Biological ageing: Statistical analysis of physical and biochemical biomarkers in UK Biobank

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For Roy, our families and our cats, who surreptitiously aged while I wrote this thesis

And in memory of my grandmother, who brought me up in her 70s and 80s, and lived to 102 but never seemed to grow old

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Abstract

Background: Age is the strongest risk factor for most chronic diseases, yet individuals age biologically at different rates. Summarising patterns of biomarkers that contribute strongly to overall and body system-specific ageing into biological and body system (bodily) ages may aid health risk communication and disease prevention. A systematic review (undertaken within this thesis) found that biological ages indicated or predicted poorer health, but few studies conducted validation or followed good practice for estimating and reporting biological ages.

Methods: Among 480,019 UK Biobank participants aged 40–70 followed up for 6–12 years via linked death registry and hospital records, analyses focused on 141,254 (29.4%) participants healthy at baseline. Sex-specific biological ages were estimated from biomarker principal components (characterised from 72 physical and biochemical biomarkers) via two main approaches: (1) the age-based Klemera Doubal method, which emphasised biomarkers strongly related to chronological age, and (2) a novel disease risk-based approach of aggregating 8 body system ages (artery, musculoskeletal, gut, cardiac, metabolic disease, inflammatory, neurological and lung ages, each estimated using Cox lasso models) using a multi-state model. The proportions of the overall biological and chronological age effects on mortality from chronic disease, age-related frailty and the 8 groups of diseases explained by bodily ages were assessed using likelihood-based measures.

Results: In healthy participants, reduced lung function, reduced kidney function, slower reaction time, lower insulin-like-growth factor 1 and lower hand grip strength strongly featured in the age-based biological age, while higher general adiposity was a shared risk factor across body system ages and therefore featured strongest in the disease risk-based biological age (together with lower central adiposity in men and lower hand grip strength in women). Although key biomarkers of body system ages were generally different from each other and from biomarkers strongly related to chronological age, biomarker patterns of body system ages apart from neurological age were moderately correlated (ρ=0.15–0.69). Gut, cardiac and neurological ages for men, and musculoskeletal and neurological

ages for women contributed substantially to the prediction of mortality and frailty in the disease risk-based biological age.

The age-based biological age overlapped with chronological age to explain two-thirds of the overall biological and chronological age effects on mortality and frailty. Biomarker constituents of the disease risk-based vs age-based biological ages alone explained 11–17% vs 2–8% of the overall effects on mortality and frailty, and the largest improvement was for frailty in healthy women. These proportions were higher when the analysis was repeated in the whole UK Biobank population (25–38% vs 15–35% for disease risk-based vs age-based biological ages). Biomarker constituents of body system ages explained 24–81% of the overall effects for their respective diseases in healthy participants.

Conclusions: Bodily ages, particularly the novel disease risk-based ages, improved our understanding of the biomarker-disease relationships represented by chronological age and improved prognosis of later life health outcomes. Biomarkers across a range of body systems described a common ageing effect and substantial proportions of age effects on later life health, supporting a broader, multi-system risk-based approach to research and prevention of diseases of ageing.

List of awards and presentations related to this thesis

Awards:

1. Nuffield Department of Population Health (NDPH) DPhil poster prize (2019)
2. NDPH DPhil scholarship (2017-2020)

Conference presentations and invited seminars:

1. Oxford Neuroimaging Statistics (NISOx) Reading Group seminar (Jun 2020, by invitation)
2. Oxford NDPH DPhil seminar (May 2020, by invitation)
3. University of Newcastle Ageing Theme seminar (Feb 2020, by invitation)
4. Oxford NDPH Symposium (Jan 2020)
5. Statistical Analysis of Multi-outcome Data Meeting (Jun 2019)
6. Oxford Primary Care Health Sciences Medical Statistics group seminar (Apr 2019, by invitation)
7. Oxford NDPH Symposium (Jan 2019)
8. Oxford Cancer Epidemiology Unit seminar (Feb 2019, by invitation)
9. Young Statisticians' Meeting (Jul 2018)
10. Oxford Medical Sciences Division DPhil Day (Jul 2018)

Part of my DPhil research also contributed to other presentations on 'Multi-state models for the analysis of multiple health outcomes' at the Oxford NDPH Symposium (Jan 2020) and 'Socioeconomic inequalities in health expectancy with and without multiple morbidities' at the Oxford Multimorbidity and Frailty Meeting (May 2018).

Poster presentations:

1. British Heart Foundation (BHF) CRE Annual Symposium (Oct 2019)
2. International Biometric Society Channel Network Conference (Jul 2019)
3. Oxford BRC Long Term Conditions & Multimorbidity/CLAHRC Networking Event (Mar 2019)
4. Gerontological Society of America Annual Meeting (Nov 2018)

Contents

List of Tables

List of Figures

“L'âge chronologique et l'âge biologique sont loin de toujours coïncider : l'apparence physique renseigne mieux que les examens physiologiques sur le nombre de nos années. Celles-ci ne pèsent pas du même poids sur toutes les épaules.”

Simone de Beauvoir, La Vieillesse

Chapter 1: Introduction

1.1 Background

Age is the strongest risk factor for most chronic diseases[1] and leading causes of death.[2] However, age is not biological, so what is it about age that predicts disease so well?

As we enter the Decade of Healthy Ageing, 2020-2030,[3] investigating how 'health' (measured in earlier life) and 'age' intertwine to precipitate age-related decline is extremely timely. The accumulation of chronic diseases over lifetimes, resulting in functional disability,[4] frailty[5] and multimorbidity[6] (the co-occurrence of 2 or more diseases in a person) is well documented worldwide. The presence of one disease can increase the risk of another, which could represent a common biological ageing effect.[7] The doubling of life expectancy in the past 2 centuries across developed countries[8] and inequalities in the prevalence[9] and incidence[10] of chronic disease multimorbidity in the UK suggest that these consequences of ageing are highly modifiable. These consequences are not attributable to a single group of diseases or health conditions – in recent years there has been growing diversity in causes of death in low mortality countries including the UK.[11]

Summarising the biological determinants of ageing into a risk score or health indicator has been proposed as a valuable way to assess differential rates of ageing among individuals and a way to investigate how these biological determinants supplement chronological age in predicting ill health.[12,13] For example, risk scores calibrated to risk of death[12,14,15] or entry into institutional care[15] have identified simple measures, such as self-reported walking pace, that are strong predictors of death in the next 5 years.[14] However, for most of these scores and indicators, their value is limited by the small window of time for intervention and prevention,[16] and findings were derived from multiple small studies (of less than 10,000 individuals) investigating a limited range of biological determinants.

