SCORE category and age-group using the meta command.

Additionally, analyses were performed within subgroups based on smoking status. In subgroups where extra co-variables were available we performed multivariable analyses of the effect of HDL cholesterol additionally adjusted for family history of CHD, triglycerides and the use of anti-hypertensive medications. The effect was also assessed within the two subgroups of those who were on anti-hypertensives agents and those who were not.

METHODS – ASSESSING THE EFFECT OF HDL CHOLESTEROL ON STROKE MORTALITY

Those with previously diagnosed myocardial infarction were excluded from these analyses, as in the original SCORE project. Data on previous stroke history were not available. However, given the age of the cohort, it is unlikely that a substantial number of the cohort have had prior cerebrovascular disease.

ICD 9 coding was used throughout. Ischemic stroke was defined as ICD 9 codes 433-438 inclusive. These are presumed to be ischemic, however there may be some haemorrhagic strokes included, due to the inclusion of the 436 code, which is not classified as ischemic or haemorrhagic. This problem has been encountered in several previous analyses also[204].

All analyses were performed separately in men and women from high (Finland, Denmark, and Britain) and low (Belgium, Spain, Italy and Germany) risk European countries. The study population was divided into HDL quintiles; the quintiles were specific to the group, e.g. women / men from high / low risk European countries. Ischemic stroke mortality rates per 1000 person years were calculated in each HDL cholesterol quintile. Ischemic stroke mortality rates were also calculated in groups with low HDL (defined as <1.3mmol/l in women and <1.0mmol/l in men) and normal HDL (defined as ≥1.3mmol/l in women and ≥ 1.0mmol/l in men).

Multivariable analyses were performed using Cox proportional hazards methods. Multivariable hazard ratios were calculated for the risks of each quintile of HDL compared to the first HDL quintile. Again these analyses were performed separately in men and women from high and low risk countries. Additionally, analyses were stratified by country. Analyses were adjusted for age alone and in a second model, adjusted for other cardiovascular risk factors – age, total cholesterol, SBP, current smoking status, diabetes and BMI.

DERIVING SCORE HDL

The same study population that was used in first HDL analysis was used for the derivation of the new function containing HDL cholesterol in addition to total cholesterol. The survival probabilities adjusted to the baseline level of risk factors at the current age and at the age in 10 years time were calculated using the Cox model. The survival probabilities for the CHD (coronary heart disease) and nCHD (non-coronary cardiovascular disease) mortality endpoints were calculated separately. These were then adjusted for the individual's risk factor levels, using the beta coefficients (equation 1). The 10 year CHD and nCHD survival probabilities were calculated by combining the current age and age+10 survival probabilities (equation 2). The adjusted survival probabilities for the two endpoints were then combined and converted to a risk of developing CVD mortality in 10 years (equation 3). Separate baseline survival curves were calculated for men and women from high and low risk cohorts. Finland and Denmark were used for the high risk cohort and Belgium, Spain and Italy were used for the low risk countries' baseline survival, as in the original SCORE project[4]. All of the cohorts were used in the calculation of the beta coefficients. Unlike the original SCORE function, the beta coefficients are gender-specific; this is because there was a substantial difference between the effect of HDL-C in women and men. Variables with non-significant beta coefficients were not included in the function.

The variables were set to the following base levels: TC: 6mmol//l, SBP: 120mmHg, HDL-C: 1mmol/l, Smoking: non-current smoker.

Two functions were derived. One, denoted HDL-C function, contained the following variables TC, HDL-C, SBP, smoking status (current vs. non-current smokers) and the other, denoted function without HDL-C, contained the same variables except for HDL-C. For both functions, age was used as the time variable and the models were stratified by cohort.

The second function was derived to compare the performance of the function with and without HDL-C. As in the original SCORE[4] diabetes was not included as a risk factor, because the European guidelines on CVD prevention those with diabetes to be already at high risk[17].

Equation 1: Survival function for CHD at current age, adjusted to risk factor levels

$$= S(age)CHD = [So(age)CHD]^{\exp((\beta chol*chol6)+(\beta sbp*sbp120)+(\beta currsmok*currsmok)+(\beta HDL\text{-}C*HDL\text{-}C1))}$$

Survival function for CHD at age in 10 years time, adjusted to risk factor levels

$$= S(age+10)CHD = [So(age+10)CHD]^{\exp((\beta chol*chol6)+(\beta sbp*sbp120)+(\beta currsmok*currsmok)+(\beta HDL\text{-}C*HDL\text{-}C1))}$$

where So = survival at the baseline level of risk factors (TC = 6mmol/l, SBP = 120mmHg, non-smoker, with or without HDL = 1mmol/l)

Repeated for the nCHD endpoint

Chol6 = cholesterol level – 6mmol/l; SBP120 = SBP level – 120mmHg; Currsmok = 1 if smoker, 0 if non-smoker; HDL-C1 = HDL-C level – 1mmol/l

Equation 2: 10 year survival probability for each endpoint (CHD and NCHD)

$= S_{10}(age)CHD = S(age+10)CHD/S(age)CHD$

$= S_{10}(age)nCHD = S(age+10)nCHD/S(age)nCHD$

Equation 3: 10 year risk of CVD (coronary and non-coronary) mortality

$= CVDRisk_{10}(age) = 1 - [S_{10}(age)CHD]*[S_{10}(age)nCHD]$

DIFFERENCES IN METHODS FROM THE ORIGINAL SCORE FUNCTION

The semi-parametric Cox proportional hazards model was used instead of the parametric Weibull model which was used in the original SCORE publication. Cox was preferred because the actual baseline survival is used as opposed to Weibull which assumes that the survival is a parametric function.

The original SCORE function which contained TC/HDL ratio as the lipid measure was derived using only 7 of the 12 cohort studies, as HDL information was only available from these studies. Although this meant that the baseline survival for the high risk chart reflected baseline rates in Norway, Finland and Denmark for the TC version, compared to Finland and Denmark alone for the TC/HDL ratio function, this made very little difference to the baseline (personal communication, Dr. Anthony Fitzgerald). Therefore, the same cohorts are used for the derivation of this function as were used for the derivation of the original TC/HDL ratio function, apart for the Scottish data, which has been dropped from the current analyses for at the request of the principal investigator of the original study.

TESTING THE PERFORMANCE OF SCORE HDL

The discrimination of the HDL-C function was compared to that of the function without HDL-C using area under receiver operating characteristic curve (AUROC). A small proportion of the dataset did not have follow-up to 10 years; in this situation we calculated Harrell's C statistic, which can account for variable follow-up times.

Recently, much attention has focused on the observation that the addition of important variables to risk function may result in very minor changes in AUROC (or Harrell's C). In terms of clinical usefulness, superiority of one risk function over another depends mainly on the ability of each to classify persons to the correct level of risk, since treatment decisions are based on this high/low risk classification. [95] Superior performance at the extremes of risk, where management decisions are already obvious, is less important. For this reason, we also compared the functions using the net reclassification index, as recently described

by Pencina et al[256], this new statistic is described in the background section above. The sensitivity and specificity of the two functions at different cut off points were also assessed.

To assess calibration of the function, in each category of risk, according to the HDL function we calculated the rate of CVD mortality (per 1000 person years) and compared this to the predicted risk (mean % 10 year risk of CVD mortality, which equates to the predicted number of CVD deaths per 100 people in 10 years or 1000 person years). This method for calculating the observed risk was preferred to the Kaplan Meyer method because many of the individuals in the Italian dataset only had follow-up complete to 6 years and therefore this cohort would not have been adequately represented in the observed 10 year risk. The ratio of the predicted to observed risk was also calculated. The Hosmer-Lemeshow goodness of fit test was calculated, overall and by tenths of the risk function. Again, the lack of complete 10 year follow-up for the Italian cohort meant that observed 10 year follow-up was not available. For this reason, the goodness of fit for the five year function was assessed – this compared the predicted 5 year risk to the observed five year risk.

PRESENTATION OF SCORE HDL

Risk charts of the HDL-C function were generated at different levels of HDL-C. An interactive electronic tool for calculating both risk functions was developed using Filemaker Pro advanced v9 software. Stata version 9 was the statistical package used throughout. Excel 2003 was used in the calculation of the net reclassification indices.

It will not be possible to include HDL cholesterol as a separate variable in the two dimensional paper charts. This has the disadvantage of complicating the process of risk estimation, because the computerized system must be used. One possibility for overcoming this problem is to include an indication of how the risk score will change if the individual has low HDL (defined as 0.8 mmol/l). The new value incorporating HDL only needs to be included on those areas of the chart where the risk score will change from low to high risk. This creates a somewhat more complicated chart but offers the advantage of taking the additional risk factor of low HDL cholesterol into account, but only where its incorporation will make a difference to the risk categorization.

HDL – RESULTS

BASELINE CHARACTERISTICS

Two thousand one hundred and ninety seven individuals were excluded due to previous diagnosis of CHD at baseline examination, this left 57,302 men and 47,659 women who had data available on HDL-C. After the

exclusion of any individuals who had missing data for any of the variables in the multivariable analyses, there were 53,000 men and 43,544 women who were included in the analysis. These were from 7 countries; the numbers included from each study and baseline characteristics for women and men respectively are shown in Table 2-9 and Table 2-10.

	Age	Years Recruited Mean follow-up	No. included No. events	HDL-C	TC	SBP	Smoke	DM	BMI
Belgium[257]	25-75	1980-84	4,754	1.54	6.09	131	17%	2%	25.93
		9.9 years	85	0.37	1.26	21			4.52
Denmark[253]	29-80	1977-90	4,970	1.61	6.11	124	47%	2%	24.23
		8.7 years	128	0.41	1.25	21			4.32
Finland[84]	24-64	1982, 1987	7,083	1.52	5.98	134	18%	2%	25.56
		11.6 years	99	0.34	1.31	21			4.45
Germany[251]	25-65	1984-85	1,937	1.64	5.92	127	22%	2%	25.91
		10.7 years	19	0.45	1.20	19			4.63
Italy[250]	20-99	1979-87	23,528	1.43	5.54	133	23%	2%	26.26
		6.4 years	87	0.36	1.20	22			4.88
Spain[252]	24-66	1986-88	1,272	1.41	5.60	120	12%	6%	26.46
		9.6 years	7	0.33	1.11	19			4.60
Total	20-99		43,544	1.49	5.75	131	24%	2%	25.87
		8.1 years	425	0.37	1.25	22			4.74

Table 2-9: Numbers and baseline characteristics for women included: (mean and standard deviation): TC (mmol/l), HDL-C (mmol/l), SBP (mmHg), BMI, Smoking (%) Diabetes (%)

	Age	Years Recruited Mean follow-up	No. included No. events	HDL-C	TC	SBP	Smoke	DM	BMI
Belgium[257]	25-75	1980-84	5,184	1.26	6.02	136	50%	2%	25.91
		9.6 years	181	0.33	1.14	18			3.53
Britain[254]	39-61	1978-80	6,985	1.15	6.28	145	51%	2%	25.44
		15.4 years	540	0.27	1.04	21			3.21
Denmark[253]	29-80	1977-90	4,907	1.33	6.11	129	57%	3%	25.41
		8.4 years	197	0.36	1.19	19			3.53
Finland[84]	24-64	1982, 1987	6,249	1.27	6.09	139	39%	2%	25.80
		11.4 years	269	0.32	1.20	17			3.21
Germany[251]	25-65	1984-85	1,948	1.32	6.10	133	39%	3%	26.91

		10.5 years	54	0.41	1.20	16			3.52
Italy[250]	19-89	1979-87	26,492	1.25	5.62	135	47%	3%	26.32
		6.5 years	374	0.34	1.19	20			3.52
Spain[252]	25-67	1986-88	1,235	1.19	5.70	123	50%	6%	25.76
		9.4 years	18	0.35	1.10	17			3.32
Total	20-89		53,000	5.86	1.25	136	47%	3%	26.03
		8.8 years	1,633	1.20	0.34	20			3.47

Table 2-10: Numbers and baseline characteristics for men included: (mean and standard deviation): TC (mmol/l), HDL-C (mmol/l), SBP (mmHg), BMI, Smoking (%) Diabetes (%)

ASSOCIATIONS BETWEEN HDL AND OTHER RISK FACTORS

There was a significant difference between the mean HDL-C in men (1.25 mmol/l) and women (1.49mmol/l), p<0.0001.

Mean HDL cholesterol levels were significantly higher in smokers than non-smokers and diabetics than non-diabetics in both men and women, as shown in Table 2-11. In men from high risk countries the mean HDL cholesterol was significantly, but marginally lower than that of men from low risk countries. In women from high risk countries, the mean cholesterol level was significantly higher than that of women from low risk countries, as shown in Table 2-11.

	Freq	Mean HDL (95%CI)	p	Freq	Mean HDL (95%CI)	p
	Women			Men		
Non-smokers	35259	1.50 (1.50 to 1.50)		29768	1.28 (1.27 to 1.28)	
Smokers	11090	1.45 (1.44 to 1.46)	<0.001	26676	1.22 (1.22 to 1.23)	<0.001
No diabetes	43131	1.49 (1.49 to 1.50)		52234	1.25 (1.25 to 1.25)	
Diabetes	1049	1.31 (1.29 to 1.34)	<0.001	1529	1.19 (1.17 to 1.21)	<0.001
High risk countries	12325	1.55 (1.55 to 1.56)		18755	1.24 (1.23 to 1.24)	
Low risk countries	35334	1.46 (1.46 to 1.47)	<0.0001	38547	1.26 (1.26 to 1.26)	<0.0001

Table 2-11: Mean and 95%CI HDL cholesterol by group (based on smoking status, diabetic status and region) and results of t tests comparing each.

Table 2-12 shows the mean level of total cholesterol, SBP, triglycerides (where this variable was available), and BMI. Table 2-12 also shows the proportion of smokers and diabetics in each HDL cholesterol quintile. In both men and women triglyceride level and BMI increased with each decrease in HDL quintile. The

proportion of smokers and diabetics increases with each decrease in HDL quintile. Mean TC level increased progressively with each increase in HDL quintile in both men and women. These trends were highly statistically significant.

In women, increasing HDL quintile was also associated with reductions in mean SBP, significant trend. This was not seen in men.

HDL quintile (n)		TC	SBP	Trigs	BMI	Smokers	Diabetes
Men							
1	12,302	5.8	136	2.8	27.0	55%	4.1%
2	11,562	5.8	136	2.1	26.4	49%	2.8%
3	10,853	5.9	136	1.9	25.9	47%	2.4%
4	11,513	5.9	136	1.7	25.7	43%	2.3%
5	11,072	6.0	137	1.4	25.1	42%	2.5%
p for trend		<0.001	NS	<0.001	<0.001	<0.001	<0.001
Women							
1	9,678	5.5	134	2.3	27.5	27%	4.8%
2	9,660	5.6	131	1.8	26.4	26%	2.4%
3	9,375	5.7	130	1.6	25.8	24%	1.9%
4	9,751	5.8	130	1.4	25.1	22%	1.5%
5	9,195	6.1	131	1.2	24.5	20%	1.3%
p for trend		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table 2-12: Mean TC (mmol/l), SBP (mmHg), triglycerises (mmol/l), age (years) & BMI levels proportion of smokers and diabetics in each HDL quintile.

These trends were also reflected in the pair-wise correlation coefficients, as shown in Table 2-13. All correlations between the HDL and the continuous risk factors were significant. Significant and meaningful inverse correlations were demonstrated between triglycerides and BMI in both men and women. There was a direct correlation between TC and HDL in both men and women.

	Correlation coefficient	P	Correlation coefficient	P
	Men		Women	
TC	0.0696	<0.001	0.1725	<0.001
SBP	0.0105	0.0126	-0.0437	<0.001
Trigs	-0.5295	<0.001	-0.5667	<0.001
BMI	-0.1947	<0.001	-0.2193	<0.001
Age	0.039	<0.001	0.0133	0.0037

Table 2-13: Correlation coefficients between HDL and other continuous risk factors

Figure 2.4 and **Figure 2.5** illustrate the linear inverse relationships between HDL cholesterol and BMI in each gender and each country.

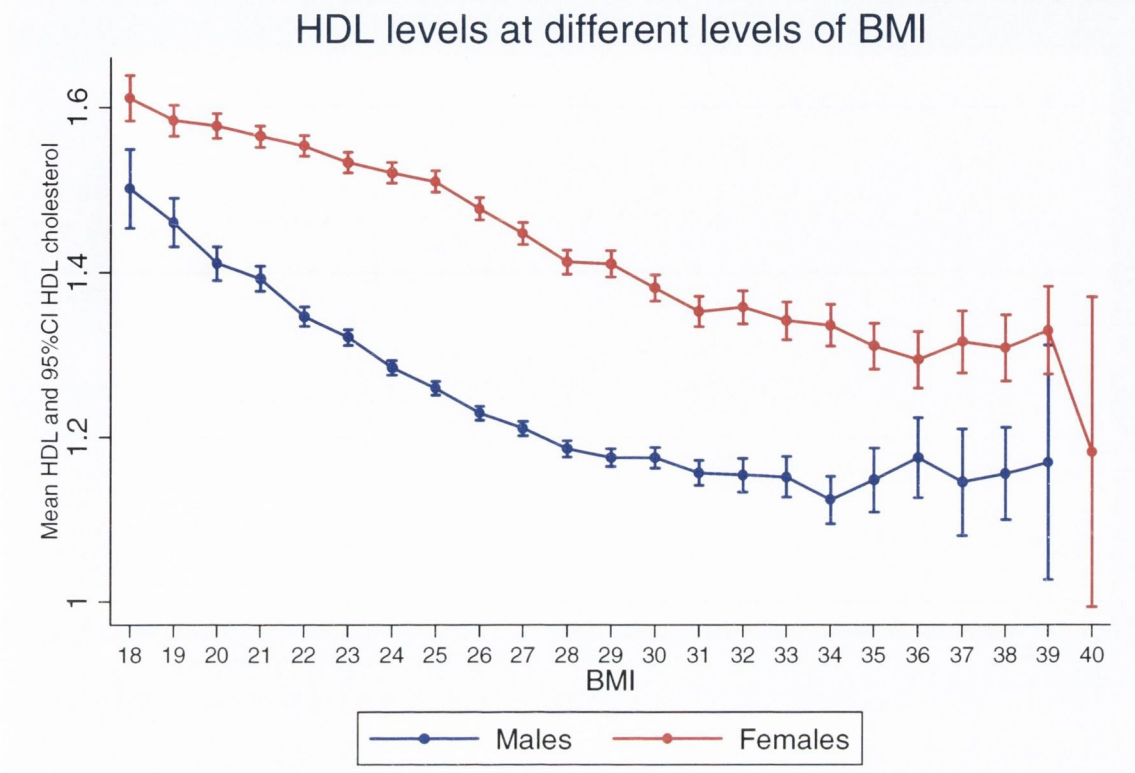


Figure 2.4: Mean (and 95%CI) HDL cholesterol at each level of BMI in men and women

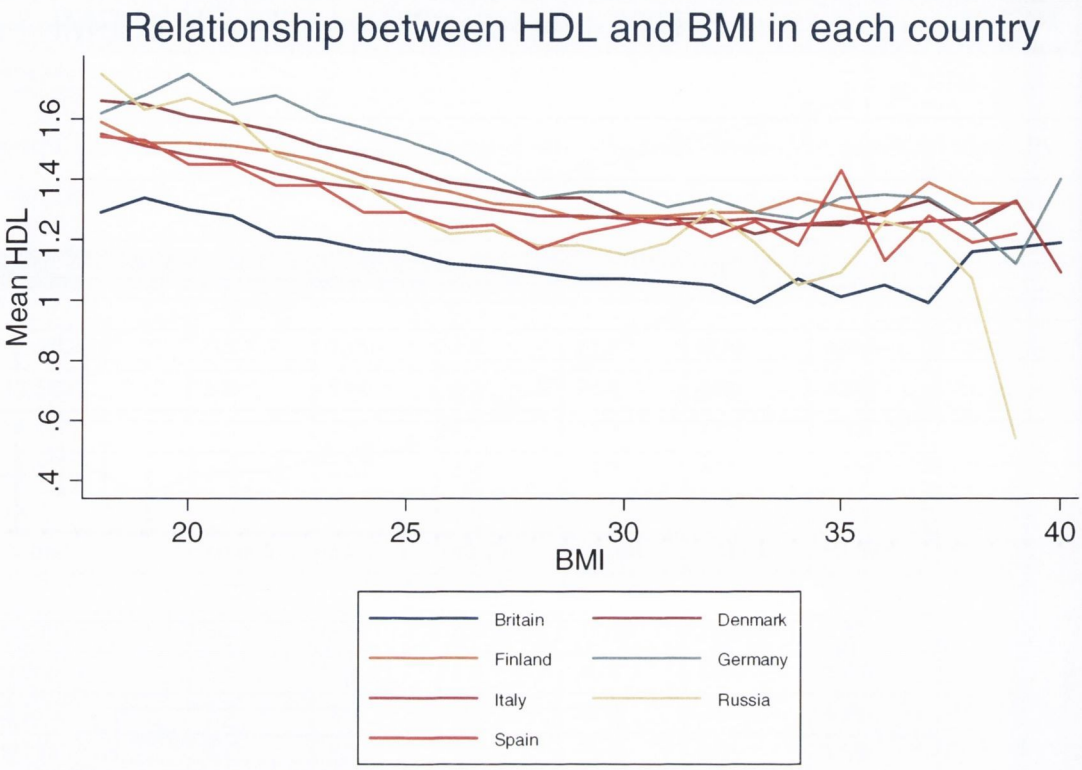


Figure 2.5: Mean HDL cholesterol at each level of BMI in each country in the SCORE dataset

The relationships between age and HDL cholesterol in men and women in each separate country are shown in **Figure 2.6**. Because the numbers from Spain and Germany are small these have not been included in the figure. In women, HDL cholesterol levels increase to approximately age 50 and decrease thereafter. In men, no consistent relationship with age can be identified.

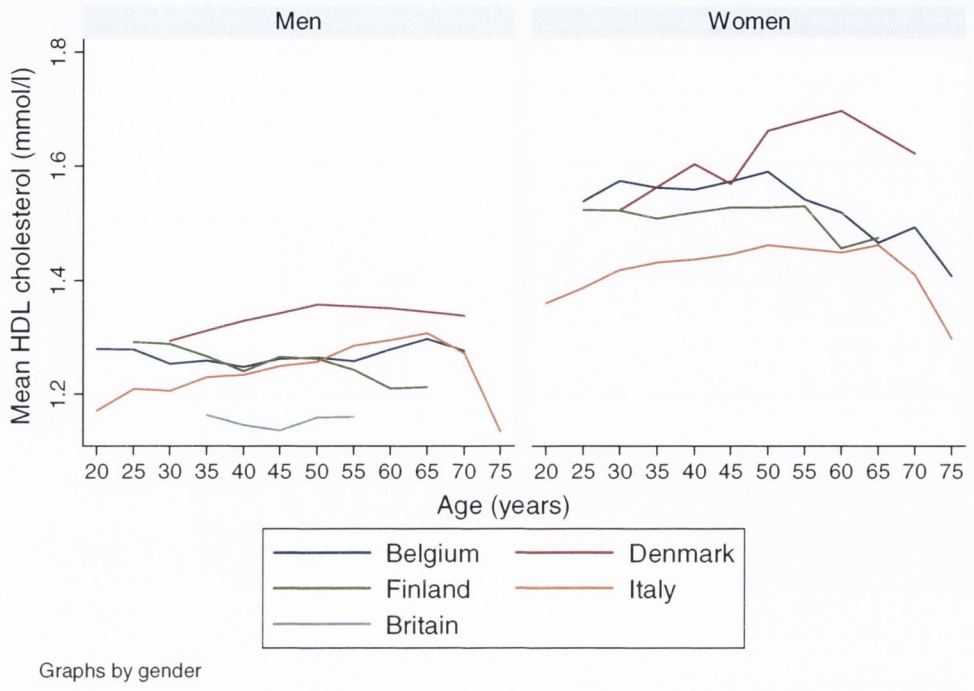


Figure 2.6: Mean HDL cholesterol at each age in men and women

EFFECT OF HDL ON CVD ENDPOINTS

The overall median observation time was 8.5 years. There were 2,198 CVD events during the observation time, 70% of which were due to fatal CHD events.

A significant inverse relationship between HDL-C quintile and CVD mortality rate was demonstrated in both men and women. Table 2-14 shows the CVD mortality rates in each HDL-C quintile.

HDL-C Quintile	HDL-C Range	N (events)	Rate (95% CI)	HDL-C Range	N (events)	Rate (95% CI)
	Women			Men		
1st	0.26 - 1.16	9678 (148)	2.00 (1.70 , 2.34)	0.05 - 0.98	12302 (528)	4.80 (4.41 , 5.23)
2nd	1.17 - 1.37	9660 (118)	1.49 (1.24 , 1.79)	0.99 - 1.14	11562 (347)	3.32 (2.99 , 3.69)
3rd	1.38 - 1.55	9375 (75)	0.98 (0.78 , 1.23)	1.15 - 1.29	10853 (319)	3.18 (2.85 , 3.55)
4th	1.56 - 1.78	9751 (73)	0.92 (0.73 , 1.16)	1.30 - 1.50	11513 (284)	2.85 (2.54 , 2.92)
5th	1.79 - 4.28	9195 (69)	0.92 (0.72 , 1.16)	1.50 - 4.86	11072 (237)	2.57 (2.27 , 2.92)

Table 2-14: CVD mortality rates (per 1000 person years) in each gender-specific HDL-C quintile

The rates in Table 2-14 are unadjusted for age. Therefore, it should be noted that the median age was very similar in each HDL-C quintile. In men, the median age was 48 years in each quintile, except the last quintile

(49 years) and in women the median age was 46 in each quintile, except the last and first quintiles (48 and 47 years respectively). Figure 2.7 and Figure 2.8 show the progressive decrease in CVD mortality rates in each HDL-C tertile in all age groups (including elderly individuals aged over 65) in women and men.

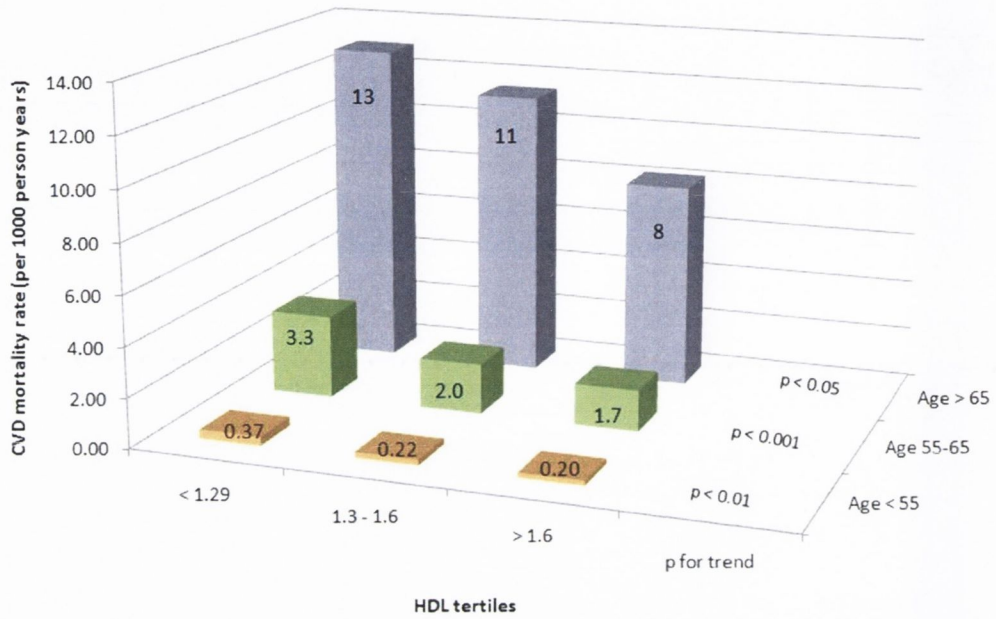


Figure 2.7: CVD mortality rates in women in each HDL tertile and age group

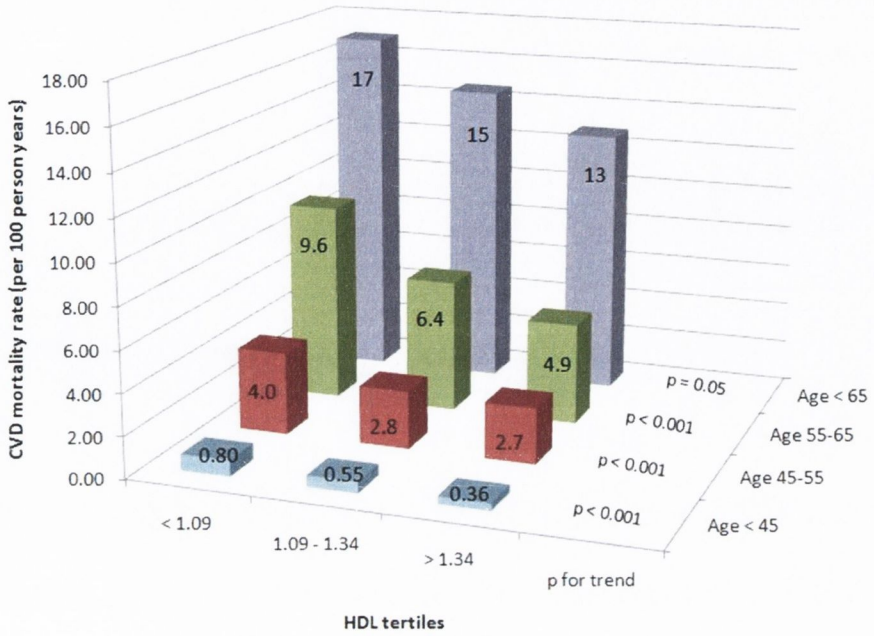


Figure 2.8: CVD mortality rates in men in each HDL tertile and age group

The inverse relationship between HDL-C and CVD mortality rate was also seen within each TC quintile in both women and men, as shown in Figure 2.9 and Figure 2.10, respectively. The rate ratio per 1 quintile increase in HDL-C, controlling for TC quintile was 0.75 (0.71 to 0.80) in women and 0.86 (0.83 to 0.89) in men. The inverse relationship between CVD mortality rate and HDL-C quintile is also seen within each SCORE category, in both women and men, as illustrated in Figure 2.11 and Figure 2.12, respectively. The rate ratio per 1 quintile increase in HDL-C, controlling for SCORE category, for each increase in HDL-C quintile was 0.82 (0.77 to 0.87) in women and 0.87 (0.50 to 0.90) in men.

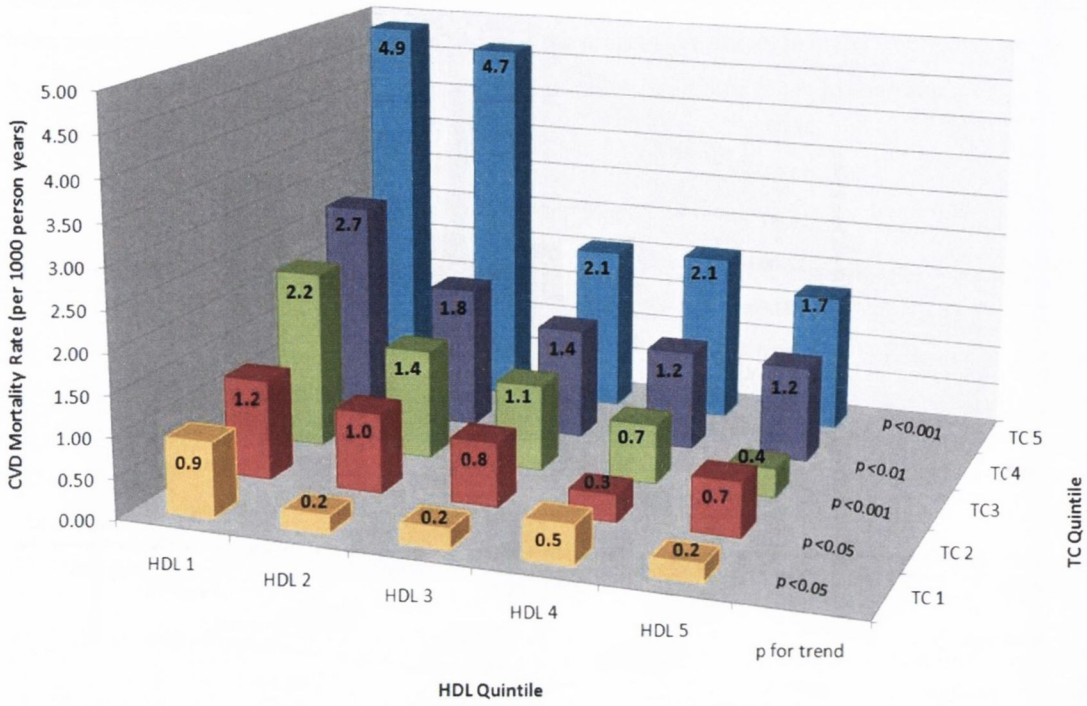


Figure 2.9: CVD mortality rates in women in each HDL quintile within each total cholesterol quintile

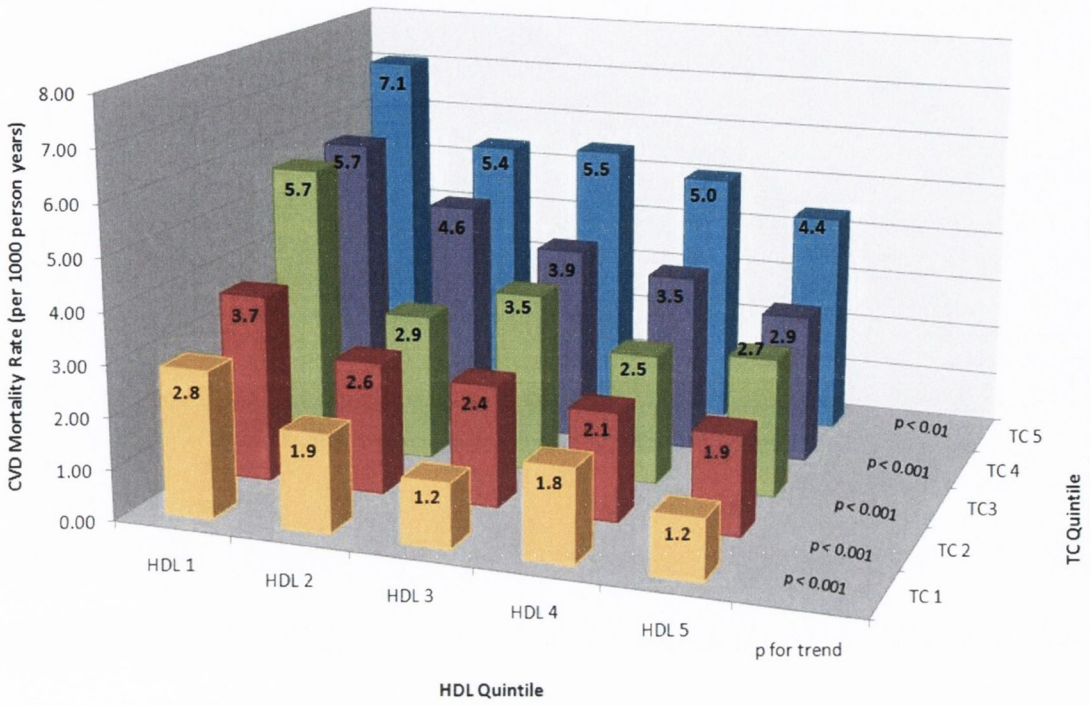


Figure 2.10: CVD mortality rates in men in each HDL quintile within each total cholesterol quintile

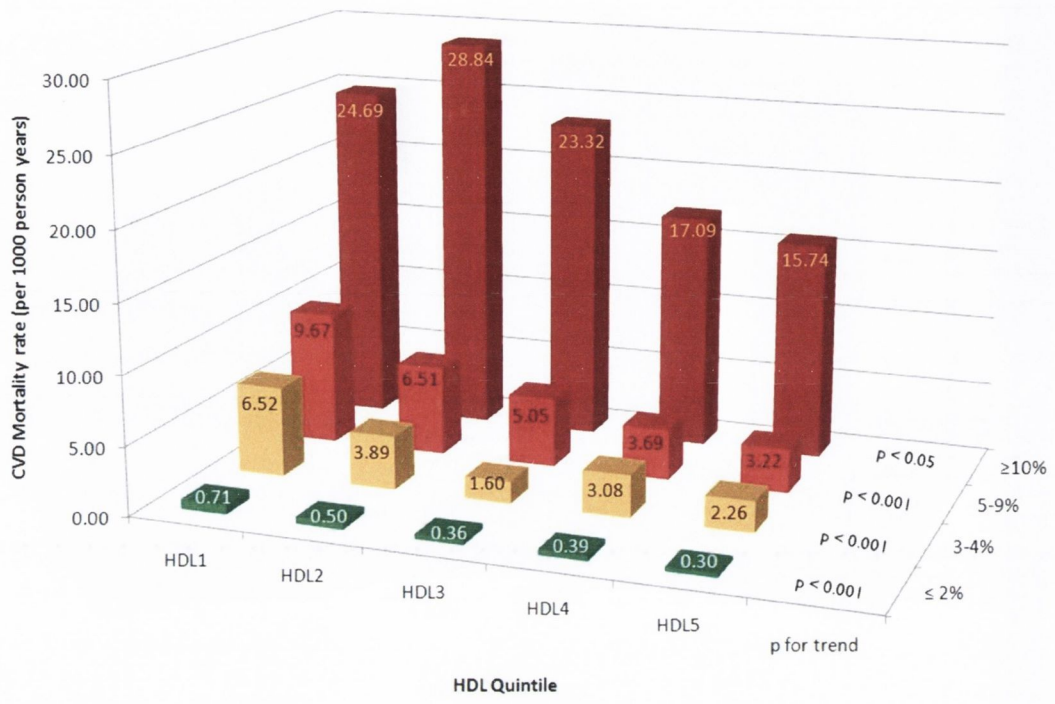


Figure 2.11: CVD mortality rates in women in each HDL quintile within each SCORE category

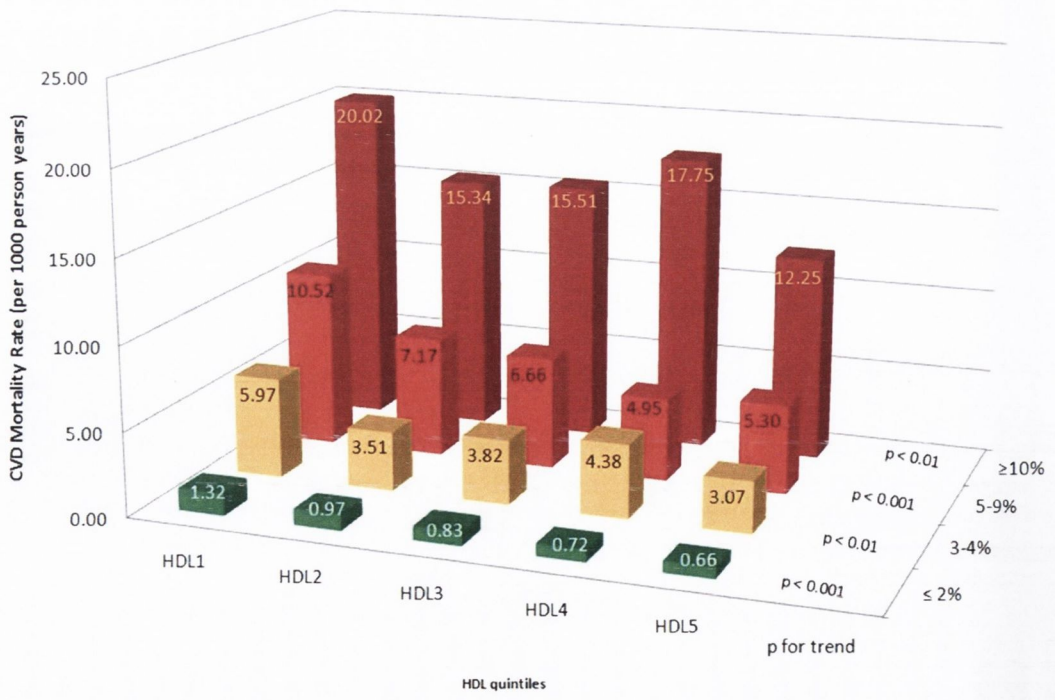


Figure 2.12: CVD mortality rates in men in each HDL quintile within each SCORE category

Table 2-15 shows the relationship of HDL-C as a continuous variable with CVD mortality and CHD mortality, both adjusted for age alone and additionally for other risk factors. HDL-C was a significant protective factor for both CVD and CHD mortality, with multivariable adjusted hazard ratios per 0.5 mmol/l increase in HDL-C of 0.76 (0.70 to 0.83) and 0.60 (0.51 to 0.69) in men and women respectively for the CVD mortality endpoint and 0.71 (0.64 to 0.78) and 0.55 (0.45 to 0.68) respectively for the CHD mortality endpoint.

Women				
Age group	CHD Mortality endpoint		CVD Mortality endpoint	
	Age-adjusted HR (95% CI)	Multivariable adjusted HR* (95%CI)	Age-adjusted HR (95% CI)	Multivariable adjusted HR* (95%CI)
<55	0.80 (0.54 to 1.2)	0.78 (0.49 to 1.22)	0.63 (0.45 to 0.88)	0.62 (0.42 to 0.91)
55-65	0.52 (0.39 to 0.69)	0.58 (0.39 to 0.69)	0.6 (0.49 to 0.74)	0.69 (0.55 to 0.87)
>65	0.56 (0.42 to 0.75)	0.49 (0.35 to 0.69)	0.62 (0.51 to 0.77)	0.53 (0.42 to 0.68)
All	0.58 (0.49 to 0.70)	0.55 (0.45 to 0.68)	0.61 (0.54 to 0.70)	0.60 (0.51 to 0.69)
Per std dev*	0.67 (0.58 to 0.76)	0.64 (0.55 to 0.75)	0.69 (0.63 to 0.77)	0.68 (0.61 to 0.76)
Men				
Age group	CHD Mortality endpoint		CVD Mortality endpoint	
	Age-adjusted HR (95% CI)	Multivariable adjusted HR* (95%CI)	Age-adjusted HR (95% CI)	Multivariable adjusted HR* (95%CI)
<45	0.58 (0.40 to 0.85)	0.66 (0.44 to 0.99)	0.60 (0.43 to 0.83)	0.67 (0.47 to 0.96)
45-55	0.76 (0.65 to 0.9)	0.81 (0.68 to 0.95)	0.81 (0.70 to 0.93)	0.85 (0.73 to 0.99)
55-65	0.60 (0.52 to 0.70)	0.63 (0.54 to 0.73)	0.66 (0.58 to 0.75)	0.7 (0.62 to 0.80)
>65	0.69 (0.53 to 0.88)	0.73 (0.55 to 0.96)	0.77 (0.64 to 0.94)	0.79 (0.64 to 0.98)
All	0.66 (0.60 to 0.73)	0.71 (0.64 to 0.78)	0.72 (0.66 to 0.78)	0.76 (0.70 to 0.83)
Per std dev†	0.76 (0.71 to 0.81)	0.79 (0.73 to 0.84)	0.80 (0.75 to 0.84)	0.83 (0.78 to 0.88)

Table 2-15: Hazard ratios (HR) for HDL-C as a continuous variable (per 0.5mmol/l increase) by age group in men and women*adjusted for age, total cholesterol, SBP, diabetes, smoking status and BMI †Standard deviation HDL-C: 0.37 (women), 0.34 (men)

The inverse relationship was robust and hazard ratios for CVD mortality remained significant in each age group in both men and women. This independent relationship was demonstrated in both elderly (>65 years) men and women. In women, the relationship was stronger, with a significant difference between the hazard ratios for HDL-C as a continuous variable for the CVD mortality endpoint. The interaction term between gender and HDL-C level was significant, p=0.012. While the effect of HDL-C on CVD mortality risk appears stronger in older women, the age by HDL-C interaction term was not significant in either gender. Additionally, tests for heterogeneity across each age group were also non-significant.

For the CHD endpoint, the independent relationship was demonstrated in all age groups, except the youngest age group in women; there were small event numbers in this group.

Table 2-16 shows the hazard ratios associated with each HDL-C quintile compared to the lowest quintile of HDL-C, adjusted for age alone and adjusted for all other CV risk factors. Multivariable adjusted hazard ratios showed a significantly increased risk of CVD mortality in each HDL-C quintile compared to the lowest quintile of HDL-C in both men and women. For example, women in the fifth quintile of HDL-C enjoyed a 64% reduced risk compared to women in the first quintile of HDL-C. For men, the same situation was associated with a 44% risk reduction.

HDL-C Quintile	Age adjusted hazard ratios (95% CI)	Multivariable adjusted hazard ratios*(95%CI)	Age adjusted hazard ratios (95% CI)	Multivariable adjusted hazard ratios* (95%CI)
	Women		Men	
1st	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
2nd	0.71 (0.55 , 0.90)	0.76 (0.58 , 0.999)	0.70 (0.61 , 0.80)	0.72 (0.62 , 0.83)
3rd	0.51 (0.39 , 0.68)	0.51 (0.37 , 0.7)	0.64 (0.55 , 0.73)	0.70 (0.61 , 0.81)
4th	0.46 (0.34 , 0.61)	0.45 (0.32 , 0.61)	0.60 (0.52 , 0.69)	0.67 (0.57 , 0.78)
5th	0.40 (0.30 , 0.54)	0.36 (0.26 , 0.52)	0.52 (0.44 , 0.61)	0.56 (0.47 , 0.66)

Table 2-16: Age adjusted and multivariable adjusted hazard ratios associated with each quintile of HDL-C compared to the lowest quintile *adjusted as in previous tables

Figure 2.13 and Figure 2.14 show the forest plots of the hazard ratios for CVD mortality per 0.5mmol/l increase in HDL-C in each separate country in women and men, respectively. The pooled estimate per 0.5mmol/l increase in HDL-C was 0.64 (0.56 to 0.74) in women and 0.76 (0.70 to 0.82) in men. There was no significant heterogeneity, p=0.639 in women and p=0.267 in men.

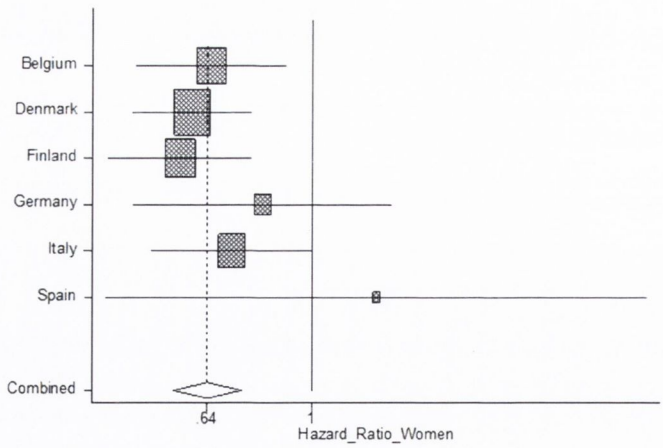


Figure 2.13: Multivariable hazard ratios (per 0.5 mmol/l increase) for CVD mortality in women in each individual country

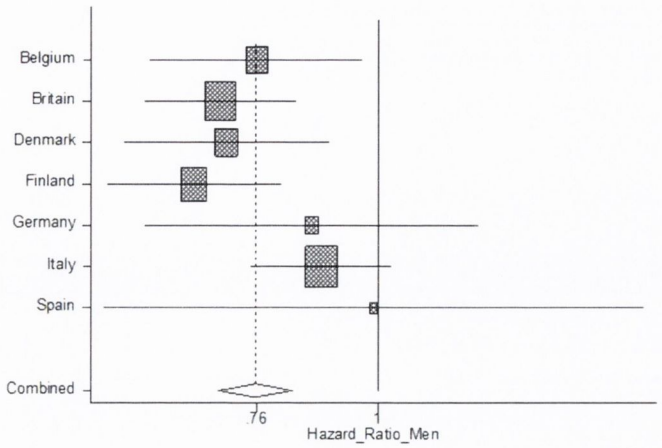


Figure 2.14: Multivariable hazard ratios (per 0.5 mmol/l increase) for CVD mortality in men in each individual country

Table 2-17 shows the age adjusted and multivariable adjusted hazard ratios for CVD mortality for HDL-C as a continuous variable in each SCORE category. The relationship was statistically significant in each group. Tests for heterogeneity in the multivariable effect of across subgroups based on SCORE category, age group and TC quintile were all non-significant.

Women		
SCORE category	Age adjusted hazard ratios (95% CI)	Multivariable adjusted hazard ratios*
≤ 2%	0.61 (0.46 to 0.80)	0.65 (0.48 to 0.88)
3-4%	0.61 (0.45 to 0.82)	0.58 (0.42 to 0.80)
5-9%	0.57 (0.42 to 0.76)	0.69 (0.51 to 0.95)
≥ 10%	0.60 (0.46 to 0.77)	0.50 (0.38 to 0.68)
Men		
SCORE Category	Age adjusted hazard ratios (95% CI)	Multivariable adjusted hazard ratios*
≤ 2%	0.58 (0.46 to 0.73)	0.59 (0.46 to 0.76)
3-4%	0.73 (0.59 to 0.89)	0.80 (0.65 to 0.99)
5-9%	0.71 (0.62 to 0.83)	0.77 (0.66 to 0.90)
≥ 10%	0.77 (0.68 to 0.87)	0.80 (0.70 to 0.92)

Table 2-17: Age adjusted and multivariable adjusted hazard ratios (per 0.5mmol/l increase in HDL-C) for CVD mortality by SCORE categories *adjustment as in previous tables

Data on triglycerides were available for 61,621 individuals. Inclusion of triglycerides in the model resulted in slight changes in the hazard ratio for HDL-C for CVD mortality in both men and women. Triglyceride level was not a significant risk factor but HDL-C remained a highly significant and important risk factor in both men and women. Data on family history were available for 33,287 individuals. When family history was included in the model it was a significant predictor of CVD mortality in men only. However, it resulted in no

difference in the hazard ratio for HDL-C in either men or women. Data on the use of anti-hypertensive medication at baseline were available for 26,259 individuals. In this subgroup, exclusion of those on anti-hypertensive mediation resulted in a higher hazard ratio for CVD mortality in women, but it remained virtually identical in men. HDL-C remained a strong protective factor in men and women who currently smoked and those who did not (all above results tabulated for men and women in Table 2-18).

	Women	Men
Group with data on triglycerides available: (n=61,621)		
Triglycerides not included in model	0.58 (0.47 to 0.72)	0.79 (0.71 to 0.89)
Triglycerides included in model	0.63 (0.49 to 0.82)	0.75 (0.66 to 0.85)
Group with data on family history available: (n=33,287)		
Family history not included in model	0.61 (0.49 to 0.75)	0.72 (0.64 to 0.81)
Family history included in model	0.61 (0.49 to 0.75)	0.72 (0.64 to 0.81)
Group with data on BP lowering medication usage: (n=26,259)		
Without exclusion of those on medication	0.65 (0.50 to 0.85)	0.73 (0.64 to 0.84)
Exclusion of those on medication	0.58 (0.40 to 0.83)	0.72 (0.63 to 0.81)
Without exclusion of those on medication but including BP treatment as a covariable in the model	0.68 (0.52 to 0.89)	0.74 (0.65 to 0.84)
Analyses by smoking subgroup (full group included)		
Current Smokers	0.48 (0.36 to 0.66)	0.77 (0.69 to 0.86)
Non-current smokers	0.64 (0.53 to 0.76)	0.75 (0.66 to 0.86)

Table 2-18: Hazard ratios for HDL-C as a continuous variable (per 0.5 mmol/l increase) with and without other CV risk factors included in the model. All hazard ratios are adjusted for age, TC, SBP, BMI, smoking status, diabetes and stratified by country

EFFECT OF HDL ON ISCHAEMIC STROKE MORTALITY

Table 2-19 shows the numbers of men and women from high and low risk European countries included in the analyses with complete data for all of the covariables and the numbers of fatal ischemic strokes which occurred during the follow-up time.

	Number included	Number fatal ischemic stroke events
Women high risk countries	12053	38
Men high risk countries	18141	105
Women low risk countries	31491	43
Men low risk countries	34859	80
Total	96544	266

Table 2-19: Numbers of participants and events in men and women from high and low risk countries

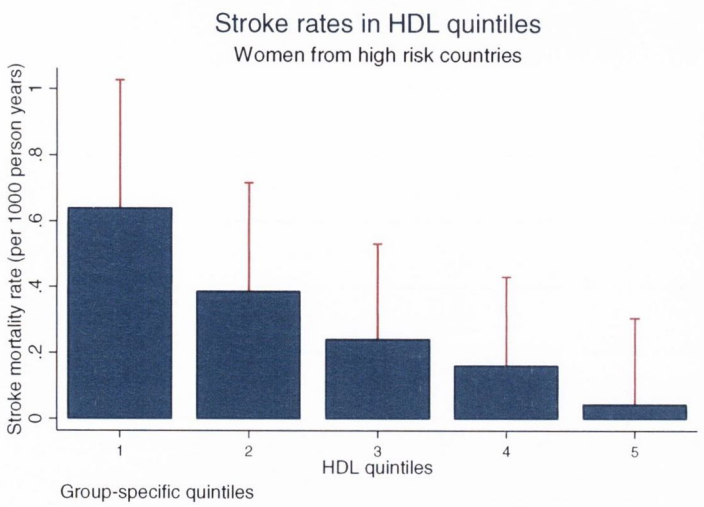
Table 2-20 shows the definitions of the quintiles in men and women from high and low risk European countries. The stroke mortality rates (per 1000 person years) in each quintile are also shown. These are illustrated in **Figure 2.15**. A strong, graded, inverse relationship between HDL levels and stroke mortality rate was seen in women from high risk countries; unadjusted rate ratio comparing 1st to 5th quintile of HDL cholesterol was 14.8 (95% CI: 2.0 to 111.4). In men from high risk countries, HDL cholesterol showed a U shaped relationship with stroke mortality rate; the highest stroke mortality was in the lowest quintile of HDL. The lowest stroke mortality rate was in the middle quintile of HDL. There was a significantly increased risk associated with the first quintile of HDL cholesterol compared to the third; rate ratio 1.98 (95%CI: 1.05 to 3.73). Both men and women from low risk countries showed no relationship between stroke rate and HDL cholesterol.

