STUDIES OF HOMOCYSTEINE AND VITAMIN D IN OLDER MEN

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THIS THESIS IS PRESENTED FOR
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WESTERN AUSTRALIAN CENTRE FOR HEALTH AND AGEING,
HARRY PERKINS INSTITUTE OF MEDICAL RESEARCH

AND

SCHOOL OF MEDICINE AND PHARMACOLOGY
THE UNIVERSITY OF WESTERN AUSTRALIA

2015
PREFACE

This thesis is presented as a collection of six scientific papers that have been published in international peer-reviewed journals. It explores the relationship between homocysteine, vitamin D, and various age-related health outcomes in older men.

A literature review and description of the study population are presented as the opening chapters. These are followed by chapters comprising each of the published works which examines a particular biomarker and health outcome in depth. The papers are presented as they appear in print, excepting some minor editorial changes to achieve consistency with prior and subsequent chapters. The thesis concludes with a discussion of the implications and future directions based on the research findings.

The papers presented in this thesis were conceived by the candidate in consultation with the candidate’s supervisors and co-authors. The precise contribution of the candidate, the candidate’s supervisors, and other co-authors, is presented at the conclusion of each paper.
STATEMENT OF ORIGINALITY

I declare that this submission is my own work. It contains no material that has been previously published, or material written by another person (except for the contributions of my co-authors, as clearly acknowledged in the text). I have obtained permission from my co-authors to include co-authored work in this thesis. No part of this thesis has been used to obtain any other degree.

________________________
Dr Yuen Yee Elizabeth Wong
(Candidate)

I confirm that the candidate has obtained permission from co-authors to include co-authored work in this thesis.

________________________
Winthrop Professor Leon Flicker
(Coordinating supervisor)
I would like to express my sincere gratitude to my supervisor, Winthrop Professor Leon Flicker, whose expertise and guidance added considerably to my research experience. I greatly appreciate his understanding and patience with me, particularly during my times of tribulation with illness and pregnancy, whilst juggling my clinical work and my PhD all at the same time. I would not have been able to complete my thesis without his kind support and mentoring. I would also like to acknowledge the important intellectual contributions of the Health In Men Study (HIMS) investigators, particularly Winthrop Professor Osvaldo Almeida, Professor Bu Yeap, Professor Graeme Hankey, Professor Paul Norman, and Professor Jonathan Golledge. I wish to thank all my colleagues at the Western Australian Centre for Health and Ageing (WACHA) for their constant support and assistance.

Special thanks go to Dr Poh-Kooi Loh and my ‘little’ brother, Kelvin, who inspired and motivated me to pursue a research career in Geriatric Medicine. I would also like to thank Associate Professor Kieran McCaul, Dr Zoe Hyde and Dr Derrick Lopez, for being ever so patient with me and providing me with invaluable statistical advice at times of critical need.

I recognise that this research would not have been possible without the men who volunteered to participate in the HIMS, as well as the research assistants who helped to collect the data. There would have been no homocysteine data to analyse without the contributions of Professor Frank van Bockxmeer. I thank the University of Western Australia and WACHA for supporting me with the scholarships.

Finally, I am forever indebted to my parents, for the unconditional love and care they have showered me throughout my very blessed life. With their constant nurture and encouragement, I was able to successfully complete my studies in medicine and serve the community constructively. To my dear husband and best friend, Kim, a very big thank you for riding this special journey with me, without whom I would not have been able to make this contribution to the world.
DEDICATION

For my parents
PUBLICATIONS COMPRISING THIS THESIS

Peer-reviewed scientific papers:


**Wong YYE**, Flicker L, Yeap BB, McCaul KA, Hankey GJ, Norman PE. Is hypovitaminosis D associated with abdominal aortic aneurysm, and is there a dose-response relationship? Eur J Vasc Endovasc Surg. 2013;45(6):657-64. (Chapter 4)


**Wong YYE**, McCaul KA, Yeap BB, Hankey GJ, Flicker L. Low vitamin D status is an independent predictor of increased frailty and all-cause mortality in older men: the Health In Men Study. J Clin Endocrinol Metab. 2013;98(9):3821-8. (Chapter 6)


AWARDS

1. Australian Postgraduate Award. 2012

2. Ad-hoc Top-up Scholarship through University of Western Australia. 2012

3. Departmental Scholarship through University of Western Australia. 2011-2012
SYNOPSIS

As the world’s population ages, there is an increased prevalence of chronic diseases, which can result in significant disability and consequently, an increased mortality rate. Effective targeting of modifiable risk factors may potentially attenuate the occurrence of these adverse events and improve the quality of life amongst older people.

Homocysteine and vitamin D status have been separately linked to several age-related declines. However, there is debate as to whether these biomarkers are merely epiphenomena or whether they do indeed play a role in mediating the development or progression of these health events that occur in old age. Existing findings from epidemiological studies and clinical trials have been inconsistent to date. The aim of this thesis was to explore whether these biomarkers were associated with adverse outcomes in several key domains, including abdominal aortic aneurysm (AAA), frailty, mortality, health-related quality of life, and cancers. The study population comprised community-dwelling men aged 70 years and over who had participated in the longitudinal, population-based Health In Men Study.

Levels of total plasma homocysteine and 25-hydroxyvitamin D [25(OH)D] were measured by immunoassays in this cohort of older men. The principle of Mendelian randomisation was also undertaken to explore the nature of associations between homocysteine and the outcomes, with the measurement of methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism by polymerase chain reaction. Outcome measures included aortic diameter (measured using an ultrasound machine), frailty (assessed with the FRAIL scale, comprising questionnaire data and physical measures), mortality (assessed by electronic record linkage), self-perceived physical health (assessed with the SF-36 Health Survey), and incident cancer diagnoses (assessed by electronic record linkage). Statistical techniques included linear and logistic regression, and Cox and competing-risks proportional hazards models.

Results of these analyses suggest that hyperhomocysteinaemia is associated with prevalent AAA, frailty, all-cause mortality, and self-perceived physical health. Using Mendelian randomisation, the MTHFR TT genotype is not associated with AAA, aortic diameter, or physical health-related quality of life, reflecting limitation of power for such analyses. Hypovitaminosis D is associated with the presence of larger AAAs, with an inverse dose-response relationship between 25(OH)D concentration and aortic diameter in those men with prevalent AAA. It is also associated with
prevalent and incident frailty, as well as an increased risk of all-cause mortality. A paradoxical association between lower 25(OH)D concentrations and reduced incidence of prostate cancer was demonstrated, whilst there is no evidence that vitamin D modulates the risk of colorectal or lung cancer in older men.

These findings suggest that hyperhomocysteinaemia and hypovitaminosis D may be deleterious to several age-related health outcomes in older men. Clinical trials are warranted to investigate whether homocysteine-lowering strategies and vitamin D supplementation can ameliorate or prevent the development of these adverse outcomes. The finding of a paradoxical association between low 25(OH)D concentration and reduced incident prostate cancer is unexpected and emphasises the need for further carefully designed studies to determine whether a causal relationship indeed exists.
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<td>1-aOHase</td>
<td>25-hydroxyvitamin D-1α-hydroxylase</td>
</tr>
<tr>
<td>1,25(OH)D3</td>
<td>1,25-dihydroxyvitamin D3</td>
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<tr>
<td>25(OH)D</td>
<td>25-hydroxyvitamin D</td>
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<td>AAA</td>
<td>Abdominal aortic aneurysm</td>
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<td>ABI</td>
<td>Ankle-brachial index</td>
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<td>AD</td>
<td>Alzheimer’s disease</td>
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<td>ADLs</td>
<td>Activities of daily living</td>
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<td>ADMA</td>
<td>Asymmetric dimethylarginine</td>
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<td>AF</td>
<td>Atrial fibrillation</td>
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<td>AIHW</td>
<td>Australian Institute of Health and Welfare</td>
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<td>aMCI</td>
<td>Amnestic MCI</td>
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<tr>
<td>ApoE</td>
<td>Apolipoprotein E</td>
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<tr>
<td>AR</td>
<td>Androgen receptor</td>
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<td>BMI</td>
<td>Body mass index</td>
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<td>BP</td>
<td>Bodily Pain</td>
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<td>BPH</td>
<td>Benign prostatic hyperplasia</td>
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<td>CABG</td>
<td>Coronary artery bypass grafting</td>
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<td>CAD</td>
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<td>CBS</td>
<td>Cystathionine β-synthase</td>
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<td>CCI</td>
<td>Charlson Co-morbidity Index</td>
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<td>CKD-EPI</td>
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<td>COPD</td>
<td>Chronic obstructive pulmonary disease</td>
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<td>COSO</td>
<td>Code of Surgical Operations</td>
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<td>CVD</td>
<td>Cardiovascular disease</td>
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<td>DSM-IV-TR</td>
<td>Diagnostic and Statistical Manual of Mental Disorders, 4th revision</td>
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<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tr>
<td>eGFR</td>
<td>Estimated glomerular filtration rate</td>
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<td>ER</td>
<td>Endoplasmic reticulum</td>
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<tr>
<td>FI</td>
<td>Frailty Index</td>
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<td>FISH</td>
<td>Fluorescence in situ hybridisation</td>
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<td>Gpx-1</td>
<td>Glutathione peroxidase-1</td>
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<td>Hcy</td>
<td>Homocysteine</td>
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<td>HHcy</td>
<td>Hyperhomocysteinaemia</td>
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<td>HIMS</td>
<td>Health In Men Study</td>
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<td>HPC</td>
<td>Hereditary prostate cancer</td>
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<td>HR</td>
<td>Hazards ratio</td>
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<tr>
<td>HRQOL</td>
<td>Health-related quality of life</td>
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<tr>
<td>hsCRP</td>
<td>High-sensitivity C-reactive protein</td>
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<tr>
<td>ICD</td>
<td>International Statistical Classification of Diseases and Related Health Problems</td>
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<td>ICD-8</td>
<td>ICD, eighth revision</td>
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<td>ICD-9</td>
<td>ICD, ninth revision</td>
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<tr>
<td>ICD-9-CM</td>
<td>ICD-9, Clinical Modification</td>
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<td>ICD-O-3</td>
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<td>ICPM</td>
<td>International Classification of Procedures in Medicine</td>
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<td>IGF</td>
<td>Insulin-like growth factor</td>
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<td>IGFBP-3</td>
<td>IGF binding protein-3</td>
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<td>LDL</td>
<td>Low-density lipoprotein</td>
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<td>MCI</td>
<td>Mild cognitive impairment</td>
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<td>MCS</td>
<td>Mental Component Summary</td>
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<tr>
<td>Mini-Cog</td>
<td>Mini Cognitive Assessment Instrument</td>
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<td>MMA</td>
<td>Methylmalonic acid</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>MMSE</td>
<td>Mini-Mental State Examination</td>
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<td>MoCA</td>
<td>Montreal Cognitive Assessment</td>
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<td>MOS</td>
<td>Medical Outcomes Study</td>
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<tr>
<td>MTHFR</td>
<td>Methylenetetrahydrofolate reductase</td>
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<tr>
<td>naMCI</td>
<td>Non-amnestic MCI</td>
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<tr>
<td>NF-κB</td>
<td>Nuclear factor κB</td>
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<td>NIA-AA</td>
<td>National Institute on Ageing – Alzheimer’s Association</td>
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<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
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<td>NO</td>
<td>Nitric oxide</td>
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<td>NSCLC</td>
<td>Non-small-cell lung cancer</td>
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<td>O$_2^-$</td>
<td>Superoxide anion</td>
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<td>OR</td>
<td>Odds ratio</td>
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<td>PAD</td>
<td>Peripheral arterial disease</td>
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<td>PCA3</td>
<td>Prostate cancer antigen 3</td>
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<td>PCR</td>
<td>Polymerase chain reaction</td>
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<td>PCS</td>
<td>Physical Component Summary</td>
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<tr>
<td>PF</td>
<td>Physical Functioning</td>
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<td>PIA</td>
<td>Proliferative inflammatory atrophy</td>
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<td>PIN</td>
<td>Prostatic intraepithelial neoplasia</td>
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<tr>
<td>PSA</td>
<td>Prostate specific antigen</td>
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<td>PTH</td>
<td>Parathyroid hormone</td>
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<td>QOL</td>
<td>Quality of life</td>
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<td>RANKL</td>
<td>Receptor activator of NF-κB ligand</td>
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<td>RCTs</td>
<td>Randomised controlled trials</td>
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<td>RP</td>
<td>Role-Physical</td>
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<td>RR</td>
<td>Relative risk</td>
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<td>S-adenosylhomocysteine</td>
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<tr>
<td>Acronym</td>
<td>Full Form</td>
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<tr>
<td>SAM</td>
<td>S-adenosylmethionine</td>
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<td>SCLC</td>
<td>Small-cell lung cancer</td>
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<td>36-item short-form Health Survey</td>
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<tr>
<td>TG</td>
<td>Triglycerides</td>
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<td>tHcy</td>
<td>Total plasma homocysteine</td>
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<tr>
<td>THF</td>
<td>Tetrahydrofolate</td>
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<tr>
<td>TIA</td>
<td>Transient ischaemic attack</td>
</tr>
<tr>
<td>UVB</td>
<td>Ultraviolet B</td>
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<tr>
<td>VDR</td>
<td>Vitamin D receptor</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
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<tr>
<td>WADLS</td>
<td>Western Australian Data Linkage System</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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CHAPTER 1

OVERVIEW AND BACKGROUND
CHAPTER 1: OVERVIEW AND BACKGROUND

1.1 Introduction

The average age of the world’s population is increasing at an unprecedented rate. This phenomenon has played a key role in the increased prevalence of chronic diseases among Australians (1). Chronic diseases can result in significant disability, and they are also leading causes of death in Australia (2).

In recognition of the importance of both extending longevity and avoiding disability throughout the life course, the National Preventative Health Taskforce was established by the Australian government with suggested strategies to improve the health of the Australian population over the next decade (3). National health priority areas, including cancer control and cardiovascular health, were identified, together with targets for preventive interventions such as smoking and obesity. The World Health Organisation (WHO) has estimated that effective targeting of risk factors through prevention could increase health life spans by up to 5 years in developed countries (4). The World Cancer Research Fund has also reported that strategic policies on diet, nutrition, and lifestyle choices can potentially reduce cancer risk (5). Scientific knowledge of the role of modifiable risk factors in relation to the ageing process has expanded significantly in recent years and it has been suggested that the appropriate management of these risk factors may reduce morbidity and mortality, improve quality of life, and reduce health service utilization in later life.

The roles of the biomarkers homocysteine and vitamin D as predictive factors for physical function and adverse health events in older people have been limited (6, 7). Large-scale cohort studies are needed to explore the associations between homocysteine and vitamin D levels with these health outcomes, and to determine the level at which they become strongly associated with ill health in older people. As these biomarkers are potentially modifiable they may represent important targets for interventions.

This thesis aims to review the important health outcomes of interest in a geriatric population. It also details the description of biomarkers homocysteine and vitamin D, as well as their relationship with the age-related health problems.
1.2 The demography of ageing

Demography is the study of the change in size, diversity, distribution and composition of human populations over time. The demographic transition is a gradual process where a society moves from high birth and mortality rates to low birth and mortality rates as a country develops from a pre-industrial to an industrialised economic system. The transition model, in general, involves four stages (8):

- In stage one, during the pre-industrial period, birth and mortality rates are high and approximately balanced. Population growth is therefore very slow.
- In stage two, as the country is developing, mortality rates are reduced dramatically due to improvements in food supply, sanitation, basic healthcare and education. Without a corresponding fall in birth rates, this results in an imbalance, with a large increase in population. The bottom of the age pyramid is widened significantly with the “population explosion”.
- In stage three, birth rates start to fall due to increased access to contraception, urbanization, increased education amongst women and transitions in values. Population growth thus begins to level off.
- In stage four, both birth and mortality rates are low. Population growth persists, with the age pyramid becoming more rectangular as those who are born during the stage two continue to age and fertility rate reduces.

Most developed countries, including Australia, are postulated to be in stage four of the demographic transition model. The age composition, like that of the world’s population, has transformed dramatically as a result of sustained low fertility and increased life expectancy. It is estimated that between 2000 and 2050, the proportion of the world’s population over 60 years will double from 11% to 22% (from 605 million to 2 billion). The number of people aged 80 years and older will likely quadruple to 395 million over the same period (9). In Australia, the older population (defined as aged 65 years and over) had increased by 29% (or about 727,000 people) and the oldest old (defined as aged 85 years and older) increased by 54% in the last decade. At 30 June 2012, 3.2 million Australians (14% of the population) were aged 65 years and over. This included 423,700 people aged 85 years and over (1.9% of the population) and 3500 people aged 100 and over (10). Women accounted for 54% of those aged 65 years and over, 65% of those aged 85 and
over, and 81% of centenarians. This is reflective of the higher life expectancy at birth for females compared to males (10).

1.2.1 Health concerns in older people

Australians enjoy one of the highest life expectancies in the world – 79.5 years for males and 84.0 years for females (1). As a consequence, various chronic health conditions tend to be more common with increasing age, which ultimately can complicate care needs and affect the quality of life. Therefore, population ageing has potential social and economic consequences which may alter the demand for services and government health spending.

1.2.1.1 Mortality

Cardiovascular disease (CVD), which encompasses ischaemic heart disease and stroke, has remained the leading cause of death worldwide, accounting for approximately 20% of deaths overall. In high-income countries, including Australia, 70% of deaths occur in people aged 70 years and over. Predominant causes of mortality in these populations are notably chronic illnesses, including CVD, cancers, dementia and chronic lung diseases (11) (Table 1.1).

Table 1.1 Leading causes of death, by sex, 2009

<table>
<thead>
<tr>
<th>Rank</th>
<th>Males Cause of death</th>
<th>% of all male deaths</th>
<th>Females Cause of death</th>
<th>% of all female deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Coronary heart disease</td>
<td>16.7</td>
<td>Coronary heart disease</td>
<td>15.3</td>
</tr>
<tr>
<td>2</td>
<td>Lung cancer</td>
<td>6.6</td>
<td>Stroke</td>
<td>9.8</td>
</tr>
<tr>
<td>3</td>
<td>Stroke</td>
<td>6.2</td>
<td>Dementia and Alzheimer’s disease</td>
<td>8.0</td>
</tr>
<tr>
<td>4</td>
<td>Chronic lower respiratory diseases</td>
<td>4.4</td>
<td>Lung cancer</td>
<td>4.4</td>
</tr>
<tr>
<td>5</td>
<td>Prostate cancer</td>
<td>4.3</td>
<td>Breast cancer</td>
<td>4.1</td>
</tr>
</tbody>
</table>

Source: Australian Bureau Statistics (12).
CVD occurs more commonly among older people, with 62% of those aged 75 and over having a cardiovascular condition compared with 5% of those aged under 45 (13). In 2009-10, CVD accounted for 11% of the problems managed by GPs (14) and 6% of all hospitalizations (15). CVD death rates increase dramatically with age. In 2009, there was a threefold increment between those aged 55-64 and those aged 65-74, and a fourfold increment between the 65-74 and 75-84 age groups. The highest CVD mortality rates occurred in those aged 85 and over (13).

Cancer is a leading cause of the total burden of disease and injury in Australia (16) and is mostly a disease of older people. In 2008, there were 112,304 new cases of cancer diagnosed in Australia. The risk of being diagnosed with cancer by the age of 75 was 1 in 3 for males and 1 in 4 for females. By age 85, it was 1 in 2 for males and 1 in 3 for females. The five most common cancers (prostate, bowel, breast, skin melanoma and lung) together accounted for 62% of all cases. In 2009-10, cancer was responsible for 1 in 10 hospitalisations in Australia. It is also the second most common cause of death, accounting for 29% of all deaths registered in Australia. The risk of dying from cancer before the age of 75 was 1 in 8 for males and 1 in 12 for females. By age 85, the risk was 1 in 4 for males and 1 in 6 for females (15).

Dementia is a significant health problem among older Australians. An estimated 298,000 Australians had dementia in 2011, of whom 92% were aged 65 years and over, and 62% were women (17). The prevalence of dementia is projected to increase in the coming decades due to population growth and ageing. In 2050, 44% of people with dementia are projected to be 85-94 years of age, compared to 36% in 2011 (17). With its progressive natural history, dementia can result in disability and increased care needs over time. Whilst dementia is one of the leading causes of death in Australia, there is, to date, no cure for this age-related medical condition (12).

Chronic respiratory diseases include a diverse range of conditions that affect the airways, in particular, asthma and chronic obstructive pulmonary disease (COPD). About 6 million Australians suffer from a chronic respiratory condition (18). In 2009, respiratory diseases accounted for 11,049 deaths in Australia, the third most common cause group of mortality (12). The median age at death for deaths due to asthma was 73.1 years for males, 80.2 years for females, and 77.9 years overall (12). Mortality rates secondary to COPD have declined
over the past few decades concomitant with declines in tobacco consumption. In 2009, the male rate had fallen to 29 per 100,000 – less than one-third of the rate in 1970. Among females, there was no reduction in death rate from this cause (19).

1.2.1.2 Chronic health conditions

Chronic health conditions are common among older people, with prevalence of up to 99% for those aged 55 years and over. Females are more likely to have at least one chronic health condition than males (78% versus 73%), which might be explained by the presence of more women in the older age ranges compared to men (1). Among community-dwelling older Australians in 2011-12, the most common chronic health conditions were reportedly short- and long-sightedness (affecting 35% and 61% of those aged 65 years and over, respectively), arthritis (49%), hypertensive disease (38%) and deafness (35%) (20).

Vision and hearing disorders are common, with age being the major contributing factor. Vision problems are more common in females (55% versus 49% in males) and people living in the least socioeconomically disadvantaged areas (54% versus 50% of those living in the most socioeconomically disadvantaged areas). Besides long- and short-sightedness, cataract, glaucoma and macular degeneration also commonly affect the older population (21). The most prevalent hearing disorder is deafness, followed by tinnitus. Hearing disorders are more common in males (17% versus 9% in females), people living outside major cities (15% versus 12% of those living in major cities), and people born in Australia (14% versus 10% of those born overseas) (15).

Arthritis involves inflammation of the joints, which can be caused by degeneration (commonest) or autoimmune conditions such as rheumatoid arthritis. Together with other musculoskeletal conditions, such as osteoporosis, back problems and gout, these conditions are large contributors to illness, pain and disability in Australia (Table 1.2). They also pose a substantial burden onto the community through extensive use of hospital and primary health care services (22).
Table 1.2   Health services for musculoskeletal conditions, 2009-10

<table>
<thead>
<tr>
<th>Problem managed</th>
<th>GP visits</th>
<th>Principle diagnosis</th>
<th>Hospitalisations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number per 100 encounters</td>
<td>Number</td>
<td>Percent</td>
</tr>
<tr>
<td>Osteoarthritis</td>
<td>2.9</td>
<td>Osteoarthritis</td>
<td>95,500</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>&lt;0.5</td>
<td>Rheumatoid arthritis</td>
<td>10,300</td>
</tr>
<tr>
<td>Back problems</td>
<td>3.3</td>
<td>Back problems</td>
<td>90,800</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>0.9</td>
<td>Osteoporosis</td>
<td>6,900</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Minimal trauma</td>
<td>86,600</td>
</tr>
<tr>
<td>All musculoskeletal</td>
<td>16.8</td>
<td>All musculoskeletal</td>
<td>459,700</td>
</tr>
<tr>
<td>conditions</td>
<td></td>
<td>conditions</td>
<td></td>
</tr>
</tbody>
</table>

Sources: Australian Institute of Health and Welfare (AIHW) (14); AIHW National Hospital Morbidity Database (23).

1.2.2  Nutrition in older people

The importance of nutrition in the maintenance of health and the prevention of disease is well recognised. Extensive studies have been conducted to investigate the role of nutritional factors in the development and progression of health outcomes specific to the older population, such as the frailty syndrome (24). Adequacy of nutritional status may be determined via interview/questionnaire surveys and/or measurement of biomarkers.

1.2.2.1  General factors affecting nutritional status in older people

Previous studies have suggested that average dietary intake levels of most nutrients are sufficient in older people (25). Nevertheless, there are many age-related physiological changes that can affect nutritional status. Older people commonly experience reduced physical activity and lower resting metabolic rate, which in turn leads to a physiological reduction in food intake in an early process of homeostatic control. The decline in energy intake is typically accompanied by a concomitant decreased intake of most other nutrients including protein and micronutrients (26). This can potentially result in a progressive weight loss or sarcopenia (27). Ageing is also associated with altered taste sensitivity, which can be due to essential changes in the sense organs or be caused by illness or medications (28). Food ingestion and mastication can be impaired by disease- or function-related factors, including poor dentition, xerostomia, dysphagia and reduced appetite. Physical and cognitive impairments can lead to difficulties in obtaining and preparing food.
Psychosocial factors including excessive alcohol consumption, bereavement and depression can result in altered pattern of dietary intake (29).

1.2.2.2 Biomarkers of nutritional exposures

Biochemical markers may be used to quantify specific nutritional status, such as protein, B-vitamins and folate, as well as vitamins C, D and E. They are measured in biologic tissues such as blood or urine, and can be direct measures of the nutrient of interest or a metabolite of the nutrient. For example, vitamin B12 can be measured directly in serum, albeit its relatively short half-life since it is water-soluble. Homocysteine, a nonprotein amino acid substrate in the B-vitamin metabolic pathway, has been shown to be inversely related to folate and B-vitamin status, and therefore may be utilised as a surrogate biochemical marker to reflect the metabolic function of B-vitamins (30, 31).

The measurement of biomarkers in human tissues attenuates the possibility of recall bias in epidemiologic studies. However, the interpretation of biomarker levels needs to take several caveats into consideration. Biomarker concentrations are dependent on various physiological bodily functions such as absorption, distribution, metabolism and excretion. In some instances, they are correlated with other serum constituents or disease processes. For example, 25-hydroxyvitamin D [25(OH)D] is metabolised in the kidneys to its active form, 1,25-dihydroxyvitamin D [1,25(OH)D3], a process which is tightly regulated by plasma parathyroid hormone (PTH), calcium and phosphorus levels. Individuals with chronic kidney disease will not be able to produce sufficient 1,25(OH)D3 (32).
1.3 Cardiovascular disease

Cardiovascular disease (CVD) relates to all diseases and conditions of the heart and blood vessels. In the following sections, the major subtypes of CVD, current evidence-based medical and surgical strategies used for treatment and secondary prevention, as well as the modifiable risk profiles are reviewed.

1.3.1 Coronary artery disease

Coronary artery disease (CAD) remains the largest single cause of death in Australia, accounting for 22,500 deaths (16% of all deaths) in 2009 (23). It results from an atherosclerotic process affecting the epicardial coronary circulation. Various theories have been postulated over years to explain the development of atherosclerosis, including theories of oxidative stress and inflammation. Hypercholesterolaemia, hypertension, diabetes mellitus, smoking and age, all of which are cardinal risk factors for the development of atherosclerotic CVD, can lead to vascular oxidative stress, via an increase in the generation of reactive oxygen species. This results in the structural and functional modification of proteins, lipids and DNA (33, 34). Together with other molecular processes, this may lead to the attraction and binding of inflammatory cells to the endothelial monolayer, which ultimately results in the formation of foam cells from macrophages with intracellular accumulation of lipids. A fibrous cap develops over an enlarging lipid core, and with ulceration and erosion, thrombus formation occurs leading to acute narrowing of the coronary artery lumen (35, 36). Over the last two decades, both experimental and clinical trials have outlined endothelial dysfunction as an important manifestation of a diseased vascular wall contributing to all stages of the atherosclerotic disease process. This is a consequence of an imbalance in endothelial injury and repair, and together with atherosclerosis, is considered as a significant prognostic indicator (37).

Chronic stable angina is a consequence of myocardial ischaemia that occurs when the supply of oxygen is unable to meet its demand. It reflects the gradual progression of CAD and medical therapy remains the cornerstone of treatment for this condition. Acute coronary syndrome develops when the atherosclerotic plaque ruptures or erodes, and in the
setting of acute coronary occlusion, there is no collateral formation to sustain a minimal level of coronary blood flow.

Optimal medical therapy may offer significant benefits in reducing symptoms of ischaemic heart disease and improving long-term prognosis, in addition to aggressive risk factor modification. This includes antiplatelet therapy, statins, angiotensin-converting enzyme and receptor inhibition, beta-blockade and/or calcium channel blockade. Up to 85% of recurrent events in patients with CAD could be prevented or delayed with the use of these medications (38, 39). Revascularisation by means of coronary artery bypass grafting (CABG) or percutaneous coronary intervention is indicated in patients with chronic stable angina refractory to medical therapy. Patients with moderate to high risk for adverse outcomes with medical therapy should be considered for treatment with CABG. Such patients include those with left main disease, severe left ventricular dysfunction and diabetes mellitus (40).

CAD is multifactorial in causation. The concept of “comprehensive cardiovascular risk” refers to and quantifies an individual’s overall risk of CVD resulting from a confluence of risk factors (41). The co-existence of multiple risk factors confers a magnified cardiovascular risk that is multiplicative rather than merely additive (42). In a large case-control study which involved 52 countries covering all continents, the INTERHEART study identified nine risk factors (namely smoking, lipids, hypertension, diabetes, obesity, diet, physical activity, alcohol consumption and psychosocial factors) that accounted for 90% of the risk of acute myocardial infarction. The two strongest risk factors, apoB/apoA1 ratio (which represents the balance of pro-atherogenic and anti-atherogenic lipoproteins) and current smoking, predicted 66.4% of all myocardial infarctions worldwide. Smoking, lipids, hypertension, diabetes and abdominal obesity accounted for 80% of the population-attributable risk (43). For risk factors such as blood pressure, there is a continuum of progressively increasing risk rather than an all-or-none relationship determined by cut-off values. In the Multiple Risk Factor Intervention Trial’s cohort study, 7.2% of myocardial infarctions occurred with systolic blood pressure ranges of ≥ 180 mmHg, which accounted for 0.9% of the study population. On the other hand, 20.7% of myocardial infarctions occurred with pressure ranges of 130-139 mmHg, which comprised 22.8% of the population (44). The “risk pyramid” implies that although individuals at the top of the

~ 9 ~
pyramid may have the highest risk of CAD, those individuals at the bottom account for the largest number of cases in the community because they constitute the largest segment of the population. Preventative strategies should therefore not only target those at the highest risk, but also the majority at lower levels of risk. Rather than focusing on unifactorial risk reduction, primary and secondary preventative measures should be veering towards multifactorial risk modification. The absolute risk prediction of each individual may be estimated by using country- or institution-specific risk charts that comprise independent predictors of CAD (45, 46) (Figure 1.1).

**Figure 1.1 Health In Men Study (HIMS) cardiovascular risk model matrix: annual incidence rate of stroke/myocardial infarction according to presence or absence of independent predictors (46)**

<table>
<thead>
<tr>
<th>Poor or fair self-perceived health</th>
<th>Waist Hip ratio ≤ 1</th>
<th>Waist Hip ratio &gt; 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBP &gt; 70 mmHg</td>
<td>DBP ≤ 70 mmHg</td>
<td>Age</td>
</tr>
<tr>
<td>Hcy&lt;15</td>
<td>Hcy≥15</td>
<td>DBP &gt; 70 mmHg</td>
</tr>
<tr>
<td>Hcy&lt;15</td>
<td>Hcy≥15</td>
<td>Age</td>
</tr>
<tr>
<td></td>
<td>85+</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>80-84</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>75-79</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>69-74</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Good or excellent self-perceived health</td>
<td>Waist Hip ratio ≤ 1</td>
</tr>
<tr>
<td>DBP &gt; 70 mmHg</td>
<td>DBP ≤ 70 mmHg</td>
<td>Age</td>
</tr>
<tr>
<td>Hcy&lt;15</td>
<td>Hcy≥15</td>
<td>DBP &gt; 70 mmHg</td>
</tr>
<tr>
<td>Hcy&lt;15</td>
<td>Hcy≥15</td>
<td>Age</td>
</tr>
<tr>
<td></td>
<td>85+</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>80-84</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>75-79</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>69-74</td>
<td>2</td>
</tr>
</tbody>
</table>

**Annual rate (per 100 person-years):**

1
2
3
4

~ 10 ~
1.3.2 Cerebrovascular disease

Cerebrovascular disease is commonly referred to as a stroke. In the 2009 Health Survey, an estimated 375,800 Australians (205,800 males and 170,000 females) had suffered a stroke at some time in their lives (18). It is the second most common cause of CVD death after CAD (15). Stroke is broadly classified as ischaemic or haemorrhagic, with ischaemic strokes accounting for the majority of strokes (>80%). Clinical features of haemorrhagic and ischaemic stroke overlap and hence, the distinction is often made with imaging. Ischaemic stroke is subdivided into small vessel occlusion (“lacunes”), large artery atherosclerosis (embolus or thrombosis) and cardioembolic. A proportion of ischaemic strokes may be cryptogenic in aetiology or consequence of other causes such as dissection or vasculitis (47).

Transient ischaemic attack (TIA) refers to acute stroke symptoms and/or signs that resolve within 24 hours. 30-40% of patients with ischaemic stroke have had preceding TIA (48). Patients who experience a stroke or TIA are at substantial risk of developing a further serious vascular event. The risks of recurrent stroke are approximately 10% in the first week, 14% in one month, and 20% by three months after onset (49, 50). Thereafter, the annual risks of recurrent stroke and myocardial infarction are around 5% and 2-3%, respectively (51).

The treatment of ischaemic and haemorrhagic stroke is dramatically different and one of the first steps in managing stroke patients is to evaluate them for the presence of haemorrhage. Acute management of an ischaemic stroke includes optimising the blood pressure to maximise perfusion to the ischaemic penumbra, giving intravenous thrombolysis to achieve early vessel recanalization and cerebral perfusion, considering intra-arterial thrombolysis to expedite the removal of thrombus, and starting antiplatelet therapy within 48 hours of stroke onset (52-55).

Despite the state-of-the-art acute interventions for stroke, there is minimal impact on overall disability subsequently. In order to effectively target primary prevention, individualised risk profiles need to be comprehensively assessed. The five major modifiable risk factors for stroke are hypertension, hypercholesterolemia, diabetes mellitus, cigarette smoking, and atrial fibrillation. There is considerable overlap with other CVD, as cerebral...
arteriosclerosis often coincides with CAD and peripheral arterial disease (PAD).

Hypertension is by far the most important risk predictor for stroke and its recurrence. A meta-analysis of multiple treatment trials revealed that every 10-mmHg increment in systolic blood pressure is associated with a 20% increased risk of stroke morbidity, and a 56% increased risk of mortality. A mean reduction of 5-6 mmHg in diastolic blood pressure correlates with a 35-40% reduction in stroke incidence (56). Based on 102 prospective studies, the Emerging Risk Factor Collaboration Study reported that diabetes mellitus is also a significant risk factor for ischaemic stroke [hazards ratio (HR) 2.27, 95% CI 1.95-2.65], haemorrhagic stroke (HR 1.56, 95% CI 1.19-2.05), all strokes (HR 2.27, 95% CI 1.95-2.65), and CAD (HR 2.00, 95% CI 1.83-2.19) (57). Nevertheless, intervention trials investigating the effects and extent of glycaemic control on stroke incidence have shown heterogeneous findings, possibly due to differing study conditions and criteria (58, 59).

Treatment of co-existing hypertension and hypercholesterolemia in patients with diabetes is highly recommended as this significantly reduces the risk of stroke (60). For individuals with normal or elevated cholesterol levels, most randomised controlled trials (RCTs) and meta-analyses have demonstrated benefits of statins in primary and secondary prevention of strokes. The effects of statins are not only consequent to a reduction in low-density lipoprotein (LDL) cholesterol levels, but also related to their modulating effects for vasoprotective, anti-inflammatory, immunological, plaque-stabilising, and vasodilatory responses (61, 62). Smokers are almost twice as likely to have an ischaemic stroke in comparison to non-smokers, and there is a dose-response relationship between the number of cigarettes smoked and the relative risk. Former smokers are also at a lower risk than current smokers, strongly implying that smokers can considerably reduce their stroke incidence by the cessation of smoking (63).

Finally, non-valvular atrial fibrillation (AF) is a major risk factor for stroke in an increasing ageing population (64). Substantial evidence indicates that warfarin is highly efficacious in preventing stroke in patients with AF, including older people of age 85 years and over (65). Co-existing major modifiable risk factors are common in people with AF and can significantly increase stroke incidence in these individuals. For example, AF confers a 5-fold increment in stroke risk compared with an approximately 3-fold risk of hypertension. For those individuals with AF and concomitant hypertension, there is an 8-fold increased
risk of stroke (64). Therefore, a holistic and multifactorial approach to management would be ideal in the realms of primary stroke prevention.

1.3.3 Peripheral arterial disease

Peripheral arterial disease (PAD) refers to the obstruction of large arteries that supply blood to the peripheries outside of the heart and brain. PAD prevalence ranges between 3% and 10%, and increases to 15-20% among individuals over 70 years of age (66, 67). PAD is a manifestation of systemic atherosclerosis. It is insidious in nature, usually having a gradual onset and long latency before symptoms arise. Careful history taking, clinical examination and measurement of the ankle-brachial index (ABI) remain the initial means of diagnosing PAD. The primary symptom of PAD of the lower extremities is claudication, which may be present in only 10% of patients (68). Claudication occurs when a stenosis in the artery restricts blood flow to the distal extremity such that the blood supply is unable to meet the increased tissue oxygen demand during exercise or exertion. PAD is associated with a high risk of CVD morbidity and mortality, and therefore evidence suggests that emphasis should be shifted to aggressive cardiovascular prevention strategies to reduce the risk of CVD complications and mortality (69, 70).

The major risk factors for PAD are similar to those associated with atherosclerosis, although the relative impact of each risk factor varies somewhat. These include male gender, cigarette smoking, advancing age, hypercholesterolemia, diabetes, and hypertension. Male gender has been reported as a PAD risk factor, since there are more men than women with PAD at a younger age group, possibly due to the greater prevalence of smoking amongst men than women. However, with the increased incidence of PAD associated with increasing age and the longer life span of women compared to men, the overall prevalence of PAD invariably becomes similar in both genders (71). Current smokers are 2.3 times more likely to develop PAD compared to non-smokers. In addition, there is a dose-response relationship with PAD risk, which increases with increasing pack-years of smoking (72). There is an association between reduced ABI with increased total cholesterol and decreased high-density lipoprotein cholesterol (HDL) concentrations, as shown in the Cardiovascular Health Study (73). Individuals with diabetes mellitus are 2-4
times more likely to develop PAD relative to non-diabetic individuals (71). For every 1% increment in glycated haemoglobin levels among individuals with type 2 diabetes, the risk of PAD increases by 28% (74). Prior history of CAD also predisposes to an increase in PAD risk by 1.5-fold (75).

The Health In Men Study (HIMS) investigators have identified a similar risk profile for prevalent claudication and incident PAD amongst older men, with age, smoking, hypertension, diabetes and history of CVD dominating (76). Likewise, the risk factors for claudication were similar in the Framingham Heart Study (77). In addition, the authors further developed an intermittent claudication risk prediction model as a guide for clinicians to educate patients on the importance of reducing their modifiable risk factors to avoid vascular complications related to PAD (Figure 1.2).

Figure 1.2  Risk prediction model: 4-year probability of intermittent claudication (77)

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Risk Factor Points</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Age, years</td>
<td>45-49</td>
</tr>
<tr>
<td>Sex</td>
<td>Female</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>&lt;170</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>Normal</td>
</tr>
<tr>
<td>Cigarettes per day, n</td>
<td>0</td>
</tr>
<tr>
<td>Diabetes</td>
<td>No</td>
</tr>
<tr>
<td>CAD</td>
<td>No</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Points</th>
<th>4-year probability</th>
<th>Points</th>
<th>4-year probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10</td>
<td>&lt;1%</td>
<td>23</td>
<td>10%</td>
</tr>
<tr>
<td>10-12</td>
<td>1%</td>
<td>24</td>
<td>11%</td>
</tr>
<tr>
<td>13-15</td>
<td>2%</td>
<td>25</td>
<td>13%</td>
</tr>
<tr>
<td>16-17</td>
<td>3%</td>
<td>26</td>
<td>16%</td>
</tr>
<tr>
<td>18</td>
<td>4%</td>
<td>27</td>
<td>18%</td>
</tr>
<tr>
<td>19</td>
<td>5%</td>
<td>28</td>
<td>21%</td>
</tr>
<tr>
<td>20</td>
<td>6%</td>
<td>29</td>
<td>24%</td>
</tr>
<tr>
<td>21</td>
<td>7%</td>
<td>30</td>
<td>28%</td>
</tr>
<tr>
<td>22</td>
<td>8%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Pharmacotherapy for PAD is similar to that as for CAD. Antiplatelet agents reduce all-cause mortality and fatal cardiovascular events in individuals with claudication (78). Lipid-lowering agents, particularly statins, improve pain-free walking distance and reduce total CVD events (70). Randomised trials have shown clear benefit of antihypertensive therapy in PAD patients, with significant risk reductions in CVD events (79, 80). Patients with PAD and diabetes mellitus should aim for optimal glycaemic control with glycated haemoglobin maintained at <7% (81). Therapy for the amelioration of claudication symptoms include cilostazol, a phosphodiesterase III inhibitor, which inhibits platelet activation and relaxes vascular smooth muscles (82). Endovascular or surgical interventions are usually considered for lifestyle limiting claudication that has not responded to conservative therapies, as well as for critical limb ischaemia (83, 84).

### 1.3.4 Aortic aneurysmal disease

Aneurysms are localised pathological dilatations of any vessel. In arteries, a diameter of 1.5 times that of the normal artery is a generally accepted definition. The natural history of most aneurysms is gradual expansion with increasing risk of rupture. Aneurysmal rupture is the tenth commonest cause of mortality in Western countries and accounts for about 2% of all deaths (85). In some cases, thrombosis, distal embolization, or both, may occur. Aneurysms are most common in the abdominal aorta in comparison to other sites such as the thoracic aorta and coronary arteries, and therefore abdominal aortic aneurysm (AAA) is the focus of review in this section (86).

The major non-modifiable risk factors for AAA include male gender, increasing age, and ethnicity. The prevalence of AAA is about 5% in men and 1% in women over 65 years of age (87). There is some evidence that AAAs are more common in Northern Europeans than Asians or Africans (88, 89). Other dominant risk factors for AAA overlap with those for CAD, including smoking, hypercholesterolemia, and hypertension. The association of ever smoking with AAA is 2.5 times greater than the association of ever smoking with CAD, and 3.5 times greater than the association with cerebrovascular disease (90). Current smoking also results in progressive AAA expansion and a greater risk of rupture (91). It has been suggested that cessation of smoking may lead to a slow decline in risk, indicating a priority in management for any individual with AAA (92).
hypercholesterolemia has been associated with an increased risk of AAA (88, 89, 93). The association between hypertension and AAA is weaker, possibly due to differing definitions of hypertension in epidemiological studies (93-95). A distinct difference in risk factor for aneurysmal versus atherosclerotic disease is the finding of a protective effect of diabetes in AAA, the cause of which remains to be established (88, 96, 97). The HIMS investigators further extended this observation by demonstrating that the negative association exists across a continuum of aortic diameters that includes the non-aneurysmal range (98).

Current management options for AAA are either surveillance or surgical repair (endovascular or open). Intervention is undertaken if the aneurysm expands to >5.5 cm or becomes symptomatic. Early elective surgery for patients with smaller AAAs has offered no survival advantage. Open surgery is associated with a moderate risk of perioperative death and complications, and also has a prolonged recovery period. Endovascular AAA repair has a main limitation of durability which warrants long-term follow-up and imaging (99-101). Henceforth, the need to identify effective risk factor modifications and medical therapies that could reduce or eliminate small AAA expansion and also improve overall clinical outcome such as reducing CVD events has become a major priority in the treatment of AAA. Definitive evidence for effective drug treatments of AAA are presently still lacking and current trials are examining the efficacy of exercise, doxycycline, angiotensin converting enzyme inhibition and mast cell stabilization in limiting AAA progression (102).
1.4 Geriatric syndromes

Geriatric syndromes are defined as “multifactorial health conditions that occur when the accumulated effect of impairments in multiple systems render an older person vulnerable to situational challenges” (103). Multiple risk factors and multiple organ systems are often involved. In this section, some clinical conditions that are commonly classified as geriatric syndromes are reviewed.

1.4.1 Frailty

Frailty is an emerging geriatric syndrome with important implications for the care of the older population. The prevalence of frailty is between 3% and 7% in older people aged 65-75 years (104). The incidence of frailty increases with age, reaching more than 32% in those aged more than 90 years (105).

1.4.1.1 Frailty and disability

Although the terms frailty and disability are commonly used interchangeably to identify vulnerable older adults, these are distinct clinical entities that are causally related (106). There is no ‘gold standard’ for the definition of frailty. An individual who has frailty is considered to have “excess vulnerability to stressors due to age-related decline in physiologic reserve across multi-systems, resulting in reduced ability to maintain or regain homeostasis after a destabilising event” (107). These physiologic systems may include neuromuscular, such as sarcopenia and reduction in muscle fiber function; osteopenia; dysregulation of the hypothalamic axis, inflammatory cascade and immunological function; and heart rate variability (108, 109). The process of frailty is thought to commence with an aggregate expression of risk from these physiologic degradations, and when the losses of reserve reach an aggregate threshold that leads to serious vulnerability, the syndrome may become evident (106). Whether frailty should be defined to be a ‘state’ as individuals transit between the robust, prefrail, and frail categories over time remains an area of controversy. Clinical manifestations of frailty largely include wasting (loss of muscle mass and power, and weight loss), loss of endurance, low activity, decreased balance and
mobility, slowed motor performance, and potentially cognitive slowness or impairment (108, 110). Disability is mostly diagnosed by self-reported difficulty in specific activities essential for independent living, including self-care and desired activities for sustenance of one’s quality of life (106). Individual diseases, co-morbid diseases or impairments, and frailty itself are identified risk factors for physical disability (111, 112). Disability may also exacerbate frailty due to decreased activity or nutritional intake. Disability and frailty not only share a causal interconnectedness with each other, but also a co-occurrence. This is illustrated by the finding of a higher proportion of frail individuals amongst those who are disabled compared to the non-disabled (106). Management of disability involves mainly rehabilitation with increased access to community and supportive services. Medical care for frail older people focuses on exploration and treatment of the pathologic causes of progressive weakness, weight loss and other hallmarks of frailty (106). Underlying pathologic causes may include health conditions such as depression and congestive cardiac failure (108).

1.4.1.2 Phenotypes of frailty

Frailty is broadly defined by a physical “sarcopenic” phenotype or a multi-domain phenotype.

The physical phenotype of frailty was initially presented by Fried et al in 2001 as an operational definition of frailty, based on their work in the Cardiovascular Health Study (113). This consists of five measurable items that are identified as characteristics of frailty, namely shrinking, weakness, exhaustion, slowness and low physical activity (Table 1.3). A person is classified as frail if three or more of these criteria are met, as intermediate if one or two of the criteria is met, and as robust if none of the criteria is met. Frailty defined by this means has been associated with several adverse health outcomes, including hospitalization, mortality, worsening mobility and falls (113).
Table 1.3 Domains and criteria for the Fried model of frailty

<table>
<thead>
<tr>
<th>Characteristics of frailty</th>
<th>Measure proposed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shrinking</td>
<td>Unintentional weight loss of ≥ 10 pounds (≥ 4.5 kg) in the previous year OR at follow-up, of ≥ 5% of body weight in previous year</td>
</tr>
<tr>
<td>Weakness</td>
<td>Grip strength in lowest 20% of the population, adjusted for gender and body mass index</td>
</tr>
<tr>
<td>Exhaustion</td>
<td>Self-reported exhaustion, according to the Center of Epidemiological Studies</td>
</tr>
<tr>
<td>Slowness</td>
<td>The slowest 20% of the population, based on time to walk 15 feet (4.6 m), adjusting for gender and standing height</td>
</tr>
<tr>
<td>Low activity</td>
<td>Weighted score of kilocalories expended per week, in lowest quintile of the population for each gender</td>
</tr>
</tbody>
</table>

Another scale was subsequently developed by Ensud et al in 2008 using data from the Study of Osteoporotic Fractures, in an attempt to simplify the definition of frailty and cater to the busy clinical setting. This consists of three criteria, namely shrinking (weight loss of ≥ 5% between examinations), exhaustion (self-reported exhaustion according to the Geriatric Depression scale) and physical function (inability to rise from chair five times without using arms). Frailty is considered when ≥ two of these components are present. This has been shown to be comparable to the Fried model of frailty in terms of its predictive properties (114). An expanded model of the physical phenotype was also assessed in the Three-City Study. When the domain of cognitive impairment was added to the frailty scale, its predictive validities for disability and mortality was improved relative to the original Fried model of frailty (115).

The multi-domain phenotype of frailty is based on the principle of accumulation of deficits that are associated with frailty. The Frailty Index (FI) was first constructed by Rockwood et al in 1999 based on identified deficits in the domains of cognition, mood, motivation, communication, mobility, balance, bowel and bladder function, activities of daily living (ADLs), nutrition, social resources, as well as several co-morbidities. The FI is mostly derived from a comprehensive geriatric assessment of the individual and is based on the concept that the more deficits a person has, the more likely the person is to be frail. It is reported as a ratio of prevalent deficits to the total number of potential deficits. The index was found to be highly predictive of mortality and institutionalisation (116). One major
limitation of the FI would be its suitability in a busy clinical setting due to the comprehensive assessment required to obtain the variables. In addition, the weighting of a variable may also need to be accounted for, given that certain deficits may be more strongly associated with adverse outcomes than others.