The UK government recently announced ambitious targets to increase healthy life expectancy by at least 5 years by 2035 while also reducing inequalities in life expectancy,[17] through predictive and personalised prevention strategies in healthcare.[17] One potential strategy is the use of biological age, a composite risk score or index estimated from measurements of biomarkers of ageing[7,18-20] and expressed in terms of an age (i.e. in years). Measuring health specifically through biological age was a key recommendation in recent reviews of ageing research.[21,22] These reviews highlighted the importance of prevention, prediction and understanding causal mechanisms[22] to tackle multimorbidity, frailty and disability.[21,22]

Biological ageing

Ageing has been defined by biologists as an "intrinsic, inevitable and irreversible age-related process of loss of viability and increase in vulnerability"[23,24] to pathology or age-related diseases.[2] Cohort studies specifically investigating ageing commenced as early as 1958 (the Baltimore Longitudinal Study of Ageing [BLSA]) but only proliferated in the early 21st century.[25] Nevertheless, an early finding from the BLSA was that its participants aged at different rates according to their biomarker measurements and psychosocial characteristics.[26]

Due to the biological complexity of ageing processes, many theories of biological ageing have been published and have continued to be refined. The earliest hypotheses and studies of ageing, published as early as 1882, concentrated on evolutionary theories and ageing on the cellular level,[27] e.g. ageing as an inevitable process due to the wearing out of tissue,[27] the 'antagonistic pleiotropy' theory (genes that have beneficial effects at younger ages are favoured, regardless of whether they cause harm at older ages), and the 'disposable soma' theory (ageing is not programmed, and happens because energy is expended more efficiently elsewhere instead of maintaining bodily functions).[26] Some later theories that captured the complexity in ageing through hypothesising dynamic networks of multiple mechanisms of ageing on multiple biological scales[28,29] were also proposed by many ageing

researchers but gained more traction with clinical ageing researchers than biologists,[29] as clinical researchers were perhaps more aware of the growing number of patients who present with different patterns of age-related diseases or multimorbidity in clinical practice.

Over the last few decades, there were unresolved debates on whether the underlying process of biological ageing was a singular or multi-system process[30] and whether biological ageing is the same as disease.[2,31] Several prominent biologists in the 1980s held the view that there is no single mechanism of ageing and relationships between physiological systems cannot be captured by a single or composite measure.[25] To highlight the diversity of the biological ageing process, Medvedev summarised more than 300 theories ranging from body system-specific biological pathways to evolutionary, genetic and mathematical theories in 1990.[32] In a similar vein, Peto and Doll argued that there is no singular process of ageing and chronological age is not a causal factor of ageing processes, with the headline "there is no such thing as ageing".[30] However, more recently, some biologists have returned to searching for specific biological mechanisms that synchronise age-related debilitation and disease.[24]

By contrast, in the past few years, gerontologists have often invoked the geroscience hypothesis: that because many chronic diseases share the same underlying major risk factor (chronological age), then intervening in the ageing process will in parallel delay the appearance or severity of these diseases.[1,33] This is still a very new area of investigation as ageing researchers still face difficulty in identifying or defining a singular biological ageing process, and this hypothesis relies on a non-biological risk factor, chronological age, to link multiple disease trajectories together.

Biomarkers of ageing

Biomarkers are commonly defined as characteristics that are "objectively measured and evaluated as indicators of normal biological processes, pathogenic processes, or pharmacological responses to therapeutic intervention".[34] Biomarkers of ageing appear to be first defined in 1988,[35] as 'a biological

parameter of an organism that either alone or in some multivariate composite will, in the absence of disease, better predict functional capability at some late age, than will chronological age'. Criteria for qualifying as ageing biomarkers were first proposed in 1988:[35] that they (1) predict the rate of ageing, or are better predictors of lifespan than chronological age, (2) monitor a basic process that underlies the ageing process, not the effects of disease, (3) can be tested repeatedly without harming the person, and (4) work in humans and animals.[35]

Research has been conducted on a wide range of biomarkers of ageing, including physical, biochemical,[20] epigenetic,[36] leucocyte telomere length[37] and more recently, imaging,[38] wearable sensor,[39] transcriptomic, metabolomic and proteomic biomarkers.[18] Biomarkers may be classified into body systems, body functions or domains in numerous ways.[7,12,19,20,40-42] A recent research programme on biomarkers for healthy ageing[7] proposed a mapping of physical and biochemical biomarkers in 5 categories of function: physiological, physical capability, endocrine, cognitive and immune, with most groups spanning multiple body systems.[7] Additionally, this programme summarised evidence on the associations of these biomarkers with age-related outcomes, which tended to be from single biomarker rather than multi-biomarker analyses, and which highlighted multiple areas where evidence was lacking.[7] A review of ageing biomarkers that systematically searched physical, biochemical and molecular ageing biomarkers found separate studies that together linked thousands of biomarkers (mostly molecular biomarkers) to ageing, and defined biomarker-based biological ages and indices as composite biomarkers.[18]

Many biological ageing studies published in the last decade have made reference to the organisation of the ageing process into 9 'hallmarks' of ageing (among these were cellular senescence, genomic instability and telomere attrition), each representing biological pathways on a sub-cellular scale.[43] A similar framework was used to define 7 'pillars' of ageing.[44] However, human biomarker research has not typically been organised by these hallmarks or pillars,[45] possibly because measuring many of these biomarkers was infeasible in large-scale human studies,[46] or because these hallmarks or pillars were not seen as relevant to clinical practice or to known modifiable biological pathways.

One of the first trials of therapies for biological ageing therefore focused on analysing blood-based biomarkers and imposed additional criteria for clinical relevance: feasibility within trial design, reliability of the biomarker assay and sensitivity to detect change.[46]

One of the first studies of biological age, a study of Hiroshima survivors in 1965, considered the use of 17 candidate biomarkers of ageing.[47] Several of these biomarkers are still measured in contemporary medical examinations or cohort studies (e.g. blood pressure, hand grip strength, lung function and blood biochemical markers), while others such as hair greying, skin elasticity and detection of electric vibrations, are no longer administered. In 1969, Comfort proposed a larger panel of 53 biomarkers of ageing spanning multiple body systems, including many of the 17 biomarkers proposed earlier, but also adding cognitive tests, biopsy- and necropsy-based biomarkers.[48]

Health systems and research funding tend to be siloed into single diseases[6] or body systems, with the result that studies of associations between single biomarkers and single diseases or body systems comprise the vast majority of epidemiological research. More recently, novel biostatistical approaches such as phenome-wide association studies[49] and high-dimensional regression models[50] have permitted single biomarker-multiple diseases and multiple biomarkers-single disease study designs respectively.