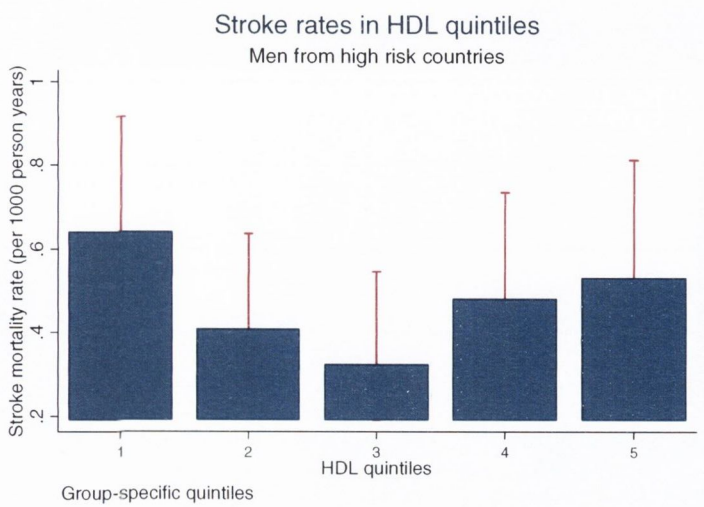
Table 2-20 shows the rates in those with low and normal HDL cholesterol levels; these are illustrated in Figure 2.16. Low HDL cholesterol levels in women from high risk countries were associated with significantly higher ischemic stroke mortality rates than normal HDL levels; unadjusted rate ratio: 3.86 (95%CI: 2.03 to 7.35)). In men from high risk countries, low Hhigher ischemic stroke mortality rates, which reached borderline statistical significance p=0.055; unadjusted rate ratio: 1.49(95%CI: 0.99 to 2.24). Both men and women from low risk European countries showed no difference in rates of ischemic stroke mortality between those with low and normal HDL cholesterol levels.

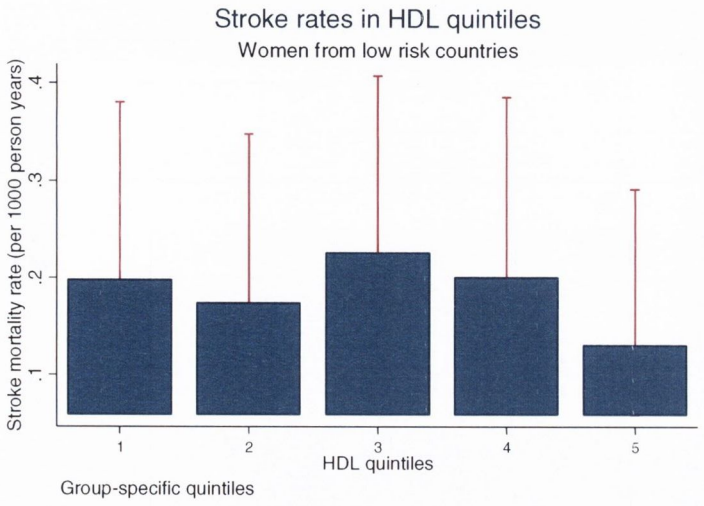
High Risk Countries								
	Women				Men			
HDL Quintile	HDL	Rate (95% CI)	Hazard ratio Model 1	Hazard Ratio Model 2	HDL	Rate (95% CI)	Hazard ratio Model 1	Hazard ratio Model 2
1	≤ 1.25	0.64 (0.40 to 1.00)	Ref	Ref	≤0.98	0.64(0.45 to 0.92)	Ref	Ref
2	1.26 –1.44	0.38 (0.21 to 0.72)	0.76	0.80	0.99 –1.12	0.41(0.26 to 0.64)	0.64	0.70
3	1.45 –1.61	0.24 (0.11 to 0.53)	0.38*	0.35*	1.13 –1.26	0.32(0.19 to 0.55)	0.53*	0.63
4	1.62 –1.84	0.16 (0.06 to 0.43)	0.26*	0.29*	1.27 –1.46	0.48(0.31 to 0.74)	0.76	0.87
5	≥1.85	0.04 (0.01 to 0.31)	0.06**	0.07**	≥ 1.47	0.53(0.35 to 0.81)	0.84	0.94
Low Risk Countries								
	Women				Men			
HDL Quintile	HDL	Rate (95% CI)	Hazard ratio Model 1	Hazard Ratio Model 2	HDL	Rate (95% CI)	Hazard ratio Model 1	Hazard ratio Model 2
1	≤ 1.13	0.20 (0.10 to 0.38)	Ref	Ref	≤0.98	0.3 (0.20 to 0.51)	Ref	Ref
2	1.14 –1.33	0.17 (0.09 to 0.35)	0.87	0.65	0.99 –1.13	0.30 (0.18 to 0.49)	0.88	0.78
3	1.34 –1.52	0.23 (0.12 to 0.41)	1.45	1.06	1.14 –1.29	0.23 (0.13 to 0.40)	0.65	0.69
4	1.53 –1.75	0.20 (0.10 to 0.39)	1.32	1.07	1.3 –1.49	0.39 (0.24 to 0.61)	1.13	1.20
5	≥1.76	0.13 (0.06 to 0.29)	0.83	0.76	≥ 1.5	0.34 (0.21 to 0.55)	0.93	1.13

Table 2-20: Stroke mortality rates and adjusted hazard ratios for the risk of stroke mortality in men and women from high and low risk countries associated with HDL cholesterol quintiles.

* p<0.05, **p<0.01 Model 1: adjusted for age only. Model 2: adjusted for age, total cholesterol, systolic blood pressure, diabetes, smoking status and body mass index


Stroke rates in HDL quintiles
Women from high risk countries
Stroke mortality rate (per 1000 person years)
0
.2
.4
.6
.8
1
1
2
3
4
5
HDL quintiles
Group-specific quintiles


Stroke rates in HDL quintiles
Men from high risk countries
Stroke mortality rate (per 1000 person years)
.2
.4
.6
.8
1
1
2
3
4
5
HDL quintiles
Group-specific quintiles


Stroke rates in HDL quintiles
Women from low risk countries
Stroke mortality rate (per 1000 person years)
.1
.2
.3
.4
1
2
3
4
5
HDL quintiles
Group-specific quintiles

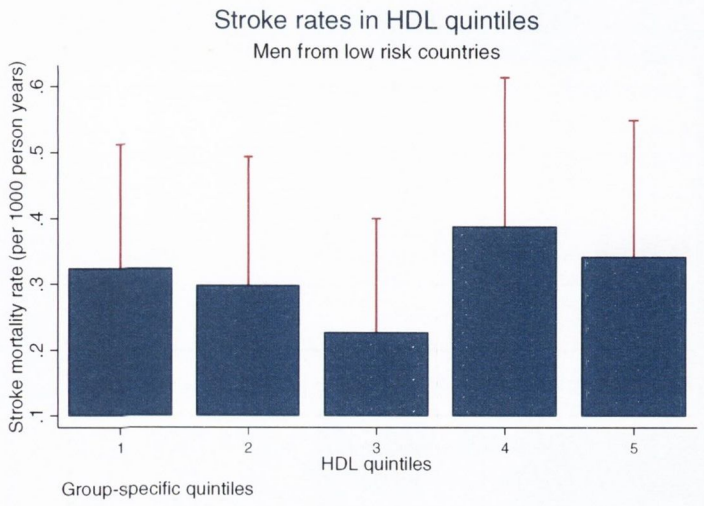

Stroke rates in HDL quintiles
Men from low risk countries
Stroke mortality rate (per 1000 person years)
.1
.2
.3
.4
.5
.6
1
2
3
4
5
HDL quintiles
Group-specific quintiles

Figure 2.15: Stroke mortality rates in each group-specific quintile of HDL cholesterol in men and women from high and low risk European regions

High Risk Countries						
	Women			**Men**		
HDLCategory	Rate (95% CI)	Hazard Ratio Model 1	Hazard Ratio Model 2	Rate (95% CI)	Hazard ratio Model 1	Hazard ratio Model 2
Low	0.67 (0.44 to 1.01)	3.83***	3.74***	0.63 (0.45 to 0.89)	1.46	1.30
Normal	0.17 (0.11 to 0.28)	1- Ref	1 - Ref	0.43 (0.34 to 0.54)	1 - Ref	1 – Ref
Low Risk Countries						
	Women			**Men**		
HDLCategory	Rate (95% CI)	Hazard ratio Model 1	Hazard Ratio Model 2	Rate (95% CI)	Hazard ratio Model 1	Hazard ratio Model 2
Low	0.19 (0.12 to 0.31)	0.91	0.94	0.32 (0.20 to 0.51)	1.12	1.07
Normal	0.18 (0.13 to 0.27)	1 –Ref	1 -Ref	0.31 (0.24 to 0.40)	1 - Ref	1 – Ref

Table 2-21: Stroke mortality rates in those with normal and low (<1.3 in women <1.0mmol/l in men) HDL levels. Adjusted hazard ratios for the risk associated with low compared to normal HDL are also shown. Model 1 & 2 as in table above

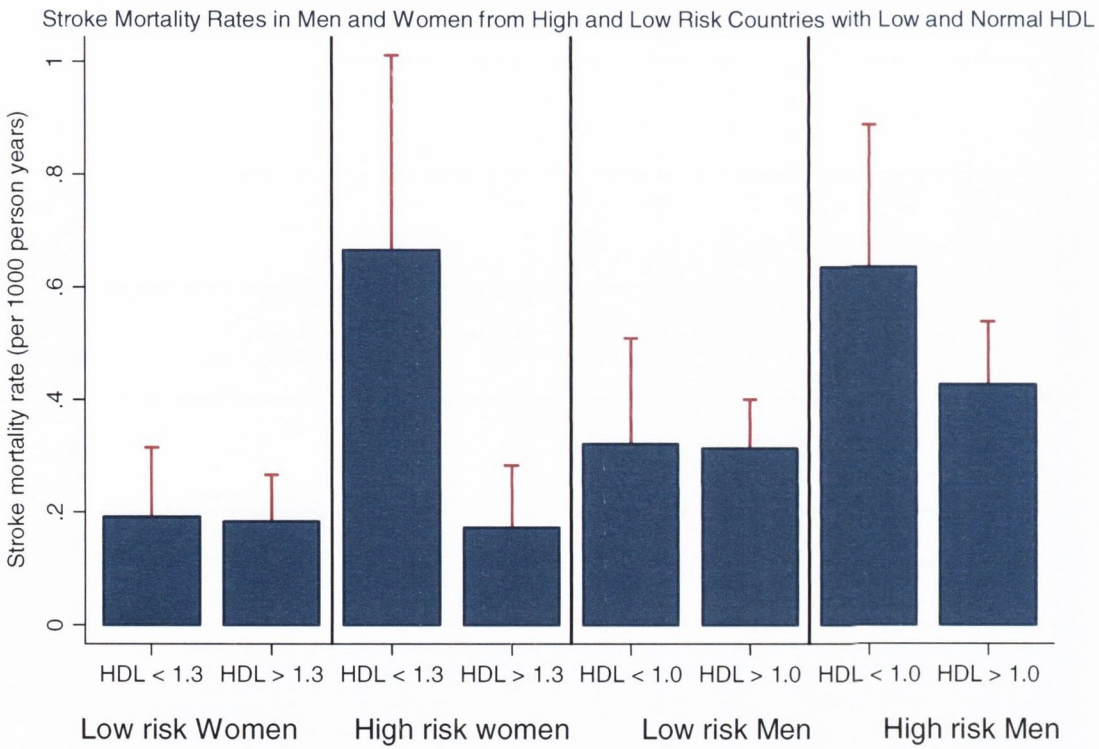


Figure 2.16: Stroke mortality rates in low and normal HDL categories in men and women from high and low risk European regions

The results of multivariable analyses on the effect of HDL cholesterol on stroke mortality mirrored those of the univariable analyses. Table 2-20 shows the age adjusted and multivariable adjusted hazard ratios for each quintile of HDL cholesterol compared to the highest HDL cholesterol quintile in women and men from high and low risk countries. A strong, graded, inverse and independent relationship is seen in women from high risk countries, with sequential increases in HDL cholesterol quintile associated with progressively a larger protective effect, as illustrated in Figure 2.17. In men from high risk countries, again, a U shaped relationship was noted, although this was non-significant, see Figure 2.17. As expected from univariable analyses, there was no clear relationship between HDL cholesterol quintile and stroke mortality in either men or women from low risk countries.

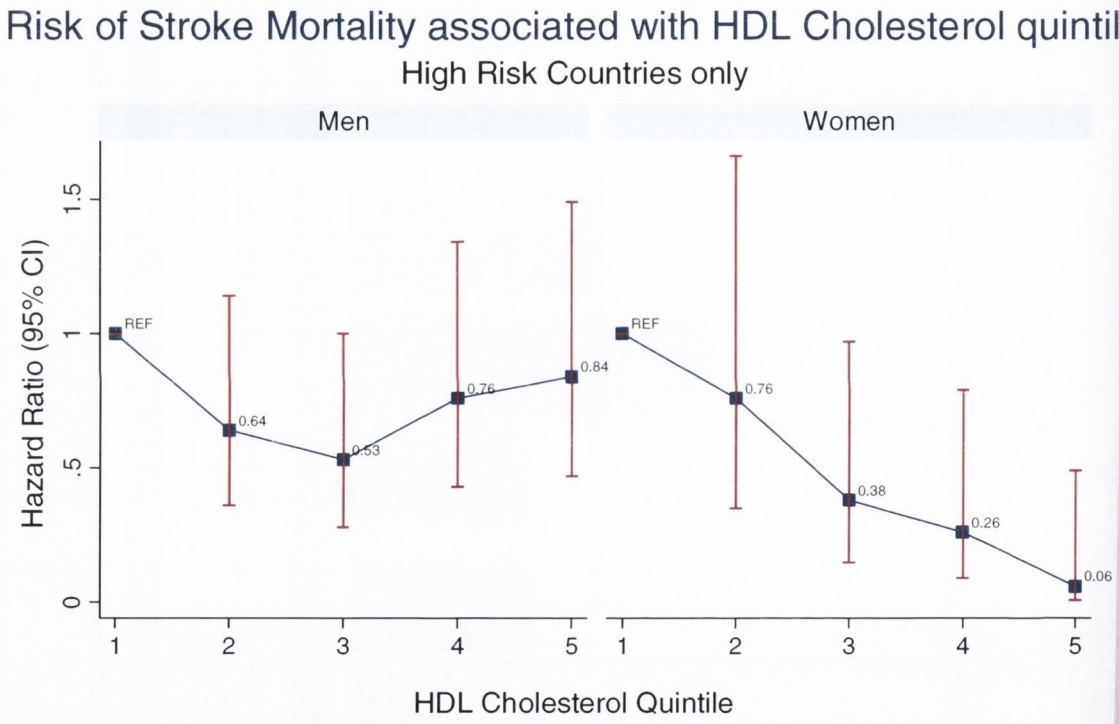


Figure 2.17: Multivariable adjusted hazard ratios for stroke mortality associated with each cholesterol quintile compared to the lowest quintile of HDL

DERIVING SCORE HDL

The CHD and nCHD survival curves, adjusted to baseline levels of the risk factors, for men and women for the HDL function are shown in **Figure 2.18** and **Figure 2.19**, respectively.

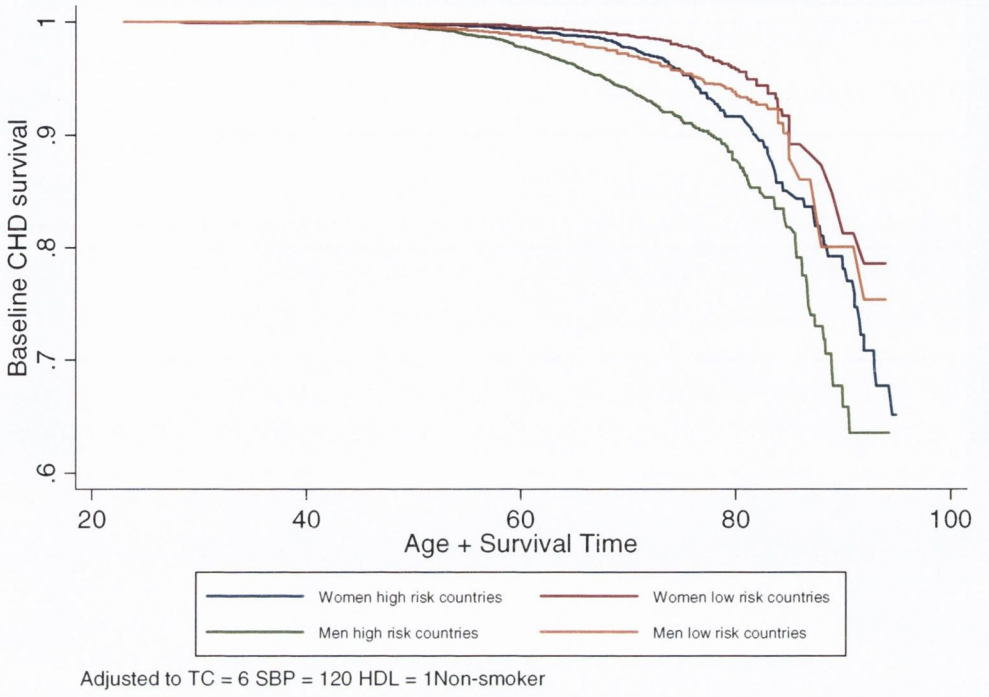


Figure 2.18: Baseline CHD survival for the HDL function, adjusted to baseline levels of risk factors

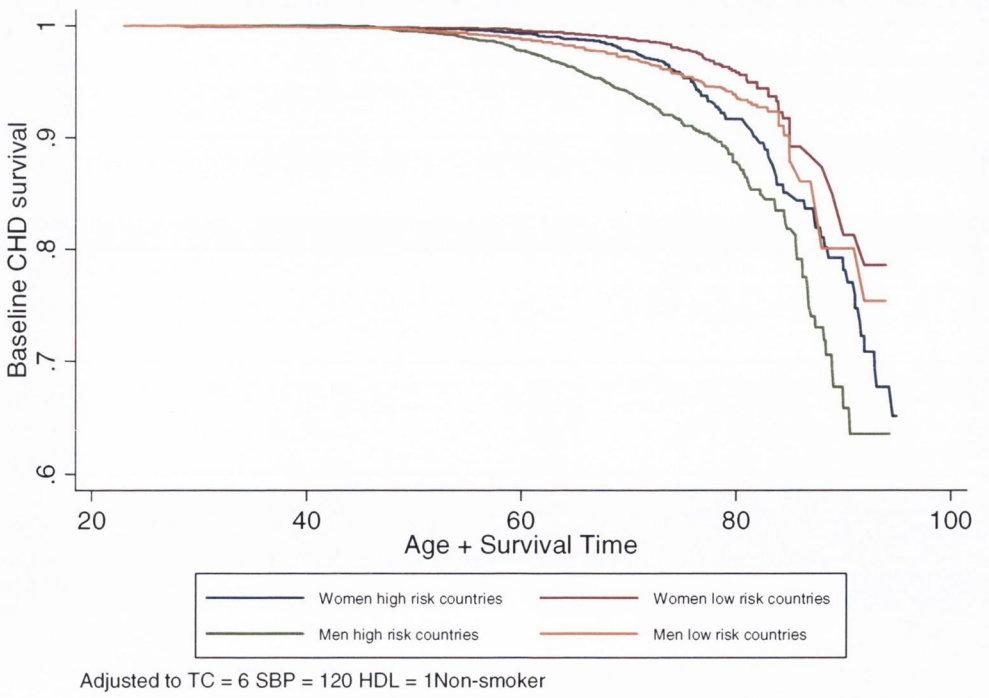


Figure 2.19: Baseline nCHD CVD survival for HDL function, adjusted to baseline level of risk factors

The beta coefficients and hazard ratios for each risk factor for both risk functions are shown in Table 2-22 and Table 2-23 respectively. Figure 2.20 and Figure 2.21 show the risk chart without HDL-C for high risk men and women; displaying the 10 year risk of CVD mortality in each risk factor combination. The risk associated with some examples of risk factor combinations is shown, firstly using the risk function without HDL-C and secondly, using the HDL-C function at 4 different HDL-C levels; 0.8, 1.0, 1.4, and 1.8 mmol/l. The examples have been selected to show how the inclusion of different levels of HDL-C can change the risk estimate in those at intermediate risk. Figure 2.22, Figure 2.23, Figure 2.24 and Figure 2.25 show, for illustration purposes only, separate risk charts, created using the HDL-C function, at four HDL-C levels; 0.8, 1.0, 1.4, and 1.8 mmol/l, for use in high risk countries.

Women				
	CHD mortality endpoint – 260 events		Non-CHD CVD mortality endpoint – 196 events	
Variable	Model without HDL-C	Model with HDL-C	Model without HDL-C	Model with HDL-C
HDL-C	-	0.63 (0.55 , 0.73)	-	0.75(0.64 , 0.88)
TC	1.19 (1.05 , 1.36)	1.26 (1.11 , 1.42)	0.84 (0.71 , 0.98)	0.87 (0.75 , 1.02)
SBP	1.32 (1.17 , 1.48)	1.28 (1.13 , 1.44)	1.47 (1.29 , 1.68)	1.45 (1.27 , 1.65)
Smoking	1.86 (1.38 , 2.50)	1.91 (1.41, 2.57)	2.23 (1.59 , 3.12)	2.28 (1.63 , 3.20)
Men				
	CHD mortality endpoint – 1,241 events		Non-CHD CVD mortality endpoint – 433 events	
Variable	Model without HDL-C	Model with HDL-C	Model without HDL-C	Model with HDL-C
HDL-C	-	0.78 (0.73 , 0.83)	-	0.94 (0.85 , 1.04)
TC	1.33 (1.26 , 1.40)	1.33 (1.26 , 1.41)	1.00 (0.90 , 1.11)	1.00 (0.91 , 1.11)
SBP	1.38 (1.32 , 1.45)	1.37 (1.31 , 1.44)	1.48 (1.36 , 1.60)	1.47 (1.36 , 1.60)
Smoking	1.84 (1.65, 2.07)	1.80 (1.60 , 2.02)	1.70 (1.40 , 2.05)	1.68 (1.39 , 2.05)

Table 2-22: Hazard ratios for SCORE HDL-C and SCORE C for men and women (per 1 standard deviation increase for continuous variables and for current smoker versus non-current smoker)

Women				
	CHD mortality endpoint		Non-CHD CVD mortality endpoint	
	Model without HDL-C	Model with HDL-C	Model without HDL-C	Model with HDL-C
HDL-C	-	-1.196 (-1.561 , -0.830)		-0.744 (-1.158 , -0.330)
TC	0.137 (0.040 , 0.234)	0.176 (0.082 , 0.271)	-0.138 (-0.258 , -0.017)	-0.103 (-0.224 , 0.018)
SBP	0.013 (0.007 , 0.018)	0.011 (0.006 , 0.017)	0.018 (0.012 , 0.024)	0.017 (0.011 , 0.023)
Smoking	0.619 (0.323 , 0.915)	0.647 (0.350 , 0.943)	0.800 (0.463 , 1.138)	0.826 (0.488 , 1.164)
Men				
	CHD mortality endpoint		Non-CHD CVD mortality endpoint	
	Model without HDL-C	Model with HDL-C	Model without HDL-C	Model with HDL-C
HDL-C	-	-0.796 (-0.989 , -0.602)	-	-0.227 (-0.526 , 0.072)
TC	0.230	0.230 (	-0.007	-0.003

	(0.185 , 0.276)	0.185 , 0.275)	(-0.091 , 0.077)	(-0.087 , 0.081)
SBP	0.017 (0.014 , 0.019)	0.017 (0.014 , 0.019)	0.020 (0.016 , 0.024)	0.020 (0.016 , 0.024)
Smoking	0.627 (0.513 , 0.742)	0.606 (0.491 , 0.720)	0.548 (0.357 , 0.740)	0.544 (0.352 , 0.736)

Table 2-23: Beta coefficients for risk factors in the HDL-C function and the function without HDL-C. Beta coefficients are per 1 mmol//increase in HDL-C and TC, per 1mmHg increase in SBP and comparing current smoker to non-current smoker

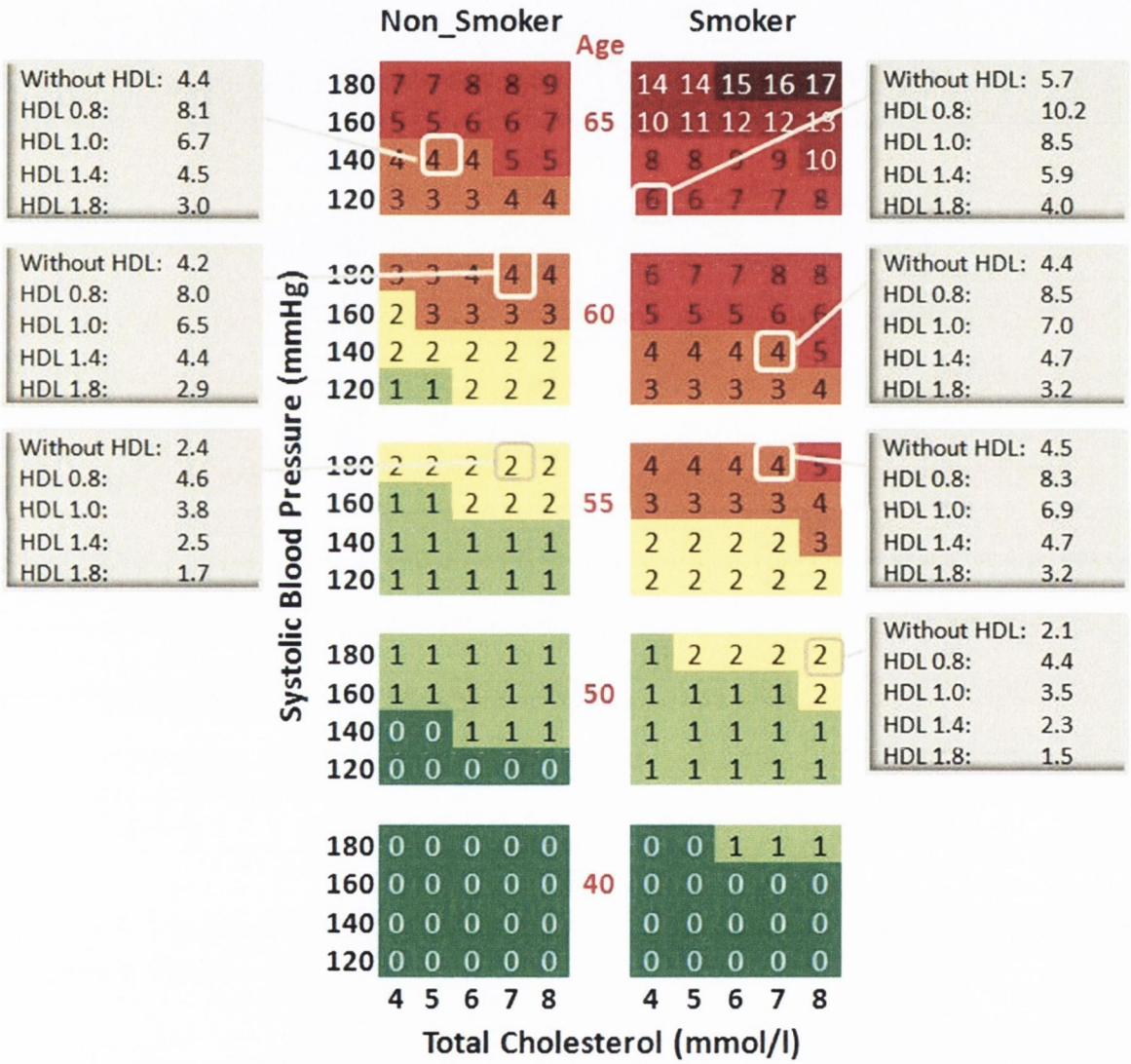


Figure 2.20: Risk function without HDL for Women from high risk European regions, with examples of the corresponding estimated risk when different levels of HDL-C are included

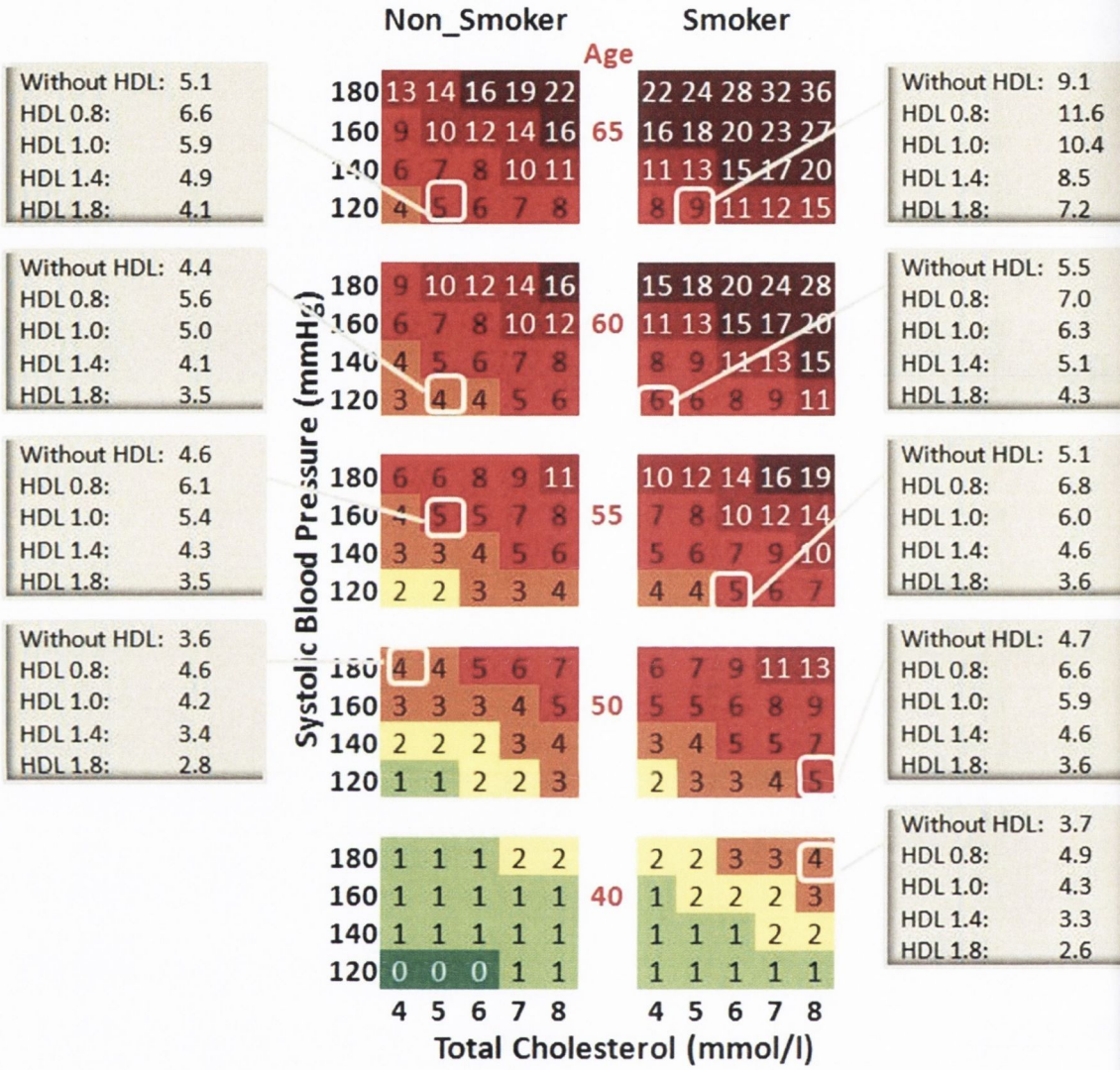


Figure 2.21: Risk function without HDL for men from high risk European regions, with examples of the corresponding estimated risk when different levels of HDL-C are included

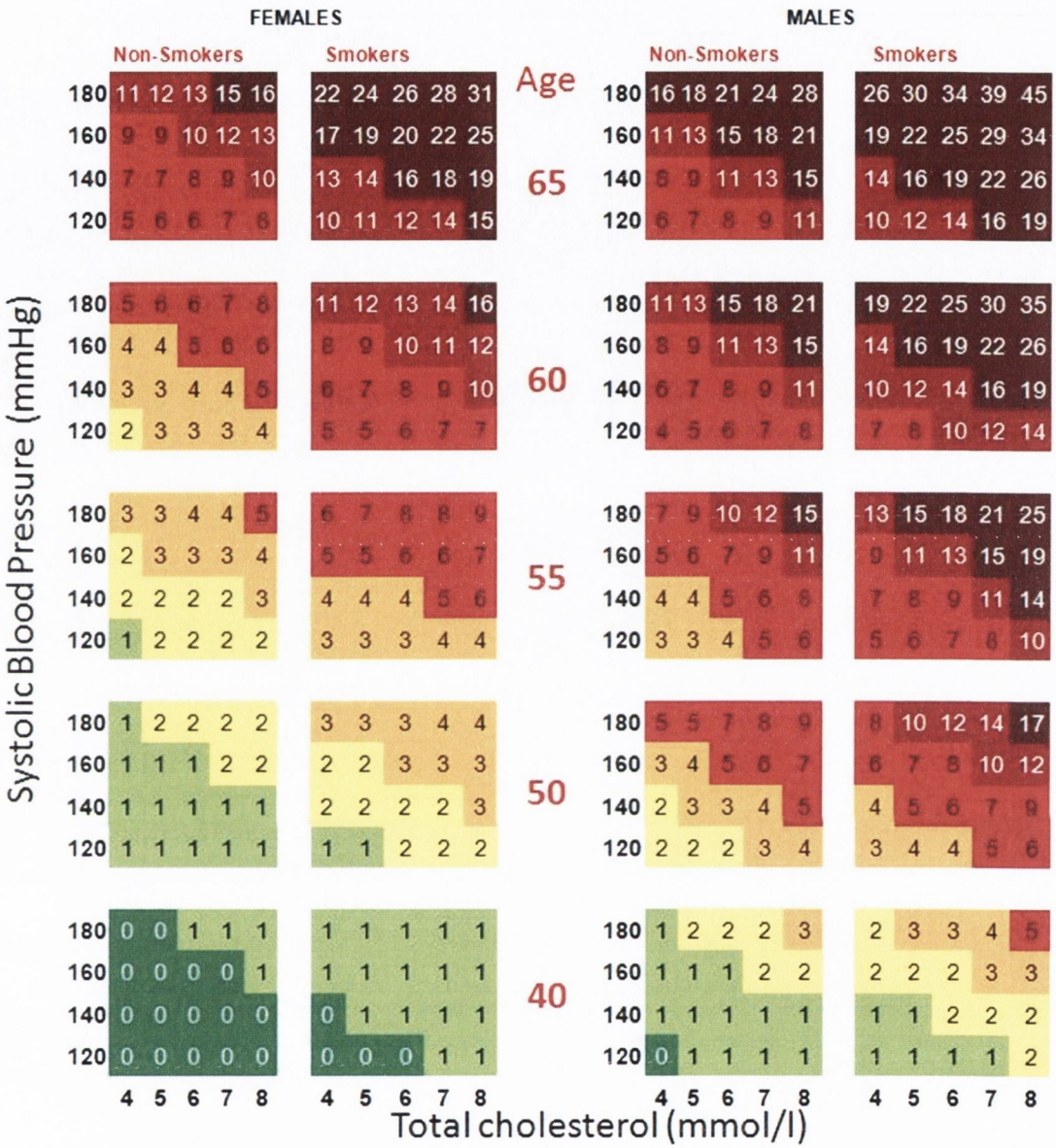


Figure 2.22: SCORE HDL for use in high risk countries - HDL = 0.8 mmol/l

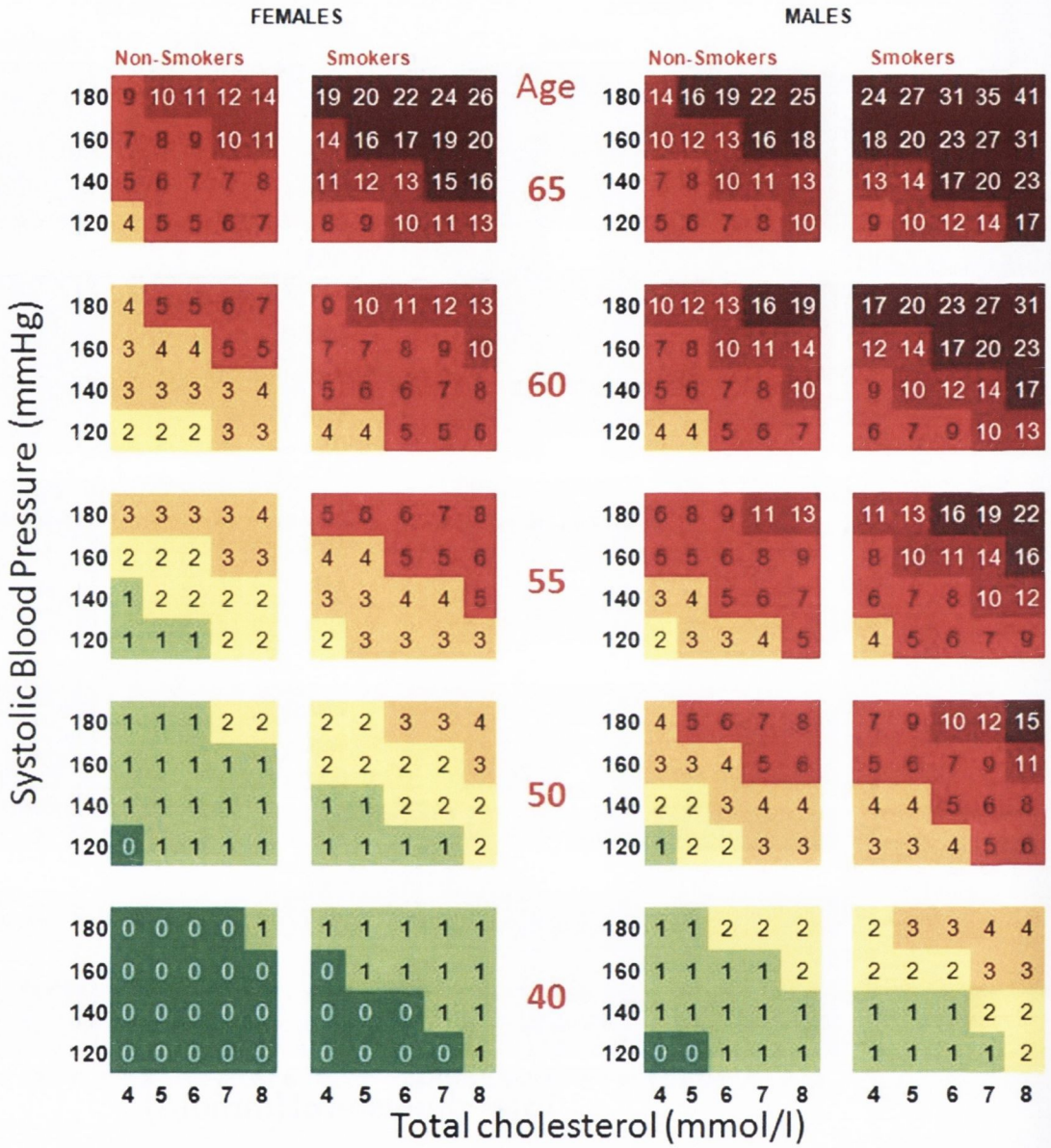


Figure 2.23: SCORE HDL for use in high risk countries - HDL = 1.0 mmol/l

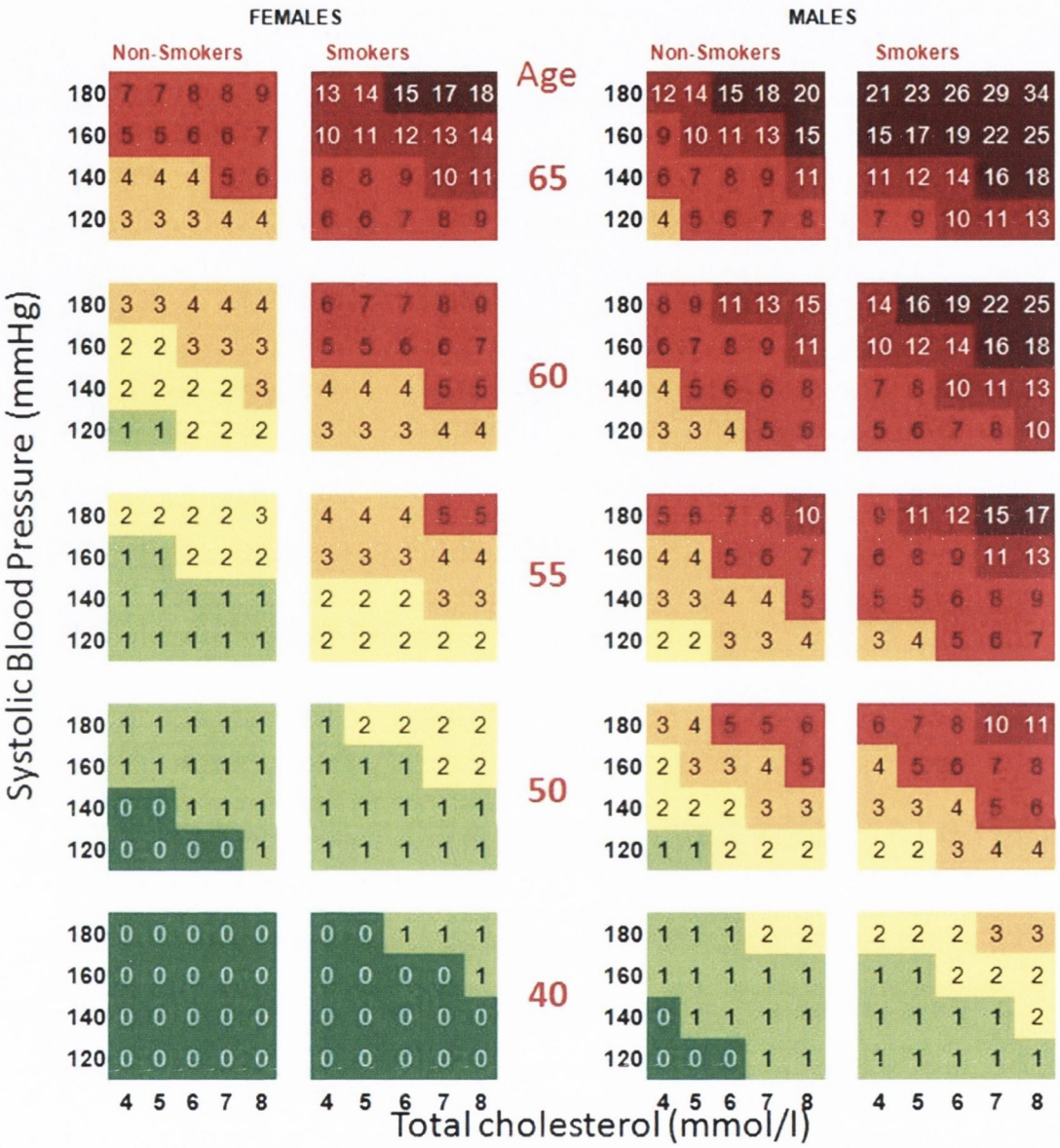


Figure 2.24: SCORE HDL for use in high risk countries - HDL = 1.4 mmol/l

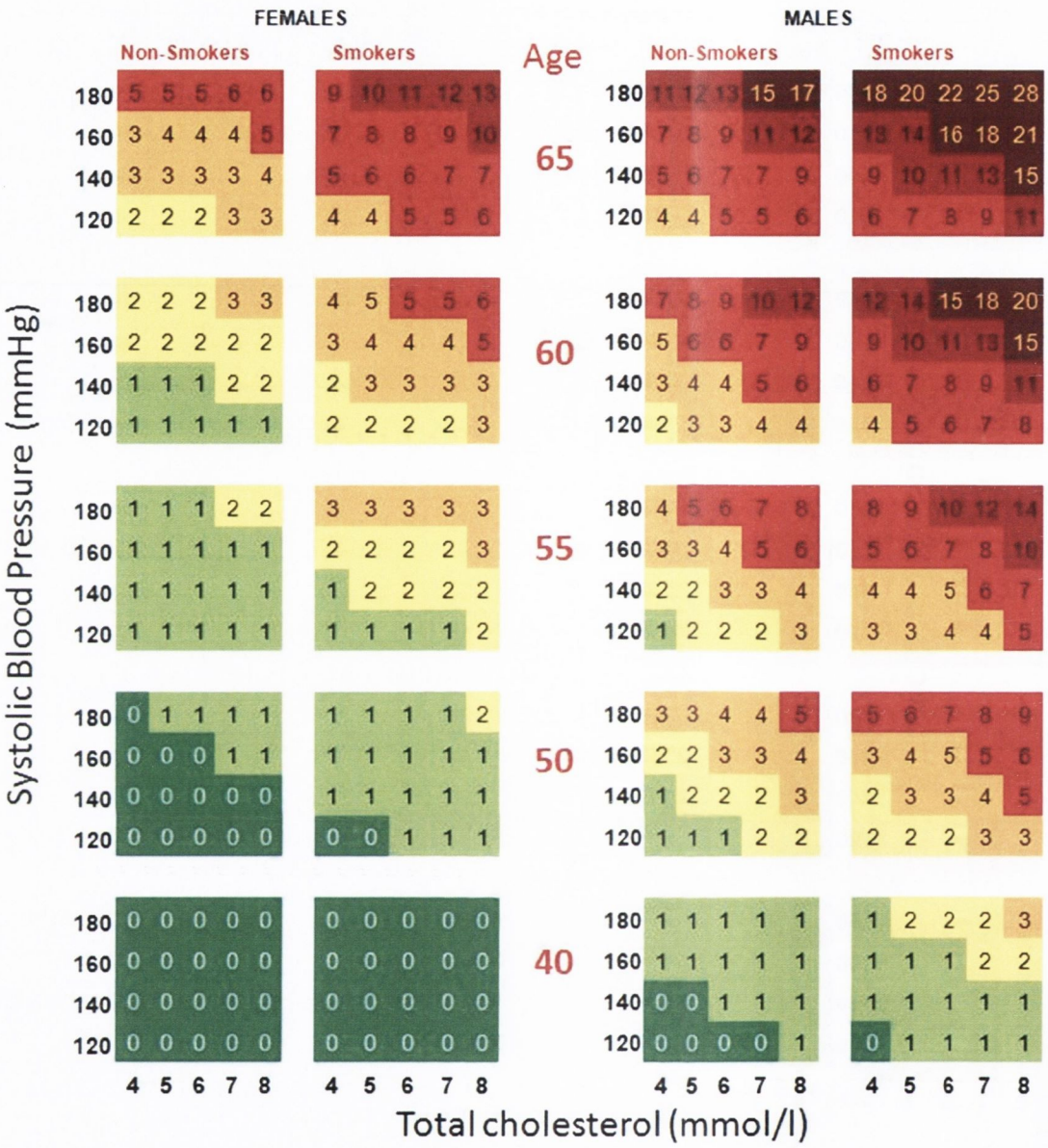


Figure 2.25: SCORE HDL for use in high risk countries - HDL = 1.8 mmol/l

TESTING THE PERFORMANCE OF SCORE HDL – COMPARING TO SCORE CONTAINING TOTAL CHOLESTEROL ALONE

DISCRIMINATION

AREA UNDER RECEIVER OPERATING CHARACTERISTIC CURVE

The AUROC in the entire group was slightly greater for the HDL-C function than the function without HDL-C at 0.814 and 0.808 respectively, (p<0.0001). Table 2-24 shows AUROCs for the entire group and for each subgroup. The greatest difference in AUROC was in high risk women, where inclusion of HDL-C in the function resulted in an AUROC increase from 0.796 to 0.829, p=0.0001. The sensitivity and specificity for the two functions at different cut points for high/low risk are shown in Table 2-25. Changes in Harrell's C statistic values for the entire group and each subgroup were very similar to the AUROC changes (see Table 2-26).

	ROC function without HDL-C	ROC HDL-C function	p value for difference
Entire group	0.808	0.814	<0.0001
Women	0.807	0.815	0.1244
Men	0.763	0.769	0.0001
Women from high risk countries	0.796	0.829	0.0001
Men from high risk countries	0.758	0.767	0.0001
Women from low risk countries	0.801	0.795	0.4516
Men from low risk countries	0.737	0.742	0.0572

Table 2-24: AUROC (Area under receiver operating characteristic curves) for CVD mortality for men and women from low and high risk countries

HDL function			Function without HDL		
Cut point	Sensitivity	Specificity	Cut point	Sensitivity	Specificity
Entire Group					
2%	90%	54%	2%	89%	54%
3%	78%	70%	3%	76%	67%
5%	55%	85%	5%	52%	85%
7%	40%	92%	7%	38%	92%
Women					
2%	65%	81%	2%	65%	80%
3%	44%	91%	3%	39%	91%
5%	19%	98%	5%	13%	98%
7%	9%	99%	7%	6%	99.5%
Men					
2%	95%	33%	2%	94%	34%
3%	84%	53%	3%	83%	54%
5%	62%	75%	5%	60%	76%
7%	46%	86%	7%	44%	87%

Table 2-25: Sensitivity and specificity at various cut points of the functions with and without HDL-C

	HDL function	Function without HDL
Women	0.897	0.869
Men	0.802	0.782
Women from high risk countries	0.914	0.878
Men from high risk countries	0.781	0.777
Women from low risk countries	0.885	0.867
Men from low risk countries	0.813	0.804

Table 2-26: Harrell's C statistic for HDL function and function without HDL in subgroups

RECLASSIFICATION

Net reclassification indices (NRI) are shown in Table 2-27. These indicate the net proportion of cases who were re-classified in the correct direction plus the net proportion of non-cases who were re-classified in the correct direction, using two categories only - high ≥5% / low <5%. In each subgroup, use of the HDL function resulted in superior risk classification, as indicated by the positive NRI for each subgroup. The highest NRI (0.115, p=0.015) was in women from high risk countries.

	NRI	P value
All	0.022	0.006
All Women	0.054	0.014
All Men	0.015	0.082
Women from high risk countries	0.115	0.015
Men from high risk countries	0.016	0.153
Women from low risk countries	0.007	0.683
Men from low risk countries	0.009	0.498

Table 2-27: Percentage in each group correctly reclassified into high/low risk of CVD – Net reclassification index

When classifying individuals into four risk categories (≤2%, 3-4%, 5-9%, ≥10%) net reclassification indices were greater;0.038 in the entire group and 0.17 in women from high risk countries. The NRIs for the 4 categories and 7 categories are shown in Table 2-28.

4* SCORE categories		
	NRI	p value
Entire cohort	0.038	0.0024
High Risk Females	0.17	0.0046
High Risk Males	0.066	0.0002
Low Risk Females	-0.014	0.7351
Low Risk Males	0.018	0.3692
7** SCORE categories		
Entire cohort	0.113	<0.0001
High Risk Females	0.368	<0.0001
High Risk Males	0.104	<0.0001
Low Risk Females	0.016	0.7701
Low Risk Males	0.048	0.0291

Table 2-28: Net reclassification indices for 4* and 7 SCORE categories** *4 categories: ≤2%, 3-4%, 5-9%, ≥10% ** 7 Categories: <1%, 1%, 2%, 3-4%, 5-9%, 10-14%, ≥15% - the colour coded categories on the SCORE charts.

Table 2-29 shows the full reclassification tables for the 4 SCORE categories to show in detail how the net reclassification indices are calculated. Men and women are included together in this example. The net reclassification index (NRI) is calculated using these reclassification tables, as described by Pencina et al[256].

The reclassification tables are constructed separately in those who develop the endpoint within the ten years (upper part of the table) and those who do not (lower part of the table). Those who develop the endpoint and move to a higher category with the HDL function are moving in the correct direction – these have been shaded in green in the table. Those who develop the endpoint moving to a lower category are moving in the incorrect direction – these have been shaded in red. Those who do not change category – across the diagonal of the table – are shaded in grey. The next step is to minus the incorrectly reclassified individuals from the correctly reclassified individuals – this gives the net number of individuals correctly reclassified in the group who did develop the endpoint. This is divided by the total number of those who developed the endpoint. The same is done for the group who do not develop the endpoint – in this case those who move to a lower category are correctly reclassified etc. The sum of the net correctly reclassified percentages in the two groups (those who did and those who did not develop the endpoint) gives the net reclassification index. For the example given below the NRI was calculated at 0.038, p =0.0024.