1.4.2 Falls and fragility fractures

Falls are important causes of disability, morbidity and mortality in an ageing population. As with other geriatric syndromes, falls commonly result from an accumulation of impairments in multiple domains. This may involve a complex interaction of intrinsic and extrinsic factors (Table 1.4). The risk of falling increases with increasing number of risk factors. Among these, a previous history of falls is the strongest risk factor for future falls, with a 55% absolute risk of falling during follow-up across studies (117).

Table 1.4 Risk factors for falls (118)

<table>
<thead>
<tr>
<th>Intrinsic factors</th>
<th>Extrinsic factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous history of falls</td>
<td>Medications (especially psychotropic, opiate-based analgesia, diuretic and antihypertensive drugs)</td>
</tr>
<tr>
<td>Gait and balance problems</td>
<td>Polypharmacy</td>
</tr>
<tr>
<td>Cognitive impairment or dementia</td>
<td>Excessive alcohol consumption</td>
</tr>
<tr>
<td>Neurological disorders (peripheral neuropathy, stroke, Parkinson’s disease, slowed reflexes, myopathy, proprioceptive impairment)</td>
<td>Environmental hazards (loose rugs, clutter, poor lighting, wet floor, unstable furniture, patient restraints)</td>
</tr>
<tr>
<td>Vestibular disorders</td>
<td></td>
</tr>
<tr>
<td>Visual impairment (glaucoma, retinopathy, macular degeneration, cataract)</td>
<td></td>
</tr>
<tr>
<td>Joint deformities (previous fractures, degeneration)</td>
<td></td>
</tr>
<tr>
<td>Postural hypotension</td>
<td></td>
</tr>
<tr>
<td>Recent illness or delirium</td>
<td></td>
</tr>
</tbody>
</table>

Fall-related injuries accounted for the majority of all hospitalised injury cases (over 70%) among Australians aged 65 and over, with female hospitalisation being more predominant (15). Of all deaths due to falls, 87% were of people aged 70 years and over (119). It is therefore important to emphasise secondary preventative strategies after identification of falls risk in an individual. These include education of the patient and caregiver, and multi-
disciplinary assessment followed by appropriate interventions targeting the identified risk factors. Based on a Cochrane Systematic Review of 111 RCTs, there is strong evidence that exercise is beneficial for community-dwelling older adults in reducing the rate of falling and falls risk, either as a multi-component group, Tai chi or individually prescribed home-based exercise (120). Vitamin D supplementation may also be considered for those individuals with a low vitamin D status to reduce fall rate (120).

Major fragility fractures include those of the forearm, vertebral body and hip, but fractures of the humerus, pelvis and tibia are not uncommon in patients with osteoporosis. The incidence of fragility fractures increases with age, and is higher in women than men. Among these fractures, hip fractures have the most clinical significance in older people, due to their higher associated morbidity and mortality rates compared to all other fragility fractures combined (121). The risk of hip fracture increases exponentially from the age of 60, with majority occurring in women because of their longer lifespan and high prevalence of osteoporosis (122). About 40% of all patients with hip fracture have experienced a prior fragility fracture (121). Hip fracture is often considered a hallmark of ageing, as it primarily affects people with the highest co-morbidities and dependence in ADLs and mobility. Mortality ranges from 5 to 10% in the first month after a hip fracture, with one-third of older people dying within a year of fracture. Poor prognostic indicators include advanced age, male gender, poorly controlled co-morbid conditions, cognitive disorders and institutionalisation (122). Peri-operative orthogeriatric input to this specific group of patients have been shown to significantly improve mortality and functional outcomes (123).

1.4.3 Cognitive impairment and dementia

Mild cognitive impairment (MCI) refers to an intermediate state between normal cognition and dementia (124). According to the Mayo criteria (125), individuals with MCI have:

- A subjective cognitive complaint that is usually corroborated by an informant,
- Preserved general cognitive function,
- Impairment of one or more of the cognitive domains (memory, attention-executive function, visuospatial skills and/or language), and
• Essentially normal ADLs.

MCI affects one-fifth of people aged 65 years and over (126). It may be classified into amnestic and non-amnestic groups, and further into single and multiple domains. Amnestic MCI (aMCI) is characterised by the presence of memory impairment, whilst non-amnestic MCI (naMCI) refers to the presence of impairment in one or more of the other cognitive domains with relative preservation of memory (124). Diagnosis of MCI is based on clinical judgement, aided by history taking, physical examination, screening mental state examination and formal neuropsychological testing.

aMCI is generally thought of as a precursor to Alzheimer’s disease (AD) and therefore is the most researched subtype (127). It is presumed that the greater the extent of additional non-memory domains, the greater the risk of progression from MCI to dementia. The development of a predictive profile for this group of individuals would be desirable, by the combination of data on clinical, genetic, neuroimaging and surrogate biomarkers. Current focus is on early detection of cognitive decline which hopefully can lead to the implementation of therapies that may potentially slow its progression. A recent systematic review by Cooper et al has concluded that while there is no pharmacological intervention that is effective for preventing the conversion of MCI to dementia, cognitive therapy in the form of memory training and stimulation, as well as recreation and social interaction, might potentially improve cognition over 6 months. It is recommended that further trials of high quality are required to investigate the potential long-term benefit of this intervention (128).

Dementia is increasingly common with advancing age, affecting nearly 1 in 4 people aged 85 years and over. An estimated 222,100 Australians (1.0%) had dementia in 2011, and this is projected to increase to more than 464,000 (1.6%) by 2031 (129). AD is the most common form of dementia in older adults. Symptoms of AD include memory loss, impaired judgement and decision-making capacity, inability to perform ADLs, and changes in behaviour, mood and personality (130).

The predictive risk factors for AD include age, female gender, low educational level, history of head injury, late-life depression, and the conventional CVS risk factors such as hypertension, diabetes, hyperlipidaemia and hyperhomocysteinaemia (130, 131).
Genetically, apolipoprotein E (ApoE) ε4 allele has been associated with late-onset disease, whereas mutations in the amyloid precursor protein, presenilin 1 and presenilin 2 are associated with autosomal dominant, early-onset cases of AD (132, 133).

Clinical evaluation for AD involves a thorough history taking and neurological examination. Performance-based screening tools that may be used for initial assessment include the Mini-Mental State Examination (MMSE) test, the Mini Cognitive Assessment Instrument (Mini-Cog) and the Montreal Cognitive Assessment (MoCA) (134-136). Organic causes which are potentially reversible or treatable need to excluded, such as delirium, depression, vitamin B12 deficiency and hypothyroidism. In particular, delirium and depression are two most likely conditions that can mimic dementia and are potentially reversible with appropriate therapy. As these conditions have considerable overlap in cognitive symptoms with dementia, determination of an underlying dementia syndrome leading to cognitive frailty inevitably becomes more obscured in their presence. Current standardised clinical criteria that allow accurate diagnoses of AD are mainly from the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) and the National Institute on Ageing – Alzheimer’s Association (NIA-AA) (137, 138).

Early and accurate diagnosis of AD is desirable so as to facilitate timely treatment of the condition and long-term care planning. The primary objectives of pharmacological interventions are to preserve cognitive and functional ability, minimise behavioural disturbances and slow disease progression. These medications include cholinesterase inhibitors and glutamate N-methyl-D-aspartate (NMDA) antagonist (139, 140). People with advancing AD may require a substantial amount of aged care and health services including community aged care packages and hospital services. In 2010, 53% of permanent residents in residential aged care facilities in Australia have had a diagnosis of dementia (141).

1.4.4 Depression

Depression has a profound negative impact on older adults. Besides causing significant emotional suffering in these individuals, this condition also reduces their quality of life and function, as well as increases both medical morbidity and mortality (142-146). In general,
clinically significant depressive symptoms prevail in approximately 8-16% of community-dwelling older people (147-149). The prevalence of major depression is 1-3% among the older population, with increased incidence among those aged 85 years and over, reaching 13% (142, 150, 151).

Late-life depression is often co-morbid with chronic illnesses. A meta-analysis by Chang-Quan et al demonstrated that, when compared with the older people without chronic disease, those with chronic disease were at a higher risk of depression [relative risk (RR) 1.53, 95% confidence interval (CI) 1.20-1.97]. In the same study, those older adults with poor self-rated health were also at higher risk for depression in comparison to those with good self-rated health (RR 2.40, 95% CI 1.94-2.97) (152). There is a high prevalence of depression among the cognitively impaired, ranging between 20% and 50% (153, 154). Among those older adults with both major depression and dementia, there is a higher incidence of impairment in ADLs, behavioural disturbances and serious wandering (155). Depression adversely influences the outcomes of co-morbid health conditions in older people, and vice versa (142, 143). There is reported higher risk of non-suicide related mortality among the older population, with the severity and duration of depressive symptoms being significant predictive factors (142, 146, 156, 157). Depression is a highly treatable illness if detected. Studies have shown that a combination of psychotherapy and pharmacotherapy would have yielded a better outcome than either therapy alone (158, 159).
1.5 Health-related quality of life

Quality of life (QOL) is defined by the WHO as “the individuals’ perception of their position in life, in the context of their culture and value systems, and in relation to their goals, expectations, standards and concerns” (160). It is a multi-dimensional concept and several key factors have been identified as important for successful ageing, including personal satisfaction, physical environment, social environment, socio-economic status, cultural factors, health status, personality and personal autonomy. Health-related quality of life (HRQOL) refers to physical and mental health perceptions in relation to the above factors (161).

Measuring QOL is pertinent as it may help determine the burden of preventable disease, injuries and disabilities, and also provide valuable insights into its relationship with reversible risk factors. On a national level, this may help monitor progress in achieving the nation’s health objectives. QOL measurements are varied. These include broad measures such as the WHOQOL-BREF, and disease-specific measures such as the European Organisation for Research and Treatment of Cancer QOL questionnaire. The 36-item short-form Health Survey (SF-36) is a HRQOL measure and therefore the focus of review in this section.

1.5.1 The SF-36 Health Survey

The SF-36 is a generic measure of the health status of an individual, unlike other measures that target a specific age, disease or treatment group. It is constructed to compare general and specific populations in areas of functional health and well-being, the relative burden of disease, and the relative benefits of alternative therapies. There are 36 questions in the health survey which yield an eight-scale profile of scores and two summary measures (Figure 1.3). All but one of the 36 items (self-reported health transition) are used to score the eight scales.

The eight health concepts measured in the SF-36 were selected from the Medical Outcomes Study (MOS) (162). Each scale is an aggregate of between two and ten items, with each item used for the scoring of only one scale. The three scales which correlate most highly with the physical component (namely, Physical Functioning, Role-Physical, and Bodily Pain) also contribute most to the scoring of the Physical Component Summary (PCS)
Validity studies have demonstrated that the three scales which contribute most to the physical component are most responsive to treatments that modify physical morbidity, such as knee replacement, hip replacement and heart valve surgery (165-167). In contrast, the scales which contribute most to the mental component respond most to therapies that target mental health (168-170). Other predictive validity studies have linked SF-36 scales and summary measures to utilization of health care services, unemployment within one year, and 5-year survival (163).
1.6 Cancers

The risk of most types of cancer increases with age. With the growth of an ageing population, the burden of cancer in older people is likely to increase. In Australia, more than 100,000 new cases of cancer are diagnosed each year, and the incidence is projected to increase by 2020. The five most common cancers together account for approximately 60% of all cases (15) (Figure 1.4). In this section, prostate, colorectal and lung cancers are reviewed.

Figure 1.4 Incidence of the five most commonly diagnosed cancers in Australia, 2008 (15)

![Graph showing the incidence of the five most commonly diagnosed cancers in Australia, 2008.](image)

1.6.1 Prostate cancer

Prostate cancer accounts for approximately 30% of cancers diagnosed each year in Australian men (171). It is the second most common cause of cancer death, after lung cancer (172). The prevalence is estimated to be around 120,000 in Australian men, and it is predicted that the number will increase to 267,000 by 2017 (173).
1.6.1.1 Risk factors

Age

One of the most significant risk factors for the development of prostate cancer is age. By the age of 75, the risk is 1 in 8 men, and the number increases to 1 in 6 by the age of 85 (172).

Geographical and ethnic variations

Residents at North America and northern European countries have a higher risk than those living in the Far East. In migration studies, the incidence of prostate cancer in men emigrating from a low- to high-risk location increases to that of the local population within two generations, implicating environmental and lifestyle-related factors contributing to prostate carcinogenesis (174-176). In the United States, African-Americans were noted to have the highest incidence of prostate cancer compared to other races, and they also have the highest mortality rate once diagnosed with prostate cancer. The reason for this ethnic variation in incidence remains to be established (177, 178).

Dietary variations

Diet is a major lifestyle-related risk factor for prostate cancer. In prospective cohort studies, increased fat and red meat consumption has been associated with an increased risk of prostate cancer, with a dose-response relationship between the amount of red meat consumption and the risk of cancer (179-181). Vegetables and antioxidants such as carotenoid lycopene (found in tomatoes), vitamin E and selenium, have been associated with reduced risk of prostate cancer (182).

Genetic predisposition

In a study of cancer risk among 44,788 pairs of twins in Sweden, Denmark, and Finland, 42% of the total prostate cancer cases were attributed to inheritance (183). Men with one affected first-degree relative (father or brother) are twice more likely to develop prostate cancer, and the risk increases with increasing number of affected family members (184). Complex segregation analyses have isolated rare autosomal dominant and X-linked alleles as accountable for the increased risk of cancer (185-188). The hereditary prostate cancer
(HPC) gene (susceptibility locus on chromosome 1) has been most thoroughly investigated, with heterogeneous findings of association (189, 190). In more recent studies, BRCA2 mutation has been found to confer a higher risk of prostate cancer in men by approximately five to seven-fold, whilst BRCA1 carriers may have approximately double the risk of prostate cancer than that observed in the general population (191, 192). Men with BRCA1 or BRCA2 germline mutations may potentially be at risk of developing highly aggressive prostate cancers with higher mortality at a younger age compared to those of sporadic cancers in the general population (193, 194).

**Prostatic inflammation**

Prostate cancers, like all carcinomas, arise in differentiated epithelial cells and/or progenitor cells through the activation of oncogenes and the loss of tumour suppressor genes (195). It has been proposed that early prostate tumorigenesis may be characterised by proliferative inflammatory atrophy (PIA), which are focal areas of atrophic lesions arising from chronic inflammation. This progresses to prostatic intraepithelial neoplasia (PIN), and subsequently carcinoma in some cases (196). The sources of inflammation may be from exposure to different infectious agents and/or ingestion of carcinogens (196-198). Somatic genomic abnormalities which are associated with PIN and prostate cancer cells have also been found in cells with PIA (199).

**Androgens**

Androgens have been shown to promote growth and proliferation of prostate cancer cells both in vitro and in vivo (200-202). On the other hand, the theory of higher endogenous testosterone levels being associated with a higher risk of prostate cancer in men remains controversial. Two prospective studies, using data from the Physicians’ Health Study and the Baltimore Longitudinal Study, have suggested a relationship between higher levels of total and free testosterone with prostate cancer, respectively (203, 204). In the HIMS, higher free testosterone concentrations are associated with incident prostate cancer (205). However, a pooled analysis of 18 prospective studies comprising 3886 cases and 6438 controls revealed that total testosterone was not associated with incident disease (206). Meta-analyses of interventional studies have also concluded that testosterone supplementation is unlikely to be associated with an increased risk (207, 208). These
findings are in stark contrast to the traditional belief that prostate cancer growth is dependent on serum testosterone level and appear paradoxical to the proven beneficial effects of androgen deprivation on cancer progression (209). A Saturation Model has been proposed to explain why prostate growth is sensitive to androgen concentrations at very low levels, but becomes insensitive to variations of androgen concentrations at higher levels: androgens exert their prostatic effects primarily by binding to the androgen receptor (AR). When maximal androgen-AR binding is achieved at serum testosterone concentrations well below the physiologic range, the presence of additional androgen produces minimal further effects (210).

1.6.1.2 Biomarkers for prostate cancer

Besides being used as a diagnostic test for prostate cancer, serum prostate specific antigen (PSA) also has a role as a biomarker for the monitoring of cancer progression and for recurrence following curative therapy (211). The diagnostic test performance characteristics of PSA are variable. Its specificity and sensitivity ranges from 20-40% and 70-90%, respectively, whilst its area-under-the-curve metric of the receiver operating characteristic curve is between 0.55 and 0.70 for its ability to identify patients with cancer (212). Several non-malignant events may elevate PSA level and result in false positives, including inflammation, infection, trauma and benign prostatic hyperplasia (BPH) (212-214). Conversely, approximately 15% of men with low PSA levels (< 4.0 ng/ml) have prostate cancer, with higher grade tumours detected in 15% of these men (215, 216). In order to achieve a balance between missing clinically important cancers and performing unnecessary biopsies or treatment, more research on prostate cancer biomarkers are essential, with a goal to introduce new investigative assays with higher cancer specificity that may supplement or replace PSA.

Among the new wave of biomarkers that are emerging as non-PSA-based diagnostic tests for prostate cancer, the most prominent ones include urinary prostate cancer antigen 3 (PCA3) and TMPRSS2-ERG measurements. PCA3 is a noncoding RNA transcript that has been shown to be elevated in > 90% of prostate cancer tissues but not in normal or BPH tissues. With its high sensitivity and specificity, it is therefore established as a robust biomarker for prostate cancer (217, 218). TMPRSS2-ERG is a gene fusion product that is specific for prostate cancer and is androgen-regulated (219). It is present in approximately
50% of prostate cancer cases and accounts for 90% of prostate cancer fusions (220). It has been associated with higher tumour stage and prostate cancer-specific mortality, as well as specific morphologic features commonly seen with aggressive cancer phenotype (221, 222). Both PCA3 and TMPRSS2-ERG tests are currently adjunctive to PSA and further head-to-head trials are required to determine the clinical performance of these measurements in the absence of PSA screening.

1.6.2 Colorectal cancer

Colorectal cancer accounts for approximately 13% of all new cancers in Australia. In 2010, there were 3982 deaths from colorectal cancer (2205 men and 1777 women), accounting for 9.3% of all cancer deaths in Australia. Despite these figures, survival rates for colorectal cancer have actually increased in the past few years, possibly as a result of early diagnosis and improved treatment. In Australia, between the periods of 1982-1987 and 2006-2010, five-year survival rate had increased from 48% to 66% (223).

The adenoma-carcinoma sequence is commonly used to describe the colorectal carcinogenesis process. Multiple molecular pathways are involved in the tumorigenesis, with chromosomal instability, allelic imbalance at chromosomal loci (including 18q), and chromosomal amplification and translocation contributing to 85% of most sporadic cases (224-226). The remaining 15% of cases have high-frequency microsatellite instability, a phenotype which results from an inability of the DNA nucleotide mismatch-repair system to correct errors during DNA replication. This is controlled by several genes (including MLH1, MSH2 and MSH6) and tumours arising from microsatellite instability are often proximal in location, poorly differentiated and mucinous in histology (227).

1.6.2.1 Risk factors

Identified risk factors for colorectal cancer include inherent/non-modifiable risk factors (age, gender, inflammatory bowel disease, family history, and hereditary syndromes) and lifestyle-related/modifiable risk factors (diet, physical inactivity, obesity, diabetes mellitus, smoking, and alcohol consumption).
Age

The incidence of colorectal cancer and its mortality rate increases with age. In 2009, the average age of colorectal cancer diagnosis in Australia was 69.3 years. The risk of developing colorectal cancer before the age of 85 was 1 in 12, and the risk of dying from colorectal cancer before this age was 1 in 45 (172).

Gender

Worldwide, and in Australia, men are at greater risk for colorectal cancer than women. In 2009, the age-standardised incidence rate was 73.0 cases per 100,000 Australian men, compared with 50.5 cases per 100,000 Australian women. Between 1982 and 2009, incidence rates for men have increased from 66.7 cases per 100,000 to 73.0 cases per 100,000 men. In contrast, the incidence rates for women have remained stable over the same period (52.0 cases per 100,000 women to 50.2 cases per 100,000 women). The age-standardised mortality rate was also higher for men than women (172).

Inflammatory bowel disease

Ulcerative colitis and Crohn’s disease account for a significant proportion of incident colorectal cancer. The risk increases with the duration of illness (2% at 10 years, 18% by 30 years), and also severity and extent of inflammation (228-230).

Hereditary syndromes and family history

About 6% of colorectal cancer cases are associated with hereditary syndromes (such as Lynch syndrome and familial adenomatous polyposis) and at least 20% are familial (having two or more first degree relatives with colorectal cancer) (231-233). Individuals with a first-degree relative with colorectal cancer are at almost twice the risk of developing colorectal cancer compared to the general population. This risk is further increased with increasing number of affected family members or if a family member is diagnosed with this disease before 60 years of age (234). Almost all colorectal tumours associated with Lynch syndrome have microsatellite instability, whilst most cases of familial adenomatous polyposis are caused by germline mutations in the APC tumour suppressor gene (231, 232).
Diet

Consumption of red meat has been found to be associated with increased risk of colorectal cancer, with the presence of a dose-response relationship. This may be related to the high-temperature cooking of meats (including barbecuing) which increases the production of polycyclic aromatic hydrocarbons and other cooking-related carcinogens (235). The relationship between consumption of fruits and vegetables and cancer risk is inconclusive (236-238). There is strong evidence that consumption of food containing dietary fibre, in particular fibre from cereals and whole grains, is protective against colorectal cancer (239). Meta-analyses have reported modest protective effects of fish consumption and increased dietary calcium intake on colorectal cancer risk (240, 241).

Physical activity

Physical activity is strongly linked to decreased risk of colorectal cancer. In a meta-analysis of 52 cohort and case-control studies, an inverse association between physical activity and colon cancer was found in both men [RR 0.76, 95% CI 0.71-0.82] and women (RR 0.79, 95% CI 0.71-0.88) (242).

Obesity

In prospective studies, increased body mass index (BMI) was associated with an increased risk of colorectal cancer for both men and women (243, 244). A meta-analysis of 29 studies reported that for every 5 kg/m² increase in BMI, there was an associated 24% increased risk of colon cancer and a 9% increased risk of rectal cancer in men, as well as a 9% increased risk of colon cancer in women (245). Obesity also increases the risk of dying from colorectal cancer (246).

Diabetes mellitus

There is a strong association between diabetes mellitus and colorectal cancer (247-250). A recent meta-analysis concluded a 38% increased risk of colon cancer and a 20% increased risk of rectal cancer in patients with diabetes compared to those without diabetes. Hyperinsulinaemia may be the link as studies have shown that insulin is an important growth factor for colonic mucosal cells and stimulates colonic tumour cells (251, 252). This is further supported by evidence between colorectal cancer risk and insulin biomarkers such
as plasma insulin-like growth factor (IGF) and IGF binding protein-3 (IGFBP-3) (253). The presence of type 2 diabetes mellitus may also increase mortality rate in patients with colorectal cancer compared to those patients with no diabetes (254).

**Smoking**

A large meta-analysis of over 100 studies has reported an 18% increased risk in colorectal cancer among smokers compared with non-smokers (RR 1.18, 95% CI 1.11-1.25) (255). There is also an increased risk of mortality from colorectal cancer among smokers. These associations are stronger for rectal cancer than colon cancer (255). Smoking has been linked to the formation and aggressiveness of adenomatous colonic polyps, and may also modify cancer risk in patients with Lynch syndrome (256, 257).

**Alcohol**

In a meta-analysis of alcohol drinking and colorectal cancer risk across 27 cohort and 34 case-control studies, the risk was increased by 21% for moderate drinkers (2-3 drinks/day) and by 52% for heavy drinkers (≥ 4 drinks/day) compared with non-drinkers and occasional drinkers. There was also a dose-response relationship (258). This elevated risk may be mediated by alcohol acting as a methyl group antagonist and interfering with folate absorption in the folate-dependent DNA methylation pathway (259, 260).

### 1.6.2.2 Biomarkers for colorectal cancer

Treatment modalities for colorectal cancer include surgery, radiotherapy, chemotherapy and adjuvant monoclonal antibodies. Parallel development of predictive molecular and clinical markers is paramount as these markers indicate the likely response to treatment. KRAS is the only validated predictive molecular marker in colorectal cancer for epidermal growth factor receptor-directed monoclonal antibodies (261). The presence of high-frequency microsatellite instability would suggest minimal benefit from adjuvant fluorouracil treatment (262). Allelic imbalance at chromosome 18q might be a poor prognostic indicator with a negative survival effect in patients with stage III microsatellite-stable colorectal cancers (263). Other extensively researched potential prognostic and predictive markers include thymidylate synthase (the primary target of the active metabolite of fluorouracil), excision-repair cross-complementing-1 (implicated in response to platinum...
drugs), vascular endothelial growth factor (VEGF) and its receptors, and the interleukins (264).

1.6.3 Lung cancer

In 2009, there were 10,193 new cases of lung cancer diagnosed in Australia, accounting for nearly 9% of all new cancers. Lung cancer is the fourth most commonly diagnosed cancer for Australian men (after prostate, bowel and melanoma of the skin) and for Australian women (after breast, bowel and melanoma of the skin). It is the leading cause of cancer death, and an estimated 13,640 Australians are anticipated to be diagnosed with lung cancer by 2020 (171, 172).

Lung cancer is commonly classified into two major groups based on histology:

- Small-cell lung cancer (SCLC) which accounts for approximately 20% of all lung cancers, and
- Non-small-cell lung cancer (NSCLC) which accounts for 80%. This include squamous cell carcinoma (30% of all lung cancers), adenocarcinoma (30%), and large-cell carcinoma (10%). Bronchoalveolar carcinoma is generally sub-classified under adenocarcinoma (265).

1.6.3.1 Risk factors

Smoking

Numerous epidemiological studies have consistently identified smoking as a major risk factor for lung cancer (266, 267). Longitudinal studies have also established the causal role of cigarette smoking in lung cancer mortality (268). Tobacco smoke contains an estimated 4000 chemical compounds, including over 60 carcinogens that can precipitate cancer formation (269). In Australia, tobacco smoking is the most predominant cause of lung cancer, accounting for about 90% of lung cancers in males and 65% in females. Compared to never smokers, smokers have a more than 10-fold risk of developing lung cancer (270). The risk is further increased with younger age at initiation of smoking, greater number of cigarettes smoked, and longer duration of smoking (271). Second-hand smoke contains the
same carcinogens as those inhaled by smokers, and epidemiological studies have concluded that passive smoking also elevates the risk of lung cancer (272). Former smokers have lower risk than current smokers, and longer duration of abstinence is associated with greater reductions in risk (273).

Age

In Australia, the occurrence of lung cancer is strongly related to age, with 84% of new lung cancers in males and 80% in females diagnosed in those aged 60 years and over (270).

Gender

Women smoking the same amount as men are twice as likely to develop lung cancer (274, 275). Between 1982 and 2007, the age-standardised incidence rate of lung cancer decreased in Australian males by 32% but increased in Australian females by 72%. Likewise, the age-standardised mortality rate from lung cancer for the males decreased by 41% while the mortality rate for females increased by 56% during the same period. The different pattern of incidence and mortality rates may reflect historical differences in smoking behaviour amongst Australian men and women (270).

Family history

Individuals with a family history of lung cancer, particularly in a first-degree relative, are at higher risk of developing lung cancer, with early age at onset (40-59 years) (276). The risk is increased with more than one affected first-degree relative (277). The increased risk may be explained by common genetic profiles among family members and shared smoking behaviours.

Prior respiratory diseases

Lung cancer risk may be modified by a prior history of respiratory diseases such as asthma, emphysema, and hay fever. In a case-control study of 1553 cases and 1375 healthy controls, emphysema was associated with an increased risk of lung cancer [odds ratio (OR) 2.87, 95% CI 2.20-3.76] while hay fever had a significant protective effect (OR 0.58, 95% CI 0.48-0.70) (278). In a meta-analysis, asthma was a significant risk factor for lung cancer among the non-smokers with a pooled risk ratio of 1.9 (95% CI 1.4-2.5) (279).
Occupational exposures

Lung cancer may result from inhalation of a range of industrial and chemical carcinogens, including asbestos, radiation, diesel exhaust fumes and certain metals such as nickel, cadmium, chromium and nickel. These compounds are commonly found in industries like mining and quarry, asbestos production, metal industries, shipbuilding, railroad equipment manufacturing, gas production, and construction (280).

1.6.3.2 Biomarkers for lung cancer

The goal in lung cancer management is to detect the disease at the earliest stage when prognosis is the most favourable. Circulating biomarkers have the best potential to be a cost-effective method for early cancer diagnosis. Several blood-based lung cancer biomarkers have been identified, including proteins, protein panels, and antibodies to tumour-associated antigens. Ostroff et al studied a panel of 12 highly discriminatory proteins (including cadherin-1, CD30 ligand, and endostatin) using an aptamer-based assay and reported the ability to distinguish lung cancers from the healthy controls with high precision and accuracy (281). In another proteomic study, Patz et al reported that serum levels of carcinoembryonic antigen, α1-antitrypsin, and squamous cell carcinoma antigen have the potential to help guide the management of indeterminate pulmonary nodules that were 7-30 mm in size (282). Higgins et al identified a variant protein, Ciz1, which was found to be elevated in patients with lung cancer (283). A panel of antibodies against 6 different proteins that are known to be involved in various stages of the carcinogenesis pathway was tested in validation trials, and is now being marketed as the EarlyCDT-Lung test for early detection of lung cancer (284).

Molecular and genetic analyses are also being conducted. Ostrow et al identified DNA methylation in tumour suppressor genes and confirmed that there were differences in methylation status in lung cancer cases versus controls. These variations in methylation can be utilised to distinguish individuals with benign solid pulmonary nodules and ground-glass opacities from those patients with lung cancer (285). Boeri et al studied the utility of microRNAs which are mostly involved in cell proliferation, apoptosis, and differentiation. The authors concluded that plasma microRNA expression levels were useful in diagnosing early-stage lung cancer as well as prognosticating the disease (286). Finally, gene
expression profiling in peripheral blood mononuclear cells can potentially distinguish patients with NSCLC from non-cancer controls with high sensitivity and specificity. Comparative expression levels may also be useful to detect disease recurrence since their levels were significantly reduced after surgical resection of the cancerous lesion (287).
1.7 Homocysteine

The homocysteine (Hcy) theory first originated in 1962 when Carson and colleagues described an excessive amount of urinary Hcy in a group of patients with mental retardation (288). This was soon attributed to a disorder of methionine metabolism and referred to as homocystinuria. In 1964, pathological findings of homocystinuria concomitant with the description of widespread vascular changes and thrombosis were published (289). In the same year, Mudd and colleagues identified the absence or diminished activity of cystathionine β-synthase (CBS) as a possible cause of abnormal metabolism of the essential amino acid methionine, resulting in an elevated concentration of Hcy in the blood as well as homocystinuria (290). Hyperhomocysteinaemia (HHcy) was first suggested as the main mediator of the vascular changes in 1969, through findings from separate autopsies undertaken in an infant with homocystinuria who died at the age of seven weeks. Mudd and colleagues discovered markedly elevated Hcy in the infant’s urine and plasma, but unlike those findings in CBS deficiency, methionine was extremely low or undetectable. This was then established to be a consequence of abnormal metabolism of vitamin B12 affecting the re-methylation of Hcy to methionine (291). The common denominator in these two disorders was the elevated Hcy in the blood. When McCully performed an autopsy on this infant, he documented widespread arteriosclerotic changes and thrombosis (292). Based on these autopsy and further experimental evidences, the ‘Hcy hypothesis’ was later formulated, proposing that HHcy might be a risk factor for atherosclerosis in the general population (293). In 1976, Wilcken and colleagues first tested this hypothesis and demonstrated that patients with coronary artery disease were more likely to have HHcy than the control patients (294).

1.7.1 Homocysteine metabolism

Hcy is a nonprotein amino acid derived from the hepatic metabolism of the essential sulphur-containing amino acid, methionine. It is a substrate for three competing metabolic pathways (295, 296) (Figure 1.5).
The methionine cycle

Dietary methionine is converted to S-adenosylmethionine (SAM) by methionine adenosyltransferase. SAM is then used by a host of methyl-transferases as a methyl donor, yielding S-adenosylhomocysteine (SAH) as a by-product. SAH, in turn, undergoes hydrolysis by SAH hydrolase to form Hcy. The methionine cycle is completed with the remethylation of Hcy back to methionine by B12-dependent methionine synthase, using 5-methyltetrahydrofolate as substrate. In an alternative remethylation route, betaine is used as the methyl donor by the enzyme betaine-homocysteine methyltransferase.

The folate cycle

In this pathway, Hcy is converted back to methionine by methionine synthase. The enzyme uses 5-methyltetrahydrofolate as the methyl-donor, and methylcobalamin (a biologically active form of vitamin B12) as catalyst. The tetrahydrofolate (THF) generated from this process reacts with serine to form 5,10-methylenetetrahydrofolate. The folate cycle is completed with the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate by B2-dependent methylenetetrahydrofolate reductase (MTHFR).

Transsulfuration of Hcy

When the remethylation is saturated, or when cysteine is required, Hcy formed in the methionine cycle enters the transsulfuration pathway where it is irreversibly converted to cysteine. This is initiated by B6-dependent CBS which catalyses the conversion of Hcy to cystathionine. The latter is then converted to cysteine by B6-dependent cystathionine γ-lase. This process is mainly limited to cells of the liver and kidneys.
In plasma, approximately 99% of Hcy is oxidised to disulfides. The vast majority is bound to proteins. Only 1% of all Hcy moieties is reduced ‘free’ Hcy. The term “total plasma Hcy” (tHcy) refers to the combined pool of all forms of Hcy in plasma (297). Among fasting individuals, tHcy > 15 µmol/l is arbitrarily classified as moderate (16-30 µmol/l), intermediate (31-100 µmol/l) and severe (> 100 µmol/l) HHcy (298).

1.7.2 Determinants of plasma homocysteine

Age and sex

Increasing age and male sex are associated with higher tHcy concentrations (299, 300). The age-dependent increase may be partly attributed to deteriorating renal function or reduced folate status due to poor eating (301, 302). The sex-related differences may be explained by the effects of sex hormones during menopause in women, or larger muscle mass in men with higher Hcy production linked to creatine-creatinine synthesis (299, 303).
Nutrition and supplements

In older people, folate and vitamin B12 levels are inversely correlated with Hcy concentrations (304). Insufficient intake of these vitamin co-factors required for Hcy metabolism may lead to HHcy. The Hordaland Hcy Study assessed nutrient intake in 12,000 participants through questionnaires and demonstrated that folate intake was a strong negative determinant of Hcy concentrations (305). The Framingham Offspring Study further investigated the relationship between Hcy and nutrient intake by using a validated food-frequency questionnaire. They found that dietary intake of B-vitamins and regular use of vitamin B supplements were inversely associated with Hcy concentrations (306). Selhub and colleagues also examined the same cohort and concluded that both intake and plasma levels of folate, vitamin B12 and vitamin B6 were primary determinants of tHcy in older adults, accounting for 67% of HHcy cases (31).

Folic acid supplementation is more effective in reducing Hcy levels than dietary folate (307). A meta-analysis of 12 intervention studies demonstrated that folic acid supplementation reduced tHcy concentrations by 25%, with similar effects in daily doses of 0.5-5 mg/day. The proportional and absolute reductions in tHcy were greater at higher pre-treatment Hcy and lower pre-treatment folate concentrations. Vitamin B12 in an average dose of 0.5 mg/day produced an additional 7% reduction in tHcy. Vitamin B6 did not have an additional effect on tHcy concentration (308). Flicker et al confirmed similar beneficial effects of B-vitamin supplementation in older men, and further demonstrated that the Hcy-lowering effect was maximal for those who had lower pre-treatment vitamin B12 concentrations (309).

Lifestyle

Smoking is positively associated with tHcy concentration (310). In particular, the Hordaland Hcy Study demonstrated a strong dose-response relationship between the number of cigarettes and tHcy levels, independent of age, sex and folate intake (305, 311).

In the same study as well as in others, heavy coffee consumption was found to be one of the strongest lifestyle determinants of Hcy in a dose-response relationship (305, 312, 313). Verheoef and colleagues investigated the cause and found that caffeine is only partly
responsible for the Hcy-increasing effect of coffee (up to 25-50%). They concluded that compounds in coffee other than caffeine might be playing an additional role (314).

The Hordaland Hcy Study demonstrated a weak inverse association between physical activity and tHcy concentration, albeit ambiguous findings in other studies (311, 315, 316). This is likely a consequence of residual confounding as physical activity is generally associated with a healthier lifestyle which can reduce tHcy concentration.

Intervention trials investigating the effect of alcohol on tHcy concentration have also yielded inconsistent findings. In a randomised, diet-controlled cross-over trial, tHcy concentration was increased after moderate consumption of red wine and spirits relative to water consumption. However, no significant effect was seen after moderate consumption of beer. This difference in effect was attributed to the relatively higher content of vitamin B6 in beer (317). In another non-randomised study, tHcy concentrations were elevated after six weeks of 30 g/day alcoholic consumption compared to those who were assigned mineral water consumption, independent of the type of alcohol (318). In an observational study, Mayer and colleagues demonstrated that beer intake was positively associated with folate and vitamin B12 concentrations, and negatively associated with tHcy concentration (319).

**Diseases and drugs**

Several diseases and drugs can influence tHcy concentrations via various mechanisms, including the inhibition of B-vitamin function, disulphide-exchange reactions, increased/decreased Hcy production, interference with renal function, and influence on hormonal status.
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<th>Diseases</th>
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<td>Renal dysfunction</td>
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<td>Cancer</td>
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<td>Psoriasis</td>
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<td>Rheumatoid arthritis</td>
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<td>Advanced insulin-dependent diabetes mellitus</td>
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<td>Inflammatory bowel disease</td>
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<td>Coeliac disease</td>
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<th>Drugs</th>
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<td>Folate antagonists (eg methotrexate, anti-convulsants, cholestyramine)</td>
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<td>Cobalamin antagonists (eg nitrous oxide, metformin)</td>
<td>Increase</td>
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<td>Vitamin B6 antagonists (eg theophylline, niacin)</td>
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<td>Sex steroids</td>
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<td>Contraceptives</td>
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<td>Estrogens (postmenopausal)</td>
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<td>Androgens</td>
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<td>Others</td>
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<td>Cyclosporin A</td>
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<td>Betaine</td>
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1.7.3 Genetics – MTHFR polymorphisms

Genetic defects that affect Hcy metabolism include mutations in gene encoding CBS, MTHFR and methionine synthase. This section focuses on an overview of the genetic mutations in MTHFR.

MTHFR is an important regulatory enzyme in the folate and Hcy metabolism that catalyses the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. By fluorescence in situ hybridisation (FISH), the human MTHFR gene has been localised to chromosomal region 1p36.3 (320). At present, a total of 34 rare but deleterious mutations in MTHFR, as well as 9 common polymorphisms have been identified. The 677 C→T variant is well-recognised as the commonest genetic cause of HHcy, and this involves the conversion of an alanine to a valine codon which subsequently encodes a thermolabile variant (321). MTHFR thermolability is inherited recessively and is present in approximately 5% of the general population. The frequency varies in populations from different geographic areas, with the highest reported so far in the Mediterranean countries and in Hispanic Americans (322, 323).

The homozygous TT genotype (677TT) is associated with reduced activity and increased thermolability of the enzyme (321). In 677TT individuals, the specific activity at 37°C is between 25% and 35% of control values, while heterozygous (677CT) individuals have values which are intermediate between controls and 677TT. The 677 C→T polymorphism is associated with mild to moderate HHcy. On average, 677TT individuals may have approximately 3.5 µmol/l or 35% higher tHcy concentrations than the wild type homozygotes (677CC) (324, 325). The influence of the 677 C→T mutation on tHcy concentrations has been shown to be predominantly dependent on the plasma folate status, as demonstrated in the NHLBI Family Heart Study cohort. Among individuals with lower plasma folate concentrations (< 15.4 nmol/l) and had the 677TT genotype, tHcy was 24% higher than those individuals with the CC genotype. For those individuals with higher folate levels ≥ 15.4 nmol/l, there was no difference in tHcy concentrations between the genotypes. These findings suggest that folate supplementation may be able to overcome the effect of the mutation and prevent HHcy in individuals with the thermolabile MTHFR (325).
Of all reported variants in MTHFR, only the 677 and 1298 substitutions have been expressed and confirmed to affect enzyme activity. The functional impact, if any, of other polymorphisms remains undetermined. The 1298 A→C is characterised by a point mutation in exon 7 which results in a glutamate to alanine substitution. Although the activity of the encoded enzyme is reduced (to 68% of the wild type enzyme), the mutated enzyme is not thermolabile and homozygotes do not have HHcy or reduced plasma folate concentration. On the other hand, individuals who are compound heterozygotes for the 1298C and 677T alleles have a biochemical profile resembling that of 677TT homozygotes, with resultant HHcy (326, 327).

### 1.7.4 HHcy and toxicity

HHcy is linked to increased athero-thrombotic events, resulting in occlusive vascular disease. The vascular pathology observed in patients with homocystinuria consists mainly of fibrous arteriosclerotic plaques with minimal lipid deposition. These plaques cause significant narrowing and thrombotic occlusions of major arteries and arterioles throughout the body, and typically occur within the first two decades in individuals with homozygous homocystinuria. In cases of moderate HHcy not related to enzyme deficiency, the development of complex atheromas occurs at a slower pace allowing opportunity for lipid deposition and more prominent degenerative changes. The observation of accelerated atherogenesis regardless of the metabolic origin of HHcy is highly supportive of Hcy toxicity as the predominant mechanism of vascular lesions (295).

**Homocysteinylation**

This process involves the covalent binding of Hcy to proteins, which then modifies the functions of both components. Targeted proteins include fibronectin, transthyretin, albumin, haemoglobin, immunoglobulins, transferrin, antitrypsin, and fibrinogen. Mechanisms of function modification are mainly via the inactivation of potentially active free thiol groups, introduction of new free thiol groups, and alteration of redox potential of the protein molecule. In addition, the modified proteins can act as neoantigens, triggering activation of the inflammatory response. These neoantigens may also induce an
autoimmune response, resulting in neoantigen-antibody interaction leading to activation of circulating macrophages, with consequent vascular endothelium damage (328).

**Endoplasmic reticulum (ER) stress**

The ER is very sensitive to intracellular Hcy or its metabolites. When intracellular Hcy is increased, the Hcy reacts with ER proteins, causing a local redox imbalance. This leads to misfolding of secretory and membrane proteins, initiating an ER stress response. As a result, dysregulation of lipid metabolism, activation of inflammatory pathways, impaired insulin signalling, and apoptotic cell death are triggered, all of which are key events in the development and progression of atherosclerosis (329-331).

**Inflammation**

In vitro studies have demonstrated Hcy-related upregulation and induction of several pro-inflammatory cytokines in cultured human endothelial cells. These include monocyte chemo-attractant protein I, and interleukin 8 which is a T-lymphocyte and neutrophil chemo-attractant (332). Their pro-inflammatory effects are mediated via Hcy-induced activation of the transcription factor nuclear factor κB (NF-κB) which induces the expression of genes coding for these cytokines (333). This process is substantiated by evidence of the in vivo activation of NF-κB and downstream inflammatory marker expression in atherosclerotic lesions of apoE-null mice with HHcy, hence supporting the concept that Hcy contributes to the development of atherosclerosis by causing vascular inflammation (334).

**Oxidative stress**

HHcy has been shown to promote endothelial dysfunction and impair endothelium-dependent vasodilation (335). One proposed mechanism of Hcy-induced oxidative stress causing cellular dysfunction is via the generation of reactive oxygen species in endothelial cells by auto-oxidation of the thiol group of Hcy (336). Another mechanism is Hcy-dependent activation of NADPH oxidase and xanthine oxidase, two pro-oxidant enzymes which can enhance the intracellular production of superoxide anion (O$_2^-$) in endothelial cells (337, 338). O$_2^-$ reacts with and reduces the availability of endothelial nitric oxide (NO) to yield peroxynitrite, a process which can result in alteration of the vascular tonus (339).
Peroxynitrite also induces thromboxane A$_2$ synthesis in both endothelial cells and platelets, leading to vasoconstriction (338). Further supportive evidence of an oxidative mechanism is suggested from studies of mice with altered cellular expression of the anti-oxidant enzyme, glutathione peroxidase-1 (Gpx-1). Mice with Gpx-1 deficiency were found to be more susceptible to endothelial dysfunction in the absence of HHcy. When HHcy was induced in these mice, endothelial dysfunction was exacerbated, implying that Hcy may play a sensitising role in conditions susceptible to a peroxide-dependent oxidative event (340). Finally, HHcy has been associated with elevated levels of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NO synthases. ADMA inhibits vascular NO production and its concentrations are correlated with endothelial dysfunction, atherosclerosis and ischaemic stroke (341-343). As such, it has emerged as a potential marker of cardiovascular risk in several observational studies (344).

1.7.5 Hcy and age-related problems

Vascular diseases

HHcy is an independent risk factor for CAD, cerebrovascular disease and PAD (345, 346). In the Zutphen Elderly Study which comprised of a population-based cohort of 878 older men (aged 64-84 years), HHcy was associated with an increased prevalence of myocardial infarction (OR 1.81, 95% CI 1.07-3.08) and stroke (OR 4.61, 95% CI 1.79-11.89) (347). Cross-sectional studies also demonstrated an increased association of PAD with elevated Hcy concentrations in older men and women (348, 349). Prospective studies further suggested that HHcy is predictive of CAD and cerebrovascular disease in older people (46, 350, 351). In addition, HHcy increases the risk of cardiovascular hospitalisation, and both cardiovascular and all-cause mortality in older adults (352-354).

Cognitive impairment and dementia

Hcy has been proposed to contribute to the pathogenesis of neurodegenerative disorders. In observational studies, patients with AD have significantly higher Hcy levels than the controls (355, 356). More convincing evidence for the association was reported by Clarke and colleagues in a case-control study of 164 patients with clinically diagnosed AD,
including 74 patients with histologically confirmed AD. The researchers discovered that patients with tHcy ≥ 14 µmol/l were 4.5 times more likely to have confirmed AD compared to those with tHcy ≤ 11 µmol/l (OR 4.5, 95% CI 2.2-9.2), adjusting for age, gender, social class, cigarette smoking and apoE-ɛ4. After three years of follow-up, radiological evidence of disease progression was noted to be greater among those with higher tHcy levels at baseline (357). Further evidence of a causative association between Hcy and AD was demonstrated in the Framingham Study involving 1092 older adults. The risk of developing AD after 8 years almost doubled in individuals with baseline tHcy concentrations > 14 µmol/l relative to those with lower tHcy concentrations (358). Similarly, in a larger longitudinal study, 4227 men aged 70 years and over from the HIMS cohort were followed up for 6 years. The hazard of dementia increases in men with higher tHcy (> 15 µmol/l). The study was, however, under-powered to investigate the effect of MTHFR genotype on dementia (359).

Several mechanisms for HHcy-related cognitive impairment have been postulated. In animal studies, Hcy impairs DNA repair and induces apoptosis in hippocampal neurons, thereby increasing their vulnerability to excitotoxic and oxidative injuries. Hcy can also be directly toxic to cultured human and murine neuronal cells (360, 361). Hcy stimulates NMDA receptors, resulting in calcium influx and excitotoxicity, which can also be enhanced by Hcy-related potentiation of glutamate neurotoxicity (362). Hcy sensitises neurons to β-amyloid toxicity and is linked to tau hyperphosphorylation (363, 364). Anatomically, HHcy has been associated with cerebral cortical and hippocampal atrophy (365, 366).

**Depression**

The association between Hcy and depression in older people has not been consistently reported in observational studies. The population-based Rotterdam Study screened 3884 older adults with a mean age of 73 years and found a weak association between elevated Hcy levels and depression after adjustment for age, gender, CVD and functional disability (367). In another population-based cohort of 4338 older people (aged 70-74 years) recruited in the Hordaland Hcy Study, tHcy (OR 1.90, 95% CI 1.11-3.25) and MTHFR TT genotype (OR 1.69, 95% CI 1.09-2.62) were significantly associated with depression after adjustment for age and gender (368). Almeida and colleagues investigated the association in 3752
community-based older men aged 70 years and over. In the HIMS, the odds of having depression is increased by 4% per 1-µmol/l increase in tHcy (OR 1.04, 95% CI 1.02-1.05) (369). The authors also performed a meta-analysis of nine cross-sectional studies including HIMS and reported a positive association between elevated tHcy and depression (OR 1.70, 95% CI 1.38-2.08). In another meta-analysis of five studies, the odds of ever having depression is increased among individuals with the homozygote TT variant in comparison to those with the CC genotype (OR 1.22, 95% CI 1.01-1.47) (369).