1.2 Biological age and other health indices

Biological age

Biological age in the most rudimentary form is a biomarker-based risk score expressed in terms of an age. There is no complete consensus on the definition of the concept of biological age,[42,51] and two commonly cited properties are: (1) it measures the "true global state" (of health) or "true life expectancy" of an individual better than chronological age,[42,51] and (2) it measures damage accumulation in individuals, and when measured longitudinally can be used to track the trajectory

of damage over time.[12,20] It can be used as a proxy measure and predictor of general health or accelerated ageing, and is generally benchmarked as being equal to chronological age on average in a population,[51,52] rather than an ideal notion of 'normal ageing'. The estimation and use of biological ages has been motivated by socioeconomic and biomedical initiatives to improve the measurement of health and ageing in populations beyond using chronological age since the 1980s.[25] In these wider contexts, chronological age was effectively a means of summarising multiple ageing processes into a single indicator of health, without utilising any other information on the underlying processes.

Alternative terms have also been used in place of 'biological age'. 'Physiological age' was used in older studies included in a review of biological age estimation methods by Jia et al,[20] such as the 1965 study of Hiroshima survivors.[47] More recently, 'ageing clocks' (originally used to describe biological ages that were either estimated from epigenetic biomarkers or estimated using deep learning methods) were treated as synonymous with biological age.[50] 'Bodily ages' has also been used as an umbrella term in one review that focused on brain ages to encompass overall biological ages and body system-specific ages such as heart age and brain age.[38]

The Jia et al methods review provided the most comprehensive summary of biological age studies to date, including over 50 studies (many of which had cohort sizes of less than 200 subjects) published in the last 60 years, although the review focused on estimation methods and included animal studies.[20] In comparison, other reviews of biological ages have had a narrower scope, focusing on biological ages estimated via deep learning methods[50,53] or on the characteristics of ages that pertain to a specific body system.[38,53-56]

Estimation of biological ages

Biological age estimation has been approached in diverse ways,[20,21,38,50,51,57,58] ranging from linearly regressing chronological age on a handful of biomarkers[47] to constructing neural network models of 10,000 timepoints of physical activity biomarker data.[59] Several estimation methods treat biological

ageing as a latent process,[20,51] where aspects of this process may be detectable and discoverable but not fully manifested via biomarker measurements and disease incidence patterns. These methods of summarising biomarkers of ageing into a biological age approach the concept of biological age 'as a unity of effects rather than a unity of causes',[60,61] and are agnostic to whether there is a singular, common ageing process or multiple ageing processes underlying the biomarker patterns analysed. Key concerns raised in the 1980s about limited statistical power to detect differences in ageing[25] are now allayed with the advent of larger and more deeply phenotyped cohort studies and biobanks, as well as advances in computing and statistics.[50]

Of the numerous estimation methods, many emphasised or selected biomarkers that were most strongly related to chronological age[20] (age-based biological ages) while others emphasised or selected those most strongly related to health outcomes[50,54,62-65] (outcome-based biological ages) and some included chronological age as a constituent 'biomarker' of the biological age while others did not.[13,50] Methods that emphasised biomarkers most strongly related to chronological age utilised chronological age as a proxy for the ageing process,[20] while those that included chronological age as a constituent 'biomarker' resulted in biological ages that were not wholly biological in their composition or substantially modifiable, and simply capitalised on the predictive power of chronological age for later life health.[13,50]

Many biological age researchers focused just on estimating age-based biological ages.[20] The Levine group, however, has progressed from estimating age-based biological ages constructed from physical and biochemical biomarkers in a US population,[12] to supplementing these biological ages with epigenetic biomarkers,[66] then developing mortality-based biological ages constructed from the same types of biomarkers (PhenoAge[62] and GrimAge[67]), and more recently investigating putative genetic determinants of biological ages.[68]

Other health indices

The most common types of health indices are disease risk scores, especially for cardiovascular disease.[69] These risk scores tend to include only well-known biomarkers for their respective diseases and non-biological risk factors such as sex, age and health behaviours.[69] Other disease indices such as the Charlson comorbidity index[70] and Cambridge Multimorbidity Score[71] capture the burden of prior disease across multiple body systems. The main distinction of biological age from these indices or risk scores is its ease of comparison with chronological age and its applicability to healthy populations.[64] Risk scores have tended to be developed with unhealthier populations (e.g. people who have specific diseases or health conditions) in mind instead of healthy populations, whereas biological age can be derived for all individuals regardless of health, and there may be a greater utility for risk prediction and preventative health interventions earlier in the life course before the onset of major disease. However, those scores that were developed in large populations to predict general health outcomes such as mortality[14,72,73] share similarities with some biological ages discussed in this thesis.

Limited research has been conducted on body system ages, which, similar to biological age, are body system-specific risk scores expressed in terms of an age. Research has mainly focused on heart,[54-56] brain[38,56] and lung[56] ages. However, the MediAge research centre in South Korea categorised physical and biochemical biomarkers into 6 body systems (cardiac, lung, liver, pancreas, kidney and body shape) prior to estimating body system ages using each group of biomarkers.[74,75] Different body system ages could be aggregated into an overall biological age, although to date this aggregation has not been discussed in published research and has only been proposed via commercial channels.[76,77]

Similar to disease risk scores, many other ageing indices are also not expressed in terms of an age. The most well-known of these indices, frailty indices and allostatic load, are based on counts of pre-selected biomarker measurements that have reached predefined thresholds for poor health in an individual (cumulative deficits indices or models), but they shared similarities with, and have been compared with, biological ages.[13,78] The frailty index[15] has been rapidly adopted in clinical settings (particularly primary and geriatric care) in recent years,[5] and measures the accumulation of multiple

types of health deficits (disease history, symptoms, functional limitations and biomarkers).[13] In contrast, biological age is estimated solely from biomarkers and can be used to identify higher-risk individuals prior to the onset of frailty.[12] Allostatic load, which was a cumulative index derived from approximately 10 biomarkers that measured the cumulative physiological burden through individuals' attempts to adapt to environmental stressors,[79,80] preceded the frailty index and motivated the development of other biological or psycho-socio-biological health indices such as the healthy ageing score,[81] healthy ageing index[82] and healthy ageing phenotype.[83] Several indices of physiological dysregulation[80,84,85] and homeostatic dysregulation[41] were motivated by the concept of allostatic load but are instead estimated from continuous biomarker measurements rather than number of biomarker measurements that were beyond the predefined thresholds, and they treated the magnitudes of deviations of biomarker levels from normal levels (normal levels were assumed to be the population means in empirical studies[41,85]) in either direction as indicators of poorer health (i.e. 'dysregulation'). Conversely, biological ages generally presumed a unidirectional association of biomarker levels with poorer health through their methods of estimation.[20]