	FUNCTION-C ≤ 2%	FUNCTION C = 2-4%	FUNCTION C = 5-9%	FUNCTION C ≥ 10%	Total
FUNCTION-H ≤ 2%	160	28	0	0	188
FUNCTION H =2-4%	36	333	26	1	396
FUNCTION H = 5-9%	0	61	247	28	336
FUNCTION H ≥ 10%	0	0	45	238	283
Total	196	422	318	267	1,203
	FUNCTION-C ≤ 2%	FUNCTION C = 2-4%	FUNCTION C = 5-9%	FUNCTION C ≥ 10%	Total
FUNCTION-H ≤ 2%	40,022	1,806	2	0	41,830
FUNCTION H = 2-4%	1,918	13,231	829	1	15,979
FUNCTION H = 5-9%	2	1,268	4,970	290	6,530
FUNCTION H ≥ 10%	0	4	468	1,733	2,205
Total	41,942	16,309	6,269	2,024	66,544

Table 2-29: Reclassification tables in those who did develop CVD mortality within 10 years (upper part) and those who did not develop the endpoint (lower part). Function C: function without HDL-C. Function H: HDL-C function

CALIBRATION

OBSERVED TO PREDICTED RATIOS

Figure 2.26 shows the observed rate of CVD mortality (per 1000 person years) and the predicted number of CVD deaths in 100 people over a 10 year period, calculated using the HDL-C function. The ratio between predicted and observed in each category is also shown on the graph. It indicates good correlation between

the observed and predicted risk in the HDL-C function. A similar correlation was seen for the function without HDL-C.

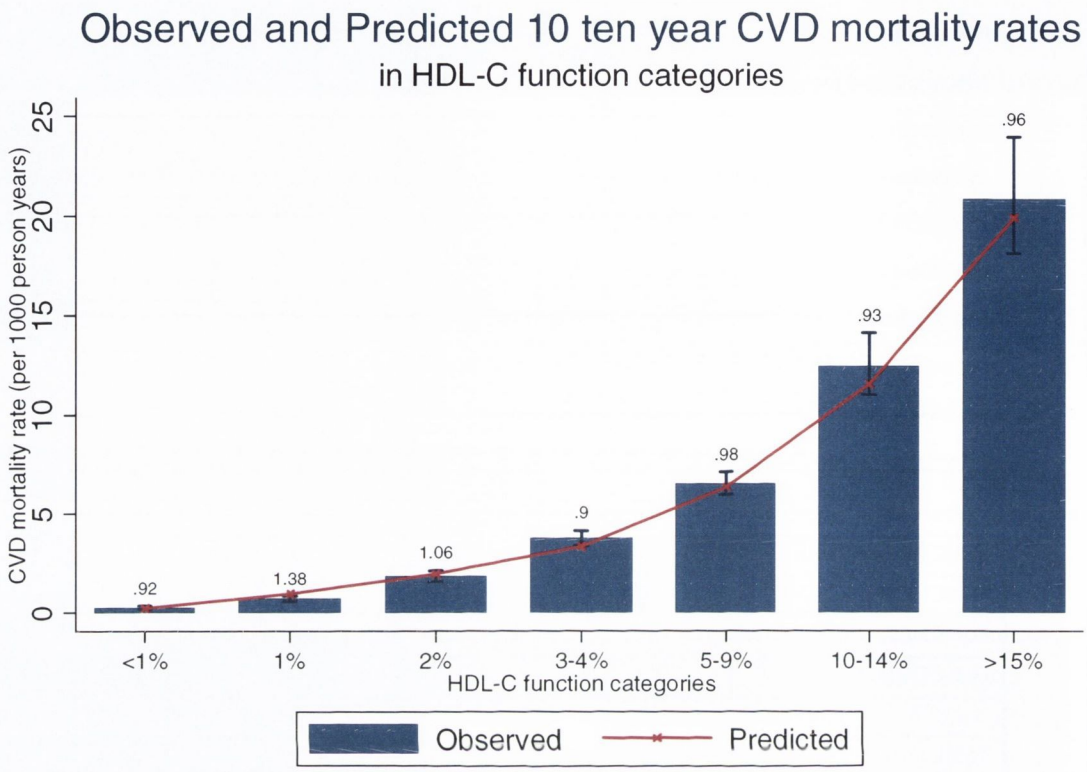


Figure 2.26: HDL risk function – Observed and predicted 10 year CVD mortality rates, by categories of risk

ASSESSING GOODNESS OF FIT

Some of the cohorts included in this analysis did not have complete follow-up to 10 years. Because Cox methods were used to derive the function this variable follow-up was taken into account in the derivation of the function. However, for testing the goodness of fit of the function 10 year observed risk was not available for all cohorts for comparing with the 10 year risk as predicted by the function. Since 5 year follow-up was complete in > 96% of individuals we derived a 5 year function also and tested the fit of this function using the Hosmer-Lemeshow goodness of fit test. There was no significant lack of fit when comparing predicted and observed 5 year survival in either women or men, at the 5% level. The calibration plot by deciles of risk function is shown in Figure 2.27 (men and women together). The "hl" command in Stata was used with the option "sample" selected; this adjusts the degrees of freedom to account for the fact that the performance of the function was tested on the derivation cohort.

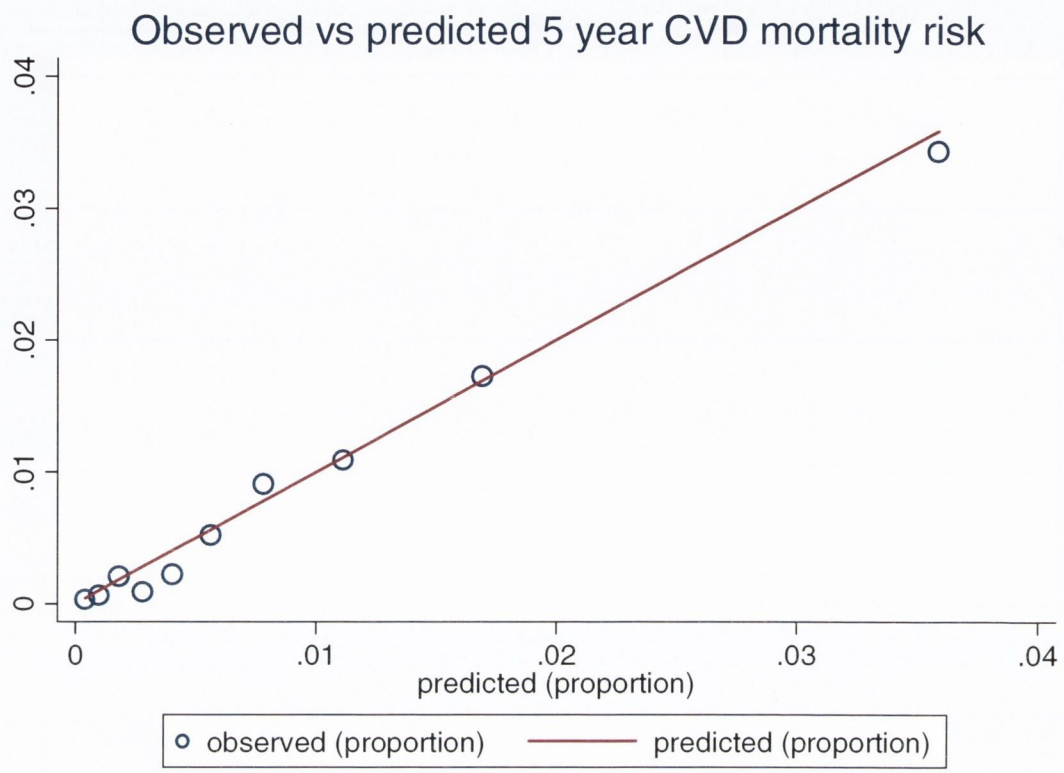


Figure 2.27: Hosmer-Lemeshow goodness of fit within each tenth of the HDL-C risk function in men and women together

The comparison of observed rate of CVD mortality to predicted 10 year risk shown in Figure 2.26 is appropriate because the observed risk is in terms of rates per 1000 person years and therefore takes the variable follow-up into account.

COMPARING PERFORMANCE OF SCORE HDL TO SCORE CONTAINING TOTAL CHOLESTEROL/ HDL CHOLESTEROL RATIO

In separate analyses, we compared a model with HDL-C and TC entered separately to one including the ratio and demonstrated that although the AUROC for the entire group did not change, there was a statistically significant improvement in likelihood ratio when the first model was used. Additionally, there was an improvement in net reclassification indices; again the largest improvement was seen in women.

Simple system for inclusion of HDL level only where HDL level will affect the risk categorization

A SCORE chart (for use in high risk countries) without HDL cholesterol included is shown in Figure 2.28. The numbers within the red squares, which are included on some of the boxes (close to the threshold for high /

low risk) indicate what the risk estimate (% 10 year risk of fatal CVD) would be if the individual had a low HDL (<0.8 mmol/l). If the red square is not shown then the individual will not change risk category if their HDL cholesterol is low. This gives an option for inclusion of this extra risk factor within a two dimensional chart. However, if does not allow for different levels of HDL to be included in the risk estimate.

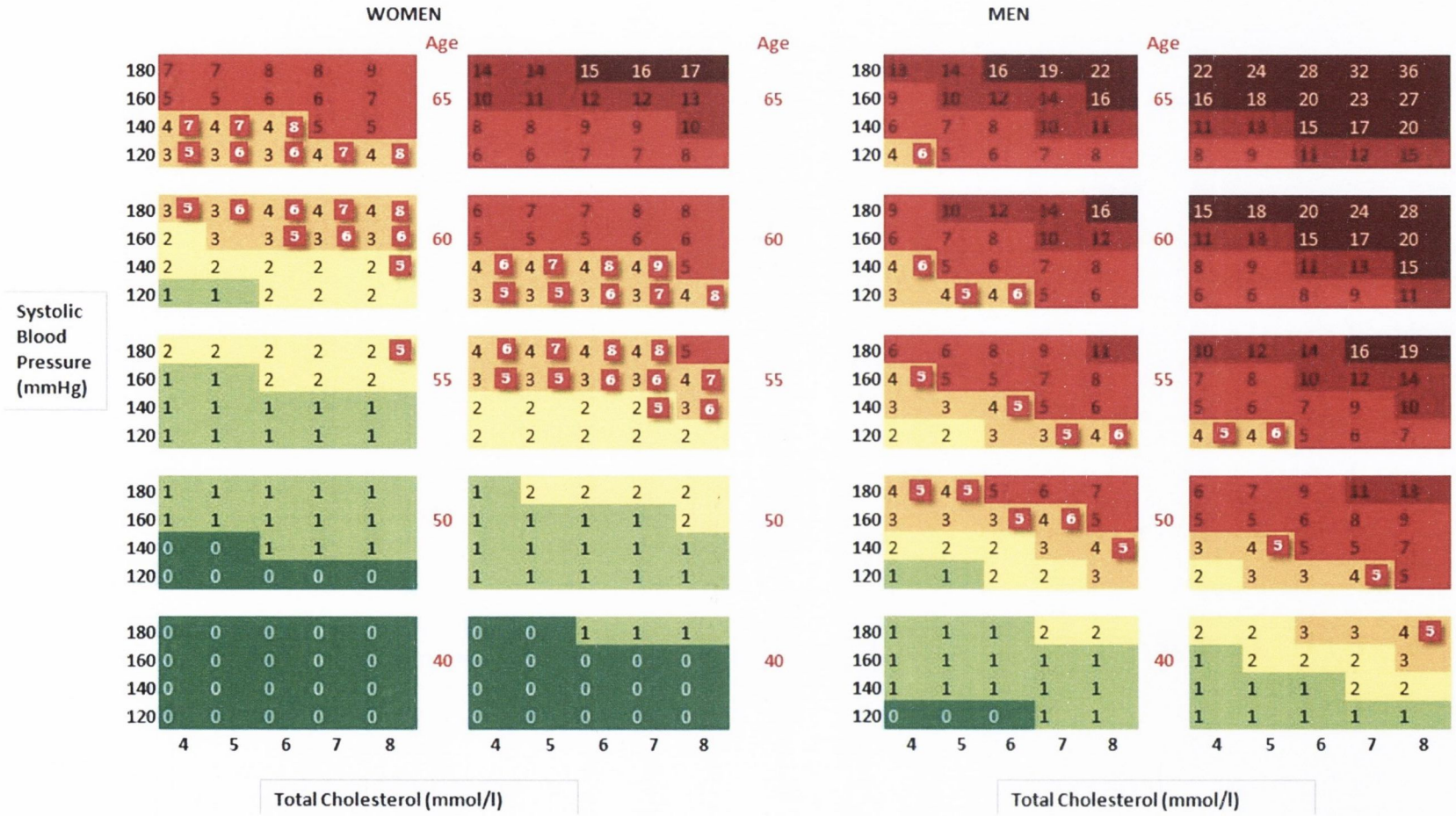

WOMEN
MEN
Age
Age
Age
Age
65
60
55
50
40
180
160
140
120
Systolic Blood Pressure (mmHg)
4 5 6 7 8
Total Cholesterol (mmol/l)
Total Cholesterol (mmol/l)

Figure 2.28: SCORE chart (for use in high risk countries) with indication of change in risk estimate if the HDL cholesterol level is 0.8mmol/l (only indicated if the risk category will change due to the inclusion of the HDL level)

SCORE HDL – INTERACTIVE COMPUTER PROGRAM

An interactive computer program for the calculation of both the function without HDL cholesterol and the function with HDL was developed. The program allows the user to enter risk factor information and automatically calculate the SCORE. The use of the Filemaker Pro Advanced software means that I have been able to develop a stand alone (run-time) version of the program. This is available on CD-ROM and can be used on any computer without the requirement for installation of the Filemaker Pro software.

The data entry screen, as shown in Figure 2.29 shows is simple to use, with clear boxes indicating where the risk factor information should be entered. On clicking the "Calculate SCOREs" icon the results screen appears – this is shown in Figure 2.30. The 10 year risk of fatal CVD based on the entered risk factors is clearly displayed. The SCORE calculated using the HDL function and using the function without HDL are both shown.

To make the program more accessible and to facilitate the use of the program in routine clinical practice, it can also be used as a database. The patient's demographic information, their risk factors and their SCOREs are stored. Records can be displayed as individual records or as a list, as shown in Figure 2.31.

A one page summary for including in the patient's medical records can be printed and additionally, a one page summary of risk factors in plain English is available. This can be given to the patient for their own records. It also includes some guideline recommended advice on improving their risk factor profile.

The program has already been used as part of a cardiovascular risk factor screening day for staff of Adelaide Meath Hospital for the purposes of data management, calculation of SCORE and communication of results to participants.

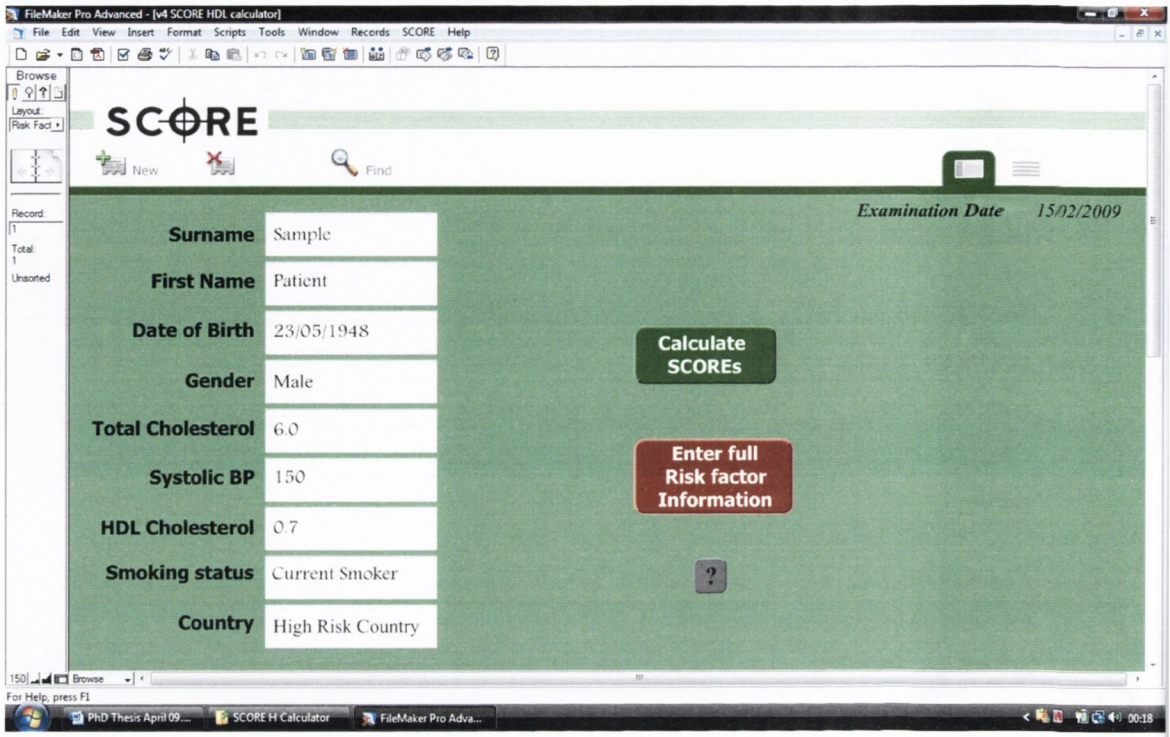


Figure 2.29: Screen shot of the data entry screen for the interactive risk calculator

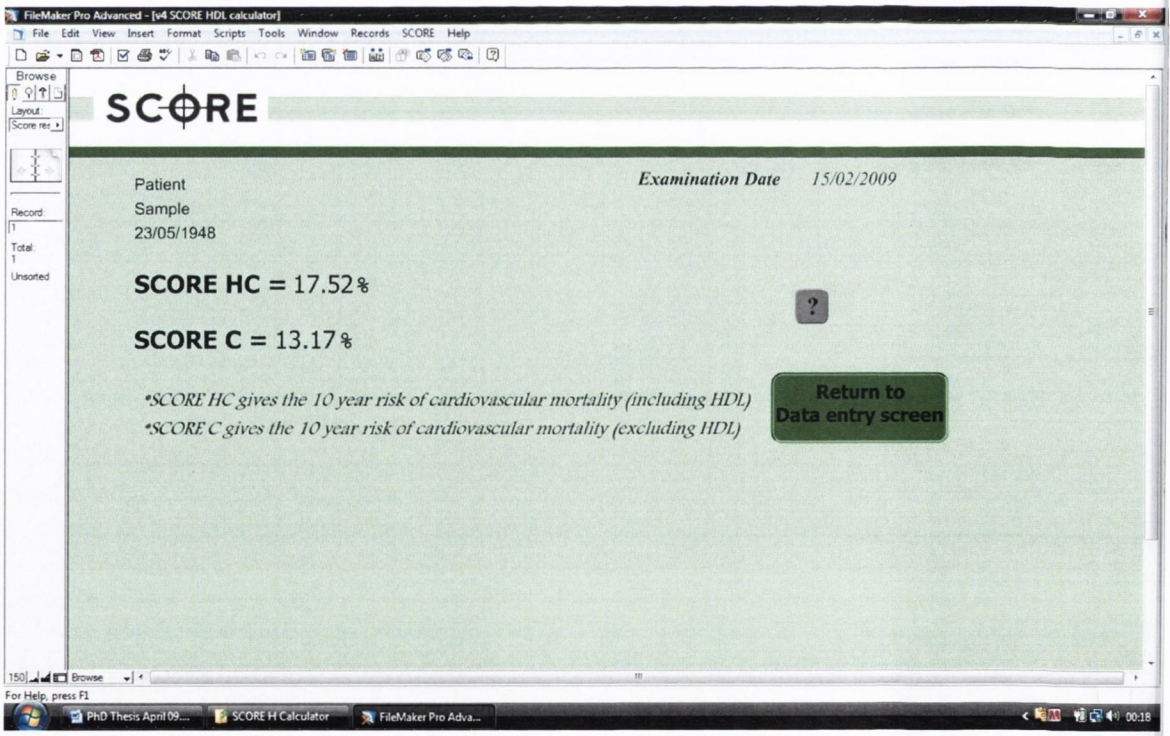


Figure 2.30: Screen shot of the results screen for the interactive risk calculator

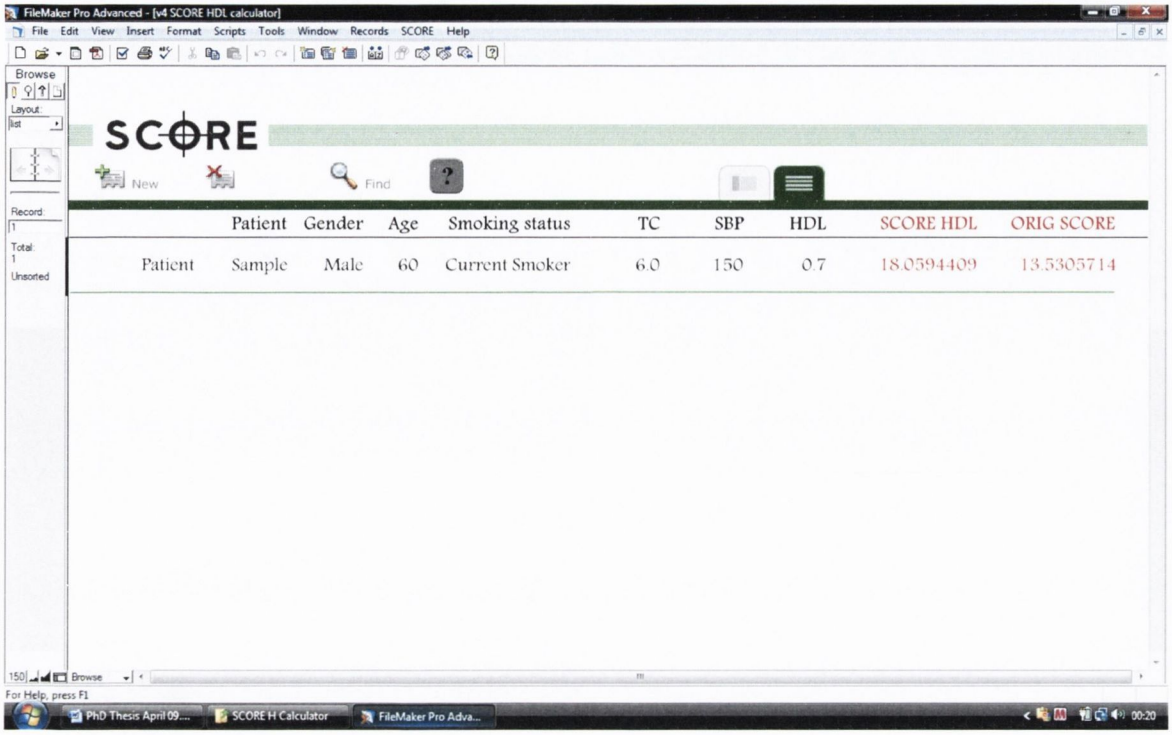


Figure 2.31: Screen shot of the database of patients and risk factors in list format

HDL – DISCUSSION

HDL – ASSOCIATIONS BETWEEN HDL CHOLESTEROL AND OTHER RISK FACTORS

HDL cholesterol was statistically significantly higher in women than men, as noted in other studies. There was no consistent pattern of HDL cholesterol levels in the high and low risk countries though, with HDL cholesterol higher in women from high risk countries and higher in men from low risk countries.

Consistent with previous studies, HDL cholesterol was significantly and importantly lower in smokers. HDL cholesterol was also linked to other components of the metabolic syndrome. Lower HDL cholesterol was found in those with diabetes and in women with increasing levels of SBP. As expected HDL cholesterol levels were inversely associated with triglyceride level. The strongest correlate of HDL cholesterol levels was BMI. Here there was a linear decrease in HDL cholesterol levels with increases in BMI. This relationship starts with BMI as low as 20 and continues throughout the level of BMI levels to 35. At this point the relationship

appears to level off, however, numbers in this BMI range are smaller, as evidenced by the widening confidence intervals in **Figure 2.4**. HDL cholesterol level decreases from 1.40 to 1.16 in men and 1.58 to 1.30 in women across this range. This linear inverse relationship with BMI was demonstrated in each individual country also.

The inter-relationships between BMI and CVD risk and between BMI and other risk factors, including HDL cholesterol, have been explored in the thesis of Dr. Alexandra Dudina[258]. It appears that while HDL cholesterol remains independently associated with CVD mortality, BMI is acting in a contrasting way. When adjusting for age and smoking alone there is a substantial risk associated with elevations in BMI. However, BMI appears to be exerting its effect through modification of other risks, including total and HDL cholesterol, blood pressure and diabetes. Once adjustment is made for these additional factors the hazard ratio for BMI becomes attenuated[258].

In men, a decrease in HDL cholesterol was noted above the age of approximately 65 years. However, in women HDL cholesterol levels appear to increase until a plateau is reached at approximately age 50 years, after which levels start to drop again. This is consistent with previous observations of the positive effect of oestrogen on HDL cholesterol levels, as the level appears to drop at the age of menopause.

Unfortunately, alcohol was not available to study in this dataset. Additionally, the correlation between waist circumference and HDL cholesterol could not be investigated.

HDL – EFFECT ON CV ENDPOINTS

The size of the SCORE data set has allowed clarification of some of the apparent inconsistencies in the relationship between HDL-C and CV risk. A strong, inverse association between HDL-C and both CVD mortality and CHD mortality has been demonstrated. The effect is robust and withstands adjustment for other CV risk factors. The effect of HDL-C has been demonstrated at all levels of TC and SCORE total CV risk. The effect of HDL-C as a risk modifier is approximately equal in each SCORE category.

Importantly, we have demonstrated that HDL-C continues to function as an important protective factor in all age groups, including those aged over 65. This is particularly important in view of the aging population and the current paucity of large studies examining the role of HDL-C in the elderly.

COMPARISON TO PREVIOUS STUDIES

The magnitude of the effect of HDL-C on CHD and CVD mortality seen in this analysis is similar to that which has been demonstrated in previous studies. The details of the previous analyses of the effect of HDL cholesterol in prospective studies is given in **Table 2-2**, with the hazard ratios converted to the unit used in this analysis; increase of 0.5mmol/l HDL-C, for ease of comparison. In our study, the hazard ratios were 0.60

(95%CI 0.51 to 0.69) in women and 0.76 (0.70 to 0.83) in men for the CVD mortality endpoint. This effect was close in magnitude to the majority of previous studies. As mentioned above 24 of 28 of them with data available demonstrated hazard ratios between 0.40 and 0.80. In a re-analysis of four American prospective studies, [135] the hazard ratio for CVD mortality was between 0.78 and 0.44 in men and between 0.48 and 0.4 in women. The corresponding hazard ratio in men the re-analysis of the British regional heart study [135] was 0.68 (coronary incidence) and in a large Norwegian study [150] 0.81 in men and 0.71 in women. The Framingham estimates were 0.67 in men and 0.53 in women[176], the Russian LRC estimates was 0.78 in men[146] and the Indiana Medical Group studies [138] demonstrated a hazard ratio of 0.80 in men and women combined.

One of the strengths of this analysis is that it forms the largest multivariable analysis of the effect of HDL-C on CV risk. The prospective studies collaboration analysis [139] and the AMORIS study [175] analyses are larger with 1.5 and 0.96 million person years of observation respectively; however, the hazard ratios for HDL-C are unadjusted for other CV risk factors.

COMPARISON TO PREVIOUS STUDIES – EFFECT OF HDL-C ON CV RISK IN THE OLDER AGE GROUP

In this analysis we have demonstrated that HDL cholesterol continues to function as an important protective factor in all age groups. Unlike what is seen with many risk factors for CVD there is no evidence of a reduction in the importance of HDL cholesterol as a protective factor with age. In fact, the hazard ratio for older women is higher than that for younger women. However, this difference did not reach statistical significance and there was no significant HDL/age interaction. It is interesting to note that in a re-analysis of the INTERHEART study which looked at the effect of different risk factors in men and women older and younger than 60 years of age[259], the protective effect of both moderate alcohol intake and physical activity was stronger in older than younger men. Additionally, both of these factors have a positive impact on HDL cholesterol.

Table 2-3 details the results and characteristics of previous prospective studies of elderly men and women which have examined the effect of HDL cholesterol on outcome. As shown in the table, there have been few previous demonstrations of the effect of HDL cholesterol on cardiovascular or coronary endpoints in older women. Those studies which have demonstrated a significant effect [188, 191, 197, 198] have generally been either solely in women with established cardiovascular disease[191] or contained a high proportion of women with pre-existing disease [188, 197, 199]. We suggest that the reason our study has demonstrated an independent effect when other studies have not, is because of lack of power in previous studies, which in general included small numbers. Several studies in the table have demonstrated significant univariable effects of HDL cholesterol[139, 181, 182] and others have shown an effect in the elderly subgroup, but have not analysed men and women separately[157, 180, 185, 195, 210].

This is only the fourth multivariable analysis of a prospective study in older women to show an independent protective effect of HDL cholesterol. Of note, none of the previous studies were restricted to those without pre-existing coronary disease as in our study. The other studies, the Rotterdam study, the CASTEL study, and the Hebrew Hospital study all contained a high proportion of women which established coronary disease (9%, 11% and up to 41% respectively) [188, 197, 198].

HDL AND RISK OF STROKE

We have demonstrated substantially different relationships between HDL and stroke mortality in men and women from high and low risk countries. In women from high risk countries, an inverse, independent, graded relationship between HDL cholesterol and stroke mortality risk was demonstrated. In men from high risk countries, the relationship appeared U shaped, with the lowest risk associated with the middle quintile of HDL and the highest risk associated with the lowest quintile of HDL cholesterol. No effect was seen in those from low risk countries. These unusual findings deserve specific consideration.

HDL AND RISK OF STROKE – COMPARISON TO PREVIOUS STUDIES

While substantial evidence is available linking HDL and protection from coronary heart disease, the effect of HDL cholesterol on stroke outcomes is less well understood. Several prospective [71, 123, 182, 204-214] and case control studies[260-262] have shown a protective effect. Others[139, 157, 216, 220], however, including the largest study to date, which was undertaken by the prospective studies collaboration[139] showed no effect of HDL cholesterol on stroke mortality, in any age group. Unfortunately, it was not possible to ascertain whether there were gender differences in the effect as the analyses were not performed separately in men and women.

We have demonstrated a strongly graded, inverse and independent relationship with stroke mortality in women from high risk countries, with no evidence of a threshold effect or increased risk at markedly elevated levels. To our knowledge, this is the first quantification of this independent, significant relationship specifically in women in a prospective study. However, most prospective studies examining the relationship specifically in women found an inverse relationship which did not remain significant after multivariate adjustment.

The observed difference in effect of HDL cholesterol on stroke outcomes in men and women is somewhat difficult to understand. Previous prospective studies have demonstrated an inverse effect, although in several the relationship was not graded and most of the risk appeared to be due to low HDL cholesterol with little further protection afforded by further increases in HDL cholesterol.

A U shaped relationship between HDL cholesterol and stroke[157, 217] and all-cause[144, 146, 263] and CVD mortality[151] [150] has previously been demonstrated, with increased risk in men with the highest levels of HDL cholesterol. Interestingly, in the large Norwegian study[150] the increased risk associated with markedly elevated HDL cholesterol was substantially greater for the CVD than the CHD mortality endpoint, suggesting that much of the excess mortality was caused by stroke.

The increased risk with high levels of HDL cholesterol may be due to confounding factors. For example, as increased alcohol intake has been associated with elevated HDL cholesterol and alcohol intake[144] was not adjusted for in this analysis, some of those with high levels of HDL cholesterol may have had excessive alcohol intake and this, as opposed to the high HDL cholesterol, may have increased their risk of stroke mortality (particularly haemorrhagic stroke). Of note though, the curvilinear relationship between HDL cholesterol and CHD remained in one analysis after adjustment for alcohol intake[151].

The inclusion of larger numbers of men with higher HDL cholesterol levels in this study may have facilitated the demonstration of an effect of markedly elevated HDL cholesterol levels previously under-recognised. For example, in the Israeli study, which demonstrated a linear protective effect of HDL cholesterol in men, the mean HDL cholesterol was 1.06 mmol/l compared to a mean of 1.24 mmol/l in men from high risk countries in this analysis[213]. Israeli men with the lowest stroke mortality rate were the highest tertile of HDL cholesterol, which was defined as >1.10 mmol/l[213]. In our analysis, the group of men from high risk countries with the lowest stroke mortality risk had similar HDL cholesterol levels (1.13 – 1.26 mmol/l), although they were the 3rd quintile.

LIMITATIONS IN THIS ANALYSIS OF HDL AND STROKE INTER-RELATIONSHIPS

Due to inclusion of the ICD9 code 436 in the ischemic stroke mortality endpoint, which is undefined cerebrovascular event, we have not been able to analyse the effect of HDL cholesterol on definite ischemic stroke. However, we have been able to exclude haemorrhagic strokes that were specifically coded as haemorrhagic. This is a problem which has been encountered in previous analyses also [264] [204]. Previously, it has been shown that ischemic stroke is more closely associated with lipid levels than haemorrhagic stroke.[212] This is logical as ischemic stroke is more likely to be associated with atherosclerosis. While haemorrhagic strokes usually only account for a small proportion of all strokes (~ 10 – 15%), as the case fatality rate is higher in haemorrhagic than ischemic stroke, it is possible that there may be a greater number of haemorrhagic strokes included because our endpoint was fatal not incident stroke. This is another possible explanation for the lack of effect of HDL cholesterol on stroke mortality in individuals from low risk countries and men from high risk countries. It is possible also that gender differences in the numbers of haemorrhagic strokes included exaggerated observed gender differences in the effect of HDL cholesterol on stroke risk.

ISCHAEMIC STROKE SUBTYPES

In this analysis we have not been able to assess the effect of HDL cholesterol on different subtypes of ischemic stroke. Previously, it has been demonstrated that HDL cholesterol is most closely linked with the atherothrombotic stroke subtype[261, 262] and to a lesser extent the lacunar subtype[262]. As our endpoint is fatal stroke, it is possible that there is over-representation of the atherothrombotic and embolic subtypes, which have higher case fatality rates, and under-representation of the lacunar subtype. This under-representation of the lacunar subtype may be contributing to the lack of a statistically significant relationship between HDL cholesterol and stroke risk in some of the subgroups of individuals.

FUTURE DIRECTIONS – EFFECT OF HDL ON STROKE

Further analyses investigating the effect of HDL cholesterol on specific subtypes of ischemic stroke would be useful, particularly if these analyses were performed separately in men and women. Further research into the effect of HDL particle size on stroke outcomes is also required, as differences in HDL particle size between men and women and between individuals from different countries may be accounting for the variation in effect of HDL cholesterol on stroke outcome demonstrated in this analysis.

HDL PARTICLE SIZE

HDL particle size was not available in this study and the inclusion of HDL particle size in future analyses may shed further light on this issue. Current research on the association between HDL particle size and CVD risk is conflicting. Evidence on the relationship between HDL cholesterol subfractions and risk of stroke specifically is virtually non-existent. Older prospective studies, in general, showed the larger HDL2 to have a greater protective effect on CHD endpoints than the smaller HDL3[265], although some also showed that HDL 3 was more protective[126, 148].

More recent analyses which involve more precise estimation of HDL particle size have shown that elevated levels of the very small dense HDL particles (HDL 3b) are associated with the presence of other risk factors, particularly components of the metabolic syndrome[266, 267] and suggest that higher proportions of small dense HDL may be responsible for the increased risk in some individuals with normal or high HDL cholesterol levels. In sharp contrast to this, a recent nested case control study on the prospective EPIC Norfolk study showed that increasing average HDL particle size was associated with increased risk of cardiac events[268]. However, assessing the *average* HDL particle size may inadequately represent the variation in levels of each HDL subtype.

The observed gender differences in the effect of HDL cholesterol may be related to gender differences in HDL particle size. Previously, it has been demonstrated that more HDL cholesterol is in the form of small

dense HDL particles in men than women[266]. Further investigation of these complicated inter-relationships is warranted.

MARKEDLY ELEVATED HDL CHOLESTEROL DUE TO CHOLESTEROL ESTER TRANSPORT PROTEIN INHIBITORS

Torcetrapib is a cholesterol ester transport protein inhibitor which is associated with dramatic increases in both HDL cholesterol levels (of up to 72%) and HDL particle size (as evidenced by disproportionate increases in HDL cholesterol compared to Apo A1 levels). It was envisaged that these high HDL cholesterol levels would protect against CVD events. However, the ILLUMINATE trial[243] which compared atorvastatin alone with atorvastatin in combination with torcetrapib resulted in increased all-cause mortality in the group given torcetrapib and consequent early termination of the trial. This has resulted in speculation that high levels of HDL cholesterol or large HDL particles are associated with worse outcomes. We have not demonstrated any increased risk associated with high or very high levels of HDL cholesterol except for the stroke mortality endpoint in men from high risk countries.

It is interesting to note that there were 6 fatal strokes in the group given torcetrapib compared to none in the atorvastatin alone group. This would be consistent with the finding in this analysis of increased risk of fatal stroke in men (from high risk countries) with the highest HDL cholesterol levels. However, it is also possible that the increase in stroke (and all-cause) mortality was related to an off-target effect of torcetrapib on aldosterone and blood pressure. No information is given on whether the excess fatal strokes occurred in men or women or the specific increases in HDL cholesterol, HDL particle or blood pressure in these individuals.

HDL - NOVEL ASPECTS

This analysis confirms the strength and gradation of the relationship between HDL-C and CVD risk. The relationship is particularly strong for the coronary heart disease mortality endpoint. The relationship is independent of other CV risk factors, including those risk factors which HDL cholesterol is associated with.

The stronger relationship in women which was been previously noted has been clarified. We have also clarified that the relationship holds at all levels of total cholesterol. As mentioned above, there has previously been some confusion regarding this. Importantly, this study population contains only those without previous evidence of CHD; therefore, we have confirmed that this relationship is important in the general population, not only those with previous CHD as some previous studies have demonstrated.

This analysis shows for the first time that the independent protective effect of HDL cholesterol on CV risk is seen at all levels of total CV risk. This suggests that incorporation of HDL cholesterol into risk estimation systems as a separate variable may improve risk estimation.

Importantly, we have also demonstrated that the independent relationship is consistent in apparently healthy older women. As discussed above there has been a lack of evidence demonstrating this relationship in previous prospective studies.

The inverse, independent and graded effect of HDL cholesterol on stroke mortality in healthy women is also novel. However, the lack of association in those from low risk European countries and the U-shaped relationship in men need further investigation.

DOES HDL FULFILL THE CAUSAL CRITERIA?

In this, the largest multivariable analysis of the effect of HDL-C on CVD, we have shown a **strong** and **graded** inverse relationship between HDL-C and CVD risk. The relationship was **independent** of all co-variables available in the dataset, although it was not possible to adjust for physical activity and alcohol. The effect was highly **consistent**; seen in both genders, all age groups and at all levels of total CV risk. Our use of prospective studies where HDL levels were measured up to 21 years before the outcome occurred indicates the appropriate **temporal sequence**. Previous studies have demonstrated **agreement across disciplines** with a similar effect seen in case-control[269], cross-sectional[270] and basic science[271] analyses. Several **plausible biological mechanisms** for the effect have been demonstrated including: reverse cholesterol transport, anti-oxidant, anti-thrombotic and anti-inflammatory effects[271], as have been discussed above.

However, there is a lack of consistency in trials assessing the **benefit of HDL elevation** on CV outcomes. While some trials have indicated a beneficial effect of pharmacologically elevating HDL-C levels [239, 246], others have shown opposite results [243]. A recent meta regression has failed to demonstrate a beneficial effect of HDL-C elevation across previous randomized controlled trials[238]. HDL-C level is also modified by lifestyle changes such as reducing overweight, increasing physical activity [272] and smoking cessation [223];, however, it has been difficult to separate the role of HDL elevation resulting from these actions from the other favourable effects these have on CV risk.

In conclusion, this analysis has added to the evidence that (low) HDL-C is a causal factor in the development of CVD; however, all criteria have not been met, as yet. Our summary of the current situation regarding evidence for HDL cholesterol as a causal factor and how this analysis has added to this evidence base is presented in **Table 2-30**. Each criterion is given a score (0 – minimum, 3 – maximum) to indicate the weight of evidence supporting fulfilment of that criterion. For comparison, total cholesterol - a well established cardiovascular risk factor is assessed in the same way.

	HDL Cholesterol	How has this analysis added?	Total Cholesterol
Biologically plausible	3	-	3
Strong	3	Clear demonstration of the magnitude	3

		of effect in both men and women	
Temporal Sequence	3	Long follow-up available in the SCORE dataset confirms that low HDL cholesterol precedes the development of CVD	3
Graded	3	Graded relationship demonstrated across HDL quintiles – no evidence of a threshold effect	3
Independent	3	Independent of conventional risk factors and also of triglycerides and family history of CHD in sensitivity analyses	3
Consistent	3	Consistency clearly demonstrated across both gender, all age groups and all levels of total CVD risk	3
Agreement between disciplines	3	The findings in this analysis of cohort data agree with previous case control, cross sectional and animal studies	3
Treatable	2	-	3
Benefit Results	1-2	-	3

Table 2-30: Summary of fulfillment of causal criteria by low HDL cholesterol, as a risk factor for CVD.

SCORE HDL

Given the clear results of the above analyses, we hypothesized that inclusion of TC and HDL-C as separate variables in the risk function would improve risk estimation. Discrimination of the function as measured using AUROC was improved by the incorporation of HDL as a separate variable. The improvement was statistically significant but modest. This is in part because the large sample size of the present study generates an impressive p value even when the observed improvement in discrimination may be of modest clinical significance.

COMPARISON TO OTHER STUDIES

Much attention has focused on the AUROC as a method for comparing the discriminative performance of two risk estimation functions[95, 256]. AUROC, which compares the tradeoff between sensitivity and specificity, was originally developed for the comparison of a diagnostic test with a gold standard. Therefore, this measure may not be the most appropriate for comparison risk estimation systems, which unlike diagnostic tests do not have yes/no answers regarding the presence or absence of disease. Because a large proportion of the ability to predict CVD outcomes is governed by gender and age alone, once even basic cardiovascular risk factors are included there is often little potential for the addition of other factors to improve risk estimation, as measured by AUROC. [16, 221] As detailed in the section on reclassification above, many previous studies have shown that the discrimination (as measured b AUROC) may not increase, although a risk factor may have an important and independent effect on the endpoint[94]. However, several studies have shown that the incorporation of extra risk factor may in fact improve risk

classification – which is particularly important clinically, as decisions are made based on these classifications, as detailed in **Table 1-7** on page 56.

NRI was used to determine the percentage that would be correctly reclassified to a different risk category using the HDL-C function [256]. Using this measure (NRI), we have demonstrated that the reclassification resulting from the incorporation of HDL-C in the risk function is in the net correct direction in all groups, when using the 2 category classification. In women from high risk countries a substantial proportion are correctly reclassified when HDL-C is included (0.115 in the 2 category classification). This clinically important NRI results mainly from a substantial number of cases being reclassified to a higher risk category. This improvement in NRI after incorporation of HDL-C has also been demonstrated by the Framingham group. [256]

COMPARING THE BETA COEFFICIENTS TO THOSE OF OTHER RISK ESTIMATION SYSTEMS.

The beta coefficients for HDL cholesterol for CHD events in men in CUORE and PROCAM were -0.011 and -0.032 per 1mg/dl increase in HDL cholesterol respectively, compared to -0.021 per 1mg/dl in this analysis, however, this was for CHD mortality not incidence. In the Framingham study the beta coefficients for log HDL (mg/dl) for CVD incidence were -0.71 and -0.93 in women and men respectively, compared to -1.34 and -0.81 in this analysis, again these are not fully comparable because the endpoint in our analysis is CVD mortality.

COMPARING RISK ESTIMATION USING TOTAL CHOLESTEROL ALONE & TOTAL AND HDL CHOLESTEROL SEPARATELY

In the original SCORE paper it was demonstrated that whether TC alone or TC/HDL ratio was included in the function made very little difference to the risk estimate; 79.0% of persons from high risk countries had the same risk estimate using the two versions and 98.2% had a risk that differed by no more than 1%. The reason for the lack of change in risk estimates seems to be related to the underlying risk of the study population. In the SCORE dataset the median age is 47 years of age, meaning that the majority of individuals are at low risk. In those at low absolute risk even factors associated with substantial relative risks will cause only minor changes in the absolute risk. In this analysis, the inclusion of HDL-C and TC as separate variables still resulted in only minor changes in absolute risk in the population overall, with only 6.5% changing their risk by 1% or more. However, the change in risk is much greater in those who have unusually high or low HDL-C levels, especially if they are already at intermediate risk, as illustrated in Figure 2.20 and Figure 2.21. Accurate risk estimation is particularly important in this intermediate risk category as this is the point at which clinical decisions regarding preventive measures are made.

WHY DOES THE INCLUSION OF THE TC/HDL RATIO IN THE ORIGINAL FUNCTION RESULT IN ONLY MINOR CHANGES IN RISK ESTIMATES?

Many epidemiological studies have demonstrated that TC/HDL ratio, as a single lipid measure has the best predictive ability for CVD outcomes. [139, 147, 172] Some of these have even found TC/HDL ratio to be superior to ratios involving apolipoproteins. [147, 172] It is difficult to understand then why risk estimates based on the SCORE function changes little when TC/HDL ratio was included instead of TC alone. It was not because HDL is not a risk factor in this population as I have demonstrated above.

I believe the reason is two-fold. Firstly, as discussed above, the calculation of the change in absolute risk in this predominantly low risk population has under-represented the impact of including TC/HDL ratio. Secondly, we have shown that entering the two lipid variables as part of a ratio does not fully represent the predictive ability of each. As detailed above, we have shown superior risk estimation using HDL cholesterol in addition to total cholesterol as opposed to as part of the ratio. This is in agreement with the findings of the prospective studies collaboration analysis which showed that although the best single lipid measure was the TC/HDL-C ratio, inclusion of the individual lipid sub-fractions separately in the model resulted in a further, if minor, improvement in predictive ability. [139]

We suggest that the reason why including HDL and TC separately results in an improved model compared to when both variables are included as part of the ratio is because entering HDL as its inverse (1/HDL) means imposing a non-linear relationship on the effect of HDL. This is demonstrated in Figure 2.32.

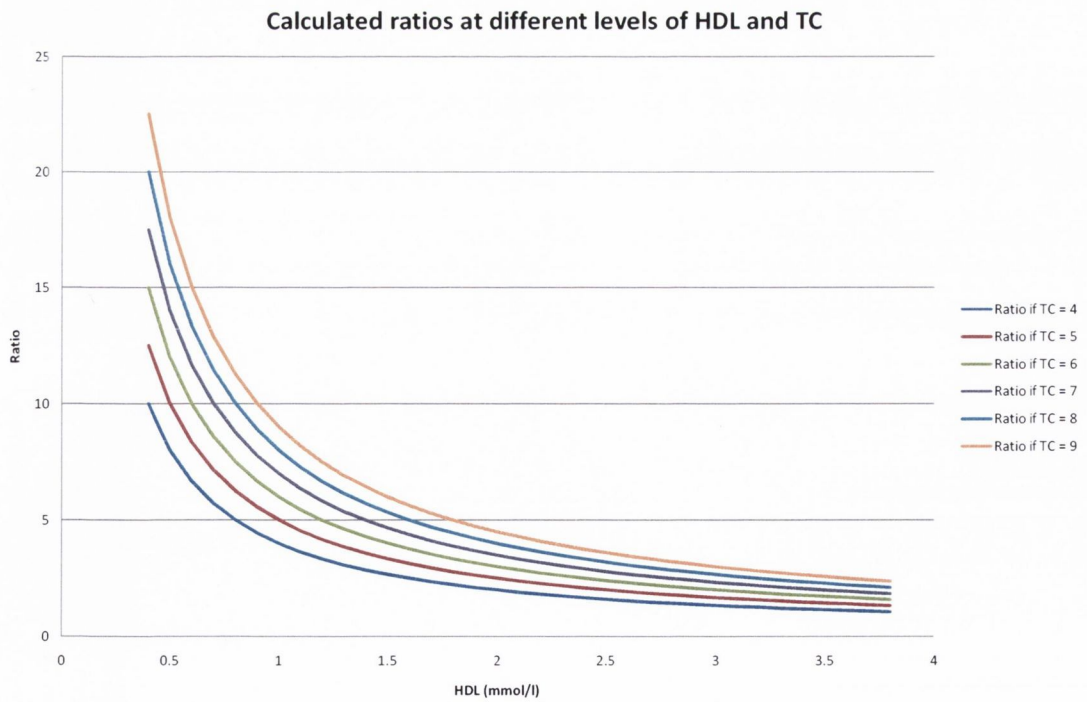


Figure 2.32: Illustration of the change in the TC/HDL ratio as HDL cholesterol level changes

It is also possible that the value of HDL (as part of the ratio) may have been underestimated in the original SCORE paper because beta-coefficients for the risk factors (including the TC/HDL ratio) were not gender-specific. Here, as shown in Table 2-23, gender specific beta coefficients were used and there were substantial differences between the HDL-C beta coefficients in men and women, especially in the case of the nCHD endpoint.

IMPLICATIONS FOR CVD PREVENTION IN CLINICAL PRACTICE

The decision regarding whether the routine use of HDL-C in risk estimation should be recommended in all or just in specific individuals is one for national and international guideline generating bodies, but our demonstration of the effect of including HDL-C will be useful. Other factors to be considered include the cost of measuring HDL-C and the need to use the computerised as opposed to the simple paper chart if HDL-C is included. We suggest that if HDL-C were incorporated into HeartScore, its inclusion would be optional, i.e. if HDL-C level was not available the risk would still be calculated using the original equation. The other option for simplifying the process is the use of the risk which indicates where low HDL cholesterol will change the risk category from low to high risk.

LIMITATIONS OF HDL-C ANALYSES

Some limitations of these analyses should be acknowledged. Laboratory methods for HDL-C measurement were not standardized between all cohorts and as single baseline measurements only were available we have not been able to adjust for regression dilution bias. However, both variation in laboratory measurements and regression to the mean would be expected to result in a dilution of the effect of HDL-C.

Some consider the use of CVD mortality only as the endpoint to be a limitation of the SCORE project. CVD mortality was specifically chosen as the endpoint because firstly it is a hard endpoint and secondly, easily standardized across countries. This also means that the country specific and updated versions of the function can be generated using easily available national mortality statistics [249, 273].

HDL – CONCLUSIONS

In summary, we have demonstrated a significant independent inverse relationship between HDL-C and CVD. The relationship was robust and significant in each age group and each SCORE category of total CV risk. There was a significant interaction between HDL-C and gender with a stronger effect in women. The indendent effect was demonstrated in each age group, including older women.

While incorporation of HDL-C as an extra variable in SCORE did not result in a meaningful improvement in risk estimation for the entire population, the option of incorporating HDL-C is important for individuals,

particularly in those close to the high/low risk threshold, in individuals who have unusually high or low HDL-C levels and in women from high risk countries.

CHAPTER 3 RESTING HEART RATE

In this section I note that resting heart rate is an easily obtained measure of CV health and review the evidence linking resting heart rate to outcome.

Specific research questions are defined on page 192.

Resting heart rate is one of the most easily obtained measures of cardiovascular health. Increased resting heart rate is associated with both increased total and cardiovascular mortality in the general as well as the coronary artery disease and hypertensive populations. Although resting pulse rate can be quickly and reliably assessed it is often overlooked as an index of cardiovascular risk. The existing evidence on the following points is discussed prior to detailing how the current analysis aims to add to this evidence:

- The evidence for an association between cardiovascular mortality and increased resting heart rate and its utility as a variable in estimation of cardiovascular risk.
- The possible mechanisms for the association between heart rate and mortality
- Whether the association is likely to be causal
- The likelihood of benefit from reducing heart rate
- The questions which remain unanswered regarding the relationship between resting heart rate and development of CVD

HEART RATE – EFFECT ON LIFE EXPECTANCY – ANIMAL STUDIES

ANIMAL STUDIES - INTER-SPECIES

Smaller mammals tend to have higher heart rates and shorter life spans than larger ones. There is a semi-logarithmic, inverse relationship between heart rate and life expectancy; as the heart rate of different mammalian species increases, the life expectancy decreases. Humans are the only exception.[274] This relationship has been graphically illustrated by Levine, as shown in Figure 3.1. One explanation for the association between body size and heart rate is that smaller body sizes require higher metabolic rates and consequent elevated heart rates.