**Physical function**

Several studies have explored the association between Hcy and physical function in older people. Cross-sectional analyses have linked poorer gait, balance and quadriceps strength to higher tHcy concentrations (370-372). In prospective cohort studies, HHcy has also been shown to be predictive of worsening mobility and physical performance in older adults (373, 374). In the MacArthur Studies of Successful Aging which studied 499 highly functioning individuals aged 70-79 years prospectively, physical performance measures of balance, gait, lower body strength and coordination, and manual dexterity were performed. With each standard deviation increment in tHcy, there was an increased risk of being in the worst quartile of decline in these physical performance measures after 28 months (adjusted OR 1.5, 95% CI 1.2-1.9) (374).

**Osteoporotic fracture**

HHcy has been proposed as a potential risk factor for osteoporosis based on the observation that patients with autosomal recessive homocystinuria have an increased prevalence of early-onset generalised osteoporosis (375). Further supportive evidence includes a more recent longitudinal study in the Netherlands involving 2406 men and women aged 55 years and over. The adjusted RR of an osteoporotic fracture is 1.4 (95% CI 1.2-1.6) for each standard deviation increase in the log-transformed tHcy (376). In the Framingham study cohort of 1999 older adults, men and women with the highest quartile of tHcy have a higher risk of hip fractures compared to the lowest quartile [risk ratios 3.84 (95% CI 1.38-10.70) and 1.92 (95% CI 1.18-3.10), respectively] (377). The mechanism was postulated to be due to Hcy interfering with the formation of intermolecular cross-links that help stabilise the collagen macromolecular network (378, 379).
1.8 Vitamin D

Low vitamin D status is prevalent in Australia and New Zealand. An estimated 30% of Australians have inadequate vitamin D status, with higher proportions in women during winter and in residents at the southern states (380-382). Other groups at greater risk of vitamin D deficiency include housebound and institutionalised older people, dark-skinned people (particularly those who are modestly dressed due to religious or cultural practices), those who regularly avoid sun exposure, and possibly people working in enclosed environment (380, 383-386).

1.8.1 Calcium and vitamin D metabolism

Vitamin D in humans may be derived from sunlight exposure, diet and supplements. Exposure to sunlight is the major determinant of vitamin D status, and efficiency of vitamin D synthesis in the skin is dependent on the amount of ultraviolet B (UVB) photons that penetrate into the epidermis. Increased skin melanin pigmentation and topical application of sunscreen can reduce vitamin D production by > 90%, as both efficiently absorb UVB photons (387, 388). Diet contributes 5-10% of the vitamin D requirements. Dietary sources of vitamin D include oily fish such as salmon and tuna, fortified dairy products, egg yolk and mushroom (389).

As shown in Figure 1.6, solar UVB radiation penetrates the skin and converts 7-dehydrocholesterol to pre-vitamin D₃, which is then rapidly converted to vitamin D₃. This is metabolised in the liver to 25(OH)D, which is the main circulating form of vitamin D and hence used to determine an individual’s vitamin D status. 25(OH)D is metabolised in the kidneys by the enzyme 25-hydroxyvitamin D-1α-hydroxylase to its biologically active form 1,25(OH)D₃. The renal production of 1,25(OH)D₃ is tightly regulated by plasma PTH, calcium and phosphorus levels. 1,25(OH)D₃ interacts with its nuclear receptor, vitamin D receptor (VDR), in the small intestinal cells and increases the absorption of calcium, which facilitates bone mineralisation and optimises muscle function. When dietary calcium is inadequate, vitamin D helps maintain calcium homeostasis by interacting with the VDR in bone osteoblasts to induce the expression of the plasma membrane protein, receptor activator of NF-κB ligand (RANKL). This binds to the RANK on the pre-osteoblasts and
leads to the formation of a mature osteoclast. Bone resorption results with the release of calcium and phosphorus into the circulation. Another function of 1,25(OH)D3 is the induced expression of enzyme 25-hydroxyvitamin D-24-hydroxylase, which catabolises both 25(OH)D and 1,25(OH)D3 into biologically inactive, water-soluble calcitroic acid (32, 389-392).

If the level of 1,25(OH)D3 is decreased, there is reduction in intestinal calcium absorption. This is immediately recognised by the calcium sensor in the parathyroid glands, resulting in increased expression, synthesis, and secretion of PTH. PTH enhances tubular absorption of calcium and stimulates the kidneys to produce more 1,25(OH)D3. PTH also enhances the expression of RANKL on osteoblasts to stimulate the formation of mature osteoclasts, which mobilise calcium stores from the bones (393, 394).

Although there is no consensus on optimal levels of 25(OH)D, vitamin D status can be defined as follows (395, 396):

- Vitamin D adequacy: 25(OH)D ≥ 50 nmol/l at the end of winter (≥ 60 nmol/l at the end of summer);
- Vitamin D insufficiency: 25-49 nmol/l;
- Vitamin D deficiency: < 25 nmol/l.
1.8.2 Osteoporosis and fractures

There is evidence that optimal mineral metabolism and bone density occurs at serum 25(OH)D concentrations of 50-60 nmol/l, which is achievable with vitamin D supplementation of 800 IU/day (395). Bischoff-Ferrari and colleagues recently undertook a pooled analysis of data involving 31,022 participants (mean age 76 years) from 11 double-
blind RCTs of oral vitamin D supplementation. The conclusion is that higher-dose vitamin D supplementation of ≥ 800 IU/day may be most beneficial in reducing fracture risk in people ≥ 65 years of age – with a 30% reduction in the risk of hip fracture (RR 0.70, 95% CI 0.58-0.86) and a 14% reduction in the risk of non-vertebral fracture (RR 0.86, 95% CI 0.76-0.96), as compared with those taking placebo or calcium alone. The data also supports a 25(OH)D concentration of > 60 nmol/l for maximal prevention of fractures (397). In the context of combined therapy with vitamin D and calcium, 25(OH)D status plays a more dominant role for the maintenance of bone health (397, 398). Sanders et al explored the effect of annual oral administration of high-dose vitamin D on the risk of falls and fractures. In this double-blind, placebo-controlled trial of over 2000 older community-dwelling women (aged 70 years and older), annual oral administration of 500,000 IU of cholecalciferol resulted in 25(OH)D levels between 90 and 120 nmol/l within 12 months, and a paradoxical increased risk of falls and fractures (399). A similar study by Smith et al yielded concordant findings (400). On the other hand, a trial of four-monthly oral administration of 100,000 IU cholecalciferol was associated with a 22% reduction (RR 0.78, 95% CI 0.61-0.99) in the risk of any first fracture, and 33% reduction (RR 0.67, 95% CI 0.48-0.93) for first hip, wrist or forearm, or vertebral fracture (401). All these findings, together with those concluded in the pooled analysis, are supportive of more frequent dosing (daily or weekly) as an effective means of reducing fracture risk.

1.8.3 Neuromuscular function and falls

Myopathy is a prominent feature of severe vitamin D deficiency and may manifest as proximal muscle weakness, diffuse muscle pain, and gait impairments such as having a waddling gait (402-404). This is likely mediated by the presence of VDR in skeletal muscles which allows the binding of the active metabolite, 1,25(OH)D3 (405). De novo protein synthesis occurs with resultant muscle cell growth (403, 406). Individuals with myopathy are plausibly more susceptible to fractures through an increased incidence of falls (403, 404, 406-408).

Cross-sectional studies have implicated low vitamin D status with improved physical performance measures and reduced risk of falls. In a Norwegian cohort study, higher
25(OH)D concentrations are correlated with better upper limb muscle strength, ability to climb stairs, physical activity, and absence of fall occurrences (409). Among the participants of the Longitudinal Aging Study Amsterdam, those with 25(OH)D levels < 25 nmol/l were more than twice likely to experience sarcopenia (defined in the study as a loss of grip strength > 40% or a loss of muscle mass > 3%), compared to those with levels > 50 nmol/l (404). Using the same study cohort, low 25(OH)D concentrations < 50 nmol/l were associated with poorer lower extremity physical performance as well as a greater decline over time, compared with participants with higher 25(OH)D levels of > 75 nmol/l (410). Other studies are also supportive of these findings (411, 412). Based on the data for lower extremity function, a desirable threshold for optimal 25(OH)D levels is suggested to be ≥ 40 nmol/l, with greatest improvement in function achievable from very low 25(OH)D concentrations of up to 50 nmol/l, and less pronounced but continuous improvement at concentrations > 50 nmol/l.

These epidemiological studies are further supported by RCTs of vitamin D supplementation. In a cohort of 148 German women (mean age 74 years) with 25(OH)D levels < 50 nmol/l, vitamin D and calcium combined therapy resulted in a 72% increase in 25(OH)D, a 9% reduction in body sway, and reduced number of falls, relative to those administered calcium monotherapy (413). Combined evidence from eight RCTs (n=2426) revealed that higher dose supplemental vitamin D (700-1000 IU/day) reduces the risk of falling by 19% (pooled RR 0.81, 95% CI 0.71-0.92; n=1921 from seven trials) compared to lower dose supplementation (< 700 IU/day). Falls were not reduced by low-dose supplemental vitamin D (pooled RR 1.10, 95% CI 0.89-1.35 from two trials), or by achieved serum 25(OH)D concentrations < 60 nmol/l (414). Whilst the evidence of vitamin D on falls prevention remains heterogeneous, the latest recommendation by the Institute of Medicine (United States) is to maintain a minimum serum 25(OH)D level of 50 nmol/l, which can be attained by administration of 800 IU/day of vitamin D (415).

1.8.4 Extra-skeletal actions of vitamin D

The effects of vitamin D are not only limited to calcium metabolism and the maintenance of bone health. Mounting epidemiological evidence suggests that vitamin D may also be
implicated in other non-skeletal biological processes, due to the presence of VDR in other tissues and organs (32, 416, 417). The VDR belongs to a superfamily of steroid hormone nuclear receptors and binds 1,25(OH)D3 with high affinity, mediating transcriptional gene regulation (32, 417-419). Genetic variants of the gene encoding the VDR have also been implicated in the associated risk of vitamin D with various illnesses such as cancers and immune disorders (420, 421). Target cells and organs which have been identified to possess this nuclear receptor include the heart, pancreas, brain, skin, gastrointestinal tract, gonads, and the immune cells (32, 416). Some of these cells and tissues also express the enzyme 25-hydroxyvitamin D-1α-hydroxylase, indicating potential paracrine or autocrine functions for 1,25(OH)D3 in the control of cell proliferation and differentiation (390, 422).

In addition, 1,25(OH)D3 and the VDR ligands mediate intracellular signalling via non-genomic pathways (423, 424). Both components have been demonstrated to exhibit potent anti-proliferative, pro-differentiative, and immuno-modulatory activities (via both genomic and non-genomic mechanisms) in several clinical and experimental settings (425). The non-calcemic actions of vitamin D analogues have indicated potential therapeutic applications of the VDR ligands in many pathologic conditions, including inflammatory diseases (rheumatoid arthritis, Crohn’s disease), dermatological conditions (psoriasis), cancers (prostate, colon, breast, skin), and autoimmune diseases (type 1 diabetes, multiple sclerosis) (389, 426-430).

**Vitamin D and CVD**

Ecological studies report an increased prevalence of cardiovascular events during winter months and with increasing distance from the equator. This has been attributed to low vitamin D status due to reduced exposure to UVB radiation in these settings (431, 432). Subsequent epidemiological studies revealed associations of hypovitaminosis D with obesity, diabetes mellitus, dyslipidaemia, endothelial dysfunction, hypertension, CVD, and cerebrovascular disease (433-436). Potential mechanisms include activation of the renin-angiotensin system, increased systemic and vascular inflammation, and adverse cardiovascular modelling (437).

RCTs of vitamin D supplementation on cardiovascular outcomes were, however, heterogeneous. A meta-analysis of 10 RCTs of vitamin D (400-8571 IU/day) ± calcium on
blood pressure in 37,162 participants revealed no significant reduction in systolic blood pressure (weighted mean difference -1.9 mmHg, 95% CI -4.2 to 0.4 mmHg) or diastolic blood pressure (weighted mean difference -0.1 mmHg, 95% CI -0.7 to 0.5 mmHg) (434). Lower 25(OH)D concentration was associated with incident CVD in 5 of 7 analyses (6 cohorts), but 4 trials found no effect of supplementation on cardiovascular outcomes (434). A recent meta-analysis of 7 prospective studies in 1214 individuals reported that low 25(OH)D levels were associated with an increased risk of stroke in comparison to higher levels (pooled RR 1.52, 95% CI 1.20-1.85) (436). On the other hand, a broader systematic review involving 51 RCTs of moderate quality that were published before August 2010 reported that vitamin D supplementation was not significantly associated with any effect on stroke, myocardial infarction, or death (438). There was also no significant association with surrogate outcomes of lipid fractions, glucose and blood pressure (438). These data are supported by a more recently published Randomised Evaluation of Calcium Or vitamin D trial comprising 5292 adults aged ≥ 70 years, in which there was no reported significant difference in cerebrovascular disease mortality, vascular mortality, or all-cause mortality amongst participants allocated vitamin D supplementation, compared to those not allocated vitamin D (439).

Vitamin D and cancer

Residents at higher latitudes are at increased risk for Hodgkin’s lymphoma as well as colon, pancreatic, prostate, ovarian, and breast cancers. They are also more likely to die from these cancers compared to those at lower latitudes (440-443). Further epidemiological studies later indicate that levels of 25(OH)D < 50 nmol/l are associated with a 30-50% increased risk of incident colon, prostate, and breast cancer, along with higher cancer-related mortality (442, 444-447). Several in vitro studies have also shown that certain cancer cells can be responsive to the anti-proliferative effects of 1,25(OH)D3 (32, 394, 416, 418, 426, 448, 449).

There is a biological plausibility to explain the relationship between vitamin D and the reduced risk of cancers. It has been demonstrated that colon, prostate, breast and other tissues express 25-hydroxyvitamin D-1α-hydroxylase which helps produce 1,25(OH)D3 locally to control cellular proliferation and differentiation, thus keeping cancer at bay (390, 425, 441, 444). In malignant cells, 1,25(OH)D3 can induce apoptosis and modulate
angiogenesis, thereby reducing the ability of the cells to survive or procreate (428, 450). Once 1,25(OH)D3 completes its mission, it initiates its own self-destruction by stimulating the CYP24 gene to catabolise it into the biologically inactive calcitroic acid (32, 390).

The relationship between vitamin D and skin cancer is complicating as they share a common key factor, UVB radiation. The same spectrum of UVB radiation that is responsible for cutaneous vitamin D synthesis also causes DNA damage that can precipitate epidermal malignancies (451). Laboratory studies suggest that vitamin D and its metabolites may reduce the risk of skin cancer by inhibiting the hedgehog signalling pathway which underlies the development of basal cell carcinoma, as well as stimulating tyrosinase activity and melanocyte maturation (452, 453). VDR-depleted normal and malignant skin cells exhibit hyper-proliferative activity and decreased apoptosis (454, 455).

In humans, observational studies on vitamin D status have been conflicting, with some reporting an association between higher vitamin D levels and increased skin cancer risk, some showing reduced risk, and others showing null association (456-459). There is also limited conclusion on the efficacy of vitamin D or its metabolites on skin cancer prevention or treatment (460-462). Residual confounding is prominent in these epidemiological studies and additional research in humans are required to better define the true relationship between vitamin D and cutaneous carcinogenesis risk.

Vitamin D and autoimmune diseases

Several epidemiological studies have revealed a similar latitudinal risk gradient for multiple sclerosis (463-466). Residents who had lived below 35 degrees latitude during the first 10 years of their lives have an approximately 50% reduction in the risk of multiple sclerosis (463, 464). Among Caucasian men and women in the United States, the risk of multiple sclerosis is significantly decreased by 41% for every 50 nmol/l increment in 25(OH)D concentration (467). In another prospective cohort study, women who had ingested ≥ 400 IU of vitamin D supplement per day had a 41% lesser risk of developing the condition, compared to those with no supplemental intake (468).

Increased cytokine production and lymphocyte proliferation have been implicated in the destruction of insulin-secreting beta-cells within the pancreas, leading to the development of type 1 diabetes mellitus. 1,25(OH)D3 acts as an immuno-modulator, offering protection...
against this illness (469). In addition, beta-islet cells also express VDR and respond to 1,25(OH)D3 by increasing insulin production (469-471). Epidemiological data have suggested that vitamin D supplementation in pregnant mothers and children reduces the risk of type 1 diabetes. In a Finnish birth-cohort study, vitamin D supplementation in pregnant mothers was associated with a reduced risk of type 1 diabetes when adjusted for neonatal, anthropometric and social characteristics (RR for regular versus no supplementation 0.12, 95% CI 0.03-0.51) (472). For the 10,366 children in the same study who had received 2000 IU of vitamin D3 per day during their first year of life, the risk of type 1 diabetes after 31 years of follow-up was reduced by approximately 80% (RR 0.22, 95% CI 0.05-0.89) (472). Hypovitaminosis D is associated with increased insulin resistance, decreased insulin production, and an increased risk of metabolic syndrome (473). The role of vitamin D in type 2 diabetes mellitus was also investigated, with a prospective study concluding that a combined daily intake of 1200 mg of calcium and 800 IU of vitamin D lowered the risk of type 2 diabetes by 33% (RR 0.67, 95% CI 0.49-0.90), in comparison to a combined daily intake of < 600 mg of calcium and < 400 IU of vitamin D (474).

Vitamin D is implicated in many other autoimmune diseases, including rheumatoid arthritis, inflammatory bowel disease, systemic lupus erythematosus, and psoriasis (475-478). One of the greatest achievements in vitamin D research is its therapeutic benefit in the treatment of psoriasis (479). Over the last few decades, it has convincingly been demonstrated that vitamin D compounds are effective and safe in the topical treatment of psoriasis. These vitamin D analogues, which include calcitriol, calcipotriol, tacalcitol and maxacalcitol, are nowadays representative of a standard therapy for this condition (480-482). The efficacy and safety of oral calcitriol as a potential therapy of psoriasis is also proven in a long-term follow-up trial (483).
1.9 Thesis hypotheses and aims

The aim of this thesis is to explore the relationship between two biomarkers, namely Hcy and vitamin D, with various age-related health outcomes, in a cohort of community-dwelling older men. The specific aims and hypotheses of each chapter are highlighted below.

Chapter 3

Aim: To determine whether tHcy is associated with AAA and aortic diameter.

Hypotheses: Elevated tHcy is associated with the presence of AAA and there is a positive dose-response relationship with abdominal aortic diameter.

Chapter 4

Aim: To determine whether vitamin D is associated with AAA and aortic diameter.

Hypotheses: Hypovitaminosis D is associated with the presence of AAA and there is an inverse relationship with AAA diameter.

Chapter 5

Aim: To determine whether tHcy is associated with frailty and mortality.

Hypotheses: Elevated tHcy is associated with increased frailty. It is also predictive of all-cause mortality, independent of frailty.

Chapter 6

Aims: To determine whether vitamin D is associated with frailty and mortality.

~ 60 ~
Hypotheses: Hypovitaminosis D is associated with increased frailty. It also predicts all-cause mortality, independent of frailty.

Chapter 7

Aims: To determine whether tHcy is associated with physical HRQOL.

Hypotheses: HHcy is associated with poorer physical HRQOL scores on the SF-36 health survey.

Chapter 8

Aims: To determine whether vitamin D is associated with incident prostate, colorectal and lung cancers.

Hypotheses: Hypovitaminosis D is associated with increased incidence of prostate, colorectal and lung cancer.
CHAPTER 2

STUDY POPULATION AND METHODOLOGY
CHAPTER 2: STUDY POPULATION AND METHODOLOGY

2.1 Study population

The HIMS originated from a randomised trial of ultrasound screening for AAAs to explore whether early detection and surveillance of this condition would reduce mortality (484). This population-based cohort study was conducted in Perth, Western Australia in 1996-99. It recruited mainly older men aged 65 years and over as AAAs are more common in males with a higher prevalence in the older age group. Since the commencement of this trial, HIMS has expanded beyond the research of AAA to include studies of other age-related health outcomes in the older men.

All participants were identified from the electoral roll via the Western Australian Data Linkage System (WADLS), which provides electronic record linkage to the state’s population health information and other data (485). As voting is compulsory in Australia, the cohort obtained is likely to be representative of community-dwelling older men. Based on sample size calculation for the trial, 41,000 men who were resident in Perth were selected, after excluding 8801 men who lived in satellite towns (486). Of these men, 2296 died prior to randomisation, leaving 38,704 men who were then randomised into screening and control arms of equal size (19,352 men in each arm). Of those men in the screening arm, 12,203 (63.1%) attended the baseline screening at a clinic between 15 April 1996 and 29 January 1999. During their clinic visit, participants underwent an ultrasound scan of their abdominal aorta, as well as completed a questionnaire. Men in the control arm of the trial were never contacted, and were instead followed-up via electronic record linkage. This initial wave of HIMS, which comprises only the men in the screening arm, is referred to as Wave 1.

Approximately five years later, 10,940 surviving men who had participated in Wave 1 were invited to participate in a follow-up survey, with the intent of leading to a broader study of ageing. Between 24 October 2001 and 17 August 2004, a total of 4263 men attended the clinic and completed the questionnaire. A further 1322 men were unable to attend the clinic, but were able to complete their questionnaires which were all returned by mail. In total, 5585 men (51.1%) participated in the second wave of HIMS, which is referred to as
Wave 2. Of the 4263 men who had attended the clinic, early morning blood samples were obtained from 4249 men.

Surviving men who had attended Wave 1 or Wave 2 were later invited to participate in the third wave of HIMS between October 2008 and April 2009. Of 7445 surviving men, 3274 (44.0%) participated in this wave and were only required to return their questionnaires by post.

A subsequent Wave 4 of HIMS was concluded and Wave 5 is currently underway. As data from these waves were not analysed as part of this thesis, they are therefore not further described here.
Men aged 65 years and older were randomly selected from electoral roll (N=49801)

8801 men lived too far away from screening clinic;
2296 men died before randomisation

38704 men were randomised

19352 men were invited to participate in HIMS

456 men were excluded from study:
- Previous scan or operation for AAA (n=328)
- < 65 years of age (n=118)
- Could not scan aorta (n=10)

19352 men were randomised as Control group: no contact were made and these men were followed up via record linkage

6693 men were unable to attend, unwell, untraceable or did not respond

12203 men attended clinic and completed Wave 1 questionnaire in 1996-1999

1263 men died before invitation to follow-up

10940 men were invited to follow-up study

155 men died after invitation

5200 men were unable to attend, unwell, untraceable or did not respond

4263 men attended clinic and completed Wave 2 questionnaire in 2001-2004;
1322 men completed Wave 2 questionnaire by postage only;
4249 of the above had blood samples collected from them;

3340 men died before invitation to follow-up

7445 men were invited to follow-up study

126 men died after invitation;
3684 men were unwell, untraceable or did not respond;
361 men withdrew from HIMS

3274 men completed Wave 3 questionnaire in 2008-2009 by postage

~ 67 ~
2.2 Data collection

Participants completed a questionnaire at each of the three waves of HIMS. The questionnaires are presented in this thesis in the appendix. During Waves 1 and 2, they were also invited to attend a clinic, during which some clinical assessments were performed. Blood samples were collected from those who attended the Wave 2 clinic.

2.2.1 Questionnaires

The Wave 1 questionnaire was adapted from the instruments used in the National Heart Foundation of Australia Risk Factor Prevalence Study, which was a series of surveys aimed to explore the prevalence and impact of cardiovascular risk factors in the community (487-489). Participants provided information on their demographics, medical history, self-reported height and weight, physical activity, tobacco use, dietary habits, alcohol use, and family medical history. Symptoms of PAD, as per the Edinburgh Claudication Questionnaire, was also incorporated (490).

In Wave 2, the questionnaire comprised a wider range of instruments than those used in Wave 1. These included the Sense of Community/Neighbourhood Satisfaction Scale; SF-36 Health Survey; 11-item Duke Social Support Index; 15-item Geriatric Depression Scale; and the Adaptability, Partnership, Growth, Affection and Resolve scale (164, 491-496). Additional questions related to PAD symptoms, tobacco use, medical history, medication use; impairments in speech, vision and hearing; sleep problems; falls and injuries; living arrangements; hobbies; and languages spoken.

The Wave 3 questionnaire assessed demographic status, carer status, medical history, medication use, tobacco use, alcohol use, falls and injuries, impairments in vision and hearing, sleep problems, dietary habits, physical activity, and self-reported height and weight. Instruments used in this Wave included the Edinburgh Claudication Questionnaire, SF-36 Health Survey, International Prostate Symptom Score, 9-item Patient Health Questionnaire depression scale, Index of Independence in ADLs (modified form), and Instrumental ADLs scale (modified form) (164, 490, 497-500). Questions relating to sexual
activity and dysfunction were adapted from the National Social Life, Health, and Ageing Project (501).

2.2.2 Clinic assessments

During Waves 1 and 2, the height and weight of clinic attendees were measured using a wall-mounted stadiometer and a digital scale, respectively. Waist and hip circumferences were measured with a measuring tape. Systolic and diastolic blood pressure at the brachial artery were measured in a sitting position using a mercury sphygmomanometer, with the average of two measurements recorded into the database.

During Wave 1, the greatest transverse and antero-posterior diameter of the abdominal aorta was measured using a Toshiba Capasee ultrasound machine with a 3.75 MHz probe (Toshiba Australia, North Ryde, Australia).

In Wave 2, 3918 men elected to undergo cognitive screening with the Standardised MMSE (502). A random sample of 700 men attending the clinic were also invited to participate in a more detailed cognitive assessment using the California Verbal Learning Test, second edition (503). Of these, 683 were successfully assessed.

2.2.3 Blood collection

Of the 4263 men who attended the Wave 2 clinic, consent was obtained from 4249 for blood collection. Fasting blood samples were collected between 0800 and 1030 hours to minimise circadian variation. Serum was prepared immediately following phlebotomy and stored at -80 °C until assayed. Assays were performed in the Biochemistry Departments of Royal Perth and Fremantle Hospitals. Of the biochemical parameters being assessed, only those analysed in this thesis are described in this section.
tHcy

The level was measured by fluorescence polarization immunoassay on an IMx analyser (504). The inter-assay coefficient of variation was 4%. Normal range of tHcy is < 15 µmol/l, as determined by the laboratory’s reference range.

MTHFR 677C→T mutation

Genomic DNA was isolated from nucleated blood cells via the Triton X-100 method. The nt677C→T mutation was determined using the polymerase chain reaction (PCR). Hinfl restriction enzyme digestion was performed directly in the PCR tube at 37 ºC for 4 hours before analysis of restriction fragments by polyacrylamide gel electrophoresis (505). Allele frequencies were estimated by gene counting, and the genotype distribution compared with those expected under Hardy-Weinberg equilibrium.

25(OH)D

Plasma 25(OH)D was measured using the automated DiaSorin “LIAISON 25(OH)D TOTAL” chemiluminescent immunoassay. This was carried out on archived serum in a series of runs performed between 2011 and 2012. The interassay coefficient of variation was 13.2% at 37.9 nmol/l and 11.3% at 131 nmol/l. The date of blood collection was documented and seasonality determined based on the period of collection.

Creatinine

Serum creatinine was measured with a Roche Hitachi 917 analyser (Roche Diagnostics). Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (506).

Glucose

This was measured with an enzymatic assay using hexokinase on the Roche Hitachi 917 analyser (Roche Diagnostic GmbH, Mannheim, Germany). The interassay precision was 2.9% at 4.8 mmol/l and 2.2% at 15.2 mmol/l. The normal range for fasting men is 3.5-5.6 mmol/l.
Lipids

Cholesterol, LDL, and triglycerides (TG) were measured using the enzymatic colourimetric assays on the Roche Hitachi 917 analyser (Roche Diagnostic GmbH, Mannheim, Germany). The interassay precisions were as follows: total cholesterol, 2.3% at 3.2 mmol/l and 2.1% at 6.7 mmol/l; LDL, 1.4% at 2.1 mmol/l and 1.7% at 4.1 mmol/l; and for TG, 4.8% at 0.9 mmol/l and 2.4% at 2.0 mmol/l. Normal fasting ranges are < 5.5 mmol/l for total cholesterol, < 3.4 mmol/l for LDL, and < 1.8 mmol/l for TG.

High-sensitivity C-reactive protein (hsCRP)

hsCRP was measured on a particle-enhanced immunonephelometry assay on a BNII analyser (Dade Behring GmbH, Birmingham, United Kingdom). The interassay precision was 8.0% at 0.36 mg/l, 4.5% at 4.13 mg/l, 5.2% at 13.5 mg/l, and 3.2% at 51.3 mg/l. The normal range for men is < 10 mg/l.

2.2.4 Electronic record linkage

The WADLS was established in 1995, and provides electronic record linkage to the state’s population health collections (485, 507). It comprises six core data elements: birth records, midwives’ notifications, cancer registrations, inpatient hospital morbidity, inpatient and outpatient mental health services data, and mortality records in Western Australia. Additional datasets include the electoral roll, presentations to hospital emergency departments, and the use of community and residential aged care services. Information utilised as part of this thesis were mainly obtained from the electoral roll, death registrations, cancer notifications, and hospital morbidity datasets.

The target population in HIMS was extracted from the electoral roll in 1995. The name, address, and date of birth of these men were obtained for the AAA trial in Wave 1, and further extracts were requested during Waves 2 and 3 to locate those men who had participated in the initial wave.

The death registry records all deaths since 1969. Only records after Wave 1 were requested for the HIMS. It includes both the text on the original dataset, plus a multiple-cause
International Statistical Classification of Diseases and Related Health Problems (ICD) coded record generated by the Australian Bureau of Statistics based on the death certificates and other data (eg, post-mortem diagnosis). Deaths occurring before 1 July 1999 were coded with the ninth revision of the ICD (ICD-9), and later deaths were coded with the tenth revision (ICD-10).

The cancer registry includes all cancer notifications in the state since 1981. Reporting of cancer and other neoplasms is mandatory in Western Australia, including all in-situ neoplasms, all malignant neoplasms of the skin (other than primary squamous or basal cell carcinomas), and all neoplasm of the central nervous system, whether malignant or benign (508). Records are coded with the International Classification of Diseases for Oncology, third edition (ICD-O-3) system.

The hospital morbidity dataset includes all separations (discharges, transfers, and deaths) from public and private hospitals in the state since 1970. Each record contains a principal diagnosis code, up to 20 secondary diagnosis codes, and up to 11 procedure codes. Records between 1 January 1970 and 31 December 1978 were coded with the eighth revision of the ICD (ICD-8) for diagnoses, and the Code of Surgical Operations (COSO) for procedures. Records between 1 January 1979 and 31 December 1987 were coded with ICD-9 and the International Classification of Procedures in Medicine (ICPM). Records between 1 January 1988 and 30 June 1999 were coded with ICD-9, Clinical Modification (ICD-9-CM). Records after June 1999 are coded with ICD-10, Australian Modification (ICD-10-AM).

Data provided by WADLS has been subjected to quality assurance processes, including periodic audits of hospital coding (485, 508). Previous validations of the WADLS hospital morbidity dataset against national registry data and patient medical charts have revealed a high positive predictive value of the WADLS primary diagnostic data, albeit less complete data for secondary comorbidities (509, 510).
2.3 Ethical approval

At all three waves of the HIMS, all participants provided written informed consent to participate. Additional consent was obtained for blood collection at Wave 2. All analyses were performed on de-identified datasets. Ethical approval was obtained from the Human Research Ethics Committee of the University of Western Australia, Confidentiality of Health Information Committee, and the Department of Health Western Australia Human Research Ethics Committee. Research protocols complied with the Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects.
CHAPTER 3

PLASMA TOTAL HOMOCYSTEINE IS ASSOCIATED WITH ABDOMINAL AORTIC ANEURYSM AND AORTIC DIAMETER IN OLDER MEN
CHAPTER 3: PLASMA TOTAL HOMOCYSTEINE IS ASSOCIATED WITH ABDOMINAL AORTIC ANEURYSM AND AORTIC DIAMETER IN OLDER MEN

This chapter was published in the Journal of Vascular Surgery:


3.1 Abstract

Objective: To determine whether plasma total homocysteine (tHcy) and the methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism are associated with abdominal aortic aneurysm (AAA) and aortic diameter.

Design: Cross-sectional study

Setting: Western Australia

Subjects: 4248 community-dwelling men aged 70-88 years.

Main outcome measures: Infrarenal aortic diameter was measured using ultrasound, tHcy measured by immunoassay and MTHFR 677T polymorphism detected by polymerase chain reaction method.

Results: In adjusted multinomial logistic regression analyses, the odds of having an AAA (aortic diameter ≥ 30 mm) for men with high tHcy (≥ 15 µmol/l) compared to those with normal tHcy (< 15 µmol/l) was 1.45 (95% CI 1.10 to 1.91). Every 5-µmol/l increment in tHcy was associated with 0.15 mm (95% CI 0.01 to 0.28) increase in mean aortic diameter. tHcy concentration was higher in MTHFR TT homozygotes compared with ‘wild type’ CC individuals. There was, however, no apparent association between MTHFR C677T
polymorphism with AAA (TT vs CC genotype: OR 0.97, 95% CI 0.72 to 1.31) or aortic diameter (TT vs CC genotype: mean increment of 0.01 mm, 95% CI -0.63 to 0.65).

Conclusions: Elevated tHcy is associated with the presence of AAA in older men. There is also a positive dose-response relationship between tHcy and abdominal aortic diameter. Longitudinal studies and clinical trials of lowering tHcy are required to assess whether these relationships are causal.
3.2 Introduction

Abdominal aortic aneurysm (AAA) affects approximately 5% of men aged 65 years and over (511). Many of the risk factors for AAA are similar to those for atherosclerosis, including age, family history, smoking, dyslipidaemia, hypertension and obesity (85, 89). AAA has traditionally been regarded as a consequence of atherosclerosis (512). However, epidemiological differences, such as the inverse association of diabetes with AAA, suggest disparate pathogenesis (98, 513). Interest in the cause of AAA has been stimulated by the current absence of an effective drug therapy which limits AAA progression (102).

Homocysteine is a non-protein amino acid derived from the hepatic metabolism (demethylation) of the essential sulfur-containing amino acid, methionine. It is a substrate for three competing metabolic pathways. It can be re-methylated to methionine through: 1) a ‘salvage pathway’ (the Folate Cycle), a process which requires folate and B-vitamins as cofactors, and 2) the hepatic betaine-homocysteine methyltransferase reaction. It can also be irreversibly converted via the transulfuration pathway in the liver and kidney to cystathionine and cysteine. Elevated plasma total homocysteine (tHcy) has been extensively correlated with vascular events, including myocardial infarction and stroke (514, 515). Its association with AAA is, however, controversial, as previous studies have provided discordant findings (516-522). The possibility of reverse causality bias and residual confounding in these studies precludes establishment of a causal link between tHcy and AAA.

The principle of Mendelian randomisation has been utilised in clinical studies to explore the nature of association between tHcy and AAA, and their results have been equivocal (516, 517, 523-525). This is based on the concept that the random assortment of alleles at the time of gamete formation creates a randomised trial of genetic polymorphisms with a greater or lower expression of the relevant protein from birth onward, and hence the relationship between the genetic variant, risk factor of interest and outcome is not susceptible to the common biases or confounding that are prevailing in observational studies (526). It has been well documented that a commonly occurring methylenetetrahydrofolate reductase (MTHFR) gene variant 677T encodes a defective enzyme with reduced catalytic activity in the formation of 5-methyltetrahydrofolate. The
latter is the methyl donor needed for the conversion of homocysteine to methionine, thereby increasing basal plasma tHcy by about 20% (321). Based on the theory of Mendelian randomisation, the MTHFR C677T genotypes should have no direct effect on AAA other than that mediated via tHcy concentration (527).

In this present study, we sought to determine whether there is any relationship between tHcy and both the presence of AAA and aortic diameter in a large cohort of community-dwelling men aged 70-88 years, while accounting for the traditional risk factors for AAA and other co-morbidities. In an attempt to further explore the potential causal role of tHcy on AAA, we also investigated the association between the MTHFR C667T polymorphism with AAA and aortic diameter in this cohort of older men.
3.3 Methods

Study design and participants

We conducted a cross-sectional study of participants from the Health in Men Study (HIMS), which has been described in detail elsewhere (528). In brief, 12,203 community-dwelling men aged 65-83 years participated in a trial of screening for AAA. Each man completed a health assessment between 1996 and 1999 (HIMS Wave 1). In 2001-2004, 5,154 men responded to the second phase of this study (HIMS Wave 2) and blood samples were collected from 4,249 of them. The Human Research Ethics Committee of the University of Western Australia approved the protocol for HIMS which was conducted in accordance with the Helsinki Declaration for Human Rights.

Outcome of interest

The abdominal aortic diameter was measured during HIMS Wave 1. The greatest transverse and anteroposterior diameter of the infrarenal aorta was measured using a Toshiba Capasee ultrasound machine with a 3.75-MHz probe (Toshiba Australia, North Ryde, New South Wales, Australia). The reproducibility of these ultrasound measurements has been previously reported (529). An AAA was considered present if the abdominal aortic diameter was 30 mm or greater.

Biochemical analyses

Blood samples were collected during Wave 2 between 0800 and 1030. Plasma was separated from the blood samples within 1 hour of collection and stored at -80°C until assayed. tHcy was measured by fluorescence polarization immunoassay on an IMx analyzer (504). The inter-assay coefficient of variation was 4%. Genomic DNA was isolated from nucleated blood cells via the Triton X-100 method, and the nt677C→T mutation was determined using the polymerase chain reaction (PCR). HinfI restriction enzyme digestion was performed directly in the PCR tube at 37°C for 4 hours before analysis of restriction fragments by polyacrylamide gel electrophoresis, as previously described (505). Allele frequencies were estimated by gene counting, and the genotype distribution compared with those expected under Hardy-Weinberg equilibrium.
Serum high-sensitivity C-reactive protein (hsCRP) was measured with assay on a BNII analyzer (Dade Behring, Birmingham, UK). Serum creatinine, glucose, cholesterol, low-density lipoprotein and triglycerides were measured with a Roche Hitachi 917 analyzer (Roche Diagnostics).

Other explanatory variables

The following variables were available from the Wave 2 assessment: age, smoking status (current, former or never smoker) and taking B-vitamin supplements or not. Data from Waves 1 and 2 were utilised to determine the prevalence of cardiovascular disease (CVD) (self-reported history of angina, myocardial infarction, heart failure, coronary artery bypass grafting, coronary angioplasty, carotid endarterectomy, aortic bypass surgery, peripheral arterial surgery or stroke, or use of anti-platelet medications for these conditions), hypertension (self-reported diagnosis, or use of anti-hypertensive medications, or measured blood pressure ≥ 140/90 mmHg), diabetes (self-reported diagnosis, or use of glucose-lowering medication, or fasting glucose of ≥7 mmol/l or non-fasting glucose of ≥11 mmol/l) and dyslipidaemia (self-reported diagnosis, or use of lipid-lowering medication, or fasting low-density lipoprotein ≥ 3.4 mmol/l, high-density lipoprotein < 0.9 mmol/l, triglycerides ≥ 1.8 mmol/l, or total cholesterol ≥ 5.5 mmol/l).

In order to calculate the weighted Charlson Co-morbidity Index (530), we obtained the health records and death certificates (until the end of Wave 2) from the Western Australian Data Linkage System (WADLS). This database provides electronic linkage to the state’s use of health services and medical morbidities (485). 17 common medical conditions that predict one-year mortality were accounted for: myocardial infarction, congestive heart failure, peripheral arterial disease, cerebrovascular disease, dementia, chronic pulmonary disease, connective tissue disease, ulcers, liver disease, diabetes (including diabetes with end organ damage), hemiplegia, renal disease, leukaemia, lymphoma, other tumours, metastatic tumours, and AIDS. The number and seriousness of these comorbid diseases were evaluated, by assigning integer weights to these conditions using adjusted relative risks and developing a composite index score that ranges from 0 to 37 (530).
Height, weight and waist circumference were measured during Wave 2 in accordance with guidelines of the International Society for the Advancement of Kinanthropometry (531). Body mass index (BMI) was calculated from height and weight in kg/m².

**Statistical analysis**

Data were analysed using Stata release 11.1 (StataCorp, College Station, TX, USA). Descriptive statistics were calculated for the demographic, lifestyle and clinical variables according to the presence or absence of AAA. tHcy was dichotomised into ‘high tHcy’ (≥ 15 µmol/l) and ‘normal tHcy’ (< 15 µmol/l) as determined by the laboratory’s reference range. Aortic diameter was divided into intervals of < 19 mm, 19-22 mm, 23-29 mm and ≥ 30 mm, based on standard deviations of logarithmic diameter (512). tHcy was modeled as categorical and continuous variables: according to whether tHcy was ≥ 15 µmol/l, per 5-µmol/l increment in tHcy, and doubling of tHcy concentration (by dividing the natural logarithm of tHcy by the natural logarithm of 2). Multinomial logistic regression analyses were performed to assess for any dose-response relationship between tHcy and aortic diameter intervals, using a reference interval of 19-22 mm. Linear regression analyses were performed with aortic diameter modeled as a continuous variable. Analyses were also repeated after logarithmic transformations of the aortic diameter due to its positively-skewed distribution. Adjustments were made for age, smoking, CVD, diabetes, hypertension, dyslipidaemia, Charlson’s comorbidity index, BMI and serum creatinine as potential confounders. The results were reported as Odds Ratio (OR) with 95% confidence intervals (95% CI). P-values < 0.05 were considered statistically significant.

Descriptive statistics were used to calculate the distribution of the MTHFR genotypes according to AAA. Analysis of variance and linear regression analysis were used to explore the association of the MTHFR genotypes with tHcy. Linear and multinomial logistic regression analyses were performed to investigate the relationship between the MTHFR polymorphism with aortic diameter and AAA, using the CC genotype and aortic diameter interval of 19-22 mm as references.
3.4 Results

Socio-demographic, clinical and biochemical characteristics of the study population according to the presence or absence of AAA are shown in Table 3.1. 4248 men, aged between 70 and 88 years, had complete data for tHcy and aortic diameter measurements. 1120 men (26.3%) had high tHcy (≥ 15 µmol/l) and the mean (± SD) tHcy concentration for the cohort was 13.4 ± 5.6 µmol/l. None of the participants had homocysteinuria. 318 men (7.5%) had AAA (aortic diameter ≥ 30 mm) and the mean (±SD) aortic diameter for the cohort was 22.8 ± 4.9 mm (range 15.9 to 79.2 mm).

Table 3.1 Demographic, lifestyle and clinical characteristics of the study population according to the presence or absence of abdominal aortic aneurysm (AAA)

<table>
<thead>
<tr>
<th></th>
<th>AAA (n=318)</th>
<th>No AAA (n=3930)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years *</td>
<td>77.7 (4.1)</td>
<td>76.5 (3.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking, n(%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>37 (11.7)</td>
<td>179 (4.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Former smoker</td>
<td>240 (75.7)</td>
<td>2371 (60.3)</td>
<td></td>
</tr>
<tr>
<td>Never smoked</td>
<td>41 (12.6)</td>
<td>1380 (35.1)</td>
<td></td>
</tr>
<tr>
<td>Taking B-vitamin supplements, n(%)</td>
<td>15 (4.7)</td>
<td>231 (5.9)</td>
<td>0.395</td>
</tr>
<tr>
<td>Cardiovascular disease, n(%)</td>
<td>208 (65.4)</td>
<td>1625 (41.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes, n(%)</td>
<td>67 (21.1)</td>
<td>596 (15.2)</td>
<td>0.005</td>
</tr>
<tr>
<td>Hypertension, n(%)</td>
<td>307 (96.5)</td>
<td>3452 (87.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dyslipidaemia, n(%)</td>
<td>262 (82.4)</td>
<td>2740 (69.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Charlson’s Index Score ≥ 5, n(%)</td>
<td>29 (9.1)</td>
<td>146 (3.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI, kg/m²*</td>
<td>27.1 (3.8)</td>
<td>26.5 (3.6)</td>
<td>0.006</td>
</tr>
<tr>
<td>Aortic diameter, mm*</td>
<td>36.4 (7.5)</td>
<td>21.8 (2.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>tHcy, µmol/l</td>
<td>15.1 (6.1)</td>
<td>13.2 (5.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>hsCRP, mg/l</td>
<td>4.9 (8.4)</td>
<td>3.7 (7.3)</td>
<td>0.008</td>
</tr>
<tr>
<td>Creatinine, µmol/l*</td>
<td>104.8 (40.9)</td>
<td>92.8 (30.6)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*mean (SD)

Association of tHcy with AAA

Table 3.2 shows an incremental association between tHcy and aortic diameter intervals (p<0.001). Adjustments were made for age, smoking, CVD, diabetes, hypertension, dyslipidaemia, Charlson’s co-morbidity index, BMI and serum creatinine. Using a reference interval of 19-22 mm, the odds of having an AAA (aortic diameter ≥ 30 mm) for
men with high tHcy (≥ 15 µmol/l) compared to those with normal tHcy (< 15 µmol/l) was 1.45 (95% CI 1.10 to 1.91). The association persisted when tHcy was modeled as continuous variables. Figure 3.1 demonstrates the odds ratio of having an AAA with changing tHcy concentrations, with tHcy entered into the models as restricted cubic splines (532).

Table 3.2  Multinomial logistic regression models\(^a\) evaluating the association of plasma homocysteine (tHcy) with aortic diameter intervals in community-dwelling older men

<table>
<thead>
<tr>
<th>Aortic diameter intervals (mm)</th>
<th>&lt; 19</th>
<th>19-22</th>
<th>23-29</th>
<th>≥ 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of men, (n)</td>
<td>440</td>
<td>2427</td>
<td>1063</td>
<td>318</td>
</tr>
<tr>
<td>tHcy, (\mu\text{mol/l})</td>
<td>12.8 ± 5.0</td>
<td>13.1 ± 5.1</td>
<td>13.8 ± 5.1</td>
<td>15.1 ± 6.1</td>
</tr>
<tr>
<td>tHcy ≥ 15 (\mu\text{mol/l})^c</td>
<td>0.76 (0.58 to 1.00)</td>
<td>Reference</td>
<td>1.20 (1.00 to 1.43)</td>
<td>1.45 (1.10 to 1.91)</td>
</tr>
<tr>
<td>Per 5-(\mu\text{mol/l}) increment in tHcy^c</td>
<td>0.90 (0.79 to 1.02)</td>
<td>Reference</td>
<td>1.06 (0.99 to 1.14)</td>
<td>1.12 (1.02 to 1.23)</td>
</tr>
<tr>
<td>Doubling of tHcy^c</td>
<td>0.76 (0.58 to 0.97)</td>
<td>Reference</td>
<td>1.14 (0.96 to 1.36)</td>
<td>1.50 (1.14 to 1.99)</td>
</tr>
</tbody>
</table>

\(^a\)Adjusted for age, smoking status, cardiovascular disease, hypertension, dyslipidaemia, diabetes, Charlson’s comorbidity index, body mass index and serum creatinine.

\(^b\)mean ± SD

\(^c\)Odds ratio (95% confidence interval) presented for high tHcy (≥ 15 \(\mu\text{mol/l}\)) relative to normal tHcy (< 15 \(\mu\text{mol/l}\)), per 5-\(\mu\text{mol/l}\) increment in tHcy and doubling of tHcy.

Figure 3.1  Odds ratio of an abdominal aortic aneurysm (aortic diameter ≥ 30 mm) with changing plasma total homocysteine (tHcy) levels

tHcy are entered into the models as restricted cubic splines (3 knots) with a reference value of 9 \(\mu\text{mol/l}\) (median of the lowest quartile of tHcy concentrations). The tHcy scale of 8 to 22 \(\mu\text{mol/l}\) relates to the range between 5\(^{th}\) and 95\(^{th}\) percentiles of values (\(n=3823\)). Dashed lines denote 95% confidence interval.
Association of tHcy with aortic diameter

When multivariate linear regression analyses were performed, men with high tHcy had 0.41 mm (95% CI 0.06 to 0.77) larger mean aortic diameter in comparison to those with normal tHcy concentrations. Every 5-µmol/l increment in tHcy was associated with 0.15 mm (95% CI 0.01 to 0.28) larger mean aortic diameter. Doubling of tHcy was associated with 0.39 mm (95% CI 0.05 to 0.73) larger mean aortic diameter. These associations persisted when aortic diameter was log-transformed (data not shown).

Sensitivity analyses

We performed a sensitivity analysis by excluding those men who reported taking B-vitamin supplements. When this was done, the odds of having an AAA for men with high tHcy and not taking B-vitamin supplements compared to those with normal tHcy were not altered to a great extent (OR 1.48, 95% CI 1.11 to 1.96). When hsCRP was included in the model of tHcy and AAA, the effect estimate remained similar (OR 1.44, 95% CI 1.09 to 1.90).

Association of MTHFR polymorphism with AAA and aortic diameter

The MTHFR polymorphism data were available for 4130 men. The distribution of the MTHFR genotypes was similar for participants with and without AAA: CC 281 (45.5%), CT 267 (43.3%) and TT 69 (11.2%) for men with AAA and CC 1549 (44.1%), CT 1566 (44.6%) and TT 398 (11.3%) for the remaining men (p=0.795). The genotype distribution was in Hardy-Weinberg equilibrium (exact test, p=0.773). Mean (± SD) aortic diameter were 24.1 ± 6.5 mm, 23.8 ± 6.1 mm and 24.1 ± 6.2 mm for men with the CC, CT and TT genotypes, respectively (F=1.08, p=0.341). As reported previously(533), the mean (± SD) tHcy concentration was 13.1 ± 5.1 µmol/l, 13.5 ± 4.9 µmol/l and 14.4 ± 9.7 µmol/l for men with the CC, CT and TT genotypes, respectively (F=10.16, p < 0.001).