Demographic concepts that are similar to biological ages, such as country-level equivalent ages of a reference 65 year old population in terms of age-related disease burden[86] and 'thanatological age' (average time to death for populations estimated from demographic risk factors),[87] and demographic indicators such as life expectancy and years of life lost, measure health at a population level rather than capturing between-individual health differences. In contrast to end-of-life health indicators, biomarker-based biological ages personalise and convey the immediacy of poor health. More subjective measures of biological age that are not based on biomarker measurements include biological ages determined from photographs,[88] own sentiment[89] and the combination of sentiments based on photographs.[90]

The diverse range of published biological ages and health indices may have resulted from the differences in motivations for tracking biological ageing. The wide range of potential objectives is summarised in the next section.

1.3 Potential objectives of estimating a biological age

The population health relevance of biological age was noted at the outset but in reality, few biological age studies have discussed potential applications in public health, and have instead focused on addressing biological and statistical objectives. Potential applications were mainly summarised from studies that proposed panels of ageing biomarkers[7,18,91] and a report on the use of risk stratification tools (biological age can be used for this purpose) in the UK National Health Service (NHS)[92] rather than from biological age studies. Biological ages could simply reflect poor health in people who have known health conditions,[62] but could be better used to detect accelerated aging in healthy people earlier in life, for primary prevention of diseases of ageing.[55] Biological or bodily ages can improve the communication of health risks to individuals, as many health conditions (such as chronic diseases) are strongly age-related.[64] Expressing a risk score in terms of an age allows individuals to easily interpret their own levels of risk relative to the expected risks for their age group, avoiding a common fatalistic perception of their levels of risk as a consequence of their chronological age. Other potential objectives, such as using biological age as a surrogate outcome in intervention studies or trials, monitoring health risks, informing policies on preventative health and health inequalities and social security planning (Table 1.3.1), require larger-scale organisational planning and investment for their implementation.

In contrast, many aims proposed by biological age studies were related to improving our biological and statistical understanding of ageing processes (epistemic aims). The most frequently reported motivations of biological ageing studies were to achieve a greater understanding of biological mechanisms of ageing (causal) and to predict health status (predictive; Table 1.3.1). In epidemiological analyses, biological age has been treated as an outcome (when assessing biological or non-biological determinants of health)[93,94] and also an exposure (when used to predict health outcomes).[12,13,42,62,78,95-99] It can also be an intermediate or surrogate outcome if these two types of analysis are combined. As a consequence of treating biological age as a 'composite biomarker',[18]

several criteria for ageing biomarkers, summarised in the Jia et al methods review,[20] are also applicable to biological ages. These criteria are related to the causal basis of ageing: improving understanding of biological mechanisms of ageing, assess the degree of similarity in risk factors of different diseases and assessing the extent of modifiability of biological age or disease risk (Table 1.3.1). Potential objectives related to prediction and interpretation could generally be summarised into the estimation of biological ages that are indicators or predictors of later life health (Table 1.3.1).

A potential objective that had not been explicitly stated in previous studies is the evaluation of chronological age as a proxy risk factor for ageing via its relationship with biological age (Table 1.3.1). This objective is related to earlier socioeconomic and biomedical motivations to improve the measurement of health and ageing beyond using chronological age,[25] and to the common use of biological age estimation methods that emphasise biomarkers that have the strongest relationships with chronological age.[20]

Commercial aims are listed in Table 1.3.1 for completeness and they may have informed general research priorities on biological ages, e.g. through commercially funded studies[98,100,101] or consulting academics.[102] These aims are irrelevant to this thesis.

1.4 Research aims and outline of the thesis

In summarising the state of research on biological ageing in previous sections, several gaps in biological age research from a population health perspective have been identified. Despite earlier motivations to use biological age as a better way to measure health than chronological age in populations, few biological age studies aimed to improve public health, e.g. through detecting accelerated ageing in individuals earlier in life and communicating health risks. In many biological age studies, chronological age was presumed to be a good proxy for biological ageing, but the relationships between chronological age and ageing biomarkers in describing and explaining a range of age-related health outcomes have not been explored in detail.

Additionally, previous biological age research was conducted on smaller cohorts with a limited number of candidate biomarkers, resulting in limited information to investigate the relative importance of the wide range of putative ageing biomarkers for multiple health outcomes in a population. Understanding biological mechanisms of ageing was a commonly stated aim of biological age studies, however the majority of these studies did not assess the associations between biomarkers or biological ages and health outcomes apart from mortality (Section 1.2). For biological age to be used in the early detection of ageing, investigating its association with end-of-life outcomes such as mortality would be less relevant than earlier life outcomes such as chronic disease incidence. With the exception of the Levine group, there was limited research on the causal relevance of constituent biomarkers for ill health, particularly as biological ages were analysed in all participants within cohort studies rather than a healthy subset of participants and could have captured biomarker patterns that were a consequence of prior disease.

The large and deeply phenotyped UK Biobank resource provides an unrivalled opportunity to conduct a synthesised investigation of the earlier stages of ageing, as the study includes an exceptional number of participants and range of biomarker measurements, and is linked to secondary care and death records with information on multiple health and disease outcomes.[103] This thesis investigates the ≈100 physical and biochemical biomarkers measured in the UK Biobank,[104] many of which are commonly measured in a clinical setting (and are therefore most applicable to population health), tend to be more modifiable than molecular and imaging biomarkers, and are similar to the less invasive biomarkers of ageing used in previous biological age studies (Section 1.2). These biomarkers spanned multiple body systems,[105] and this thesis was partly motivated by the wish to systematically organise these biomarkers by their relationships with body system-specific health outcomes, after assessing their measurement reliability and their patterns of age-related changes. Other types of biomarkers, such as genetic, heart and brain image, and activity sensor biomarkers, were also available in the UK Biobank through specific access requests[104] and were not used in this thesis.