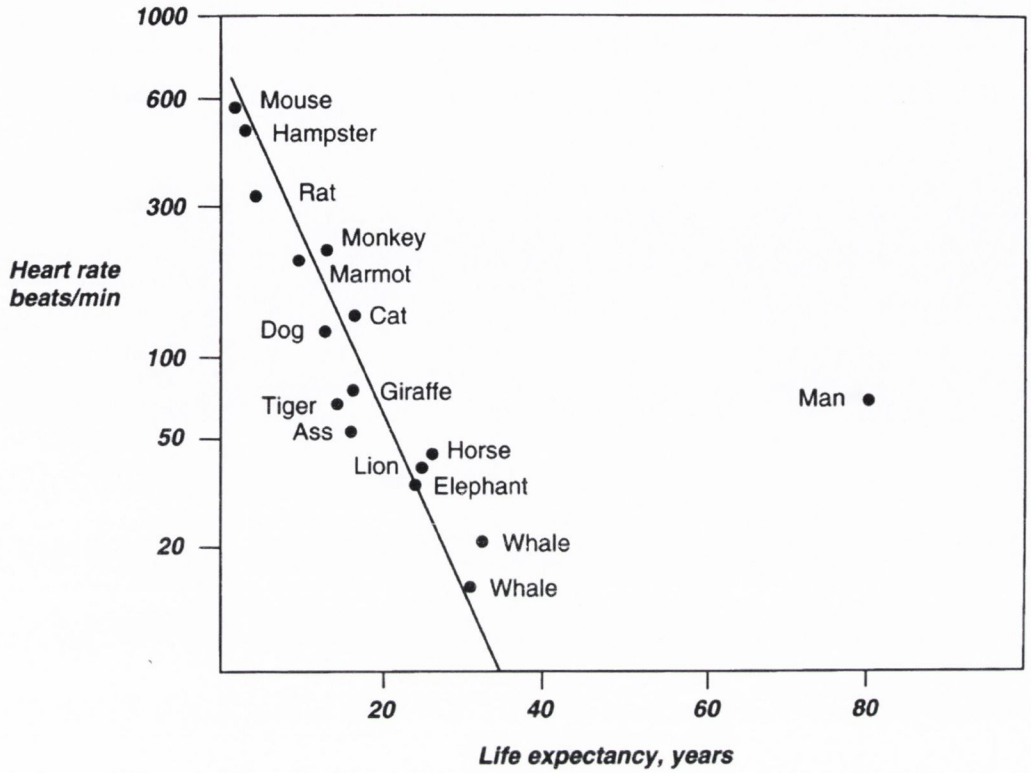


Figure 3.1: Relationship between average heart rate in mammalian species and their life expectancies, from [274] with permission

The number of heart beats in a lifetime is constant amongst the various species of mammal within one order of magnitude, average 7.4 +/- 5.6 x 10^8, despite a 40 fold difference in life expectancy. The number of heart beats per lifetime in each of the species of mammal in plotted against life expectancy in **Figure 3.2**.

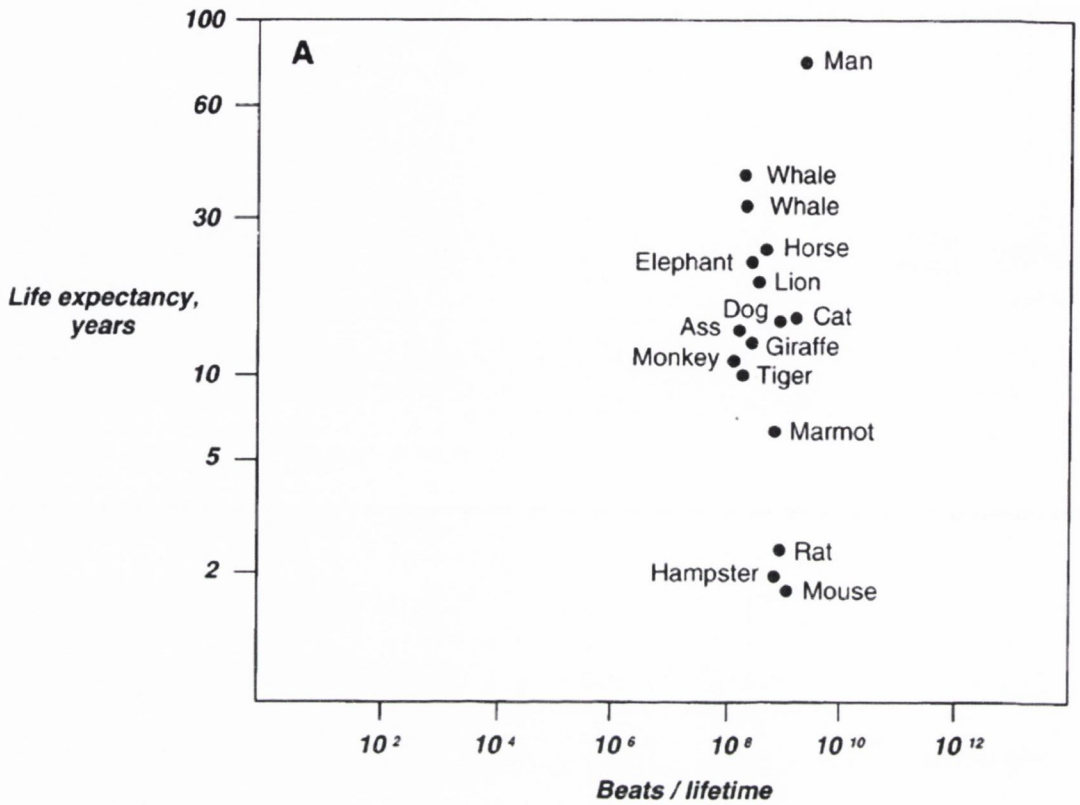


Figure 3.2: Average number of heart beats per lifetime in mammalian species, from [274] with permission

ANIMAL STUDIES – INTRA-SPECIES

If inter-species differences in heart rate determine longevity, the next question is whether heart rate also determines disease and life expectancy within a species. Kaplan et al studied the effect of propranolol on coronary artery atherosclerosis (CAA) in monkeys. [275] Fifteen monkeys were given propranolol mixed with an atherogenic diet and 15 controls were given an atherogenic diet only. All of the monkeys were examined for CAA after 26 months. Previous studies had shown socially dominant monkeys to be predisposed to the development of atherosclerosis when fed an atherogenic diet. Here, the dominant monkeys in the control group exhibited significantly exacerbated CAA. However, treated dominant monkeys did not develop exacerbated CAA. (Mean atherosclerosis 0.71mm2 in dominant untreated and 0.23mm2 in dominant treated (0.43 and 0.30 in treated and untreated subordinates respectively)). These effects were independent of blood lipids, blood pressure and resting heart rate. The authors conclude that propranolol reduces the development of atherosclerosis in behaviourally predisposed monkeys.

A similar study has been performed by Beere et al. [276] The effect of lowered heart rate achieved by means of sinoatrial node ablation on monkeys fed on atherogenic diet was assessed. The extent of coronary

atherosclerosis after 6 months was compared to that seen in control monkeys who underwent a sham operation. The monkeys with sinoatrial ablation had significantly lower heart rates, as seen on telemetry and significantly less atherosclerosis formation. The average lesion area in the low heart rate group was only one third of that seen in the high heart rate group, despite no difference in blood lipids, blood pressure or body weight.

HEART RATE – EFFECT ON MORTALITY AND CVD ENDPOINTS – EPIDEMIOLOGICAL STUDIES

EPIDEMIOLOGICAL STUDIES ON THE EFFECT OF HEART RATE – IMPORTANT POTENTIAL CONFOUNDERS TO CONSIDER

Many factors may potentially confound the relationship between cardiovascular risk and heart rate. Hypertension and heart rate are intrinsically linked; therefore it is important to include this as a covariate in the multivariate analysis. Another possible confounder is exercise, as exercise may contribute some of its beneficial effect on cardiovascular risk through heart rate reduction, but also has many other proven beneficial effects independent of heart rate, such as elevation of high density lipoprotein cholesterol.

EPIDEMIOLOGICAL STUDIES EXAMINING THE EFFECT OF HEART RATE ON OUTCOME IN THE GENERAL POPULATION

Several longitudinal studies have examined the effect of increasing heart rate on coronary heart and cardiovascular disease in the general population as well as subgroups of people including those with coronary artery disease, the elderly, hypertensives and diabetics. **Table 3-1** represents a summary of each of the main studies which have examined the effect of heart rate on cardiovascular endpoints in the general population. Specific note has been made in **Table 3-1** to indicate which of the studies have exercise/physical activity included as a covariate. Also, the odds ratio or relative risk has only been reported if it continued to be significant after multivariate analysis, which includes systolic blood pressure, unless otherwise stated.

Name and Year	Study Population	Number and Gender	Follow-up	Age Group	End Points	Effect of Elevated Heart Rate	Qualification of effect (CVD Mortality) (multivariate)	Covariates included/ Other
Flipiopvsky [277] 1992	Paris Prospective Study	4,907 men	17 years	Middle aged	Total mortality CV Mortality	Potential indep RF Not Sisnificant		Sports activity and BMI included
Jouven [278] 1996	Paris prospective Study	7,746 men	22 years	43-52	Fatal MI Sudden death	Indep RF Indep RF	1.22 (1.12-1.49) for each increase of 10.2bpm	No exercise covariate
Benetos[279] 1999	French IPC	12,123 men 7,263 women	17 years	40-69	Total Mortality CV Mortality	Preditor in both genders. Indep RF in men only	61-80: 1.35 (1.01-1.80) 81-100: 1.44 (1.04-2) >100: 2.18 (1.37-3.47) Compared to <60 Men only	Physical activity included. Subgroup analyses - effect persisted in: HTN and non-HTN, >65 and <65
Kristal-Boneh[280] 2000	CORDIS	3,527 men	8 years		CV Mortality	Indep RF	1.95 (1.1-3.8) >70 compared to <70	Sports activity included. Many haem factors included
Seccareccia[281] 2001	MATISS	2,533 men	9.7 years		CV Mortality	Indep RF	2.54 (1.25-5.16) >70 compared to <70	Arm circumference and adjusted FEV considered surrogates for exercise
Sharper[282] 1993	Brit Reg Heart Study	7,735 men	8 years	40-69	CHD Mortality Sudden Death	Indep RF Indep RF	3.3 (1.4-7.8) 5.2 (1.4-18.7) >90 compared to <60	Physical activity included. Persisted in subgroup HTN vs.non-HTN
Kannel[283] 1987	Framingham	5,070 men and women	30 years	35-64 and 65-94	CV mortality (2 year incidence)	Indep in men not women Related univariate	Standardised regression coefficients for effect of heart	No exercise/fitness covariate Effect persisted in men after exclusion of those with

Name and Year	Study Population	Number and Gender	Follow-up	Age Group	End Points	Effect of Elevated Heart Rate	Qualification of effect (CVD Mortality) (multivariate)	Covariates included/ Other
						in women also (gradients steeper in men)	rate on 2 year CVD mortality: Men (35-64) 0.288 (p<0.001) Men (65-94) 0.147 (p<0.05)	interim CAD development
Gillum[284] 1991	NHANES	5,136 white men and women	9.9 years		CV Mortality (whites)	RF in both – univariate Multivariate men only	White Men: 1.44 (1.08-1.92)	Authors report that inclusion of BMI and physical activity did not change results, figures not given
		859 black men and Women	10.3 years		CV Mortality (blacks)	RF in both – univariate Multivariate black women only	Black Women: 3.03 (1.46-6.28) >84 compared to <74)	
Reunanen [285] 2000		5598 men 5119 women	23 years	30-59	Total Mortality CV Mortality	Univariate assoc Univariate assoc but assoc no significant after addition of SBP to model		
Dyer [286] 1980	3 Chicago industry studies	1233 men	15 years	40-59	CHD	Not significant on multivariate analysis Indep RFin 2 of 3 studies		No exercise covariate
		1899 men	17 years	40-55				
		5784 men	5 years	45-64	Sudden death			
Greenland[287] 1999	Chicago Heart Assoc Detection	18,787 men 14,994 women	22 years	18-74 – divided into 3 age groups	CHD Mortality	Increased HR signifcantly associated with	RR per each increase of 12bpm (1	No exercise covariate

Name and Year	Study Population	Number and Gender	Follow-up	Age Group	End Points	Effect of Elevated Heart Rate	Qualification of effect (CVD Mortality) (multivariate)	Covariates included/ Other
	Project in Industry					CHD mortality in men 18-39 and 40-59, women 40-59. Only remained after addition of SBP to model in women 40-59 and men 18-39	standard deviation) Men 18-39: 1.20(1.02-1.42) Women 40-59: 1.13 (1.01-1.28)	
Okamura 2004		3856 men 4944 women	16.5years	30-59 Over 60	CV Mortality	Indep RF men 30-59 Indep RF women 30-59	Men: 2.55 (95%CI 1.22-5.31) comparing >74bpm to <60bpm Women: 3.61 (95%CI 1.34-9.72) comparing 70-77bpm to <60bpm	Albumin included No exercise covariate
Tverdal 2008[288]	Norwegian surveys	180353 Men 199490 Women	Mean 12.6 years	Mean age 41.4 years	CVD mortality	Independent RF for all cause mortality both genders. Independnet RF for CVD mortalirty in men only	<65 bpm compared to > 95 bpm: Men: 1.37 (1.19 to 1.59) Women: 0.78 (0.53 to 1.15)	No exercise covariable
Hsia 2009[289]	Women's Health Initiative	129 135 women	Mean 7,8 years	Mean age 62 years	Coronary events	Independent RF in women but blood pressure only	1.26 (1.11 to 1.42) comparing >76 bpm ≤62	Physical activity included & caffeine intake – but blood pressure only included as a

Name and Year	Study Population	Number and Gender	Follow-up	Age Group	End Points	Effect of Elevated Heart Rate	Qualification of effect (CVD Mortality) (multivariate)	Covariates included/ Other
						included as a dichotomous variable	bpm	dichotomous variable

Table 3-1: Epidemiological Studies investigating the relationship between heart rate and CVD endpoints in the general population

EPIDEMIOLOGICAL STUDIES – GENERAL POPULATION- MEN

Heart rate has been demonstrated to be an independent risk factor for the development of CVD in men, in the majority of the studies listed in **Table 3-1**. In a minority of the studies the effect was no longer significant after multivariate adjustment. This was the case in the Chicago Heart Association Detection in Industry study by Greenland et al[287] (except for the male subgroup aged 18-39) , the 3 Chicago Industry studies by Dyer et al[286], the first of the Paris Prospective studies by Flipipovsky et al [277] and the study in the Finnish population by Reunanen et al [285]. In the latter study, the authors point out that the association lost statistical significance after the addition of systolic blood pressure; this also occurred in the study by Greenland et al. In the NHANES study the effect of elevated heart rate on CVD mortality is significant on multivariate analysis in white, but not black men [284]. In another large study the hazard ratio for resting heart rate was attenuated by the addition of other risk factors, but it remained a statistically significant risk factor[288].

The finding of the association becoming non-significant with the addition of SBP into the model has prompted some to postulate that the effect of heart rate on CVD mortality may be mediated through hypertension. Two sources of evidence would dispute this. The first is the evidence from the other studies, in which heart rate continued to have an effect when SBP was included as a covariate. There are eight of these listed in **Table 3-1**. The second piece of evidence comes from investigations of the relationship specifically within the hypertensive population – both in studies specifically limited to the hypertensive individuals and in sub-group analyses. Shaper et al [282] and Benetos et al [279] performed such subgroup analyses in hypertensive and non-hypertensive groups and concluded that heart rate remained an independent risk factor in both (see **Figure 3.4** also). In an observational study restricted to hypertensive men, Gillman et al demonstrated an odds ratio for CVD mortality of 1.48 (95%CI 1.05-2.09) per 40bpm increase in heart rate, which was independent of other risk factors. [290] A large study by Thomas et al of 60,343 hypertensive men, showed the relative risk associated with heart rate greater than 80 to heart rate less than 80 to be 1.48 (95%CI 1.22-1.78) in men under 65 and 1.32 (95%CI 1.11-1.56) in men over 65 [291].

Of the eight studies in which heart rate is shown to be an independent risk factor, five contain physical activity (or surrogate markers for this) as a covariate in the multivariate analysis. This suggests that the effect of elevated heart rate on cardiovascular risk is not merely as a result of elevated heart rate acting as a proxy for sedentary lifestyle. However, it should be remembered that each of these included only a dichotomous indicator of physical activity and therefore may not have represented the full spectrum of physical fitness.

The odds ratios and relative risks shown in **Table 3-1** associated with increasing heart rate suggest that the risk of CVD mortality associated with increasing heart rate is both strong and graded. This is illustrated in **Figure 3.1**, which shows the adjusted relative risks of IHD and sudden death associated with various

categories of heart rate, as demonstrated in the study by Shaper et al [282]. The authors of the Framingham study also demonstrated a graded relationship and commented that there was no indication of any critical or threshold values which could be labeled as safe or hazardous[283].

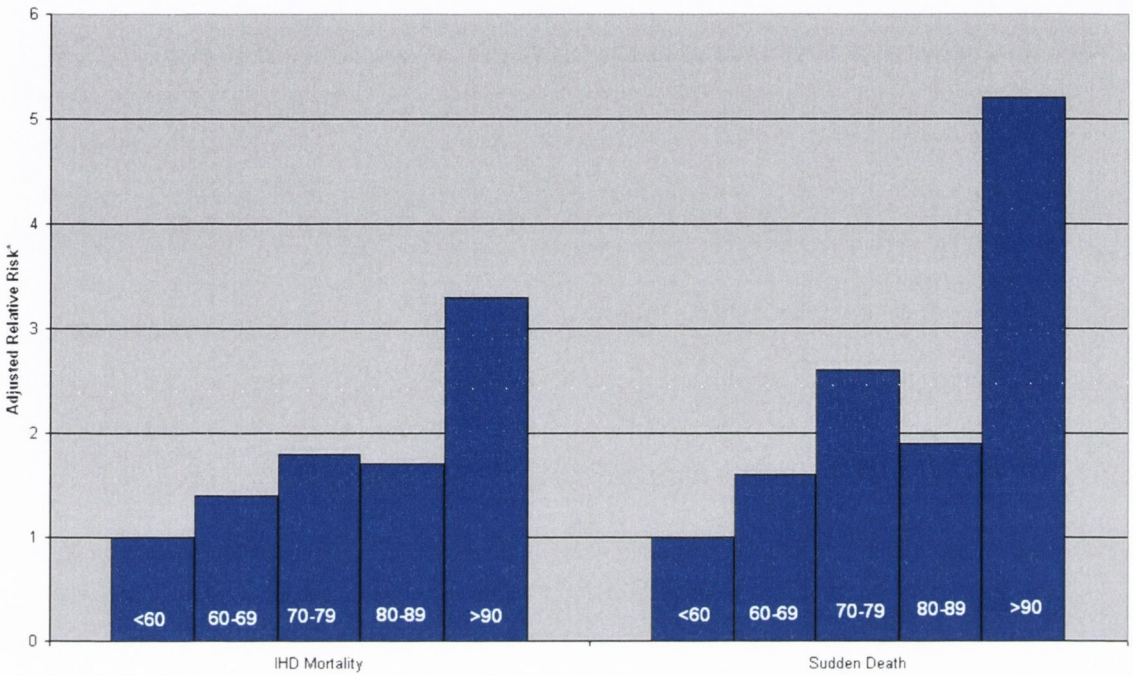


Figure 3.3: Graded relationship between increasing heart rate and outcome in men in British Regional Heart Study, from [282]

Another important factor to consider when assessing whether a risk factor is causal is the appropriate temporal sequence. This applies particularly in the case of heart rate where undiagnosed or sub-clinical heart disease may be causing an elevated heart rate, possibility due to compensation for a reduction in left ventricular systolic function. In this way reverse causality could be accounting for the association. In an attempt to rule out this possibility, the authors of the Framingham study re-analysed the relationship after exclusion of those who developed CHD in the interim [283] . The effect remained significant in this re-analysis.

EPIDEMIOLOGICAL STUDIES – GENERAL POPULATION- WOMEN

In women, the relationship between heart rate as a cardiovascular risk is less clear. In general, it is more difficult to demonstrate associations of risk factors to cardiovascular events and mortality in women. This may be in part because women develop cardiovascular disease on average 10 years later than men. This means that, at any given age, a longer follow-up time will be required for sufficient events to occur to demonstrate a significant relationship. Also, many of the older studies, especially those based on cohorts

derived from industry, did not include women. Of the studies listed in **Table 3-1**, only the Framingham study[283], the French IPC study[279], the NHANES study[284], the Chicago Heart Association Detection Project in Industry[287], the study by Reunanen et al[285], the large Norwegian study[288] and the Women's Health Initiative study[289] included women.

The Framingham study showed that total and cardiovascular death were, in general, related to increasing heart rate at all ages[283]. The risk gradient associated with increasing heart rate was steeper in men than in women for both total and cardiovascular mortality. After adjustment for other cardiovascular risk factors, the relationship to total, but not cardiovascular mortality, remained independent, in women. Of the studies listed in **Table 3-1**, only the NHANES study[284] (in black women only) and the Chicago study[287] (in women aged 40-59), actually demonstrated an independent effect in women. The analysis of the Women's Health Initiative by Hsia et al specifically set out to investigate whether RHR was an independent risk factor in healthy women, due to the previous lack of evidence for this, but it cannot be considered a true demonstration of this independence since hypertension was only adjusted for as a dichotomous variable and SBP as a continuous variable was not included in the model[289].

A study by Perk et al is interesting in this regard because it included women who were over the age of 70 at study entry and followed them for 6 years. [292] Four hundred and twenty two people were included. Only total mortality was analysed. The odds ratio comparing heart rate greater than 77bpm to less than this was 3.37 (95%CI 0.96-11.8) for total mortality, adjusted for previous CVD, hypertension, anaemia, congestive heart failure, smoking and level of exercise. This was statistically significant when those on B-blockers were removed from the analysis. (OR:8.5 (95%CI 1.19-60.1). The association was not significant in men and it should be noted that again blood pressure was adjusted for as a dichotomous rather than continuous variable.

Chang and colleagues studied the effect of increasing heart rate on mortality in a group of disabled (mobility or self care difficulty with mini mental test score > 18), older (>65) women). [293] The women were from the Women's Health and Aging Study 1 (WHAS1). The hazard ratio for total mortality comparing a heart rate >90 to 60-89, was 2.0 (95%CI 1.2-3.3). This was adjusted for age, disease status, cardiovascular risk factors, physical activity, and physical and pulmonary function. The same analysis was repeated in the subgroup with no previous clinical or electrocardiographic evidence of ischaemic heart disease. The hazard ratio here was 2.3 (95% CI 0.98 – 5.3), however it did not reach statistical significance. This lead to the suggestion that subclinical heart failure could be causing some of the effect and that controlling for ejection fraction would have been useful.

Gillman studied the association between increased heart rate and cardiovascular mortality in a group of 2,037 men and 2,493 women from the Framingham study who had hypertension [290]. On univariate analysis, increased heart rate was associated with increased risk of cardiovascular and all cause mortality

over the follow-up period of 34 years. After adjustment for age, SBP, cholesterol, cigarette smoking, glucose intolerance and left ventricular hypertrophy, increased heart rate was significantly associated with all cause mortality in men and women, but with cardiovascular mortality in men only.

PREDICTIVE ABILITY OF ELEVATED HEART RATE IN THE ELDERLY

Results of studies investigating the effect of heart rate as a risk factor in the older age group have been very variable. As discussed above some studies have shown an effect in older women[292] or in older women with pre-existing CHD[293]. The Cardiovascular Study in the Elderly (CASTEL, [187]) evaluated the effect of increasing heart rate on cardiovascular mortality over 12 years of observation in 763 men and 1175 women over the age of 65. An elevated heart rate was found to be an independent predictor of cardiovascular mortality in older men but not in older women. The relative risk of cardiovascular mortality in men associated in the fifth quintile of heart rate compared to the 3 intermediate quintiles was 1.38 (95%CI: 0.94 – 2.03, p = 0.005). This was adjusted for age, BMI, TC, HDL-C, triglycerides, glucose, uric acid, creatinine, FEV1, CHD, CHF, diabetes, hypertension, intermittent claudication, history of CVA, sedentariness, alcohol intake, smoking and regular medication.

Palatini et al addressed whether this association held in hypertensive elderly patients.[294] In a group of elderly people (1557 men, 3138 women) with untreated systolic hypertension, elevated heart rate was associated with both total and cardiovascular mortality. Multivariable analysis was only undertaken in men and women combined. Comparing those with heart rate >79bpm to those less than this, the hazard ratios were 1.88 (95%CI 1.33-2.67, p<0.01) and 1.60 (95%CI 0.99 – 2.58, p=0.05) for total and cardiovascular mortality, respectively. These figures were adjusted for sex, age, smoking, drinking status, SBP, previous CVD, diabetes, haemoglobin. Further adjustment for other lipid and haematological parameters as well as BMI did not change the results. Interestingly, ambulatory heart rate measurement did not add prognostic information to that provided by clinic heart rate. The authors comment that the univariable results were similar in older men and older women.

The Chicago Heart Association Study listed in **Table 3-1** included a group of men and women aged 60-74 years [287]. Elevated heart rate was not an independent risk factor in this age group. The Framingham study also analysed the effect by age groups (34-64 and 65-94) and the independent association seen held in men in both groups.

As demonstrated above, the results of studies investigating the effect in women and in the elderly are not in complete agreement. Differences in study populations, lengths of follow-up, adjustment for potential confounders and methods for endpoint ascertainment may be responsible for the different results and conclusions.

THE EFFECT OF ELEVATED HEART RATE ON RISK OF SUDDEN DEATH IN THE GENERAL POPULATION

Some of the studies in **Table 3-1** have looked specifically at the risk of sudden death associated with elevated heart rate. The mechanisms by which elevated heart rate predisposes to sudden death are unclear. An obvious explanation would be that an elevated heart rate is associated with other risk factors including physical inactivity, lipoprotein abnormalities and previous cardiovascular health. However, in the case of the study by Shaper et al[282], all of these were included in the multivariate analysis. Here an adjusted relative risk for sudden death of 5.2 (95%CI 1.4-18.7) comparing those with heart rate <90bpm to heart rate >90bpm was demonstrated. A subgroup analysis in those with and without hypertension was also performed. Elevated heart rate was associated with an increased risk of sudden death in both groups, as shown in **Figure 3.4.** The combination of elevated heart rate and hypertension gave rise to a relative risk of 6.0 (95% CI 2.4-15.2). The effect of elevated heart rate on increasing risk of sudden death was also demonstrated in men in the CASTEL study[187] and in men in the Framingham study[283], as described above.

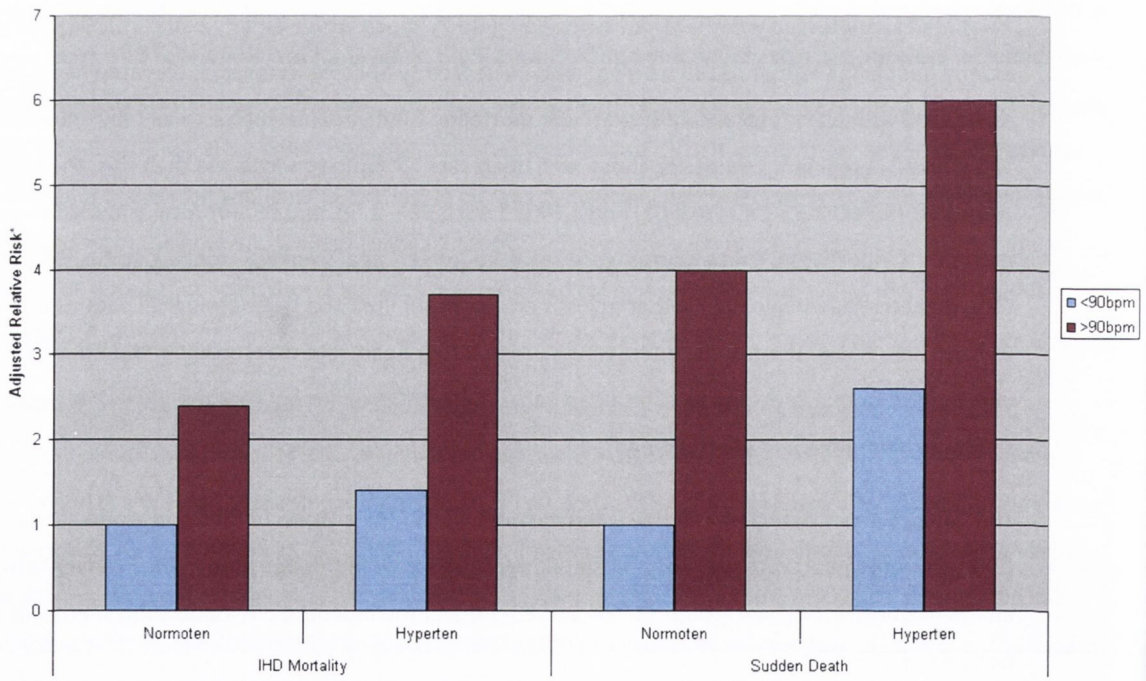


Figure 3.4: Effect of elevated heart rate (<90 bpm) on IHD mortality and sudden death in hypertensive and non-hypertensive men in the British Regional Heart Study, from [282]

EPIDEMIOLOGICAL STUDIES - EFFECT OF ELEVATED HEART RATE IN CHD PATIENTS

Table 3-2 describes the studies which have examined the effect of elevated heart rate in people who have pre-existing CHD. These studies show considerable heterogeneity in methods with some looking at the effect on 30 day or in hospital mortality in patients directly after acute myocardial infarction and others looking at long term prognosis in those with stable CHD.

One important factor in assessing the relationship in this population is the fact that larger infarcts with consequent reduction in left ventricular function will be associated with increased heart rate. Therefore any relationship between elevated heart rate and poorer outcome could be confounded by this; elevated heart rate could be acting as a proxy for reduced left ventricular function. The correlation between worsening degrees of heart failure and increasing heart rate is well established[295]. Some of the studies include ejection fraction as a covariate in the multivariate analysis; note has been made of this in **Table 3-2**.

Author and Year	Study Group	Number	Follow-up	Endpoint	When heart rate taken	Odds Ratio/Relative Risk	Covariates	Subgroup analyses/Other
Hathaway[296] 1998	GUSTO-I trial	1081 patients Acute STEMI	30 days	30 day mortality	Admission heart rate – independent risk factor, U-shaped relationship	1.49 (1.41-1.59) 84bpm compared to 60bpm	Ejection fraction or infarct size are not included in multivariate analysis, Killip class is included.	Includes a nomogram for predicting outcome, heart rate is a variable.
Copie[297] 1996		579 Acute MI	2 years	Sudden Death Non-sudden CV Death	Mean predischarge heart rate on 24hr monitor	HR superior to LVF in predicting sudden death, HR variability superior for sensitivity >40%, same for sensitivity <40%. Non-sudden death, all three predict equally		
Disegni[298] 1995	SPRINT	1044 patients with MI	1 year	In-hospital mortality 1 year mortality	Admission heart rate independent RF for both	In-hosp mortality: 1.36 (1.08-1.72) 1 year mortality 1.45 (1.15-1.84) per 15bpm increase	Severity of heart failure, cardiomegaly on chest x-ray, 4x normal limit of CPK and LDH included as covariates	Subgroups: assoc significant only in mild CHF. Excess 1 year mortality in HR >90, identical in men and women
Diaz[299] 2005	CASS	24,913 stable CHD (proven or suspect) Men and women analysed together	14.7 years	CV Mortality	Baseline HR	1.31 (1.15-1.48) >83 compared to <62	Recreational activity Diuretics B-blockers Antiplatelet meds Lipid-lowering meds No. diseased coronary vessels Ejection fraction	Extensive subgroup analysis for total mortality: assoc held in all groups: men vs women, <65 vs >65, HTN vs non-HTN, DM vs non-DM, BMI>27 vs BMI<27, EF<50% vs >50%, B-blockers vs.

Author and Year	Study Group	Number	Follow-up	Endpoint	When heart rate taken	Odds Ratio/Relative Risk	Covariates	Subgroup analyses/Other
								non B-blockers
Wong[295] 1989	Framingham	464 male and 233 female patients post MI	9.7 years	Reinfarction CHD Mortality	Heart rate approx 1 year after MI	Elevated HR significantly increased risk of coronary death, not reinfarction on univariate but not multivariate analysis		
Hjalmarson[300] 1990		1,807 patients less than 12 hours post MI	At least 3 months (1,410 – 1 year)	All Cause Mortality	Admission HR	Independent RF – no RR P= 0.004	Degree of heart failure, Age, Max BUN, Max CK, Prev AMI, Prev CHF	

Table 3-2: Epidemiological studies examining the effect of heart rate on outcomes in individuals with established coronary heart disease

Firstly, let us consider the studies looking at heart rate in acute MI patients. DiSegni et al assessed the effect of admission heart rate in 1044 acute MI patients.[298] Admission heart rate was shown to be an independent risk factor for in hospital and 1 year mortality. An increase of 15bpm in admission heart rate was associated with a hazard ratio of 1.36 (95%CI 1.08-1.72). This was independent of factors including, previous MI, diabetes, SBP, anterior MI, severity of CHF, cardiomegaly on CXR, CPK elevation (4xupper limit), LDH elevation (4xupper limit). While clinical markers of severity of heart failure were included, ejection fraction was not. Interestingly, in subgroup analyses of none, mild, moderate and severe CHF, elevated heart rate was only significantly associated with increased mortality in mild CHF. Hathaway et al studied admission heart rate in a similar number of acute MI patients. Again, elevated HR was an independent risk factor for 30 day mortality. However, only a clinical marker of heart failure (Killip class) was used as a covariate in the multivariate analysis. In summary, elevated admission heart rate is clearly related to increased short and intermediate term outcome in acute MI patients, but whether this association could be due to confounding is uncertain.

A study by Diaz et al of a large number of stable patients with proven or suspected coronary heart disease[299] is noteworthy for several reasons. Twenty-four thousand nine hundred and thirteen subjects were followed for a median of 14 .7 years. Several factors were included as covariates including ejection fraction as a continuous variable, number of vessels affected and several medical treatments as detailed in **Table 3-2**. The hazard ratio for HR >83bpm compared to < 62bpm was 1.31 (99%CI 1.15-1.48), indicating elevated heart rate is an independent risk factor for cardiovascular death in the long term in this population. Extensive subgroup analysis was done on the relationship between total mortality and elevated heart rate. The association held in each, including those with ejection fraction greater and less than 50% and those on beta-blockers and not on beta-blockers. In a previous smaller study, Wong demonstrated that elevated heart rate was associated with increased risk of CHD mortality but not non-fatal re-infarction in the long term.[295] This relationship was not statistically significant in multivariate analysis.

HEART RATE – SCIENTIFIC MECHANISMS

It has been suggested that the association between elevated heart rate and both vascular and non vascular mortality may be explained by lack of physical fitness and poor general health. However, many of the studies quoted above which have shown an effect have adjusted for these possible confounders. This, coupled with the fact that numerous trials have shown a benefit from heart rate reduction in those with CHD, suggest the need to look for mechanisms whereby heart rate may relate to the development of disease and hence prognosis directly.

The mechanism by which heart rate lowering protects the myocardium is not yet fully elucidated. However, it has been postulated that a low heart rate may be exerting its effect in a number of ways including protecting from ischaemia and arrhythmias as well as protecting from atherogenesis and plaque rupture

Heart rate is a critical determinant of myocardial oxygen consumption in patients with CHD. Other factors are contractility and end systolic stress. Beta-blockers, because they have negative inotropic as well as negative chronotropic effects, reduce myocardial oxygen demand through all of these mechanisms. Therefore, we cannot conclude that the benefits of beta-blockade are directly due to heart rate reduction (as will be discussed below).

Reduction of heart rate and consequent reduction of myocardial oxygen demand is one of the main proposed mechanisms for the beneficial effect of heart rate reduction in CHD patients. Additionally, reduction of heart rate results in prolongation of diastole with consequent enhancement of myocardial perfusion.

It has been demonstrated that the autonomic nervous system plays a critical role in the genesis of sudden cardiac death. Sympathetic activation is known to promote the occurrence of life-threatening ventricular arrhythmias, whereas increased vagal tone exerts a protective and anti-fibrillatory effect.[301, 302] This is likely a factor in the particularly strong association between elevated heart rate and sudden death.

Elevated heart rates have been shown to increase the progression for atherosclerosis in both humans and animals, as described above. A mechanism for this may be the haemodynamic effects of increased heart rate. It is known that heart rate, along with systemic blood pressure, modulates flow velocity and shear stress oscillation. Alterations in both of these haemodynamic forces may predispose to the development of coronary atherosclerosis. It also seems logical that higher heart rates predispose to the formation of atheroma simply increasing the number of shear stresses to which the artery is exposed per minute.

It has been suggested that elevated heart rate may be involved in coronary plaque disruption; the central pathophysiological mechanism underlying acute coronary syndromes and the progression of coronary atherosclerosis. Heidland et al retrospectively analysed 106 patients who underwent coronary angiography twice within 6 months. [303] Fifty-three patients were patients with initially smooth stenoses, who developed plaque disruption by the time of the second angiogram. These were matched with 53 individuals with initially smooth stenoses who did not have evidence of coronary plaque disruption on the second examination. Logistic regression identified positive associations between plaque disruption and elevated heart rate (as measured on 24 hour monitor at time of first angiogram), left ventricular mass above 270g and a negative association with the use of β-blockers. It should be noted that other cardiovascular risk factors were not included in the multivariate model. The authors point out that blood pressure was similar in both groups initially, but higher in the disruption group at the time of the second angiogram.

One of the Chicago studies showed a U-shaped relationship between heart rate and sudden death[286], indicating that bradycardia is also associated with sudden death; this is probably as a result of conduction abnormalities. Baseline ECG conduction abnormalities were excluded from the analysis in many of the other studies including CASTEL[187] and presumably for this reason, did not show this association with bradycardia.

HEART RATE – ASSOCIATIONS WITH OTHER RISK FACTORS INCLUDING METABOLIC SYNDROME

Singh et al [304] and Martin et al [305] have studied the relationship between other cardiovascular risk factors and elevated resting heart rate. Singh found that both genetic and environmental factors (including body mass index, systolic and diastolic blood pressure, smoking, and alcohol consumption) are associated with elevated resting heart rate. Martin et al has estimated the heritability of resting heart rate to be 26%, and obtained significant evidence of linkage for resting heart rate on chromosome 4q. These authors also demonstrated that those with elevated resting heart rate, especially females, tend to have higher glucose and insulin levels. Their mean values for waist circumference, triglycerides, body mass index, systolic and diastolic blood pressure also tended to be higher combined with lower HDL cholesterol levels. This complex is easily recognized as the metabolic syndrome, prompting the question - is elevated heart rate part of this syndrome?

A prospective study by Shigetoh and colleagues showed an independent association between higher heart rates (>80 bpm) at baseline and the future development of obesity, diabetes and insulin resistance after 20 years of follow-up [306]. This group had previously demonstrated the cross-sectional association between elevated heart rates and components of the metabolic syndrome.

A recent review by Cook et al has suggested the possibility that reduced bioavailability of nitric oxide may be involved in the pathogenesis elevated resting heart rate.[307] Much evidence exists which describes the role of nitric oxide as a mediator of cardiac autonomic control. For example, it has been demonstrated in both animals and humans that nitric oxide augments vagal tone and has an inhibitory effect on the sympathetic nervous system.[308-310] Given the previous demonstration of an association between elevated heart rates and sympathetic overactivity[311], the possibility of decreased bioavailability of nitric oxide as an aetiological factor in the pathogenesis of elevated heart rates seems reasonable. Furthermore, a substantial body of evidence links sympathetic overactivity to insulin resistance and the metabolic

syndrome[312], which adds weight to speculation that elevated heart rate is related to metabolic syndrome.

Clearly, elevated heart rates are associated with other cardiovascular risk factors, including the components of the metabolic syndrome. However, much work is still required to dis-entangle the complicated inter-relationships between heart rate, sympathetic overactivity and the bioavailability of nitric oxide and to investigate whether these could be related to the pathogenesis of the metabolic syndrome. The possibility also exists that the relationship is confounded by the fact that those with higher heart rates tend to be less physically active – a characteristic known to be associated with the development of the metabolic syndrome.

DOES HEART RATE REDUCTION RESULT IN CLINICAL BENEFITS?

As discussed above, elevated heart rates are related to future development of CHD. However, in order to assess if the relationship is causal it is necessary to examine whether treatment of the risk factor (heart rate reduction) as associated with clinical benefits.

B-BLOCKADE IN CORONARY ARTERY DISEASE PATIENTS

Beta adrenergic blocking drugs are now established therapy in both stable coronary heart disease and heart failure. One of these main mechanisms of action is heart rate reduction. Both the European and American guidelines[313] on the treatment of stable angina stress the importance of B-blocker therapy patients post MI. The American guidelines specifically suggest the up-titration of the dose of B-blocker to achieve a resting heart rate of 55-60bpm, or less than 50 in severe angina provided there are no contraindications. [313] The use of beta-blockade in post MI and heart failure patients is considered a class 1 recommendation with a level of evidence of A.

A recent meta-regression analysis of the effects of different B-blockers on mortality in patients post myocardial infarction found non-significant benefits of acute treatment, but a significant 24% relative risk reduction in mortality with long-term secondary preventive treatment, see **Table 3-3**. [314]

A meta-analysis of the effect of β-blocker therapy in stable heart failure (mainly NYHA class II or III) was undertaken by Brophy et al in 2001. [315] Twenty-two randomised placebo controlled trials were identified, all of which had mortality as an outcome and a follow-up period of greater than 3 months. Trials using β-blockers with intrinsic sympathomimetic activity were excluded. Most of the trials used metoprolol,

bisoprolol or carvedilol. Total mortality and rate of hospitalisation were significantly reduced in the group receiving β-blocker therapy, see **Table 3-3** .

Outcome Measure	Post myocardial Infarction[314]	Congestive Cardiac Failure[315]
Odds ratio for total mortality	0.76 (95%CI 0.70, 0.83)	0.65 (95%CI 0.53, 0.80)
Lives saved per 100 treated for 1 year	1.3 (95%CI 0.7, 1.8)	3.8 (95%CI 2.1, 5.3)

Table 3-3: Results of meta-analyses of effects of Beta Blocker therapy in post myocardial infarction and congestive cardiac failure patients

We should now examine the evidence that the beneficial effect of beta-blockade in these two patient populations is due, at least in part, to heart rate reduction.

A study by Thackray et al published in 2006 is particularly interesting as the objective of the study was to determine whether the beneficial effect of B-blockers on ventricular function in patients with cardiac failure is mediated through reduction of heart rate.[316] A group of 49 pacemaker-dependent patients, with symptomatic left ventricular dysfunction (EF 26%+/-9% at baseline) were randomized to either lower rate (60bpm) pacing or higher rate (80bpm) pacing. All of the patients were receiving beta-blocker treatment. Mean LV end-diastolic and systolic volumes increased with higher-rate versus lower rate pacing, whereas LV ejection fraction declined. All of these results were statistically significant. The authors concluded that reversal of beta-blocker-induced bradycardia has deleterious effects on ventricular function, suggesting heart rate reduction is an important mediator of their effects. It should be noted that the numbers completing the study protocol were small, 12 in higher rate group and 13 in the lower rate group.

A recent meta-regression by Cucherat et al investigated the effect of heart rate reducing medication (beta-blockers and calcium channel blockers) in patients post MI on the risk of all-cause mortality, cardiac death, sudden death and reinfarction [317]. Of 25 randomized controlled trials evaluating the effect of long term B-blocker or calcium channel blocker treatment in patients post MI, 17 gave information on both heart rate reduction and mortality. Trials achieving greater reductions in heart rate were associated with greater reductions in risk. The inverse relationship between heart rate reduction and log odds ratio was statistically significant for each of the endpoints: all-cause mortality, cardiac death, sudden death and reinfarction. The meta-regression illustrating the relationship between all-cause mortality and magnitude of heart rate reduction is shown in **Figure 3.5**. Each 10bpm reduction in the heart rate is estimated to reduce the odds ratio of cardiac death by about 26%.

In an earlier analysis, Kjekshus [318] also noted an approximately linear relationship between the mean reduction in heart rate achieved in each trial of beta-blockade in post MI patients and beneficial effect of treatment on long-term outcome, in terms of both mortality and recurrent MI.

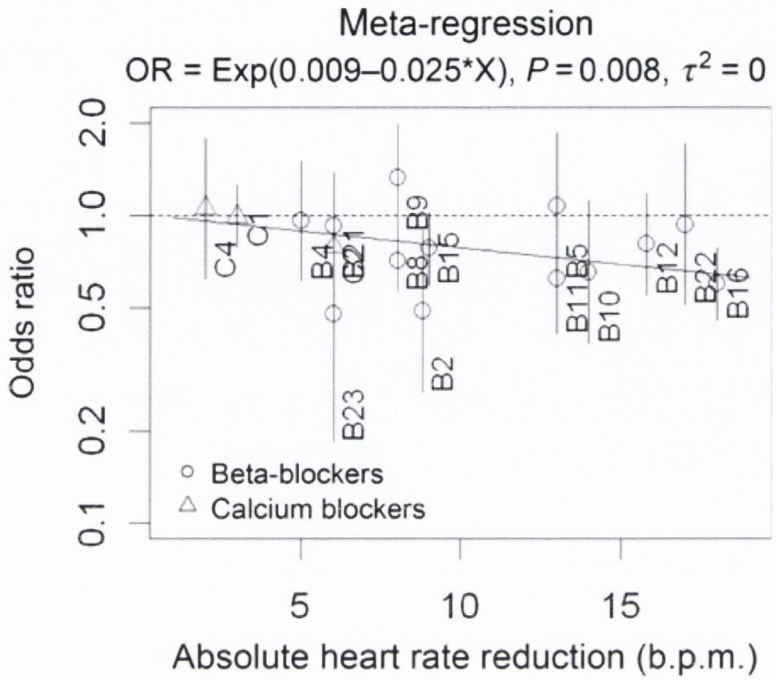


Figure 3.5: Meta-regression - effect of beta-blocker or calcium channel blocker therapy on all-cause mortality

EFFECT OF PURE HEART RATE REDUCTION BY IVABRADINE - A NEW SELECTIVE IF INHIBITOR

I_f, a mixed Na and K inward current activated by hyperpolarisation and modulated by the autonomic nervous system, is one of the most important ionic currents for regulating pacemaker activity in the sinoatrial node. Ivabradine is a novel specific heart rate lowering agent that acts in sinoatrial node cells by selectively inhibiting the pacemaker I_f current in a dose-dependent manner. It is unique in that it appears to be a pure heart rate lowering medication without other effects.

The BEAUTIFUL trial [319] recently added to the evidence for the benefit of heart rate reduction in stable coronary artery disease patients with left ventricular systolic dysfunction (ejection fraction < 40%). The 10,917 individuals included were randomized to pure heart rate reduction with ivabradine or placebo. Ivabradine reduced heart rate by 6 bpm (SE 0.2) at 12 months. Of note, a similar percentage of the placebo and active treatment groups were on beta-blocker treatment. While treatment with ivabradine did not affect the primary endpoint of the trial, in a pre-specified subgroup with baseline heart rates > 70 bpm, the secondary endpoint of risk of hospitalization for fatal or non-fatal myocardial infarction was significantly reduced (0.64, 95% CI 0.49-0.84, p=0.001) over a median follow-up period of 19 months.

This suggests that heart rate reduction in this patient population results in reduced risk of recurrent MI, irrespective of the method of heart rate reduction. The benefit of heart rate reduction in the general population has not yet been investigated.

Is the relationship between heart rate and CVD and total mortality one of cause and effect?

Based on the evidence presented above, a summary of how well heart rate fulfills each criterion for causality is presented in **Table 3-4**. Each criterion is given a score (0 – minimum, 3 – maximum) to indicate the weight of evidence supporting fulfillment of that criterion. For comparison, total cholesterol - a well established cardiovascular risk factor is assessed in the same way.

	Heart Rate	Total Cholesterol
Biologically plausible	2	3
Strong	3	3
Temporal Sequence	2	3
Graded	3	3
Independent	2	3
Consistent	1-2	3
Agreement between disciplines	2	3
Treatable	3	3
Benefit Results	1-2	3

Table 3-4: Fulfilment of causal criteria by heart rate as a risk factor for CVD

ROLE OF RESTING HEART RATE IN RISK ESTIMATION SYSTEMS

Currently, total risk estimation systems are used widely in clinical practice in guiding primary prevention measures. For example, the SCORE and Heart Score risk estimation systems[4] are recommended by the European guidelines on CVD prevention. The question arises as to whether resting heart rate should be included in risk estimation systems. The major components of current risk estimation systems- smoking, blood cholesterol and blood pressure- are not only risk factors but are modifiable risk factors whose modification has been proven to be associated with benefit, in the general population. Resting heart rate does not fall into this category. However, inclusion in risk estimation systems could still be warranted if shown to improve risk estimation, particularly if it improves risk classification in those at the borderline of high / low risk.

Recently, attention has focused on simplifying risk estimation systems. Systems have been developed which estimate risk based on only non-laboratory measured variables. These have the advantage of improving accessibility and cost effectiveness of risk estimation. They may be particularly useful in regions of the world with reduced access to medical facilities. In general, to date, these have focused on the inclusion of BMI in place of lipid variables. A further step in simplifying the process would be to substitute RHR for the blood pressure variable. RHR as a variable which is measured particularly easily is an ideal candidate for

inclusion in these simplified risk estimation systems. Such a system could be used outside the clinic, because the only equipment required is a weighing scales, or possibly even in the individual's own home as a form of self-assessment of risk.

HEART RATE – UNANSWERED QUESTIONS REGARDING RESTING HEART RATE

- Is there an independent effect of RHR on outcomes in women in the general population
- Is the effect of RHR on outcome independent of risk factors, especially systolic blood pressure as a continuous variable and physical fitness
- Is the effect of RHR due to a confounding effect of those with other co-morbidities and chronic diseases tending to have higher RHRs?
- Is the temporal sequence of the relationship between RHR and outcome consistent with a causal relationship or is the effect due to reverse causality?
- Can inclusion of RHR in risk estimation systems result in meaningful improvements?
- Does reduction of RHR in the general population, by conservative or pharmacological means, result in a benefit in terms of CVD and life expectancy?

HEART RATE – SPECIFIC RESEARCH QUESTIONS

1. What is the effect of resting heart rate level on risk? – including total mortality, cardiovascular mortality, coronary heart disease mortality and stroke mortality
2. What is the relationship between resting heart rate and non-fatal events?
3. Does the effect apply equally in men and women?
4. Does the effect apply equally in those with and without pre-existing hypertension?
5. Is this effect independent of the effect of other CV risk factors, particularly other conventional cardiovascular risk factors, measures of physical fitness and measures of general fitness and co-morbidities?
6. What other factors are associated with elevated resting heart rate?
7. Are the criteria for causality met?
8. Will development of a SCORE risk estimation system with resting heart rate as an additional variable result in a significant improvement in our ability to estimate risk of CVD? Improvement would be assessed based on measures of calibration, discrimination and the numbers corrected classified into high and low risk groups.
9. What level of risk estimation can be provided by a risk estimation system containing only easily measured variables such as heart rate, BMI, age, sex and smoking status?

HEART RATE – STUDY POPULATION

FINRISK – SAMPLING, SURVEY AND DATA COLLECTION METHODS

FINRISK is a large prospective population-based observational study[84]. Full details of the methodology have been described elsewhere [84]. Briefly, collection of baseline data began in 1972. Subsequent studies began in 1977, 1982, 1987, 1992, 1997, 2002 and 2007. Initially, surveys were conducted in the North Karelia and Kuopio provinces, with inclusion of southwestern Finland, northern Finland and the capital region in later years. Random, independent and representative population samples were drawn from population registers. Initially, those aged 30 to 59 years were recruited. Subsequently, the age range was extended, with the 1997 and subsequent studies including individuals aged 25 to 74. Only the 1977-1997 surveys are included in this report.

The survey methods followed the WHO MONICA protocol from 1982[255]; these were comparable with the methods used in 1972 and 1977. The detailed methodology has been discussed elsewhere[84, 255].

The surveys included a self-administered questionnaire, which included questions on medical history, health behaviours including medications, smoking history and physical activity. For this analysis, current smokers were defined as currently smoking or having quit less than six months previously; non-current smokers were defined as those who never smoked and those who quit smoking more than six months previously.

A validated[320] assessment of leisure time physical activity was employed. Participants were asked the following question: How much do you exercise and stress yourself physically in your leisure time? The answers were coded as follows:

1. In my leisure time I read, watch television, and work in the household with tasks which do not make me move much and which do not physically tax me
2. In my spare time I walk, cycle or exercise otherwise at least 4 hours per week. This includes walking fishing and hunting, light gardening etc. but excludes travel to work.
3. In my spare time I exercise to maintain my physical condition, for example, running, jogging, skiing, gymnastics, swimming, playing ball games or I do heavy gardening or the like for at least 3 hours per week.
4. In my spare time I regularly exercise competitive-wise several times a week, orienteering, skiing, swimming, playing ball games or other heavy sports.

For the purpose of these analyses, answers three and four were combined, due to small numbers (particularly of women) in the fourth category. Leisure time activity was chosen over activity at work as this has been shown to correlate better with CVD risk factors[321].

Participants also underwent an examination including measurement of weight, height and blood pressure. Resting heart rate (RHR) was measured by palpation of the radial artery pulsation over 30 seconds, in the sitting position after 5 minutes rest. Waist circumference was measured also from 1987 onwards. A venous blood sample was taken for measurement of total cholesterol and additionally HDL cholesterol from 1982 onwards. Triglycerides were also measured in the 1972, 1992 and later surveys. All cholesterol determinations were made in the same central laboratory.

Follow-up to end of 2003 is available and was collected in accordance with MONICA methodology[322]. The follow-up was based on the mortality register by Statistics Finland, which is linked to the risk factor surveys using social security numbers assigned to every citizen of Finland.