Both linear and multinomial logistic regression analyses showed no apparent association between MTHFR polymorphism with aortic diameter and AAA (Table 3.3). The odds ratio of having an AAA was 0.95 (95% CI 0.79 to 1.15) for men with the CT genotype and 0.97 (95% CI 0.72 to 1.31) for men with the TT genotype, in comparison to those with the CC genotype. Men with the CT genotype had a reduction of 0.29 mm (95% CI -0.69 to 0.12) in
mean aortic diameter. On the other hand, men with the TT genotype had an increment of 0.01 mm (95% CI -0.63 to 0.65) in diameter.

Table 3.3 Multinomial logistic regression models evaluating the association of the methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism with aortic diameter intervals in community-dwelling older men

<table>
<thead>
<tr>
<th>MTHFR C677T polymorphisms</th>
<th>Aortic diameter intervals (mm)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 19</td>
<td>19-22</td>
<td>23-29</td>
<td>≥ 30</td>
</tr>
<tr>
<td>-CT genotype</td>
<td>1.18 (0.94 to 1.48)</td>
<td>Reference</td>
<td>0.99 (0.84 to 1.16)</td>
<td>0.95 (0.79 to 1.15)</td>
</tr>
<tr>
<td>-TT genotype</td>
<td>0.91 (0.62 to 1.33)</td>
<td>Reference</td>
<td>1.10 (0.86 to 1.41)</td>
<td>0.97 (0.72 to 1.31)</td>
</tr>
</tbody>
</table>

aOdds ratio (95% confidence interval) presented for CT and TT genotypes relative to CC genotype.
3.5 Discussion

Our results provide further evidence for an association between elevated tHcy and the presence of AAA, independent of the traditional CVD risk burden and other co-morbidities. The risk of having an AAA for men with high tHcy is 45% higher in comparison to those with normal tHcy concentrations. tHcy is also correlated with abdominal aortic diameter in a positive dose-response relationship, with every 5-µmol/l increment in tHcy being associated with 0.15 mm larger mean aortic diameter. These findings suggest that elevated tHcy could either be an epiphenomenon or have a role in the development of an AAA in ageing men.

The strengths of this study include the large sample size of robustly characterised, population-based community-dwelling older men. We were able to comprehensively adjust for potential confounders in our multivariate analyses, including age, smoking, CVD and risk factors which have established associations with both tHcy and AAA. The addition of other variables that might modify the relationship, including statin and antiplatelet therapies did not significantly alter the effect estimates. Measurement of the aortic diameter was performed in a standardised procedure using ultrasound.

A major limitation would be the fact that blood samples were obtained from the participants several years (5.7 ± 0.9 years) after baseline aortic diameter measurements. It is possible that the aortic diameters in our cohort would have increased by a small margin in the time interval (93). However, it is unlikely that the small number of interval cases of AAA would have substantially altered the results. The cross-sectional design of our study and the lack of serial assessments of tHcy concentration or aortic diameter also preclude determination of causality. Our findings could reflect reverse causality, with higher co-morbidities in men having AAA at baseline leading to less physical activity and poorer diet, and consequently higher tHcy concentrations. Our findings are conservative due to a possible ‘healthy survivor’ effect. Participants who had responded for the Wave 2 assessment were younger in age (p<0.001), more physically active (p<0.001) and had less self-reported co-morbidities (p<0.001) during Wave 1 compared to the non-respondents of the follow-up study. This self-selection of participants might have biased our findings towards lower tHcy concentrations in our cohort compared to the non-respondents and therefore move our
results towards null hypothesis, leading to an underestimation of the magnitude of the association between tHcy with AAA. Finally, our data for B-vitamin supplementation was based on self-reported history. By not having access to B-vitamin concentrations, we were therefore unable to definitively account for possible effects due to prevailing folate concentration.

Homocysteine influences a range of molecular pathways of potential relevance to the pathogenesis of AAA, some of which may be different from those implicated in atherosclerosis. Experimentally, homocysteine induces endothelial dysfunction and matrix remodeling in part by stimulating the secretion of matrix metalloproteases and release of reactive oxygen species which are implicated in AAA pathogenesis (534-536). Immune mechanisms may also be important in AAA formation (537, 538). Homocysteine stimulates chemokine/cytokine secretion from cultured human monocytes and has been implicated in suppressing regulatory T-cell function (539, 540). Homocysteinylation, which is a manifestation of homocysteine toxicity, is characterised by a non-enzymatic chemical reaction that can cause structural protein inactivation with consequent destruction of the aortic wall (541). A recent animal study has demonstrated that hyperhomocysteinaemia contributes to the development of AAA via aortic adventitial inflammation, a process which is reversible with folic acid supplementation (542). Although we have demonstrated a statistically significant quantitative association between elevated tHcy with the enlargement of the abdominal aortic diameter by mere fractions of a millimeter, these small differences are of limited clinical significance for an individual person. Despite this, findings from previous experimental studies have indicated that this association is biologically plausible, suggesting the possibility that homocysteine-lowering strategies could limit AAA formation in humans.

To our knowledge, this is the largest epidemiological study to demonstrate an independent association between elevated tHcy with the presence of AAA and aortic diameter. Brunelli et al were the first to conduct a case-control study of 58 men and reported a six-fold increase in the likelihood of having AAA with hyperhomocysteinaemia (OR 6.0, 95% CI 1.2 to 29.6) (517). Subsequent studies further implicated tHcy in the pathophysiology of AAA (516, 520, 543-545). Researchers also found that patients with AAA had lower vitamin B12 levels, which are important in the regulation of tHcy concentrations (519). On
the other hand, discrepant results have emerged from other studies which may be limited by patient selection and residual confounding (518, 521, 522). Further exploration of this relationship with adequately powered longitudinal studies and clinical trials would be required to have an improved understanding of any role homocysteine metabolism plays in AAA pathogenesis.

In our study, tHcy concentrations were elevated with the MTHFR 677T variant compared with the 677C or ‘wild’ allele. There was, however, no obvious association between the T allele and the presence of AAA and aortic diameter. We performed a retrospective power calculation (80% power with an alpha set at 0.05) and deduced that the study would require over a million participants (approximately 300 000 men with the TT genotype) to detect an effect size of 0.03 mm increase in mean aortic diameter associated with a 1-µmol/l increment in tHcy (effect size derived from our multivariate linear regression analysis). Similarly, other previous studies would have been clearly underpowered to establish an association (516, 517, 523-525). When a meta-analysis of these studies was conducted (approximately 2300 subjects in total), an increased risk of AAA associated with the T allele variant (risk ratio 1.14, 95% CI 1.08 to 1.21) was obtained (546). This is supportive of a causal link between hyperhomocysteinaemia and AAA.

In conclusion, elevated tHcy is associated with the presence of AAA in older men, independent of the traditional CVD risk burden and other co-morbidities. There is also a positive dose-response relationship between tHcy and abdominal aortic diameter, suggesting a role of tHcy in AAA development in ageing men. However, Mendelian randomisation to the MTHFR TT genotype is not associated with AAA or aortic diameter, despite higher tHcy. The discordance in these findings may reflect confounding or random error. Longitudinal studies and clinical trials of lowering tHcy are required to assess whether these relationships are causal.
3.6 **Author contributions**

Study conception and design: YW, LF, PN

Acquisition of data: LF, GH, FB, BY, PN

Statistical analyses: YW, KM

Interpretation of analyses: YW, JG, LF, KM, GH, FB, BY, PN

Drafting of manuscript: YW

Critical revision of manuscript: JG, LF, KM, GH, FB, BY, PN

Final approval of manuscript: All authors
CHAPTER 4

IS HYPOVITAMINOSIS D ASSOCIATED WITH ABDOMINAL AORTIC ANEURYSM, AND IS THERE A DOSE-RESPONSE RELATIONSHIP?
CHAPTER 4: IS HYPOVITAMINOSIS D ASSOCIATED WITH ABDOMINAL AORTIC ANEURYSM, AND IS THERE A DOSE-RESPONSE RELATIONSHIP?

This chapter was published in the European Journal of Vascular and Endovascular Surgery:


4.1 Abstract

Objective: To investigate the association between plasma 25-hydroxyvitamin D [25(OH)D] concentrations with the presence of abdominal aortic aneurysm (AAA) and aortic diameter.

Design: An observational study of 4233 community-dwelling men aged 70-88 years, who participated in a randomised controlled trial of screening for AAA.

Methods: Infrarenal aortic diameter measured by ultrasound and 25(OH)D by immunoassay.

Results: There were 311 men (7.4%) with AAA (defined as aortic diameter ≥ 30 mm). Multivariable models were adjusted for age, smoking, cardiovascular disease, hypertension, diabetes, dyslipidemia, body mass index and serum creatinine concentration. Amongst men with the lowest 25(OH)D quartile of values compared with the highest quartile, the adjusted odds ratio of having an AAA increased in a graded fashion from 1.23 (95% CI 0.87 to 1.73) for AAA ≥ 30 mm to 5.42 (95% CI 1.85 to 15.88) for AAA ≥ 40 mm. Similarly, there was a dose-response relationship between 25(OH)D concentrations and the size of the AAA: every 10-nmol/l decrease in 25(OH)D levels was associated with 0.49 mm (95% CI 0.11 to 0.87) increase in mean aortic diameter.

Conclusions: Low vitamin D status is associated with the presence of larger AAA in older men, and there is a graded inverse relationship between 25(OH)D concentrations and AAA.
diameter. Further research is needed to clarify the mechanisms underlying these associations.
4.2 Introduction

Vitamin D and its metabolites are known to have significant roles in the maintenance of calcium and bone homeostasis. In recent years, they have also been extensively researched for their pleiotropic effects on biological processes other than skeletal health. Epidemiological studies have suggested that hypovitaminosis D might be a contributory factor to cardiovascular disease (CVD) including ischemic heart disease, peripheral arterial disease (PAD) and stroke (547). It is also linked to major traditional CVD risk factors, such as hypertension, dyslipidaemia and obesity (548), as well as CVD mortality (549). Postulated mechanisms for these presumed cardiovascular effects include endothelial dysfunction, inflammation, reduced vessel compliance, detrimental effects via bone proteins such as osteoprotegerin, as well as dysregulation of the renin-angiotensin system (550-554). On the other hand, a recent systematic review and meta-analysis of vitamin D intervention trials failed to demonstrate a significant reduction in CVD events with vitamin D supplementation, thus implying that hypovitaminosis D might be an epiphenomenon, rather than as an etiological factor for CVD (438).

Aneurysmal arterial disease is pathologically characterised by extensive disruption of the extracellular matrix with the loss of medial elastin and consequent reduction in tensile strength (555). This results in vascular wall weakening and progressive localised dilatation. Abdominal aortic aneurysm (AAA) and occlusive CVD share many risk factors, including age, male gender, genetic predisposition, smoking, hypertension, hypercholesterolemia and obesity (85, 89). As AAA is also independently associated with a higher risk of incident CVD events and mortality, it has been traditionally considered as a manifestation of atherosclerosis (512). However, there are important differences between aneurysmal and occlusive CVD suggesting disparate pathogenesis. The most notable difference is the inverse association between diabetes and AAA (98).

Vitamin D influences a range of molecular pathways of potential relevance to the pathogenesis of AAA (556). As the relationship between vitamin D status and AAA is unknown, we sought to determine whether there is any relationship between plasma concentration of vitamin D and both the presence and diameter of AAA. This was undertaken in a large cohort of men aged 70-88 years.
4.3 Methods

Study design and participants

We conducted a cross-sectional observational study of participants from the Health in Men Study (HIMS), which has been described in detail elsewhere (486, 528). In brief, approximately 40000 men aged 65 years and older were randomised to the screening and control arms of the trial of screening for AAA. 12203 men participated in screening and completed a health assessment between 1996 and 1999 (HIMS Wave 1). In 2001-2004, 5585 men responded to the second phase of this study (HIMS Wave 2) and blood samples were collected from 4249 of them. The Human Research Ethics Committee of the University of Western Australia approved the protocol for HIMS and informed consent was obtained from the participants.

Outcome of interest

The abdominal aortic diameter was measured during HIMS Wave 1. The greatest transverse and anteroposterior diameter of the infrarenal aorta was measured using a Toshiba Capasee ultrasound machine with a 3.75-MHz probe (Toshiba Australia, North Ryde, New South Wales, Australia). The reproducibility of these ultrasound measurements has been previously reported (529). An AAA was considered present if the abdominal aortic diameter was 30 mm or greater.

Explanatory variables

Using a combination of data collected at Waves 1 and 2, the following variables were available: age, smoking status (current, former or never smoker during Wave 2), physical activity (≥ 150 minutes of moderate to vigorous exercise in a usual week during Wave 1), CVD (self-reported history of angina, myocardial infarction, heart failure, coronary artery bypass grafting, coronary angioplasty, carotid endarterectomy, aortic bypass surgery, peripheral arterial surgery or stroke during Wave 1 or 2), hypertension (self-reported diagnosis, or use of anti-hypertensive medications, or measured average blood pressure > 140/90 mmHg), diabetes (self-reported diagnosis, or use of glucose-lowering medication, or a fasting glucose of ≥ 7 mmol/l, or non-fasting glucose ≥ 11 mmol/l) and dyslipidaemia (self-reported diagnosis, or use of lipid-lowering medication, or fasting low-density...
lipoprotein $\geq 3.4$ mmol/l, high-density lipoprotein $< 0.9$ mmol/l, triglycerides $\geq 1.8$ mmol/l, or total cholesterol $\geq 5.5$ mmol/l). Height, weight and waist circumference were measured during Wave 2 in accordance with guidelines of the International Society for the Advancement of Kinanthropometry (531). Body mass index (BMI) was calculated from height and weight in kg/m$^2$.

Validation of CVD variable

In addition to data from the questionnaire, the Western Australian Data Linkage System (WADLS) (485) was used to identify men with any admissions to hospital for CVD by the end of Wave 2 screening. This system links together data from the state cancer registry, death registry and hospital morbidity data system. The admissions were identified using the relevant diagnoses and procedure codes from the International Classification of Diseases (ICD). The predictive utility of CVD for all-cause mortality was tested in our cohort by utilising mortality records up to 31st December 2010.

Biochemical analyses

Blood samples were collected during Wave 2 between 0800 and 1030. Plasma was separated from the blood samples within 1 hour of collection and stored at -80°C until assayed. Plasma 25-hydroxyvitamin D [25(OH)D], an established marker of vitamin D status, was measured using the automated DiaSorin “LIAISON 25(OH)D TOTAL” chemiluminescent immunoassay, which was carried out on archived serum in a series of runs performed between 2011 and 2012. The interassay coefficient of variation was 13.2% at 37.9 nmol/l and 11.3% at 131 nmol/l. Serum high-sensitivity C-reactive protein (hsCRP) was measured with assay on a BNII analyzer (Dade Behring, Birmingham, UK). Serum creatinine, glucose, cholesterol, low-density lipoprotein and triglycerides were measured with a Roche Hitachi 917 analyzer (Roche Diagnostics).

Statistical analysis

Data were analysed using Stata release 11.1 (StataCorp, College Station, TX, USA). Descriptive statistics were calculated for the demographic, lifestyle and clinical variables according to the presence or absence of AAA. A Cox proportional hazards model was used to test the association between CVD and mortality. Adjustments were made for age,
smoking, hypertension, diabetes, dyslipidaemia, BMI and creatinine. The association between 25(OH)D and AAA was investigated in three different ways: according to 25(OH)D quartiles of values (using the highest quartile as reference), per 10-nmol/l decrease in 25(OH)D concentration, and by halving of 25(OH)D concentration (by dividing the natural logarithm of 25(OH)D by the natural logarithm of 0.5). To investigate the relationship between 25(OH)D with the presence of AAA, logistic regression analyses were performed with 25(OH)D modeled as continuous and categorical variables. The associations were explored for the presence of any AAA (aortic diameter ≥ 30 mm) and larger AAAs (diameter ≥ 35 mm and ≥ 40 mm, respectively). To determine the relationship between 25(OH)D and aortic diameter, linear regression was used with aortic diameter modeled as a continuous variable in aneurysmal (aortic diameter ≥ 30 mm) and non-aneurysmal (< 30 mm) ranges. Adjustments were made for age, smoking, CVD, diabetes, hypertension, dyslipidaemia, BMI and serum creatinine as potential confounders. The results were reported as Odds Ratios (OR) and Hazard Ratio (HR) with 95% confidence intervals (95% CI). P-values < 0.05 were considered statistically significant.
4.4 Results

A flow chart detailing disposition of the study participants is shown in Figure 4.1. Men who had died prior to Wave 2 follow-up or did not attend due to various reasons were older in age (p<0.001), more likely to be smokers (p<0.001) and self-report a history of cardiovascular disease (p=0.025), hypertension (p<0.001) and diabetes (p<0.001) during Wave 1, in comparison to those men who subsequently responded in Wave 2. The demographic, clinical and biochemical characteristics of men with and without AAA are demonstrated in Table 4.1. 4233 men, aged 70-88 years, had complete data for 25(OH)D levels, aortic diameter and co-morbidities. The mean (± SD) 25(OH)D concentration for the cohort was 68.3 ± 23.3 nmol/l and the quartiles of values correspond to 10.0-52.8 nmol/l (median 42.3 nmol/l), 52.9-67.3 nmol/l (median 60.3 nmol/l), 67.4-81.6 nmol/l (median 73.8 nmol/l) and 81.7-238.4 nmol/l (median 93.7 nmol/l), respectively. 311 men (7.4%) had an AAA (aortic diameter ≥ 30 mm), 120 men (2.8%) had AAA ≥ 35 mm and 66 men (1.6%) had AAA ≥ 40 mm. The mean (± SD) aortic diameter for the cohort was 22.8 ± 4.9 mm (range 15.9 to 79.2 mm).
Figure 4.1  Study flow diagram of Health In Men Study (HIMS)

Men aged 65 years and older were randomly selected from electoral roll (N=49801)

38704 men were randomised

19352 men were invited to participate in HIMS

19352 men were randomised as Control group: no contact were made and these men were followed up via record linkage

8801 men lived too far away from screening clinic; 2296 men died before randomisation

456 men were excluded from study:
- Previous scan or operation for abdominal aortic aneurysm (n=328)
- < 65 years of age (n=118)
- Could not scan aorta (n=10)

6693 men were unable to attend, unwell, untraceable or did not respond

12203 men attended clinic and completed Wave 1 questionnaire in 1996-1999

1263 men died before invitation to follow-up

10940 men were invited to follow-up study

155 men died after invitation

5200 men were unable to attend, unwell, untraceable or did not respond

4263 men attended clinic and completed Wave 2 questionnaire in 2001-2004; 1322 men completed Wave 2 questionnaire by postage only; 4249 of the above had blood samples collected from them; 4233 men had complete data for 25-hydroxyvitamin D concentrations, aortic diameter and comorbidities
Table 4.1 Demographic, lifestyle and clinical characteristics of the study population according to the presence or absence of abdominal aortic aneurysm (AAA)

<table>
<thead>
<tr>
<th></th>
<th>AAA (n=311)</th>
<th>No AAA (n=3922)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, years</strong> *</td>
<td>77.7 (3.6)</td>
<td>76.5 (3.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Smoking, n(%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>37 (11.9)</td>
<td>178 (4.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Former smoker</td>
<td>234 (75.5)</td>
<td>2371 (60.5)</td>
<td></td>
</tr>
<tr>
<td>Never smoked</td>
<td>39 (12.6)</td>
<td>1373 (35.0)</td>
<td></td>
</tr>
<tr>
<td><strong>Physical activity, n(%)</strong></td>
<td>56 (18.0)</td>
<td>901 (23.0)</td>
<td>0.044</td>
</tr>
<tr>
<td><strong>Cardiovascular disease, n(%)</strong></td>
<td>182 (58.5)</td>
<td>1165 (29.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Diabetes, n(%)</strong></td>
<td>67 (21.5)</td>
<td>596 (15.2)</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>Hypertension, n(%)</strong></td>
<td>247 (79.7)</td>
<td>2971 (77.1)</td>
<td>0.292</td>
</tr>
<tr>
<td><strong>Dyslipidemia, n(%)</strong></td>
<td>255 (82.0)</td>
<td>2773 (69.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>BMI, kg/m²</strong></td>
<td>27.0 (3.8)</td>
<td>26.5 (3.6)</td>
<td>0.010</td>
</tr>
<tr>
<td><strong>Waist circumference, cm</strong></td>
<td>101.8 (10.6)</td>
<td>99.0 (10.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Aortic diameter, mm</strong></td>
<td>36.2 (7.5)</td>
<td>21.8 (2.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>25(OH)D, nmol/l</strong></td>
<td>65.0 (22.8)</td>
<td>68.6 (23.3)</td>
<td>0.010</td>
</tr>
<tr>
<td><strong>hsCRP, mg/l</strong></td>
<td>4.9 (8.5)</td>
<td>3.7 (7.3)</td>
<td>0.007</td>
</tr>
<tr>
<td><strong>Creatinine, µmol/l</strong></td>
<td>104.8 (40.9)</td>
<td>92.9 (30.7)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*mean (SD)

Predictive utility of CVD for all-cause mortality

In the multivariable Cox proportional hazards model, CVD predicted all-cause mortality (HR 1.53, 95% CI 1.36 to 1.73).

Association of 25(OH)D with AAA

In multivariable logistic regression analysis (Table 4.2), the adjusted OR of having an AAA for men with the lowest 25(OH)D quartile of values compared with the highest quartile was 1.23 (95% CI 0.87 to 1.73). This association became statistically significant and stronger in a graded fashion for larger AAAs, with an OR of 2.02 (95% CI 1.13 to 3.61) for AAA ≥ 35 mm, and OR 5.42 (95% CI 1.85 to 15.88) for AAA ≥ 40 mm. When 25(OH)D was modeled as continuous variables, there was a graded increase in association with larger AAAs: for every 10-nmol/l decrease in 25(OH)D concentration, the odds was increased by 14% (OR 1.14, 95% CI 1.04 to 1.24) for having AAA ≥ 35 mm, and by 23% (OR 1.23, 95% CI 1.08
to 1.39) for having AAA ≥ 40 mm. Figure 4.2 demonstrates the odds ratio of having an AAA ≥ 40 mm with changing 25(OH)D levels.

Table 4.2 Multivariable logistic regression models\(^1\) evaluating the association of 25-hydroxyvitamin D [25(OH)D] with the presence of abdominal aortic aneurysm (AAA) in community-dwelling older men

<table>
<thead>
<tr>
<th></th>
<th>AAA ≥ 30 mm (n=311)</th>
<th>AAA ≥ 35 mm (n=120)</th>
<th>AAA ≥ 40 mm (n=66)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
<td>OR</td>
</tr>
<tr>
<td>25(OH)D(^*)</td>
<td>1.03</td>
<td>0.98 to 1.09</td>
<td>1.14</td>
</tr>
<tr>
<td>Halving of 25(OH)D</td>
<td>1.16</td>
<td>0.92 to 1.44</td>
<td>1.60</td>
</tr>
<tr>
<td>25(OH)D quartile 1</td>
<td>1.23</td>
<td>0.87 to 1.73</td>
<td>2.02</td>
</tr>
<tr>
<td>25(OH)D quartile 2</td>
<td>0.84</td>
<td>0.58 to 1.22</td>
<td>1.90</td>
</tr>
<tr>
<td>25(OH)D quartile 3</td>
<td>1.20</td>
<td>0.85 to 1.70</td>
<td>1.41</td>
</tr>
<tr>
<td>25(OH)D quartile 4</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval.
\(^*\)Odds ratio per 10-nmol/l decrease in 25(OH)D concentration.
\(^1\)Adjusted for age, smoking, cardiovascular disease, hypertension, dyslipidaemia, diabetes, body mass index and serum creatinine.

The quartiles for 25(OH)D corresponded to values of: 10.0-52.8 nmol/l (median 42.7 nmol/l), 52.9-67.3 (median 60.3 nmol/l), 67.4-81.6 nmol/l (median 73.8 nmol/l) and 81.7-238.4 nmol/l (median 93.7 nmol/l), respectively.

Figure 4.2 Odds ratio of an abdominal aortic aneurysm ≥ 40 mm with changing serum 25-hydroxyvitamin D [25(OH)D] levels

25(OH)D are entered into the models as restricted cubic splines (3 knots) with a reference value of 93.7 nmol/l (median value of the highest quartile). The 25(OH)D scale of 30 to 110 nmol/l relates to the range between 5\(^{th}\) and 95\(^{th}\) percentiles of values (n=3810). Dashed lines denote 95% confidence interval.
Association of 25(OH)D with aortic diameter

We explored the association of 25(OH)D with abdominal aortic diameter by stratifying the aortic diameter into aneurysmal (≥ 30 mm) and non-aneurysmal (< 30 mm) ranges (Table 4.3). Adjustments were made for age, smoking, CVD, hypertension, diabetes, dyslipidaemia, BMI and serum creatinine. There was no statistically significant association between 25(OH)D and aortic diameters in the non-aneurysmal range. For men with aortic diameters ≥ 30 mm, every 10-nmol/l decrease in 25(OH)D concentration was associated with 0.49 mm (95% CI 0.11 to 0.87) increase in mean aortic diameter. For those men with the lowest 25(OH)D quartile of values compared with the highest quartile, there was an associated 3.06 mm (95% CI 0.70 to 5.41) increment in aortic diameter. The associations between 25(OH)D and aortic diameter in the aneurysmal range persisted when aortic diameter was log-transformed. Every 10-nmol/l decrease in 25(OH)D concentration was associated with 0.01 mm (95% CI 0.00 to 0.02) increase in log-transformed aortic diameter. For those men with the lowest 25(OH)D quartile of values compared with the highest quartile, there was an associated 0.08 mm (95% CI 0.02 to 0.13) increment in log-transformed aortic diameter.

Table 4.3 Linear regression models evaluating the association of 25-hydroxyvitamin D [25(OH)D] with abdominal aortic diameter in aneurysmal and non-aneurysmal ranges in community-dwelling older men

<table>
<thead>
<tr>
<th>Aortic diameter &lt; 30 mm</th>
<th>Aortic diameter ≥ 30 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude Mean (95% CI)</td>
</tr>
<tr>
<td>25(OH)D</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>0.01 (-0.04 to 0.03)</td>
</tr>
<tr>
<td>Halving of 25(OH)D</td>
<td>-0.03 (-0.18 to 0.12)</td>
</tr>
<tr>
<td>25(OH)D quartile 1</td>
<td>-0.09 (-0.32 to 0.13)</td>
</tr>
<tr>
<td>25(OH)D quartile 2</td>
<td>0.05 (-0.17 to 0.28)</td>
</tr>
<tr>
<td>25(OH)D quartile 3</td>
<td>-0.03 (-0.26 to 0.19)</td>
</tr>
<tr>
<td>25(OH)D quartile 4</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Abbreviation: 95% CI, 95% confidence interval.
†Regression coefficient per 10-nmol/l decrease in 25(OH)D concentration.
‡Adjusted for age, smoking, cardiovascular disease, hypertension, diabetes, dyslipidaemia, body mass index and serum creatinine.

The quartiles for 25(OH)D corresponded to values of: 10.0-52.8 nmol/l (median 42.7 nmol/l), 52.9-67.3 (median 60.3 nmol/l), 67.4-81.6 nmol/l (median 73.8 nmol/l) and 81.7-238.4 nmol/l (median 93.7 nmol/l), respectively.
Subgroup analyses

Vitamin D supplementation might influence 25(OH)D concentration and these data were available at the Wave 2 assessment. Ten men with AAA and 90 men without AAA had reported taking these supplements (p=0.303). We performed a sensitivity analysis of the association between 25(OH)D and AAA by excluding men taking supplements. The effect estimate for the presence of an AAA in men with the lowest 25(OH)D quartile of values compared with the highest quartile was not significantly affected (OR 1.18, 95% CI 0.84 to 1.67). For AAA ≥ 35mm and AAA ≥ 40mm, the ORs were 1.95 (95% CI 1.08 to 3.50) and 5.56 (95% CI 1.90 to 16.32), respectively. Additional adjustments for physical activity, waist circumference and hsCRP also did not appreciably alter the association of 25(OH)D with AAA (data not shown).

When seasonality was added to the multivariable logistic regression models, the odds of having an AAA in men with the lowest 25(OH)D quartile of values compared with the highest quartile was 1.27 (95% CI 0.90 to 1.81). For AAA ≥ 35mm and AAA ≥ 40mm, the ORs were 1.92 (95% CI 1.07 to 3.47) and 4.69 (95% CI 1.59 to 13.85), respectively. For blood samples collected during the summer/autumn and winter/spring calendar periods, the lowest quartile of 25(OH)D values corresponded to 58.8 nmol/l and 48.0 nmol/l, respectively. When multivariable logistic regression analysis was performed using 25(OH)D collected during summer/autumn, the odds of having an AAA in men with the lowest 25(OH)D quartile of values compared with the highest quartile was 1.27 (95% CI 0.88 to 1.83). When this was repeated for 25(OH)D collected during winter/spring, the OR remained similar (OR 1.25, 95% CI 0.90 to 1.73). When the analyses were repeated for AAA ≥ 35mm and AAA ≥ 40mm stratified by the season of blood sample collection, the associations persisted.
4.5 Discussion

In this population-based sample of older men, there is an association between low 25(OH)D concentration and the presence of larger AAA, which is independent of traditional cardiovascular risk factors. In men with AAA, 25(OH)D concentration is also inversely correlated with abdominal aortic diameter in a dose-response relationship.

The strengths of this study include the large sample size of well-characterised community-dwelling older men. Measurement of the aortic diameter was performed in a standardised procedure using ultrasound. We acknowledge several limitations in this study. The observational design of our current study and the fact that blood samples were obtained from the participants several years (5.7 ± 0.9 years) after baseline aortic diameter measurements preclude determination of causality over time. We did not have data on calcium or parathyroid hormone, which can influence vitamin D metabolism. Aortic diameters in our cohort were likely to have increased by a small margin between the period of baseline measurement and blood sampling (93). However, we have assumed in this study that the small number of interval cases of AAA would not have substantially altered the results or introduced any bias. The self-selection of participants in subsequent follow-up suggests a possible ‘healthy survivor’ effect which might have biased our findings towards higher 25(OH)D concentrations in our cohort compared to the non-respondents. This could have moved the results of our study towards null hypothesis and lead to an underestimation of the association between low 25(OH)D with AAA. Our findings were therefore conservative. The study was limited to men and we were therefore unable to generalise our findings to women, although the prevalence of AAA is about 5 times greater in men than women (85).

To our knowledge, this is the first study to demonstrate an independent relationship between low vitamin D status with the presence and size of AAA in older men. Van de Luijtgaarden et al have recently reported on the vitamin D status in 236 patients with either thoracic or abdominal aneurysm (557). Although 9.7% of these patients with aortic aneurysm had vitamin D levels ≤ 25 nmol/l (‘severely deficient’), the lack of a control group and the combining of patients with thoracic and abdominal aneurysms make this study inconclusive with regards to the strength and independence of any association.
between vitamin D status and AAA presence and size (557). Several previous studies have attempted to explore the relationship between vitamin D status and vascular integrity which is conceivably impaired in AAA (558). AAA is characterised by reduced elastin concentration relative to collagen in the aortic wall (85), also considered to be the pathophysiology in arterial stiffness with reduced vascular compliance (559). Previous observational studies have associated low vitamin D status with increased arterial stiffness (551, 560-563). Other clinical studies have also reported on the observed association between 25(OH)D and PAD, an alternative model of vascular disease which shares similar yet distinct pathogenesis as AAA (547, 564-568). Our results extend these findings and further support the relevance of vitamin D to large artery disease in humans.

In experimental studies, 1,25(OH)D3 binds to the vitamin D receptor and modulates the expression of multiple proteins relevant to the arterial wall, including vascular endothelial growth factor (VEGF) and matrix metalloproteinase type 9 (556). Vitamin D exerts vasoprotective effects through the inhibition of VEGF-induced endothelial cell proliferation, the anti-proliferative effect on vascular smooth muscle cell, and the suppression of angiogenesis via induction of apoptosis (450, 569). It also modulates the inflammatory cascade by inhibiting the production of pro-inflammatory cytokines (interleukin-6 and interleukin-8) and the expression of adhesion molecules in human arterial endothelial cells (570). Finally, vitamin D plays a fundamental immune modulating role by augmenting T-helper 2 cell responses via selective cytokine production, a condition that predominates in AAA formation (571, 572).

Our prevalence of AAA was 7.4%, which was relatively high in comparison to those derived from other large AAA screening programs (511). There are a number of possible reasons: men aged up to 83 years, rather than the usual 65-74 years, were included in our study (aortic diameter increases with age), and the fact that measurement of aortic diameter was performed using ‘outer to outer’ wall (this gives the largest possible diameter). The relatively lower proportions of larger AAAs may be explained by these cases being offered surgery prior to the blood testing phase of the study. The inverse relationship between diabetes and AAA has been widely reported, albeit inconclusively due to statistically non-significant findings in some studies (573). Although we have found a positive association between AAA and the presence of diabetes by the end of Wave 2 screening, a previous
analysis using data in Wave 1 reported an inverse correlation between serum glucose and aortic diameter in non-diabetic men (98). Despite demonstrating seasonal variation in plasma 25(OH)D concentrations, this has not altered the association between low vitamin D status with the presence of larger AAA in our cohort of older men.

In conclusion, we found an inverse relationship between vitamin D status and the presence of larger AAA, irrespective of traditional CVD and associated risk burden, in community-dwelling older men. There is also an inverse dose-response association between 25(OH)D concentrations and the size of AAA, suggesting a role of vitamin D in the severity of aneurysmal arterial disease. Further prospective observational studies are needed to clarify the mechanisms underlying these associations and to explore the feasibility of interventional studies with abdominal aortic diameter as an endpoint.
4.6 Author contributions

Study conception and design: YW, LF, PN

Acquisition of data: LF, GH, PN

Statistical analyses: YW, KM

Interpretation of analyses: YW, LF, BY, KM, GH, PN

Drafting of manuscript: YW

Critical revision of manuscript: LF, BY, GH, PN

Final approval of manuscript: All authors
CHAPTER 5

HOMOCYSTEINE, FRAILTY, AND ALL-CAUSE MORTALITY IN OLDER MEN:
THE HEALTH IN MEN STUDY
CHAPTER 5: HOMOCYSTEINE, FRAILTY, AND ALL-CAUSE MORTALITY IN OLDER MEN: THE HEALTH IN MEN STUDY

This chapter was published in the Journals of Gerontology (Medical Sciences):

5.1 Abstract

Background: Frailty and hyperhomocysteinaemia are common in the older population. Our objectives were to determine whether elevated homocysteine (tHcy) is associated with frailty and mortality.

Methods: We conducted a prospective cohort study. tHcy was measured by immunoassay in 4248 community-dwelling men aged 70-88 years. Frailty was assessed with the FRAIL scale. Mortality was determined from the death registry.

Results: At baseline, 1117 men (26.3%) had high tHcy (≥ 15 µmol/l) and 685 (16.2%) were frail (i.e. having 3 or more deficits in the FRAIL scale). There were 749 deaths during a follow-up duration of 5.1 ± 1.3 years. In cross-sectional analysis, high tHcy was associated with increased prevalent frailty (OR 1.49, 95% CI 1.22 to 1.81) after adjusting for confounding factors. After a period of 5.3 ± 0.8 years, the longitudinal relationship between high tHcy and frailty was weakened in multivariate analysis (OR 1.25, 95% CI 0.95 to 1.65). When assessing the relationship between tHcy and incident frailty, the odds of being frail at follow-up for men with high tHcy and having zero deficit at baseline (i.e. FRAIL scale=0) were 1.59 (95% CI 0.88 to 2.89) in adjusted analysis. High tHcy also predicted all-cause mortality (HR 1.25, 95% CI 1.06 to 1.48) after adjusting for frailty and other covariates.
Conclusions: Hyperhomocysteinaemia is associated with the prevalence of frailty. It is also predictive of all-cause mortality, independent of frailty. Our results suggest that the association between tHcy and mortality is largely not mediated through the occurrence of frailty.
5.2 Introduction

Frailty is becoming increasingly common as the world’s population ages. It is defined as “a state of excess vulnerability to stressors due to age-related decline in physiologic reserve across multisystems, resulting in reduced ability to maintain or regain homeostasis after a destabilising event” (107). Differing conceptual approaches have been applied to describe this phenomenon, including incorporation of physical characteristics and function (113), and utilising a combination of clinical deficits and co-morbidity domain (574). The FRAIL scale was subsequently developed, which incorporates the above two approaches (575, 576). Frailty has been reported to independently predict risks of adverse health outcomes including falls, disability, institutionalization, health-related quality of life, and mortality (577-580).

Various factors are thought to mediate the development of frailty, such as advanced age and medical co-morbidities (581). The physiological correlates of frailty have also been explored, with no conclusive evidence of association between biomarkers and frailty to date (582). Homocysteine, a B-vitamin metabolite, is one possible candidate that may underlie the development of the frailty syndrome. This is a sulfur amino acid whose metabolism stands at the intersection of 2 pathways: remethylation, which requires folic acid and B12 coenzymes, and transsulfuration, which requires pyridoxal-5’-phosphate, the B6 coenzyme. Total plasma homocysteine (tHcy) has been shown to be inversely related to the intake and plasma levels of folate and B-vitamins (31), and as such, may be used as a surrogate biochemical marker to reflect their metabolic function (30). At the cellular level, sufficient stores of B-vitamins are essential for “one-carbon” transfer metabolisms, and their deficiencies may result in mitochondrial dysfunction with deleterious changes in cellular function (583). These could conceivably cause muscle weakness and atrophy, leading to sarcopenia with progressive physical decline (584). At the molecular level, B-vitamin deficiency may be mediated via hyperhomocysteinaemia through mechanisms of oxidative stress (585), or by homocysteinylation (328) which involves covalent binding of tHcy to proteins. These modified proteins or neoantigens can trigger the inflammatory cascade, resulting in vascular endothelium damage and subsequently vascular events, further leading to functional decline and frailty. Homocysteine-induced endothelial dysfunction can occur through different mechanisms, via atherosclerotic plaque formation and increased risk of
thromboembolic events (586). All these biological pathways could lead to a multisystem
decline due to destabilization of the neuromuscular and metabolic balance. In addition,
severe hyperhomocysteinaemia can cause endoplasmic reticulum stress leading to cellular
growth arrest and apoptosis (331), and ultimately accelerated ageing (587). This can result
in a higher risk of mortality, thus making this biochemical marker an important target for
investigating this adverse health outcome in older people. As frailty also predicts survival
(588), its influence should not be ignored whilst elucidating the biologic association of
tHcy with mortality.

In this study, we sought to determine if elevated tHcy is associated with frailty and
mortality in later life. We addressed these aims by investigating the cross-sectional and
longitudinal relationship between tHcy and frailty (measured by the FRAIL scale) using a
large cohort of community-dwelling men aged 70-88 years, as well as the longitudinal
relationship between elevated tHcy and all-cause mortality, taking into account the possible
mediating effect of frailty.
5.3 Methods

Study design and participants

We conducted a prospective cohort study, using participants from the Health in Men Study (HIMS), which has been described in detail elsewhere (528). In brief, 12203 community-dwelling men aged 65-87 years sampled from the electoral roll of Australia completed a health assessment between 1996 and 1999 (HIMS Wave 1). In 2001-2004, 10940 men were invited to participate in the second phase of this study (HIMS Wave 2) and blood samples were collected from 4249 of them. In 2008-2009 (HIMS Wave 3), 7445 surviving men were mailed a third questionnaire, of which 3274 responded. The Human Research Ethics Committee of the University of Western Australia approved the protocol for HIMS which was conducted in accordance with the Helsinki Declaration for Human Rights.

Outcome of interest

Frailty was assessed during Waves 2 and 3 with a FRAIL scale (575, 576). This consists of 5 domains: fatigue, resistance, ambulation, illness, and loss of weight. A score of 1 for each domain indicates its presence, and a score of 0 indicates its absence. Responses to the SF-36 Health Survey (589) during Waves 2 and 3 were used to assess symptoms of fatigue (worn out or feeling tired most of the time), resistance (inability to climb a flight of stairs) and ambulation (inability to walk 100m). A score of 1 was recorded for illness if the participant reported having more than five of the following during Waves 2 and 3 respectively: arthritis, diabetes, angina or myocardial infarction, hypertension, stroke, asthma, chronic bronchitis, emphysema, osteoporosis, colorectal cancer, skin cancer, depression or an anxiety disorder, Alzheimer’s disease or other dementia, or leg ulcers. Participants scored 1 for weight loss if their weight decreased by more than 5% between Waves 1 and 2, and between Waves 2 and 3. We considered participants to be frail if they scored a total of three or more in these domains (i.e. FRAIL scale ≥ 3). This approach has been validated by analysing the predictive utility of the scale for all-cause mortality and disability (577, 590).
Records of all hospital admissions and mortality were obtained from the Western Australian Data Linkage System (WADLS) (485), which links together records from the mental health data, cancer registry, death registry and hospital morbidity data.

**Explanatory variables**

The following socio-demographic variables were collected from participants: age (difference in years between date of assessment and date of birth), education (completed high school or better by Wave 1), living circumstance (living alone or in residential aged care facility during Waves 2 and 3) and smoking status (classified as never, former or current smoker during Waves 2 and 3). We identified cardiovascular disease, hypertension, diabetes and dyslipidaemia as potential confounders in the relationship between tHcy and mortality, and hence further elaborated the prevalence of these comorbid diseases from self-reported, clinical and biochemical data available from Waves 1, 2 and 3. Cardiovascular disease was present when the participant reported having a history of angina, myocardial infarction, heart failure, coronary artery bypass grafting, coronary angioplasty, carotid endarterectomy, aortic bypass surgery, peripheral arterial surgery or stroke, or use of medications at the time of assessment for these conditions. Men were considered to have hypertension if they reported having the condition or use of anti-hypertensive medications or had measured blood pressure of equal to or greater than 140/90 mmHg. Men who were diagnosed with diabetes, reported use of glucose-lowering medication, or had a fasting or non-fasting glucose of ≥7 mmol/l or ≥11 mmol/l respectively, were considered to have diabetes. Men who self-reported the condition or use of lipid-lowering medication, or had fasting low-density lipoprotein of 3.4 mmol/l or higher, high-density lipoprotein less than 0.9 mmol/l, triglycerides of 1.8 mmol/l or higher, or total cholesterol of 5.5 mmol/l or higher were considered to have dyslipidaemia.

Clinical information from WADLS were collected from 1990 to the time of blood sampling and the weighted Charlson Comorbidity Index calculated (530). The latter takes into account 17 common medical conditions that predict one-year mortality: myocardial infarction, congestive heart failure, peripheral arterial disease, cerebrovascular disease, dementia, chronic pulmonary disease, connective tissue disease, ulcers, liver disease,
diabetes (including diabetes with end organ damage), hemiplegia, renal disease, leukaemia, lymphoma, other tumours, metastatic tumours, and AIDS.

Biochemical analyses and other measures

Blood samples were collected during Wave 2 between 0800 and 1030. Plasma was separated from the blood samples within 1 hour of collection and stored at -80°C until assayed. tHcy was measured by fluorescence polarization immunoassay on an IMx analyzer (504) and dichotomised into ‘high tHcy’ (≥ 15 µmol/l) and ‘normal tHcy’ (< 15 µmol/l) as determined by the laboratory’s reference range. The inter-assay coefficient of variation was 4%.

Serum creatinine, glucose, cholesterol, low-density lipoprotein and triglycerides were measured with a Roche Hitachi 917 analyzer (Roche Diagnostics). High-sensitivity C-reactive protein (hsCRP) was measured with assay on a BNII analyzer (Dade Behring, Birmingham, UK). Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (591).

Height, weight and blood pressure were measured by trained research assistants during Wave 2. Height and weight were self-reported during Wave 3. Body mass index (BMI) was calculated from height and weight in kg/m².

Statistical analysis

Data were analysed using Stata release 11.1 (StataCorp, College Station, TX, USA). Descriptive statistics were calculated for the demographic, lifestyle and clinical variables in Waves 2 and 3 according to the FRAIL scale. The association between tHcy and frailty were investigated in three different ways: according to whether tHcy was ≥ 15 µmol/l, per 5-µmol/l increment in tHcy, and by doubling of tHcy concentration (by dividing the natural logarithm of tHcy by the natural logarithm of 2). To determine the cross-sectional and longitudinal relationship between tHcy and frailty, logistic regression analyses were used. Adjustments were made for age, education, living circumstance, smoking and renal function (using eGFR as proxy in this study). A sensitivity analysis was performed to determine whether exclusion of men who had a history of cardiovascular disease would affect the cross-sectional association. Logistic regression analyses were repeated for
incident cases of frailty during Wave 3 for those men with zero deficit at baseline (i.e. FRAIL scale =0 during Wave 2). The association between high tHcy and individual components of the FRAIL scale during Wave 3 was tested using multivariate logistic regression analyses.

Cox proportional hazards models and Cuzik’s test for trend were used to explore the association between tHcy and all-cause mortality. Adjustments were made for age, education, living circumstance, smoking, cardiovascular disease, diabetes, hypertension, dyslipidaemia, Charlson’s comorbidity index, renal function and baseline frailty status. The results were reported as Odds Ratio (OR) or Hazards Ratio (HR) with 95% confidence intervals (95% CI). P-values < 0.05 were considered statistically significant.
5.4 Results

A flow chart detailing disposition of the study participants is shown in Figure 5.1. Men who had died prior to Wave 2 follow-up or did not attend due to various reasons were older in age (p<0.001), more likely to be current or former smokers (p<0.001), and self-report a history of cardiovascular disease (p=0.025), hypertension (p<0.001) and diabetes (p<0.001) during Wave 1, in comparison to those men who subsequently responded in Wave 2. Similarly, men who had died prior to Wave 3 follow-up or had not attended to the questionnaire were older in age (p<0.001) and more likely to be current or former smokers (p<0.001) in Wave 2, compared to those men who had completed the questionnaire in Wave 3. However, there was no statistically significant difference in their Charlson’s comorbidity indices (p=0.155).
Men aged 65 years and older were randomly selected from electoral roll and invited to participate in HIMS (N=19352)

7149 men were excluded from study, out of town, unwell, untraceable or did not respond

12203 men attended clinic and completed Wave 1 questionnaire in 1996-1999

1418 men died before follow-up in Wave 2; 5200 men were unable to attend, unwell, untraceable or did not respond

4263 men attended clinic and completed Wave 2 questionnaire in 2001-2004
1322 men completed Wave 2 questionnaire by postage only
4249 of the above had blood samples collected from them

3340 men died before invitation to follow-up in Wave 3

7445 men were invited to participate in Wave 3

126 men died after invitation; 4045 men were unable to attend, unwell, untraceable or did not respond

3274 men completed Wave 3 questionnaire in 2008-2009, of which 1824 men had complete data on frailty components, comorbidities and plasma homocysteine levels

~ 122 ~
The socio-demographic, clinical and biochemical characteristics of the study population during Waves 2 and 3 according to FRAIL scale are shown in Table 5.1. tHcy levels were available for 4248 men, aged between 70 and 88 years, during Wave 2. 1117 men (26.3%) had high tHcy (≥ 15 µmol/l) and the mean (± SD) tHcy concentration for the Wave 2 cohort was 13.4 ± 5.6 µmol/l. 4227 men had complete data for frailty and were thus the focus of our cross-sectional analysis. 685 (16.2%) of these men were frail (i.e. having 3 or more deficits). After a follow-up period of 5.3 ± 0.8 years (Wave 3), 237 men (34.6%) who were frail at baseline died, compared to 498 men (14.0%) who were non-frail (i.e. having less than 3 deficits) (p<0.001). Of those participants who responded to Wave 3, 1824 had complete data for frailty, comorbidities and tHcy levels at baseline. They were similar in age, smoking and comorbidity status compared to the rest of the Wave 3 cohort who were not included in our longitudinal analysis. 461 men (25.3%) during Wave 3 were frail, out of which 131 (28.4%) had high tHcy.
In univariate cross-sectional logistic regression analyses (Table 5.2), high tHcy was associated with increased odds of being frail (OR 2.11, 95% CI 1.78 to 2.51). The association persisted after adjusting for age, education, living circumstance, smoking and eGFR (OR 1.49, 95% CI 1.22 to 1.81). When men with a history of cardiovascular disease were excluded from the models, the associations persisted in univariate (OR 2.03, 95% CI 1.56 to 2.63) and multivariate (OR 1.43, 95% CI 1.06 to 1.93) analyses. When modeled as continuous variables, elevated tHcy continued to be associated with prevalent frailty.
In longitudinal logistic regression analyses (Table 5.2), high tHcy was associated with increased odds of being frail after a period of 5.3 ± 0.8 years (OR 1.66, 95% CI 1.30 to 2.12). The association was weakened after adjusting for age, education, living circumstance, smoking and eGFR (OR 1.25, 95% CI 0.95 to 1.65). When assessing the longitudinal relationship between tHcy and incident frailty, only 809 men with FRAIL scale = 0 during Wave 2 were included in the analyses. The odds of being frail at follow-up for these men with high tHcy were 1.89 (95% CI 1.11 to 3.22). After adjusting for potential confounders, the odds were reduced to 1.59 (95% CI 0.88 to 2.89). The association between high tHcy and individual components of the FRAIL scale during Wave 3 was tested using multivariate logistic regression analyses. High tHcy at baseline predicted the ambulation (OR 1.32, 95% CI 1.01 to 1.72) component. There was no statistically significant association with the fatigue (OR 1.21, 95% CI 0.96 to 1.53), resistance (OR 1.07, 95% CI 0.84 to 1.37), illness (OR 1.07, 95% CI 0.77 to 1.48) and weight loss (OR 1.10, 95% CI 0.85 to 1.42) components.