This thesis therefore focuses on an epidemiological investigation of physical and biochemical biomarkers of biological ageing in healthy participants within the prospective UK Biobank cohort. In summary, the thesis uses statistical models to systematically assess and summarise these candidate biomarkers into biomarker-based biological ages, in order to investigate the key biomarkers of ageing and to supplement chronological age in predicting later life health outcomes.

The objectives of this thesis, based on the observational study design and the large number of candidate biomarkers, were therefore most closely linked to 3 of the potential epistemic objectives of estimating a biological age (objectives 1–3), and they bore 2 potential public health objectives in mind (objectives 4–5):

1. Using biomarker measurements to supplement chronological age in the prediction of health outcomes
2. Evaluating chronological age as a proxy for biological ageing
3. Assessing the degree of similarity in biological risk factors of different diseases
4. Earlier detection of health risks in individuals – through the analysis of risks of poor health in later life within a healthy subpopulation, and the use of estimation methods for biomarker-based biological ages
5. Communication of health risks – through the provision of individualised biological and body system ages that were easily benchmarked against chronological age.

To achieve these aims, this thesis consisted of the following stages:

Stage 1: Investigating and implementing reliable approaches for estimating and validating biomarker-based biological ages in the UK Biobank

- Systematic review of the literature on large scale, validated biological age studies in humans (Chapter 2)
- Identifying a reference healthy subpopulation in the UK Biobank (Chapter 3)

- Phenotyping biomarker measurements and health outcomes in the UK Biobank (Chapter 3)
- Developing biostatistical methods for the estimation and validation of biological ages (Chapter 4)

Stage 2: Identifying key biomarker constituents of the estimated biological ages

- Biological ages were estimated via 2 approaches:
    1. Established age-based approach: emphasising biomarkers most strongly related to chronological age (Chapter 5)
    2. Novel 2-step disease risk-based approach: estimating body system ages that emphasised biomarkers most strongly related to disease risk for multiple specific disease clusters (Chapter 6) and aggregating these body system ages into a biological age (Chapter 7)
- Putative associations between key biomarkers and diseases were compared to evidence from previous epidemiological studies (Chapters 5 and 6)

Stage 3: Assessing the extent to which key biomarkers of different adverse health outcomes (such as age-related chronic diseases, mortality and frailty) were similar

- Investigating the key biomarkers of body system ages, which summarised biomarker patterns most predictive of disease risk in several body systems (Chapter 6)
- Assessing the relative weights of these body system ages when aggregated into a disease risk-based biological age (Chapter 7)

Stage 4: Assessing the prognostic capability of bodily ages for these health outcomes

- Investigating whether previously published (large scale and validated) biological ages were shown to be prognostic for or indicative of health outcomes (Chapter 2)

- Comparing the predictive power of bodily ages for the incidence of each health outcome during follow up via linked hospital and death records (Chapters 5–7)
- Assessing the overlaps between bodily and chronological ages in predicting these outcomes (Chapters 5–7).

Through findings for the later stages (stages 2–4), this thesis also aimed to inform priorities for future research on biological determinants of ageing.

1.5 The role of the author and related publications

At the time of writing, one manuscript drafted from the analysis of an age-based biological age in the UK Biobank (described in Chapters 3–5) has been accepted (pending minor revisions) at the Journals of Gerontology Series A: Biological Sciences and Medical Sciences. A second manuscript drafted from the systematic review of biological ages in Chapter 2 is nearly ready for submission to a journal and has had its review protocol registered on PROSPERO. These manuscripts have been reviewed by my co-authors (particularly my supervisors), and one co-author, Jong-Wook Ban, additionally contributed to the systematic review as the second reviewer. My co-authors for these papers read and provided advice on the content and methodology.

These planned publications are:

1. Chan MS, Arnold M, Offer A, Hammami I, Mafham M, Armitage J, Perera R, Parish S. Biological age in UK Biobank: biomarker composition and prediction of mortality and hospital admissions. Accepted pending minor revisions at J Gerontol A Biol Sci Med Sci. (medRxiv preprint DOI: 10.1101/2019.12.12.19014720)
2. Chan MS, Ban J, Arnold M, Perera R, Parish S. Systematic review of large-scale studies of biological ageing in humans. In preparation. (PROSPERO protocol: CRD42019079241)

Further manuscripts will be drafted based on the research in the later chapters of the thesis. Research for the systematic review (Chapter 2) has also informed my contributions to 2 manuscripts under review entitled ‘Biomarkers in the prediction of multimorbidity: scoping review’ (authors: EA Spencer, GA Ford, MS Chan, R Perera and CJ Heneghan; medRxiv preprint DOI: 10.1101/2020.11.25.20214999) and ‘Design, methods, and reporting of impact studies of cardiovascular clinical prediction rules were suboptimal: A systematic review’ (authors: J-W Ban, MS Chan, TB Muthee, A Paez, R Stevens and R Perera), which do not form part of this thesis.

Chapter 1 tables and figures

Table 1.3.1: Summary of potential aims of estimating a biological age, by type of application

Potential aims of estimating a biological age
Public health: • Earlier detection of high risk individuals for preventative or targeted interventions[16,33,92] • Communication of health risks to individuals[64] • Used as a surrogate outcome in intervention studies or trials[33,50] • Aid health systems' monitoring of individuals' overall health risks (Suggested for biomarker panels[18]) • Inform policies on preventative health and health inequalities[25] • Social security planning and budgeting (if used to predict healthcare utility)[42] **Epistemic:** *Related to causality* • Greater understanding of or new discoveries of biological mechanisms of ageing (Suggested for biomarker panels[7,18,45,91]) • Assess the degree of similarity in risk factors of different diseases (Suggested for personalised medicine in general[92]) • Monitor a basic mechanism of the aging process and not an effect of disease[7,35,96] • Assess extent of modifiability of biological age or disease risk, based on biomarker composition (Suggested for biomarker panels[45]) *Related to prediction of health outcomes* • Predict healthspan or lifespan[18,57,78,91,106] • Predict disease or health status better than chronological age [12,18,20] *Related to interpretation of biological age* • Reflect physiological function[20,47,91,96,107] • Evaluate chronological age as a proxy risk factor for ageing, via its relationship with biological age • Standardise the method of assessing biological ageing[52] **Commercial:** • Help to identify and prioritise drug targets[50] • Incentivise customers to stay healthy, and help companies estimate insurance premiums[108] • Health monitoring or real time health coaching for customers[102,109]

Chapter 2: Systematic review of large-scale biological age studies

2.1 Introduction

Research into biological ageing has generally focused on improving our biological and statistical understanding of ageing processes rather than informing or improving public health (Section 1.3). At the same time, biological age formed from biomarkers was identified as a promising approach for detecting and summarising differential rates of biological ageing between individuals (Section 1.2). For biological age to be used as more than a research tool, for example as a means of risk communication[56] or as supplementary information on early detection or prognosis of disease in a clinical setting[20] (Section 1.3), an initial requirement is that its association with poor health should be clearly demonstrated.