HEART RATE METHODS

END POINT DEFINITIONS

The eight, ninth and tenth revision of the International Classification of Diseases (ICD) were used to identify CHD mortality, defined as ICD 9 codes 410-414 and CVD mortality, which included in addition 401-409, 426 – 443, 798.1 and 798.2, with exclusion of the following definitely non atherosclerotic causes of death: 426.7, 429.0, 430.0, 432.1, 437.3, 437.4, 437.5. The corresponding ICD8 and 10 codes were used. These correspond to the definition of endpoints in the SCORE project[4]. Nonfatal myocardial infarction (MI) was defined as ICD9 codes 410-411 and corresponding ICD8 and 10 codes. The endpoint any CHD event includes both nonfatal MI and CHD mortality.

EXCLUSIONS

Those with previous myocardial infarction were excluded as in the SCORE project. Those with pre-existing angina were excluded due to the possibility that they would have been on treatment with heart rate modifying medications. Those with previously diagnosed heart failure also were excluded from the analysis as reduced left ventricular function may cause an elevation in heart rate, which would confound the role of RHR in determining CVD risk. Those on antihypertensive medications were also excluded, because these medications may have modified RHR.

STATISTICAL METHODS - ASSOCIATIONS BETWEEN HEART RATE AND OTHER RISK FACTORS

The mean levels of each continuous risk factor (total cholesterol, HDL cholesterol, systolic blood pressure, waist circumference (where available), body mass index and triglycerides (where available) was calculated within each quintile of HDL cholesterol. The p value for the trend across resting heart rate quintiles was calculated to assess if there was a statistically significant association between resting heart rate and other risk factors. The percentage of smokers, individuals with diabetes and individuals in each category of physical activity was also assessed within each resting heart rate quintile.

STATISTICAL METHODS - EFFECT ON ENDPOINTS

UNIVARIABLE ANALYSIS OF THE EFFECT OF RESTING HEART RATE

The population was divided into gender specific quintiles of RHR. The rates (per 1000 person years) were calculated within each gender specific quintile and category of RHR, defined as ≤ 60, 60-90 and > 90 bpm, separately in men and women. The endpoints analyses were: CVD mortality, CHD mortality, total mortality, and fatal or nonfatal CHD event.

MULTIVARIABLE ANALYSIS OF THE EFFECT OF RHR

The effect of RHR as a continuous variable (per 15 bpm increase) on each endpoint was calculated using Cox proportional hazards methods. All of the analyses were performed separately in men and women and stratified by year of study and area of Finland. The first model included study years 1977-1997. The co-variables introduced into the model were: age, smoking status (current or non-current smoker), systolic blood pressure, total cholesterol, self-reported diabetes, body mass index (BMI) and physical activity (categorical variable). HDL cholesterol was only available as a risk factor in study years 1982 to 1997. A second model (model 2) included study years 1982-1997 and HDL cholesterol was included as an additional co-variable; the observation time for this analysis was shorter. The time variable was time from study entry to first event. In the case of nonfatal MI individuals were considered to have left the study after the first event.

The above analyses were repeated for the effect of heart rate as a categorical variable. Categories were defined as: RHR < 60, 60-90, > 90.

The above analyses were undertaken using the entire length of the follow-up, up to 21 years. In separate analyses, the follow-up was truncated to assess the effect of heart rate on outcomes in the shorter term.

To assess if heart rate was functioning as a marker of pre-existing subclinical disease, the analyses were repeated with events occurring in the first two years excluded from the dataset. The effect on hazard ratio for heart rate as a continuous variable for CVD mortality after the addition of each co-variable was also assessed to determine the level of confounding of the heart rate relationship causes by each other CV risk factor.

To assess for an interaction effect between gender and heart rate, men and women were included together and an interaction term included in the model. The multivariable adjusted effect on heart rate on CVD mortality was assessed in all strata of physical activity. To allow sufficient numbers for this analysis, men and women were analysed together and gender was included as a risk factor.

To examine whether heart rate was merely functioning as a marker of pre-existing co-morbidities, we repeated the analyses (CVD mortality endpoint only) having excluded all those with any of the following co-morbidities: asthma, emphysema, rheumatoid arthritis, or cancer. The effect of RHR on CVD mortality was also examined in those with and without baseline elevated SBP.

In survey years 1992 and 1997 additional variables were available. Waist circumference was available in both 1992 and 1997. Triglyceride measurement was also available in 1997.To assess the effect of additionally controlling for these variables we performed sensitivity analyses restricted to years 1992 and 1997 (model 3 – to assess addition of waist circumference) and 1997 alone (model 4 – to assess addition of both triglycerides and waist circumference).

All statistical analyses were undertaken using Stata version 9.

INCORPORATION INTO RISK ESTIMATION SYSTEMS

The 1977-1997 surveys were included in derivation dataset for the new functions as these have RHR available and sufficient follow-up. The end point definitions used were the same as those used in the SCORE project[4], as described above. The functions for estimation of the CHD mortality and the non-coronary CVD mortality risks were calculated separately and combined, as in the original SCORE project. Exclusions have been described above.

STATISTICAL METHODS –DERIVATION OF FUNCTIONS CONTAINING RESTING HEART RATE

A function for estimation of 10 year risk of CVD mortality was derived for the study population, using Cox proportional hazards model. The function was derived by combining the baseline survival and the beta-coefficients for each of the risk factors, as in the SCORE project. The methods for derivation of the risk function have been detailed in the HDL methods section above. Age was included as a risk factor in this function as opposed as the time variable in the original SCORE function. The functions were derived

separately in men and women to assess whether inclusion of RHR resulted in a greater improvement in either gender.

The variables included in the function were the same as in the original SCORE function – total cholesterol, systolic blood pressure and current smoking status, plus RHR as a continuous variable. A second function was also derived, exactly the same as the first expect, without including heart rate. This was for comparisc with the function including RHR, in order to assess the improvement in risk estimation afforded by the incorporation of heart rate.

Previous analyses of the effect of RHR have shown that RHR has a stronger effect on CVD mortality in 10 year follow-up when compared to longer observation periods[288], therefore we have truncated follow-u at 10 years for these analyses.

STATISTICAL METHODS – TESTING AND INTERNAL VALIDATION OF THE FUNCTIONS CONTAINING HEART RATE

The performance of the two risk estimation functions (with and without RHR included) will be compared based on:

- Discrimination (Area under receiver operating characteristic curve and Harrell's C statistic)
- Observed to predicted ratios
- Goodness of fit testing – Hosmer Lemeshow test
- Sensitivity and specificity at various cut points for high risk
- Net reclassification index[256]

Because only 6 year follow-up is included for the study year 1997, this year is excluded from the dataset when assessing goodness of fit, reclassification and observed to predicted ratios.

Additionally, we assessed the improvement in discrimination after incorporation of RHR as an additional variable in a simpler function including only easily measured variables: age, gender, smoking status and BM (analysed as a quadratic variable). Simple risk estimation charts containing only these variables were created and the discrimination and calibration of the function was assessed as above.

HEART RATE RESULTS

BASELINE CHARACTERISTICS

Thirty-nine thousand nine hundred and thrity-three individuals had data available on heart rate. These were from five of the six FINRISK study years, as heart rate was not recorded in the 1972 survey. Exclusion of those with previous myocardial infarction, angina, heart failure or pharmacological treatment for hypertension resulted in a total of 7,318 individuals being removed from the dataset. There were 30,151 individuals (14,673 men and 15,478 women) who had data available for all of the co-variables in multivariable model 1. Of these 21,842 (10,512 men and 11,330 women) additionally had data available on HDL cholesterol; these are included in multivariable model 2. The baseline characteristics of those in both multivariable models are shown in **Table 3-5**.

Women																	
	Model 1								Model 2								
	TC	*SBP*	*Smokers*	*DM*	*BMI*	*Min PA*	*Mod PA*	*Heavy PA*	*TC*	*SBP*	*Smokers*	*DM*	*BMI*	*HDL*	*Min PA*	*Mod PA*	*Heavy PA*
1	5.7	131	18%	1%	24.9	27%	52%	21%	5.5	130	20%	1%	25.0	1.56	24%	52%	24%
2	5.7	132	20%	1%	25.0	31%	52%	17%	5.5	131	22%	1%	25.0	1.55	28%	54%	18%
3	5.8	134	21%	1%	25.3	32%	52%	16%	5.6	133	23%	1%	25.3	1.54	30%	54%	16%
4	5.9	136	21%	1%	25.4	36%	51%	13%	5.7	135	23%	1%	25.4	1.53	34%	52%	14%
5	6.0	142	23%	2%	25.7	38%	53%	9%	5.8	141	25%	2%	25.7	1.53	35%	55%	10%
Total	5.8	134	20%	1%	25.3	32%	52%	16%	5.6	134	22%	1%	25.2	1.54	30%	53%	17%
Men																	
	Model 1								Model 2								
	TC	*SBP*	*Smok*	*DM*	*BMI*	*Min PA*	*Mod PA*	*Heavy PA*	*TC*	*SBP*	*Smok*	*DM*	*BMI*	*HDL*	*Min PA*	*Mod PA*	*Heavy PA*
1	5.9	136	30%	1%	25.5	17%	44%	38%	5.6	136	27%	1%	25.6	1.30	15%	43%	41%
2	5.9	139	36%	1%	25.9	23%	50%	26%	5.8	138	34%	1%	26.0	1.29	23%	50%	27%
3	6.1	140	41%	2%	26.0	27%	52%	21%	5.8	139	38%	2%	26.1	1.26	26%	52%	22%
4	6.1	143	49%	2%	26.3	31%	52%	17%	5.9	142	47%	2%	26.4	1.26	31%	51%	17%
5	6.2	148	54%	2%	26.3	35%	53%	12%	6.0	147	53%	2%	26.6	1.26	34%	53%	13%
Total	6.0	141	41%	2%	26.0	27%	50%	23%	5.8	140	39%	2%	26.1	1.28	25%	50%	25%

Table 3-5: Baseline characteristics in each quintile of resting heart rate in men and women included in models 1 and 2

TC: mean total cholesterol SBP: mean systolic blood pressure Smokers: Percentage of current smokers DM: Percentage with diabetes BMI: mean body mass index PA: physical activity Min: minimal Mod: Moderate HDL: mean high density lipoprotein cholesterol

ASSOCIATIONS BETWEEN HEART RATE AND OTHER RISK FACTORS

As shown in **Table 3-5**, there was a trend towards higher total cholesterol levels, higher blood pressures, higher rates of smoking, higher BMI, higher levels of physical inactivity, lower levels of heavy physical activity and lower HDL cholesterol levels with increasing heart rate quintile. This was seen in both men and women.

EFFECT OF RESTING HEART RATE ON ENDPOINTS

UNIVARIABLE ANALYSES

In men, on univariable analysis CVD, CHD and total mortality rates increased with each successive increase in heart rate quintile. The effect was strong and graded, with a rate ratio of 2.02 (1.53 to 2.68) comparing the fifth to the first quintile of heart rate, for the CVD mortality endpoint. For the nonfatal MI endpoint, the relationship was not as strong or graded and the relationship between all CHD events (fatal and nonfatal) was intermediate. The rates are shown in **Table 3-6** and illustrated in **Figure 3.6**.

In women, on univariable analysis, the association was less strongly graded especially for the CHD mortality endpoint. However, there was a significantly increased rate of CVD, CHD and total mortality in the fifth quintile of heart rate compared to the first; the rate ratio for the CVD mortality endpoint was similar to that seen in men - 2.20 (1.78 to 2.67). Again, the strength of the relationship reduced when examining nonfatal MI rate. These rates are not adjusted for age; there was a slight increase in median age as heart rate quintile increased, as shown in **Table 3-6**.

HR Quin	HR Range	N	Age	CVD mortality Rate	CHD mortality Rate	Total mortality rate	Fatal or nonfatal CHD event
Women							
1	≤ 64	4,361	41	1.1 (0.9 to 1.4)	0.6 (0.5 to 0.9)	3.7 (3.3 to 4.2)	1.6 (1.3 to 1.9)
2	66 – 68	2,443	42	1.5 (1.2 to 1.9)	1.0 (0.7 to 1.4)	4.7 (4.1 to 5.4)	2.1 (1.7 to 2.6)
3	70 – 74	3,258	42	1.4 (1.2 to 1.8)	1.0 (0.8 to 1.3)	4.4 (3.9 to 5.0)	2.1 (1.8 to 2.6)
4	76 – 80	2,929	43	1.8 (1.5 to 2.2)	0.9 (0.7 to 1.2)	5.3 (4.7 to 5.9)	2.4 (2.0 to 2.9)
5	≥ 82	3,154	43	2.2 (1.9 to 2.7)	1.5 (1.2 to 1.9)	6.9 (6.2 to 7.6)	3.1 (2.6 to 3.6)
Men							
1	≤ 60	3,366	41	2.6 (2.2 to 3.0)	1.9 (1.6 to 2.3)	7.0 (6.3 to 7.7)	4.6 (4.1 to 5.2)
2	62 – 66	2,846	42	3.2 (2.7 to 3.7)	2.2 (1.8 to 2.7)	8.2 (7.4 to 9.1)	5.6 (4.9 to 6.3)
3	68 – 72	3,487	42	3.9 (3.4 to 4.4)	2.6 (2.2 to 3.0)	10.2 (9.4 to 11.1)	6.9 (6.3 to 7.7)
4	74 – 80	2,870	42	4.1 (3.6 to 4.7)	3.0 (2.5 to 3.5)	11.5 (10.5 to 12.5)	6.2 (5.6 to 7.0)
5	≥ 82	2,831	43	5.6 (5.0 to 6.3)	4.4 (3.8 to 5.0)	14.1 (13.1 to 15.3)	8.0 (7.2 to 8.8)

Table 3-6: CVD, CHD and total mortality rates (unadjusted) in each gender-specific quintile of heart rate in men and women

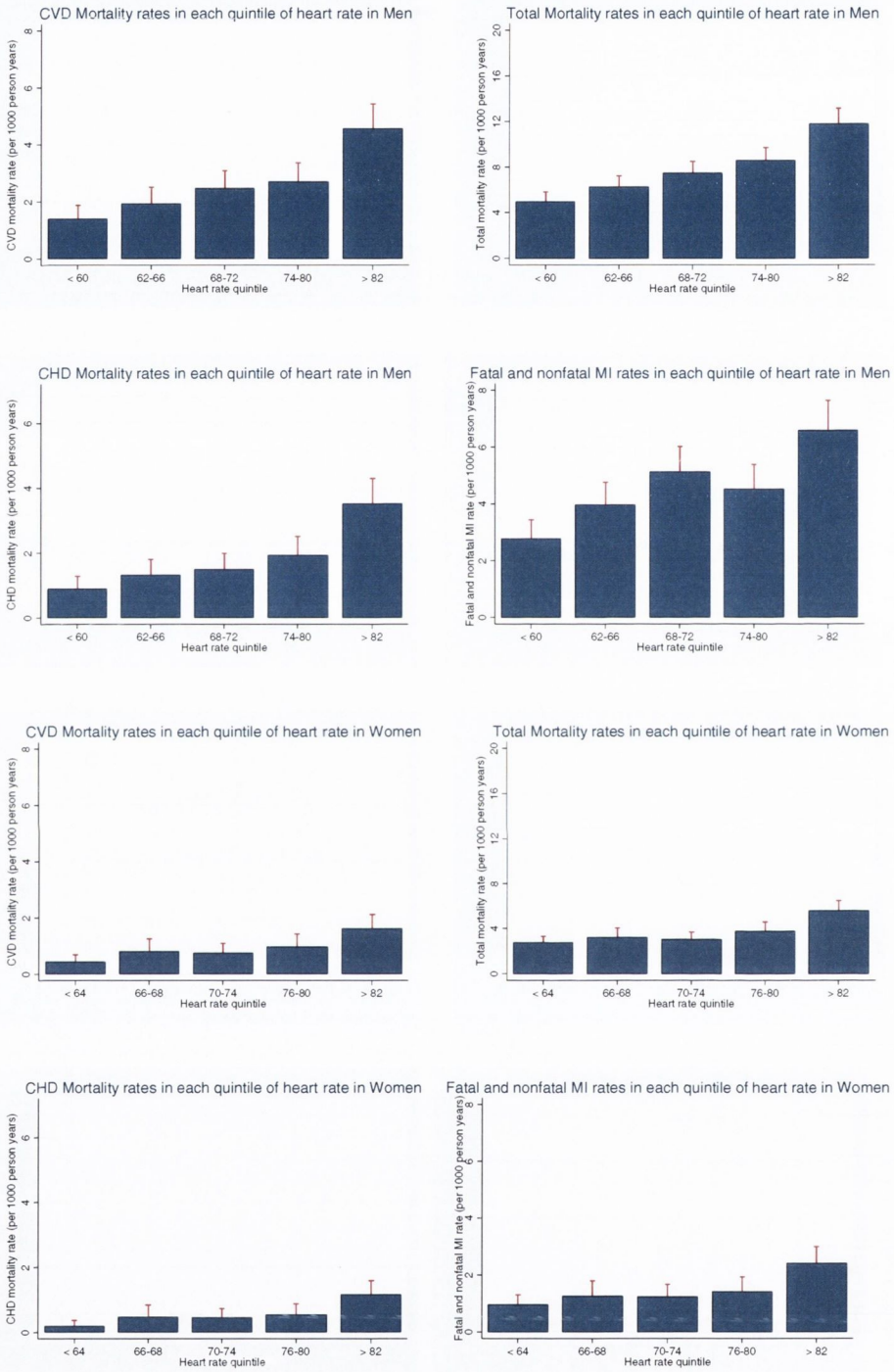


Figure 3.6: Rates of each endpoint in each quintile of RHR in men and women

On univariable analysis of the rates in each heart rate category, rates increased in each category in both men and women, as shown in **Table 3-7** and illustrated in **Figure 3.7**. Rate ratios comparing < 60 to >90 bpm were 2.67 (2.10 to 3.40) for men and 2.32 (1.54 to 3.49) for women for the CVD mortality endpoint, 2.77 (2.10 to 3.64) for the CHD mortality endpoint in men and very similar (2.79 (1.70 to 4.58)) in women. Rate

ratios were slightly lower for both genders for the total mortality endpoint (2.19 in women and 2.28 in men). As seen in the quintile analyses, rate ratios for the nonfatal MI endpoint were lower and those for both fatal and nonfatal MI were intermediate.

Category	N	Age	CVD mortality Rate	CHD mortality Rate	Total mortality Rate	Fatal and nonfatal CHD events
Women						
< 60	2,421	42	1.1 (0.8 to 1.5)	0.7 (0.5 to 1.0)	3.7 (3.2 to 4.4)	1.6 (1.3 to 2.1)
60 -90	13,194	43	1.6 (1.4 to 1.8)	1.0 (0.8 to 1.1)	4.9 (4.6 to 5.2)	2.2 (2.0 to 2.4)
> 90	1,124	45	2.6 (1.9 to 3.4)	1.9 (1.4 to 2.7)	8.2 (7.0 to 9.6)	3.8 (3.0 to 4.7)
Men						
< 60	3,520	41	2.6 (2.2 to 3.0)	1.9 (1.6 to 2.3)	7.0 (6.3 to 7.7)	4.6 (4.1 to 5.2)
60 – 90	11,457	43	3.9 (3.6 to 4.2)	2.8 (2.5 to 3.0)	10.5 (10.0 to 11.0)	6.5 (6.1 to 6.9)
> 90	1,119	44	6.9 (5.7 to 8.2)	5.4 (4.4 to 6.6)	15.9 (14.1 to 17.8)	8.6 (7.3 to 10.1)

Table 3-7: CVD, CHD and total mortality rates in each heart rate category in men and women

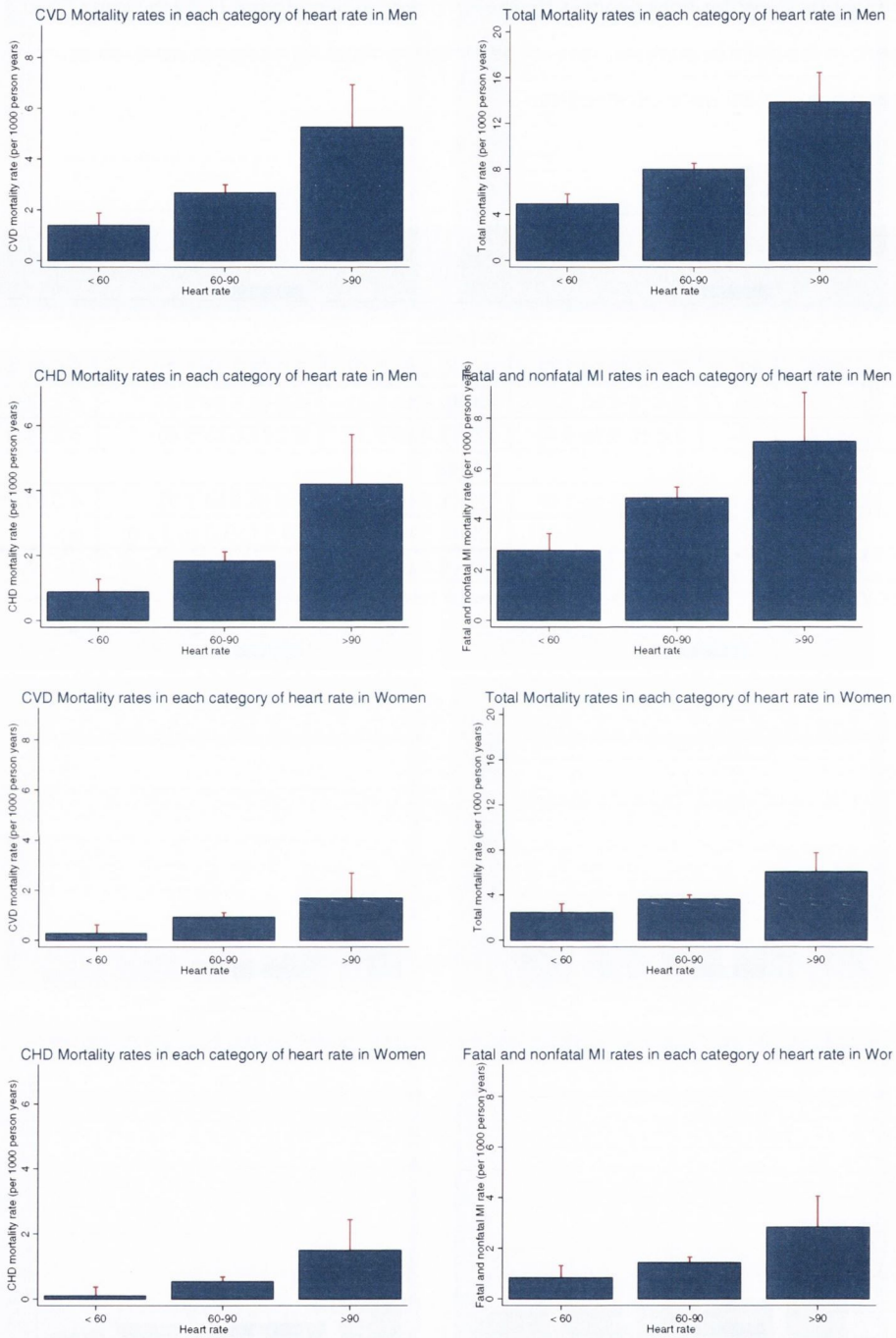


Figure 3.7: Rates of each endpoint in each category of RHR in men and women

MULTIVARIABLE ANALYSES

In multivariable analyses (**Table 3-8**), heart rate as a continuous variable remained a significant predictor of CVD mortality after full adjustment in model 1 – hazard ratio 1.13 (1.001 to 1.27) and 1.19 (1.09 to 1.28) per

15 bpm increase in heart rate in women and men respectively for the CVD mortality endpoint. Additionally, heart rate remained a risk factor after the inclusion of HDL cholesterol in the model (model 2). It should be noted that the follow-up period is longer in model 1 than model 2, which is likely the reason for the higher hazard ratio in model 2, as discussed below.

	Model 1 (age-adjusted only)	Model 1 (adjusted for other CV RF*)	Model 2 (adjusted for other CV RF* & HDL-C)
Women			
Number included (events)	15,478	15,478	11,330
Hazard ratio for CVD mortality	1.30 (1.15 to 1.46)	1.13 (1.001 to 1.27)	1.32 (1.08 to 1.60)
Hazard ratio for CHD mortality	1.35 (1.16 to 1.56)	1.17 (1.01 to 1.36)	1.50 (1.18 to 1.91)
Hazard ratio for total mortality	1.26 (1.18 to 1.35)	1.18 (1.10 to 1.26)	1.21 (1.09 to 1.34)
Hazard ratio for fatal and nonfatal CHD events	1.26 (1.14 to 1.39)	1.12 (1.01 to 1.23)	1.20 (1.03 to 1.40)
Men			
Number included	14,673	14,673	10,512
Hazard ratio for CVD mortality	1.41 (1.31 to 1.52)	1.19 (1.09 to 1.28)	1.24 (1.11 to 1.40)
Hazard ratio for CHD mortality	1.46 (1.34 to 1.60)	1.23 (1.12 to 1.35)	1.34 (1.17 to 1.53)
Hazard ratio for total mortality	1.35 (1.29 to 1.41)	1.19 (1.13 to 1.25)	1.19 (1.11 to 1.28)
Hazard ratio for fatal and nonfatal CHD events	1.23(1.16 to 1.31)	1.06 (0.99 to 1.13)	1.06 (0.97 to 1.17)

Table 3-8: Age and multivariable adjusted hazard ratios for each endpoint for heart rate as a continuous variable (per 15 bpm increase)

As shown in **Table 3-8**, the hazard ratio for heart rate was attenuated after the addition of the other CV risk factors. For example, in women in model 1 the hazard ratio for CVD mortality decreased from 1.30 in the model adjusted for age only to 1.14 in the fully adjusted model. Most of the attenuation occurred with the introduction of systolic blood pressure into the model. However, in men particularly, introduction of the smoking variable also attenuated the hazard ratio as did introduction of the physical activity variable in both genders (full data shown in **Table 3-9**).

	Women				Men			
	Model 1	*Model 2*	*Model 3*	*Model 4*	*Model 1*	*Model 2*	*Model 3*	*Model 4*
None	1.40***	1.58***	1.73***	1.60	1.41***	1.50***	1.48***	1.49***
Age	1.30***	1.45***	1.71**	1.58	1.41***	1.50***	1.53***	1.56**
SBP	1.19**	1.37**	1.59*	1.50	1.28***	1.38***	1.42***	1.50**
TC	1.18**	1.37**	1.60*	1.51	1.27***	1.36***	1.41***	1.49**
Smoke	1.17**	1.33**	1.56*	1.35	1.20***	1.27***	1.31**	1.41*
Diabetes	1.15*	1.32**	1.57*	1.38	1.20***	1.26***	1.31**	1.41*
BMI	1.15*	1.32**	1.56*	1.37	1.20***	1.26***	1.32**	1.41*
HDL		1.37**	1.53*	1.28		1.26***	1.32**	1.42*
Waist			1.53*	1.26			1.29*	1.38*
Trigs				1.26				1.40*
Phys_act	1.13*	1.32**	1.50*	1.19	1.19***	1.24***	1.29*	1.42*
Number	15478	11330	7863	5536	14673	10512	7109	5038
Events	397	140	39	16	881	372	130	58

Table 3-9: Hazard ratio for CVD mortality for heart rate as a continuous variable (per 15 bpm increase) with sequential addition of covariables into the model

In model 2, the addition of HDL cholesterol as an extra co-variable did not result in any change to the hazard ratio for heart rate or its statistical significance. Further adjustment for waist circumference (models 3 and 4) and triglycerides (model 4) where these variables were available made no substantial difference to the hazard ratios, although due to small event numbers, particularly in women in these subgroups the hazard ratios did not remain significant, as shown in **Table 3-9**.

As shown in **Table 3-8**, heart rate also remained an independent predictor of the other mortality endpoints (CHD and total mortality). Conversely, while heart rate was associated with increased risk of nonfatal MI in age-adjusted analyses, the relationship was substantially weaker than for the mortality endpoints and attenuated further, losing statistical significance, with adjustment for other risk factors.

The increase in effect of heart rate as a risk factor with decreasing observation times is shown in **Table 3-10** .

To investigate whether elevated heart rate was an independent risk factor or merely a marker of subclinical disease I re-analysed the effect including only fatal events which occurred in the two years after the initial examination date. This resulted in virtually no difference in the hazard ratios for heart rate as a continuous variable as shown in **Table 3-11**.

	Women	Men
Full observation time (27 years)	1.17 (1.04 to 1.32)	1.20 (1.11 to 1.30)
15 years	1.27 (1.05 to 1.55)	1.22 (1.09 to 1.36)
10 years	1.45 (1.10 to 1.92)	1.30 (1.13 to 1.49)
7 years	1.45 (0.99 to 2.13)	1.39 (1.16 to 1.65)
5 years	1.62(0.96 to 2.73)	1.44 (1.15 to 1.79)

Table 3-10: Effect of varying observation time on hazard ratio for heart rate as a continuous variable (per 15 bpm increase), adjusted for age, TC, SBP and smoking

	Model 1 (age-Adjusted only)	Model 1 (adjusted for other CV RF*)	Model 2 (adjusted for other CV RF* & HDL-C)
Women			
Number included	15478 (389)	15478 (389)	11330 (138)
Hazard ratio for CVD mortality	1.29 (1.14 to 1.45)	1.12 (1.00 to 1.27)	1.28 (1.05 to 1.56)
Men			
Number included	14673 (844)	14673 (844)	10512 (349)
Hazard ratio for CVD mortality	1.41 (1.31 to 1.52)	1.19 (1.10 to 1.29)	1.27 (1.12 to 1.43)

Table 3-11: Age and multivariable adjusted hazard ratios for heart rate as a continuous variable, per 15 bpm increase (excluding events occurring within the first 2 years of observation)

The effect of heart rate category on risk of each endpoint was also calculated as this is particularly clinically relevant. The age-adjusted and multivariable adjusted hazard ratios for heart rate categories 60-90bpm and >90 bpm are shown in **Table 3-12**.

		Model 1 (age-adjusted only)	Model 1 (adjusted for other CV RF*)	Model 2 (adjusted for other CV RF* & HDL-C)
Women				
CVD mortality	≤ 60	Reference	Reference	Reference
	62 – 90	1.36 (0.96 to 1.92)	1.37 (0.97 to 1.94)	2.62 (1.15 to 5.99)
	>90	2.07 (1.34 to 3.20)	1.48 (0.94 to 2.31)	3.07 (1.19 to 7.94)
CHD mortality	≤ 60	Reference	Reference	Reference
	62 – 90	1.29 (0.84 to 1.99)	1.32 (0.85 to 2.05)	4.35 (1.06 to 17.86)
	>90	2.48 (1.46 to 4.19)	1.82 (1.06 to 3.13)	7.63 (1.71 to 34.07)
Total mortality	≤ 60	Reference	Reference	Reference
	62 – 90	1.21 (1.01 to 1.45)	1.18 (0.98 to 1.41)	1.30 (0.98 to 1.73)
	>90	1.94 (1.53 to 2.45)	1.62 (1.27 to 2.06)	1.71 (1.18 to 2.49)
Fatal and nonfatal CHD events	≤ 60	Reference	Reference	Reference
	62 – 90	1.21 (0.92 to 1.59)	1.16 (0.88 to 1.53)	1.45 (0.89 to 2.36)
	>90	1.91 (1.34 to 2.71)	1.37 (0.95 to 1.97)	1.98 (1.08 to 3.62)
Men				
CVD mortality	≤ 60	Reference	Reference	Reference
	62 – 90	1.47 (1.22 to 1.77)	1.18 (0.97 to 1.42)	1.34 (0.96 to 1.87)
	>90	2.61 (2.02 to 3.37)	1.57 (1.20 to 2.05)	1.94 (1.27 to 2.97)
CHD mortality	≤ 60	Reference	Reference	Reference
	62 – 90	1.38 (1.11 to 1.71)	1.11 (0.89 to 1.38)	1.49 (0.99 to 2.25)
	>90	2.74 (2.04 to 3.66)	1.67 (1.23 to 2.26)	2.38 (1.43 to 3.96)
Total mortality	≤ 60	Reference	Reference	Reference
	62 – 90	1.47 (1.31 to 1.62)	1.23 (1.09 to 1.38)	1.23 (1.03 to 1.46)
	>90	2.28 (1.94 to 2.68)	1.58 (1.34 to 1.87)	1.77 (1.38 to 2.26)
Fatal and nonfatal CHD events	≤ 60	Reference	Reference	Reference
	62 – 90	1.44 (1.25 to 1.66)	1.18 (1.02 to 1.36)	1.34 (1.06 to 1.70)
	>90	1.87 (1.51 to 2.32)	1.24 (0.99 to 1.54)	1.42 (1.02 to 1.99)

Table 3-12: Age and multivariable adjusted hazard ratios for each endpoint associated with heart rate categories.

There was no significant interaction between gender and effect of heart rate on CVD mortality. Interaction terms were insignificant in both model 1 (p=0.410) and model 2 (p=0.742). Heart rate remained a predictor of CVD mortality in both the inactive and the moderately active strata of physical activity. However, statistical significance was lost in those who undertook heavy physical activity (event numbers in this group were small). Fully adjusted hazard ratios per 15 bpm increase in heart rate were: 1.12 (1.01 to 1.24), 1.18 (1.08 to 1.30) and 1.23 (0.98 to 1.55) respectively for the 3 physical activity strata (model 1 – men and women combined). A similar pattern was seen in model 2 – shown in **Table 3-13**.

	Women		Men	
With those with co-morbidities excluded				
	Model 1	Model 2	Model 1	Model 2
Hazard ratio for CVD mortality	1.13 (0.99 to 1.28)	1.18 (1.09 to 1.29)	1.28 (1.03 to 1.59)	1.23 (1.08 to 1.39)
By physical activity stratum (men and women analysed together)				

	Model 1	Model 2
Light activity	1.12 (1.01 to 1.24)	1.24 (1.06 to 1.45)
Moderate activity	1.18 (1.08 to 1.30)	1.30 (1.13 to 1.50)
Heavy activity	1.23 (0.98 to 1.55)	1.11 (0.79 to 1.58)

Table 3-13: Effect of heart rate (per 15 bpm increase) on CVD mortality, with those with co-morbidities excluded and in each physical activity stratum.

After exclusion of those with co-morbidities (as listed above), the relationship between heart rate and CVD mortality remained essentially the same in both models. However, statistical significance was lost in women in model 1 (p=0.063). Hazard ratios per 15 bpm increase in heart rate were 1.13 (0.99 to 1.28) and 1.18 (1.09 to 1.29) in women and men respectively in model 1. Corresponding hazard ratios for model 2 were 1.28 (1.03 to 1.59) and 1.23 (1.08 to 1.39) in women and men, respectively.

Analyses by blood pressure subgroup showed a stronger effect of heart rate in those with baseline blood pressure less than 140mmHg in both men and women. As shown in **Table 3-14**, hazard ratios (adjusted for age, SBP, TC and smoking) were significant in all blood pressure subgroups except in women with elevated baseline blood pressure in model 1.

	Baseline SBP ≤ 140mmHg	Baseline SBP > 140mmHg
Women (Model 1)	1.37 (1.05 to 1.79)	1.07 (0.94 to 1.23)
Women (Model 2)	1.50 (1.001 to 2.26)	1.28 (1.02 to 1.62)
Men (Model 1)	1.39 (1.19 to 1.63)	1.13 (1.02 to 1.24)
Men (Model 2)	1.31 (1.01 to 1.72)	1.23 (1.08 to 1.40)

Table 3-14: Hazard ratios for CVD mortality for heart rate as a continuous variable (per 15 bpm increase) in men and women with high and normal baseline systolic blood pressure (SBP)

To allow comparison between elevated heart rate as a risk factor and other risk factors in this dataset the hazard ratios for the CVD mortality for the other risk factors (multivariable-adjusted, including resting heart rate) are shown in **Table 3-15**. The group used for this analysis are those included in model 2 above.

Risk Factor	Hazard ratio for CVD mortality in Women	Hazard ratio for CVD mortality in Men
SBP (per 1mmHg increase)	1.012 (1.004 to 1.019)	1.015 (1.010 to 1.021)
Total chol (per 1mmol/l increase)	1.02 (0.89 to 1.18)	1.15 (1.05 to 1.26)
Current smoking	2.37 (1.56 to 3.60)	2.29 (1.85 to 2.84)
Diabetes	4.12 (2.23 to 7.61)	2.24 (1.46 to 3.45)
BMI (per 1 unit increase)	1.01 (0.97 to 1.04)	1.04 (1.01 to 1.07)
HDL Chol (per 1 mmol/l increase)	0.41 (0.24 to 0.71)	0.97 (0.71 to 1.34)
Moderately active compared to inactive	0.58 (0.40 to 0.83)	0.78 (0.63 to 0.98)
Heavily active compared to inactive	0.79 (0.42 to 1.47)	0.77 (0.53 to 1.10)

Table 3-15: Multivariable adjusted (including RHR) hazard ratios for CVD mortality for other risk factors

Table 3-16 shows the hazard ratios for the variables included in the function and the beta coefficients. The 10 year survivals centered at age = 40, TC = 6mmol/l SBP = 120mmHg +/- RHR = 60bpm for the function with and without RHR are also shown in **Table 3-16**.

	Men		Women	
	RHR function	Function without RHR	RHR function	Function without RHR
Age (per year)	0.093	0.092	0.120	0.119
TC (per mmol/l)	0.278	0.278	0.179	0.191
SBP(per 1mmHg)	0.018	0.021	0.018	0.021
Current smoker vs. Non-current smoker	0.709	0.774	1.089	1.136
RHR (per 1 bpm)	0.017	-	0.018	-
Baseline 10 year survival	0.9970373	0.9965994	0.9995711	0.9994714

Table 3-16: Beta coefficients and hazard ratios for the variables included in the function with and without RHR and baseline survivals centered at age = 40, TC = 6mmol/l SBP = 120mmHg +/- RHR = 60bpm

AUROCs and Harrell's C statistics are shown in **Table 3-17** for the function with and without RHR as a continuous variable in men, women and the entire group. The function including RHR provided superior discrimination compared to the function without RHR, with an improvement in AUROC from 0.8794 to 0.8814. However, this modest improvement did not reach statistical significance, except in women, as shown in **Table 3-17**. Goodness of fit testing showed reasonable fit of the RHR function in both men and women, with no significant lack of fit; (p = 0.1452 in men and p = 0.7372 in women). There was no evidence of superior calibration of either function.

	Men	Women	Entire group
RHR function	0.8440 (0.8394)	0.8816 (0.8799)	0.8814(0.8767)
Function without RHR	0.8420 (0.8360)	0.8739 (0.8727)	0.8794(0.8739)
Improvement in AUROC	0.0020, p=0.3915	0.0077, p= 0.0236	0.0020, p=0.174
Improvement in Harrell's C	0.0034	0.0072	0.0028

Table 3-17: AUROC (Area under receiver operating characteristic curves) in women and men on incorporation of RHR into risk function (Harrell's C statistic shown in brackets)

Observed to predicted ratios were very similar for the RHR function and the function without RHR; 0.99 and 1.02 in men and women respectively. The predicted to observed ratios for each of the age groups for both functions are shown in **Table 3-18** – none differ by more than 0.02 and there is no consistent superiority of one function over another.

	Men		Women	
	RHR function	Function without RHR	RHR function	Function without RHR
All ages	0.99	0.98	1.02	1.03
Under 40	1.40	1.40	1.48	1.48

40-45	0.96	0.94	0.87	0.86
45-50	0.74	0.74	0.84	0.85
50-55	0.91	0.91	1.09	1.08
55-60	1.06	1.04	1.18	1.19
Over 60	0.96	0.96	0.91	0.91

Table 3-18: Predicted to observed ratios for the RHR function and the function without RHR

Net reclassification indices indicated no statistically significant improvement in classification using the function including RHR, for the overall population; with a NRI of 0.0025, non-significant.

Table 3-19 shows the sensitivity and specificity of the RHR function and the function without RHR at different thresholds for high risk. There were only minor differences in sensitivity and specificity between the two functions.

	RHR function		Function without RHR	
Cut point	Sensitivity	Specificity	Sensitivity	Specificity
2%	83.3	79.4	83.3	79.0
3%	67.7	87.1	67.3	87.1
5%	45.8	93.6	45.8	93.7

Table 3-19: Sensitivity and specificity of the RHR and function without RHR at different thresholds for high risk.

INCORPORATION INTO RISK ESTIMATION SYSTEMS – SIMPLIFIED SYSTEM

Risk estimation charts for the simple function containing RHR are shown in **Figure 3.8.**. **Table 3-20** shows the beta coefficients and baseline survivals for the simpler risk estimation function. BMI was shown to have a J shaped relationship with CVD mortality in women, with a more linear relationship in men. Therefore, the quadratic version of the BMI variable was included for women only.

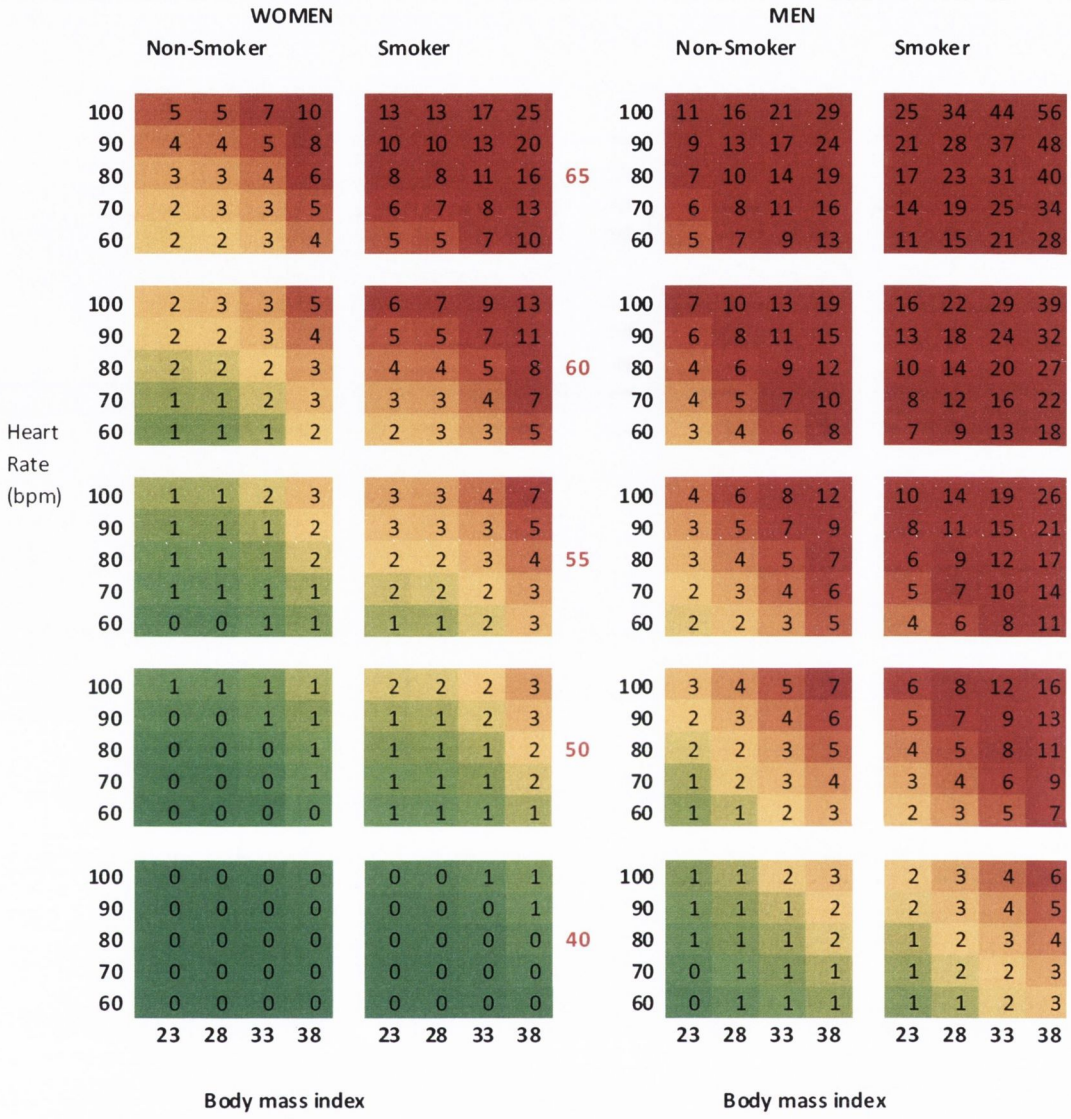


Figure 3.8: Simple risk chart including only easily measured variables - gender, age, smoking status, resting heart rate and BMI

	Men		Women	
	RHR function	Function without RHR	RHR function	Function without RHR
Age (per year)	0.1010975	0.1020107	0.1415269	0.143448
BMI continuous	0.0640427	0.0691234	-0.0408001	-0.0419021
BMI quadratic term	-	-	0.00408001	0.0044115
Current smoker vs. Non-current smoker	0.7762996	0.8736721	0.9315619	0.9837828
RHR (per 1 bpm)	0.0228724	-	0.0244292	-
Baseline 10 year	0.9969311	0.9961686	0.9994194	0.9991891

survival				

Table 3-20: Beta-coefficients for risk factors and baseline survivals for the simple scores with and without RHR included

The simple function including RHR resulted in good discrimination and calibration, as summarized in **Table 3-21**. The AUROC for the simple function containing RHR was 0.8638, an improvement from 0.8576 for the function without RHR, p=0.0076. The addition of RHR to the function also resulted in an improvement in risk classification, with a net reclassification index of 0.14, p<0.01. However, these AUROCs were still slightly inferior to the function containing lipid and blood pressure variables.

	Men		Women	
	Simple Score with RHR	Simple Score without RHR	Simple Score with RHR	Simple Score without RHR
Discrimination				
AUROC	0.8196	0.8121, p=0.036	0.8667	0.8547, p=0.0
Harrell's C	0.8212	0.8131	0.8672	0.8541
Calibration				
Predicted to Observed ratios	1.06	0.95	1.05	0.99
Hosmer Lemeshow statistic p value	0.2413	0.6542	0.9242	0.5623

Table 3-21: Measures of discrimination and calibration in Simple score with and without RHR included

HEART RATE – DISCUSSION

MAIN FINDINGS – ADDING TO EVIDENCE ON THE FULFILLMENT OF CAUSAL CRITERIA FOR THE RELATIONSHIP BETWEEN RESTING HEART RATE AND MORTALITY

This analysis has clearly demonstrated the substantial role of elevated RHR as a risk factor for CVD, CHD and total mortality. We have confirmed the strength of the relationship. For example, in men in model 2 (shorter follow-up), heart rate >90 bpm compared to heart rate < 60 bpm was associated with an almost 2 fold increased risk of CVD mortality. This was after adjustment for all other risk factors and is similar in magnitude to the risk associated with current smoking in this group. In women, the same situation was shown to be associated with a 3 fold increase in risk. These estimates are similar to those seen for approximately equal comparisons of heart rate categories in other studies, which ranged from 1.5 to 3.3 fold increased risk in the higher heart rate category[279-284, 323] as shown in Table 3-1.

We have confirmed the graded nature of the relationship, particularly in men. The risk seems to increase more steeply in the highest quintile of heart rate, in line with the findings of previous studies. Previously, there was inconsistency regarding the effect in women, with earlier studies, which may have been underpowered, showing either an insignificant or less important effect in women than men. Only 4 studies in the general population have actually shown a significant relationship between CVD or CHD outcomes in

women [284, 287, 289, 323] and of note one of these[289] did not adjust for systolic blood pressure as a continuous variable. We have demonstrated that RHR functions as a significant independent predictor of mortality outcomes in women as well as men, with no evidence of a gender interaction. Thus, our analysis adds to the scientific understanding of RHR as a risk factor in women.

Stroke has previously been shown to have a weaker association with elevated heart rate[288{Hsia, 2009 #245, 289]. Since stroke represents a greater proportion of CVD deaths in women than men this may be accounting for some of the reason for the weaker association in previous studies and may have contributed to the lack of power of some studies to detect a significant independent effect in women[288]. This effect was seen in this analysis also, with a stronger effect of resting heart rate on CHD mortality than CVD mortality, which includes fatal strokes.

Previously, there was doubt concerning the independence of the effect, particularly regarding possible confounders such as blood pressure, physical activity and co-morbidities[288]. This analysis has confirmed that RHR is strongly related to other risk factors including smoking, SBP, BMI, total cholesterol, HDL cholesterol (inversely) and physical activity levels. Due to these associations the effect of RHR on CVD endpoints attenuated on addition of the other CV risk factors to the model.

However, despite the clear demonstration of the association between RHR and other risk factors, the effect of RHR on CVD endpoints remained statistically significant even after inclusion of all of the risk factors including physical activity as a categorical variable and persisting within strata of physical activity. Additionally, the relationship remained after exclusion of those with co-morbidities, which could potentially have been confounding the relationship. Additionally, the effect of RHR was not substantially altered by the addition of waist circumference and triglycerides into the model in subgroups where these variables were available. This is the fourth largest study of the effect of RHR on outcome in the general population. It is important to note that in the only three larger studies[287-289], either did not adjust for systolic blood pressure or the relationship did not persist once other CV risk factors were introduced into the model, either in women[288] or in both genders[287].

Pre-existing or even subclinical CVD may lead to elevations in heart rate. In this way reverse causality could account for the relationship between heart rate and the future development of CVD. This makes our demonstration of the persistence of the relationship once events occurring in the first 2 years had been removed relevant in demonstrating that an elevated RHR precedes the development of disease and not vice versa.

As mentioned above, without a RCT showing a benefit of heart rate reduction in the general population causality cannot conclusively be proven. A recent European Society of Hypertension statement[324] suggested the next priority would be a RCT of anti-hypertensive therapy (with heart rate neutral effects)

combined with either a pure heart rate-reducing agent (e.g. I_f blocker) or placebo in hypertensive population with elevated heart rate. We have shown that elevations in heart rate may have a more powerful effect in individuals who are normotensive at baseline, as demonstrated by others also[288]. This suggests that such a RCT should be extended to non-hypertensive individuals with elevated heart rates. To definitely address the question a RCT of pure-heart rate reduction in individuals at high total CVD risk and with elevated RHR would be appropriate; this would have the advantage of not being confounded by additional effects of beta-blockade.

Heart rate seemed to be a stronger risk factor in the group with a shorter follow-up time. This finding was consistent across a range of observation times, but the reason for this situation is unclear. One may postulate that reverse causality is responsible. However, we have shown this to be unlikely by the persistence of a strong effect after removal of events occurring within the first 2 years. The increase in relative importance of stroke (on which elevated RHR has a weaker effect) is a likely explanation, which has been suggested previously[288]. In an aetiological analysis, such as this, considering the shorter follow-up time seems more reasonable, since many of the individuals will have been started on anti-hypertensive medication within 10 years, this will also have reduced RHR in many cases. Alternatively, this observation may be related to the mechanism by which elevated heart rate exerts its deleterious effect. Previously, it has been shown that elevated heart rates are a stronger risk factor for sudden deaths, probably by predisposing to arrhythmia [278, 282]. Possibly, individuals who have elevated heart rates over long periods of time are less vulnerable to arrhythmogenic effect.

The demonstration of a more important effect of heart rate on mortality (CVD, CHD and total) than nonfatal ischemic events is in line with previous studies in men that have shown heart rate to be a stronger predictor of sudden ischemic heart disease death[278, 282]. This suggests that one of the important mechanisms of RHR as a risk factor is through pre-disposition to arrhythmia, which would increase the case-fatality of ischemic events.

STRENGTHS AND LIMITATIONS OF THIS ANALYSIS

The main strengths of this analysis are the large size of the dataset, prospective design, the standardization of the methods for data collection and follow-up, the long observation time (up to 27 years) and the representativeness of the sample of the general population. The number of covariables available has allowed us to adjust for many of the factors previously considered potential confounders of the relationship. We have not been able to adjust for exercise capacity as measured by cardiopulmonary testing between heart rate and CVD. However, the physical activity questions included in the FINRISK study have been validated in this population and has been shown to correlate with predicted maximal oxygen uptake[320].

Heart rate variability and heart rate response to exercise are also associated with CVD outcomes [325]. Information on these variables was not available for analysis in this dataset. Additionally, only baseline resting heart rates measurements were available, therefore we have not been able to adjust for regression dilution bias or to assess the effect of change in heart rate during the observation time on outcome, which has recently been shown to be an important predictor of CVD death in men[325]. However, lack of adjustment for regression dilution bias would be expected to underestimate the effect of RHR on outcome.

IMPLICATIONS FOR MANAGEMENT OF CVD RISK

This analysis should highlight to clinicians involved in primary prevention that both men and women with elevated RHR should be considered at higher risk than indicated by SCORE or Framingham risk estimates. Like many other risk factors for CVD, heart rate is a marker of increased risk, but not considered a target for intervention at present[17]. Lowering of total CVD risk through more intensive attention to other CV risk factors is logical, as are conservative measures to lower heart rate, including out ruling secondary causes of tachycardia and avoidance of factors know to elevate heart rate including excessive use of caffeine, psychological stress and physical inactivity[17]. Whether benefit will result from lowering of elevated heart rates can only be assessed in a randomized controlled trial. This analysis signals the need for consideration of such a trial.