Table 5.2 Univariate and multivariate logistic regression analyses of associations between elevated homocysteine (tHcy) and frailty (FRAIL scale ≥ 3) during HIMS Wave 2 and 3

<table>
<thead>
<tr>
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<th>Cross-sectional analyses</th>
<th>Longitudinal analyses</th>
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<tbody>
<tr>
<td></td>
<td>Univariate</td>
<td>Adjusted(^b)</td>
</tr>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>tHcy ≥ 15 µmol/l(^a)</td>
<td>2.11 (1.78 to 2.51)</td>
<td>1.49 (1.22 to 1.81)</td>
</tr>
<tr>
<td>Per 5-µmol/l increment in tHcy</td>
<td>1.38 (1.28 to 1.49)</td>
<td>1.20 (1.11 to 1.29)</td>
</tr>
<tr>
<td>Doubling of tHcy</td>
<td>2.38 (2.00 to 2.84)</td>
<td>1.67 (1.37 to 2.05)</td>
</tr>
</tbody>
</table>

Abbreviations: OR, Odds ratio; 95% CI, 95% confidence interval.
\(^a\)Odds ratio presented for high tHcy (≥ 15 µmol/l) in comparison with normal tHcy (< 15 µmol/l).
\(^b\)Adjusted for age, education, living circumstance, smoking and renal function (eGFR).
Among those participants who had data for tHcy levels during Wave 2, 749 (17.6%) men subsequently died during a mean follow-up duration of 5.1 ± 1.3 years (range 0.1 to 7.2 years). Men who died were older (p<0.001), had more co-morbidities (p<0.001) and had higher tHcy levels (15.1 ± 8.1 µmol/l versus 13.0 ± 4.8 µmol/l, p<0.001) than those who were alive by the end of the study. There was a graded association between tHcy and all-cause mortality, as shown in Figure 5.2 (z=8.9, p<0.001). This association was tested with multivariate Cox proportional hazards models (Table 5.3). After adjusting for age, education, living circumstance, smoking, cardiovascular disease, diabetes, hypertension, dyslipidaemia, Charlson co-morbidity index, renal function and frailty status at baseline, high tHcy continued to predict all-cause mortality (HR 1.25, 95% CI 1.06 to 1.48). When tHcy was included as quantitative variables, the associations remained significant.

Figure 5.2  Univariate Cox proportional hazards model exploring total plasma homocysteine (tHcy) levels and association with all-cause mortality.

The tHcy scale of 8 to 22 µmol/l relates to the range between 5th and 95th percentiles of values (n=3823). tHcy is entered as restricted cubic spline. Reference value for hazard ratio is 15 µmol/l. Dashed lines denote 95% confidence interval.
Table 5.3  Univariate and multivariate Cox proportional hazards models of associations between elevated homocysteine (tHcy) and all-cause mortality after 5.1±1.3 years

<table>
<thead>
<tr>
<th></th>
<th>Univariate</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>HR (95% CI)</td>
<td>HR (95% CI)</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>tHcy ≥ 15 µmol/l²</td>
<td>1.75</td>
<td>1.50</td>
<td>1.30</td>
<td>1.25</td>
</tr>
<tr>
<td></td>
<td>(1.51 to 2.03)</td>
<td>(1.29 to 1.74)</td>
<td>(1.10 to 1.54)</td>
<td>(1.06 to 1.48)</td>
</tr>
<tr>
<td>Per 5-µmol/l increment in</td>
<td>1.14</td>
<td>1.12</td>
<td>1.11</td>
<td>1.11</td>
</tr>
<tr>
<td>tHcy</td>
<td>(1.11 to 1.17)</td>
<td>(1.09 to 1.16)</td>
<td>(1.06 to 1.15)</td>
<td>(1.07 to 1.16)</td>
</tr>
<tr>
<td>Doubling of tHcy</td>
<td>1.89</td>
<td>1.60</td>
<td>1.41</td>
<td>1.37</td>
</tr>
<tr>
<td></td>
<td>(1.65 to 2.17)</td>
<td>(1.38 to 1.86)</td>
<td>(1.18 to 1.67)</td>
<td>(1.15 to 1.65)</td>
</tr>
</tbody>
</table>

Abbreviations: HR, Hazards ratio; 95% CI, 95% Confidence interval.
Model 1: adjusted for age.
Model 2: adjusted for age, education, living circumstance, smoking, cardiovascular disease, diabetes, hypertension, dyslipidaemia, Charlson co-morbidity index, renal function (eGFR).
Model 3: adjusted for age, education, living circumstance, smoking, cardiovascular disease, diabetes, hypertension, dyslipidaemia, Charlson co-morbidity index, renal function (eGFR), frailty status at baseline.
²Hazards ratio presented for high tHcy (≥ 15 µmol/l) in comparison with normal tHcy (< 15 µmol/l).
5.5 Discussion

Our study has demonstrated an association between elevated tHcy and prevalent frailty, independent of age and other known confounding factors. We also demonstrated that elevated tHcy levels are predictive of all-cause mortality, independent of frailty status and of other covariates. Although tHcy is likely to play some role in the development of frailty in older men, frailty is unlikely to be a major mediator of the association between tHcy and all-cause mortality.

This study, to our knowledge, is the first to investigate the relationship between tHcy and frailty in a large cohort of community-dwelling older men. Previous studies have explored the relationship between B-vitamins and metabolites with frailty, which resulted in mixed findings. Investigators of the Italian InCHIANTI study (592) found that low folate intake was independently associated with frailty (OR 1.84; 95% CI 1.14 to 2.98). Biochemical marker levels were, however, not analysed or correlated. Using cross-sectional data from the combined Women’s Health and Ageing Study (WHAS I and II), Michelon et al (24) found a higher prevalence of vitamin B12 deficiency among the frail community-dwelling older women compared to the non-frail, but no apparent association between frailty and serum levels of B-vitamins. Semba et al (593) analysed the relationship prospectively using a subset of this cohort (WHAS I) and concluded that there was no association between the B-vitamins and incident frailty after 3 years of follow-up. Analyses from the combined WHAS cohort were further race-stratified by Matteini et al (594) and investigations limited to Caucasian women. There were higher proportions of vitamin B12 deficiency and elevated methylmalonic acid (MMA) among the Caucasian women compared to the African American women, and these biomarkers were subsequently found to be related to frailty in Caucasian women (OR 0.69, 95% CI 0.49 to 0.99 for quantitative vitamin B12 levels; and OR 1.34, 95% CI 1.00 to 1.80 for MMA). 54 (9.8%) of the Caucasian women presented with hyperhomocysteinaemia (defined as > 13.9 µmol/l in the study), and there was no association between tHcy and frailty in adjusted analysis (OR 1.08; 95% CI 0.72 to 1.62). This is the only study to date that had explored the relationship between tHcy and frailty, and as the authors noted, should be extended to other races and gender to better elucidate the metabolic pathways of frailty. It may also have lacked statistical power to detect small to moderate effect changes.
Our finding that men with low and very high BMIs (<18.5 and ≥ 30 kg/m², respectively) showed increased frailty was consistent with a previous study conducted by Hubbard et al that utilised the English Longitudinal Study of Ageing cohort (595). Sarcopenia, arbitrarily defined as a loss of muscle protein mass and function, plays a predominant role in the pathogenesis and development of frailty (584). An increase in body fat mass may obscure the loss of muscle tissue, a condition termed as “sarcopenic obesity” which is related to physical disability (596). Hence, the weight loss component of our FRAIL scale may be of less significance in the operational definition of sarcopenia and frailty. About 19.8% of our participants met our weight loss criterion, compared with 43.2% for the fatigue domain and 29.9% for the resistance domain, both of which were measurements of physical health and function.

Despite demonstrating that high tHcy levels predicted the ambulation component of the FRAIL scale, we were unable to definitively exclude reverse causality where hyperhomocysteinaemia may be a consequence of poor physical health. To further eliminate the possibility of tHcy being a marker for a vascular event causing frailty (515), we performed a sensitivity analysis after excluding all men with a history of cardiovascular disease. The association between tHcy and frailty in the cross-sectional analysis persisted, suggesting a possible direct effect of tHcy in the pathogenesis of the frailty syndrome in ageing men. The hypothesis of tHcy-induced inflammation as a mechanism of physical decline (597) was tested when hsCRP was added to the fully adjusted model for frailty. The effect estimates were altered minimally (data not shown), implying that the relationship between tHcy and frailty in our cohort may be independent of the inflammatory pathway.

Frailty is known to be a dynamic condition, as frail older individuals can become non-frail, and pre-frail older individuals are more likely than non-frail individuals to transit to the full frailty syndrome (113). We did not establish strong associations between tHcy and frailty in our longitudinal analyses, and it is possible that we might have missed transitions of the frailty state that occurred during shorter intervals than our follow-up duration. We refined our analysis by excluding men who were pre-frail (i.e. having 1 or 2 deficits) and frail at baseline, and derived a stronger relationship between high tHcy and incident frailty in those men with zero deficit at baseline. Statistical power was reduced with the smaller number of men, and hence the wider confidence interval.
Our finding that elevated tHcy predicted all-cause mortality complements and extends those of prior epidemiological studies (353, 354, 598-600) and suggests that lowering tHcy levels may potentially reduce mortality risk, regardless of the frailty status. On the other hand, a meta-analysis of large randomised trials has not indicated a beneficial effect of B-vitamin therapies on vascular events or mortality in people at risk of or with established cardiovascular disease (601). It has been argued that these findings may be limited by study methodology and patient selection (602, 603), hence supporting the need for further observational and clinical trials with a view to developing appropriate primary preventive strategies. To address the possibility of reverse causality due to pre-existing ill health that might have led to elevated tHcy levels, we repeated the analyses after excluding those men who died within 6 months from baseline. The association with all-cause mortality persisted (OR 1.24, 95% CI 1.04 to 1.47), suggesting that tHcy might be a risk factor rather than a biomarker of this adverse outcome.

The strengths of this study include our large sample size of population-based community-dwelling men at baseline, with a wide range of tHcy concentrations and high prevalence of hyperhomocysteinaemia to investigate our hypotheses. The focus on this well-established cohort of older men aged 70 years and above was highly relevant in the study of frailty and mortality, with these men being at the highest risk for these adverse health outcomes, and from whom a wealth of clinical information was available. However, limitations include our reliance on self-reported weight data at Wave 3 which could possibly lead to an under- or over-estimation of its value, and hence misclassification bias of the FRAIL scale. The problems in using the SF-36 health survey among older adults have been discussed previously (604), and the measurement of frailty based on its components might be subjected to potential recall bias and day-to-day variation. We did not have access to B-vitamin concentrations for our cohort, and thus were unable to exclude the possibility of effect modification by prevailing folate concentrations. 60 (8.8%) men who were frail at baseline reported taking B-vitamin supplements at the time of assessment, compared to 181 (5.1%) men who were non-frail (p<0.001). We repeated our cross-sectional analysis after excluding those men who took B-vitamin supplements and found that the effect estimate of high tHcy on frailty remained essentially unchanged (OR 1.55, 95% CI 1.26 to 1.90). The self-selection of study participants might have biased our findings towards lower tHcy and
less frailty compared to the non-respondents, hence limiting the generalisability of our
study results to the total population in Australia. As we have pointed out, men who did not
respond or had died prior to the follow-up were older in age, more likely to be smokers or
ex-smokers, and had more comorbidities compared to those who had responded. This is
likely to move our results towards the null hypothesis and lead to an underestimation of the
association between elevated tHcy and increased risk of frailty. Interpretation of our results
will need to take this caveat into consideration.

In conclusion, hyperhomocysteinaemia is associated with the prevalence of frailty. Our
attempt to demonstrate a longitudinal relationship did not yield a significant correlation
between tHcy and incident frailty. Hyperhomocysteinaemia is predictive of all-cause
mortality, independent of the baseline frailty status. Our results suggest that the association
between tHcy and mortality is largely not mediated through the occurrence of frailty.
5.6 Author contributions

Study conception and design: YW, LF

Acquisition of data: OA, GH, LF

Statistical analyses: YW, KM

Interpretation of analyses: YW, OA, KM, BY, GH, LF

Drafting of manuscript: YW

Critical revision of manuscript: OA, BY, GH, LF

Final approval of manuscript: All authors
CHAPTER 6

LOW VITAMIN D STATUS IS AN INDEPENDENT PREDICTOR OF INCREASED FRAILTY AND ALL-CAUSE MORTALITY IN OLDER MEN: THE HEALTH IN MEN STUDY
CHAPTER 6: LOW VITAMIN D STATUS IS AN INDEPENDENT PREDICTOR OF INCREASED FRAILTY AND ALL-CAUSE MORTALITY IN OLDER MEN: THE HEALTH IN MEN STUDY

This chapter was published in the Journal of Clinical Endocrinology and Metabolism:

Wong YYE, McCaul KA, Yeap BB, Hankey GJ, Flicker L. Low vitamin D status is an independent predictor of increased frailty and all-cause mortality in older men: the Health In Men Study. J Clin Endocrinol Metab. 2013;98(9): 3821-8

6.1 Abstract

Context and objective: Hypovitaminosis D and frailty are common in the older population. We aimed to determine whether 25-hydroxyvitamin D [25(OH)D] concentrations are associated with frailty and mortality.

Design: Prospective cohort study.

Setting and participants: 4203 older men aged 70-88 years in Perth, Western Australia

Main outcome measures: 25(OH)D was measured by immunoassay. Frailty was assessed with the 5-point FRAIL (Fatigue, Resistance, Ambulation, Illness and Loss of weight) scale. Mortality was determined from the death registry via the Western Australian Data Linkage System.

Results: At baseline, 676 (16.1%) men were frail, as defined by having ≥3 deficits (FRAIL scale ≥ 3). In multivariate cross-sectional analysis, low vitamin D status, defined by the lowest quartile of 25(OH)D values (<52.9 nmol/l), was associated with increased prevalent frailty (odds ratio [OR] 1.96, 95% confidence interval [CI] 1.52 to 2.52) in comparison to the highest quartile of 25(OH)D values (>81.6 nmol/l). After a mean period of 5.3 years, the adjusted odds ratio of being frail at follow-up for men with low vitamin D and having zero deficit at baseline (FRAIL scale = 0) was 1.56 (95% CI 1.07 to 2.27). Low vitamin D...
also predicted all-cause mortality over a period of up to 9.2 years (hazards ratio 1.20, 95% CI 1.02 to 1.42), independent of baseline frailty and other covariates.

**Conclusion:** Hypovitaminosis D is associated with prevalent and incident frailty in older men. Hypovitaminosis D also predicts all-cause mortality, independent of frailty. The association between vitamin D and mortality is not solely dependent on the occurrence of frailty.
6.2 Introduction

Hypovitaminosis D is widespread in the older population (605). A systematic review by Holick et al reported that the prevalence of low vitamin D status amongst community-dwelling older adults ranges between 20-100% (605). Studies conducted in Australasia also revealed a higher risk of vitamin D deficiency in older people who were hospitalised or residing in residential facilities (383, 606).

The frailty syndrome, which commonly precedes deleterious health outcomes in older people (113), has been linked to various biomarkers (577, 607). Although the term frailty is an evolving concept, there is, to date, a lack of consensus regarding the definition and a standardised assessment tool for the purposes of clinical practice and research (575). The physical phenotype of frailty, conceptualised and operationalised by Fried et al, has predominated recent literature and is based on 5 measurable characteristics, namely unintentional weight loss, self-reported exhaustion, weakness of grip strength, slow walking speed, and low physical activity (113). Expanded models, which included additional domains such as cognitive impairment, diseases and geriatric syndromes, subsequently emerge and constitute the “frailty index” (608). This is operationalised as a risk index, with improved predictive validities for adverse health outcomes (608, 609).

Recent investigations have suggested a potential etiological role for vitamin D in the development of frailty, albeit not consistently (610-616). Both hypovitaminosis D and frailty have also been linked to increased mortality (113, 617), but it is uncertain whether either or both are causal risk factors for mortality. A clear independent relationship between vitamin D status with frailty and mortality has yet to be established in epidemiological studies, due to limitations of residual confounding, reverse causality bias and the possibilities of publication and citation biases. The diversity of frailty model components, the differing definitions of vitamin D insufficiency and different laboratory methods may also in part explain the divergence of findings in studies examining the association of vitamin D with frailty and mortality.

In this study, we explored the cross-sectional and longitudinal relationships between vitamin D and frailty in a large cohort of older men aged 70-88 years. We aimed to minimise potential confounding and reverse causality bias by adjusting for all potential
confounding factors and by seeking consistency of associations between vitamin D with prevalent and incident cases of frailty. We also explored the longitudinal relationship between low vitamin D and all-cause mortality, whilst accounting for the effect of frailty.
6.3 Methods

Study design and participants

We conducted a prospective cohort study, using participants from the Health in Men Study (HIMS), which has been described in detail elsewhere (89, 528). In brief, approximately 40000 men aged 65 years and older were randomised to the screening and control arms of the trial of screening for abdominal aortic aneurysm. 12203 men participated in screening and completed a health assessment between 1996 and 1999 (HIMS Wave 1). In 2001-2004, 5585 men responded to the second phase of this study (HIMS Wave 2) and blood samples were collected from 4249 of them. In 2008-2009 (HIMS Wave 3), 7445 surviving men were mailed a third questionnaire, of which 3274 responded. The Human Research Ethics Committee of the University of Western Australia approved the protocol for HIMS and informed consent was obtained from the participants.

Outcomes of interest

Frailty was assessed during Waves 2 and 3 with the FRAIL scale. As described previously (577, 607), this consists of 5 domains: Fatigue, Resistance, Ambulation, Illness, and Loss of weight. A score of 1 for each domain indicates its presence, and a score of 0 indicates its absence. Responses to the SF-36 Health Survey (589) during Waves 2 and 3 were used to assess symptoms of fatigue (worn out or feeling tired most of the time), resistance (inability to climb a flight of stairs) and ambulation (inability to walk 100m). A score of 1 was recorded for illness if the participant reported having >5 of the following during Waves 2 and 3 respectively: arthritis, diabetes, angina or myocardial infarction, hypertension, stroke, asthma, chronic bronchitis, emphysema, osteoporosis, colorectal cancer, skin cancer, depression or an anxiety disorder, Alzheimer’s disease or other dementia, or leg ulcers. Participants scored 1 for weight loss if their weight decreased by >5% between Waves 1 and 2, or between Waves 2 and 3. We considered participants to be frail if they scored a total of ≥3 in these domains (i.e. FRAIL scale ≥ 3). This approach has been validated by analysing the predictive utility of the scale for all-cause mortality and disability (577).
Mortality data was obtained from the Western Australian Data Linkage System (WADLS) (485), which links together records from the mental health data, cancer registry, death registry and hospital morbidity data.

Explanatory variables

The following variables were collected from participants: age, education (completed high school or better by Wave 1), living circumstance (living alone or in residential aged care facility during Waves 2 and 3), smoking status (never, former or current smoker during Waves 2 and 3), physical activity (≥ 150 minutes of moderate to vigorous exercise in a usual week during Waves 1 and 3), vitamin supplementation (taking multivitamin, calcium or vitamin D supplementation during Waves 2 and 3), cardiovascular disease (self-reported history of angina, myocardial infarction, heart failure, coronary artery bypass grafting, coronary angioplasty, carotid endarterectomy, aortic bypass surgery, peripheral arterial surgery or stroke during Waves 1, 2 and 3), hypertension (self-reported history or use of anti-hypertensive medication or had average measured blood pressure > 140/90 mmHg), diabetes (self-reported history or use of glucose-lowering medication or had a fasting or non-fasting glucose ≥7 mmol/l or ≥11 mmol/l respectively), and dyslipidaemia (self-reported history or use of lipid-lowering medication or had fasting low-density lipoprotein ≥3.4 mmol/l, high-density lipoprotein <0.9 mmol/l, triglycerides ≥1.8 mmol/l or total cholesterol ≥5.5 mmol/l). Clinical information from WADLS were collected from 1990 to the time of blood sampling and the weighted Charlson Comorbidity Index calculated (530). The latter takes into account 17 common medical conditions that predict one-year mortality: myocardial infarction, congestive heart failure, peripheral arterial disease, cerebrovascular disease, dementia, chronic pulmonary disease, connective tissue disease, ulcers, liver disease, diabetes (including diabetes with end organ damage), hemiplegia, renal disease, leukaemia, lymphoma, other tumours, metastatic tumours, and AIDS. Height, weight and blood pressure were measured by trained research assistants during Wave 2. Height and weight were self-reported during Wave 3. Body mass index (BMI) was calculated from height and weight in kg/m².
Biochemical analyses

Blood samples were collected during Wave 2 between 0800 and 1030. Plasma was separated from the blood samples within 1 hour of collection and stored at -80°C until assayed. Plasma 25-hydroxyvitamin D [25(OH)D], an established marker of vitamin D status, was measured using the automated DiaSorin “LIAISON 25(OH)D TOTAL” chemilumininescent immunoassay, which was carried out on archived serum in a series of runs performed between 2011 and 2012. The interassay coefficient of variation was 13.2% at 37.9 nmol/l and 11.3% at 131 nmol/l. The date of blood collection was documented and seasonality determined: summer (December-February), autumn (March-May), winter (June-August) and spring (September-November). Serum creatinine, glucose, cholesterol, low-density lipoprotein and triglycerides were measured with a Roche Hitachi 917 analyzer (Roche Diagnostics). Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation (591).

Statistical analysis

Analyses were performed using Stata release 11.1 (StataCorp, College Station, TX, USA). Descriptive statistics were calculated for the demographic, lifestyle and clinical data collected by the end of HIMS Wave 2, according to vitamin D status. The association between 25(OH)D and frailty was investigated in three different ways: per 10-nmol/l decrease in 25(OH)D concentration, by halving of 25(OH)D concentration (dividing the natural logarithm of 25(OH)D by the natural logarithm of 0.5), and according to 25(OH)D quartiles of values (using the highest quartile as reference). To determine the cross-sectional relationship between vitamin D and frailty, logistic regression analyses were used. Adjustments were made for age, education, living circumstance, smoking, physical activity, taking vitamin supplementation, renal function (using eGFR) and seasonality. A sensitivity analysis was performed, via the exclusion of men who had a history of cardiovascular disease. Logistic regression analyses were repeated for incident cases of frailty during Wave 3 for those men with zero deficit at baseline (i.e. FRAIL scale=0 during Wave 2). The associations between vitamin D and individual components of the FRAIL scale during Waves 2 and 3 were tested using multivariate logistic regression analyses.
Cox proportional hazards models were used to explore the association between vitamin D and all-cause mortality. Adjustments were made for age, education, living circumstance, smoking, cardiovascular disease, diabetes, hypertension, dyslipidaemia, Charlson’s comorbidity index, BMI, renal function, seasonality and baseline frailty status. The results were reported as Odds Ratio (OR) or Hazards Ratio (HR) with 95% confidence intervals (95% CI). P-values < 0.05 were considered statistically significant.
6.4 Results

The socio-demographic, clinical and biochemical characteristics of the study population by the end of HIMS Wave 2, according to vitamin D status, are shown in Table 6.1. 4203 men, aged 70-88 years, had complete data for 25(OH)D levels and frailty and were thus the focus of the cross-sectional analysis. The mean (± SD) 25(OH)D concentration for the cohort was 68.3 ± 23.3 nmol/l and the quartiles of values correspond to 10.0-52.8 (median 42.3 nmol/l), 52.9-67.3 nmol/l (median 60.3 nmol/l), 67.4-81.6 nmol/l (median 73.8 nmol/l) and 81.7-238.4 nmol/l (median 93.7 nmol/l), respectively. Sixty-five men had 25(OH)D concentration of >120 nmol/l and 10 of them (15.6%) were taking vitamin supplementation (p=0.094). They were younger in age (p<0.001) and more likely to be physically active (p=0.002) compared to those with 25(OH)D < 120 nmol/l. There was no statistically significant difference in their smoking habit (p=0.654), prevalence of cardiovascular disease (p=0.210) and number of comorbidities (p=0.290). Ten men had 25(OH)D >180 nmol/l and one of them (10%) was on vitamin supplementation (p=0.946).

676 (16.1%) of these men were frail (ie, having ≥ 3 deficits) at baseline. After a follow-up period of 5.3 ± 0.8 years (HIMS Wave 3), 1817 men who had responded to this wave had complete data for 25(OH)D at baseline and frailty. They were similar in age, smoking and comorbidity status compared with the rest of the Wave 3 cohort who were not included in this longitudinal analysis. 459 men (25.3%) during Wave 3 were frail, out of which 131 (28.5%) had low vitamin D (ie, 25(OH)D <52.9 nmol/l).
Table 6.1 Demographic, lifestyle and clinical characteristics of the study population by the end of HIMS Wave 2, according to vitamin D status

<table>
<thead>
<tr>
<th></th>
<th>25(OH)D in lowest quartile&lt;sup&gt;a&lt;/sup&gt; (n=1048)</th>
<th>25(OH)D in higher quartiles&lt;sup&gt;b&lt;/sup&gt; (n=3155)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, years&lt;sup&gt;c&lt;/sup&gt;</strong></td>
<td>76.9 (3.8)</td>
<td>76.4 (3.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Completed high school or better, n (%)</strong></td>
<td>531 (50.7)</td>
<td>1634 (51.8)</td>
<td>0.494</td>
</tr>
<tr>
<td><strong>Lived alone or in residential aged care facility, n (%)</strong></td>
<td>224 (21.4)</td>
<td>483 (15.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Smoking, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoked</td>
<td>328 (31.3)</td>
<td>1075 (34.0)</td>
<td>0.006</td>
</tr>
<tr>
<td>Former smoker</td>
<td>648 (61.8)</td>
<td>1937 (61.4)</td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>72 (6.9)</td>
<td>143 (4.5)</td>
<td></td>
</tr>
<tr>
<td><strong>Physical activity, n (%)</strong></td>
<td>186 (17.7)</td>
<td>769 (24.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Taking vitamin supplementation, n (%)</strong></td>
<td>87 (8.3)</td>
<td>305 (9.7)</td>
<td>0.188</td>
</tr>
<tr>
<td><strong>Cardiovascular disease, n (%)</strong></td>
<td>315 (30.0)</td>
<td>1024 (32.4)</td>
<td>0.139</td>
</tr>
<tr>
<td><strong>Hypertension, n (%)</strong></td>
<td>821 (79.3)</td>
<td>2375 (76.5)</td>
<td>0.065</td>
</tr>
<tr>
<td><strong>Diabetes, n (%)</strong></td>
<td>221 (21.1)</td>
<td>462 (14.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Dyslipidaemia, n (%)</strong></td>
<td>746 (71.2)</td>
<td>2230 (70.7)</td>
<td>0.757</td>
</tr>
<tr>
<td><strong>Charlson’s index score ≥ 5, n (%)</strong></td>
<td>66 (6.3)</td>
<td>103 (3.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>FRAIL scale ≥ 3, n (%)</strong></td>
<td>227 (21.7)</td>
<td>449 (14.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Presence of FRAIL scale component, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>508 (48.5)</td>
<td>1298 (41.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Resistance</td>
<td>372 (35.5)</td>
<td>872 (27.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ambulation</td>
<td>238 (22.7)</td>
<td>510 (16.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Illness</td>
<td>70 (6.7)</td>
<td>148 (4.7)</td>
<td>0.012</td>
</tr>
<tr>
<td>Loss of weight</td>
<td>207 (19.8)</td>
<td>624 (19.8)</td>
<td>0.985</td>
</tr>
<tr>
<td><strong>BMI, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;18.5 kg/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>13 (1.2)</td>
<td>14 (0.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>18.5-24.9 kg/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>304 (29.0)</td>
<td>1118 (35.4)</td>
<td></td>
</tr>
<tr>
<td>25.0-29.9 kg/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>531 (50.7)</td>
<td>1606 (50.9)</td>
<td></td>
</tr>
<tr>
<td>≥30 kg/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>200 (19.1)</td>
<td>417 (13.2)</td>
<td></td>
</tr>
<tr>
<td><strong>25(OH)D, nmol/l</strong></td>
<td>40.8 (9.2)</td>
<td>77.5 (18.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>eGFR, ml/min/1.73 m&lt;sup&gt;2&lt;/sup&gt;</strong></td>
<td>70.7 (16.0)</td>
<td>70.5 (14.9)</td>
<td>0.641</td>
</tr>
<tr>
<td><strong>Season of blood collection, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summer/Autumn (Dec-May)</td>
<td>329 (31.4)</td>
<td>1682 (53.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Winter/Spring (Jun-Nov)</td>
<td>719 (68.6)</td>
<td>1473 (46.7)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Low vitamin D status defined as 25(OH)D in the lowest quartile of values (10.0-52.8 nmol).

<sup>b</sup>Remaining men defined by the combination of the higher quartiles of values as a single category (52.9-238.4 nmol/l).

<sup>c</sup>Mean (SD).
Cross-sectional analyses between 25(OH)D and prevalent frailty during Wave 2

In multivariate cross-sectional logistic regression analyses (Table 6.2), low vitamin D was associated with increased odds of being frail, in comparison to the highest quartile of 25(OH)D values (OR 1.96, 95% CI 1.52 to 2.52). Adjustments were made for age, education, living circumstance, smoking, physical activity, taking vitamin supplement, renal function and seasonality. When men with a history of cardiovascular disease were excluded from the model, the association persisted (OR 1.55, 95% CI 1.10 to 2.18). Every 10 nmol/l decrease in 25(OH)D concentration was associated with an increased adjusted odds of 1.12 (95% CI 1.07 to 1.17) in prevalent frailty.

Longitudinal analyses between 25(OH)D and incident frailty during Wave 3

When assessing the longitudinal relationship between vitamin D and incident frailty (Table 6.2), only 1625 men with FRAIL scale=0 during Wave 2 were included in the analyses. The odds of being frail at follow-up for these men with low vitamin D were 1.56 (95% CI 1.07 to 2.27), in comparison to the highest quartile of 25(OH)D values. When modeled as continuous variables, lower 25(OH)D concentrations continued to be associated with incident frailty. Figure 6.1 demonstrates the odds of prevalent and incident frailty with changing 25(OH)D concentrations.
Table 6.2 Logistic regression analyses of associations between low vitamin D status and frailty (FRAIL scale ≥ 3)

<table>
<thead>
<tr>
<th></th>
<th>Prevalent frailty</th>
<th>Incident frailty^c</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude OR (95% CI)</td>
<td>Adjusted OR^b (95% CI)</td>
</tr>
<tr>
<td>Per 10-nmol/l decrease in 25(OH)D</td>
<td>1.13 (1.09 to 1.17)</td>
<td>1.12 (1.07 to 1.17)</td>
</tr>
<tr>
<td>Halving of 25(OH)D</td>
<td>1.68 (1.45 to 1.95)</td>
<td>1.63 (1.39 to 1.92)</td>
</tr>
<tr>
<td>25(OH)D quartile 1^a</td>
<td>2.03 (1.61 to 2.58)</td>
<td>1.96 (1.52 to 2.52)</td>
</tr>
<tr>
<td>25(OH)D quartile 2</td>
<td>1.40 (1.10 to 1.80)</td>
<td>1.42 (1.10 to 1.84)</td>
</tr>
<tr>
<td>25(OH)D quartile 3</td>
<td>1.23 (0.95 to 1.58)</td>
<td>1.22 (0.94 to 1.58)</td>
</tr>
<tr>
<td>25(OH)D quartile 4</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; OR, Odds ratio; 95% CI, 95% confidence interval.
^a25(OH)D quartiles of values corresponded to thresholds of 10.0-52.8 nmol/l, 52.9-67.3 nmol/l, 67.4-81.6 nmol/l, 81.7-238.4 nmol/l, respectively.
^bAdjusted for age, education, living circumstance, smoking, physical activity, taking vitamin supplement, renal function (eGFR) and seasonality.
^cOnly men with FRAIL scale = 0 during Wave 2 were included in the analyses.
Figure 6.1  Odds ratio of prevalent and incident frailty during Wave 2 (A) and Wave 3 (B), respectively, with changing serum 25-hydroxyvitamin D [25(OH)D] levels.

25(OH)D are entered into the models as restricted cubic splines (3 knots) with a reference value of 50.0 nmol/l. Dashed lines denote 95% confidence interval.
Analyses between 25(OH)D and individual components of the FRAIL scale

The associations between lower 25(OH)D concentrations and individual components of the FRAIL scale during Waves 2 and 3 were tested using multivariate logistic regression analyses (Table 6.3). Lower 25(OH)D levels at baseline almost consistently predicted the fatigue, resistance and ambulation components.

Table 6.3 Multivariate logistic regression analyses\(^a\) of associations between low vitamin D status and the components of FRAIL scale during HIMS Waves 2 and 3

<table>
<thead>
<tr>
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<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>1.08 (1.05 to 1.11)</td>
<td>1.06 (1.02 to 1.11)</td>
</tr>
<tr>
<td>Resistance</td>
<td>1.08 (1.04 to 1.11)</td>
<td>1.06 (1.02 to 1.11)</td>
</tr>
<tr>
<td>Ambulation</td>
<td>1.09 (1.05 to 1.13)</td>
<td>1.08 (1.03 to 1.14)</td>
</tr>
<tr>
<td>Illness</td>
<td>1.08 (1.02 to 1.16)</td>
<td>1.03 (0.97 to 1.10)</td>
</tr>
<tr>
<td>Weight loss</td>
<td>0.99 (0.96 to 1.02)</td>
<td>1.05 (1.00 to 1.10)</td>
</tr>
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</table>

Per 10-nmol/l decrease in 25(OH)D:

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<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>1.42 (1.25 to 1.61)</td>
<td>1.18 (0.98 to 1.43)</td>
</tr>
<tr>
<td>Resistance</td>
<td>1.41 (1.23 to 1.61)</td>
<td>1.36 (1.12 to 1.64)</td>
</tr>
<tr>
<td>Ambulation</td>
<td>1.46 (1.25 to 1.70)</td>
<td>1.37 (1.10 to 1.70)</td>
</tr>
<tr>
<td>Illness</td>
<td>1.40 (1.09 to 1.81)</td>
<td>1.19 (0.92 to 1.54)</td>
</tr>
<tr>
<td>Weight loss</td>
<td>1.02 (0.88 to 1.18)</td>
<td>1.23 (1.01 to 1.50)</td>
</tr>
</tbody>
</table>

Halving of 25(OH)D:

<table>
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<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>1.67 (1.34 to 2.01)</td>
<td>1.33 (1.01 to 1.75)</td>
</tr>
<tr>
<td>Resistance</td>
<td>1.65 (1.35 to 2.02)</td>
<td>1.70 (1.29 to 2.25)</td>
</tr>
<tr>
<td>Ambulation</td>
<td>1.58 (1.25 to 2.00)</td>
<td>1.56 (1.13 to 2.14)</td>
</tr>
<tr>
<td>Illness</td>
<td>1.59 (1.07 to 2.36)</td>
<td>1.42 (0.97 to 2.07)</td>
</tr>
<tr>
<td>Weight loss</td>
<td>0.92 (0.74 to 1.16)</td>
<td>1.30 (0.97 to 1.74)</td>
</tr>
</tbody>
</table>

Low vitamin D\(^b\):

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>1.16 (1.25 to 1.58)</td>
<td>1.16 (1.25 to 1.56)</td>
</tr>
<tr>
<td>Resistance</td>
<td>1.17 (1.35 to 1.56)</td>
<td>1.17 (1.35 to 1.56)</td>
</tr>
<tr>
<td>Ambulation</td>
<td>1.17 (1.25 to 1.56)</td>
<td>1.17 (1.25 to 1.56)</td>
</tr>
<tr>
<td>Illness</td>
<td>1.16 (1.25 to 1.56)</td>
<td>1.16 (1.25 to 1.56)</td>
</tr>
<tr>
<td>Weight loss</td>
<td>1.16 (1.25 to 1.56)</td>
<td>1.16 (1.25 to 1.56)</td>
</tr>
</tbody>
</table>

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; OR, Odds ratio; 95% CI, 95% confidence interval. **Bold:** P-value <0.05.

\(^a\)Adjusted for age, education, living circumstance, smoking, physical activity, taking vitamin supplement, renal function (eGFR) and seasonality.

\(^b\)Low vitamin D status defined as 25(OH)D in the lowest quartile of values (i.e. < 52.9 nmol/l) in comparison to the highest quartile of values (i.e. > 81.6 nmol/l).
Analyses between 25(OH)D and all-cause mortality

Among those participants who had data for 25(OH)D levels during Wave 2, 1144 men (27.2%) subsequently died during a mean follow-up duration of 6.7 ± 1.8 years (range 0.6-9.2 years). 322 men (47.6%) who were frail at baseline died, compared with 822 men (23.3%) who were nonfrail (ie, having < 3 deficits; p<0.001). Men who died were also older (p<0.001), had more comorbidities (p<0.001) and had lower 25(OH)D concentrations (66.2 ± 23.2 nmol/l vs 69.1 ± 23.3 nmol/l, p<0.001) than those who were alive by 31st December 2010. As shown in Figure 6.2, the inverse association between 25(OH)D and all-cause mortality was apparent only at lower levels of 25(OH)D (≤ 50 nmol/l). This association was tested with multivariate Cox proportional hazards models (Table 6.4). After adjusting for age, education, living circumstance, smoking, cardiovascular disease, diabetes, hypertension, dyslipidaemia, Charlson’s comorbidity index, BMI, renal function, seasonality and baseline frailty status, low vitamin D continued to predict all-cause mortality (HR 1.20, 95% CI 1.02 to 1.42). Exclusion of men who took vitamin supplementation did not alter the effect estimate substantially (HR 1.22, 95% CI 1.03 to 1.45). After excluding those men who died within 6 months from baseline, the association of low vitamin D with all-cause mortality persisted (HR 1.18, 95% CI 1.00 to 1.40). When 25(OH)D was included as quantitative variables, the associations remained significant.

Figure 6.2  Univariate Cox proportional hazards model exploring the association between serum 25-hydroxyvitamin D [25(OH)D] levels and all-cause mortality (n=4229)

25(OH)D is entered as restricted cubic spline (3 knots) with a reference value of 50 nmol/l. Dashed lines denote 95% confidence interval.
Table 6.4  Univariate and multivariate Cox proportional hazards models of associations between vitamin D status and all-cause mortality after 6.7 ± 1.8 years

<table>
<thead>
<tr>
<th></th>
<th>Univariate HR (95% CI)</th>
<th>Model 1 HR (95% CI)</th>
<th>Model 2 HR (95% CI)</th>
<th>Model 3 HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per 10-nmol/l decrease in 25(OH)D</td>
<td>1.05 (1.03 to 1.08)</td>
<td>1.04 (1.02 to 1.07)</td>
<td>1.05 (1.02 to 1.08)</td>
<td>1.04 (1.01 to 1.07)</td>
</tr>
<tr>
<td>Halving of 25(OH)D</td>
<td>1.31 (1.18 to 1.45)</td>
<td>1.25 (1.12 to 1.38)</td>
<td>1.27 (1.13 to 1.42)</td>
<td>1.21 (1.08 to 1.35)</td>
</tr>
</tbody>
</table>

| 25(OH)D quartile 1<sup>a</sup> | 1.29 (1.10 to 1.51) | 1.23 (1.05 to 1.45) | 1.24 (1.05 to 1.46) | 1.20 (1.02 to 1.42) |
| 25(OH)D quartile 2          | 1.00                 | 1.00                 | 1.00                 | 1.00                 |
| 25(OH)D quartile 3          | 0.98 (0.83 to 1.15)  | 0.98 (0.83 to 1.15)  | 0.98 (0.83 to 1.17)  | 0.99 (0.84 to 1.17)  |
| 25(OH)D quartile 4          | 0.96 (0.81 to 1.14)  | 0.97 (0.82 to 1.15)  | 0.95 (0.82 to 1.15)  | 0.99 (0.83 to 1.17)  |

Abbreviations: HR, Hazards ratio; 95% CI, 95% Confidence interval; 25(OH)D, 25-hydroxyvitamin D.

Model 1: adjusted for age.
Model 2: adjusted for age, education, living circumstance, smoking, cardiovascular disease, diabetes, hypertension, dyslipidaemia, Charlson co-morbidity index, body mass index, renal function (eGFR), seasonality.
Model 3: adjusted for all covariates in Model 2, and for frailty status at baseline.

<sup>a</sup> 25(OH)D quartiles of values corresponded to thresholds of 10.0-52.8 nmol/l, 52.9-67.3 nmol/l, 67.4-81.6 nmol/l, 81.7-238.4 nmol/l, respectively.
6.5 Discussion

This study demonstrated that older men with low vitamin D status of < 50 nmol/l are nearly twice as likely to be frail in comparison to those with higher vitamin D levels > 80 nmol/l. For older men who are nonfrail at baseline and have low vitamin D levels, there is a greater than 50% increased risk of becoming frail at five years. Hypovitaminosis D is also an independent predictor of all-cause mortality, regardless of frailty status or other comorbidities. These findings imply that vitamin D may be a contributor in the development of frailty, although frailty is unlikely to play a major role in the association between vitamin D and all-cause mortality.

The strengths of this study include our large sample size of population-based men at baseline, with a wide range of 25(OH)D concentrations to investigate our hypotheses. Our results were robust with the modeling of 25(OH)D as both a categorical and continuous variable. The focus on this established and well-characterised cohort of older men aged 70 years and above was highly relevant in the study of frailty and mortality, with these men being at the highest risk for these health outcomes. However, there were some limitations in this study, including our reliance on self-reported weight data at Wave 3 which could possibly lead to an under- or over-estimation of its value, and hence misclassification bias of the FRAIL scale. Problems in using the SF-36 health survey among older adults have been discussed previously (604), and the measurement of frailty based on its components might be subjected to potential recall bias and day-to-day variation. We did not have calcium and parathyroid hormone data, which may influence vitamin D metabolism. The self-selection of study participants might have biased our findings towards higher 25(OH)D levels and less frailty compared to the non-respondents, hence limiting the generalisability of our study results to the total population of men in Australia. Men who did not respond or had died prior to Wave 2 follow-up were older in age (p<0.001), more likely to be current or former smokers (p<0.001), and had more self-reported comorbidities (p<0.001) during Wave 1 compared to the non-respondents of the follow-up study. This is likely to bias our results towards the null hypothesis and lead to an underestimation of the association between vitamin D and frailty. Interpretation of our results will need to take this caveat into consideration.
Our findings that hypovitaminosis D is associated with frailty are complementary and extend those found in the Longitudinal Ageing Study Amsterdam (616). In that study, frailty was defined as three out of nine frailty indicators (low BMI, low peak expiratory flow, cognitive functioning, poor distant vision and hearing problems, incontinence, low sense of mastery, depressive symptoms and physical activity) and serum 25(OH)D was measured by a competitive binding protein assay. The odds of being frail at baseline for 25(OH)D < 25 nmol/l vs > 50 nmol/l was 2.60 (95% CI 1.60 to 4.21). Low 25(OH)D was also associated with incident frailty (OR 2.04, 1.01 to 4.13) (616). Other epidemiological studies utilising differing frailty definitions and 25(OH)D measurement methods have also reported consistent findings of a significant association between low vitamin D status and prevalent frailty in older men (610-612, 614, 615). When the relationship was examined prospectively in the Osteoporotic Fractures in Men study, there was no apparent association between low vitamin D status and frailty after 4.6 years (612). This discrepancy in findings may be explained by reduced statistical power.

We demonstrated that low vitamin D is associated with and predictive of the fatigue, resistance and ambulation domains of the FRAIL scale, all of which are measures of the physical health component of the SF-36 (589) as well as manifestations of sarcopenia (584). This is in line with the relevant biological pathways through which vitamin D may mediate the development of frailty. The active metabolite, calcitriol, is known to initiate muscle protein synthesis and enhance the contractile properties of muscle (405). Calcitriol also influences calcium homeostasis via a nongenomic mechanism with consequent increased muscle strength (411). Low vitamin D status has been associated with sarcopenia, muscle weakness, poor physical performance and increased falls (411, 618-620), all of which may accelerate the frailty process. Vitamin D also downregulates inflammatory markers such as interleukin-12, and its deficiency may result in increased pro-inflammatory cytokines which can impact on muscle strength and performance (597, 621).

Our findings also suggest that low vitamin D status of < 50 nmol/l may be detrimental to survival in older men, an association which has been consistently reported in other prospective cohort studies (617, 622, 623). The recruitment age groups in these studies were younger (ranged between 20 and 70 years) and despite emerging evidence which suggested a U-shaped association between vitamin D status and mortality (624-626), we
did not demonstrate a higher risk of mortality with increased 25(OH)D concentrations in our study population of older men. This is possibly due to the limited number of participants with higher levels of 25(OH)D. In a prospective, community-based cohort study conducted in the United States, low 25(OH)D concentrations were associated with increased risk of adverse clinical events (composite clinical outcome of hip fracture, myocardial infarction, cancer and death) in older adults aged ≥ 65 years (HR 1.24, 95% 1.09 to 1.42) (627). To test the possibility that pre-existing ill-health might have led to restricted outdoor activity and subsequent hypovitaminosis D (reverse causality), we repeated our analyses with exclusion of men who died within 6 months from baseline. The association of low vitamin D with all-cause mortality persisted, suggesting that hypovitaminosis D might be a risk factor rather than an epiphenomenon of this adverse outcome. Although vitamin D supplementation increases 25(OH)D concentrations (628), clinical trials on survival has not supported the effect of vitamin D alone (629, 630). Further clarification and investigation on the long term benefits of vitamin D supplementation and the association with different baseline vitamin D levels would be warranted, which provides rationale for future large intervention studies.

In conclusion, hypovitaminosis D is associated with prevalent frailty and predictive of incident frailty in older men. It is also predictive of all-cause mortality, independent of the baseline frailty status. Although we have shown a significant association in our longitudinal analyses, a causal relationship cannot be inferred. Adequately powered randomised clinical trials focusing on physical performance and frailty will be warranted to definitively conclude on the potential benefit of vitamin D supplementation as a risk reduction strategy. Our results suggest that the association between vitamin D and mortality is largely not mediated through the occurrence of frailty.
6.6 Author contributions

Study conception and design: YW, LF

 Acquisition of data: LF, GH

 Statistical analyses: YW, KM

 Interpretation of analyses: YW, KM, BY, GH, LF

 Drafting of manuscript: YW

 Critical revision of manuscript: JG, BY, GH, LF

 Final approval of manuscript: All authors
CHAPTER 7

ELEVATED HOMOCYSTEINE IS ASSOCIATED WITH POORER SELF-PERCEIVED PHYSICAL HEALTH IN OLDER MEN: THE HEALTH IN MEN STUDY
7.1 Abstract

Objectives: To determine the relationship between high total homocysteine (tHcy) and self-perceived physical health, by investigating the associations between tHcy, the methylenetetrahydrofolate reductase (MTHFR) 677T polymorphism and physical health-related quality of life (HRQOL).

Study design: We conducted a cross-sectional study using a cohort of 4248 community-dwelling men aged 70-88 years.

Main outcome measures: In addition to clinical determinants of physical health, tHcy was measured by immunoassay, the MTHFR 677T polymorphism was detected by a polymerase chain reaction (PCR)-based method, and physical HRQOL were assessed with the SF-36 Health Survey.

Results: In multiple regression analyses, the odds of being in the lowest quartile of the physical component summary (PCS) scores (i.e. < 35) was 1.47 (95% CI 1.21 to 1.78) for men with high tHcy (≥ 15 µmol/l), after adjusting for age, smoking, history of hazardous alcohol use, polypharmacy, prevalent falls and weighted Charlson co-morbidity index. When history of hypertension, heart disease, stroke, arthritis and osteoporosis were included in place of the Charlson’s index, the result was unchanged (OR 1.45, 95% CI 1.20 to 1.75). Men with the MTHFR TT homozygosity had significantly higher tHcy
concentration than those with the CC genotype (mean difference of 1.38 µmol/l, 95% CI 0.77 to 1.99). However, there was no apparent association between the MTHFR polymorphism and PCS.

**Conclusion:** Elevated tHcy is associated with poorer self-perceived physical health in community-dwelling older men. The results of this study support further longitudinal investigations to assess this relationship prospectively.
7.2 Introduction

Maintaining or improving health-related quality of life (HRQOL) has been identified as a major priority, and hence an important health outcome in older people. From the perspective of older individuals, quality of life is not only determined by treatment of their specific disease, but also their ability to function and remain independent at acceptable levels. Self-rated HRQOL has been reported to independently predict risk of mortality (631), particularly the physical function subscale of the SF-36 Health Survey (589, 632). The physical components of the SF-36 are shown to be positively correlated with objective physical performance measures in community-dwelling older adults and as such, may be used as proxy of self-perceived physical health (633).