Few studies have compared biological ages developed using different biomarkers or estimation methods (Section 1.2), and even fewer discussed potential applications in public health (Section 1.3), unlike studies of established risk scores for specific diseases.[56,110] To date, biological age has been used in one trial, as a secondary endpoint in a study on 25% caloric restriction in humans,[33] and there are no known implementation studies. On the other hand, specific body system ages based on cardiovascular, respiratory and physical activity risk factors have been used in several trials or implementation studies.[56] The lack of trials or implementation studies for an overall biological age may be reflective of limited readily-accessible evidence on its clinical utility.

Several comparative studies of biological ageing scores[12,42,57] have assessed the association of these scores with health outcomes or their predictive power of health outcomes, and a recent literature review (Jia et al)[20] surveyed methods of estimating biological age. That methods review did not report its search strategy and included more than 50 studies of biological age with a large range of cohort sizes and settings. Many of the included studies had cohort sizes of less than 200 subjects, not all adults or humans. It conveyed the notion that biological age research was carried out for

mechanistic (biological and statistical) purposes rather than practical (clinical and public health) purposes, and specifically focused on assessing the statistical properties of estimation methods instead of comparing the resulting biological ages and the utility and predictive power of the methods. It described four statistical methods that have been commonly used to create a biological age based on biomarkers and suggested that the Klemera Doubal method (KDM; which derived a weighted sum of biomarker principal components based on their linear relationship with chronological age),[51] published in 2006, was the best of these, based on its statistical properties.[20] This was corroborated with results from the quantitative studies, based on the relative predictive power of biological ageing scores for health outcomes.[12,42,57] Similar to findings from a review of cardiovascular clinical prediction rules,[110] studies cited in the Jia et al review did not justify why new biological ages are needed or give reference to existing biological ages. Previous systematic reviews or protocols for systematic reviews of studies on biological age were not identified.

To conduct a comprehensive assessment of biological ages and their relevance to clinical practice and public health, this review focused on studies of adult humans, that are large-scale, both in terms of population size and the range of biomarkers (across body systems) considered in biological age estimation. It assessed empirical aspects of biological age estimation and validation in these studies, rather than the statistical or methodological aspects of biological age estimation, which had been discussed in the Jia et al review.[20] This review aimed to: (1) map the different cohorts, study designs, constituent biomarkers and methods used, to compare the merits of the biological ages, and (2) summarise the aims of studies estimating and validating biological ages and assess how they achieved their aims, to give contextual information on the applicability of a biological age and the selection of study design and methods to public health and clinical practice.

2.2 Methods

The study protocol for this review was prospectively registered on the PROSPERO international prospective register of systematic reviews, with registration number CRD42019079241 (Appendix 2.1).

Search strategy

Ovid Medline and EMBASE databases were searched for published articles between 1 January 2007 and 25 February 2019. No language restrictions were applied. The search strategy is detailed in Appendix 2.2.

The search terms consisted of three main components, 'topic', 'method' and 'constituents', all of which had to be present in each article within its title, abstract, subject headings or heading words (Table A2.1):

1. Topic – includes free text 'bio* age' and 'bio* ag?ing', and related terms gathered from an initial systematic search
2. Method – includes free text 'estimat*', 'measure*' and 'predict*'
3. Constituents – includes free text 'biomarker*', 'biological marker*' and 'variable*', and MeSH term 'Biomarkers/' (Medline) or 'Biological markers/' (EMBASE)

Inclusion criteria

Studies were included if they met the following inclusion criteria: observational cohort studies of at least 550 individuals aged 30 years and above, with biological ages developed from at least 3 clinical biomarkers (to capture biomarkers across a range of systems), where biological age is expressed in terms of an age and is validated against at least one health outcome (Table A2.2 and Appendix 2.1). A power calculation (described in Appendix 2.2) estimated that the cohort size should be ≥550 to detect a small effect size when fitting a linear multivariable regression of biological age with the minimum number of biomarkers (3 biomarkers). The age threshold (30 years and above) was

determined based on changes in biomarker values more likely relating to age-related decline rather than early-life development.

In this review, clinical biomarkers are defined as non-genetic and non-epigenetic predictors of individuals' health status, excluding predictors directly related to the presence or absence of any health outcome, health behaviours, socioeconomic factors, environmental factors, sex and chronological age. To avoid circularity, health outcomes do not include those that are mainly defined based on levels of constituent biomarkers, such as hypertension, hypercholesterolaemia, diabetes and chronic kidney disease and obesity. Biological ages were intended to be reflective of a general rather than an organ- or disease-specific ageing process, and should capture more than one specific body system or the interaction between two body systems. Correspondingly, eligible biological ages in studies should include at least 3 biomarkers, to capture a wide enough range of biomarkers across domains[7] or physiological systems.[45]

Validation could be carried out through measuring health outcomes cross-sectionally or longitudinally, and there was no pre-specified method for validation. If the analysis was longitudinal, there was no restriction on length of follow-up period or event rate.

The reference list of the Jia 2017 methods review[20] was assessed for relevant studies, and a list of 9 studies that met the inclusion criteria were identified from this review[20] and initial searches (Appendix 2.2). This list was used to assess the systematic search for specificity.

Data extraction and analysis

The titles and abstracts of all articles were initially screened independently by two reviewers, MSC and J-WB. All articles accepted at the initial screening underwent full text screening independently by two reviewers. Each article was treated as a single and separate study, unless it reused biological ages described in other included articles without modification.