INCORPORATION OF HEART RATE INTO RISK ESTIMATION SYSTEMS – MAIN FINDINGS AND IMPLICATIONS

In this analysis we have shown that while the addition of RHR as a continuous variable to the variables currently included in the SCORE risk estimation function improves risk estimation, the difference is not statistically significant and unlikely to be clinically meaningful in the population as a whole. This lack of improvement in AUROC for risk functions after the addition of an important independent variable has previously been seen for other variables including multiple biomarkers[94], HDL cholesterol[256, 326] and ethnicity[47]. This is probably related to the fact that age and gender alone provide a high AUROC and the potential for improving AUROC beyond this is limited. Additionally, as most of the population will have RHRs close to the mean the number of individuals whose risk estimate changes substantially in the overall population will be low. Nevertheless, the minority of individuals, who have a high RHR, may be exposed to a considerable increase in risk.

Recently, there has been increasing interest in the use of risk estimation systems which use only easily measured variables. These systems make risk estimation more accessible and cost effective. Recently, the Framingham[45] group and the NHANES[82] group have shown little reduction in predictive ability when lipid measures were replaced by BMI. We suggest that RHR would be a particularly useful measure to

include in such a system, because it is extremely easily measured and has no associated costs. As shown above, this function performed well. The AUROC was significantly improved on addition of RHR to this simple function, although, it did not reach the AUROC of the function containing RHR in addition to lipid measures and blood pressure. The choice of BMI as the other factor in the simple score was influenced by the fact that it is a readily available measurement, is modifiable through alterations in lifestyle, and reductions have been shown to favorably affect other risk factors[327].

It is possible that part of the reason that inclusion of RHR in the simple risk estimation system results in an appreciable improvement is due to a combination of its independent effect on CVD risk and its association with other risk factors. Our cross-sectional analysis of the association between heart rate and other CV risk factors showed that individuals with higher RHRs tend to have higher systolic blood pressure, total cholesterol, triglycerides, waist circumference and lower levels of physical activity and HDL cholesterol. Others have also commented on the association between higher RHRs and components of the metabolic syndrome[307], as discussed above.

This dataset is very appropriate for assessing the value of including RHR in risk estimation systems because The National FINRISK Study[84] forms a large proportion of the dataset used for derivation of the SCORE function for use in high risk countries[4]. One of the limitations of this analysis is that we have validated the function internally only. External validation is essential for the evaluation of new risk estimation functions. However, it is unlikely that the risk function would perform inferiorly on the population from which it was derived and therefore, our conclusion of the minor improvement in risk estimation on incorporation of RHR is not biased by this. Heart rate post exercise and heart rate variability were not available in this cohort. These measures may have had more predictive ability than RHR alone[277], however, the use of such measures would complicate rather than simplify risk estimation.

HEART RATE – CONCLUSIONS

This large prospective cohort study confirms the strong and graded relationship between resting heart rate and CVD, CHD and mortality endpoints. We have clarified a number of previously disputed issues relating to fulfilment of the causal criteria, specifically independence, appropriate temporal sequence and the consistency of the relationship in women. We have demonstrated, for the first time that RHR is a risk factor independent of a validated measure of physical activity. Additionally, we have shown elevated RHR to be a stronger risk factor for the development of fatal as opposed to nonfatal MI, compatible with the possibility of a pro-arrthymogenic mechanism. The consistency of the effect between men and women has not been clearly demonstrated previously.

RHRs greater than 90 bpm have been shown to be associated with at least a doubling of risk – a similar effect to smoking. This highlights the need to consider tachycardia as an additional risk factor when estimating risk in the context of primary prevention.

Inclusion of RHR in risk estimation systems which already contain blood pressure and lipid measurements does not result in an appreciable improvement in their performance. However, in part due to its association with other risk factors, inclusion in simple risk estimation systems containing only easily measured variables results in useful improvements. As a measure which is simple and inexpensive to obtain, RHR is particularly suitable for inclusion in this type of risk estimation system. Our presentation of a risk estimation system including only non-laboratory, non-clinic based measures provides an opportunity for enhancing the cost effectiveness and accessibility of risk estimation.

CHAPTER 4 SCORE O.P. – RISK ESTIMATION IN OLDER PERSONS

In this section, I review the evidence for the role of risk factors in the older age group and the benefits of risk factor reduction for the prevention of CVD in this group. A discussion of the limitations of current risk estimation systems in the elderly leads on to the rationale and hypothesis for this analysis which aims to provide an improved method for CVD risk estimation in older people.

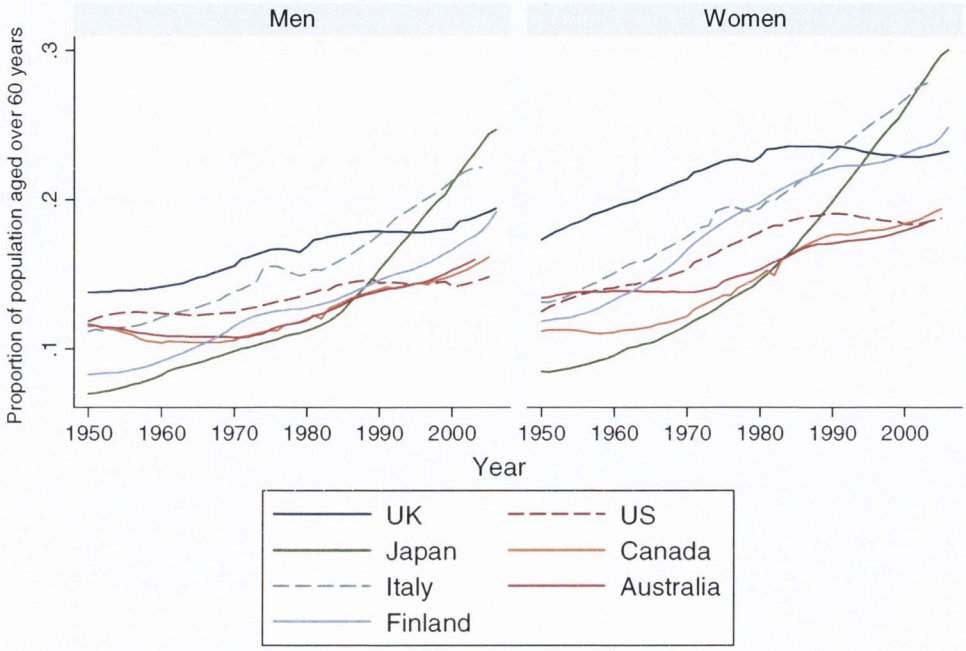
The specific research questions are outlined on page 224.

THE AGING POPULATION

Over the last 50 years, the world's population has been aging. This global and progressive phenomenon has substantial societal implications, particularly with regard to the need for medical care. Between 1950 and 2000, the number of persons aged over 60 years tripled to 600 million worldwide and this number is expected to triple again in the next 50 years to 2 billion[328]. The rate of increase is currently fastest in the developed world, with the proportion of older individuals expected to rise from one fifth in 2000 to one third in 2050. Even in the developing world the proportion is expected to increase from 8% in 2000 to 20% in 2050[328].

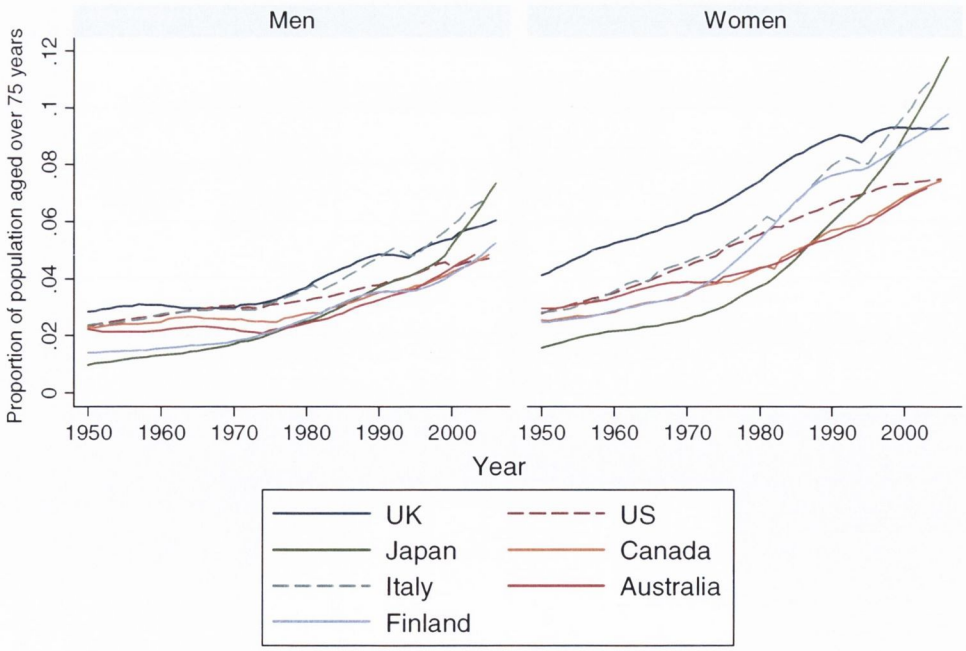
Figure 4.1 and **Figure 4.2** show the change in proportion of population aged over 60 years and 75 years, respectively. These figures have been calculated using the population figures from the WHO statistics

website. Unfortunately, population figures for the developing countries are very limited.



Source: WHO Website

Figure 4.1: Change in proportion of the population aged over 60 years in developed countries



Source: WHO Website

Figure 4.2: Change in proportion of the population aged over 75 years in developed countries

CARDIOVASCULAR DISEASES IN THE ELDERLY

Cardiovascular diseases account for the majority of deaths in this age group. This increase in the proportion of total deaths caused by CVD is shown in **Figure 4.3**. These figures are from the 2006 Irish central statistics office database. **Figure 4.3** also shows how the proportion of CVD accounted for by cerebrovascular disease also increases in older age groups.

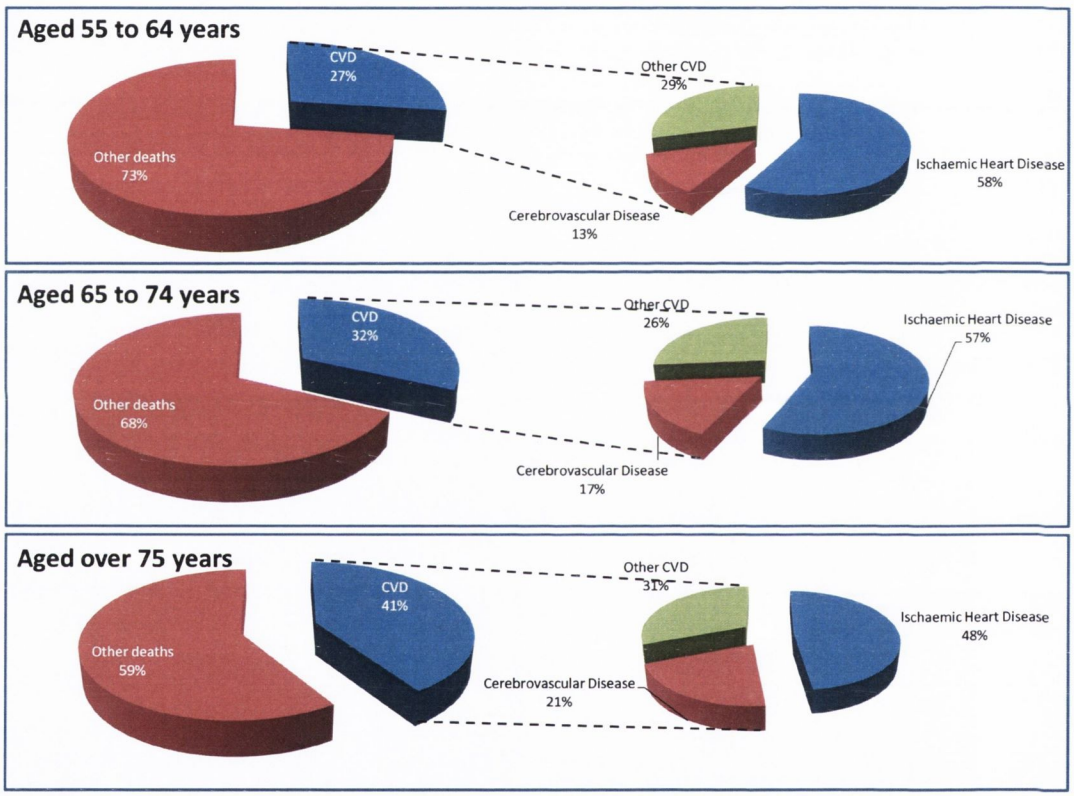


Figure 4.3: The proportion of deaths accounted for by CVD and the proportion of CVD accounted for by ischaemic heart disease and cerebrovascular disease in each of the three age groups.

This increase in the proportion of cerebrovascular disease in the older age group is important in terms of risk of assessment of CV risk because risk factors may have differing effects on the coronary heart disease and cerebrovascular disease endpoints.

Prevention of CVD in this age group is also particularly important as the consequences of CVDs cause significant and long lasting disabilities, particularly heart failure and disability due to stroke[273]. These

have significant health economic consequences, which will increase further with the rising proportion of older individuals in the population. .

RISK FACTORS IN THE ELDERLY

There is substantial evidence indicating that most conventional risk factors continue to function in the older age group[259, 329-331]. Novel risk factors also including homocysteine[72] and multiple biomarker scores[179] also function. In some cases these could be seen as markers of subclinical disease.

In general, prospective studies assessing the importance of risk factors for CVD in the elderly have been small. The prospective studies collaboration is an exception to this. However, only lipid measures and BMI have been assessed to date and these have not been adjusted for the presence of multiple other risk factors[139, 332]. This lack of power may have been responsible for the inconsistent results regarding which factors remain important. The greatest consistency is for the continued effect of elevated SBP, elevated blood glucose and low levels of physical activity[190, 333-336]. Many studies have shown total cholesterol not to be a risk factor in the elderly[182, 186, 334]. However, others have shown a continued effect[120, 189, 337], including the prospective studies collaboration which showed an effect even in the very old(80-89 years)[139].

In a re-analysis of the INTERHEART case control study all nine variables: Apo B/Apo A1 ratio, hypertension, physical activity, smoking, fruit and vegetable intake, psychosocial, diabetes, abdominal obesity and alcohol, were still associated with risk of MI in those aged over 60. Although, all except physical activity and alcohol in men were associated with weaker effects[15].

Most studies assessing the differential effects of risk factors in older and young have shown that relative risks associated with CV risk factors tend to reduce as people age[15, 51, 139, 332, 337].

This reduction in relative risk does not mean that risk factors are of lesser importance as people age. Because the absolute risk of CVD increases dramatically with increasing age, as illustrated in **Figure 4.4** below, the excess or attributable risk associated with CV risk factors tends to increase in older age groups. This increase in excess risk and decrease in relative risk with age is illustrated in **Figure 4.5** and **Table 4-1**. For this example, the risk associated with being in the fourth quartile of SBP as compared with the first in different age groups in the SCORE cohort is illustrated.

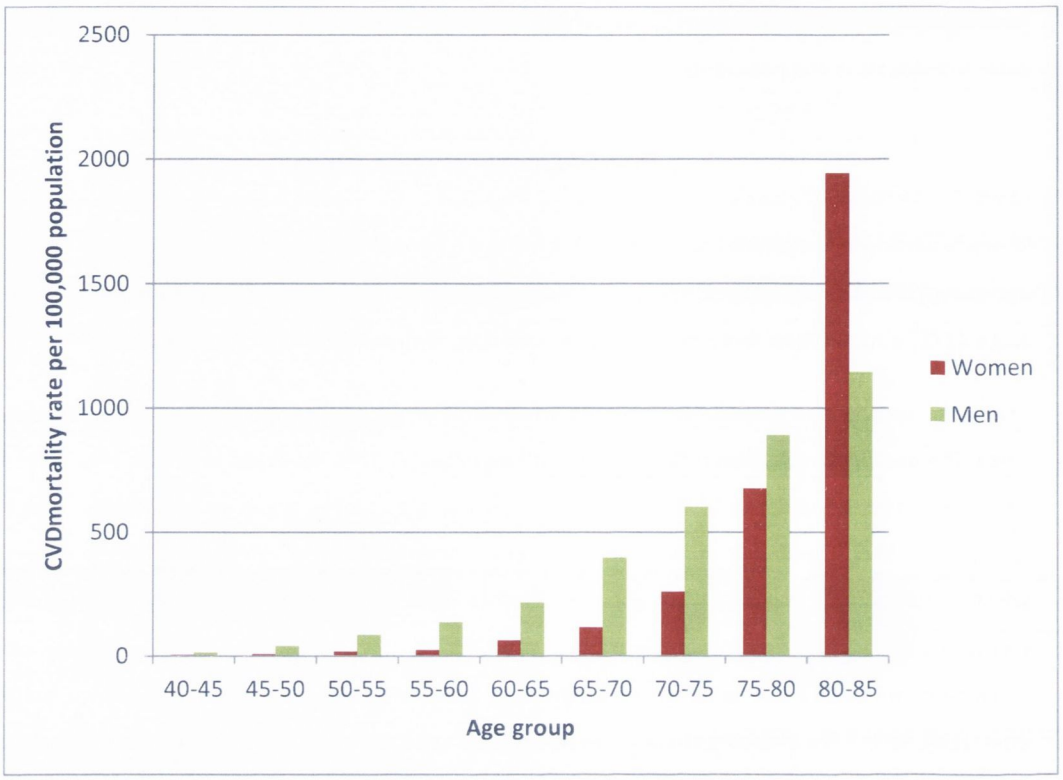


Figure 4.4: CHD mortality rates per 100,000 population in Ireland in 2006 in each age group

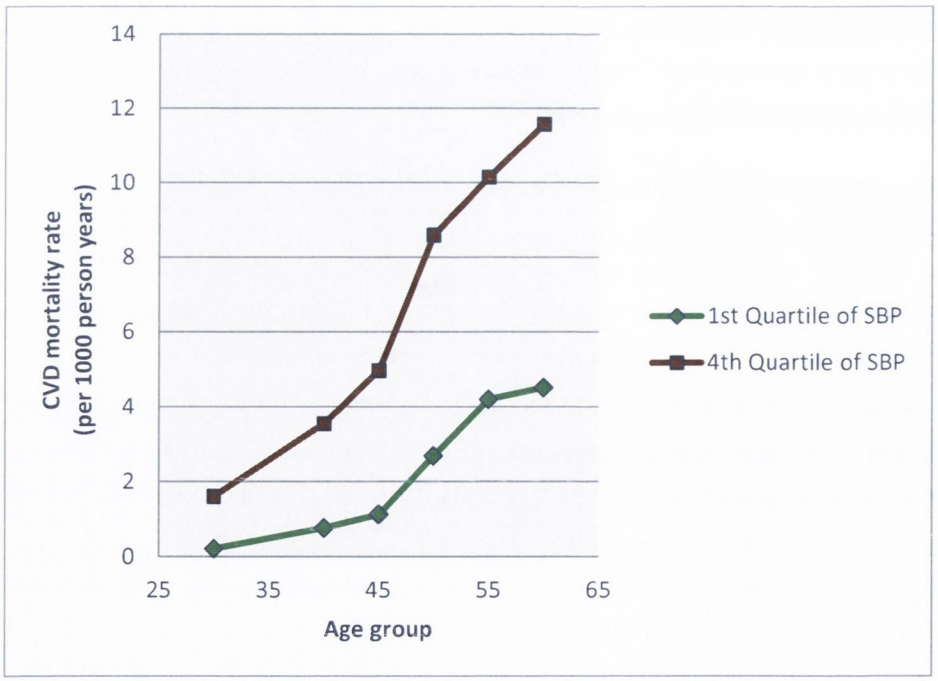


Figure 4.5: CVD mortality rates in the 1st and 4th quartiles of SBP in different age groups in the SCORE cohort

Age group	Relative risk	Excess risk*
30	7.6	1.4
40	4.6	2.8
45	4.4	3.8
50	3.2	5.9
55	2.4	6.0
60	2.6	7.1

Table 4-1: Relative and excess risk associated with 4th compared to 1st quartile of SBP in different age groups. * excess risk equals difference in CVD mortality rate (per 1000 person years)

PREVENTION OF CVD IN THE ELDERLY – RISK FACTOR MODIFICATION

Traditionally, randomized controlled trials related to CVD have focused on the younger and middle-aged age groups. This has led to a lack of evidence regarding the benefits of both acute treatments and preventive measures of CVD in this age group. As highlighted in a recent statement on treatment of coronary disease in older individuals by the American Heart Association[338], specific trials in the elderly or at least the inclusion of older subgroups are particularly important since there may be differential effects of treatments in this age group. This may occur due to increasing co-morbidities and varying pharmacodynamics in the older age group. Elderly specific endpoints also require attention, for example, frailty, cognitive function and maintenance of independence[338].

However, more recently, trials specifically in the older age group and subgroups analyses are becoming available. There is now randomized controlled trial evidence demonstrating the benefit of modification of risk factors in the older age group, including lipid modification[339] and smoking cessation[340, 341]. In the case of hypertension this applies not only to over 65s,[342] but has recently been extended to the very old[343].

For example, the HYVET trial of indapamide versus placebo for the treatment of systolic hypertension in those aged over 80 years demonstrated antihypertensive therapy to be associated with a 30% reduction in risk of stroke, 21% reduction in risk of death from any cause and a 64% reduction in heart failure[343].

With regard to prevention of stroke, which becomes more common with aging, the use of anticoagulation in the presence of atrial fibrillation[344] and carotid endarterectomy for secondary prevention in those with significant carotid stenosis have also been shown to be beneficial.

Lifestyle modifications, especially those focusing on increasing physical fitness, have also been shown to result in benefit in the older age group[345]. Programs which increase physical fitness, including cardiac rehabilitation, also result in important non-CVD related benefits in the elderly in terms of preventing

osteoporsosis and reducing frailty which often leads to reductions in independence and quality of life in older people.

Therefore, CVD should be considered preventable even in this age group and efforts should be made to increase usage of preventive measures in this population[346]. Increasing the evidence base for these measures as well as increasing awareness of the proven benefits would assist this.

RISK ESTIMATION IN OLDER PERSONS – LIMITATIONS OF CURRENT SYSTEMS AND POSSIBILITIES FOR OVERCOMING THESE

In order for evidence based guidelines on CVD prevention to be applied to the older age group, accurate risk estimation systems for this age group are required. However, at present there are a number of difficulties with the accuracy of risk estimation in this age group. Current risk estimation systems vary in the age ranges to which they apply. Most can be used up to age 75 years[45-48]. However, the SCORE function concentrates on the middle-aged group and is only recommended for use in the 40 to 65 year age range[4].

The accuracy of risk estimation has recently been shown to be substantially lower in older compared to middle aged individuals[72, 179]. The best demonstration of risk estimation in the elderly to date has been undertaken by Zethelius and colleagues[179]. In a function based on the Uppsala Longitudinal Study of Adult Men reasonable discrimination of a risk function containing conventional risk factors for prediction of CVD mortality, initially healthy men aged over 65, was demonstrated, with an AUROC of 0.688. The addition of four biomarkers (troponin I, N-terminal pro-brain natriuretic peptide, cystatin C, and C-reactive protein) resulted in an improvement in risk estimation with an increase in the AUROC to 0.748. It should be noted however, that these results are based on internal validation only.

An analysis of the performance of the Framingham function in initially healthy 85 year old individuals in the Leiden 85 plus study showed very poor discrimination with an AUROC of only 0.53, which was not significantly different from 0.50; (95% CI: 0.43 to 0.64)[72]. A function derived from the Leiden 85 plus study and containing the same conventional risk factors resulted in a similarly low AUROC. In fact, out of a number of risk factors and biomarkers only homocysteine was a significant predictor of CVD mortality in this group. It should be remembered that age, which is the strongest contributor to the discrimination of virtually all risk functions, could not function in this cohort because all of the group were the same age at baseline. This contributes to the remarkably low AUROC. The dataset for this study was small; 302 participants with 35 events.

Another study from the Netherlands in individuals aged over 70 years showed similarly poor discrimination with AUROCs of 0.55 and 0.60 for the PROCAM and Framingham functions respectively for the prediction of CVD mortality[347]. These investigators found an improvement in discrimination (AUROC 0.74) when

interleukin 6, CRP and carotid plaque burden where included in addition to conventional risk factors. However, this was also an internal validation.

One possible reason for the reduced accuracy of current risk estimation systems is that most of these systems were derived from cohorts of primarily middle-aged people, with older individuals under-represented in the derivation cohorts. The same beta-coefficients for the risk factors were applied at all age ranges meaning that the risks associated with risk factors in younger individuals have been extrapolated to the older age groups. As outlined above, substantial evidence points to the fact that although most conventional risk factors still function in the older age group, the relative importance of the risk factors changes with age[259] and therefore this use of the same beta coefficients in all age groups may be inappropriate. As demonstrated in the INTERHEART study, several risk factors assume lesser importance, however, others may be more important predictors of events, for example, moderate alcohol consumption and physical activity were significantly more important protective factors for myocardial infarction in men over 60 years than in men under 60 years [259].

Under-prescription of guideline recommended preventive measures in the elderly is a recognized problem[348, 349]. An interesting study by Ko et al demonstrated that not only did the probability of statin prescription decrease by 6.4% with each 1 year increase in age but those at highest baseline risk were the group least likely to receive statin therapy[350]. Part of this may be due to the paucity of RCTs focusing specifically on the effects of medication in the older age group. In the case of CVD prevention, primary prevention measures should be based on an assessment of the individual's level of total CVD risk. Current risk estimation systems are either non-applicable[4] to or inaccurate[72] in older individuals. This may be contributing to reluctance to prescribe these preventive measures in older individuals.

SCORE O.P. will be an extension of the SCORE system for use in the older age group (age 65 to 75 years). The function will be derived entirely from a cohort aged over 65 years at baseline. The system will utilise only the risk factors which remain significant predictors of CVD in the older age groups and will eliminate the problem of applying beta coefficients derived from studies of younger individuals to older individuals. The hypothesis is that the use of the specific risk factors which are most important in the older age group and the age-specific beta coefficients may result in an improvement in risk estimation.

SCORE O.P. – SPECIFIC RESEARCH QUESTIONS

1. To examine the effect of risk factors on both CHD and non-CHD CVD mortality in the older and younger age groups
2. To examine the proportion of CVD mortality caused by CHD and stroke in different age groups
3. To derive a function for the estimation of CVD risk in the older age group, called SCORE O.P.

4. In line with the above hypothesis, this risk estimation system would be derived specifically and exclusively from longitudinal data from the over 65 year old age group.
5. The derivation dataset will include the original SCORE dataset over 65 year olds and additionally a large Norwegian dataset will be added. To facilitate this, the baseline survival (both CHD and nCHD) of the Norwegian cohort will need to be examined in order to assess the most appropriate method for incorporating this newer data.
6. To internally validate this new risk estimation function in the dataset from which it was derived
7. To compare the performance of this risk estimation function with the performance of the original SCORE, in order to test the hypothesis of superior risk estimation in a function derived specifically from the older age group
8. To examine the most appropriate method for dealing with blood pressure lowering treatment in risk estimation systems in the older age group

SCORE O.P. – STUDY POPULATION

The original SCORE function was derived from a pooled dataset of 12 European cohort studies. This pooled dataset included over 205,000 individuals, representing 2.1 million person years of observation[4]. Of these 12 studies, three had data available for individuals aged over 65 years – Denmark, Italy and Belgium. The dataset used for the derivation of SCORE O.P. included the 6,154 individuals aged over 65 years from these three studies[250, 253, 257], to which was added data from a large cohort of 40,825 individuals aged 65 years and above from the Cohort of Norway (CONOR) prospective study [351].

Table 4-2 gives details of the population, recruitment and sampling, years of recruitment and number included for each of the individual studies in the dataset used for this analysis.

Country	Study	Recruitment and sampling	Participants aged ≥65 years	Years recruited
Belgium	Belgian Interuniversity Research on Nutrition and Health (BIRNH) [257]	Population – Stratified random sample based on National Belgian Registry	1,389	1980-1984
Denmark	The Glostrup Population Studies [253]	Population – Random sample & birth cohort – pooled	1,336	1977 1983-1984 1991-1992
Italy	Risk factors and life expectancy pooling	Population – random samples using either	3,429	1983 – 1987

	project (RIFLE) [250] – SCORE O.P. includes only 23 of 52 original cohorts	electoral registers or local population register offices		
Norway	Cohort of Norway (CONOR) [351]	Population – Random samples based on unique personal identification number	40,825	1994 – 2003

Table 4-2: Participating projects in the SCORE O.P. dataset

Detailed methods of the data collection methods, follow-up methods and case ascertainment for the individual studies have been published elsewhere[250, 253, 257, 351].

The endpoints were defined as in the original SCORE project, as described above.

SCORE O.P. – METHODS

DIFFERENTIAL EFFECT OF RISK FACTORS IN OLDER AND YOUNGER AGE GROUPS

Cox proportional hazards model was used to assess the effect of the following risk factors on CHD mortality and non-CHD CVD mortality:

- Age (as a continuous variable)
- Total cholesterol (as a continuous variable, per 1mmol/l increase)
- SBP (as a continuous variable, per 10mmHg increase)
- HDL cholesterol (as a continuous variable, per 0.5mmol/l increase)
- Current smoking status, versus non-current smoking status
- Diabetes (either self-reported or fasting glucose > 7mmol/l)
- Body mass index (as a continuous variable, per 5 unit increase)

This was performed separately in older and younger men and women from the SCORE dataset. Only countries which included older individuals were included. The analyses were stratified by country. It is noted that the older and younger individuals from Norway were from two different cohort studies. A hazard ratio for each endpoint, both adjusted for age only and adjusted additionally for each of the other risk factors listed was calculated.

PROPORTIONS OF CVD DEATHS CAUSED BY CHD AND STROKE IN DIFFERENT AGEGROUPS

Using the entire SCORE O.P. dataset and data on younger age groups from the corresponding countries in the original SCORE dataset, the rates of CHD mortality and stroke mortality per 1000 person years were calculated. The ratio of stroke mortality to CHD mortality in each age group was examined.

EXPLORATORY ANALYSES FOR INCORPORATION OF NORWEGIAN DATA

ASSESSING THE BASELINE SURVIVAL

To assess the most appropriate method for incorporating the newer Norwegian data with the original SCORE dataset the baseline survival of the Norwegian dataset was studied and compared with the baseline survival of the high and low risk cohorts in the original SCORE dataset, which contained over 65 year olds (low risk - Belgium and Italy; high risk - Denmark). The Kaplan Meyer survival curves for the two endpoints CHD and nCHD mortality were studies separately. These baseline survivals were adjusted to a baseline level of the risk factors (age 65 years, total cholesterol = 6mmol/l, SBP = 120mmHg, no diabetes, non-current smoker).

ASSESSING FOR DIFFERENCES IN RELATIVE RISK ASSOCIATED WITH RISK FACTORS

Gender specific hazard ratios for each risk factor for CVD mortality were calculated using Cox proportional hazards model. This was conducted separately in the Norwegian cohorts and the original SCORE cohorts combined (all limited to the over 65 year old age group). Adjustment was made for the other risk factors and the analyses were stratified by country.

EXPLORATORY ANALYSES ON DIFFERENT METHODS FOR HANDLING THOSE ON BP TREATMENT

Information on baseline use of anti-hypertensives was only available in the Norwegian cohort. The following sensitivity analyses were conducted to determine the bias in risk estimation caused by not considering the use of blood pressure lowering medication and also to determine the best methods for inclusion of BP lowering treatment as an extra variable. Only the Norwegian cohort was used for this analysis.

Three separate risk functions were derived as follows:

- Norwegian cohort split in two based on the use of blood pressure lowering treatment and the function derived separately in each
    - This should represent the most accurate option because the higher risk associated with use of blood pressure lowering medication is taken into account, as well as, the specific hazard ratios for SBP and other risk factors in those on and not on anti-hypertensives

- Function derived ignoring whether individuals were on blood pressure lowering treatment or not
- Function derived in the Norwegian cohort as a whole, but with anti-hypertensive use included as a separate risk factor

The correlation between the risk factor estimates obtained using each of the three methods was assessed by calculating correlation coefficients. This was done separately in men and women and in those on and not on anti-hypertensive medication at baseline. The performance of the three functions was compared using internal validation and the discrimination (AUROC) and calibration (Hosmer-Lemeshow and observed to predicted risks) of the three different functions was assessed. SCORE O.P. charts for men and women were compared for each of the three options for handling BP treatment.

The fully adjusted hazard ratios for each of the risk factors were calculated separately in men and women on and not on anti-hypertensive treatment.

DERIVATION OF SCORE O.P.

Two versions of the function were developed as in the original SCORE project, one for use in high risk European regions and the other for use in low risk regions. To determine whether the more recent data from Norway should be included as a high or low risk country I constructed survival curves for both endpoints, adjusted to baseline levels of total cholesterol, HDL cholesterol and systolic blood pressure and non-smoker, non-diabetic status for the countries originally included in the high and low risk functions and compared the adjusted survival curve of the Norwegian cohort to these.

The statistical methods used were similar to those of the original SCORE project[4]. The derivation dataset contained only individuals aged over 65 years. Cox proportional hazards model was used to derive a function for estimating 10 year risk of CVD mortality. A function for estimating 5 year risk was also developed. The analyses were conducted separately in men and women and the analyses were stratified by region. In this way, the baseline survivals were both gender and region-specific. Gender specific beta coefficients were calculated but the same beta coefficients were used for both high and low risk regions.

The baseline survival was combined with the beta coefficients for each of the risk factors which remained significant predictors of the endpoints, as shown in equation 4-1, to give the estimate of 10 year risk. Risk factors which remained significant in the two multivariable models were included.

Equation 4-1:

$$10 \text{ year risk of CVD mortality} = 1 - [So^{\exp((\beta chol * chol6)+(\beta sbp * sbp120)+(\beta currsmok * currsmok) + (\beta hdl * hdl1)+(\beta diabetes * diabetes))}]$$

If Chol6 = cholesterol level – 6mmol/l; SBP120 = SBP level – 120mmHg; Currsmok = 1 if smoker, 0 if non-smoker; hdl1 = HDL-C – 1; diabetes = 1 if diabetic or = 0 if non-diabetic

And So(10) = baseline 10 year CVD survival, adjusted to the baseline level of the risk factors

To calculate the risk of 5 year CVD mortality, the baseline survival to So(5) is inserted.

TESTING THE PERFORMANCE OF NEW FUNCTION

The performance of the function was tested in terms of discrimination including area under receiver operating characteristic curve and Harrell's C statistic, which is more reliable in the situation of variable follow-up. The sensitivity, specificity and predictive values of the function at different cut-off points for high/low risk were also calculated. The calibration of the function was assessed in terms of predicted to observed ratios and Hosmer Lemeshow goodness of fit testing. Hosmer Lemeshow testing was only possible for the five year function because complete follow-up to ten years was not available for some of the cohorts.

INTERNAL VALIDATION – SENSITIVITY ANALYSIS

To test the hypothesis that using the specific risk factors which were particularly important in the older age group and deriving the beta coefficients for these risk factors specifically from the older age group improves risk estimation, we compared the discrimination of the original SCORE function and SCORE O.P. in terms of AUROC and predicted to observed ratios. The test dataset contained only those aged over 65 years. Because the CONOR cohort was not included in the derivation cohort of the original SCORE function, the Norwegian data had to be excluded from the test dataset for this particular sensitivity analysis. Inclusion of the CONOR data in the test dataset would have resulted in an inequitable "home advantage" for the SCORE O.P. function compared to the original SCORE function.

All statistical analyses were performed using Stata 9.

SCORE O.P. - RESULTS

SCORE O.P. – BASELINE CHARACTERISTICS

Twenty thousand, one hundred and twenty-one women and 20,704 men were included in the analysis. Median follow-up was 7.8 years for women and 6.8 years for men. One thousand, one hundred and fifty-

four fatal CHD events occurred in men 842 fatal CHD events occurred in women during the follow-up period. The corresponding figures for the nCHD CVD mortality endpoint were 1,111 and 1,037.

Baseline characteristics for the entire group and subdivided by cohort and gender are given in **Table 4-3**. The rates of CHD and nCHD CVD mortality in each country are also shown in **Table 4-3**. Of note the CHD mortality rate of Norway is approximately half that of Denmark, even though originally, both countries were considered high risk European regions, for SCORE. This is because of the later baseline of the Norwegian cohort, which has been added for SCORE O.P.

Women					
	Belgium	Denmark	Italy	Norway	Total
Number	619	683	1,680	17,139	20,121
No. events CHD	30	53	26	733	842
No. events nCHD CVD	25	47	20	1019	1111
Rate CHD (per 1000 person yr)	4.5	11.7	2.9	5.5	5.5
Rate nCHD CVD (per 1000 person yr)	4.4	10.4	1.9	7.7	7.2
Baseline years	1980-1984	1977-1991	1984-1987	1994-2003	
Median followup (yr)	10.1	8.8	6.0	7.9	7.8
Median age (years)	69.4	70.3	67.0	73.6	72.9
Age range (years)	65-75	69-80	65-99	65-99	65-99
% DM	6%	6%	6%	7%	7%
% Smokers	4%	34%	9%	16%	16%
Mean TC (mmol/l)	7.0	6.9	6.2	6.8	6.7
Mean HDL (mmol/l)	1.5	1.6	1.4	1.6	1.5
Mean SBP (mmHg)	151	150	154	155	155
Men					
	Belgium	Denmark	Italy	Norway	Total
Number	770	653	1,749	17,532	20,704
No. events CHD	40	67	65	982	1154
No. events nCHD CVD	43	35	30	929	1037
Rate CHD (per 1000 person yr)	6.1	17.7	5.9	8.1	8.1
Rate nCHD CVD (per 1000 person yr)	6.6	9.2	2.7	7.6	7.3
Baseline years	1980-1984	1977 1991	1984-1987	1994-2003	
Median followup (yr)	10.1	6.2	6.0	6.8	6.8
Median age (years)	69.6	70.3	67.0	72.3	71.6
Age range (years)	65-75	69-80	65-93	65-101	65-101
% DM	4%	7%	6%	7%	7%
% Smokers	49%	51%	32%	23%	26%
Mean TC (mmol/l)	6.2	6.1	5.7	6.1	6.0
Mean HDL (mmol/l)	1.3	1.3	1.3	1.4	1.3

Mean SBP (mmHg)	145	147	150	150	149

Table 4-3: Baseline characteristics and numbers included for each of the countries included in the analysis

RESULTS – DIFFERENTIAL EFFECTS OF RISK FACTORS IN OLDER AND YOUNGER PEOPLE

Table 4-4 and **Table 4-5** show the hazard ratios in younger and older men and women for the endpoints CHD mortality and nCHD CVD mortality respectively. To summarise the effects of the risk factors in the older age group, several risk factors remain independently associated with CHD mortality in the older age group. These are: age, total cholesterol, systolic blood pressure, HDL cholesterol (as discussed in section 1), current smoking and the presence of diabetes. This is seen in both men and women, although total cholesterol is a stronger risk factor in older men than older women and HDL cholesterol was shown to be a stronger protective factor in older women than older men. In both older men and older women BMI, as a continuous variable, did not remain statistically significant as a risk factor once adjusted for the presence of other risk factors.

Comparing the risk factors for CHD mortality in younger and older groups in all cases there is a reduction in hazard ratio for each of the risk factors. The main exception to this is age itself. Each increase of 1 year in baseline age was associated with a higher hazard ratio in both older men and women, compared to their younger counterparts. Additionally, HDL cholesterol was a stronger protective risk in older than younger women, however, this difference did not reach statistical significance.

Looking at the risk factors for nCHD CVD mortality, total cholesterol did not prove to be an independent risk factor in any subgroup and HDL cholesterol was only a protective factor in older women. Increasing BMI was associated independently with increasing risk of nCHD CVD mortality in younger but not older individuals. Current smoking, diabetes, and systolic blood pressure were independent risk factors in all subgroups, but again the hazard ratios were attenuated in the older age group. As with CHD mortality, the relationship between age and nCHD CVD mortality was stronger in older than younger men and women.

Age adjusted hazard ratios				
	Women		Men	
	Younger (<65 years)	Older (≥65 years)	Younger (<65 years)	Older (≥65 years)
Age (per 1 year increase)	1.12 (1.09 to 1.15)	1.16 (1.15 to 1.18)	1.11 (1.10 to 1.12)	1.12 (1.11 to 1.14)
SBP (per 10mmHg increase)	1.29 (1.22 to 1.37)	1.08 (1.05 to 1.12)	1.24 (1.21 to 1.28)	1.10 (1.07 to 1.13)
Total Chol (per 1 mmol/l increase)	1.40 (1.32 to 1.50)	1.11 (1.05 to 1.18)	1.31 (1.27 to 1.36)	1.23 (1.17 to 1.30)
Current Smoker	2.80 (2.10 to 3.72)	1.80 (1.49 to 2.17)	2.39 (2.09 to 2.72)	1.72 (1.51 to 1.95)
Diabetes	9.03 (5.60 to 14.56)	2.66 (2.19 to 3.22)	3.10 (2.39 to 4.02)	1.80 (1.50 to 2.17)
BMI (per 5 unit increase)	1.22 (1.06 to 1.41)	1.04 (0.97 to 1.13)	1.29 (1.19 to 1.41)	1.08 (0.99 to 1.18)
HDL (per 0.5mmol/l increase)	0.59 (0.40 to 0.87)	0.53 (0.38 to 0.73)	0.73 (0.61 to 0.87)	0.73 (0.57 to 0.94)
Multivariable adjusted hazard ratios				
	Women		Men	
	Younger (<65 years)	Older (≥65 years)	Younger (<65 years)	Older (≥65 years)
Age (per 1 year increase)	1.07 (1.04 to 1.10)	1.16 (1.15 to 1.18)	1.09 (1.08 to 1.10)	1.13 (1.12 to 1.14)
SBP (per 10mmHg increase)	1.26 (1.19 to 1.34)	1.08 (1.05 to 1.11)	1.22 (1.18 to 1.25)	1.08 (1.06 to 1.11)
Total Chol (per 1 mmol/l increase)	1.35 (1.26 to 1.45)	1.12 (1.06 to 1.18)	1.26 (1.21 to 1.30)	1.22 (1.16 to 1.29)
Current Smoker	2.93 (2.19 to 3.92)	1.96 (1.62 to 2.37)	2.45 (2.15 to 2.80)	1.82 (1.60 to 2.08)
Diabetes	7.28 (4.47 to 11.87)	2.73 (2.24 to 3.32)	2.55 (1.96 to 3.31)	1.88(1.56 to 2.27)
BMI (per 5 unit increase)	1.06 (0.91 to 1.23)	1.02 (0.94 to 1.10)	1.15 (1.06 to 1.26)	1.08 (0.99 to 1.17)
HDL (per 0.5mmol/l increase)	0.61 (0.41 to 0.91)	0.49 (0.35 to 0.68)	0.74 (0.62 to 0.89)	0.73 (0.57 to 0.95)

Table 4-4: Hazard ratios for CHD mortality different risk factors for older and younger individuals

Age adjusted hazard ratios				
	Women		Men	
	Younger (<65 years)	Older (≥65 years)	Younger (<65 years)	Older (≥65 years)
Age (per 1 year increase)	1.15 (1.12 to 1.19)	1.18 (1.17 to 1.19)	1.13 (1.11 to 1.15)	1.15 (1.14 to 1.16)

SBP (per 10mmHg increase)	1.30 (1.22 to 1.39)	1.08 (1.05 to 1.11)	1.34 (1.28 to 1.41)	1.07 (1.04 to 1.10)
Total Chol (per 1 mmol/l increase)	1.11 (0.98 to 1.25)	0.94 (0.89 to 0.99)	1.13 (1.04 to 1.23)	0.97 (0.91 to 1.02)
Current Smoker	2.68 (1.90 to 3.77)	1.46 (1.22 to 1.74)	1.62 (1.29 to 2.04)	1.69 (1.48 to 1.94)
Diabetes	4.84 (2.51 to 9.35)	1.95 (1.62 to 2.35)	2.75 (1.70 to 4.46)	1.62 (1.32 to 1.99)
BMI (per 5 unit increase)	1.41 (1.20 to 1.65)	0.91 (0.85 to 0.97)	1.49 (1.28 to 1.73)	1.06 (0.97 to 1.16)
HDL (per 0.5mmol/l increase)	0.70 (0.48 to 1.03)	0.68 (0.49 to 0.94)	0.92 (0.71 to 1.20)	0.93 (0.69 to 1.25)
Multivariable-adjusted hazard ratios				
	Women		Men	
	Younger (<65 years)	Older (≥65 years)	Younger (<65 years)	Older (≥65 years)
Age (per 1 year increase)	1.12 (1.08 to 1.15)	1.17 (1.16 to 1.18)	1.10 (1.08 to 1.13)	1.15 (1.14 to 1.16)
SBP (per 10mmHg increase)	1.27 (1.19 to 1.36)	1.09 (1.06 to 1.12)	1.32 (1.26 to 1.39)	1.07 (1.04 to 1.10)
Total Chol (per 1 mmol/l increase)	1.01 (0.89 to 1.15)	0.94 (0.89 to 0.98)	1.05 (0.97 to 1.15)	0.96 (0.91 to 1.02)
Current Smoker	3.09 (2.18 to 4.36)	1.48 (1.24 to 1.78)	1.81 (1.44 to 2.29)	1.77 (1.54 to 2.03)
Diabetes	3.46 (1.77 to 6.75)	1.97 (1.62 to 2.38)	2.12 (1.30 to 3.45)	1.62 (1.31 to 1.99)
BMI (per 5 unit increase)	1.26 (1.07 to 1.49)	0.88 (0.82 to 0.94)	1.28 (1.10 to 1.49)	1.07 (0.97 to 1.17)
HDL (per 0.5mmol/l increase)	0.85 (0.57 to 1.25)	0.58 (0.41 to 0.82)	1.02 (0.78 to 1.32)	0.94 (0.69 to 1.27)

Table 4-5: Hazard ratios for nCHD CVD mortality for different risk factors in older and younger individuals

PROPORTION OF CVD DEATHS CAUSED BY CORONARY HEART DISEASE, STROKE AND PERIPHERAL VASCULAR DISEASE

These analyses were conducted using the full dataset to explore the different proportions of deaths caused by coronary disease and stroke in older and younger individuals. The proportion of total deaths caused by stroke increased with increasing age, as shown in **Figure 4.6**. The proportion caused by coronary disease also increased. Also illustrated in **Figure 4.6** is the increasing proportion of cardiovascular deaths which are caused by stroke with advancing age. This was seen in both men and women from high and low risk countries.

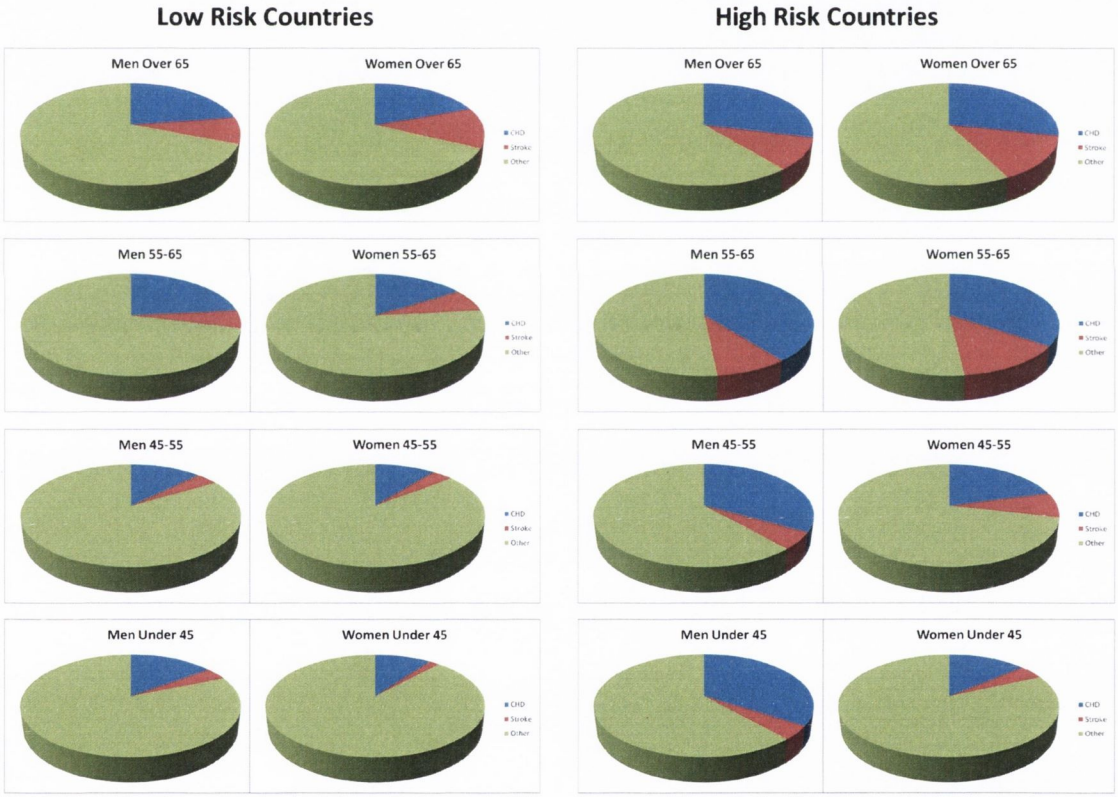


Figure 4.6: Proportions of total deaths caused by stroke and coronary heart disease in different age groups

Figure 4.7 and **Figure 4.8** illustrate in individuals from high and low risk countries respectively, the coronary heart disease and stroke mortality rates in different age groups. The number represents the ratio between stroke and

coronary mortality. In general, there is a trend towards increasing ratio of stroke to coronary deaths with increasing age group and in all subgroups the ratio is highest in the oldest age group.

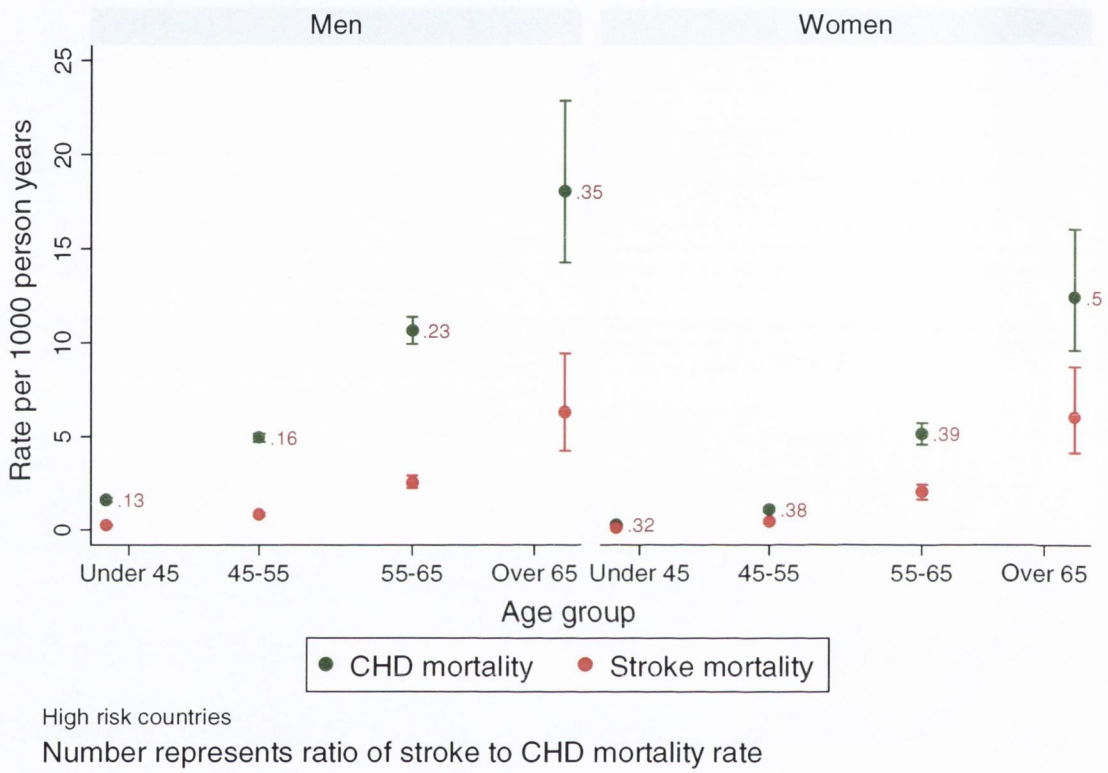


Figure 4.7: Coronary heart disease and stroke mortality rates in different age groups in individuals from high risk countries

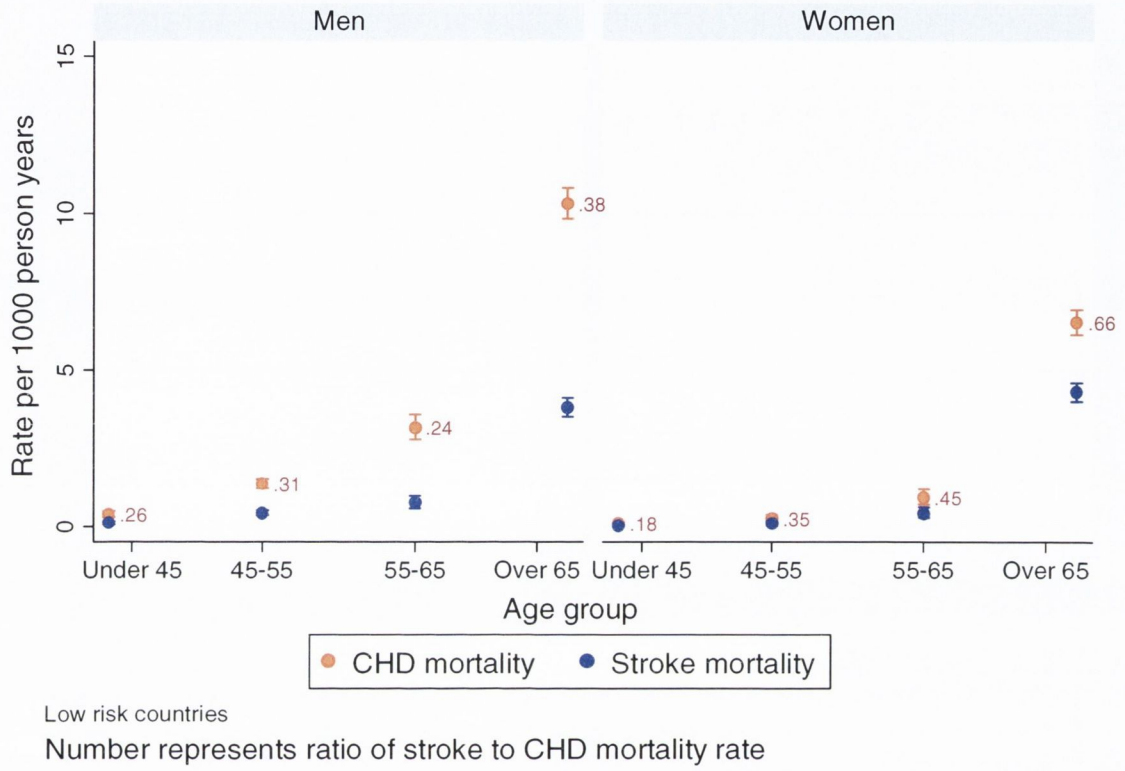


Figure 4.8: Coronary heart disease and stroke mortality rates in different age groups in individuals from low risk countries

EXPLORATORY ANALYSES FOR THE INCORPORATION OF THE MORE RECENT NORWEGIAN DATA WITH THE SCORE DATASET

ASSESSING THE BASELINE SURVIVAL IN NORWEGIAN AND ORIGINAL SCORE COHORTS

The baseline survivals for the original low and high risk SCORE cohorts and the Norwegian cohort in men and women are shown in **Figure 4.9** and **Figure 4.10** respectively for the CHD mortality endpoints. The baseline survival for men and women respectively for the nCHD mortality endpoints are shown in **Figure 4.11** and **Figure 4.12**.