Previous studies have explored associations of disease-specific biochemical markers with HRQOL (634, 635), but few have investigated the direct relationship between homocysteine and HRQOL. Homocysteine is an amino acid intermediate formed during the metabolism of methionine, a process which requires B-vitamins as cofactors. Total plasma homocysteine (tHcy) has been shown to be inversely related to folate and B-vitamin status (31) and therefore may be used as a surrogate biochemical marker to reflect the metabolic function of B-vitamins (30). At the cellular level, sufficient stores of B-vitamins are essential for “one-carbon” transfer metabolisms, and their deficiencies may result in mitochondrial dysfunction with deleterious changes in cellular function (583). These could conceivably cause muscle weakness and atrophy, leading to progressive physical decline. At the molecular level, B-vitamin deficiency may be mediated via hyperhomocysteinaemia through mechanisms of oxidative stress (585), or by homocysteinylation (328) which involves covalent binding of tHcy to proteins. These modified proteins or neoantigens can trigger the inflammatory cascade, resulting in vascular endothelium damage and subsequently cerebro- or cardiovascular events, leading to functional and physical deterioration. tHcy-induced endothelial dysfunction can also occur through different mechanisms, via atherosclerotic plaque formation and increased risk of thromboembolic events (586). This may in turn explain the increase in white matter hyperintensities and their correlations with impaired cognitive and lower extremity function (636). The direct neurotoxic effects by tHcy via excitotoxicity (637) and apoptosis (638) may explain the pathogenesis of neurodegenerative diseases which can affect physical function. tHcy may
play a causal role in these associated pathologies leading to physical decline, or be a biomarker for other underlying processes which mediate these disorders. In the presence of plausible biological pathways, it is therefore an important target for investigating poor physical health and function in older people. In this study, we hypothesised that men with elevated tHcy levels would have lower scores in the physical SF-36 components that indicate poorer self-perceived physical health, relative to those men with normal tHcy levels.

In an attempt to explore the potential causal role of tHcy on poor physical health, we also investigated the association between the methylenetetrahydrofolate reductase (MTHFR) polymorphism and physical HRQOL. This gene has been established as an important genetic determinant of tHcy via the 677C→T polymorphism which generates the 677T variant. This results in a thermolabile enzyme that is less effective in the remethylation of homocysteine to methionine and consequently, a 20% increase in basal plasma tHcy (321). Previous studies have indicated that the TT variant of the MTHFR genotype may be associated with higher risk of vascular events, including coronary heart disease (639) and ischaemic strokes (640). Based on these findings, we hypothesised that men with the MTHFR TT genotype would have lower scores in the physical SF-36 components, relative to those having the CC genotype.
7.3 Methods

Study design and participants

We conducted a cross-sectional study using participants from the Health in Men Study (HIMS), which has been described in detail elsewhere (528). In brief, 12203 community-dwelling men aged 65-87 years sampled from the electoral roll of Australia completed a health assessment between 1996 and 1999 (i.e. HIMS Wave 1). Between 2001 and 2004, surviving men (n=9718) were invited to participate in the second phase of this study (i.e. HIMS Wave 2) and blood samples were collected from 4249 of them. The latter are the focus of this study. The Human Research Ethics Committee of the University of Western Australia approved the protocol for HIMS which was conducted in accordance with the Helsinki Declaration for Human Rights.

Outcome of interest

HRQOL was assessed during Wave 2 using the Medical Outcomes Study Short-Form (SF-36) Health Survey (589). This yields two component summaries, which in turn are composed of eight subscales of health profile. Each subscale is measured on a scale of 0 to 100, with higher scores representing better health. Standardised scores for the physical component summary (PCS) were calculated using weights from the Australian population norms (641). The Physical Functioning (PF), Role-Physical (RP) and Bodily Pain (BP) scales, together with the PCS, have been shown to be the most valid physical health measures, since they are most responsive to treatment that target physical morbidity (642).

Explanatory variables

The following information was collected: age (difference in years between the date of assessment in Wave 2 and the participant’s date of birth), highest level of education attained (completed or not high school) and smoking status (non-smokers, former smokers and current smokers). Information on alcohol use (hazardous alcohol use defined as consumption of 14 or more standard drinks per week) and physical activity (vigorous and/or non-vigorous exercises in a usual week) were retrieved from the Wave 1 assessment. The prevalence of medical conditions was determined from self-reported data from Waves 1 and 2. An affirmative answer to the question in Wave 2: “In the last 12 months, have you...
had a fall to the ground (not including stumbles or trips)?” was considered indicative of prevalent falls. Polypharmacy was defined as a daily intake of ≥ 5 medicines at the time of Wave 2 assessment. Records of all hospital admissions from 1990 to Wave 2 were obtained from the Western Australian Data Linkage System (WADLS), which provides electronic linkage to the state’s use of health services and medical morbidities (485). The health morbidities of participants were coded and the weighted Charlson Co-morbidity Index calculated (530). The latter takes into account 17 common medical conditions that predict one-year mortality: myocardial infarction, congestive heart failure, peripheral arterial disease, cerebrovascular disease, dementia, chronic pulmonary disease, connective tissue disease, ulcers, liver disease, diabetes (including diabetes with end organ damage), hemiplegia, renal disease, leukaemia, lymphoma, other tumours, metastatic tumours, and AIDS.

Biochemical analyses and other measures

Fasting blood samples were collected between 0800 and 1030 during Wave 2. Plasma was separated from the blood samples within 1 hour of collection and stored at -80°C until assayed. tHcy was measured by fluorescence polarization immunoassay on an IMx analyzer (504). The inter-assay coefficient of variation was 4%. Genomic DNA was isolated from nucleated blood cells via the Triton X-100 method, and the nt677C→T mutation was determined using the polymerase chain reaction (PCR). HinfI restriction enzyme digestion was performed directly in the PCR tube at 37°C for 4 hours before analysis of restriction fragments by polyacrylamide gel electrophoresis, as previously described (505). Allele frequencies were estimated by gene counting, and the genotype distribution compared with those expected under Hardy-Weinberg equilibrium.

The blood samples were also used to measure serum creatinine, glucose, cholesterol, low-density lipoprotein and triglycerides with a Roche Hitachi 917 analyzer (Roche Diagnostics). Serum thyroid-stimulating hormone (TSH) was measured using an Elecsys 2010 immuno-analyzer (Roche Diagnostics Australia, Castle Hill, NSW, Australia). Serum high-sensitivity C-reactive protein (hsCRP) was measured with assay on a BNII analyzer (Dade Behring, Birmingham, UK). Estimated glomerular filtration rate (eGFR) was
calculated using the Cockcroft-Gault equation: \[((140 – age) \times wt (kg))/(\text{plasma creatinine} \times 0.8136)\].

Height (to the 0.5 cm), weight (to 0.2 kg) and blood pressure were measured by a trained research assistant during Wave 2. Body mass index (BMI) was calculated from height and weight in kg/m².

**Statistical analysis**

Data were analysed using Stata release 11.1 (StataCorp, College Station, TX, USA). Low physical SF-36 scores were defined as the lowest quartile of values in this study, which corresponded to thresholds of PF < 55, RP < 25, BP < 51 and PCS < 35. The higher quartiles of values were combined into a single category and used as a reference for comparison. Descriptive statistics were calculated for the demographic, lifestyle and clinical risk factors according to PCS scores. tHcy was dichotomised into ‘high tHcy’ (≥ 15 µmol/l) and ‘normal tHcy’ (< 15 µmol/l) as determined by the laboratory’s reference range. The association between tHcy and PCS were investigated in three different ways: according to whether tHcy was ≥ 15 µmol/l, per 5-µmol/l increment in tHcy, and by doubling of tHcy concentration (by dividing the natural logarithm of tHcy by the natural logarithm of 2). To determine the relationship between tHcy and PCS, linear and logistic regression analyses were used, with PCS modeled as a continuous and categorical variable (low PCS versus non-low PCS), respectively. A number of potential confounders were identified from the demographic, lifestyle and clinical information that we had collected. We tested their interaction with tHcy individually. A model containing the covariate, tHcy, and an interaction term between the two was fitted and the significance of the interaction term assessed. Covariates that showed significant interaction with tHcy were retained for inclusion in the final model. For the remaining covariates, their roles as confounders of the tHcy effect were assessed by comparing the adjusted effect of tHcy with its unadjusted effect. Any covariate which altered the tHcy effect by 4% or more was treated as a potential confounder and retained for inclusion in the final model. Final modeling was an iterative process of adding these covariates to a base model of tHcy and PCS. Two approaches were used to incorporate co-morbidity as a covariate in our analyses. The first model included adjustments for prevalent medical conditions that would have an effect on
physical health, whilst the second model included adjustments for weighted Charlson’s index (< 5 vs ≥ 5).

To compare the effect estimate of tHcy on PCS to the effect estimates of other covariates and biomarkers, such as age and hsCRP, on PCS, a linear regression model that included the demographic, lifestyle, comorbidity, clinical and biochemical variables in this study was performed. Supplementary analyses were also undertaken after excluding men who reported taking B-vitamin supplements, which may influence the tHcy and B-vitamin levels. The results were reported as Odds Ratio (OR) with 95% confidence intervals (95% CI). P-values < 0.05 were considered statistically significant.

Descriptive statistics were used to calculate the distribution of the MTHFR genotypes according to PCS. Logistic regression analyses were performed to investigate the relationship between MTHFR and PCS, using the CC genotype as reference. Adjustments were made as per the tHcy models. Analysis of variance and linear regression analysis were used to explore the association of the MTHFR genotypes with tHcy.
7.4 Results

tHcy levels were available for 4248 men, aged between 70 and 88 years. 1117 men (26.3%) had high tHcy (≥ 15 µmol/l) and the mean (± SD) tHcy concentration for the cohort was 13.4 ± 5.6 µmol/l. Socio-demographic, clinical and biochemical characteristics of the study population according to PCS are shown in Table 7.1.
<table>
<thead>
<tr>
<th>Demographic, lifestyle and clinical characteristics of the study population according to physical component summary (PCS) scores</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic</strong></td>
</tr>
<tr>
<td>Age, years&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Completed high school or better, n(%)</td>
</tr>
<tr>
<td><strong>Lifestyle</strong></td>
</tr>
<tr>
<td>Smoking, n(%)</td>
</tr>
<tr>
<td>Never smoked</td>
</tr>
<tr>
<td>Former smoker</td>
</tr>
<tr>
<td>Current smoker</td>
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<tr>
<td>History of hazardous alcohol use, n(%)</td>
</tr>
<tr>
<td>History of being physically active, n(%)</td>
</tr>
<tr>
<td><strong>Co-morbidities</strong></td>
</tr>
<tr>
<td>Stroke, n(%)</td>
</tr>
<tr>
<td>Diabetes, n(%)</td>
</tr>
<tr>
<td>Hypertension, n(%)</td>
</tr>
<tr>
<td>Arthritis, n(%)</td>
</tr>
<tr>
<td>Heart disease, n(%)</td>
</tr>
<tr>
<td>Asthma, n(%)</td>
</tr>
<tr>
<td>COPD, n(%)</td>
</tr>
<tr>
<td>Osteoporosis, n(%)</td>
</tr>
<tr>
<td>Depression, n(%)</td>
</tr>
<tr>
<td>Dementia or memory problems, n(%)</td>
</tr>
<tr>
<td>Prevalent falls, n(%)</td>
</tr>
<tr>
<td>Fracture in previous 12 months, n(%)</td>
</tr>
<tr>
<td>Polypharmacy, n(%)</td>
</tr>
<tr>
<td>Weighted Charlson’s Index Score ≥ 5, n(%)</td>
</tr>
<tr>
<td><strong>Clinical measurements&lt;sup&gt;c&lt;/sup&gt;</strong></td>
</tr>
<tr>
<td>BMI, kg/m&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>SBP, mmHg</td>
</tr>
<tr>
<td>DBP, mmHg</td>
</tr>
<tr>
<td><strong>Biochemical measures&lt;sup&gt;c&lt;/sup&gt;</strong></td>
</tr>
<tr>
<td>tHcy, µmol/l</td>
</tr>
<tr>
<td>Cholesterol, mmol/l</td>
</tr>
<tr>
<td>LDL, mmol/l</td>
</tr>
<tr>
<td>TG, mmol/l</td>
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<tr>
<td>Glucose, mmol/l</td>
</tr>
<tr>
<td>TSH, mU/l</td>
</tr>
<tr>
<td>hsCRP, mg/L</td>
</tr>
<tr>
<td>GFR, ml/min</td>
</tr>
</tbody>
</table>

<sup>c</sup>mean (SD)
Men with high $\text{tHcy}$ had a mean (± SD) PCS score of 40.6 ± 11.0, whilst those with normal $\text{tHcy}$ ( < 15 µmol/l) had a mean PCS score of 43.9 ± 10.2. Using linear regression analyses, the difference in mean PCS was -3.26 points (95% CI -3.98 to -2.55), which remained significant (-1.21 points; 95% CI -1.91 to -0.51) after adjustment for age, smoking, history of hazardous alcohol use, prevalent falls, polypharmacy, history of hypertension, heart disease, stroke, arthritis and osteoporosis (first model). The association also persisted (-1.45 points; 95% CI -2.23 to -0.67) after adjustment for age, smoking, history of hazardous alcohol use, prevalent falls, polypharmacy and the weighted Charlson’s index (second model). Every 5-µmol/l increment in $\text{tHcy}$ was associated with a reduction of 0.34 points in the predicted PCS (95% CI -0.64 to -0.05) in the first model, and a reduction of 0.50 points (95% CI -0.82 to -0.18) in the second model. With a doubling of $\text{tHcy}$, there were associated reductions of 0.77 points (95% CI -1.46 to -0.09) and 1.05 points (95% CI -1.81 to -0.29) in the predicted PCS, respectively, after adjustment for the same confounders. In univariate and multivariate logistic regression analyses, participants with elevated $\text{tHcy}$ were more likely to be in the lowest quartile of PCS scores (i.e. < 35), as shown in Table 7.2. Figure 7.1 demonstrates the risk of having PCS in the lowest quartile varies with changing $\text{tHcy}$ levels.
Table 7.2  Univariate and multivariate logistic regression analyses of associations between high homocysteine (tHcy) and low physical SF-36 scores

<table>
<thead>
<tr>
<th></th>
<th>Crude OR (95% CI)</th>
<th>Model 1 OR (95% CI)</th>
<th>Model 2 OR (95% CI)</th>
<th>Model 3 OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>tHcy ≥ 15 µmol/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF</td>
<td>2.08 (1.79 to 2.41)</td>
<td>1.61 (1.35 to 1.92)</td>
<td>1.40 (1.16 to 1.70)</td>
<td>1.46 (1.20 to 1.77)</td>
</tr>
<tr>
<td>RP</td>
<td>1.55 (1.33 to 1.82)</td>
<td>1.40 (1.17 to 1.67)</td>
<td>1.27 (1.05 to 1.53)</td>
<td>1.26 (1.04 to 1.53)</td>
</tr>
<tr>
<td>BP</td>
<td>1.30 (1.12 to 1.51)</td>
<td>1.22 (1.03 to 1.45)</td>
<td>1.09 (0.91 to 1.31)</td>
<td>1.12 (0.93 to 1.35)</td>
</tr>
<tr>
<td>PCS</td>
<td>1.94 (1.67 to 2.26)</td>
<td>1.63 (1.37 to 1.95)</td>
<td>1.45 (1.20 to 1.75)</td>
<td>1.47 (1.21 to 1.78)</td>
</tr>
</tbody>
</table>

Per 5-µmol/l increment in tHcy

<table>
<thead>
<tr>
<th></th>
<th>OR (95% CI)</th>
<th>OR (95% CI)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PF</td>
<td>1.38 (1.29 to 1.49)</td>
<td>1.22 (1.13 to 1.33)</td>
<td>1.40 (1.16 to 1.70)</td>
</tr>
<tr>
<td>RP</td>
<td>1.21 (1.13 to 1.29)</td>
<td>1.15 (1.07 to 1.24)</td>
<td>1.27 (1.05 to 1.53)</td>
</tr>
<tr>
<td>BP</td>
<td>1.11 (1.05 to 1.18)</td>
<td>1.08 (1.01 to 1.16)</td>
<td>1.09 (0.91 to 1.31)</td>
</tr>
<tr>
<td>PCS</td>
<td>1.25 (1.17 to 1.34)</td>
<td>1.15 (1.06 to 1.24)</td>
<td>1.07 (0.98 to 1.16)</td>
</tr>
</tbody>
</table>

Doubling of tHcy

<table>
<thead>
<tr>
<th></th>
<th>OR (95% CI)</th>
<th>OR (95% CI)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PF</td>
<td>2.19 (1.99 to 2.55)</td>
<td>1.62 (1.35 to 1.94)</td>
<td>1.36 (1.11 to 1.66)</td>
</tr>
<tr>
<td>RP</td>
<td>1.66 (1.43 to 1.94)</td>
<td>1.48 (1.23 to 1.78)</td>
<td>1.32 (1.09 to 1.59)</td>
</tr>
<tr>
<td>BP</td>
<td>1.26 (1.09 to 1.46)</td>
<td>1.20 (1.01 to 1.43)</td>
<td>1.04 (0.87 to 1.25)</td>
</tr>
<tr>
<td>PCS</td>
<td>1.78 (1.53 to 2.08)</td>
<td>1.48 (1.23 to 1.77)</td>
<td>1.23 (1.01 to 1.49)</td>
</tr>
</tbody>
</table>

Abbreviations: PF, Physical Function; RP, Role-Physical; BP, Bodily Pain; PCS, Physical component summary; OR, Odds ratio; 95% CI, 95% confidence interval.

Model 1: adjusted for age, smoking and history of hazardous alcohol use.
Model 2: adjusted for age, smoking, history of hazardous alcohol use, polypharmacy, prevalent falls, hypertension, heart disease, stroke, arthritis, osteoporosis.
Model 3: adjusted for age, smoking, history of hazardous alcohol use, polypharmacy, prevalent falls, weighted Charlon’s index group.

Low physical SF-36 scores defined as lowest quartiles of values, corresponding to thresholds of PF < 55, RP < 25, BP < 51, PCS < 35.

Odds ratio presented for high tHcy (≥ 15 µmol/l) in comparison with normal tHcy (< 15 µmol/l).

When high-sensitivity C-reactive protein (hsCRP) was included: OR 1.44 (95% CI 1.19 to 1.74) in model 2 and OR 1.45 (95% CI 1.20 to 1.77) in model 3.
Figure 7.1  Odds ratio of having physical component summary (PCS) scores in lowest quartile of values (i.e. < 35) with changing plasma total homocysteine (tHcy) levels

The tHcy scale of 8 to 22 µmol/l relates to the range between 5th and 95th percentiles of values (n=3823). Dashed lines denote 95% CI.

Other covariates including education, history of being physically inactive, diabetes, asthma, chronic obstructive pulmonary disease, dementia, depression and fractures in previous 12 months, BMI, systolic and diastolic blood pressure and GFR were investigated and found to have minimal confounding effect on PCS. They were therefore not included in the final models. There were no significant interactions between the variables and tHcy.

In the linear regression model which included the variables and biochemical markers as listed in Table 7.1, the beta-coefficients of tHcy and other covariates, including hsCRP, age and smoking, on PCS were -0.06 (P<0.001), -0.07 (P<0.001), -0.15 (P<0.001) and -0.07 (P<0.001), respectively. A total of 246 (5.8%) men reported taking B-vitamin supplements. Mean (± SD) tHcy concentration was 11.7 ± 3.8 µmol/l in men who took B-vitamin supplements and 13.5 ± 5.7 µmol/l in men who did not take the supplements. When repeat analyses were performed after excluding those men who reported taking B-vitamin supplements, the effect estimates on PCS remained largely unchanged (data not shown).
The results for the MTHFR polymorphism were available for 3812 men. The distribution of the MTHFR genotypes was similar for participants in the lowest quartile of PCS scores and the remaining men: CC 412 (44.4%), CT 424 (45.7%) and TT 91 (9.8%) for men in the lowest quartile and CC 1258 (44.3%), CT 1256 (44.2%) and TT 329 (11.6%) for the remaining men ($x^2=2.32$, $P=0.313$). The genotype distribution was in Hardy-Weinberg equilibrium (exact test, $P=0.212$). Mean (± SD) PCS score were 43.0 ± 10.7, 42.6 ± 10.6 and 43.7 ± 10.3 for men with the CC, CT and TT genotypes, respectively ($F=1.90$, $P=0.150$). The odds ratio of being in the lowest quartile of PCS scores for men with the TT genotype was 0.86 (95% CI 0.67 to 1.11). When adjusted for age, smoking, history of hazardous alcohol use, prevalent falls, polypharmacy and the weighted Charlson’s index, the effect estimate remained unchanged. Mean (± SD) tHcy concentration was 13.1 ± 5.1 µmol/l, 13.5 ± 4.9 µmol/l and 14.4 ± 9.7 µmol/l for men with the CC, CT and TT genotypes, respectively ($F=10.16$, $P < 0.001$). Compared with the reference CC genotype, mean tHcy was 0.43 µmol/l (95% CI 0.05 to 0.82) and 1.38 µmol/l (95% CI 0.77 to 1.99) higher amongst men with CT and TT genotypes, respectively.
7.5 Discussion

In this population-based sample of older men, elevated tHcy was associated with poorer self-perceived physical health, independent of age, smoking, history of hazardous alcohol use, prevalent falls, polypharmacy, hypertension, heart disease, stroke, arthritis, osteoporosis, and weighted Charlson’s co-morbidity index. The risk of having poorer physical HRQOL was increased by at least 45% when tHcy concentration is 15 µmol/l or higher. In our study, men with the MTHFR TT homozygosity had significantly higher tHcy concentration than the CC genotype. However, there was no apparent association between the MTHFR polymorphism and physical HRQOL.

This study, to our knowledge, is the first to investigate the cross-sectional relationship between tHcy and self-reported physical health using a large cohort of community-dwelling older men. Previous studies, mostly intervention trials in people with established vascular diseases, have yielded mixed findings. In a randomised trial by Hvas et al (643), 140 non-hospitalised individuals over 70 years of age were assigned vitamin B12 injection treatment or placebo. Despite vitamin B12 therapy lowering plasma methylmalonic acid and tHcy levels, the effect on self-reported health was equivocal. In another study that involved increasing vitamin B-related dietary intake in pre-dialysis patients with chronic renal failure, the intervention improved the physical SF-36 scores, despite persisting elevated tHcy levels (644). In a trial by Tsarouhas et al (645), 28 patients with stable chronic heart failure were randomised, with prescribed exercise regime to the study group versus no exercise in the control group. The intervention led to reductions in tHcy and improved perceived quality of life, particularly the physical component score. These inconclusive findings are likely limited by the small sample sizes, patient selection and residual confounding. To date, no studies have been sufficiently powered to specifically investigate the direct relationship between tHcy and HRQOL, as well as the effect of tHcy-lowering treatment on changes in HRQOL.

The strengths of this study include our large sample size of 4248 community-dwelling men, with a wide range of tHcy concentrations and high prevalence of hyperhomocysteinaemia to investigate our hypotheses. As a guide to interpreting the impact of tHcy on HRQOL, we compared the effect estimate of tHcy to those associated with other covariates and
biomarkers in the same group of men using the linear regression model of the PCS. The effect of tHcy on PCS was noted to be comparable to the effect of hsCRP, an inflammatory biomarker that has been shown to be predictive of later life mortality (646). For every one standard deviation increment in hsCRP, there was an associated 0.07-standard deviation reduction in the predicted PCS (b = -0.07 versus -0.06 for tHcy). The physiological relevance of the impact on self-reported physical health associated with high versus normal tHcy has not yet been determined. Hence, we compared the magnitude of the effect of tHcy on PCS against those of age and specific illnesses. A difference of 3.26 points in PCS is similar to the observed difference between the two oldest age strata in Australian men (641), which implies that an intervention that improves PCS by this magnitude might result in approximately five years’ “reduction in ageing”. For every 5-µmol/l increase in tHcy, there is a 7% increased risk of having low PCS scores in our cohort, which is comparative to a 60% higher risk of coronary artery disease in men and a 0.5 mmol/l increment in cholesterol level (345). Having low physical SF-36 scores would be almost comparable to the burden of developing symptomatic left ventricular systolic dysfunction or New York Heart Association (NYHA) class III, conditions which are known to be associated with poor prognosis (647).

In our study, there was no obvious association between the MTHFR 677T polymorphism and physical HRQOL. The most likely explanation would be the significant lack of power to reliably estimate the reduction in PCS in men with and without the TT mutation. Based on retrospective power calculations, to detect an effect size of 0.10 point reduction in PCS associated with a 1-µmol/l increment in tHcy (effect size derived from our cross-sectional analyses), one million participants (approximately 108000 with the TT genotype) would be required (80% power with an alpha set at 0.05).

We acknowledge several limitations in this study. The cross-sectional nature of our study precludes determination of causality over time and thus, our findings could reflect reverse causality, with poorer physical health leading to less physical activity, poorer diet, increased alcohol consumption, and consequently higher tHcy levels. We did not have updated data on alcohol use and physical activity during Wave 2 and consequently cannot dismiss the possibility that these activities might have changed over time or during the follow-up interval. Our analyses assumed that engagement in such activities would have
been relatively stable between Waves 1 and 2, or at least that their impact would not have substantially altered the outcomes of the study. The interpretation of our findings must take such a caveat into account. The problems in using the SF-36 health survey among older adults have been discussed previously (604). Although this questionnaire was self-administered by our participants, they were inspected immediately after completion by our research assistants and missing data were clarified with the participants. The mean physical SF-36 scores in our cohort were higher than those observed in the general Australian population of similar age ranges (641), suggesting that our findings might be generalised to the healthier section of the population. The self-selection of participants in Wave 2 might have biased our findings towards lower tHcy and better physical health compared to the non-respondents, which would move the results towards the null hypothesis and lead to an underestimation of the association between high tHcy and poorer physical HRQOL. Finally, we did not have access to B-vitamin concentrations for our cohort, and thus were unable to exclude the possibility of effect modification by prevailing folate concentrations. We repeated our analyses after excluding men who reported taking B-vitamin supplements and found that the effect estimates on PCS remained essentially unchanged.

We conclude that elevated tHcy is associated with poorer self-perceived physical health in community-dwelling older men, which suggests a potential role of tHcy on physical health in ageing men. An attempt to explore a causal relationship by using the principles of Mendelian randomization did not yield a significant correlation between the MTHFR polymorphism and HRQOL, although this is likely to reflect limitations of power for such analyses. The results of this study support further longitudinal investigations to assess this relationship prospectively.
7.6 **Author contributions**

Study conception and design: YW, LF

Acquisition of data: OA, GH, FB, LF

Statistical analyses: YW, KM

Interpretation of analyses: YW, OA, KM, BY, GH, LF

Drafting of manuscript: YW

Critical revision of manuscript: OA, BY, GH, FB, LF

Final approval of manuscript: All authors
CHAPTER 8

IN OLDER MEN, LOWER PLASMA 25-HYDROXYVITAMIN D IS ASSOCIATED WITH REDUCED INCIDENCE OF PROSTATE, BUT NOT COLORECTAL OR LUNG CANCER
CHAPTER 8: IN OLDER MEN, LOWER PLASMA 25-HYDROXYVITAMIN D IS ASSOCIATED WITH REDUCED INCIDENCE OF PROSTATE, BUT NOT COLORECTAL OR LUNG CANCER

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8.1 Abstract

Context and objective: Prostate, colorectal and lung cancers are common in men. In this study, we aimed to determine whether vitamin D status is associated with the incidence of these cancers in older men.

Design: Prospective cohort study.

Setting and participants: 4208 older men aged 70-88 years in Perth, Western Australia

Main outcome measures: Plasma 25-hydroxyvitamin D [25(OH)D] concentration was measured by immunoassay. New diagnoses of prostate, colorectal and lung cancers were determined via electronic record linkage.

Results: During a mean follow-up of 6.7 ± 1.8 years, there were 315, 117 and 101 new diagnoses of prostate, colorectal and lung cancer. In multivariate competing risks proportional hazards models, every 10 nmol/l decrease in 25(OH)D concentration was associated with a 4% reduction in prostate cancer incidence (sub-hazard ratio [SHR] 0.96, 95% confidence interval [CI] 0.92-1.00). Every halving of 25(OH)D concentration was associated with a 21% reduction in incident prostate cancer in multivariate analysis (SHR 0.79, 95% CI 0.63-0.99). Following exclusion of prostate cancer cases diagnosed within 3
years of blood sampling, low 25(OH)D < 50 nmol/l was associated with lower incident prostate cancer, and higher 25(OH)D > 75 nmol/l was associated with higher incidence, when compared to the reference range 50-75 nmol/l, respectively (p = 0.027). Significant associations were also observed when 25(OH)D was modeled as a quantitative variable. No associations were observed between plasma 25(OH)D concentration with incidence of colorectal or lung cancer.

**Conclusion:** Lower levels of vitamin D may reduce prostate cancer risk in older men. By contrast, levels of vitamin D did not predict incidence of colorectal or lung cancers. Further studies are needed to determine whether a causal relationship exists between vitamin D and prostate cancer in ageing men.
8.2 Introduction

Prostate, colorectal and lung cancers are common in older males (648). Important risk factors include increased age, smoking and physical inactivity. Previous studies have linked vitamin D deficiency to the risk of cancer, but the role of vitamin D in cancer pathogenesis is currently controversial.

Many experimental studies have documented pivotal roles of vitamin D in cancer genesis and progression. Its anti-carcinogenic qualities have been attributed to its active metabolite, 1,25-dihydroxyvitamin D3 [1,25(OH)D3], which exerts its influence via 2 pathways: the genomic and non-genomic (rapid) pathways. The genomic pathway requires the binding of 1,25(OH)D3 to the vitamin D receptor (VDR), which regulates transcription of genes involved in numerous cellular processes relevant for anti-cancer effects (649). The non-genomic pathway involves binding of 1,25(OH)D3 to the VDR, leading to intracellular signaling, rapid activation of cellular ion channels, and subsequent protection of DNA integrity (650). 1,25(OH)D3 also has an immune-modulatory effect which impede the development of malignancy (651). Despite accumulating evidence from experimental studies suggesting that low vitamin D status might be a causal risk factor for cancer, a recent systematic review of prospective cohort studies have reported no association between elevated vitamin D concentrations and lower risks of most cancers, excepting colorectal cancer (652). Similarly, prospective studies on vitamin D and cancer mortality as well as vitamin D and survival in cancer patients have yielded inconsistent findings (653). Randomised controlled trials of vitamin D supplements have failed to show anti-cancer effects, possibly due to methodological limitations and inadequate statistical power (654). Further observational and interventional studies are therefore warranted to clarify the potential role of vitamin D on cancer incidence.

In this study, we examined the relationship between vitamin D status and the incidence of prostate, colorectal and lung cancers in men aged 70-88 years. The primary circulating form of vitamin D, plasma 25-hydroxyvitamin D [25(OH)D] was measured as an indicator of vitamin D status. We tested the hypothesis that vitamin D level at baseline would predict the incidence of specific cancer types in a large population-based cohort of older men.
8.3 Methods

Study population

We conducted a prospective cohort study of participants from the Health in Men Study (HIMS), which has been described in detail elsewhere (528). In brief, approximately 40,000 men residing in Perth, Western Australia, were randomly selected from the electoral roll. These men, aged 65-83 years, were randomised to the screening and control arms of a trial of screening for abdominal aortic aneurysm. 12,203 men participated in the screening and completed a health assessment between 1996 and 1999 (HIMS Wave 1). In 2001-2004, 5,585 men responded to the second phase of this study (HIMS Wave 2) and blood samples were collected from 4,249 of them. More than 95% of the participants were Caucasian. The Human Research Ethics Committee of the University of Western Australia approved the protocol for HIMS and written informed consent was obtained from the participants.

Outcomes of interest

Cancer diagnoses and mortality information were obtained from the Western Australian Data Linkage System (WADLS), which links together data from the state cancer registry, death registry and hospital morbidity data system (485). Notification of cancer is mandatory in Western Australia and the International Classification of Diseases for Oncology (ICD-O-3) is used for cancer coding. For our analyses, we considered topography codes C33 and C34 to indicate lung cancer; C18, C19, C20 and C21 to indicate colorectal cancer; and C61.9 to indicate prostate cancer. For incident cancer cases, we included only primary invasive malignancies detected after the date of blood sampling and before December 31, 2010. Metastases, neoplasms in which primary or metastatic status was uncertain, neoplasms of unknown behaviour and in situ carcinomas were all excluded.

Explanatory variables

Using a combination of data collected at Waves 1 and 2, the following variables were available: age at Wave 2, education (completed high school or better by the end of Wave 1), living circumstance (living alone or in residential aged care facility during Wave 2), smoking status (current, former or never smoker during Wave 2), and taking calcium and vitamin D supplements during Wave 2 (yes or no). During Wave 1, the participants were...
asked whether they had done any vigorous exercise (apart from work) in a usual week, that would make them breathe harder or puff and pant (such as fast walking, jogging, aerobics, vigorous swimming, vigorous cycling, tennis, football, and squash). Physical activity was defined as ≥ 150 min of vigorous exercise in a usual week.

In order to calculate the weighted Charlson Co-morbidity Index (CCI) (530), we obtained the health records and death certificates from WADLS and evaluated the number and seriousness of the comorbid diseases. The Charlson’s Index takes into account 17 common medical conditions that predict one-year mortality: myocardial infarction, congestive heart failure, peripheral arterial disease, cerebrovascular disease, dementia, chronic pulmonary disease, connective tissue disease, ulcers, liver disease, diabetes (including diabetes with end organ damage), hemiplegia, renal disease, leukaemia, lymphoma, other tumours, metastatic tumours, and AIDS (530).

Height and weight were measured in accordance with guidelines of the International Society for the Advancement of Kinanthropometry(531). Body mass index (BMI) was calculated from height and weight in kg/m².

Biochemical analyses

Blood samples were collected during Wave 2 between 0800 and 1030. Plasma was separated from the blood samples within 1 hour of collection and stored at -80°C until assayed. As described previously (655), we measured 25(OH)D using the automated DiaSorin “LIAISON 25(OH)D TOTAL” chemilumininescent immunoassay. This was carried out on archived plasma aliquots in a series of runs performed between 2011 and 2012. The interassay coefficient of variation was 13.2% at 37.9 nmol/l and 11.3% at 131 nmol/l. The date of blood collection was documented and seasonality determined: summer (December-February), autumn (March-May), winter (June-August) and spring (September-November). Serum creatinine was measured with a Roche Hitachi 917 analyzer (Roche Diagnostics).

Statistical analyses

Data were analysed using Stata release 11.1 (Stata Corp, College Station, TX, USA). Descriptive statistics were calculated for the demographic, lifestyle and clinical variables.
according to the presence or absence of each cancer of interest. Men who reported taking calcium and vitamin D supplements were excluded from all analyses. The associations between 25(OH)D and incident cancer were explored by competing risk analyses (656). This approach was being considered due to the fact that in epidemiological studies, patients dying from non-cancer causes are usually considered as controls. These individuals might in reality be susceptible to biomarker abnormalities or to the development of cancer. The association between biomarker and cancer incidence might as a result be unrecognised due to their premature non-cancer mortality. Incident cancer was reported as sub-hazard ratio (SHR) with 95% confidence intervals (95% CI).

We defined lower vitamin D status as 25(OH)D < 50 nmol/l, a threshold used widely by experts to indicate vitamin D deficiency (428). Higher vitamin D status was defined as > 75 nmol/l, a point from which parathyroid hormone levels plateau to a steady state (657). The associations between plasma 25(OH)D concentration and cancer incidence was investigated in three different ways: according to whether 25(OH)D was < 50 nmol/l or > 75 nmol/l (using 50-75 nmol/l as reference), per 10-nmol/l decrease in concentration, and by halving of 25(OH)D. We transformed 25(OH)D by dividing the natural logarithm of 25(OH)D by the natural logarithm of 0.5. After this transformation, a one-unit change corresponds to a halving of the level of 25(OH)D.

To explore whether the associations between 25(OH)D and specific incident cancers were curvilinear, we entered 25(OH)D into the models as restricted cubic splines. The associations appeared curvilinear and were subsequently modeled with this approach. When 25(OH)D is modeled as categorical variables, the reported p-values test the null hypothesis that the SHRs are all equal to 1. When 25(OH)D is modeled as quantitative variables, the reported p-values test the null hypothesis that there is no linear trend between 25(OH)D and incident cancer. We performed univariate and multivariate analyses, adjusting for age, education, living circumstance, smoking status, physical activity, CCI, BMI, creatinine, seasonality and previous diagnosis of cancer (other than the cancer of interest). 397 men had a previous diagnosis of prostate cancer and were excluded from the incident prostate cancer analyses. 138 and 27 men had previous diagnoses of colorectal and lung cancer respectively, and were excluded from the incident colorectal and lung cancer analyses.
analyses, respectively. To minimise the possibility of reverse causality and ascertainment bias, we repeated the univariate and multivariate analyses after excluding the incident cases diagnosed within 3 years of blood sampling. P-values < 0.05 were considered statistically significant.
8.4 Results

The demographic, lifestyle and clinical characteristics of the study population, according to the presence or absence of incident cancers of interest, are shown in Table 8.1. 25(OH)D was available for 4233 men. 907 men (21.4%) had 25(OH)D concentration < 50 nmol/l, 1834 men (43.3%) had 25(OH)D concentration between 50 and 75 nmol/l, and 1492 men (35.3%) had 25(OH)D > 75 nmol/l. Men with 25(OH)D < 50 nmol/l were older in age compared to those with 25(OH)D > 75 nmol/l (77.4 years vs 76.9 years, p=0.003). The former were more likely to be current or former smokers (p=0.005), and also had higher number of co-morbidities (p<0.001). Detailed descriptive statistics of 25(OH)D data are published elsewhere (658).
Table 8.1 Demographic, lifestyle and clinical characteristics of the study population (excluding men who took calcium and vitamin D supplements) by the end of HIMS Wave 2, according to the presence or absence of incident cancer

<table>
<thead>
<tr>
<th></th>
<th>Prostate cancer</th>
<th>Colorectal cancer</th>
<th>Lung cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes (n=295)</td>
<td>No (n=3190)</td>
<td>P value</td>
</tr>
<tr>
<td>Age, years*</td>
<td>76.8 ± 3.6</td>
<td>77.0 ± 3.6</td>
<td>0.394</td>
</tr>
<tr>
<td>Completed high school or better, n (%)</td>
<td>158 (50.2)</td>
<td>1687 (50.2)</td>
<td>0.453</td>
</tr>
<tr>
<td>Lived alone or in residential aged care facility, n (%)</td>
<td>61 (19.4)</td>
<td>590 (16.8)</td>
<td>0.237</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoked</td>
<td>115 (36.5)</td>
<td>1159 (32.9)</td>
<td>0.431</td>
</tr>
<tr>
<td>Former smoker</td>
<td>184 (58.4)</td>
<td>2172 (61.7)</td>
<td>0.87</td>
</tr>
<tr>
<td>Current smoker</td>
<td>16 (5.1)</td>
<td>190 (5.4)</td>
<td>0.224</td>
</tr>
<tr>
<td>Physical activity, n (%)</td>
<td>82 (26.0)</td>
<td>787 (22.4)</td>
<td>0.135</td>
</tr>
<tr>
<td>CCI ≥ 5, n (%)</td>
<td>4 (1.3)</td>
<td>130 (3.7)</td>
<td>0.025</td>
</tr>
<tr>
<td>BMI, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 18.5 kg/m²</td>
<td>1 (0.3)</td>
<td>24 (0.7)</td>
<td>0.885</td>
</tr>
<tr>
<td>18.5-24.9 kg/m²</td>
<td>111 (35.2)</td>
<td>1206 (34.4)</td>
<td>0.434</td>
</tr>
<tr>
<td>25.0-29.9 kg/m²</td>
<td>156 (49.5)</td>
<td>1771 (50.5)</td>
<td>0.202</td>
</tr>
<tr>
<td>≥ 30 kg/m²</td>
<td>47 (14.9)</td>
<td>505 (14.4)</td>
<td>0.012</td>
</tr>
<tr>
<td>25(OHD)D, &lt; 50 nmol/l</td>
<td>55 (18.6)</td>
<td>704 (22.1)</td>
<td>0.224</td>
</tr>
<tr>
<td>50-75 nmol/l</td>
<td>124 (42.0)</td>
<td>1372 (43.0)</td>
<td>0.433</td>
</tr>
<tr>
<td>&gt;75 nmol/l</td>
<td>116 (39.3)</td>
<td>1114 (34.9)</td>
<td>0.304</td>
</tr>
<tr>
<td>25(OH)D, nmol/l</td>
<td>70.9 ± 22.1</td>
<td>68.3 ± 23.6</td>
<td>0.065</td>
</tr>
<tr>
<td>Creatinine, µmol/l</td>
<td>91.3 ± 21.8</td>
<td>94.1 ± 32.8</td>
<td>0.139</td>
</tr>
<tr>
<td>Season of blood collection, n (%)</td>
<td>157 (49.8)</td>
<td>1672 (47.5)</td>
<td>0.423</td>
</tr>
<tr>
<td>Summer/Autumn (Dec-May)</td>
<td>158 (50.2)</td>
<td>1849 (52.5)</td>
<td>0.265</td>
</tr>
<tr>
<td>Winter/Spring (Jun-Nov)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Continuous data presented as mean ± standard deviation and categorical data as n (%).
The participants were followed-up for a mean duration of 6.7 ± 1.8 years (range 0.1-9.2 years), comprising 25 723, 27 359 and 28 089 person-years for prostate, colorectal and lung cancers, respectively. During this period, 315 men were diagnosed with prostate cancer, 117 with colorectal cancer, and 101 with lung cancer. After exclusion of all men taking calcium and vitamin D supplements as well as new cancer diagnoses occurring within 3 years of blood sampling, there were 155 men diagnosed with prostate cancer, 60 with colorectal cancer and 60 with lung cancer during follow-up. There were 943 competing risk events (death from any cause) in men included in prostate cancer analyses, 1071 in colorectal cancer analyses, and 1065 in lung cancer models.

Association between 25(OH)D and incident prostate cancer

As illustrated in Table 8.2, every 10 nmol/l decrease in 25(OH)D concentration was associated with a 4% reduction in prostate cancer incidence, after adjustment for age, education, living circumstance, smoking status, physical activity, CCI, BMI, creatinine, seasonality and previous diagnosis of cancer (other than prostate) (SHR 0.96, 95% CI 0.92-1.00). Similarly, every halving of 25(OH)D concentration was associated with a 21% reduction in incident prostate cancer after adjustment for other risk factors (SHR 0.79, 95% CI 0.63-0.99). The association was weakened when 25(OH)D was modeled as categorical variables in the competing risk analyses (Table 8.2). To address the possibility of reverse causality, we excluded cases diagnosed within 3 years of blood sampling (Table 8.3). In multivariate analysis, low 25(OH)D concentration of < 50 nmol/l was associated with lower incident prostate cancer (SHR 0.76, 95% CI 0.46-1.23) and higher 25(OH)D concentration of > 75 nmol/l was associated with higher incidence (SHR 1.39, 95% CI 0.98-1.97), when compared to the reference range of 50-75 nmol/l, respectively (p = 0.027). Significant associations were also observed when 25(OH)D was modeled as a quantitative variable: lower 25(OH)D was associated with reduced incidence of prostate cancer in fully-adjusted analyses (per 10 nmol/l decrease: SHR 0.91, 95% CI 0.86-0.96; per halving of 25(OH)D: SHR 0.58, 95% CI 0.42-0.80).
Association between 25(OH)D and incident colorectal cancer

In both univariate and multivariate models (Table 8.2), there was no association between 25(OH)D concentration and incident colorectal cancer. Older age (SHR 1.20, 95% CI 1.11-1.31) and former smoking (SHR 1.68, 95% CI 1.06-2.66) were associated with increased incidence of colorectal cancer in all multivariate models. When cases diagnosed within 3 years of blood sampling were excluded from the models, no association between 25(OH)D and incident colorectal cancer was found (Table 8.3).

Association between 25(OH)D and incident lung cancer

In both univariate and multivariate models (Table 8.2), there was no apparent association between 25(OH)D concentration and incident lung cancer. Older age (SHR 1.12, 95% CI 1.05-1.19), current smoking (SHR 38.9, 95% CI 11.50-131.89) and former smoking (SHR 12.8, 95% CI 4.03-40.67) were associated with increased risks of lung cancer in all multivariate models. When cases diagnosed within 3 years of blood sampling were excluded from the models, no association between 25(OH)D and incident lung cancer was found (Table 8.3).
Table 8.2 Competing risks proportional hazards models exploring associations between vitamin D and incident cancers

<table>
<thead>
<tr>
<th></th>
<th>Prostate cancer (n=295)</th>
<th>Colorectal cancer (n=102)</th>
<th>Lung cancer (n=93)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Univariate</td>
<td>Multivariate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SHR</td>
<td>95% CI</td>
<td>P value</td>
</tr>
<tr>
<td>25(OH)D (nmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 50</td>
<td>0.88</td>
<td>0.64-1.22</td>
<td>0.373</td>
</tr>
<tr>
<td>50-75</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>&gt;75</td>
<td>1.11</td>
<td>0.86-1.43</td>
<td>0.048</td>
</tr>
<tr>
<td>Per 10-nmol/l decrease in 25(OH)D</td>
<td>0.96</td>
<td>0.92-1.00</td>
<td>0.048</td>
</tr>
<tr>
<td>Halving of 25(OH)D</td>
<td>0.78</td>
<td>0.63-0.97</td>
<td>0.028</td>
</tr>
</tbody>
</table>

Abbreviations: SHR, sub-hazard ratio; 95% CI, 95% confidence interval; 25(OH)D, 25-hydroxyvitamin D

*Adjusted for age, education, living circumstance, smoking status, physical activity, Charlson Comorbidity Index, body mass index, creatinine, seasonality, and previous diagnosis of cancer (other than the cancer of interest) before blood sampling.
Table 8.3  Competing risks proportional hazards models exploring associations between vitamin D and incident cancers after exclusion of cases diagnosed within 3 years of blood sampling

<table>
<thead>
<tr>
<th></th>
<th>Univariate</th>
<th></th>
<th></th>
<th>Multivariate&lt;sup&gt;a&lt;/sup&gt;</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SHR</td>
<td>95% CI</td>
<td>P value</td>
<td>SHR</td>
<td>95% CI</td>
<td>P value</td>
</tr>
<tr>
<td><strong>Prostate cancer</strong></td>
<td></td>
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<td></td>
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<tr>
<td>(n=155)</td>
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<tr>
<td>25(OH)D (nmol/l)</td>
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<tr>
<td>&lt; 50</td>
<td>0.74</td>
<td>0.46-1.19</td>
<td>0.010</td>
<td>0.76</td>
<td>0.46-1.23</td>
<td>0.027</td>
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<tr>
<td>50-75</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>&gt;75</td>
<td>1.43</td>
<td>1.02-2.01</td>
<td>0.1</td>
<td>1.39</td>
<td>0.98-1.97</td>
<td>0.001</td>
</tr>
<tr>
<td>Per 10-nmol/l decrease in 25(OH)D</td>
<td>0.90</td>
<td>0.85-0.95</td>
<td>&lt;0.001</td>
<td>0.91</td>
<td>0.86-0.96</td>
<td>0.001</td>
</tr>
<tr>
<td>Halving of 25(OH)D</td>
<td>0.56</td>
<td>0.41-0.77</td>
<td>&lt;0.001</td>
<td>0.58</td>
<td>0.42-0.80</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Colorectal cancer</strong></td>
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<td></td>
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<tr>
<td>(n=60)</td>
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<tr>
<td>&lt; 50</td>
<td>1.01</td>
<td>0.53-1.92</td>
<td>0.722</td>
<td>1.02</td>
<td>0.53-1.93</td>
<td>0.758</td>
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<td>1</td>
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<tr>
<td>&gt;75</td>
<td>0.80</td>
<td>0.44-1.45</td>
<td>0.415</td>
<td>0.80</td>
<td>0.43-1.50</td>
<td>0.946</td>
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<td>Per 10-nmol/l decrease in 25(OH)D</td>
<td>1.00</td>
<td>0.90-1.12</td>
<td>0.937</td>
<td>1.00</td>
<td>0.89-1.13</td>
<td>0.946</td>
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<tr>
<td>Halving of 25(OH)D</td>
<td>1.01</td>
<td>0.62-1.65</td>
<td>0.663</td>
<td>1.02</td>
<td>0.60-1.74</td>
<td>0.946</td>
</tr>
<tr>
<td><strong>Lung cancer</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>(n=60)</td>
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<tr>
<td>25(OH)D (nmol/l)</td>
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<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>&lt; 50</td>
<td>1.20</td>
<td>0.61-2.33</td>
<td>0.663</td>
<td>1.07</td>
<td>0.54-2.10</td>
<td>0.654</td>
</tr>
<tr>
<td>50-75</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;75</td>
<td>1.30</td>
<td>0.73-2.31</td>
<td>0.411</td>
<td>1.31</td>
<td>0.73-2.35</td>
<td>0.227</td>
</tr>
<tr>
<td>Per 10-nmol/l decrease in 25(OH)D</td>
<td>0.96</td>
<td>0.86-1.07</td>
<td>0.93</td>
<td>0.83-1.05</td>
<td>0.022</td>
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<tr>
<td>Halving of 25(OH)D</td>
<td>0.80</td>
<td>0.49-1.32</td>
<td>0.844</td>
<td>0.72</td>
<td>0.42-1.22</td>
<td>0.218</td>
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Abbreviations: SHR, sub-hazard ratio; 95% CI, 95% confidence interval; 25(OH)D, 25-hydroxyvitamin D

<sup>a</sup>Adjusted for age, education, living circumstance, smoking status, physical activity, Charlson Comorbidity Index, body mass index, creatinine, seasonality, and previous diagnosis of cancer (other than the cancer of interest) before blood sampling.
Figure 8.1 further illustrates the SHR of incident prostate, colorectal and lung cancer, excluding cancers diagnosed within 3 years of blood sampling, across concentrations of 25(OH)D. Men with 25(OH)D levels < 75 nmol/l had a lower SHR for incident prostate cancer, while 25(OH)D levels were not associated with risk of colorectal or lung cancer.