Biological ages have been used as indicators of baseline health (diagnostic) or to predict later life health outcomes or profiles (prognostic), and its constituent biomarkers tend to be measured in clinical or other health settings.[20] Hence, guidelines for systematic reviews of clinical prediction models were used to appraise biological age studies: the PRISMA checklist for systematic reviews,[111] the CHARMS checklist for data extraction from clinical risk prediction models[112] and the PROBAST checklist for risk of bias assessment in clinical risk prediction models[113] (the original CHARMS and PROBAST checklists are in Table A2.7 and Figure A2.1 respectively in Appendix 2.3). The CHARMS and PROBAST checklists were the only known tools for their respective purposes, and were recommended by the Cochrane Prognosis Methods Group for use in systematic reviews of prognosis studies.[114] The risk of bias assessment was conducted on the best-performing biological age and health outcome combination in each study, if this was not clear then first eligible biological age mentioned and the first eligible outcome mentioned in the study (including in the abstract) was assessed. There are no other known guidelines relevant to the reporting and assessment of biological age studies. The CHARMS checklist included items on cohort, biomarker, model and health outcome characteristics (e.g. study dates, location, sample size, number and type of biomarkers, outcome definition, estimation method used and method used for validation; Table A2.7). Data on the following items additional to the CHARMS checklist were also extracted: study aims, how these aims were addressed, mentions of clinical or public health applications, cohort age range, and predictive power. Data extraction and risk of bias assessment were carried out independently by the two reviewers. Any disagreements in the process were proposed to be resolved by a third independent reviewer (RP), although none were identified.

The candidate biomarkers and the biomarkers selected for use in the biological age in each study were tabulated in order to investigate the key biomarkers of biological age, and the extent of biomarker availability in different population or cohort study settings. The tabulated biomarkers were grouped by body system, based on common clinical biomarker groupings, and the grouping procedures used in the Jia 2017 review[20] and a table of biochemical biomarkers measured by the UK

Biobank.[105] The biomarker grouping was reviewed by a clinician (J-WB). Quantitative summaries of study characteristics were included where the data extracted allowed this to occur. For all included studies, a narrative analysis, specifically an inductive analysis of the themes identified in the extracted results, was conducted.

2.3 Results

A flow diagram of the search results and screening process is presented in Figure 2.3.1. Of the 859 studies identified in the database searches, 49 articles were accepted after title and abstract screening and 15 studies were included after screening and deduplication. All included studies were published since 2012. Eight of the 9 key studies that had been identified prior to the database searches were included after screening, and one of these 8 studies[57] was excluded during deduplication (Figure 2.3.1). The remaining study[62] that was not detected by the systematic search did not use the term 'biological age' or related terms (Appendix 2.2) in its title, abstract, subject headings or heading words (Section 2.2), but instead used the term 'phenotypic age'.

All articles in languages other than English had English abstracts and were excluded at title and abstract screening stage, except for one article in Chinese excluded at full text screening, where the screening was done by the first reviewer who had a working knowledge of Chinese. The 33 excluded studies and the reasons for their exclusion are listed in Table A2.3. Most of these 33 studies were excluded because their biological ages were not expressed in terms of an age, or their biological ages were not validated against an outcome other than chronological age (Table A2.3).

The Belsky 2018 study[57] met the inclusion criteria after full text screening, but used the same biological age derived in the Belsky 2015 study[16] by the same lead author, and was excluded to avoid duplication. Even though the excluded study compared several biological ageing scores, no additional eligible biological ages were used in this study and no additional health outcomes were used in the validation. The remaining 15 studies were included in the systematic review.

The aims and the key characteristics of the included studies were summarised in Tables 2.3.1 and 2.3.2 respectively. A quantitative summary of the data was not possible due to heterogeneity in biomarkers, estimation methods, validation outcomes and validation methods, except for a summary of hazard ratios (HRs) per year of biological age, for incident health outcomes for four studies[12,13,99,106] (Figure 2.3.2). The risk of bias assessment was summarised in Figure 2.3.3.

Aims addressed and proposed clinical or public health applications

The aims of all studies were research-focused and related to the capability of biological age or its biomarkers as a measure of ageing: predicting health outcomes,[12,13,16,78,95,98,99,106] validating a previously-estimated biological age,[12,19,95,96,106] comparing biological ages and health indices estimated using different methods,[12,13,96] assessing temporal changes in biological age,[16,97] identifying biomarkers[19,95,96] and classifying individuals by biological age[115] (Table 2.3.1). Four of these 15 studies had specific research aims that could inform clinical practice or public health: investigating differences in cardiovascular risk scores for people with different degrees of disabilities and/or Social Capital (having 3 or more friends);[116] using biological age in the evaluation and management of health;[117] investigating weathering (premature decline in health as a consequence of social and environmental disadvantage) and its relationship with race and psychosocial factors;[94] and estimating the contribution of changes in smoking, obesity and medication use to biological age.[97] The 11 studies that proposed clinical or public health applications (Table 2.3.1) did not provide details on how biological age would be implemented in practice. Hollar 2015[116] was one of the 11 studies that did not provide these details, but validated a heart age that had already been recommended for use by clinical guidelines. The proposed applications related to: risk stratification of individuals,[12,95,96] measuring the effectiveness of anti-ageing interventions,[12,16,98,99] health risk assessment[106,116] or screening,[95] and health risk communication through promotion of exercise[115] or clinical counselling.[116]

All studies addressed their stated aims, apart from Kang 2017[117] where the 'clinical value' of the biological age was part of the study's aims and was not defined (Table 2.3.1). Within studies' aims, the 'accuracy'[19] and 'usefulness'[12] of a biological age and a 'true biological age'[95] were presumed to be addressed, but were not defined. Only one study[13] sought to investigate the relationship between biological and chronological age.

Cohort characteristics

The sizes of the study cohorts used in model development ranged from 912[96] to 469,754,[106] and the sizes of validation cohorts ranged from 266[19] to 557,940.[106] US[12,78,94,97-99] and South Korean[95,96,106,117] cohorts were most frequently studied, Canadian cohorts were studied in 2 articles,[13,98] and Russian,[98] Chinese,[19] Polish[115] and New Zealand[16] cohorts were studied in one article each. Five studies used US cohorts recruited by the same study (NHANES) to develop or validate biological ages,[12,78,97,98,116] and the degree of overlap between cohorts in the same countries was not clear. For the only multi-national study,[98] biological ages were estimated in each population separately, and the biological ages were validated in different populations, allowing only for differences in the chronological ages of the populations. Sex-stratified analyses were conducted in 5 studies,[12,95,106,116,117] while 2 studies analysed single-sex cohorts.[96,115] Six studies used nationally representative cohorts of the US population[12,78,97-99,116] and the South Korean female population.[96]

While the inclusion criterion for age ranges was 30+ years, age ranges for all cohorts included adults aged 38+ years, except for the younger New Zealand study[16] where participants were followed up between the ages of 26-38 years (Table 2.3.2). Baseline dates, follow up dates and number of outcome events are listed in Table A2.4.