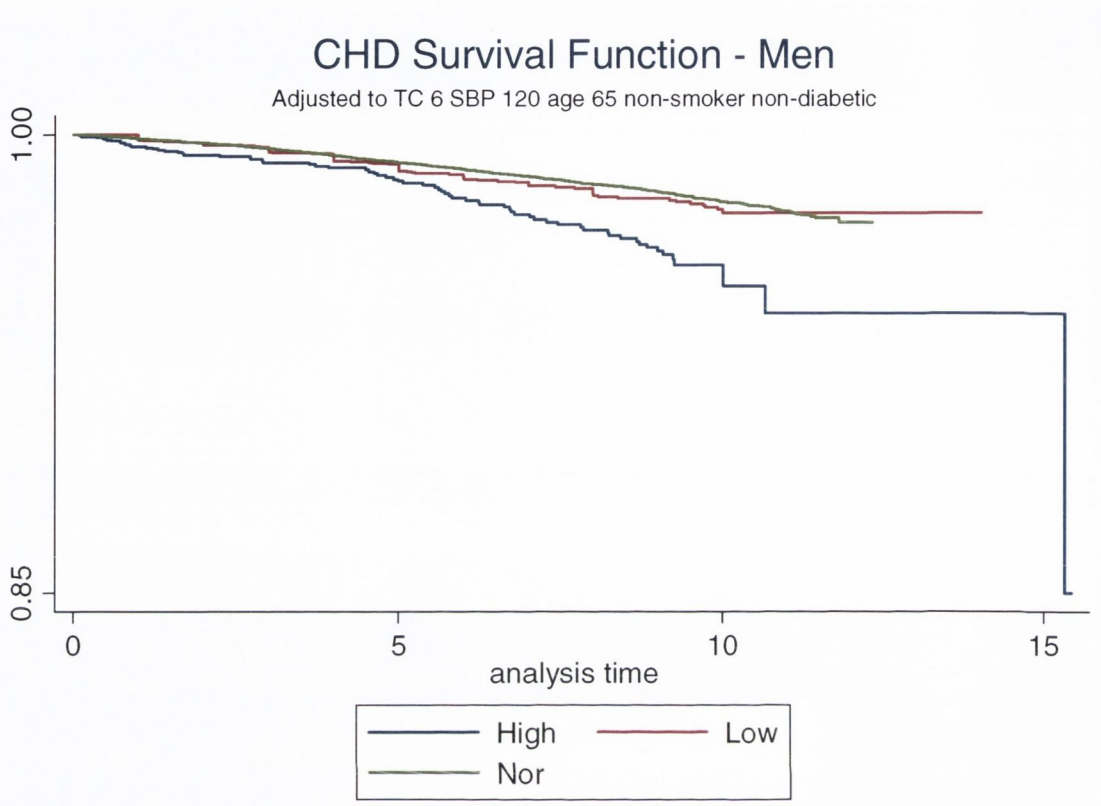


Figure 4.9: Baseline survival curve for CHD mortality in men from the original SCORE high and low risk cohorts and the Norwegian cohort

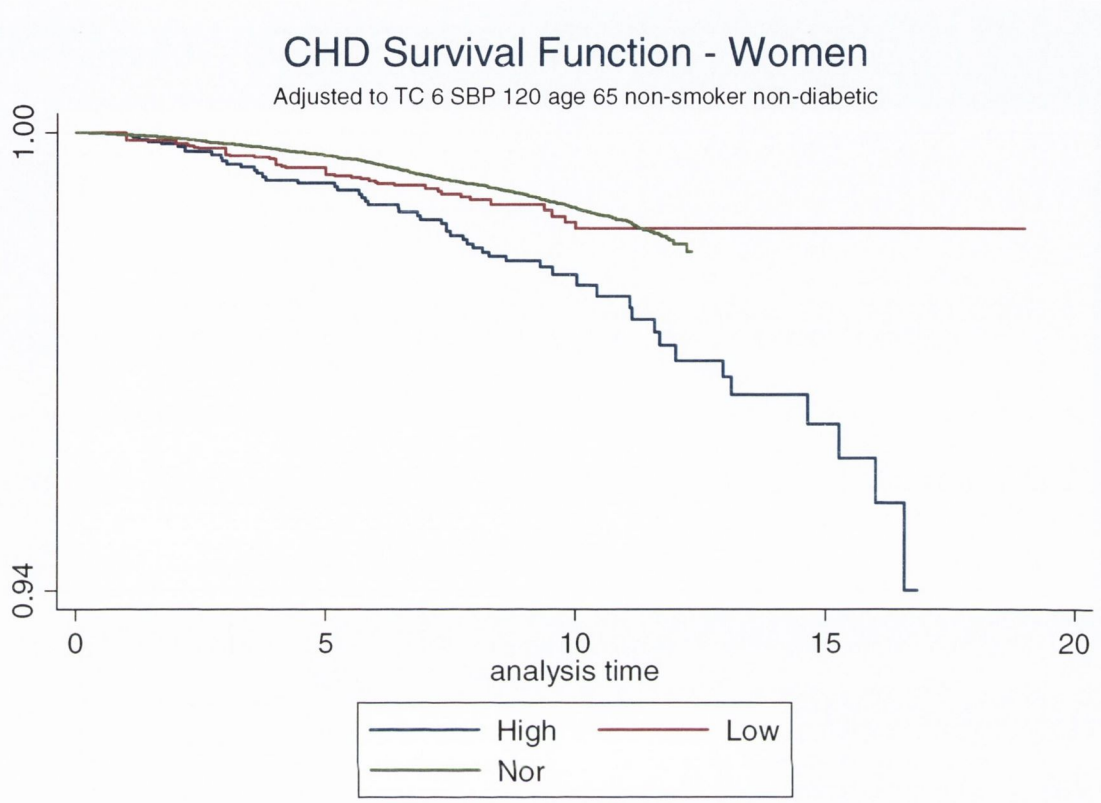


Figure 4.10: Baseline survival curve for CHD mortality in women from the original SCORE high and low risk cohorts and the Norwegian cohort

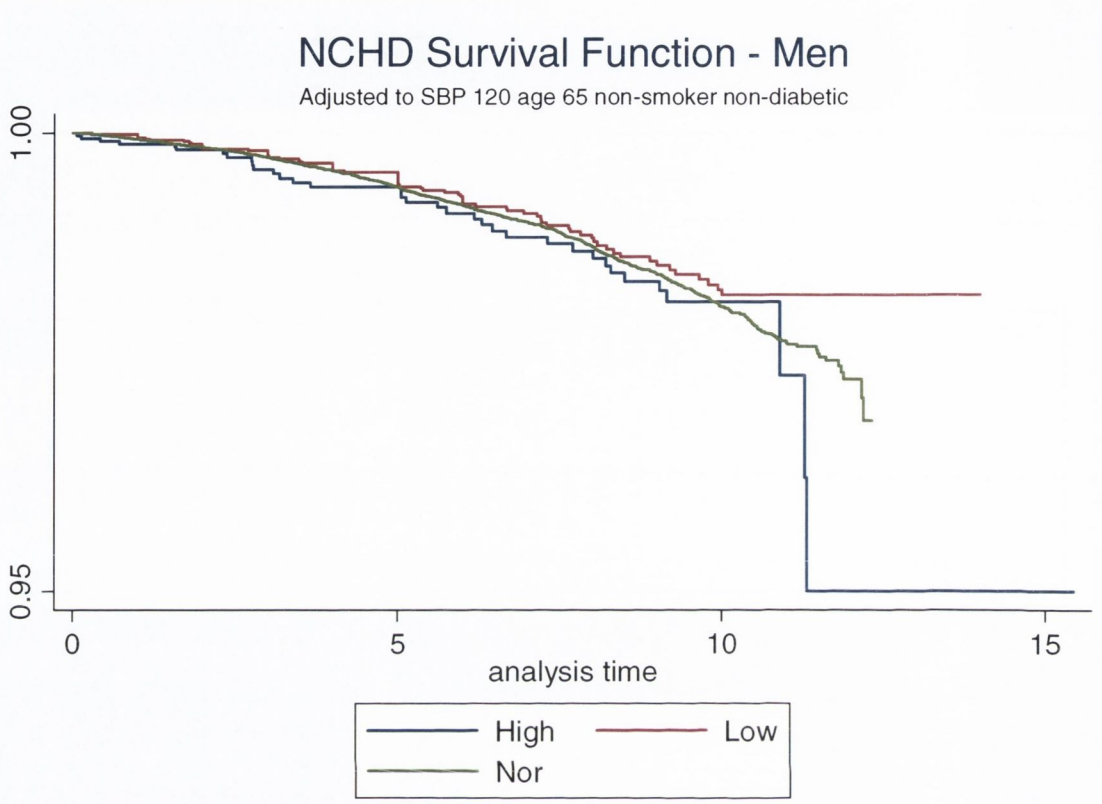


Figure 4.11: Baseline survival curve for nCHD CVD mortality in men from the original SCORE high and low risk cohorts and the Norwegian cohort

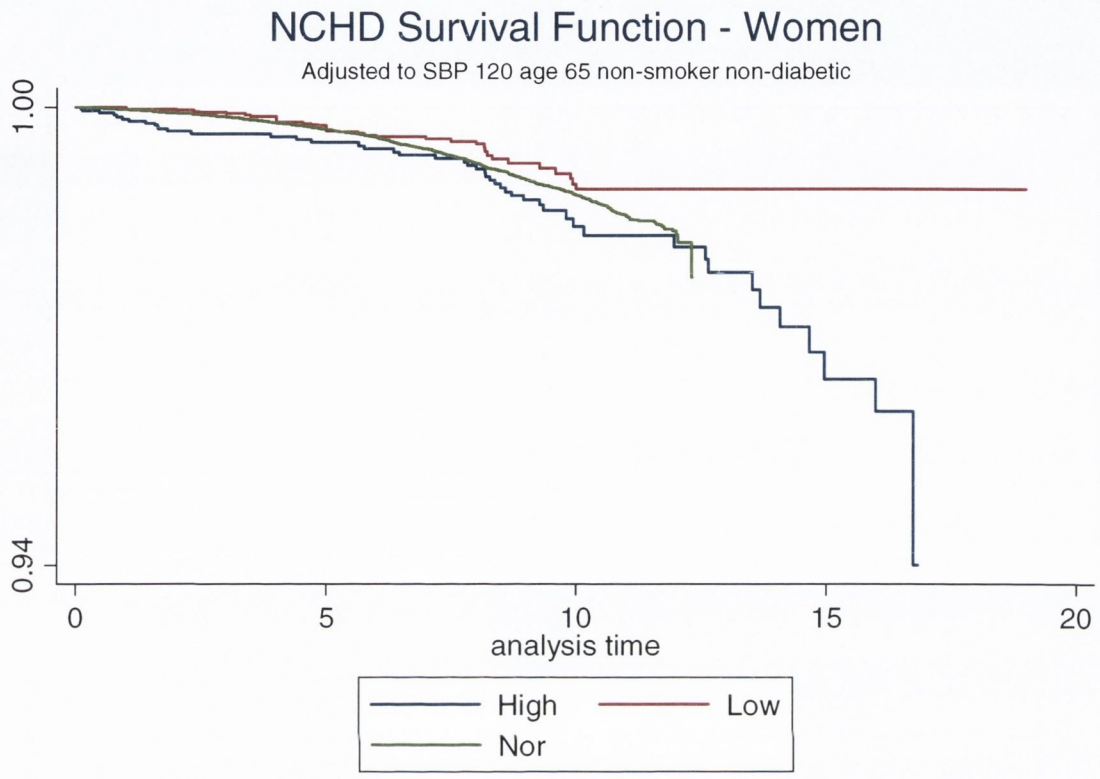


Figure 4.12: Baseline survival curve for nCHD CVD mortality in women from the original SCORE high and low risk cohorts and the Norwegian cohort

As illustrated in the baseline survivals curves, the baseline for the Norwegian cohort was closest to the low risk original SCORE countries for the CHD mortality endpoint for both men and women. For this reason, Norway was included as a low risk cohort. For the nCHD CVD mortality endpoint the baseline survivals were similar for all three groups in both men and women. However, to keep the methods as compatible with the original SCORE methods as possible, separate baseline survivals were used for high and low risk cohorts for the nCHD CVD mortality endpoint also, with Norway included as a low risk country.

ASSESSING THE EFFECT OF RISK FACTORS IN THE NORWEGIAN AND THE ORIGINAL SCORE COHORTS

Table 4-6 shows the fully adjusted hazard ratios for those in the Norwegian cohort and those in the original SCORE cohort. The hazard ratios for most of the risk factors were very similar. The only statistically significant difference was a stronger protective effect of HDL cholesterol in women from the original SCORE cohorts

compared to the Norwegian women. In men from Norway, there was a non-significantly lower hazard ratio for SBP.

	Men		Women	
	Norway	Not Norway	Norway	Not Norway
SBP (per 10mmHg)	1.06(1.05 to 1.09)	1.10 (1.05 to 1.16)	1.07 (1.05 to 1.03)	1.07 (1.01 to 1.15)
Total Cholesterol (per 1mmol/l)	1.11 (1.07 to 1.16)	1.12 (1.01 to 1.24)	1.02 (0.98 to 1.06)	0.99 (0.87 to 1.12)
HDL Cholesterol (per 1 mmol/l)	0.71 (0.62 to 0.80)	0.67 (0.47 to 0.97)	0.77 (0.69 to 0.87)	0.34 (0.22 to 0.53)
Current Smoking	1.80 (1.63 to 1.99)	1.62 (1.27 to 2.06)	1.75 (.153 to 2.00)	1.51 (1.04 to 2.20)
Diabetes	1.72 (1.49 to 1.99)	1.83 (1.22 to 2.75)	2.14 (1.87 to 2.46)	1.57 (0.97 to 2.53)
Age (per 1 year)	1.14 (1.13 to 1.15)	1.13 (1.10 to 1.16)	1.17 (1.16 to 1.18)	1.15 (1.13 to 1.18)

Table 4-6: Adjusted hazard ratios for CVD mortality in men and women in the Norwegian and original SCORE cohorts

DERIVING THE SCORE O.P. FUNCTION

Only the variables which remained statistically significant predictors of CHD and nCHD CVD mortality on multivariable analysis were included in the SCORE O.P. CHD and nCHD CVD mortality models respectively. This meant that total cholesterol, HDL cholesterol, diabetes, smoking status, systolic blood pressure and age were included in the CHD mortality model and the same variables, with the exception of total cholesterol, were included in the nCHD CVD mortality model.

Table 4-7 shows the beta coefficients for each of the risk factors which remained statistically significant predictors of the CHD and nCHD CVD mortality endpoints. **Table 4-7** also shows the adjusted baseline survivals to 10 and 5 years for men and women for the CHD and nCHD CVD mortality functions for the high and low risk functions. When these figures are inserted into equation 4-1 the SCORE O.P. risk can be calculated for any combination.

CHD Mortality endpoint			
Women – CHD		Men – CHD	
SBP	0.007 (0.004 to 0.010	SBP	0.008 (0.005 to 0.010
TC	0.121 (0.067 to 0.174	TC	0.210 (0.159 to 0.261
HDL	-0.559 (-0.732 to -0.385	HDL	-0.411 (-0.574 to -0.248
Smoking	0.629 (0.443 to .815	Smoking	0.584 (0.458 to 0.709
Diabetes	0.832 (0.638 to 1.027	Diabetes	0.610 (0.425 to 0.794

Age	0.147 (0.136 to 0.159	Age	0.118 (0.107 to 0.129
Baseline High	0.980247	Baseline High	0.950969
Baseline Low	0.990066	Baseline Low	0.977713
Non CHD CVD Mortality endpoint			
Women – nCHD		Men – nCHD	
SBP	0.007 (0.005 to 0.010	SBP	0.007 (0.004 to 0.009
HDL	-0.150 (-0.292 to -0.008	HDL	-0.284 (-0.454 to -0.114
Smoking	0.428 (0.251 to 0.604	Smoking	0.567 (0.434 to 0.700
Diabetes	0.654 (0.474 to 0.835	Diabetes	0.475 (0.270 to 0.681
Age	0.162 (0.152 to 0.172	Age	0.138 (0.127 to 0.149
Baseline High	0.984806	Baseline High	0.98063
Baseline Low	0.988839	Baseline Low	0.980119

Table 4-7: SCORE O.P. - Beta coefficients and baseline survival to 5 and 10 years for men and women

Examples of SCORE O.P. charts for men and women for use in high risk European regions are shown in **Figure 4.13**, for illustration purposes only. Because only 5 variables can be accommodated in the two dimensional paper charts these charts assume a HDL of 1.2 in men and 1.4 in women and non-diabetic status. However, inclusion of the SCORE O.P. function in the HeartScore system would enable incorporation of all of the variables.

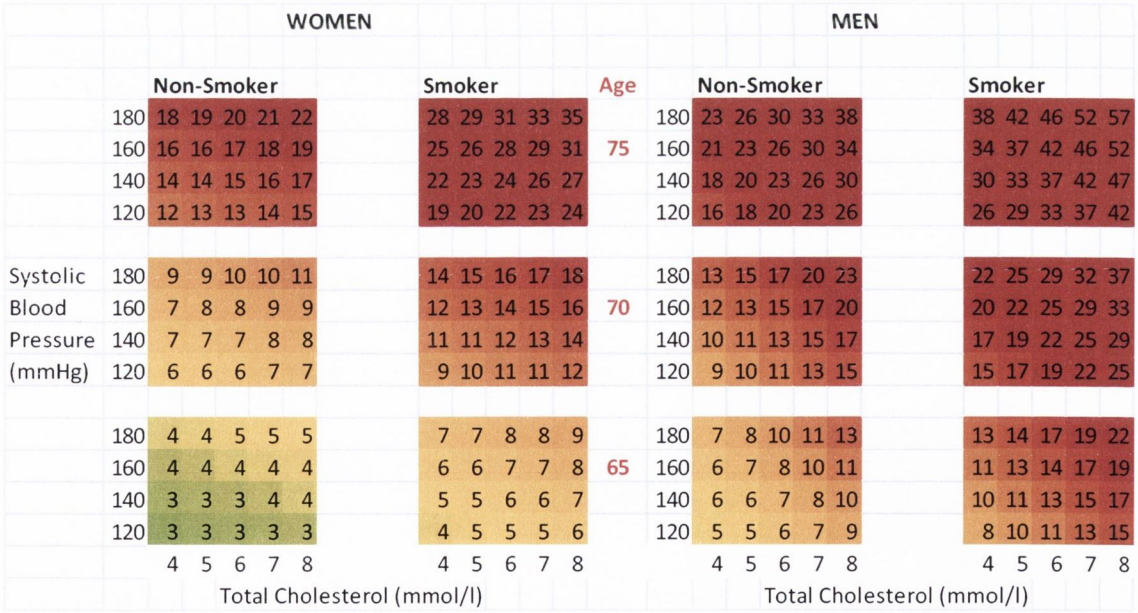


Figure 4.13: SCORE O.P. charts for men and women from high risk European countries

On internal validation the SCORE O.P. function demonstrated good discrimination, with an AUROC of 0.74 (0.73 to 0.75) in the overall group. The AUROC and Harrell's C statistics for the function SCORE O.P. are shown in **Table 4-8**. The summary calibration results – Hosmer-Lemeshow tests and predicted to observed ratios are also shown in **Table 4-8**. The function resulted in slight overestimation of risk; this occurred mainly in those at highest risk. **Figure 4.14** demonstrates graphically the predicted and observed rates of CVD mortality at each quintile of the risk function. **Figure 4.15**, **Figure 4.16** and **Figure 4.17** show the goodness of fit in each tenth of the risk function in men and women, women alone and men alone, respectively.

	Women	Men	All
AUROC	0.78 (0.76 to 0.79)	0.70 (0.69 to 0.71)	0.74 (0.73 to 0.75)
Harrell's C statistic – CHD	0.761	0.693	
Harrell's C Statistic – nCHD CVD	0.770	0.706	
Hosmer Lemeshow (p value) - five year function	17.16 (0.071)	22.70 (0.0119)	24.33 (0.0068)
Predicted / Observed ratios	1.03	1.05	1.04

Table 4-8: Internal validation results (discrimination and calibration) for SCORE O.P.

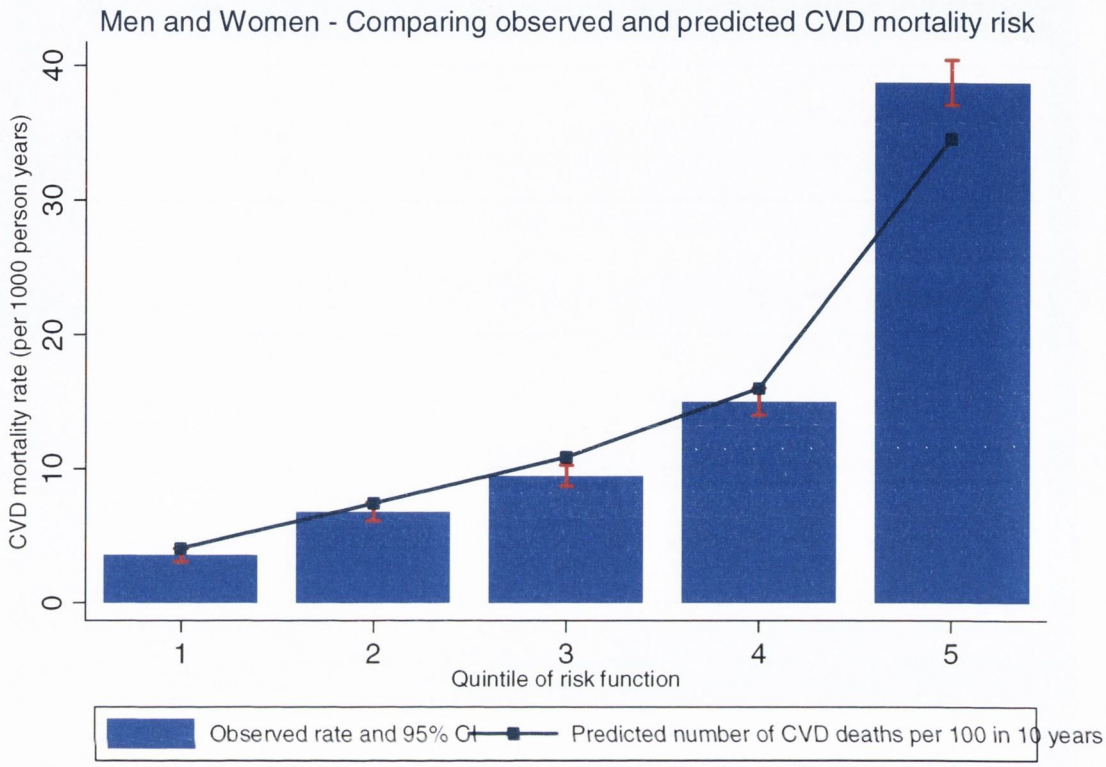


Figure 4.14: Predicted and observed rates of CVD mortality in each quintile of SCORE O.P. risk function

Figure 4.15: Goodness of fit in men and women in tenths of SCORE O.P. function (5 year predicted compared to 5 year observed risk)

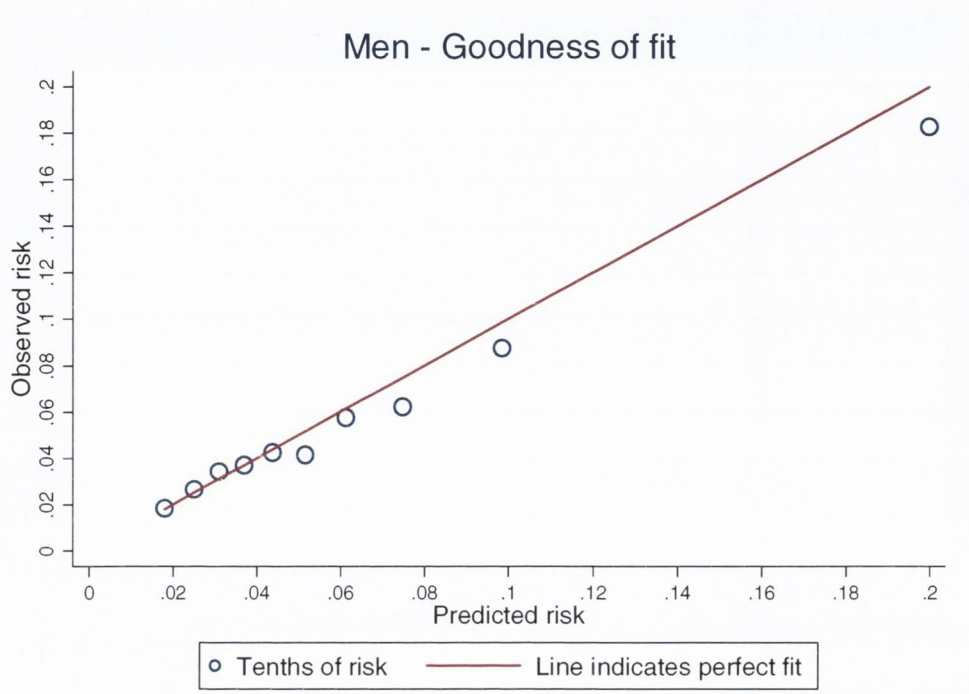


Figure 4.16: Goodness of fit in tenths of SCORE O.P. risk function in men (Predicted 5 year risk compared to observed 5 year risk)

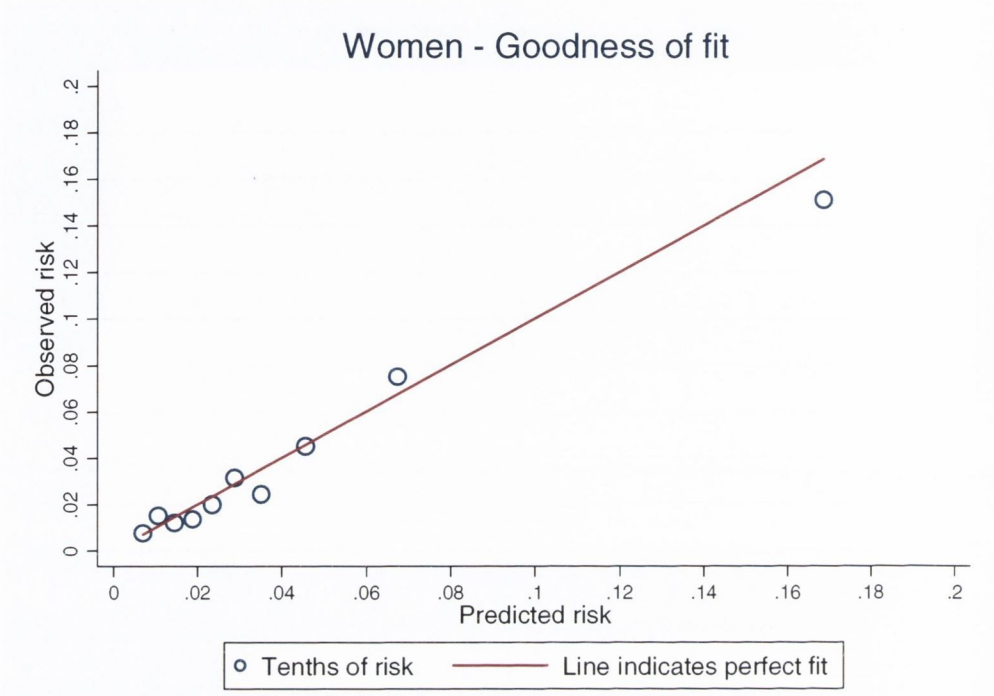


Figure 4.17: Goodness of fit in tenths of SCORE O.P. function (5 year predicted risk compared to 5 year observed risk)

INTERNAL VALIDATION – SENSITIVITY ANALYSIS

Table 4-9 shows the AUROC and Hosmer Lemeshow goodness of fit testing (5 year function) for both SCORE O.P. and the original SCORE function in the over 65 year old age group. As mentioned above only the countries (Italy, Belgium, and Denmark) included in both the original SCORE derivation dataset and the SCORE O.P. dataset were included in this test dataset. This ensured that both functions were internally validated on a dataset which was included in the derivation cohort for the function. As shown in the AUROC figures, discrimination was significantly better in the SCORE O.P. function. Calibration was also marginally improved.

	AUROC		Hosmer Lemeshow (p)	
	Men	Women	Men	Women
SCORE O.P. function	0.7036	0.7919	9.98 (0.4423)	15.26 (0.1228)
Original SCORE function	0.6849	0.7436	16.64 (0.0826)	20.96 (0.0214)
p for difference	0.05	<0.001	-	-

Table 4-9: Performance of SCORE O.P. compared to the original SCORE function in over 65 year olds

EXPLORATORY ANALYSES ON METHODS FOR INCLUSION OF BASELINE BP LOWERING MEDICATION

Table 4-10 shows the fully adjusted hazard ratios for CVD mortality in those on and not on BP lowering medications, separately in men and women. The risk associated with SBP as a continuous variable was reduced in those on anti-hypertensive medications. There were some differences in the effects of the other risk factors also, including reduced risk associated with diabetes in men in the presence of anti-hypertensive treatment. Additionally, in women there were opposite effects of total and HDL cholesterol, with total cholesterol associated with no increased risk in women not on anti-hypertensives and HDL cholesterol not associated with a protective effect in women on anti-hypertensives.

	Men		Women	
	On BP lowering treatment	Not on BP lowering treatment	On BP lowering treatment	Not on BP lowering treatment
Systolic blood pressure (per 10mmHg increase)	1.02 (0.99 to 1.05)	1.07 (1.04 to 1.10)	1.04 (1.01 to 1.07)	1.08 (1.05 to 1.11)
Total cholesterol (per 1 mmol/l increase)	1.11 (1.04 to 1.18)	1.13 (1.07 to 1.19)	1.08 (1.02 to 1.14)	0.97 (0.92 to 1.02)
HDL cholesterol (per 1 mmol/l increase)	0.67 (0.54 to 0.83)	0.77 (0.66 to 0.91)	0.98 (0.83 to 1.17)	0.71 (0.61 to 0.83)
Smoking	1.97 (1.65 to 2.34)	1.86 (1.65 to 2.11)	1.94 (1.56 to 2.41)	1.81 (1.52 to 2.15)
Diabetes	1.36 (1.09 to 1.69)	1.92 (1.57 to 2.34)	2.09 (1.74 to 2.51)	1.98 (1.59 to 2.47)
Age (per 1 year increase)	1.14 (1.13 to 1.16)	1.14 (1.13 to 1.15)	1.16 (1.15 to 1.18)	1.18 (1.17 to 1.19)

Table 4-10: Adjusted hazard ratios for CVD mortality associated with each risk factor in those on and not on anti-hypertensive medications

Table 4-11 shows the correlation coefficients between each of the three functions. The correlation was high between all three and this was demonstrated in subgroups based on gender, and antihypertensive use also.

Correlation coefficients		
	Function A	Function 3
Function 3	0.974	
Function 4	0.991	0.970

Table 4-11: Correlation coefficients between each of the three functions

Table 4-12 shows the discrimination of each of the three functions. All three performed very similarly, with no difference in AUROCs when corrected to two decimal places. However, there was a marginal improvement in discrimination of the 1st function compared to the 3rd, which was marginally better than the 2nd.

AUROC	1st – derived separately in those on and not on BP lowering meds	2nd – use of BP lowering meds not considered	3rd – use of BP lowering meds included as risk factor
All	0.744	0.738	0.742
Men	0.709	0.701	0.708
Women	0.776	0.771	0.774
On anti-hypertensives	0.718	0.715	0.715
Not on anti-hypertensives	0.748	0.749	0.747

Table 4-12: Area under receiver operating characteristic curve (AUROC) in each of the three functions

Regarding calibration, based on observed to predicted ratios, none of the three functions could be considered superior. Based on goodness of fit testing, in men there was no difference, and in women there was a minor inferiority of the 2nd function compared to the other two functions.

When comparing the charts for men and women based on the three different functions, the charts for the 1st and the 3rd functions (assuming lack of use of BP lowering medications) resulted in very similar charts. However, the charts derived from the 2nd version of the function resulted in slightly higher risk estimates, because no adjustment is made in this version for use of blood pressure lowering medication.

SCORE O.P. – DISCUSSION

DIFFERENTIAL EFFECTS OF RISK FACTORS IN OLDER AND YOUNGER PEOPLE

This analysis has shown that several conventional risk factors continue to function in the older age group, both for coronary disease and other types of CVD. In keeping with the findings of other published studies, the relative risks associated with these are weaker[15, 51, 139, 332]. Other prospective studies in older individuals which have analysed the multivariable effect of risk factors have in general included small numbers and may have been underpowered. This is substantially the largest multivariable analysis of the effect of CV risk factors in the elderly.

Unfortunately, in this analysis we have been limited in the availability of 65 year old age group, with the majority of individuals aged between 65 and 75 years at baseline. In the presence of the aging population, it would be especially useful to examine the effects of risk factors in the very elderly. Our numbers in this age group were too small to allow this analysis.

It is interesting to note that HDL cholesterol is one variable which appears to have a similar effect on CHD mortality in older and younger individuals. Other studies have shown physical activity to be a particularly important predictor in the elderly[259, 333].. Given that HDL cholesterol is positively related to physical activity, some of the effect may be related to this association. Additionally, in the INTERHEART study, moderate alcohol intake, which is also positively related to HDL cholesterol, was shown to be a stronger protective factor in older than younger men[259]. The differences in effects of risk factors in older and younger individuals have implications for risk estimation systems in the elderly.

WHY SHOULD RELATIVE RISK ASSOCIATED WITH RISK FACTORS DECREASE WITH AGE?

One possible explanation is a survivor effect. All those in the older age group have already survived free of CHD to age 65, regardless of their risk factors. It is possible then that we are looking at a privileged group of individuals who are less susceptible to the deleterious effects of the risk factors. This would be consistent with genetic factors influencing how an individual responds to environmental factors.

Some of the differences in hazard ratios for CVD mortality could be related to the fact that stroke becomes relatively more common with age. For this reason then factors such as total cholesterol would have a weaker effect on the combined CVD mortality endpoint. However, as noted in this analysis, there are differences even when the endpoint is limited to CHD mortality.

From **Table 4-1** on page 222, it is apparent that the decrease in hazard ratio for diabetes is considerably higher in younger than older individuals. Unfortunately, in this dataset we have not been able to separate type 1 diabetes from type 2 diabetes. However, it is likely that there are proportionately more type 1 diabetic patients in the younger age group. Therefore, diabetic patients in the younger age group would have had a longer duration of diabetes and, especially considering the early baseline of these younger cohorts, worse glycaemic control.

Similarly, hypertension becomes more prevalent with increasing age and therefore those who were older at baseline were on average more likely to have had hypertension for a shorter period of time. There would, however, have been more of them.

In the case of smoking the opposite could occur. Since people in general tend to start smoking early in life, approximately age 20, current smokers who are older at the baseline of the study would have an extended duration of smoking. In this way age could be acting as a surrogate for exposure time. If this were the case one would expect to see a stronger relationship with smoking in older than younger individuals, which was not observed in this analysis. It is possible that other factors related to smoking behaviour are also having an impact. For example, it is possible that older smokers tend to consume lower quantities of cigarettes.

In the case of total and LDL cholesterol, blood levels of these variables can reduce towards the end of life and in association with other disease states, including infection and neoplasia. It has often been suggested that the reduction in effect of cholesterol in older people is occurring because of a dilution in effect caused by the positive association between low total cholesterol and all-cause mortality.

ELEVATED BODY WEIGHT AS A RISK FACTOR - SPECIAL CONSIDERATIONS IN OLDER INDIVIDUALS

In the case of body mass index there may be other considerations. The work of my colleague, Dr. Alexandra Dudina, using the SCORE dataset has demonstrated that BMI predominantly exerts its effect on CVD risk through hazardous modification of other CV risk factors, mostly notably, by increasing total cholesterol, reducing HDL cholesterol, increasing blood pressure and increasing risk of diabetes. This suggestion that BMI is not having a direct effect but working through its action on other variables would fit with the reduction in effect seen in older age groups. For example, a high BMI in a 40 year old individual will have 20 years longer to cause disadvantageous changes in risk factors than the same elevation of BMI in a 60 year old. Also supporting this are previous prospective studies which showed that higher body weight has a stronger effect with longer follow-up periods.

Repeated measurements were not available in this study. However, it would be interesting to analyse the effects of change in body weight at different ages. For example, 65 year olds who are overweight could reflect two types of people, those who have been overweight all their lives – in these people elevated body weight would be expected to be deleterious and those who were of normal weight until 10 years before and then slowly gained weight – elevated body weight here would be associated with less risk. Conversely, those older people who were normal or underweight at study entry may have been overweight previously and lost weight as a consequence of co-morbid illness. This would again tend to weaken the observed relationship between body weight and CVD.

Dr. Dudina has also shown that the effect of BMI on risk decreases with age, not just in the two age groups as I have demonstrated here, but also sequentially across four increasing age groups[352]. This effect was also demonstrated in the prospective studies collaboration analysis of the effect of BMI on CVD outcomes[332].

After adjustment for the other risk factors increasing BMI was actually associated with a protective effect in older women. This is likely due to confounding by the decrease in weight which may occur in those with progressing subclinical disease or other co-morbidities.

AGE – RISK FACTOR OR EXPOSURE TIME?

In all risk estimation systems age is the strongest predictor of subsequent events[3, 4, 46, 47]. However, as demonstrated in **Table 4-4** and **Table 4-5** the effect of age increases with increasing age, hence the importance of including the age-specific beta coefficient for age as a risk factor. In terms of CVD, some consider age less as a risk factor in its own right and more so as exposure time to the other risk factors. This increasing duration of exposure combined with rising rates of apoptosis as people age is presumably the main reason why mortality increases with age.

The methods of the original SCORE function provide an innovative approach to this situation. Age is taken as the time variable in the model[4]. In this way the effect of age on risk is included in the baseline and since the baseline is specific to each age, in this way the effect of age on risk is allowed to vary at each baseline age.

The baseline survival is calculated as the probability of survival to the baseline age+10, minus the probability of survival to the baseline age. Because of the design of SCORE O.P., in that it only contains data from individuals aged over 65 years at baseline, this approach was not feasible, since I would not have been able to calculate probability of survival to age 65 without individuals aged under this in the dataset. However, inclusion of age as a risk factor in SCORE O.P. is acceptable since the age-specific beta coefficients for age have been used. It should be noted that Framingham also represents some of the variability of age as a risk factor by including it as a logarithmic variable[3].

INCREASE IN PROPORTION OF STROKE COMPARED TO CHD IN OLDER AGE GROUP

Cardiovascular disease deaths make up a progressively larger proportion of total deaths with increasing age. Our demonstration of this in the SCORE dataset (**Figure 4.6** on page 234) is in line with what we have demonstrated based on recent Irish mortality statistics (**Figure 4.3**). Moreover, we have demonstrated an increase in the proportion of cardiovascular deaths caused by stroke in older compared to younger individuals. This has important implications for prevention of CVD in the elderly and for risk estimation in the elderly.

In terms of estimating CVD risk in the elderly, risk factors which have a stronger effect on the stroke endpoint will assume greater importance. For example, total cholesterol will become less important and systolic blood pressure will assume greater importance, as occurred in the SCORE O.P. function.

The demonstration of the increasing proportion of CVD deaths caused by stroke in the elderly highlights the need for physicians to pay particular attention to ensuring implementation of guidelines for stroke prevention in this population. Preventive measures, particularly anti-hypertensives have been shown to be particularly effective for stroke prevention in this age group.[343, 353] Especially in the presence of an aging population, preventing stroke manifestations is crucial. Non-fatal stroke may lead to substantial disability which reduces independence resulting in increasing requirement for long-term care. This has obvious economic implications for society.

SCORE O.P. - RISK ESTIMATION IN OLDER PEOPLE

We have demonstrated that several conventional risk factors continue to predict CVD in the older age group and that total CVD risk can be accurately estimated in this age group. The main difference between our function and that of other risk estimation functions is that the selection of risk factors to be included and the beta coefficients or relative risk weightings assigned to each of the risk factors have been based on analyses restricted to the older age group. This is the first risk estimation system for use specifically in older men and women that has been derived from the age group to which it is to be applied. We have demonstrated that this change to the methods results in superior risk estimation in this age group.

One other risk function has been derived in older persons. However, the derivation dataset for this function contained men only[179]. Additionally, because the authors have not provided the figures required for calculating risk estimates this function cannot be used in daily clinical practice. Moreover, this function was derived from only men aged 74 years at baseline and therefore cannot be routinely applied because age cannot be included in the estimate. However, this research does support our conclusions by demonstrating an AUROC of 0.688 (when conventional risk factors alone are included). Under normal circumstances this would indicate reasonable discrimination, however, in a cohort restricted to one age at baseline this AUROC suggests very good discrimination. This function by Zethelius and colleagues gave the best discrimination in an analysis of older individuals (prior to SCORE O.P.) and of note here again the function was derived specifically from the older age group.

COMPARING SCORE O.P. WITH THE ORIGINAL SCORE FUNCTION AND FRAMINGHAM.

The original SCORE function provided risk estimation up to the age of 65 years. SCORE O.P. has been developed to estimate risk up to the age of 80. At age 65 the two charts overlap and the risk estimates can be compared. Looking at the age 65 band in the SCORE O.P. charts (Figure 4.13 page 242). and the original SCORE charts (**Figure 1.9** page 42) the risk estimates appear to be much lower using SCORE O.P. However, the baseline risk is very similar in both charts. For example, using SCORE O.P. a 65 year-old woman with TC 4.0 mmol/l and SBP 120 mmHg and non-smoking has a 2.7% 10-year risk of fatal CVD; the risk estimate using SCORE for the same individual is 2.0%. For men the corresponding figures are 4.8% and 4.4% respectively. When one takes into consideration that the SCORE O.P. charts assume beneficial levels of two other risk factors, which are not included in original SCORE (mean HDL cholesterols and non-diabetic status) it is apparent that the risk estimate at the baseline level of risk factors is actually higher in SCORE O.P. The reason for this is that the low levels of risk factors are having less of a protective effect in SCORE O.P. This is because the risk factors in general have a weaker effect in the older age group.

The reason that the SCORE O.P. charts look as if they underestimate risk in older people is because when risk factors are present the added risk (in addition to the baseline risk) due to these is less in SCORE OP compared to SCORE. In general, when comparing two risk charts the automatic way to do it is to look at the top right hand box. This is probably a particularly ineffective method, given the very small number of individuals who would actually have this combination of risks (male smoker with TC 8mmol/l and SBP 180mmHg). When looking at the risk estimate for this combination of risk factors the difference between SCORE O.P. and SCORE is marked (47% versus 22% respectively). This is because the SCORE chart over-estimates the risk associated with risk factors in the older age group because the beta coefficients for the risk factors have not been calculated using data from older persons but the entire group, which contains primarily middle aged individuals. This difference between relative risks associated with risk factors has been demonstrated in our analysis as well as in other studies[139, 259].

Other risk estimation systems also over-estimate risk in older individuals by using the same beta coefficients throughout. Table 4-13 details the risk estimates given by the 2008 version of the Framingham function[3] and compares them to the risk estimates given by the SCORE O.P. function. It is important to note that the two functions cannot be compared directly, because Framingham is estimating risk of events, whereas SCORE O.P. estimates risk of CVD death. However, we can compare the increase in estimate when multiple risk factors are included. In SCORE O.P. addition of the three risk factors (TC 8mmol/l, SBP 180mmHg and smoker) results in a 4.4 fold increase in risk in men and 3 fold in women. In Framingham, however, the estimate increases 5.5 fold in men and 9 women in women. This is clearly an overestimation of risk in older individuals with multiple risk factors and is likely the reason for the poor discrimination of current risk estimation systems in older

individuals. I have shown in this analysis that the inclusion of the relative risks which are more appropriate results in an improvement in discrimination.

	SCORE O.P. risk estimate (of CVD death in 10 year period)	Framingham risk estimation (of CVD events in 10 year period)
65 year old man non-smoker, non-diabetic, SBP 120mmHg, TC 4mmol/l, HDL 1.2mmol/l, non-diabetic	5%	13%
65 year old woman non-smoker, non-diabetic, SBP 120mmHg, TC 4mmol/l, HDL 1.2mmol/l, non-diabetic	3%	5%
65 year old man smoker, non-diabetic, SBP 180mmHg, TC 8mmol/l, HDL 1.2mmol/l, non-diabetic (top right corner of chart)	22%	71%
65 year old woman smoker, non-diabetic, SBP 180mmHg, TC 8mmol/l, HDL 1.2mmol/l, non-diabetic (top right corner of chart)	9%	47%

Table 4-13: SCORE O.P. and Framingham estimates of risk in 65 year old men and women with different combinations of risk factors

CLINCIAL IMPLICATIONS OF OVER-ESTIMATION OF RISK IN OLDER PEOPLE

This over-estimation of risk in currently used risk estimation systems[3, 36, 46, 47] has important implications for clinical practice. Looking at the original SCORE charts in the age 65 years age group, particularly all men in high risk regions aged over 65 years would be considered high risk and warrant intensive preventive modifications. As discussed below, the use of preventive medications may be particularly hazardous in the older age group. Therefore, rectification of the current over-estimation of risk in older people is particularly important.

Over-medication of older individuals is a particular problem and it is well recognised that many avoidable deaths and hospitalisations in older people occur because of appropriate prescribing in this group. Cardiovascular medications can be associated with significant adverse effects in older people. For example, statins have been shown to be associated with increasing frequency of muscle and hepatic toxicity in older people[354]. A recent study by Rogers et al showed that 14% of hospital admissions in older people were associated with drug side effects[355]. Importantly, 69% of the offending medications were cardiovascular. It is of paramount importance then that the right individuals are identified for aggressive risk factor reduction – these are those older people who are at highest risk. This ensures the best benefit to risk ratio.

Ensuring that those who derive the greatest benefit are prescribed preventive therapies also improves cost effectiveness of preventive strategies.

COMPARISON WITH OTHER STUDIES

Previous assessments of the Framingham and other risk estimation functions in the older age group have been limited, but in general have shown poor discrimination in this age group. For example, the AUROC for Framingham in the Leiden plus cohort was 0.53 (95%CI: 0.43 to 0.64) [72]. Another study from the Netherlands in individuals aged over 70 years showed similarly poor discrimination with AUROCs of 0.55 and 0.60 for the PROCAM and Framingham functions respectively for the prediction of CVD mortality[347]. Even risk functions derived from the same data they were tested in, an approach that is comparable to our analysis, did not perform well in this age group[72, 179].

To date, risk functions which have been derived sorely from the older age group are very limited. One exception is an analysis of prediction of CVD in the elderly in the Uppsala Longitudinal Study of Adult Men[179]. Their risk function containing conventional risk factors resulted in an AUROC of 0.688 in men aged over 65 years on internal validation. However, this function is only applicable to older men and the authors have not provided a means for using of this function to estimate risk in older people in clinical practice, as discussed above.

OTHER APPROACHES TO IMPROVING RISK ESTIMATION IN THE ELDERLY

Some studies have shown an improvement in risk estimation in the elderly when biomarkers and markers of sub-clinical disease are added to risk estimation[179, 347]. However, the addition only resulted in a maximum AUROC of 0.74, similar to that of the present study. We believe that the measurement of these biomarkers may both complicate and reduce the cost effectiveness of risk estimation. On this basis, our approach to improving risk estimation in the older age group may be preferable.

Another approach to risk estimation in the elderly is the inclusion of interactions between age and several other risk factors, as in the second version of QRISK[47]. This allows for some of the difference in effect of risk factors at different ages and may result in superior risk estimation in older age groups; however, this issue has not been examined to date.

IMPLICATIONS FOR THE PREVENTION OF CVD IN THE ELDERLY IN CLINICAL PRACTICE

The recent demonstrations in randomised controlled trials of the morbidity and mortality benefits of preventive measures in older individuals, even in the very old[343], have substantial implications for the prevention of CVD in this age group. Guidelines on prevention of CVD in clinical practice recommend the use of risk estimation systems so that preventive measures can be targeted towards those at highest risk[17, 39, 56, 237]. Therefore, it is clearly important to have a system which estimates risk accurately in this age group[356]. We believe this work adds to currently available evidence in this area.

This analysis raises some questions about the primary prevention of CVD in the elderly. All thresholds for high/ low risk are arbitrary. To decide on the most appropriate threshold, the point at which the projected benefits of the intervention are balanced against the potential risks should be established. Clearly, both of these components will vary in different age groups and therefore, the current use of one arbituary threshold for high/ low risk in all age groups is clearly not ideal[357]. The most appropriate threshold for high risk would depend on the risk benefit ratio and the resources available. We suggest that this could be investigated by re-analysing the results of randomised controlled trials of preventive measures and calculating the number needed to treat for each preventive measure in each risk category. Our newly derived function presents an opportunity for this risk stratification.

The increasing frequency of adverse effects in the older age group will always be a concern. This well-founded uneasiness of physicians regarding the provision of preventive medications in older people may be reduced by the increasing number of randomized controlled trials showing that the benefits outweigh the risks once they are provided for those who are most likely to benefit[193, 343]. SCORE O.P., which provides reliable estimates of absolute risk in this group, should also help in this regard.

Other potential avenues for reducing adverse effects focus on improving the delivery of care in older people[355]. These approaches are complementary to our strategy which focuses on improving risk stratification. Studies have shown that geriatric evaluation and management in outpatient clinics significantly reduces the rate of adverse effects associated with medication - adjusted relative risk 0.65 ;(95% confidence interval: 0.45 to 0.93).[358]. Additionally, this approach also increased the rate at which appropriate guideline recommended medication were prescribed. Increasing use of this approach should therefore be encouraged and holds potential for improving management of prevention in older individuals. Adherence to statins in older people, which has previously been shown to be as low as 25%[359], could also be improved.

There is still substantial debate surrounding who should provide care for older people. Clearly, geriatricians who specialise dealing with the problems associated with aging, one of which is poly-pharmacy, are best equipped to manage primary prevention prescribing in frail older people, as illustrated in the research quoted above[358].

However, in the majority of older people, general practitioners are the most appropriate providers of primary prevention, since they are in close and continuous contact with their patients. As such they can avail of opportunistic health promotion and can closely monitor for adverse effects associated with pharmacotherapy where this is deemed appropriate.

SCORE O.P. – PRACTIAL CONSIDERATIONS FOR RISK ESTIMATION IN OLDER INDIVIDUALS

SCORE O.P. charts are shown in Figure 4.13 on page 242 above. These charts are shown for illustration purposes and assume a non-diabetic status and HDL cholesterol levels of 1.2 mmol/l in men and 1.4 mmol/l in women. However, the SCORE O.P. function contains more than the five variables which can be accommodated in a two dimensional chart. In order to present SCORE O.P. in this format multiple charts at different levels of HDL cholesterol and diabetic status would need to be developed. Clearly, this complex system would not encourage its use in daily clinical practice. I, therefore, suggest that instead of producing SCORE O.P. charts, the function should be integrated into the existing HeartScore program which is managed by the European Society of Cardiology and currently available on the internet.