**Figure 8.1** Univariate competing risks proportional hazards models exploring associations between 25-hydroxyvitamin D [25(OH)D] concentrations with incident prostate, colorectal and lung cancers (excluding cancers diagnosed within 3 years of blood sampling).

25(OH)D were entered into the models as restricted cubic splines, with reference value for sub-hazard ratio (sub-HR) of 75 nmol/l.
8.5 Discussion

In this prospective cohort study of older men aged 70 years and over, lower 25(OH)D levels were associated with reduced incidence of prostate cancer. Men with plasma 25(OH)D < 50 nmol/l had a lower incidence of prostate cancer in comparison to men with 25(OH)D concentrations in the range of 50-75 nmol/l. There were no significant associations between 25(OH)D with incident colorectal and lung cancers in older men.

Our observation that lower 25(OH)D concentrations are associated with reduced risk of prostate cancer is concordant with some of the findings derived from a longitudinal nested case-control study conducted by Tuohimaa et al (659). This study explored the association between vitamin D and prostate cancer risk (622 prostate cancer cases identified) in Nordic men aged 40-58 years at onset. The authors reported that in middle-aged Norwegian and Swedish men, increased cancer risk was observed for the highest compared to the lowest quintile of 25(OH)D values (i.e. ≥ 80 versus ≤ 19 nmol/l; odds ratio [OR] 1.4, 95% CI 0.9-2.1 and OR 1.7, 95% CI 0.7-3.9, respectively). When the Finnish study data was included in analyses, a similar risk pattern persisted [25(OH)D concentration ≥ 80 nmol/l versus 40-60 nmol/l; OR 1.7, 95% CI 1.1-2.4] (659). Subsequent findings from the Health Professionals Follow-up Study reportedly showed that men with 25(OHD) levels < 37.5 nmol/l had significantly lower risk of poorly differentiated prostate cancers than men with higher levels (OR 0.42, 95% CI 0.23-0.73) (660). A prospective study by Ahn et al comprising 749 cases of prostate cancer also detected a significant trend of association between increasing quintiles of 25(OH)D levels with increased risk of aggressive disease (661). Our study indicates that the paradoxical association of lower vitamin D levels with reduced incidence of prostate cancer extends to older men. These findings are contrary to the predominating hypothesis that vitamin D might be beneficial in terms of protecting against cancer risk.

The paradoxical association might be explained by the presence of intraprostatic synthesis of 1,25(OH)D3 from 25(OH)D by normal human prostate cells. This occurs via the expression of 25-hydroxyvitamin D-1a-hydroxylase (1-aOHase) which is diminished in prostate cancer cells (662). The autocrine synthesis of 1,25(OH)D3 provides a mechanism by which local exposure to increased 25(OH)D could inhibit the growth of prostate cancer.
When 1-aOHase expression is diminished in prostate cancer cells, the cancer growth-inhibitory response by 25(OH)D is reduced (663). Whitlatch et al demonstrated that transfection of the 1-aOHase cDNA into prostate cancer cells with null 1-aOHase expression effectively restores the antiproliferative activity of 25(OH)D in the transfected cells, further supporting the causal association between loss of the enzyme activity with prostate carcinogenesis (664). Therefore, the effect of local synthesis of 1,25(OH)D3 in the prostate might not be captured in epidemiological studies based on circulating levels of vitamin D. This phenomenon might help to explain the heterogeneous conclusions in other studies exploring the relationship between vitamin D with prostate cancer (445, 665-668).

Whilst there is lack of conclusive evidence on the benefit of vitamin D supplementation in the development of prostate cancer, previous studies on the effect of pre-existing prostate cancer have so far produced ambiguous results (669, 670). A research team in the United States explored the influence of vitamin D3 supplementation at 4000 IU daily for one year on the outcome of early stage, low-risk prostate cancer (Gleason score ≤ 6, prostate-specific antigen [PSA] ≤ 10, clinical stage T1c or T2a). More than half of the study subjects remained stable or improved with supplementation, compared to a fifth of the control group who did not receive supplementation (p=0.025). Conversely, vitamin D3 supplementation did not benefit 40% of the subjects in this open-label clinical trial (669). Another study involves the randomization of 37 patients with histologically proven adenocarcinoma of the prostate who had selected prostatectomy as primary therapy. Calcitriol was administered to the treatment group at 0.5 µg/kg per week for a 4-week period prior to surgery. When prostatectomy specimens were processed and analysed, VDR expression was significantly reduced in samples from calcitriol-treated patients (p=0.004) but there was no statistically significant difference in the fraction of cells expressing the specific molecules involved with cell-cycle regulation and proliferation (670). With differing model studies and methodologies yielding inconsistent observations, further carefully planned clinical trials of adequate power are warranted to determine whether vitamin D supplementation could alter prostate cancer progression.

In our cohort of older men whose baseline ages ranged between 70 and 88 years, we did not find any significant association between 25(OH)D levels and incident colorectal cancer. The results from previous observational studies have been inconsistent.
participants in these studies were mostly younger, with the oldest participant being < 80 years of age. In a large nested case-control study involving more than 500 000 participants from 10 western European countries (1248 cases of incident colorectal cancer), lower levels of 25(OH)D were associated with higher colorectal cancer risk and higher levels of 25(OH)D associated with lower colorectal cancer risk, in comparison to a pre-defined mid-level concentration of 25(OH)D (50-75 nmol/l). The association was also noted to be stronger in the colon versus the rectum (671). Results from other studies were also suggestive of a protective effect of vitamin D on colorectal cancer (672, 673). On the other hand, research by Otani et al (674) and Braun et al (675) did not establish an association. In a 2011 meta-analysis of 9 studies comprising 2767 cases and 3948 controls, an inverse association between 25(OH)D levels and colorectal cancer risk was reported (676). Several reasons for the discrepancy in findings from these epidemiological studies have been postulated, including residual confounding, the lack of definitive cut-off points for the categories of plasma 25(OH)D levels, and the possibility of publication bias in systematic reviews as small studies with null results might not be accepted for publication. To address these limitations, randomised controlled trials have been conducted, with the largest study involving 36 282 postmenopausal women (677). Over a seven year period, the incidence of invasive colorectal cancer did not differ between women assigned to calcium plus vitamin D supplementation and those assigned to placebo (168 versus 154 cases; hazard ratio 1.08, 95% CI 0.86-1.34)(677). Similarly, a smaller study of 2686 participants suggested no benefit of vitamin D treatment (401). Further research is however needed with more focus on males, and consideration given to increasing the power of future trials, lengthening follow-up, as well as administrating moderate doses of vitamin D in order to generate a clear contrast in 25(OH)D levels between the treatment and control groups.

Prospective cohort studies on the association between 25(OH)D levels and incident lung cancer have also yielded divergent results. Our findings of no significant association are consistent with those derived from a Finnish cohort study of 6937 men and women, from which 122 incident lung cancer cases were identified after a maximum follow-up period of 24 years. After adjustment for age, sex, marital status, educational level, BMI, alcohol consumption, smoking and season of baseline 25(OH)D measurement, the relative risk (RR) for the highest versus lowest tertile of 25(OH)D values was 0.72 (95% CI 0.43-1.19).
When the analyses were stratified by gender, 25(OH)D was significantly associated with lung cancer incidence among women (RR 0.16, 95% CI 0.04-0.59) but not among men (RR 1.03, 95% CI 0.59-1.82) (678). In another case-control study (500 incident lung cancers) involving Finnish male smokers aged 55 and 62 years, no apparent association was observed when using season-specific and season-standardised 25(OH)D measures in the analyses (679). Similarly, a population-based cohort study conducted by Ordonez-Mena et al in Southwest Germany did not yield any significant association in multivariate models (680). On the other hand, a sub-analysis in a Danish population of middle-aged men and women has reported a significant risk of lung cancer for a one-unit (2.5 nmol/l) reduction in 25(OH)D concentration (hazard ratio 1.19, 95% CI 1.09-1.31). Whilst the analysis did not show a significant interaction of 25(OH)D with gender on the risk of tobacco-related cancers in this study, interaction on the risk of lung cancer risk specifically was not further explored (681). Despite inconclusive studies, a role for vitamin D in the development or progression of lung cancer remains plausible as the metabolically active 1,25(OH)D3 has been demonstrated in animal models to have inhibitory actions on the metastasis and angiogenesis in lung cancer cells (682).

The strengths of our study include the large population-based sample, availability of a wide range of 25(OH)D concentrations to investigate our hypotheses, and adjustment for competing risks in our analyses. Our focus on this well-characterised cohort of older men aged 70 years and above was highly relevant in the study of cancers, with older age being an established risk factor for these adverse health outcomes. However, there were some limitations in this study, including a single blood sample, and the absence of calcium and parathyroid hormone data which might influence vitamin D metabolism. We did not have updated data on physical activity during Wave 2 and therefore cannot dismiss the possibility that this might have altered over time or during the follow-up interval. Our analyses assumed that engagement in physical activity would have been relatively stable between Waves 1 and 2, or at least that its impact would not have substantially altered the outcomes of the study. The interpretation of our findings must take such a caveat into account. There was also limited information on cancer grade or family history of cancer. We were unable to explore the effects of increased PSA testing in our population leading to possible diagnosis of subclinical or low-grade prostate cancer. Prostate cancer diagnoses
within our cohort were mostly based on histopathology and we were thus unable to exclude the possibility of false negatives in our data, although this would likely introduce bias towards the null hypothesis. In a recent population-based analysis of PSA screening in Australian men, 66% of overall PSA testing was reportedly conducted in men < 65 years of age. One prostate cancer was detected per every 44.5 men who underwent PSA testing (2.2%), and the utilization of PSA tests for detection of prostate cancer decreased with increasing age (683). It is therefore likely that we have attained a near-complete capture of endpoints (at least for prostate cancer) via electronic record linkage. Finally, the results obtained with regards to colorectal and lung cancers in this cohort of older men may not be easily generalised to women.

In conclusion, our study suggests that higher levels of vitamin D may be associated with increased prostate cancer risk. We found no evidence that vitamin D levels modulate the risk of colorectal or lung cancer in older men. Of note, men with 25(OH)D levels < 50 nmol/l had a lower incidence of prostate cancer, yet levels above this threshold are recommended for bone health in older people (684). Therefore, further carefully designed studies on vitamin D and the incidence of prostate cancers are warranted to determine whether a causal relationship exists.
8.6 Author contributions

Study conception and design: YW, LF, BY, GH

Acquisition of data: LF, BY, GH

Statistical analyses: YW, ZH, KM

Interpretation of analyses: YW, ZH, KM, BY, JG, GH, LF

Drafting of manuscript: YW

Critical revision of manuscript: ZH, BY, JG, GH, LF

Final approval of manuscript: All authors
CHAPTER 9

SUMMARY AND CONCLUSIONS
CHAPTER 9: SUMMARY AND CONCLUSIONS

9.1 Summary of findings

The aim of this thesis is to explore the relationships between biomarkers and various age-related health outcomes in community-dwelling older men. These health outcomes are diverse and include AAA, frailty, all-cause mortality, physical HRQOL, and cancers of the prostate, colorectal system, and lung. The biomarkers of interest in this thesis are specifically, Hcy and vitamin D. This chapter summarises the main findings, with focuses on the newly generated hypotheses and derived conclusions.

In chapters 3 and 4, the biochemical predictors of AAA prevalence and progression are explored in older men. HHcy is associated with the presence of AAA in older men, after comprehensively accounting for potential confounders including the traditional CVD risk factors that have established associations with tHcy and AAA (chapter 3). There is also a positive dose-response relationship between tHcy and abdominal aortic diameter. These findings are suggestive that Hcy may play a role in the development of AAA amongst those ageing men without previous CVD or associated risk factors. They imply that Hcy-lowering strategies could potentially limit AAA formation in this group of individuals. On the other hand, Mendelian randomisation to the MTHFR TT genotype is not associated with AAA or aortic diameter, despite higher tHcy associated with this variant. The discordance in these findings may reflect limitation of power for such analyses.

Low 25(OH)D concentration is associated with the presence of larger AAA ≥ 35 mm in ageing men (chapter 4). The association is stronger for AAA ≥ 40 mm. There is no association between 25(OH)D and aortic diameters in the non-aneurysmal range. On the other hand, there is an inverse dose-response association between 25(OH)D concentration and aortic diameter in those men with prevalent AAA. These results imply that vitamin D supplementation to those men with existing aneurysmal arterial disease may potentially ameliorate the severity of the condition, albeit not playing a major role in the formation of AAA.

Chapters 5 and 6 explore the relationship between biomarkers and frailty as well as all-cause mortality. In chapter 5, HHcy is associated with the prevalence of frailty. The effect
of tHcy in the pathogenesis of the frailty syndrome is likely direct in nature, and not consequent of a prior vascular event or an inflammatory process. When associations between tHcy and individual components of frailty were explored in longitudinal models, high tHcy predicted the ambulation component solely, suggesting that men with impaired mobility are most likely to benefit from Hcy-lowering intervention. HHcy is also predictive of all-cause mortality, independent of the baseline frailty status. This implies that the association between tHcy and mortality is largely not mediated through the occurrence of frailty.

Similarly, hypovitaminosis D is associated with prevalent frailty and predictive of incident frailty in ageing men (chapter 6). Older men with low vitamin D status of < 50 nmol/l are nearly twice as likely to be frail in comparison to those with higher vitamin D levels > 80 nmol/l. For older men who are nonfrail at baseline and have low vitamin D levels, there is a > 50% increased risk of becoming frail at five years. Low vitamin D is predictive of the fatigue, resistance and ambulation components of frailty, suggesting that older men with poor physical health and performance, as well as those with sarcopenia, are most likely to benefit from vitamin D supplementation. Hypovitaminosis D also increases the risk of all-cause mortality, and the association is largely not mediated through the occurrence of frailty.

In chapter 7, the relationship between tHcy and self-perceived physical health was explored, by investigating the associations between tHcy, the MTHFR 677T polymorphism and physical HRQOL. Men with elevated tHcy have lower scores in the physical components of the SF-36 Health Survey, the impact of which is comparable to an inflammatory process or the burden of developing CAD and associated cardiovascular risk factors. This finding not only suggests a potential role of Hcy-lowering therapy in the improvement of physical health in ageing men, but also that it may possibly lead to a few years’ “reduction in ageing”. Again, the lack of association between the MTHFR polymorphism and HRQOL is likely the consequence of inadequate statistical power for such analyses.

Finally, associations between vitamin D status and incident prostate, colorectal, and lung cancer were assessed in chapter 8. Contrary to the predominant hypothesis that vitamin D
might be beneficial in terms of protecting against cancer risk, a paradoxical association between lower 25(OH)D concentrations and reduced incidence of prostate cancer was demonstrated. On the other hand, there is no evidence that vitamin D levels modulate the risk of colorectal or lung cancer in older men. These findings may reflect a limitation in epidemiological studies to accurately capture the cancer growth-inhibitory activity of 25(OH)D within prostate cells, the effect of which is diminished when prostate carcinogenesis occurs.
9.2 Strengths and limitations

The analyses presented in this thesis have several noteworthy strengths. Firstly, the HIMS participants were recruited via random sampling and are likely to be representative of community-dwelling older men. This enhances the external validity of the research findings. The large sample size provided excellent power to detect associations between the biomarkers and the outcomes of interest, including the use of longitudinal models as a means to determine a temporal relationship. The use of electronic record linkage increases the accuracy of the co-morbidity data and minimise the effects of recall, response, and non-response biases. The ability to comprehensively adjust for potential confounders in the multivariate analyses is greatly enhanced. The focus on this well-characterised cohort of older men aged 70 years and above is highly relevant to this research, with older age being an established risk factor for all the health outcomes of interest. The analyses are robust with the modelling of tHcy and 25(OH)D concentrations as both categorical and continuous variables.

However, some limitations must also be acknowledged, with majority of these being addressed in the discussion sections of the preceding chapters. The observational nature of the research limits the ability to infer causality. Having a single blood sample precludes the ability to determine if the levels of biomarkers would have been altered over time or during the follow-up interval. The fact that blood samples were obtained from the participants several years after baseline aortic diameter measurements further precludes determination of a causal relationship between the biomarkers and AAA. Data on some intermediary metabolites which may influence the Hcy and vitamin D metabolism pathways are not available for analyses. The findings from the analyses are likely conservative due to a possible “healthy survivor” effect. Those men who had responded for assessment during Wave 2 were younger and more physically active during Wave 1 compared to the non-respondents of the follow-up study. This can potentially bias the results towards the null hypotheses, therefore leading to an underestimation of the magnitude of the associations between the biomarker and health outcomes. Finally, the studies were limited to men, thereby limiting generalisability of most of the findings to women.
9.3 Implications and future directions

The findings of this thesis suggest that HHcy and hypovitaminosis D may be deleterious to several age-related health outcomes in older men. Clinical trials are warranted to investigate whether Hcy-lowering strategies and vitamin D supplementation can ameliorate or prevent the development of these adverse outcomes. The finding of a paradoxical association between low 25(OH)D concentration and reduced incident prostate cancer is unexpected and implies that hypovitaminosis D might be an epiphenomenon, rather than an etiological risk factor for carcinogenesis. Of note, men with 25(OH)D levels < 50 nmol/l have a lower incidence of prostate cancer, and yet levels above this threshold are recommended for optimal bone health in older people. Therefore, these results emphasise the need for further carefully designed studies to determine whether a causal relationship indeed exists between vitamin D and incident prostate cancers.
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WESTERN AUSTRALIAN ABDOMINAL AORTIC ANEURYSM PROJECT
Peripheral Vascular Diseases Program

Consent and forwarding of results

I consent to have the tests performed at the clinic and I understand that 2 copies of the results of my assessment will be available (one for my own records and one for my doctor). If the results of my scan are abnormal, a copy will be sent directly to my doctor.

I may be referred to an exercise program in the community.

I further understand that information, measurements and ultrasound scans collected in the course of the study will be used for research purposes, the results of which will be published in scientific journals or reports in such a way that individual participants cannot be identified.

I also understand that my own answers in this questionnaire and the results of my tests will not be released to anyone, other than my own doctor, without my specific permission.

Signature: ___________________ Date: __/__/199

Jan '97
Instructions

• The questions below will help us find out who is more likely to suffer an aneurysm.
• Please answer by circling the appropriate number or by writing your answer in the space provided.
• Please write your answers in BLOCK LETTERS.
• If you are uncertain about the answer to any of the questions, please ask the receptionist to help you.
• Please do not write in the far right hand column of each page. It is for Office use only.

GENERAL INFORMATION

Q.1 What is your date of birth? _____/_____/19_____
   day  month  year

Q.2 What is your current marital status?
   (Please circle one number only)
   1. Never married
   2. Now married
   3. Separated but not divorced
   4. Divorced
   5. Widowed
   6. De Facto (living as married)

Q.3 In which country were you born?
   (Which state or territory if born in Australia.)
   ____________________________________________

Q.4 How many years have you lived in Australia?
   _____ years
Q.5 What is the highest level of education you have completed?  
(Please circle one number only)

1. Never attended school
2. Primary school
3. Some high school
4. Completed high school (Year 12 or equivalent)
5. Completed university or other tertiary degree

MEDICAL INFORMATION

We would now like to ask you some questions about your medical history.

Q.6 Have you ever been told that you have any of the following?  
(Please circle one number on each line)

Aneurysm of the aorta .......................... 1. Yes 2. No
Bronchitis ........................................ 1. Yes 2. No
Asthma or bronchial asthma .................. 1. Yes 2. No
High blood pressure ............................ 1. Yes 2. No
Angina .............................................. 1. Yes 2. No
Stroke .............................................. 1. Yes 2. No
High cholesterol ................................. 1. Yes 2. No
High triglycerides (blood fats) ............... 1. Yes 2. No
Heart attack........................................ 1. Yes 2. No
Heart bypass surgery........................... 1. Yes 2. No
Heart balloon angioplasty...................... 1. Yes 2. No

Q.7 Do you get pain or discomfort in your leg(s) when you walk?

1. Yes  2. No  3. I am unable to walk
If NO, please go to Question 13
Q.7a  How far can you walk at an ordinary pace on level ground before this pain stops you?
1. Less than 100 yards
2. 100 yards to a quarter mile
3. Over a quarter mile

Q.8  Does this pain ever begin when you are standing still or sitting?
1. Yes  2. No

Q.9  Do you get this pain if you walk uphill or hurry?
1. Yes  2. No

Q.10  Do you get this pain when you walk at an ordinary pace on level ground?
1. Yes  2. No

Q.11  What happens to this pain if you stand still?
1. It usually continues for more than 10 minutes
2. It usually disappears in 10 minutes or less

Q.12  Where do you get this pain or discomfort?
Mark the place(s) with X on the diagram below.

Q.13  Has a doctor or nurse ever told you that you have diabetes?
1. Yes  2. No (Go to Q.18)

Q.14  IF YES, how old were you when you were first told?
   ____ years old

Q.15  Have you ever been given advice or treatment for diabetes or sugar trouble?
1. Yes  2. No (Go to Q.18)
Q.16  **IF YES**, how old were you when this advice or treatment was first given?

____ years old

Q.17  What kind of advice or treatment are you having for diabetes or sugar trouble **NOW?**  *(Please circle *one* number only)*

1. Diet advice *only*
2. Tablets *only*
3. Insulin injections *only*
4. Diet advice and tablets
5. Diet advice and injections

Q.18  Have you ever had a hernia in the groin?

1. Yes  2. No

**In the following section we would like to know about treatment you received in the last month.**

Q.19  In the last month have you been taking tablets for blood pressure?

1. Yes  2. No

Q.20  In the last month have you been taking tablets or other treatment for angina?

1. Yes  2. No

Q.21  In the last month have you been on aspirin tablets to prevent or treat heart disease?

1. Yes  2. No

Q.22  In the last month have you taken medication prescribed by a doctor to lower your blood cholesterol?

1. Yes  2. No

Q.23  In the last month have you been on a diet prescribed by a doctor or dietitian to lower your blood cholesterol?

1. Yes  2. No
HEIGHT AND WEIGHT

Please estimate if you do not know exactly

Q.24 How tall are you without shoes?

_______ centimetres

or

_______ feet _______ inches

Q.25 How much do you weigh without clothes and shoes?

_______ kilograms

or

_______ stone _______ pounds

EATING HABITS

Q.26 How many times a week do you eat meat, including sausages, devon, polony, salamis, meat pies, hamburger or bacon (but not including fish)?

1. Six or more times a week
2. Three to five times a week
3. Once or twice a week
4. Less than once a week
5. Never

Q.27 How often do you eat fish?

1. Six or more times a week
2. Three to five times a week
3. Once or twice a week
4. Less than once a week
5. Never
Q.28 What type of milk do you usually drink or use in cooking or in tea and coffee?

1. Condensed
2. Full cream (normal milk)
3. Sometimes full cream and sometimes reduced fat
4. Reduced fat
5. Skim or none
6. Other (please describe)_________________________

Q.29 Do you add salt to your food after it is cooked?

1. Rarely or never
2. Sometimes
3. Almost always or always

RECREATION, SPORT AND HEALTH FITNESS

This section asks about any vigorous exercise you do for recreation or health and fitness in a usual week. Vigorous exercise makes you breathe harder or puff and pant.

Q.30 In a usual week do you do any vigorous exercise (apart from your work) that makes you breathe harder or puff and pant? (eg. fast walking, jogging, aerobics, vigorous swimming, vigorous cycling, tennis, football, squash, etc.)

1. Yes 2. No (Go to Q.32)

Q.31 IF YES, please estimate the TOTAL TIME that you spend exercising vigorously in a usual week.

____ / ____ per week

hours / minutes

In this section we would like to find out about any non-vigorous exercise you do for recreation or health and fitness. Non-vigorous exercise only includes activities that do not make you breathe harder or puff and pant.
Q.32 In a usual week do you do any non-vigorous exercise for recreation or health and fitness? (eg. slow walking, slow cycling, Tai Chi, yoga, etc)

1. Yes 2. No (Go to Q.34)

Office use only

Q.33 IF YES, please estimate the TOTAL TIME that you spend doing non-vigorous exercise in a usual week?

____ / ____ per week
hours / minutes

SMOKING

In this section we want to know about your smoking habits.

Q.34 Have you ever smoked cigarettes, cigars or a pipe regularly?

1. Yes 2. No (Go to Q.40)

Q.35 IF YES, at what age did you start to smoke regularly?

______ years of age

Q.36 What is the most you have ever smoked for as long as one year?

___ manufactured cigarettes a day
___ grams “hand-rolled” cigarette tobacco per week
(A 1¾ ounce pouch of cigarette tobacco equals 50 grams)
___ cigars per week
___ grams pipe tobacco per week

Q.37 How often do you smoke now?

1. Every day (Go to Q.39)
2. Not every day (Go to Q.39)
3. Not at all
Q.38 IF NOT AT ALL, when did you give up smoking?

_____ / _____ /19_____  
day  month  year

Please Go to Question 40

Q.39 If you smoke now, how much do you smoke?

_____ manufactured cigarettes a day

_____ grams “hand-rolled” cigarette tobacco per week  
(A 1¾ ounce pouch of cigarette tobacco equals 50 grams)

_____ cigars per week

_____ grams pipe tobacco per week

PASSIVE SMOKING

In this section we would like to know if you were exposed to other people's tobacco smoke in the last 10 years.

Q.40 For how many years out of the last 10 have you been exposed to other people's smoke? (Please write the number of years for each of the following places.)

At home:  _____ years (out of the last 10)
At work:   _____ years (out of the last 10)
At social venues:  _____ years (out of the last 10)
In motor vehicles:  _____ years (out of the last 10)

Q.41 During the years that you were exposed, for how many hours each week were you exposed to other people’s smoke? (Please write the number of hours per week for each of the following statements.)

At home:  _____ hours in a usual week
At work:   _____ hours in a usual week
In social venues:  _____ hours in a usual week
In motor vehicles:  _____ hours in a usual week
In this section we want to know about your usual drinking habits.

Q.42 Have you ever drunk alcohol?
1. Yes  2. No (Go to Q.45)

Q.43 Have you drunk alcohol in the last year?
1. Yes  2. No (Go to Q.45)

Q.44 IF YES, how many “standard” drinks do you have each day in a usual week?

(1 “standard” drink = 1 middy of full-strength (5%) beer, or 1 pub measure of spirits, or 1 glass of sherry or port, or 1 glass of wine or 1.5 middles of Swan Gold or 2 middles of Toohey’s 2.2 or 5 middies of Swan Light.)

___drinks on Monday
___drinks on Tuesday
___drinks on Wednesday
___drinks on Thursday
___drinks on Friday
___drinks on Saturday
___drinks on Sunday
### FAMILY HISTORY

**Q.45** Did your **father** have any of the following?  
*Please circle a number on EACH line*

<table>
<thead>
<tr>
<th>Condition</th>
<th>Option 1</th>
<th>Option 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart attack or coronary thrombosis</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Stroke</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Aortic aneurysm</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Sudden death</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

**Q.46** Did your **mother** have any of the following?  
*Please circle a number on EACH line*

<table>
<thead>
<tr>
<th>Condition</th>
<th>Option 1</th>
<th>Option 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart attack or coronary thrombosis</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Stroke</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Aortic aneurysm</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Sudden death</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

**Q.47** How many of your **brothers** are alive?  
......... brothers

**Q.48** How many of your **brothers** have died?  
......... brothers

**Q.49** Altogether, how many of your brothers have had an aortic aneurysm?  
......... brothers

**Q.50** How many of your **sisters** are alive?  
......... sisters

**Q.51** How many of your **sisters** have died?  
......... sisters

**Q.52** Altogether, how many of your sisters have had an aortic aneurysm?  
......... sisters

**Q.53** Are you a twin?  
1. Yes  2. No

### OCCUPATION

**Q.54** What was the main kind of industry or business that you were in during your working life?

_________________________________
Q.55 What was the title of your occupation in your main job in life? (Please state your rank if in the armed services and your official designation)
Full job title: ____________________________________

Q.56 Please describe the main tasks and duties of that occupation.
Job description: ____________________________________
__________________________________________________

THANK YOU FOR YOUR HELP
1. Height__________cm

2. Weight__________kg

3. Waist circumference:
   1st measure__________cm
   2nd measure__________cm

4. Hip circumference:
   1st measure__________cm
   2nd measure__________cm

5. Maximum external diameter of infrarenal aorta
   ……..mms

6. Aortic image recorded on video? 1. Yes 2. No

7. Video counter for this man started at ……….?

8. Scan code……………

9. Blood pressure observer__________________________

10. Sphygmomanometer_____________________________

11. Blood pressures:
    1st reading: 2nd reading
    Systolic_______ mmHg  Systolic_______ mmHg
    Diastolic_______ mmHg  Diastolic_______ mmHg

12. Doppler pressures:

<table>
<thead>
<tr>
<th>Arm</th>
<th>Dorsalis Pedis</th>
<th>Posterior Tibial</th>
<th>Peroneal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

INSTRUCTIONS

Thank you for filling out this form. We realize it is quite long. Take your time – there is no need to complete it all in one sitting.

This form includes some questions that were in the form you filled out the last time we saw you. This is because we wish to find out if there have been any changes over the last few years.

Please answer by circling the number that applies to you or by writing your answer in the space provided.

Please write your answers in BLOCK LETTERS

If you are unsure about any questions, please leave them blank and ask when you come to the clinic.

Please do not write in the far right hand column of each page.

Reference number: □□□□□□

Your contact telephone number: ________________
(Please circle ONE number for EACH question below)

1. **Do you get pain or discomfort in your leg(s) when you walk?**
   1. Yes  
   2. No  
   3. I am unable to walk

   *If NO or unable, please go to Question 8*

2. **How far can you walk at an ordinary pace on level ground before this pain stops you?**
   1. Less than 100 yards
   2. 100 yards to a quarter of a mile
   3. Over a quarter of a mile

3. **Does this pain ever begin when you are standing still or sitting?**
   1. Yes  
   2. No

4. **Do you get this pain if you walk uphill or hurry?**
   1. Yes  
   2. No

5. **Do you get this pain when you walk at an ordinary pace on level ground?**
   1. Yes  
   2. No

6. **What happens to this pain if you stand still?**
   1. It usually continues for more than 10 minutes
   2. It usually disappears in 10 minutes or less

7. **Where do you get this pain or discomfort?**

   Please mark the place(s) with X on the diagram below

   ![Diagram of legs](image)
8. **In the last 5 years** have you ever been told for the first time by a doctor that you have any of the following conditions? **Office use only**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Answer</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Arthritis (including osteoarthritis, rheumatoid arthritis)</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>b. Diabetes (High blood sugar)</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>c. Angina</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>d. Hypertension (High Blood Pressure)</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>e. Stroke</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>f. Heart attack</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>g. Asthma</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>h. Chronic bronchitis</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>i. Emphysema</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>j. Osteoporosis</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>k. Prostate cancer</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>l. Bowel cancer</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>m. Malignant melanoma</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>n. Other skin cancer</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>o. Depression</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>p. Anxiety or Nervous disorder</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>q. Alzheimer’s Disease</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>r. Dementia or Memory Problems</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>s. Leg ulcers</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

9. Have you developed any other major problems with your health **in the last 5 years**?

   1. Yes
   2. No

If *Yes*, please give details:

   ____________________________________________
   ____________________________________________
   ____________________________________________
   ____________________________________________

3
10. **Have you ever had any of the following operations?**

(Please circle ONE answer on each line)

<table>
<thead>
<tr>
<th>Operation</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>a  Open heart surgery</td>
<td></td>
<td></td>
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<tr>
<td>b  Carotid artery surgery (carotid endarterectomy)</td>
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<td></td>
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<tr>
<td>c  Bypass surgery to the aorta or leg arteries</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d  Abdominal surgery including keyhole surgery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>e  Hip or knee replacement surgery</td>
<td></td>
<td></td>
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<tr>
<td>f  Any lung surgery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>g  Any brain surgery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>h  Prostate surgery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>i  Major neck surgery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>j  Surgery on the oesophagus (gullet)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

k  Have you had any other operations? Yes  No
If Yes, please give details.

<table>
<thead>
<tr>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>

11. **(Please circle ONE answer)**

Have you ever suffered a blow on the head which caused you to become unconscious? Yes  No

12. **(Please circle ONE answer on each line)**

Were you born prematurely? Yes  No  Don’t know
Were you under-weight at birth?  Yes  No  Don’t know

If your father is no longer alive, how old was he when he died?  Age:  _______ Don’t know

If your mother is no longer alive, how old was she when she died?  Age:  _______ Don’t know

13. Treatment for EMOTIONAL HEALTH.
(Please circle ONE answer on each line)

1. Have you ever had treatment for an emotional or nervous illness (such as depression)?
   1. Yes  2. No  (Go to Q.14)

   If YES, What type of treatment did you have?
   (Please circle all the numbers that apply)

   1. Psychological help
   2. Medication
   4. Other
      Please describe: __________________________

2. Are you having treatment for an emotional or nervous illness (such as depression) now?
   1. Yes  2. No

14. STROKE

   a) Have you ever been told (or even thought) that you have had a stroke?
      (i.e. the sudden onset of a loss of function of a particular part of the body.)
      (Please circle ONE answer only)
      1. Yes  2. No  (If NO please go to Q 15)

   b) If YES when did the first of these episodes occur?
      Month_________ Year______________

   c) When did the most recent episode occur?
      Month_________ Year______________

   d) Did you see a Doctor about this most recent episode?
      (Please circle ONE answer only)
      1. Yes  2. No

5
Confidential

If YES, please give the name of the Doctor__________________

e) Were you admitted to hospital following this most recent episode?  
If YES, please give name of the hospital____________________

15. Please list all the tablets, inhalers and other medicines you are currently taking. Please include any non-prescription medicine you may have obtained from a chemist or supermarket.

<table>
<thead>
<tr>
<th>Name of drug or medication</th>
<th>How long have you been taking this tablet or medicine? (Please write the numbers)</th>
<th>Weeks</th>
<th>Months</th>
<th>Years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

16. Are you able to take medications without help in the right dose at the right time?  
(Please circle ONE answer only)

1. Yes  2. No  3. Not on medication
SMOKING

17. Have you ever smoked cigarettes, cigars or a pipe regularly?

1. Yes  2. No (Go to Q. 23)

18. IF YES, at what age did you start to smoke regularly?

_____________ years of age

19. What is the most that you have ever smoked for as long as one year?

____________ manufactured cigarettes a day

____________ grams “hand-rolled” cigarette tobacco per week

(A 1¾ ounce pouch of cigarette tobacco equals 50 grams)

____________ cigars per week

____________ grams pipe tobacco per week

20. How often do you smoke now?

1. Every day (Go to Q. 22)
2. Not every day (Go to Q.22)
3. Not at all

21. IF NOT AT ALL, when did you give up smoking?

__/__/____
day month year

Please go to Q 23.
22. If you smoke now, how much do you smoke?

___________ manufactured cigarettes a day

___________ grams “hand-rolled” cigarette tobacco per week

(A 1¾ ounce pouch of cigarette tobacco equals 50 grams)

___________ cigars per week

___________ grams pipe tobacco per week

23. In general do you prefer to see a male doctor?

(Please circle ONE number ONLY)

1      Yes always
2      Yes, but only for certain things
3      No
4      I don't mind

24. In the LAST 12 MONTHS, have you:

(Please circle ONE number on EACH line)

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slipped, tripped, or stumbled? (not including falls to the ground)</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Had a fall to the ground? (does not include stumbles/trips)</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Been injured as a result of a fall?</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Needed to seek medical attention (eg. Doctor, hospital) for an injury from a fall?</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Had any other injury from an accident at your home?</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Broken or fractured any bone?</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>
25. Thinking about YOUR OWN HEALTH CARE, how would you rate the following (now)?

*(Please circle ONE number on EACH line)*

<table>
<thead>
<tr>
<th>Access to medical specialists if you need them</th>
<th>Excellent</th>
<th>Very Good</th>
<th>Good</th>
<th>Fair</th>
<th>Poor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

| Access to a hospital if you need it            | 1         | 2         | 3    | 4    | 5    |

| Access to after-hours medical care             | 1         | 2         | 3    | 4    | 5    |

| Access to a GP who bulk bills                  | 1         | 2         | 3    | 4    | 5    |

| Hours when a GP is available                   | 1         | 2         | 3    | 4    | 5    |

| Number of GPs you have to choose from          | 1         | 2         | 3    | 4    | 5    |

| Ease of seeing the GP of your choice           | 1         | 2         | 3    | 4    | 5    |

26. How would you rate the cost to you of your LAST visit to a general practitioner?

*(Please circle ONE number ONLY)*

1 No cost to me
2 Excellent
3 Very good
4 Good
5 Fair
6 Poor
27. **Do you have:**

(Please circle ONE number on EACH line)

<table>
<thead>
<tr>
<th>Difficulty seeing newspaper print, even with glasses?</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Difficulty recognising people across the road, even with glasses?</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Difficulty in hearing a conversation, even with a hearing aid?</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Difficulty speaking?</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

28. Are you able to manage your own money by writing cheques and paying bills?

1. Yes       2. No

29. When you travel around your neighbourhood, does someone have to help you because of your health?

(Please circle ONE number ONLY)

<table>
<thead>
<tr>
<th>1</th>
<th>Yes, all of the time</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Yes, most of the time</td>
</tr>
<tr>
<td>3</td>
<td>Yes, some of the time</td>
</tr>
<tr>
<td>4</td>
<td>Yes, a little of the time</td>
</tr>
<tr>
<td>5</td>
<td>No, none of the time</td>
</tr>
</tbody>
</table>

30. Do you have any of these sleeping problems?

(Please circle ONE number on EACH line)

<table>
<thead>
<tr>
<th>Waking up in the early hours of the morning</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lying awake for most of the night</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Taking a long time to get to sleep</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Worry keeping you awake at night</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sleeping badly at night</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>
### 31. What do you think about the neighbourhood in which you live?
How much do you agree with the following statements?

(Please circle ONE number on EACH line)

<table>
<thead>
<tr>
<th>Statement</th>
<th>Strongly disagree</th>
<th>Disagree</th>
<th>Neutral</th>
<th>Agree</th>
<th>Strongly agree</th>
</tr>
</thead>
<tbody>
<tr>
<td>I would be really sorry if I had to move away from the people in my neighbourhood</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I have a lot in common with people in my neighbourhood</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I generally trust my neighbours to look out for my property</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>People in my neighbourhood make it a difficult place to live</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I am good friends with many people in my neighbourhood</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I like living where I live</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I have little to do with people in my neighbourhood</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>My neighbours treat me with respect</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Children are safe walking around my neighbourhood during the day</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I get involved with most local issues</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>People in my neighbourhood are very willing to help each other out</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>If I no longer lived here, hardly anyone around here would notice</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>It is safe to walk around my neighbourhood at night</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>
32. **With whom do you live now?**  
(Please circle ONE answer)

1. Living alone
2. Living with spouse only
3. Living with other relatives (includes with or without spouse)
4. Living with other persons (non-relatives)
5. Nursing home or hostel
6. Other (specify): __________________________

33. **In what kind of building do you live now?**  
(Please circle ONE answer)

1. Own home with 2 or more steps
2. Own home with 1 or no steps
3. Flat or Unit with 2 or more steps
4. Flat or Unit with 1 or no steps
5. Elderly unit – unsupervised
6. Elderly unit – supervised
7. Nursing home or hostel
8. Other (specify): __________________________
34. Please, read the following statements carefully and circle ONE number on EACH line that best describes the way you have been feeling OVER THE PAST MONTH

<p>| | | | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hardly ever</td>
<td>Some of the time</td>
<td>Always</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>I am satisfied that I can turn to my family for help when something is troubling me</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>I am satisfied with the way my family talks over things with me and shares problems with me</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>I am satisfied that my family accepts and supports my wishes to take on new activities or directions</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>I am satisfied with the way my family expresses affection and responds to my emotions, such as anger, sorrow, or love</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e</td>
<td>I am satisfied with the way my family and I share time together</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>f</td>
<td>I am satisfied that I can turn to my friends for help when something is troubling me</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g</td>
<td>I am satisfied with the way my friends talk over things with me and share problems with me</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>h</td>
<td>I am satisfied that my friends accept and support my wishes to take on new activities or directions</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i</td>
<td>I am satisfied with the way my friends express affection and respond to my emotions, such as anger, sorrow, or love</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>j</td>
<td>I am satisfied with the way my friends and I share time together</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
35. In general, would you say your health is:

(Please circle ONE number only)

1 Excellent
2 Very good
3 Good
4 Fair
5 Poor

36. Compared with one year ago, how would you rate your health in general now?

(Please circle ONE number only)

1 Much better now than one year ago
2 Somewhat better now than one year ago
3 About the same as one year ago
4 Somewhat worse now than one year ago
5 Much worse now than one year ago

37. During THE PAST 4 WEEKS, have you, AS A RESULT OF YOUR PHYSICAL HEALTH, had any of the following problems with your work (including your work outside the home and housework) or other regular daily activities?

(Please circle ONE answer on each line)

<table>
<thead>
<tr>
<th>Problem</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cut down on the amount of time you spent on work or other activities</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Got less done than you would like</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Were limited in the kind of work or other activities you did</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Had difficulty doing the work or other activities (for example, it took extra effort)</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>
38. The following questions are about activities you might do during a typical day. Does YOUR HEALTH NOW LIMIT YOU in these activities? If so, how much?

(Please circle ONE number on EACH line)

<table>
<thead>
<tr>
<th>Activity</th>
<th>Yes, limited a lot</th>
<th>Yes, limited a little</th>
<th>No, not limited at all</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIGOROUS activities such as running, lifting heavy objects, participating in strenuous sports</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>MODERATE ACTIVITIES, such as moving a table, pushing a vacuum cleaner, bowling or playing golf</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Lifting or carrying groceries</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Climbing SEVERAL flights of stairs</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Climbing ONE flight of stairs</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Bending, kneeling or stooping</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Walking <strong>100 yards</strong></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Walking <strong>half a mile</strong></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Walking <strong>more than a mile</strong></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Bathing or dressing yourself</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

39. How much PHYSICAL pain have you had during the PAST 4 WEEKS?
(Please circle ONE number only)

1  No bodily pain in the last four weeks
2  Very mild
3  Mild
4  Moderate
5  Severe
6  Very severe
40. During the PAST 4 WEEKS, have you had any of the following problems with your work or other regular daily activities AS A RESULT OF ANY EMOTIONAL PROBLEMS (such as feeling depressed or anxious)?

(Please circle ONE answer on each line)  

<table>
<thead>
<tr>
<th>Cut down on the amount of time you spent on work or other activities</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Got less done than you would like</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Did not do work or other activities as carefully as usual</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

41. During the PAST 4 WEEKS, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours or groups?

(Please circle ONE number only)  

<table>
<thead>
<tr>
<th>Not at all</th>
<th>Slightly</th>
<th>Moderately</th>
<th>Quite a bit</th>
<th>Extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

42. During the past four weeks, how much did PAIN interfere with your normal work (including both work outside the home and housework)?

(Please circle ONE number only)  

<table>
<thead>
<tr>
<th>Not at all</th>
<th>A little bit</th>
<th>Moderately</th>
<th>Quite a bit</th>
<th>Extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>
43. For each question below please choose ONE number on each line which is the closest to the way you have been feeling over the PAST 4 WEEKS.
(Please circle ONE number on EACH line)

<table>
<thead>
<tr>
<th>Question</th>
<th>All of the time</th>
<th>Most of the time</th>
<th>A good bit of the time</th>
<th>Some of the time</th>
<th>A little of the time</th>
<th>None of the time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Did you feel full of life?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Have you been a very nervous person?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Have you felt so down in the dumps that nothing could cheer you up?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Have you felt calm and peaceful?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Did you have a lot of energy?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Have you felt down?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Did you feel worn out?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Have you been a happy person?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Did you feel tired?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>
44. **During the PAST 4 WEEKS, how much of the time has your PHYSICAL HEALTH OR EMOTIONAL PROBLEMS interfered with your social activities (like visiting friends, relatives, etc)?**  
*(Please circle ONE number only)*

1. All of the time  
2. Most of the time  
3. Some of the time  
4. A little of the time  
5. None of the time  

45. **How TRUE or FALSE is EACH of the following statements for you?**  
*(Please circle ONE number on EACH line)*

<table>
<thead>
<tr>
<th>Statement</th>
<th>Definitely true</th>
<th>Mostly true</th>
<th>Don’t know</th>
<th>Mostly false</th>
<th>Definitely false</th>
</tr>
</thead>
<tbody>
<tr>
<td>I seem to get sick a little more easily than other people</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I am as healthy as anybody I know</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I expect my health to get worse</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>My health is excellent</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Question</td>
<td>Hardly ever</td>
<td>Some of the time</td>
<td>Most of the time</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
<td>-------------</td>
<td>------------------</td>
<td>------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>49. Does it seem that your family and friends (people who are important to you) understand you?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50. Do you feel useful to your family and friends?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>51. Do you know what is going on with your family and friends?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>52. When you are talking with your family and friends, do you feel you are being listened to?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>53. Do you feel you have a definite role in your family and among your friends?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>54. Can you talk about your deepest problems with at least some of your family and friends?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### 55. *(Please circle ONE number in each line)*

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Are you basically satisfied with your life?</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Have you dropped many of your activities and interests?</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Do you feel that your life is empty?</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Do you often get bored?</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Are you in good spirits most of the time?</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Are you afraid that something bad is going to happen to you?</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Do you feel happy most of the time?</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Do you feel helpless?</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Do you prefer to stay at home, rather than going out and doing new things?</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Do you feel you have more problems with your memory than most?</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Do you think it is wonderful to be alive?</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Do you feel pretty worthless the way you are now?</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Do you feel full of energy?</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Do you feel that your situation is hopeless?</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Do you think that most people are better off than you are?</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>
56. Please list your Hobbies now:

_____________________________________________________________________
_____________________________________________________________________
_____________________________________________________________________

57. Which language did you first learn as a child?
(Please circle ONE number only)
1. English If Yes, please go to Question 60
2. Other Please specify: ________________

58. How often do you speak this language now?
(Please circle ONE number ONLY)
1. Daily
2. Several times a week
3. At least once each week
4. Several times a month
5. At least once each month
6. Less often than monthly

59. With whom do you speak this language?
(Please circle ONE number on EACH line)

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wife or partner</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Children</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Other close relatives</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Friends</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Other (eg work)</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>
60. How often do you use a personal computer?

(Please circle ONE answer only)
1 Never (Go to Q. 62)
2 At least every day
3 At least every week
4 Less than every week

61. What do you use a personal computer for?
(Please circle ONE number on EACH line)

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
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<tr>
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<td>Games</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
</tr>
</tbody>
</table>
62. We would be grateful if you would supply the names, addresses and contact numbers of at least two other people who might help us find you if you move house. These contacts should be younger relatives or close friends who do not live at the same address as yourself.

(a)
Name: __________________________
Address: __________________________

Telephone: __________________________

(b)
Name: __________________________
Address: __________________________

Telephone: __________________________

Thank you for your help.

⇔ Please bring this questionnaire with you when you come to the clinic.
OFFICE USE ONLY - Clinical data

Ref No: ____________________________

Date    __/__/____

Height: ___________ cms (measure to 0.5 cm)

Weight: ___________ kgs (measure to 0.2 kg)

Waist: ___________ cms (measure to 0.5 cm)

Hips: ___________ cms (measure to 0.5 cm)

First blood pressure: SBP/DBP ___________ mm Hg (measure to 2 mm Hg)

Second blood pressure: SBP/DBP ___________ mm Hg (measure to 2 mm Hg)

Blood pressure observer: ____________________________

Sphygmomanometer: ____________________________

Blood sample  1. Yes  2. No

Fasted:  1. Yes  2. No

Agrees to DNA extraction  1. Yes  2. No

Requests blood result  1. Yes  2. No

SMMSE score: (WORLD) _______/30

SMMSE score: (SERIAL 7) _______/30

Doppler pressures:

<table>
<thead>
<tr>
<th>Arm</th>
<th>Dorsalis pedis</th>
<th>Posterior Tibial</th>
<th>Peroneal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right</td>
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<tr>
<td>Left</td>
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</tbody>
</table>

DOPPLER  Yes    No    WORD RECALL  Yes    No    AAA  Yes    No

The Health In Men Study is a project of:

School of Population Health
University of Western Australia
35 Stirling Highway
Crawley, Western Australia 6009
Australia

and is funded by the National Health and Medical Research Council.
HEALTH IN MEN STUDY

STUDY INFORMATION

Thank you for taking part in this very important study called the Health In Men Study (HIMS for short), which started in 1996 as part of the Abdominal Aortic Aneurysm Screening Study.

The HIMS questionnaire aims to gather information about the health of men over the age of 70 years in Western Australia, and what factors predict healthy ageing. The study is supported by the National Health and Medical Research Council (NHMRC).

For further information about this study, please contact Professor Leon Flicker or a study representative on 9224 2855.

COMPLETION INSTRUCTIONS

Please use a BLACK pen

Please shade the circles completely

If you make a mistake or want to change any of your shaded responses, please place a cross through the incorrect response X and shade the correct response ●

For written responses, please cross it out and write your new response just above or below the one you have crossed out

If you feel uncomfortable about answering any question, you can leave it blank and move on to the next one.

When you have completed the questionnaire, please post it back to us in the reply-paid envelope without folding or bending it. No stamp is needed.

YOUR DETAILS

PLEASE NOTE: the section below will be detached to ensure that your details will remain private and confidential at all times and your choice to participate further in this study is voluntary.

First Name

Surname

Street Address

Suburb

State

Postcode

Telephone Number

Date of birth: Day / Month / Year

Today's date: Day / Month / Year
I have read the information provided about the HEALTH IN MEN STUDY and any questions I have asked have been answered to my satisfaction. I agree to take part in this study, realising that I may withdraw at any time without giving a reason and without affecting my future medical treatment. I consent to the following:

1. To complete the HEALTH IN MEN QUESTIONNAIRE to the best of my ability.

2. To take part in a telephone interview(s), if requested. This will include a simple memory test. I may also be asked some questions about anxiety symptoms.

3. To the information provided in this questionnaire being compared with previous questionnaires I have completed for this study.

4. To my answers being linked to any other information about my health such as admissions I have had to hospital, and any use of community services to help support me at home or in residential care.

5. To the information being used in the HEALTH IN MEN STUDY, and in future projects in the same general area of research, provided they have been approved by an ethics review body.

I have been advised as to what information is being collected, what the purpose is, and what will be done with the information upon completion of the research. I understand that all the information I give will remain confidential and be stored securely. I agree that research information gathered for the study may be published provided my name or other identifying information is not used.

Name (Participant)

Please print participant's name in BLOCK letters

Signature (Participant) ___________________________ Date _____ / _____ / 20_____

The Human Research Ethics Committee at the University of Western Australia requires that all participants are informed that, if they have any complaint regarding the manner in which a research project is conducted, it may be given to Professor Leon Flicker (telephone number 9224 2855) or, alternatively to the Secretary, Human Research Ethics Committee, Registrar’s Office, University of Western Australia, 35 Stirling Highway, Crawley, WA 6009 (telephone number 6488 3703). All study participants will be provided with a copy of the Information Sheet and Consent Form for their personal records.
## DEMOGRAPHIC CHARACTERISTICS

1. **What is your marital status now?**
   - ○ Married
   - ○ Divorced
   - ○ Defacto
   - ○ Widowed
   - ○ Separated
   - ○ Never married

2. **Have you ever been widowed?**
   - ○ Yes
   - ○ No (Go to question 4)

3. **What was the date of bereavement?**
   If you have been widowed more than once, please write the most recent date.
   - [ ] / [ ] / [ ]
   - Day
   - Month
   - Year

4. **With whom do you live now? (Please select **ONE** response only)**
   - ○ Living alone
   - ○ Living with spouse or partner only
   - ○ Living with other relatives (with or without spouse or partner)
   - ○ Living with other persons (non-relatives)
   - ○ Living permanently in a residential aged care facility (hostel or nursing home)
   - ○ Other (Please specify below)

5. **In what kind of building do you live now? (Please select **ONE** response only)**
   It does not matter whether the building is owned, being purchased or is a private or public rental (eg, Homeswest)
   - ○ Separate house
   - ○ Semi-detached duplex, row, town or terrace house etc.
   - ○ Flat, unit or apartment
   - ○ Independent living unit in a retirement village
   - ○ Residential aged care facility - hostel or low level care
   - ○ Residential aged care facility - nursing home or high level care
   - ○ Other (Please specify below)
6. How many stairs or steps are in the building where you live now?
   - No stairs or steps
   - 1 stair or step
   - 2 or more stairs or steps

7. Which language did you first learn as a child?
   - English (Go to question 10)
   - Other (Please specify below)
     [ ]_________ [ ]_________ [ ]_________ [ ]_________ [ ]_________ [ ]_________ [ ]_________ [ ]

8. How often do you speak this language now? (Please select ONE response only)
   - Daily
   - Several times each week
   - At least once each week
   - Several times a month
   - At least once each month
   - Less often than monthly

9. With whom do you speak this language? (Please answer ALL questions)

   a. Wife or partner
      ☐ Yes ☐ No

   b. Children
      ☐ Yes ☐ No

   c. Other close relatives
      ☐ Yes ☐ No

   d. Friends
      ☐ Yes ☐ No

   e. Other (eg, work, health professional, carer)
      ☐ Yes ☐ No

10. Have you ever served in the armed forces of Australia, New Zealand, the United Kingdom or another Commonwealth country?
    - Yes
    - No
11. Do you have a carer? This may be a family member, friend or neighbour who provides regular and sustained assistance to you without payment other than a pension or benefit. It does not include an Agency or other paid worker.
   ○ Yes
   ○ No (Go to question 15)

12. Does your main carer live with you? If you have more than one carer, answer in terms of the carer who gives you the most support.
   ○ Yes
   ○ No

13. Who is your main carer? This is the person who gives you the most support. (Please select ONE response only)
   ○ Spouse or partner
   ○ Son or daughter
   ○ Son-in-law or daughter-in-law
   ○ Grandchild(ren)
   ○ Other relative
   ○ Friend
   ○ Neighbour

14. Who are your other carers? (Please choose ALL that apply)
   ○ Not applicable, no other carer(s)
   ○ Spouse or partner
   ○ Son or daughter
   ○ Son-in-law or daughter-in-law
   ○ Grandchild(ren)
   ○ Other relative
   ○ Friend
   ○ Neighbour
15. With what person do you share the closest (most satisfying) emotional relationship now in terms of social support, friendship / companionship / confidante, love and affection? (Please select ONE response only)

○ Not applicable, no close relationships (Go to question 18)
○ Spouse or partner
○ Son or daughter
○ Son-in-law or daughter-in-law
○ Grandchild(ren)
○ Other relative
○ Friend
○ Neighbour

16. How often do you usually have contact with this person (regardless of who initiates it)? Includes telephone calls, visits, letters, etc. (Please select ONE response only)

○ Daily
○ Weekly
○ Fortnightly
○ Monthly
○ Less often than monthly

17. Where does this person live? (Please select ONE response only)

○ We live together in the same dwelling
○ In the same neighbourhood but not the same dwelling as me
○ In a different metropolitan neighbourhood in Western Australia
○ In rural Western Australia
○ Interstate
○ Overseas

HEIGHT & WEIGHT

18. How tall are you without shoes now?

\[ \text{centimetres} \quad \text{OR} \quad \text{feet} \quad \text{AND} \quad \text{inches} \]

19. How much do you weigh without clothes or shoes now?

\[ \text{kilograms} \quad \text{OR} \quad \text{stone} \quad \text{AND} \quad \text{pounds} \]
### HEALTH CONDITIONS

#### 20. Have you ever been told by a doctor that you have any of the following conditions? (Please answer EACH question)

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>a.</td>
<td><strong>Arthritis</strong> <em>(including osteoarthritis &amp; rheumatoid arthritis)</em></td>
</tr>
<tr>
<td>b.</td>
<td><strong>Osteoporosis</strong></td>
</tr>
<tr>
<td>c.</td>
<td><strong>Angina</strong></td>
</tr>
<tr>
<td>d.</td>
<td><strong>Stroke</strong></td>
</tr>
<tr>
<td>e.</td>
<td><strong>Heart attack</strong> <em>(sudden onset)</em></td>
</tr>
<tr>
<td>f.</td>
<td><strong>Heart failure</strong></td>
</tr>
<tr>
<td>g.</td>
<td><strong>Atrial fibrillation</strong></td>
</tr>
<tr>
<td>h.</td>
<td><strong>Other problems with irregular heart beat</strong></td>
</tr>
<tr>
<td>i.</td>
<td><strong>Obstructive sleep apnoea</strong> <em>(eg, requires use of a CPAP machine)</em></td>
</tr>
<tr>
<td>j.</td>
<td><strong>Asthma</strong></td>
</tr>
<tr>
<td>k.</td>
<td><strong>Chronic bronchitis</strong> <em>(ie, continues for 3 months or more &amp; keeps coming back)</em></td>
</tr>
<tr>
<td>l.</td>
<td><strong>Emphysema</strong></td>
</tr>
<tr>
<td>m.</td>
<td><strong>Prostate cancer</strong></td>
</tr>
<tr>
<td>n.</td>
<td><strong>Other prostate problems</strong> <em>(eg, benign or noncancerous enlargement)</em></td>
</tr>
<tr>
<td>o.</td>
<td><strong>Bowel cancer</strong></td>
</tr>
<tr>
<td>p.</td>
<td><strong>Melanoma</strong></td>
</tr>
<tr>
<td>q.</td>
<td><strong>Other skin cancer</strong> <em>(eg, basal or squamous cell carcinoma)</em></td>
</tr>
<tr>
<td>r.</td>
<td><strong>Alzheimer's disease or dementia</strong></td>
</tr>
<tr>
<td>s.</td>
<td><strong>Leg ulcers</strong></td>
</tr>
<tr>
<td>t.</td>
<td><strong>Glaucoma</strong></td>
</tr>
<tr>
<td>u.</td>
<td><strong>Cataracts</strong></td>
</tr>
<tr>
<td>v.</td>
<td><strong>Macular degeneration</strong> <em>(loss of central vision)</em></td>
</tr>
<tr>
<td>w.</td>
<td><strong>Epilepsy, seizures or fits</strong></td>
</tr>
<tr>
<td>x.</td>
<td><strong>Parkinson's disease</strong></td>
</tr>
<tr>
<td>y.</td>
<td><strong>Thyroid problems</strong></td>
</tr>
<tr>
<td>z.</td>
<td><strong>Irritable bowel syndrome or colitis or diverticular disease</strong></td>
</tr>
</tbody>
</table>
21. How often have you had any of the following problems in the **last 12 months**?  
(Please answer EACH question)

<table>
<thead>
<tr>
<th>a. Stiff or painful joints</th>
<th>Never</th>
<th>Rarely</th>
<th>Sometimes</th>
<th>Often</th>
</tr>
</thead>
<tbody>
<tr>
<td>b. Back pain</td>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
</tr>
<tr>
<td>c. Problems with one or both feet</td>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
</tr>
<tr>
<td>d. Breathing difficulty</td>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
</tr>
<tr>
<td>e. Indigestion or heartburn</td>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
</tr>
<tr>
<td>f. Chest pain</td>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
</tr>
<tr>
<td>g. Constipation</td>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
</tr>
<tr>
<td>h. Poor memory</td>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
</tr>
<tr>
<td>i. Dizziness, loss of balance</td>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
</tr>
<tr>
<td>j. Difficulty swallowing</td>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
</tr>
<tr>
<td>k. Problems with teeth or gums</td>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
</tr>
<tr>
<td>l. Anxiety or panic attacks</td>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
</tr>
</tbody>
</table>

22. Have you consulted an 'alternative health practitioner' (eg, herbalist, chiropractor, naturopath, acupuncturist, etc) for your own health in the **last 12 months**?  
○ Yes  
○ No

23. Do you get pain or discomfort in your leg(s) when walking?  
○ Yes (Please answer questions 24 to 29)  
○ No (Go to question 30)  
○ I am unable to walk (Go to question 30)
24. How far can you walk at an ordinary pace on level ground before this pain stops you?
   ○ Less than 100 yards
   ○ 100 yards to a quarter of a mile
   ○ Over a quarter of a mile

25. Does this pain ever begin when you are standing still or sitting?
   ○ Yes
   ○ No

26. Do you get this pain if you walk uphill or hurry?
   ○ Yes
   ○ No

27. Do you get this pain when you walk at ordinary pace on level ground?
   ○ Yes
   ○ No

28. What happens to this pain if you stand still?
   ○ It usually continues for more than 10 minutes
   ○ It usually disappears in 10 minutes or less

29. Where do you get this pain or discomfort? Please mark the place(s) with X on the diagram below.

   [Diagram]

   For office use only
   ○ FR1  ○ FR2  ○ FR3
   ○ FL1  ○ FL2  ○ FL3
   ○ BL1  ○ BL2  ○ BL3
   ○ BR1  ○ BR2  ○ BR3
30. Have you ever been told by a doctor that you have had a stroke?
   ○ Yes (Please answer questions 31 to 34)
   ○ No (Go to question 35)

31. When did the first stroke episode occur?
   Month / Year

32. When did the most recent stroke episode occur?
   Month / Year

33. Did you see a doctor about this most recent episode?
   ○ Yes
   ○ No

34. Were you admitted to hospital following this most recent episode?
   ○ Yes (Please answer question 34.1)
   ○ No (Go to question 35)

34.1 If you answered 'Yes', what hospital were you admitted to?

   | | | | | | | | | | | | | | | |
35. Have you ever been told by a doctor that you have hypertension (high blood pressure)?
   - Yes (Please answer questions 36 to 39)
   - No (Go to question 40)

36. Please state your age when you were first told that you had hypertension (high blood pressure).
   [ ] Years

37. Have you ever been given advice or treatment for your hypertension?
   - Yes
   - No

37.1 If you answered 'Yes', what kind of advice or treatment were you given?
   (Please answer EACH question)

<table>
<thead>
<tr>
<th></th>
<th>Tablets</th>
<th>Yes</th>
<th>No</th>
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<tr>
<th></th>
<th>Diet</th>
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<th>No</th>
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<tr>
<th></th>
<th>Exercise</th>
<th>Yes</th>
<th>No</th>
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<td>c</td>
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<th></th>
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<tr>
<td>d</td>
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</table>

38. Please state your age when you were first given advice or treatment for hypertension.
   [ ] Years

39. What kind of advice or treatment are you following now for hypertension?
   (Please answer EACH question)

<table>
<thead>
<tr>
<th></th>
<th>None</th>
<th>Yes</th>
<th>No</th>
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<tr>
<th></th>
<th>Tablets</th>
<th>Yes</th>
<th>No</th>
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<th>Diet</th>
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<th></th>
<th>Exercise</th>
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<tr>
<th></th>
<th>Other (Please specify below)</th>
<th>Yes</th>
<th>No</th>
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<td>e</td>
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</table>
**DIABETES**

40. Have you ever been told by a doctor that you have diabetes (high sugar levels)?
   - Yes (Please answer questions 41 to 44)
   - No (Go to question 45)

41. Please state your age when you were first told that you had diabetes (high sugar levels).

42. Have you ever been given advice or treatment for your diabetes?
   - Yes
   - No

42.1 If you answered 'Yes', what kind of advice or treatment were you given?
   (Please answer EACH question)

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Tablets</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>b. Diet</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>c. Exercise</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>d. Insulin injections</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>e. Other (Please specify below)</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>f. Other (Please specify below)</td>
<td>[ ]</td>
<td>[ ]</td>
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</tbody>
</table>

43. Please state your age when you were first given advice or treatment for diabetes.

44. What kind of advice or treatment are you following now for diabetes?
   (Please answer EACH question)

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
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</thead>
<tbody>
<tr>
<td>a. None</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>b. Tablets</td>
<td>[ ]</td>
<td>[ ]</td>
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<tr>
<td>c. Diet</td>
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<td>[ ]</td>
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<tr>
<td>d. Exercise</td>
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<td>[ ]</td>
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<tr>
<td>e. Insulin injections</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>f. Other (Please specify below)</td>
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<td>[ ]</td>
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</tbody>
</table>
45. Have you ever been told by a doctor that you have hyperlipidaemia (high levels of cholesterol, lipids, triglycerides or fats in the blood)?
   - Yes (Please answer questions 46 to 49)
   - No (Go to question 50)

46. Please state your age when you were first told that you had hyperlipidaemia (high levels of cholesterol, lipids, triglycerides or fats in the blood)?

47. Have you ever been given advice or treatment for your hyperlipidaemia?
   - Yes
   - No

47.1 If you answered 'Yes', what kind of advice or treatment were you given? (Please answer EACH question)

<table>
<thead>
<tr>
<th></th>
<th>a. Tablets</th>
<th></th>
<th>b. Diet</th>
<th></th>
<th>c. Exercise</th>
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<th>d. Other (Please specify below)</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

48. Please state your age when you were first given advice or treatment for high levels of cholesterol, lipids, triglycerides or fats in the blood?

49. What kind of advice or treatment are you following now for high levels of cholesterol, lipids, triglycerides or fats in the blood? (Please answer EACH question)

<table>
<thead>
<tr>
<th></th>
<th>a. None</th>
<th></th>
<th>b. Tablets</th>
<th></th>
<th>c. Diet</th>
<th></th>
<th>d. Exercise</th>
<th></th>
<th>e. Other (Please specify below)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

Table: HIGH CHOLESTEROL OR BLOOD FATS

45. Have you ever been told by a doctor that you have hyperlipidaemia (high levels of cholesterol, lipids, triglycerides or fats in the blood)?

46. Please state your age when you were first told that you had hyperlipidaemia (high levels of cholesterol, lipids, triglycerides or fats in the blood)?

47. Have you ever been given advice or treatment for your hyperlipidaemia?

47.1 If you answered 'Yes', what kind of advice or treatment were you given? (Please answer EACH question)

48. Please state your age when you were first given advice or treatment for high levels of cholesterol, lipids, triglycerides or fats in the blood?

49. What kind of advice or treatment are you following now for high levels of cholesterol, lipids, triglycerides or fats in the blood? (Please answer EACH question)
50. Have you ever been told by a doctor that you have depression?
   ○ Yes (Please answer questions 51 to 54)
   ○ No (Go to question 55)

51. Please state your age when you were first told that you had depression?
   □ □ Years

52. Have you ever been given advice or treatment for your depression?
   ○ Yes
   ○ No

   52.1 If you answered 'Yes', what kind of advice or treatment were you given?
       (Please answer EACH question)

   | a. Tablets | ○ Yes | ○ No |
   | b. Exercise | ○ Yes | ○ No |
   | c. Psychological treatment or counselling | ○ Yes | ○ No |
   | d. Electro convulsive therapy (ECT) | ○ Yes | ○ No |
   | e. Other (Please specify below) | ○ Yes | ○ No |

53. Please state your age when you were first given advice or treatment for depression.
   □ □ Years

54. What kind of advice or treatment are you following now for depression?
   (Please answer EACH question)

   | a. None | ○ Yes | ○ No |
   | b. Tablets | ○ Yes | ○ No |
   | c. Exercise | ○ Yes | ○ No |
   | d. Psychological treatment or counselling | ○ Yes | ○ No |
   | e. Electro convulsive therapy (ECT) | ○ Yes | ○ No |
   | f. Other (Please specify below) | ○ Yes | ○ No |
## FALLS & INJURIES

55. In the last 12 months, have you:  
(Please answer EACH question)

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Slipped, tripped or stumbled? (not including falls to the ground)</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>b. Had a fall to the ground? (does not include stumbles or trips)</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>c. Been injured as a result of a fall?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>d. Had any other injury from an accident at your home (eg, burns, cuts, bruises)</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>e. Broken or fractured any bones.</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

## SENSORY FUNCTIONING

56. Do you ever have:  
(Please answer EACH question)

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Difficulty seeing newspaper print, even with glasses?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>b. Difficulty recognising people across the road, even with glasses?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>c. Difficulty in hearing a conversation, even with a hearing aid?</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

You have now completed the first part of the questionnaire.

This would be a good time to take a break. It would also be helpful if you used this time to gather all of your medicines and vitamins etc, in preparation for the questions that will be asked in the next section.

The following section of the questionnaire will continue to ask questions about your health, mood, lifestyle and medications. If you feel uncomfortable about answering a question, then please just leave it blank and move on to the next one.

Please try to finish the questionnaire if you can.
GENERAL HEALTH

57. In general, would you say your health is...
(Please select ONE response only)
○ Excellent ○ Very good ○ Good ○ Fair ○ Poor

58. Compared to one year ago, how would you rate your health in general now? Would you say it is...
(Please select ONE response only)
○ Much better now than one year ago ○ Somewhat better now than one year ago
○ About the same as one year ago ○ Somewhat worse now than one year ago
○ Much worse now than one year ago

59. The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?
(Please answer EACH question)

a. VIGOROUS ACTIVITIES, such as running, lifting heavy objects, participating in strenuous sports
   ○ Yes, limited a lot ○ Yes, limited a little ○ No, not limited at all

b. MODERATE ACTIVITIES, such as moving a table, pushing a vacuum cleaner, bowling or playing golf
   ○ Yes, limited a lot ○ Yes, limited a little ○ No, not limited at all

c. Lifting or carrying groceries
   ○ Yes, limited a lot ○ Yes, limited a little ○ No, not limited at all

d. Climbing SEVERAL flights of stairs
   ○ Yes, limited a lot ○ Yes, limited a little ○ No, not limited at all

e. Climbing ONE flight of stairs
   ○ Yes, limited a lot ○ Yes, limited a little ○ No, not limited at all

f. Bending, kneeling or stooping
   ○ Yes, limited a lot ○ Yes, limited a little ○ No, not limited at all

g. Walking MORE THAN ONE kilometre
   ○ Yes, limited a lot ○ Yes, limited a little ○ No, not limited at all

h. Walking HALF a kilometre
   ○ Yes, limited a lot ○ Yes, limited a little ○ No, not limited at all

i. Walking 100 metres
   ○ Yes, limited a lot ○ Yes, limited a little ○ No, not limited at all

j. Bathing or dressing yourself
   ○ Yes, limited a lot ○ Yes, limited a little ○ No, not limited at all
60. During the **past 4 weeks**, have you had any of the following problems with your work (including your work outside the home and housework) or other regular daily activities **AS A RESULT OF YOUR PHYSICAL HEALTH**? (Please answer **EACH** question)

<table>
<thead>
<tr>
<th></th>
<th>Cut down on the amount of time you spent on work or other activities</th>
<th>〇 Yes</th>
<th>〇 No</th>
</tr>
</thead>
<tbody>
<tr>
<td>a.</td>
<td>Accomplished less than you would like</td>
<td>〇 Yes</td>
<td>〇 No</td>
</tr>
<tr>
<td>b.</td>
<td>Were limited in the kind of work or other activities</td>
<td>〇 Yes</td>
<td>〇 No</td>
</tr>
<tr>
<td>c.</td>
<td>Had difficulty performing the work or other activities</td>
<td>〇 Yes</td>
<td>〇 No</td>
</tr>
<tr>
<td>d.</td>
<td>(for example, it took extra effort)</td>
<td>〇 Yes</td>
<td>〇 No</td>
</tr>
</tbody>
</table>

61. During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **AS A RESULT OF ANY EMOTIONAL PROBLEMS** (such as feeling depressed or anxious)? (Please answer **EACH** question)

<table>
<thead>
<tr>
<th></th>
<th>Cut down on the amount of time you spent on work or other activities</th>
<th>〇 Yes</th>
<th>〇 No</th>
</tr>
</thead>
<tbody>
<tr>
<td>a.</td>
<td>Accomplished less than you would like</td>
<td>〇 Yes</td>
<td>〇 No</td>
</tr>
<tr>
<td>b.</td>
<td>Didn't do work or other activities as carefully as usual</td>
<td>〇 Yes</td>
<td>〇 No</td>
</tr>
</tbody>
</table>

62. During the **past 4 weeks**, to what extent has your **PHYSICAL HEALTH OR EMOTIONAL PROBLEMS** interfered with your normal social activities with family, friends, neighbours or groups?

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>〇 Not at all</th>
<th>〇 Slightly</th>
<th>〇 Moderately</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>〇 Quite a bit</td>
<td>〇 Extremely</td>
<td></td>
</tr>
</tbody>
</table>

63. How much **BODILY PAIN** have you had during the **past 4 weeks**?

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>〇 No bodily pain</th>
<th>〇 Very mild</th>
<th>〇 Mild</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>〇 Moderate</td>
<td>〇 Severe</td>
<td>〇 Very severe</td>
</tr>
</tbody>
</table>

64. During the **past 4 weeks**, how much did **PAIN** interfere with your normal work (including both work outside the home and housework)?

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>〇 Not at all</th>
<th>〇 A little bit</th>
<th>〇 Moderately</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>〇 Quite a bit</td>
<td>〇 Extremely</td>
<td></td>
</tr>
</tbody>
</table>
65. For each question, please give one answer that comes closest to the way you have been feeling during the past 4 weeks:
(Please answer EACH question)

a. Did you feel full of life?
   ○ All of the time ○ Most of the time ○ A good bit of the time
   ○ Some of the time ○ A little of the time ○ None of the time

b. Have you been a very nervous person?
   ○ All of the time ○ Most of the time ○ A good bit of the time
   ○ Some of the time ○ A little of the time ○ None of the time

c. Have you felt so down in the dumps that nothing could cheer you up?
   ○ All of the time ○ Most of the time ○ A good bit of the time
   ○ Some of the time ○ A little of the time ○ None of the time

d. Have you felt calm and peaceful?
   ○ All of the time ○ Most of the time ○ A good bit of the time
   ○ Some of the time ○ A little of the time ○ None of the time

e. Did you have a lot of energy?
   ○ All of the time ○ Most of the time ○ A good bit of the time
   ○ Some of the time ○ A little of the time ○ None of the time

f. Have you felt down?
   ○ All of the time ○ Most of the time ○ A good bit of the time
   ○ Some of the time ○ A little of the time ○ None of the time

g. Did you feel worn out?
   ○ All of the time ○ Most of the time ○ A good bit of the time
   ○ Some of the time ○ A little of the time ○ None of the time

h. Have you been a happy person?
   ○ All of the time ○ Most of the time ○ A good bit of the time
   ○ Some of the time ○ A little of the time ○ None of the time

i. Did you feel tired?
   ○ All of the time ○ Most of the time ○ A good bit of the time
   ○ Some of the time ○ A little of the time ○ None of the time
66. During the past 4 weeks, how much of the time has your PHYSICAL HEALTH OR EMOTIONAL PROBLEMS interfered with your social activities (like visiting friends, relatives, etc)?

- All of the time
- Most of the time
- Some of the time
- A little of the time
- None of the time

67. How true or false is each of the following statements to you? (Please answer EACH question)

a. I seem to get sicker a little easier than other people
   - Definitely true
   - Mostly true
   - Definitely false
   - Most of the time
   - None of the time

b. I am as healthy as anybody I know
   - Definitely true
   - Mostly true
   - Definitely false
   - Most of the time
   - None of the time

c. I expect my health to get worse
   - Definitely true
   - Mostly true
   - Definitely false
   - Most of the time
   - None of the time

d. My health is excellent
   - Definitely true
   - Mostly true
   - Definitely false
   - Most of the time
   - None of the time

SLEEPING

68. Do you have any of these sleep problems? (Please answer EACH question)

a. Waking up in the early hours of the morning
   - Yes
   - No

b. Lying awake for most of the night
   - Yes
   - No

c. Taking a long time to get to sleep
   - Yes
   - No

d. Worry keeping you awake at night
   - Yes
   - No

e. Sleeping badly at night
   - Yes
   - No

f. Excessive daytime sleepiness
   - Yes
   - No
### GENERAL HEALTH

**69. Over the past month, how often have you had a sensation of not emptying your bladder completely after you finish urinating?**
- Not at all
- About half the time
- Less than 1 in 5 times
- More than half the time
- Less than half the time
- Almost always

**70. Over the past month, how often have you had to urinate again less than two hours after you finished urinating?**
- Not at all
- About half the time
- Less than 1 in 5 times
- More than half the time
- Less than half the time
- Almost always

**71. Over the past month, how often have you found you stopped and started again several times when you urinated?**
- Not at all
- About half the time
- Less than 1 in 5 times
- More than half the time
- Less than half the time
- Almost always

**72. Over the past month, how difficult have you found it to postpone urination?**
- Not at all
- About half the time
- Less than 1 in 5 times
- More than half the time
- Less than half the time
- Almost always

**73. Over the past month, how often have you had to push or strain to begin urination?**
- Not at all
- About half the time
- Less than 1 in 5 times
- More than half the time
- Less than half the time
- Almost always

**74. Over the past month, how many times did you most typically get up to urinate from the time you went to bed until the time you got up in the morning?**
- Not at all
- About half the time
- Less than 1 in 5 times
- More than half the time
- Less than half the time
- Almost always

**75. If you were to spend the rest of your life with your urinary condition the way it is now, how would you feel about that?**
- Delighted
- Pleased
- Mostly satisfied
- Mixed - about equally satisfied & dissatisfied
- Mostly dissatisfied
- Unhappy
- Terrible
SEXUAL RELATIONSHIPS

Now we would like to ask you about sexual relationships you may have had. By 'sex' or 'sexual activity', we mean any mutually voluntary activity with another person that involves sexual contact, whether or not intercourse or orgasm occurs.

76. For some people sex is an important part of their lives and for others it is not very important at all. How important a part of your life would you say that sex is now?

- Extremely
- Very
- Moderately
- Somewhat
- Not at all

77. How many people, including men and women, have you had sexual activity with in the last five years, even if only one time?

<table>
<thead>
<tr>
<th>Number of People</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

78. During the last 12 months, how often did you have sex?

- Did not have sex (Go to question 80)
- At least once a week
- Two to three times a month
- Once a month or less

79. During the last 12 months, would you say that you had sex:

- More often than you would like?
- About as often as you would like? (Go to question 81)
- Less often than you would like?

80. If you did not have sex in the last 12 months, why not? (Please answer EACH question)

<table>
<thead>
<tr>
<th>Reason</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. I was not interested</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. My partner was not interested</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. Physical problems or limitations I had</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d. Physical problems or limitations my partner had</td>
<td></td>
<td></td>
</tr>
<tr>
<td>e. My children or other family members would not approve</td>
<td></td>
<td></td>
</tr>
<tr>
<td>f. I was grieving</td>
<td></td>
<td></td>
</tr>
<tr>
<td>g. I did not have a partner</td>
<td></td>
<td></td>
</tr>
<tr>
<td>h. Other (Please specify below)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
81. Sometimes people go through periods in which they are not interested in sex or are having trouble with sexual gratification. During the last 12 months, has there ever been a period of several months or more when you: (Please answer EACH question)

a. Lacked interest in having sex?
   - No
   - Yes, and it bothered me
   - Yes, but it did not bother me

b. Were unable to climax (experience an orgasm)?
   - No
   - Yes, and it bothered me
   - Yes, but it did not bother me

c. Came to a climax (experienced an orgasm) too quickly?
   - No
   - Yes, and it bothered me
   - Yes, but it did not bother me

d. Experienced physical pain during intercourse?
   - No
   - Yes, and it bothered me
   - Yes, but it did not bother me

e. Found sex unpleasurable (even if it was not painful)?
   - No
   - Yes, and it bothered me
   - Yes, but it did not bother me

f. Felt anxious just before having sex about your ability to perform sexually?
   - No
   - Yes, and it bothered me
   - Yes, but it did not bother me

g. Had trouble gaining or maintaining an erection?
   - No
   - Yes, and it bothered me
   - Yes, but it did not bother me
82. In the last 12 months, have you ever avoided sex because of any of the problems included in questions 81a to 81g?
   ○ No
   ○ Yes
   ○ Not applicable, no problem(s)

83. In the last 12 months, have you ever talked with your doctor about the problems included in questions 81a to 81g?
   ○ No
   ○ Yes
   ○ Not applicable, no problem(s)

84. Which of the following statements best describes your sexual attraction? (Please select ONE response only)
   ○ I have felt sexually attracted only to females, never to males
   ○ I have felt sexually attracted to both females and males
   ○ I have felt sexually attracted only to males, never to females
   ○ I have never felt sexually attracted to anyone at all

85. Which of the following statements best describes your sexual experiences? (Please select ONE response only)
   ○ I have had sexual experiences only with females, never with males
   ○ I have had sexual experiences with both females and males
   ○ I have had sexual experiences only with males, never with females
   ○ I have never had any sexual experiences with anyone at all
### MOOD

86. Over the past two weeks, how often have you been bothered by any of the following? (Please answer EACH question)

   a. Little interest or pleasure in doing things
      - Not at all
      - Several days
      - A week or more
      - Nearly every day

   b. Feeling down, depressed or hopeless
      - Not at all
      - Several days
      - A week or more
      - Nearly every day

   c. Trouble falling or staying asleep, sleeping too much
      - Not at all
      - Several days
      - A week or more
      - Nearly every day

   d. Feeling tired or having little energy
      - Not at all
      - Several days
      - A week or more
      - Nearly every day

   e. Poor appetite or eating too much
      - Not at all
      - Several days
      - A week or more
      - Nearly every day

   f. Feeling bad about yourself, that you are a failure, or have let yourself or your family down
      - Not at all
      - Several days
      - A week or more
      - Nearly every day

   g. Trouble concentrating on things such as reading the newspaper or watching television
      - Not at all
      - Several days
      - A week or more
      - Nearly every day

   h. Moving or speaking slowly that other people could have noticed. Or the opposite, feeling so fidgety or restless that you have been moving around a lot more than usual
      - Not at all
      - Several days
      - A week or more
      - Nearly every day

   i. Thoughts that you would be better off dead or of hurting yourself in some way
      - Not at all
      - Several days
      - A week or more
      - Nearly every day

87. How difficult have these problems made it for you to do your work, take care of things at home, or get along with other people?

   - Not difficult at all
   - Somewhat difficult
   - Very difficult
   - Extremely difficult
88. How many times did you do each type of activity LAST WEEK? Only count the number of times when the activity lasted for 10 minutes or more. If you did NOT do an activity, please write "0" in the box.

a. **Walking briskly** (for recreation or exercise or to get from place to place)
   
<table>
<thead>
<tr>
<th>Times in the last 7 days</th>
</tr>
</thead>
</table>

b. **Moderate leisure activity** (like social tennis, golf, bowls, recreational swimming, dancing)
   
<table>
<thead>
<tr>
<th>Times in the last 7 days</th>
</tr>
</thead>
</table>

c. **More vigorous leisure activity** (that makes you breathe harder or puff and pant)
   
<table>
<thead>
<tr>
<th>Times in the last 7 days</th>
</tr>
</thead>
</table>

d. **Vigorous household or garden chores** (that make you breathe harder or puff and pant)
   
<table>
<thead>
<tr>
<th>Times in the last 7 days</th>
</tr>
</thead>
</table>

89. If you add up all the times you spent in each activity LAST WEEK, how much time did you spend ALTOGETHER doing each type of activity? If you did NOT do an activity, please write "0" in the box.

a. **Walking briskly** (for recreation or exercise or to get from place to place)
   
<table>
<thead>
<tr>
<th>hrs</th>
<th>mins</th>
</tr>
</thead>
</table>

b. **Moderate leisure activity** (like social tennis, golf, bowls, recreational swimming, dancing)
   
<table>
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<tr>
<th>hrs</th>
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c. **More vigorous leisure activity** (that makes you breathe harder or puff and pant)
   
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<th>hrs</th>
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</table>

d. **Vigorous household or garden chores** (that make you breathe harder or puff and pant)
   
<table>
<thead>
<tr>
<th>hrs</th>
<th>mins</th>
</tr>
</thead>
</table>
DIETARY HABITS

90. How many times a week do you eat meat, including sausages, devon, polony, salamis, meat pies, hamburger or bacon (but not including fish)?

- Six or more times a week
- Once or twice a week
- Never
- Three to five times a week
- Less than once a week

91. How often do you eat fish?

- Six or more times a week
- Once or twice a week
- Never
- Three to five times a week
- Less than once a week

92. What is the main type of milk that you usually use?

- Whole or full cream milk
- Skim milk
- Soy milk
- Do not use milk
- Low or reduced fat milk
- Evaporated or sweetened condensed milk
- Other milk

93. How often do you add salt to your food after it is cooked?

- Rarely or never
- Sometimes
- Almost always or always

94. What do you spread on bread?

- Always butter
- Butter or margarine
- No spread at all
- Always margarine
- Other

95. How many serves of vegetables do you usually eat each day? This includes fresh, frozen and tinned vegetables. A serve = half a cup of cooked vegetables or a cup of salad vegetables.

- Do not eat vegetables
- 4 serves
- 1 serve
- 5 serves
- 2 or 3 serves
- 6 or more serves

96. How many serves of fruit do you usually eat each day? This includes fresh, frozen and tinned fruit. A serve = one medium piece or two small pieces of fruit or one cup of diced pieces.

- Do not eat fruit
- 4 serves
- 1 serve
- 5 serves
- 2 or 3 serves
- 6 or more serves
97. Which of the following best describes your tobacco smoking status now? (Please select ONE response only)
   - Daily smoker
   - Not a daily smoker, but at least a once a week smoker
   - Irregular smoker (less than weekly)
   - Ex-smoker (but at least 100 cigarettes or tobacco products in your entire life) (Go to question 99)
   - Never smoker (or fewer than 100 cigarettes or other tobacco products in your entire life) (Go to question 100)

98. If you smoke now, how much do you smoke? (Please answer EACH question)
   a. Manufactured cigarettes daily
   b. Grams of 'hand-rolled' cigarette tobacco per week (a 1 & 3/4 ounce pouch of cigarette tobacco equals 50 grams)
   c. Cigars per week
   d. Grams of pipe tobacco per week.

99. How old were you when you last stopped smoking regularly (that is, at least once a day)? (Please select ONE response only)
   - Not applicable (I am still a daily smoker or I have never smoked daily)
   - Specify your age in years when you last stopped smoking regularly
   - Specify how many years ago you last stopped smoking regularly (eg '07' for 7 years ago)
   - Specify the year when you last stopped smoking regularly (eg, 2003)
ALCOHOL USE

100. Have you ever drunk alcohol?
   ○ Yes
   ○ No

101. Have you drunk alcohol in the last year?
   ○ Yes
   ○ No

102. Do you drink at least weekly now?
   ○ Yes
   ○ No (Go to question 103)

102.1. If you answered 'Yes', how many 'standard' drinks do you have each day in a usual week?
   (Please provide an answer for EACH day. If you did NOT have a drink, please write "0" in the box for that day.)

   1 'standard' drink equals:
   ● 1 middy of full-strength (5%) beer, or
   ● 1 pub measure of spirits, or
   ● 1 glass of sherry or port, or
   ● 1 glass of wine, or
   ● 1.5 middies of Swan Gold, or
   ● 2 middies of Toohey's 2.2, or
   ● 5 middies of Swan Light

   Drinks on Monday
   Drinks on Tuesday
   Drinks on Wednesday
   Drinks on Thursday
   Drinks on Friday
   Drinks on Saturday
   Drinks on Sunday
In the last month have you had any difficulty in completing any of the following activities? 'Difficulty' includes needing to take extra time, changing the activity or using a device to help you. If you require any type of assistance from another person (eg, setting up, encouragement, standby supervision), then mark 'Unable to do without help' as your answer. (Please answer EACH question)

<table>
<thead>
<tr>
<th>Activity</th>
<th>No difficulty</th>
<th>Some difficulty</th>
<th>Major difficulty</th>
<th>Unable to do without help</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Grooming</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eg, washing face, cleaning teeth, brushing hair, shaving</td>
<td>○</td>
<td>○</td>
<td>○</td>
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<tr>
<td>b. Eating normal food (not just soft food)</td>
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<tr>
<td>eg, cutting up food, spreading butter, feeding</td>
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<tr>
<td>c. Bathing or taking a shower</td>
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<tr>
<td>eg, adjusting water, lathering, getting in and out, drying</td>
<td>○</td>
<td>○</td>
<td>○</td>
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<tr>
<td>d. Dressing your upper body</td>
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<tr>
<td>eg, selecting clothes, fastening buttons &amp; zips, fitting special aids</td>
<td>○</td>
<td>○</td>
<td>○</td>
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</tr>
<tr>
<td>e. Dressing your lower body</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eg, selecting clothes, fastening buttons &amp; zips, fitting special aids, tying shoelaces</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
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<tr>
<td>f. Getting up from a chair (without supervision or assistance)</td>
<td>○</td>
<td>○</td>
<td>○</td>
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<tr>
<td>g. Walking inside the house</td>
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<tr>
<td></td>
<td>○</td>
<td>○</td>
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<tr>
<td>h. Using the toilet</td>
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<tr>
<td>eg, undressing, sitting or standing, wiping, &amp; redressing</td>
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<td>○</td>
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<td>i. Shopping for personal items or groceries</td>
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<tr>
<td>(assuming you have transportation)</td>
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<td>○</td>
<td>○</td>
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<tr>
<td>j. Doing light housework</td>
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<tr>
<td>eg, washing dishes, dusting</td>
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</tbody>
</table>
**104. Do you use any of the following aids for getting around? (Please answer EACH question)**

<table>
<thead>
<tr>
<th><strong>a. Motorised scooter</strong></th>
<th>○ Yes</th>
<th>○ No</th>
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</thead>
<tbody>
<tr>
<td><strong>b. Wheelchair (motorised or not)</strong></td>
<td>○ Yes</td>
<td>○ No</td>
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<tr>
<td><strong>c. Walking frame (eg, Zimmer frame)</strong></td>
<td>○ Yes</td>
<td>○ No</td>
</tr>
<tr>
<td><strong>d. Walking stick, podstick or pylon</strong></td>
<td>○ Yes</td>
<td>○ No</td>
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<tr>
<td><strong>e. Four-point stick or quad-stick</strong></td>
<td>○ Yes</td>
<td>○ No</td>
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<tr>
<td><strong>f. Crutch or crutches</strong></td>
<td>○ Yes</td>
<td>○ No</td>
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<tr>
<td><strong>g. Other (please specify below)</strong></td>
<td>○ Yes</td>
<td>○ No</td>
</tr>
</tbody>
</table>

**105. What is your main (or most common) means of transport?**

- ○ Car (you drive)
- ○ Car (someone else drives)
- ○ Taxi
- ○ Bus
- ○ Tram or train
- ○ Other
106. Do you have any of the following problems with transport? (Please answer EACH question)

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
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</thead>
<tbody>
<tr>
<td>a. Problems with getting to places at night</td>
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<tr>
<td>b. Problems with getting to local shops &amp; services</td>
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<tr>
<td>c. Problems with getting beyond your local neighbourhood</td>
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</table>

107. Which of the following groups have you sought help or advice from in the last 6 months? It does not include informal help provided by an unpaid carer such as a family member. (Please answer EACH question)

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
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</thead>
<tbody>
<tr>
<td>a. Food services or preparation (eg, Meals on Wheels, at home or in a centre)</td>
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<td>b. Domestic services (eg, housecleaning, laundry, bill paying)</td>
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<td>c. Home maintenance services (eg, odd jobs, gardening)</td>
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<tr>
<td>d. Personal care services (eg, daily self-care tasks such as eating, bathing, dressing, toileting)</td>
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<td>e. Respite services (at home, residential aged care facility, hospital)</td>
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<td>f. Social support (provided on a one-to-one basis)</td>
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<td>g. Social groups (eg, centre-based day care, Senior Citizens Centre, friendship, craft, exercise or church groups)</td>
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<td>h. Support &amp; advisory groups (eg, Arthritis Foundation, Alzheimer's Association, AdvoCare, carer support group)</td>
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<tr>
<td>i. Nursing care (at home, in a centre or other location)</td>
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<td>j. Allied health care (eg, physiotherapy, occupational therapy, podiatry, dietitian, diabetes educator)</td>
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<td>k. Counselling or other mental health services</td>
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<td>l. Ambulance service</td>
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<tr>
<td>108. Initial Surname</td>
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<td>Dr</td>
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<tr>
<td>Street Address</td>
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<td>Suburb</td>
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<td>State</td>
<td>Postcode</td>
<td></td>
</tr>
<tr>
<td>Telephone Number</td>
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</tbody>
</table>

108.1 Would you be happy for us to contact your doctor about your results if necessary?

- Yes
- No
109. Please list all of your current medicines prescribed by a doctor. If possible, please copy the name of each medicine exactly as it appears on the package or container. Include tablets, capsules, aspirin, liquids or syrups or mixtures, puffers, sprays, nebulisers, creams, ointments, pastes, patches, suppositories, injections, etc taken now.

109.1 Do you currently take any medications prescribed by a doctor?
- Yes (Please provide details below)
- No (Go to question 110)

<table>
<thead>
<tr>
<th>a. Name of medication</th>
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</table>

Approximately how long have you been taking this medication?
- weeks
- months
- years

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<th>b. Name of medication</th>
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</table>

Approximately how long have you been taking this medication?
- weeks
- months
- years

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<tr>
<th>c. Name of medication</th>
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</table>

Approximately how long have you been taking this medication?
- weeks
- months
- years

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<tr>
<th>d. Name of medication</th>
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</table>

Approximately how long have you been taking this medication?
- weeks
- months
- years

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<tr>
<th>e. Name of medication</th>
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Approximately how long have you been taking this medication?
- weeks
- months
- years
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<th></th>
<th>Name of medication</th>
<th>Approximately how long have you been taking this medication?</th>
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<td>f.</td>
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<td>weeks OR months OR years</td>
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<td>g.</td>
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<td>i.</td>
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<td>j.</td>
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<td>weeks OR months OR years</td>
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<td>l.</td>
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<td>weeks OR months OR years</td>
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<td></td>
<td>Name of medication</td>
<td>Approximately how long have you been taking this medication?</td>
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<td>m.</td>
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<td>weeks OR months OR years</td>
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</tbody>
</table>
### CURRENT MEDICATIONS - PHARMACY (NO PRESCRIPTION)

110. Please list all of your current medicines, vitamins, etc bought from a pharmacy (chemist) without a prescription. If possible, please copy the name of each medicine exactly as it appears on the package or container. Include tablets, capsules, aspirin, liquids or syrups or mixtures, puffers, sprays, nebulisers, creams, ointments, pastes, patches, suppositories, etc taken now.

110.1 Do you currently take any medicines, vitamins etc bought from a pharmacy (chemist) without a prescription?

- **Yes (Please provide details below)**
- **No (Go to question 111)**

#### a. Name of medication

<table>
<thead>
<tr>
<th>Name of medication</th>
<th>weeks</th>
<th>OR</th>
<th>months</th>
<th>OR</th>
<th>years</th>
</tr>
</thead>
</table>

Approximately how long have you been taking this medication?

<table>
<thead>
<tr>
<th>weeks</th>
<th>OR</th>
<th>months</th>
<th>OR</th>
<th>years</th>
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</table>

#### b. Name of medication

<table>
<thead>
<tr>
<th>Name of medication</th>
<th>weeks</th>
<th>OR</th>
<th>months</th>
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Approximately how long have you been taking this medication?

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<th>weeks</th>
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#### c. Name of medication

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<tr>
<th>Name of medication</th>
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Approximately how long have you been taking this medication?

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#### d. Name of medication

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<th>Name of medication</th>
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Approximately how long have you been taking this medication?

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#### e. Name of medication

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</table>
CURRENT MEDICATIONS - HEALTHFOOD STORE OR SUPERMARKET

111. Please list all of your current medicines, vitamins, etc bought from a healthfood shop or supermarket. If possible, please copy the name of each medicine exactly as it appears on the package or container. Include tablets, capsules, aspirin, liquids or syrups or mixtures, puffers, sprays, nebulisers, creams, ointments, pastes, patches, suppositories, etc taken now.

111.1 Do you currently take any medicines, vitamins etc bought from a healthfood shop or supermarket?

- ○ Yes (Please provide details below)
- ○ No (Go to question 111)

<table>
<thead>
<tr>
<th>Name of medication</th>
<th>Approximately how long have you been taking this medication?</th>
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</table>
112. If there is ANYTHING else that you would like to tell us about changes in your health (especially in the LAST 3 YEARS) please write on the lines below.
HELP US KEEP IN TOUCH

113. We would be grateful if you would supply the contact details of at least two people who might help us to find you if you move house. These contacts should be younger relatives or close friends who do not live at the same address as you.

Contact 1:

First Name

Surname

Street Address

Suburb

State

Postcode

Telephone Number

Contact 2:

First Name

Surname

Street Address

Suburb

State

Postcode

Telephone Number

THANK YOU

Thank you for taking the time to complete this questionnaire. You are a valuable contributor to research on men's health. If you have any questions about this questionnaire, you can contact us by telephoning 9224 2855 visiting our web page at http://www.wacha.org.au/ or writing to us at

Health in Men Study
WA Centre for Health & Ageing (M573)
University of Western Australia
35 Stirling Highway
Crawley, Perth, WA 6009, Australia
If you are concerned about any aspects of your health and would like some help, then please contact your general practitioner for advice.

If you are feeling troubled now and would like someone to talk to, confidential telephone counselling is available from Lifeline on 131 114 (24-hours a day).

Lifeline's service is just the cost of a local call, but additional charges may apply to calls from pay phones, mobiles or some home phone plans.

When you have completed the questionnaire, please post it back to us in the reply-paid envelope without folding or bending it. No stamp is needed.

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