LIMITATIONS AND FURTHER WORK

The most important limitation of the study is the use of internal validation only. The next important aspect of this project is external validation. The performance of the SCORE O.P. function will be tested in an external dataset and additionally compared to the Framingham function[45] and also to the original SCORE function[4], extrapolated to the older age group. This will enable further testing of the hypothesis that derivation of the function specifically from the older age group results in improved risk estimation in the elderly.

A function containing the option of including whether the individual is on anti-hypertensives would be useful. Unfortunately, all of the cohorts used here did not have this information and therefore I have not been able to include it in SCORE O.P. However, I have demonstrated in the sensitivity analyses above that its inclusion results in only minor changes in risk estimations and accuracy of the function.

This work is also limited by the number of variables which were available to be assessed for inclusion in risk estimation. A number of extra variables were available in the Norwegian cohort, including heart rate, waist and hip circumference. Incorporation of these did not substantially improve the system. However, physical fitness or physical activity level has been shown to be a good predictor of outcome in older people[360, 361]. Assessment of the value of inclusion of this variable was not possible in this cohort. I suggest that this would be a particularly useful factor to include because it is an easily measured and modifiable variable. Future work by the

SCORE group will focus on this. It is likely that this variable functions as a risk factor in two ways, firstly because physical activity is protective against CVD but secondly because reductions in physical activity occur in those with co-morbid conditions and in those with un-recognized CVD. In this way physical inactivity could be acting as a marker of subclinical disease. These inter-relationships require detailed exploration.

Another important step in risk stratification in the elderly is the provision of accurate systems for estimating dementia risk. We have shown that with advancing years stroke becomes relatively more important than coronary heart disease. However, in the oldest old, dementia is the most significant problem. It represents an especially attractive target for prevention, since it is associated not only with high personal cost for the individual but also considerable economic implications for the society as a whole. It is not just vascular dementia, but also Alzheimer dementia which has been associated with conventional CVD risk factors[362]. Furthermore, it has been suggested that preventive therapies including statins have potential for the prevention of dementia, although this evidence is not consistent and limited to observational studies[363]. This heralds the need for the development of reliable systems for estimating absolute risk of dementia. In conjunction with this, randomized controlled trials specifically designed to investigate prevention of dementia are required. Unfortunately, dementia as an endpoint was not available in the dataset used here and so estimation of dementia risk was not feasible as part of this work.

SCORE O.P. - CONCLUSIONS

In this the largest multivariable analysis of the effect of conventional risk factors on CVD endpoints in older people I have demonstrated that independent effects of several conventional risk factors remain in the older age group. However, in agreement with other studies, the relative risk associated with them is in general weaker than that seen in younger individuals. Yet, the excess risk associated with risk factors increases, due to the substantial increase in absolute risk. Endpoints also differ in older people, with stroke representing an increasing proportion of CVD deaths as people age. These two observations led to the rationale for SCORE O.P. – that a risk estimation system derived specifically from older individuals would function better in this age group.

The derivation and internal validation of SCORE O.P. has demonstrated that accurate risk estimation is possible in older persons, using only conventional risk factors.

This is the first risk estimation system which has been developed specifically for the estimation of risk in older men and women and as such adds to the current evidence in this area. We have shown superior risk estimation to previous systems with an AUROC of 0.74.

SCORE O.P. does not extrapolate the effect of risk factors from younger individuals to older individuals as in previous systems. Of critical importance, we have used the age-specific beta coefficients for the risk factors. This change in methodology may account for the superior results demonstrated here. The revised and more accurate risk estimates may protect older individuals from over-medication. The next step will be to extend the validation process by using an external data set.

OVERALL DISCUSSION

The prevention of cardiovascular disease (CVD) in clinical practice requires a system for the accurate estimation of the risk of future development of CVD. This facilitates risk stratification so that those at highest risk, who will benefit most from preventive measures, can be selected for the most intensive risk factor modifications. This thesis provides an exploration of a number of issues related to risk estimation, with particular reference to SCORE, the risk estimation system recommended by the European guidelines on CVD prevention. SCORE has been in use since 2003 and is a robust estimation system based on prospective data from over 200,000 European individuals. A number of issues remain however which represent opportunities for refinement of risk estimation, a crucial element of disease prevention. My specific focus has been on improving accuracy through incorporation of newer risk factor including HDL cholesterol and resting heart rate, investigating whether simplified risk estimation systems can still provide accurate risk estimates and exploring methods for improving risk estimation in older individuals.

HDL Cholesterol

The role of HDL cholesterol as an independent protective factor for the development of CVD has been confirmed. I have demonstrated that this protective role applies equally in all age groups, at all total cholesterol levels and all levels of total CVD risk. The effect of HDL cholesterol in older women from the general population had not been conclusively demonstrated previously. This work demonstrates the independent protective effect of HDL in this group; with each 0.5mmol/l increase in HDL cholesterol level associated with a xx% reduction in risk of CVD mortality.

Arising from the above, a new SCORE function incorporating HDL cholesterol as a separate variable was developed. This resulted in improved risk estimation, although, the improvement in discrimination for the population as a whole was modest. For those at borderline risks of risk and for those with unusually high or low levels of HDL cholesterol, the inclusion resulted in a considerable change in the risk estimate.

In summary, for the individual, and the clinician who treats the individual, the option of including HDL cholesterol in the risk calculation is a valuable addition. Equally importantly, though, it has been demonstrated that for the entire population, whose HDL cholesterol levels are close to the mean, the inclusion of HDL cholesterol causes only minor changes in the estimate. This may be an important consideration when developing a screening program for the detection and treatment of those at high risk of CVD. Future work will focus on the incorporation of this new risk function into HeartScore – although it would be as an optional extra only; the SCORE could still be calculated accurately without measurement of HDL cholesterol. In this way the

facility for including HDL cholesterol is incorporated without introducing complexity, which could negatively impact on usage of the system.

Resting Heart Rate & Simplifying risk estimation

Resting heart rate was shown to be an important independent risk factor for CVD, particularly fatal CHD events. Of note the relationship was equally powerful in women and men from the general population. The relationship was independent of conventional risk factors and of a validated measure of physical activity. A RHR > 90 bpm was associated with a 2 fold increased risk of CVD mortality in men and a 3 fold increased risk in women, when compared to a RHR of < 60 bpm. This risk was similar in magnitude to the risk associated with current smoking.

When examining the role of RHR in risk estimation though, there was no advantage associated with incorporating RHR as an extra variable, either in the entire population or in those at borderline risk, as demonstrated by the low net reclassification index.

Improving the accuracy of risk estimation systems is an important issue and I have assessed the value of incorporating HDL cholesterol and resting heart rate into the current SCORE system. However, one of the great strengths of SCORE is the simplicity and easy distribution of the two-dimensional paper chart. At the European level, electronic or web-based delivery systems are not universally available and the value of incorporating extra variables in terms of improved accuracy has to be balanced against the complexity which it introduces.

Without an effective implementation strategy guidelines on CVD prevention are of no value. Recently, there has been increasing interest in simplified risk estimation systems. These enhance the cost-effectiveness and accessibility of risk estimation. The ability to calculate risk without the use of laboratory measurement may have potential for augmenting usage of these systems in clinical practice. Consequently, this would facilitate implementation of guidelines on CVD prevention.

A simple risk estimation system, which contained only easily measured variables –(age, gender, smoking status and body mass index) was developed. The addition of RHR to this system resulted in an improvement in discrimination and risk classification. Simple risk estimation systems, such as this, can estimate risk remarkably accurately – AUROC was 0.82 in men 0.86 in women.

A further consideration is which variables are most appropriate. RHR has the advantage of being an objective measurement. The disadvantage is that RHR has not been definitively proven to be a causal factor. No evidence is available as yet that reduction in RHR, either to pharmacological or lifestyle means, results in reduced CVD risk. Future work will focus on investigating the potential for self-assessed measures of physical activity as a variable for simplified risk estimation systems.

Risk Estimation in Older People

The continuing trend towards aging of the population globally places increased emphasis on the need for preventing CVD in this age group. While cardiovascular diseases account for the vast majority of deaths in older people, it is also critical to address the substantial burden of morbidity related to CVDs, particularly heart failure and disability after stroke, and the related economic and social consequences. This aspect of CVD contributes considerably to deteriorations in quality of life and independence which may occur with advancing years. Modification of risk factors in the elderly is known to be beneficial; however, at present risk estimation in the older age group is inaccurate. This limits potential for establishing an effective high risk preventive strategy in this age group.

As previously demonstrated, most conventional risk factors continued to function in the older age group. However, the relative risks associated were generally lower in the older age group. An exception was HDL cholesterol, which in women was a stronger protective factor for CVD mortality than in younger women. The hypothesis that incorporation of these age specific risk factor weightings would result in improved risk estimation in older people proved to be correct – with an improvement in discrimination from AUROC 0.68 to 0.70 in men and AUROC0.74 to 0.78 in women. This is the best discrimination of a risk estimation system in older people published to date.

The newly derived SCORE O.P. considerably reduces the problem of over estimation of risk in older people. This has substantial clinical implications as it will result in less individuals being selected for pharmacological modification of the risk factors. Hence, cost associated with over-medication of older people, both monetary and in terms of adverse effects, will be reduced. Meanwhile, those who will gain most will be accurately targeted for intensive risk factor reduction.

To summarise, I believe this work has added to the available evidence regarding CVD risk factors and risk estimation in a number of ways. Several issues regarding the roles of HDL cholesterol and resting heart rate as risk modifiers have been addressed and clarified. Risk estimation systems have been progressed by the provision of a risk estimation system which incorporates HDL cholesterol, the development of a simple risk estimation systems which accurately estimates risk and the creation of the first risk estimation system derived specially form this age group and incorporation age-specific risk coefficients as opposed to extrapolating from younger individuals.

This work has been built on the foundation of the remarkable contribution of the authors of the original SCORE paper. Correspondingly, I hope that this work allows potential for further development of SCORE. Some future avenues to be explored include the development of simple risk estimation systems incorporating physica

activity measures, the investigation of the most appropriate risk threshold to be considered high risk in older people and the investigation as part of a randomised controlled trial of whether reduction of resting heart rate results in benefits in an asymptomatic population.

OVERALL CONCLUSIONS

In this thesis, I have examined many of the limitations of current risk estimations systems and provided potential solutions to these, with particular reference to improvements of SCORE. I have also explored in detail relationships between HDL cholesterol and resting heart rate and CVD risk, including clarification of many previous inconsistencies. This thesis adds to current knowledge in the area of CV epidemiology and prevention in the following ways:

- It demonstrates the independent protective effect of HDL cholesterol in the largest analysis of its kind and extends this to older women. Previous studies showing this were small and included high numbers of women with pre-existing CVD.

- A risk estimation function containing HDL cholesterol as an extra variable, SCORE HDL, is derived and internally validated. This resulted in a modest improvement in accuracy of the function for the entire group. However, for those close to the threshold of high or low inclusion of HDL cholesterol can improve risk classification. This is especially important since treatment decisions are based on risk categorisation. This suggests that HDL cholesterol should be provided as an optional variable in HeartScore, the interactive electronic version of SCORE.

- It clarifies a number of issues relating to the role of elevated heart rate as a risk factor in the healthy population, specifically the independent effect in women, independence from physical activity and co-morbidities. It demonstrates that RHR is unlikely to be acting as a marker of subclincial disease suggesting that the temporal sequence would be consistent with a causal relationship. The observed stronger effect of elevated RHR on fatal than non-fatal endpoints suggests that predisposition arrhythmia may be involved in the mechanism of effect

- An accurate risk estimation system is developed which includes only easily measured variables. Incorporation of RHR improved the accuracy of this system. This may improve cost effectiveness and accessibility of risk estimation.

    Potentially, this system could be self administered. This would give the individual an indication that full risk factor profiling with their family doctor should be undertaken.

- It establishes the risk associated with several conventional risk factors in the elderly, in the largest multivariable analysis of this age group to date.
- The first risk estimation system specifically derived from older men and women is presented. This system resulted in better discrimination than that demonstrated in any previously published analysis.

The resolution of some of the limitations of risk estimation systems may improve their clinical utility, thereby promoting wider usage of risk estimation systems and augmenting implementation of guidelines on CVD prevention.

Future work will focus on external validation of these newly derived risk functions. Once this is completed they can be incorporated into HeartScore, which will enhance both accessibility and clinical application.

LIST OF FIGURES

LIST OF TABLES

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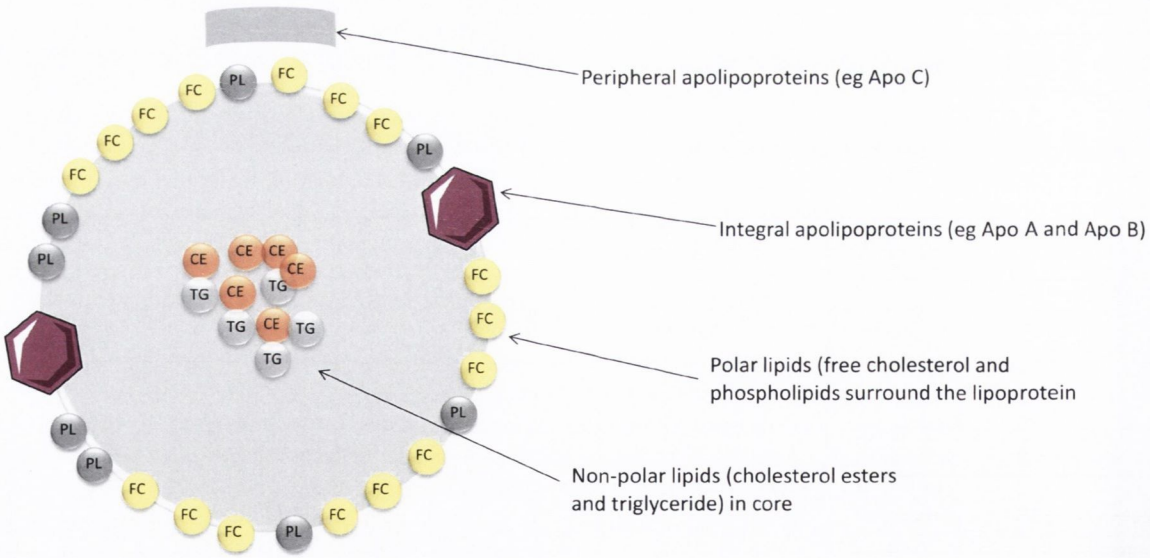
APPENDIX HIGH DENSITY LIPOPROTEIN CHOLESTEROL BIOCHEMISTRY, METABOLISM & SCIENTIFIC MECHANISMS FOR PROTECTIVE EFFECT

LIPID METABOLISM

STRUCTURE & FUNCTION OF LIPOPROTEINS

Plasma lipoproteins are macromolecular complexes of lipids and proteins which function in the transport of lipids in the circulation. They are classified by density and electrophoretic mobility. The non-polar lipids (i.e. cholesterol ester, triglycerides) reside in the core, surrounded by more polar components (e.g. free cholesterol, phospholipids, proteins). The proteins, termed apolipoproteins, play an important role in lipoprotein metabolism and have diverse functions, including the recognition of the lipoproteins by various receptors. The generalized structure of lipoproteins is shown in Appendix figure 1



Appendix figure 1: Generalized structure of a lipoprotein

The main classes of lipoproteins are, in order of their size: chylomicrons, very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), low density lipoproteins (LDL) and high density lipoproteins (HDL).

Atherogenic lipoproteins contain mainly chylomicrons, VLDL, IDL and LDL particles contain apolipoproteins B – either B46 (chylomicrons) or B100 (VLDL, IDL and LDL). The main

apolipoproteins in HDL particles is ApoA1 and ApoA2. Other apolipoproteins include ApoC, ApoE and ApoH.

Chylomicrons mainly transport dietary lipids in the form of triglyceride to the blood, through the lymphatic system. They are degraded by lipoprotein lipase and the remnants are taken up by the liver through recognition of ApoE.

VLDLs are also large lipoproteins containing mainly triglyceride. These are formed by the liver and carry endogenous lipid. Nascent VLDLs are transformed into mature VLDLDs by interacting with HDLs. The action of lipoprotein lipase on VLDLs causes a reduction in size and the formation of IDLs. The IDLs are then acted on by hepatic triglyceride lipase to produce LDLs. LDL cholesterol is either delivered to the peripheral tissues or back through the LDL receptor on the liver for excretion in bile. LDL cholesterol is required by the peripheral tissues for normal functions including normal cell membrane function, however, when excessive amounts are present in the peripheral blood it may become oxidized and enter macrophages in the vessel walls, forming foam cells and leading to atherosclerosis.

HDL cholesterol provides a system for returning cholesterol to the liver from the peripheral tissues. This prevents excessive accumulation of cholesterol, which can lead to atherosclerosis – as described above. This process of reverse cholesterol transport will be discussed in detail below.

HDL METABOLISM

THE STRUCTURE OF HIGH DENSITY LIPOPROTEIN CHOLESTEROL - APOLIPOPROTEINS

Apolipoprotein A1 (ApoA1) is the main apolipoprotein associated with HDL, making up 70% of the apolipoprotein content. It is present on virtually all HDL particles. Deletion of the ApoAI gene in mice results in greatly decreased levels of HDL cholesterol and atherosclerosis-prone mice with absent ApoAI production develop significantly increased atherosclerosis, indicating the importance of Apo A1 for normal functioning of HDL cholesterol.

ApoAI is produced by both the liver and the intestine. However, the relative contribution of each is unknown. About 20% of the apolipoprotein in HDL is apolipoprotein AII. It is also secreted from the liver and is found on approximately 50% of circulating HDL particles; these are known as ApoAI/ApoAII HDL particles.

Rate of production as opposed to clearance is more important in determining circulating levels of ApoAII. The opposite is true of ApoAI production/clearance balance. However, within phenotypically narrow groups (such as those within a narrowly defined body weight, TG or insulin sensitivity range) the rate of ApoAI production also seems to be important in determining ApoAI levels. ApoAI is clearly important in the protective function of HDL cholesterol, as discussed below. However, the exact role of ApoAII is less clear at present.

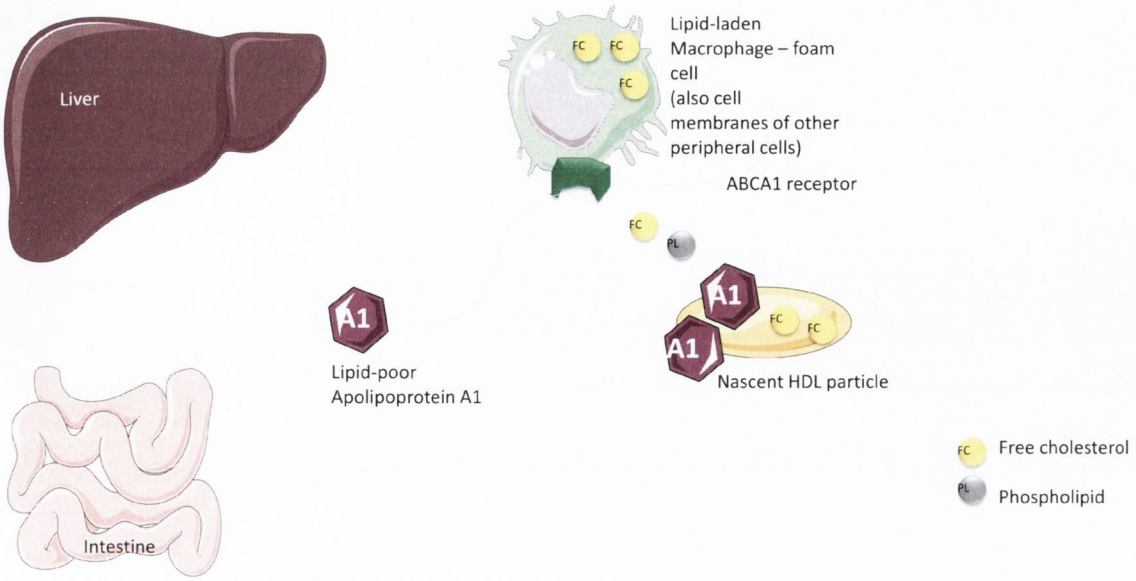
Peroxisome proliferator-activated receptor (PPAR) alpha and gamma agonists and alcohol have been shown to increase HDL cholesterol in association with increased levels of ApoAII. However, the effect on atherosclerosis of increasing HDL cholesterol through increased production of ApoAII in humans is currently unknown.

HDL – SCIENTIFIC MECHANISMS FOR PROTECTIVE EFFECT

REVERSE CHOLESTEROL TRANSPORT

CONVERSION OF LIPID POOR APO A1 TO NASCENT HDL PARTICLES

The metabolism of HDL cholesterol begins with the production of Apo A1 by the liver and intestine. Once this lipid-poor ApoA1 enters the bloodstream it rapidly acquires free cholesterol from cell membranes of peripheral cells, including lipid laden macrophages or foam cells. Transfer of free cholesterol from the peripheral cells onto lipid-poor ApoA1 is facilitated by the ABCA1 receptor on the peripheral cell. The acquisition these free cholesterol by the lipid-poor ApoA1 results in a change in shape in the HDL particle, which becomes discoid. These particles are known as nascent HDL and they retain the pre-beta motility on electrophoresis. This process is illustrated in Appendix figure 2.

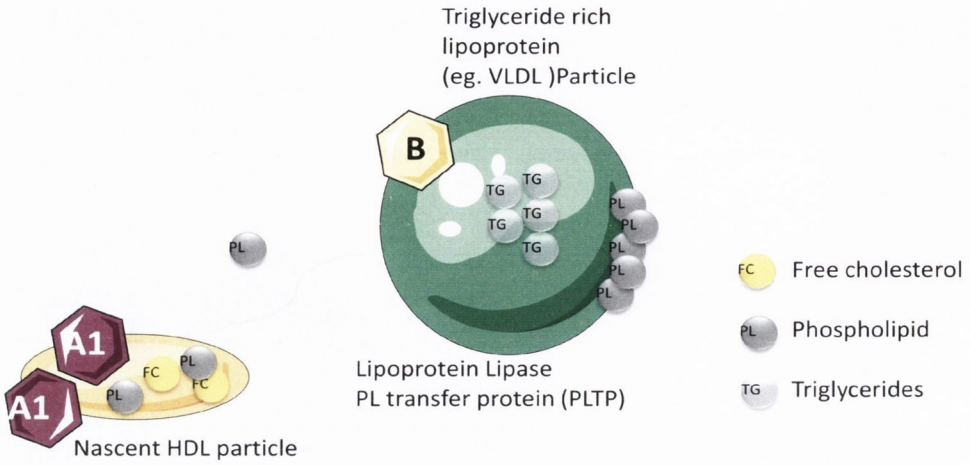


Appendix figure 2: Conversion of lipid-poor apolipoprotein A1to nascent HDL particle

Tangier disease is caused by a genetic absence of the ABCA1 receptor, which results in markedly reduced levels of ApoAI and HDL cholesterol. The rate of production of ApoAI in Tangier disease is normal, but without undergoing lipidation, the ApoAI is rapidly catabolised. Heterozygotes for the mutation in the ABCA1 gene also generally have HDL cholesterol levels less than the 5th percentile. However, as low HDL cholesterol in combination with the insulin resistance syndrome is much more common than isolated familial hypoalphalipoproteinaemia, these mutations are probably accounting for the minority of cases of low HDL cholesterol.

ACQUISITION OF PHOSPHOLIPID BY NASCENT HDL CHOLESTEROL

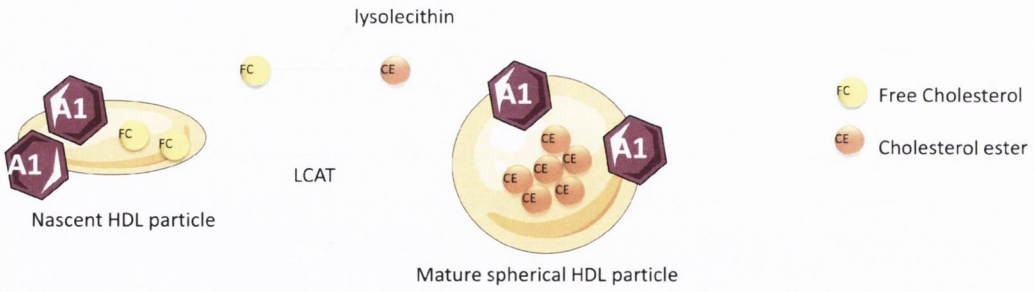
Nascent HDL also acquires lipid, especially phospholipids from triglyceride rich lipoproteins. This occurs during lipoprotein lipase mediated hydrolysis of their triglyceride core. The shed surface phospholipids and apolipoproteins are then incorporated into HDL particles, as illustrated in Appendix figure 3. Lipoprotein derived PLs are transferred onto HDL by the PL transfer protein (PLTP). An exchange of lipoproteins also occurs.



Appendix figure 3: Further lipidation of nascent HDL particle

CONVERSION OF NASCENT HDL PARTICLES TO MATURE SPHERICAL HDL PARTICLES

Lecithin: Cholesterol Acyltransferase (LCAT) is an enzyme found in plasma which catalyses the esterification of the free cholesterol present in the nascent HDL particle to cholesterol esters. 2 acyl groups are transferred from lecithin to the free cholesterol with the formation of lysolecithin and cholesterol esters. Because these cholesterol esters are hydrophobic they move to the centre of the HDL particle, resulting in a change in the shape of the particle, which becomes spherical and has alpha motility on electrophoresis. This process is illustrated in Appendix figure 4. Most of the HDL cholesterol in plasma is in the form of these mature, spherical particles.

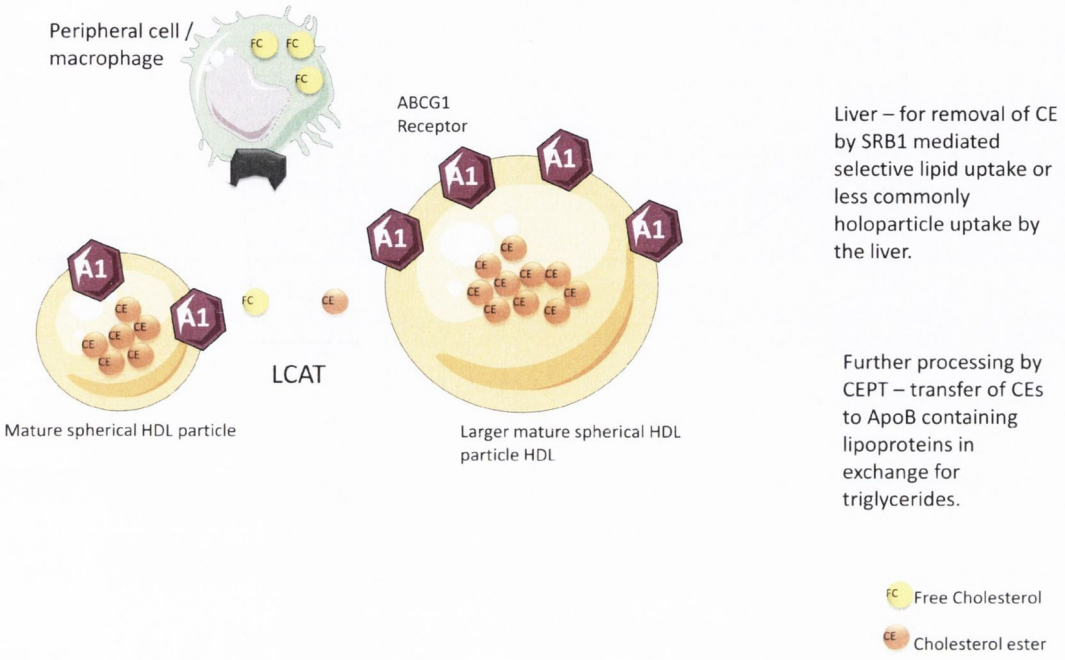


Appendix figure 4: Conversion of the nascent HDL particle to the mature spherical HDL particle

FURTHER REMODELLING AND PROCESSING OF THE SPHERICAL HDL PARTICLE

The smaller mature HDL particle is converted to the larger HDL particle by continued conversion of FC to CE. The mature HDL particles can continue to gather free cholesterol from peripheral cells. This transfer is medicated by the ABCG1 receptor, as illustrated in Appendix figure 5. ABCG1 knockout mice have accumulation of cholesterol in macrophages and over-expression of ABCG1 results in protection of tissues from cholesterol accumulation. Macrophage SR-B1 receptors may

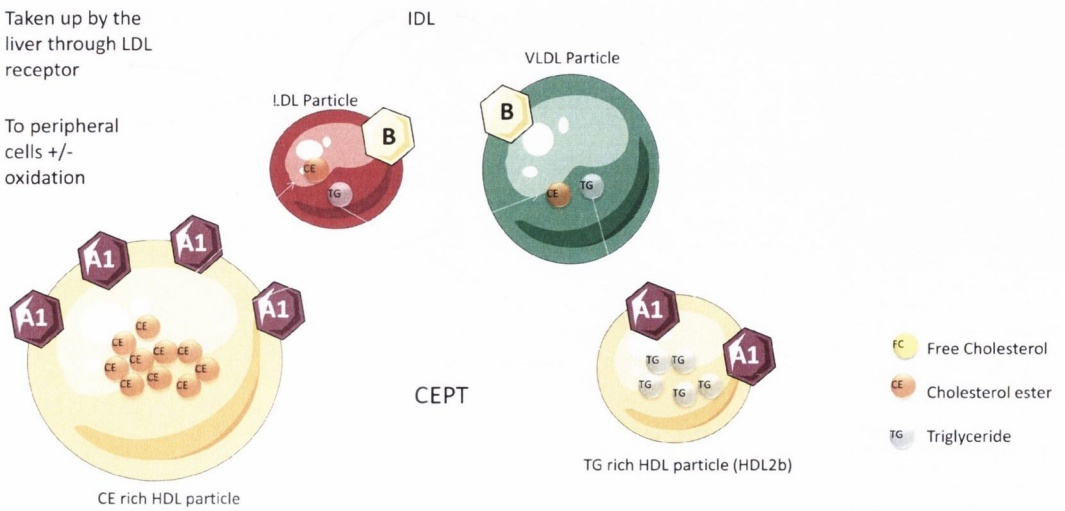
also be involved in the transfer of cholesterol from the macrophage to the mature spherical HDL particle.



Appendix figure 5: Further processing of the mature HDL particle

REMODELLING OF HDL BY CHOLESTEROL ESTER TRANSPORT PROTEINS

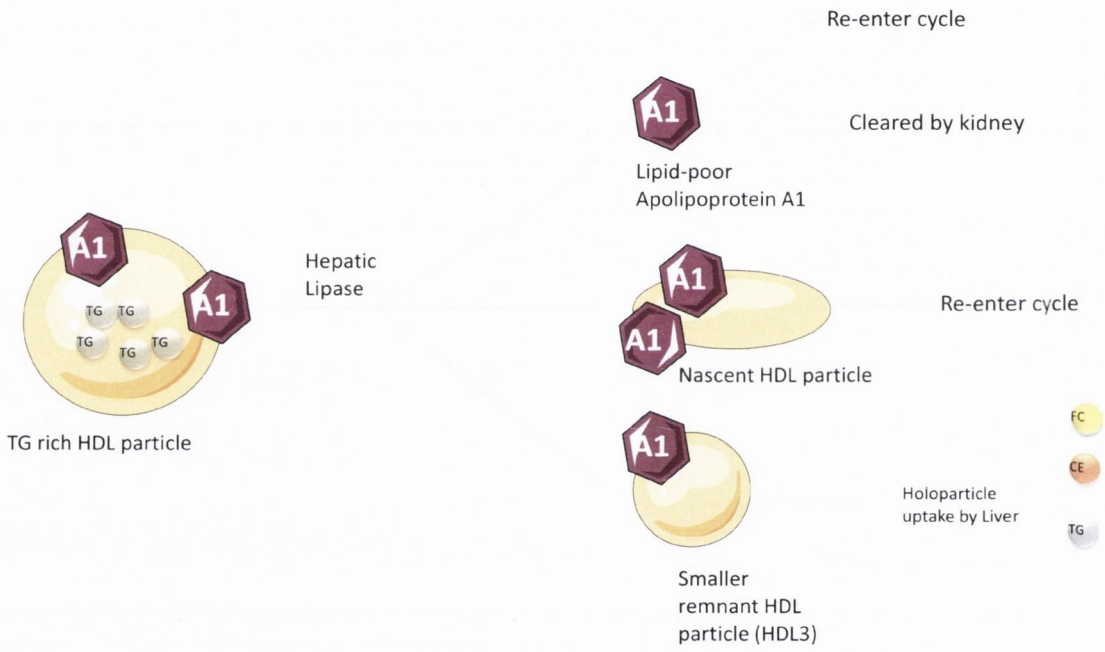
Cholesterol ester transport proteins (CEPTs) facilitate the transfer of CEs in HDL cholesterol to VLDL and LDL particles in exchange for triglycerides. The net effect of CEPT is reduction in size and number of HDL particles and enrichment of HDL particles with triglycerides, along with CE depletion. The CE-enriched LDL particles may then enter the circulation, possibly becoming oxidised or may be taken up by the liver thought the LDL receptor, as illustrated in Appendix figure 6.



Appendix figure 6: Re-modeling of HDL by cholesterol ester transport proteins

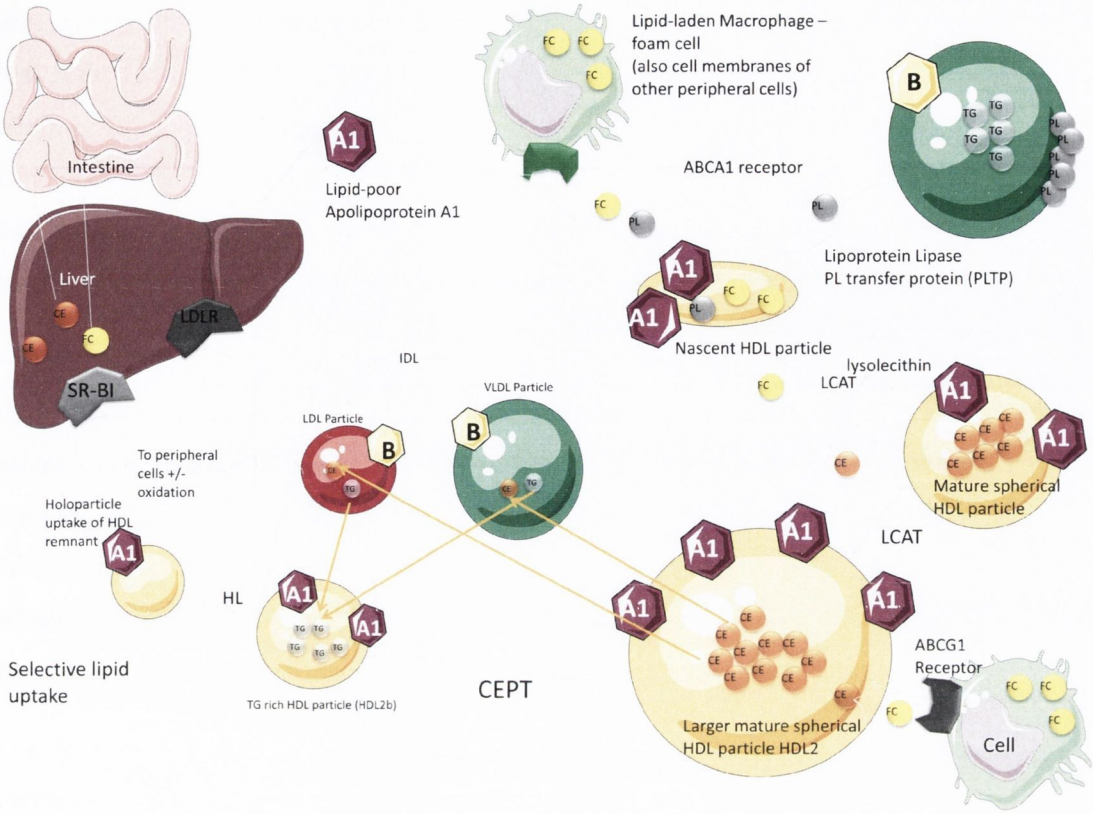
REMODELLING OF HDL BY HEPATIC LIPASE

Hepatic lipase is responsible lipolysis of HDL particles to smaller HDL remnant particles, lipid poor AI, which may re-enter the cycle or be cleared by the kidney, or nascent discoid HDL particles, which can re-enter the cycle by accepting cholesterol effluxed from peripheral cells again. The remnant particles are taken up by the liver through holoparticle uptake, as illustrated in Appendix figure 7. Endothelial lipase is also thought to have a similar activity – hydrolysis of HDL particles. However, it appears to also have a function related to Apo B containing lipoproteins.



Appendix figure 7: Re-modeling of HDL by hepatic lipase

The entire reverse cholesterol transport process is illustrated in **Appendix figure 8.**



Appendix figure 8: The process of reverse cholesterol transport

ANTI-INFLAMMATORY EFFECT

Atherogenesis is now accepted as an inflammatory process, as discussed above. HDL particles have been proposed to have a number of anti-inflammatory properties which contribute to their protective effect[1].

An important step in atherogenesis is the migration of monocytes and their adhesion to activated endothelial cells. Adhesion molecules, whose production is stimulated by pro-inflammatory cytokines (including TNFα and IL-1), are central to the initiation and continuation of this process. These include ICAM, VCAM and E-Selectin. HDL particles have been shown to inhibit the adhesion molecule-mediated recruitment of monocytes the vascular wall by reducing expression of these proteins, possibly by reducing transcription[2]. Importantly, the concentration of HDL cholesterol required to effect this reduction in adhesion molecules was within the physiological range. In vivo animal studies have demonstrated the same effect when recombinant HDL was infused[3], although the results of these animal experiments have not been entirely consistent[4].

In vitro studies have demonstrated the ability of HDL to prevent the transmigration of monocytes in response to oxidized LDL cholesterol[5].

HDL from different subjects appears to differ in ability to inhibit TNFα mediated expression of VCAM[6]. A proposed reason for this is the variation in proportions of different sizes and subtypes of HDL between individuals, however, either this nor diversity in triglyceride concentration, apolipoproteins or cholesterol esters appear to account for the differences[6]. However, studies using recombinant HDL particles with different forms of phospholipid have shown the adhesion factor-inhibiting function of HDL particle to be affected by differences in subtypes of these phospholipids[7].

C reactive protein (CRP), an acute phase reactant, is known to be associated with increased risk of atherosclerosis. Whether CRP contributes to the atherosclerotic inflammatory process or is merely a marker of the ongoing inflammatory process is still under considerable debate. However, in vitro experiments involving incubation of vascular cells with CRP have shown that its presence is associated with an increase in monocyte chemotactic factor (MCP-1), adhesion molecules and reduction of NO. Wadham et al demonstrated a reduction in the mediated increase in adhesions molecules when cells which incubated with HDL cholesterol[8].

An interesting analysis by Ansell et al showed that when comparing CHD patients without low HDL and healthy controls the anti-inflammatory properties of HDL differentiated better between the cases and controls than the absolute level of HDL. To determine the anti-inflammatory effect of the HDL in both groups the investigators incubated cells with HDL from cases and controls and studied the effect of the HDL on induction of monocyte chemotactic activity by LDL cholesterol. They demonstrated reduced anti-inflammatory activity of HDL from cases when compared with controls[9]. This suggests that some individuals who develop CHD in spite of normal or high HDL cholesterol levels may have a subtype of HDL which is dysfunctional in terms of its anti-inflammatory ability.

Further evidence for the effect of HDL on inhibiting expression of adhesion molecules comes from the demonstration of higher ICAM and E selectin levels in humans with low HDL cholesterol levels compared to those with normal or high levels[10]. Furthermore, in 20 individuals with low HDL cholesterol treatment with fenofibrate reduces ICAM and E-selectin levels[10].

ANTI-OXIDANT EFFECT

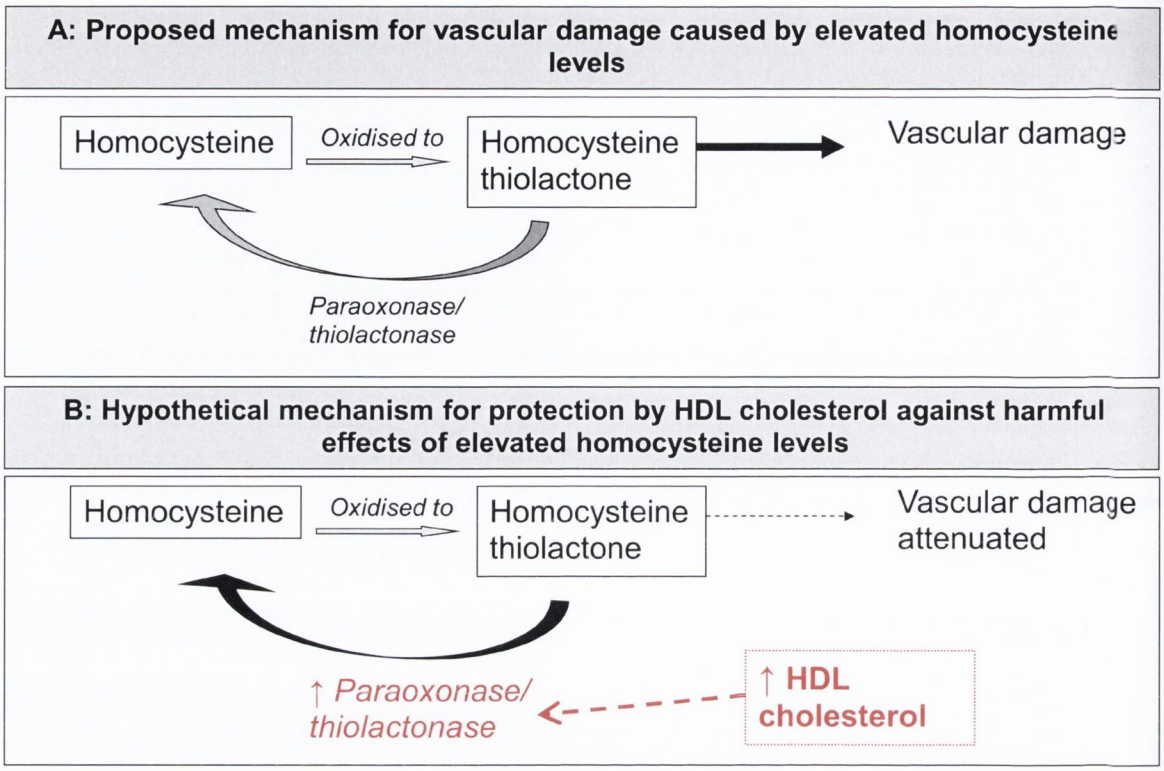
Another mechanism through which HDL cholesterol has been proposed to protect against atherosclerosis is through anti-oxidant properties. These have been demonstrated for both ApoA1 and ApoA2 which inhibit the oxidation of LDL cholesterol particles, an early step in atherogenesis.

HDL particles also carry two enzymes involved in preventing oxidation – paraoxonase 1 (PON1) and paraoxonase 3. Both are thought to have anti-oxidant properties and to prevent the oxidation of LDL cholesterol particle. An in vitro experiment, endothelial cells were incubated with HDLs containing paraoxonase and HDLs deficient in paraoxonase. Those cells incubated with paraoxonase deficient HDLs were demonstrated to have an increase in oxidation of LDL particles[11].

A recognized downstream effect of oxidation of LDL is the upregulation of endothelial cell monocyte chemotactic factor (MCP-1) production, which is a key step in the initiation of atherogenesis. Endothelial cells were incubated with oxidized LDL particles with and without human HDL and PON1. In the presence of HDL and PON1 there was a significant reduction in the production of MCP-1 compared to control endothelial cells[11]. This suggests that as well as preventing the oxidation of LDL cholesterol, HDL and PON1 also reduce the harmful effects of oxidized LDL particles.

In a study of women with low HDL, those who also have elevated levels of LDL cholesterol were shown to have higher expression of the CCR2 receptor (which mediates the binding of monocytes to MCP-1 receptor). Treatment of these individuals with estrogen increased the TC/HDL ratio and also reduced the expression of CCR2[12].

Another suggested effect of this enzyme is the decrease in conversion (oxidation) of homocysteine to its harmful metabolite homocysteine thiolactone, hereby diminishing the atherogenic effect of hyperhomocysteinaemia[13]. This potential mechanism is illustrated in Appendix figure 9.



Appendix figure 9: Hypothesis suggesting protection from harmful effects of hyperhomocysteinaemia by paraoxonase

PROMOTION OF ENDOTHELIAL FUNCTION

Nitric oxide (NO) is a potent vasodilator, which is released by normally functioning endothelial cells.

The decrease in bioavailable NO is a key step in the atherogenic process. This also contributes to the process of atherosclerosis through increases in platelet aggregation and promotion of vascular smooth muscle cell proliferation.

HDL cholesterol promotes the bioavailability of NO through a variety of mechanisms. These have mainly been demonstrated on cell culture and animal studies and need further clarification in humans. The main method through which HDL cholesterol increases the bioavailable supply of NO is through favorably affecting both localization and release of endothelial nitric oxide synthease (eNOS) from the endothelial cells. eNOS is known to promote the release of NO through the enzymatic control of the conversion of L-arginine to L-citrulline[14].

eNOS is usually located in cholesterol-enriched caveolae of endothelial cells. This system of eNOS localization is part of the normal signaling process for eNOS. Oxidised LDL cholesterol mediated changes in the cholesterol milleu of these caveolae result in disruption of normal activity of eNOS. HDL cholesterol antagonizes this effect of oxidized LDL through supply of cholesterol esters

thereby increasing the activity of the enzyme[14]. HDL cholesterol is also a direct agonist of eNOS – this is thought to be mediated through the SR-B1 receptor[15].

Additionally, HDL cholesterol promotes normal function of the endothelial cell layer by attenuating apoptosis of and promoting the integrity and migration of the endothelial cells[14].

ANTI-THROMBOTIC EFFECTS

HDL has been proposed to have a number of anti-thrombotic effects[14]. Favorable effects on two elements of Virchow's triad have been proposed – blood stasis, increased blood viscosity. The other component is mechanical – endothelial injury.

Prostacyclin is an endogenous vasodilator, increased plasma concentrations of which enhance blood flow. It is thought that HDL cholesterol increases circulating levels of prostacyclin[16]. The mechanisms through which HDL increases bioavailable NO, as described above, also lead to an increase in blood flow and therefore an anti-thrombotic effect.

HDL cholesterol reduces blood viscosity through a number of mechanisms. The fibrinolytic balance of the blood is controlled by the balance between tissue plasminogen activator (TPA), which usually removed intravascular fibrin and its endogenous inhibitor plasminogen activator inhibitor (PA1). HDL cholesterol is thought to promote fibrinolysis through the up-regulation of tissue plasminogen activator (TPA) and the down-regulation of plasminogen activator inhibitor 1 (PAI-1)[14, 17].

As discussed above HDL cholesterol reduces the production of P-selectin and E-selectin, these are pro-thrombotic factors and are found on the surface of both platelets and endothelial cells. HDL cholesterol has also been shown to reduce the production of tissue factor[14].

HDL cholesterol has also been shown in animal studies to enhance the inactivation of purified coagulation factor Va by activated protein C and protein S. HDL cholesterol also appears to increase the concentration of thrombomodulin, which is another anti-coagulant factor[14].

HDL cholesterol has also been shown to be associated inversely with platelet aggregation. This is thought to be due to both a direct effect, through downregulating the release of platelet activating factor, through down-regulating thromboxane A2 (HDL2 more effective than HDL3) and up-regulating prostacylin production[17].

Again, these effects have mainly been demonstrated in cell culture and animal studies. In humans, many studies have demonstrated associations between higher levels of HDL cholesterol and

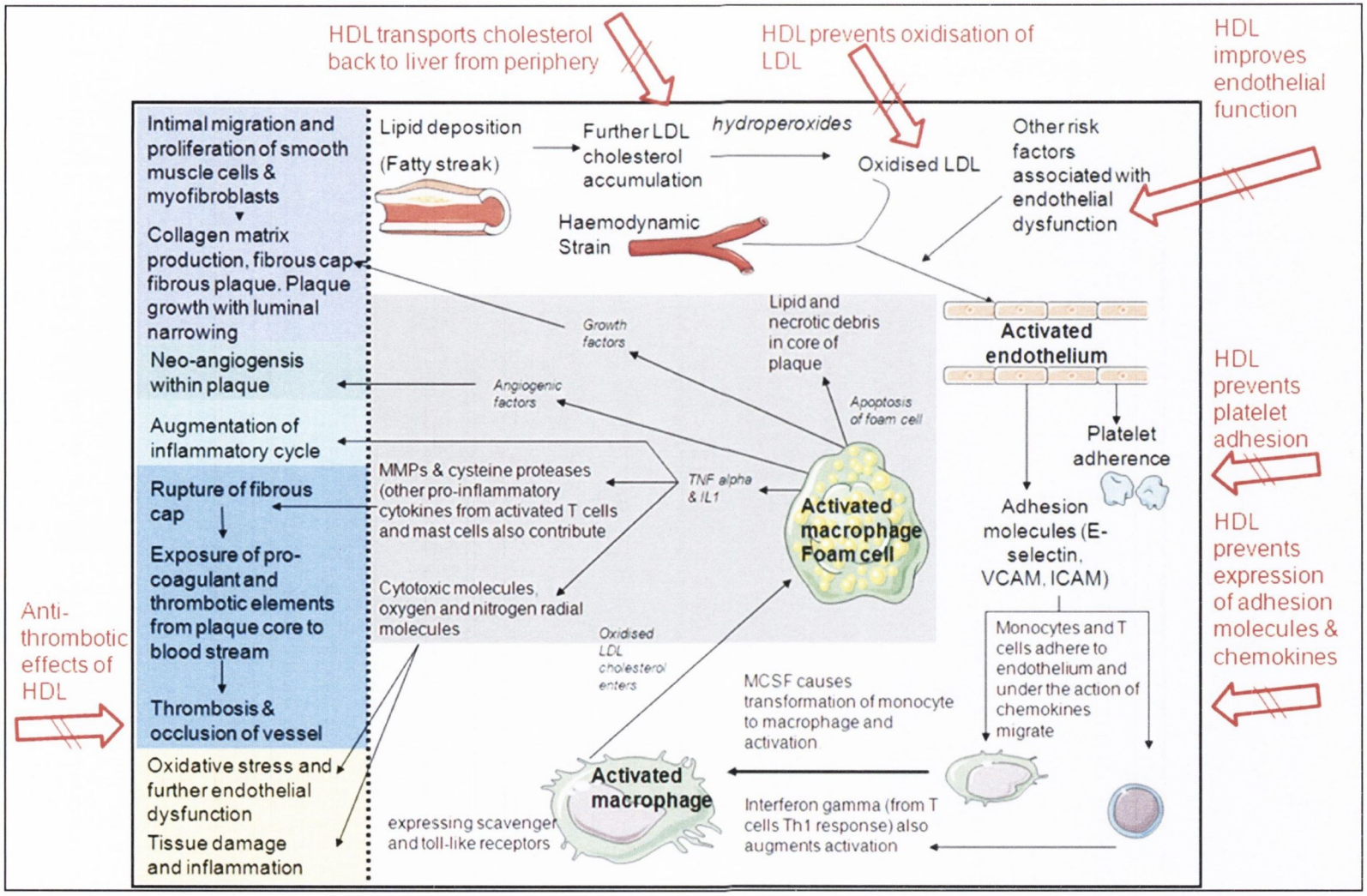
favorable changes in components of the clotting system have been described, as discussed below, it should be remembered there these associations do not definitively determine a causal relationship between HDL and anti-thrombotic mechanisms. This is because there could be another as yet unidentified factor causing both effects and confounding the relationship. However, in the presence of the basic science evidence described above, an anti-thrombotic effect of HDL cholesterol in humans certainly appears likely.

Elevated levels of PA1 and consequence predisposition towards reduced fibrinolysis is often associated the metabolic syndrome and low HDL cholesterol levels. Low HDL cholesterols correlate with elevated PA1 levels in humans [18].

HDL2 cholesterol levels have been shown to be inversely associated with platelet aggregability on multivariable analysis[19]. HDL cholesterol levels have also been shown to correlate with fibrinogen levels[20]. Nicotinic acid which is known to elevate HDL levels has also been shown to reduce fibrinogen levels[21].

The different anti-inflammatory, anti-oxidant and cholesterol efflux mechanisms of HDL cholesterol appear to be reasonably independent of each other. Evidence for this stems from the fact that lipid free Apo A1 is the preferred acceptor of cholesterol through the ABCA1 transporter, whereby lipid free Apo A1 has not been shown to exert an effect on adhesion molecules – this process can be as easily achieved by ApoA1 combined with phospholipid as by native HDL particles. Furthermore, most of the anti-oxidant property of HDL cholesterol seems to be related to paraoxonase, although ApoA1 and ApoA2 do appear to have some intrinsic anti-oxidant properties also.

The atheroprotective actions of HDL cholesterol are summarized graphically in Appendix figure 10.



Appendix figure 10: The atherosclerotic process - proposed protective effects of HDL cholesterol

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