Cohort inclusion criteria and how missing biomarker measurements were dealt with in individual studies are also listed in Table A2.4. Neither baseline nor follow up dates were reported in 2

studies,[19,98] nor in the validation cohorts of 2 studies.[96,106] Whether exclusions of participants or biomarker measurements were made was not clear in 2 studies[95,96] (Table A2.4).

The populations studied appeared to be complete case studies or defined based on the availability of biomarker measurements, except in 5 studies: 3 did not give sufficient information,[16,19,116] one imputed population averages,[106] and one imputed missing biomarker values using regression[98] (Table A2.4). The bias introduced by using only complete cases in representative cohorts was assessed in one study, where sensitivity analysis using multiple imputation of missing biomarker measurements did not change its conclusions.[94] Levels of ascertainment were not discussed in the remaining 9 studies that did not use nationally representative cohorts. One study[96] excluded individuals with diagnoses of medical conditions to avoid potential confounding, and another adjusted for prior cardiovascular disease and cancer in the prediction of subsequent mortality.[99]

Constituent biomarkers

The candidate and selected biomarkers in each study, categorised by functional group, are listed in Table 2.3.3. Candidate biomarker panel sizes ranged from 3[116] to 85,[19] while the number of selected biomarkers ranged from 3[116] to 19.[98] The number of selected biomarkers was not closely related to the cohort size of the study (Table 2.3.2). The South Korean study that had the largest cohort size (its development cohort had 469,754 individuals) had 60 biomarkers[106] (but did not list the 45 biomarkers that were not selected), while one of the smallest studies of a cohort of 1373 Chinese adults had 85 candidate biomarkers[19] (and did not list 14 of these biomarkers; Table 2.3.2).

Biomarkers were pre-selected if they had larger correlations with chronological age in the majority of the studies,[12,13,16,19,78,94,96,97,115] if they were less correlated with other selected biomarkers in 2 studies,[96,106] and based on clinical advice in 2 studies[94,99] (Table A2.4). The choice of biomarkers in 3 studies[16,78,97] was based on the precedent Levine 2013 study[12] and pre-selection criteria was not clear for 2 studies.[98,117] Nine studies selected all of their candidate biomarkers[16,78,94,97-99,106,115,116]

(Tables 2.3.2 and 2.3.3) although only 3 of these studies were solely external validation studies.[16,78,116] All studies that constructed multiple biological ages used a single panel of biomarkers, except for the Murabito 2018 study,[99] which used 2 different panels of biomarkers for clinical and inflammatory biological ages, which only overlapped for C-reactive protein.

In 3 studies,[19,106,115] candidate biomarkers were incompletely reported, and 3 studies[19,96,117] reported the numbers of biomarkers in their analysis inconsistently. Unreported biomarkers were included and noted in the number of candidate biomarkers in Table 2.3.2 but omitted from Table 2.3.3. Candidate biomarkers excluded from the estimation model tended to belong to the same functional group as those that were selected (Table 2.3.3), and 2 studies[19,95] had biomarker exclusion criteria based on biomarker intercorrelation (Table A2.4).

All studies considered and selected biomarkers across multiple body systems except Golab 2016,[115] which used only musculoskeletal biomarkers, and Hollar 2015,[116] which used only cardiovascular biomarkers (Table 2.3.3). There was substantial overlap between studies for blood-based candidate and selected biomarkers. Physical biomarkers that indicated respiratory and musculoskeletal function were measured in approximately half of the included studies, haematology was measured in 6 of the studies and cognitive function tests were conducted in 2 studies (Table 2.3.3). The choice of candidate biomarkers in each study appeared to be dependent on the availability of biomarker measurements.

One aim of this review was to investigate clinical biomarkers of biological ages, however Hollar's[116] biological age included almost as many non-clinical biomarkers (chronological age, sex, smoking status) as clinical biomarkers. The KDM biological age (briefly described below and more completely in Chapter 4) used in 4 studies[12,16,78,97] included chronological age as a constituent 'biomarker', and one study discussed concerns regarding the treatment of chronological age as a 'biomarker'.[13]

Biological age methods

The main methods proposed and used by the included studies were the KDM,[12,13,16,78,94,96,97,99] principal component analysis (PCA; taking biological age as the first or first few principal components from an analysis including chronological age as a biomarker)[12,19,95,96,106,117] and multiple linear regression (MLR; regressing chronological age on biomarkers)[12,96] methods. These methods were based on linear combinations of biomarker measurements and were 3 of the 4 methods discussed in the Jia 2017 methods review.[20] The 4th method in Jia's review, the Hochschild method,[118] was not used or considered by any of the included studies, possibly as it required additional qualitative analysis of non-biological risk factors for mortality in the reference population (such as life expectancy in the region of residence). One included study[115] used the Borkan and Norris method,[119] which is mathematically a rudimentary form of the KDM. Biological ages estimated using deep neural networks were developed and cross-validated in one study[98] and biological ages based on the General Cardiovascular Risk Profile (GCRP) points-based system[120] were externally validated in another study.[116] Two included studies compared eligible biological ages estimated using multiple methods: KDM, PCA and MLR.[12,96] Biological ageing scores developed using Mahalanobis distance (7 studies), frailty index (2 studies) and information theoretic (2 studies) methods were excluded at full text screening, because these scores were not expressed in terms of an age (Table A2.3). Within the included studies, the KDM biological ages reported in 2 studies[13,78] met the inclusion criteria, however the frailty indices, allostatic load and risk scores reported in the same studies were excluded from consideration. Measures of change in biological age over time, which were investigated in addition to the biological ages in 2 studies,[16,97] were also excluded from consideration as they were not expressed in terms of an age, and were therefore not directly comparable with cross-sectional biological ages.

Only 4 studies[12,78,96,97] stated any assumptions of the methods used, and none attempted to fulfil or check these assumptions. Studies cited the original papers that described the estimation methods, but no open-source code was made available or referenced. For the more novel Deep Neural Network

method,[98] hyperparameters, system and hardware requirements were reported. Despite the focus of this review on a multi-system biological age, 3 studies that met the inclusion criteria described body system-specific ages, a heart age,[116] an inflammatory biological age[99] and a metabolic syndrome age.[117]

Validation of health outcomes

The included studies used 5 main validation methods: (1) Discriminative ability in predicting the outcome (area under the receiver operator characteristic curve (AUC)),[12,13,78] (2) hazard ratios (HR) for biological age from survival models,[12,13,78,98,99,106] (3) comparison of health profiles between biological age groups,[115] (4) comparison of biological ages in healthier vs unhealthier populations,[19,94-97,116,117] and (5) coefficients of correlations between biological age and outcomes.[16] Both HRs and discrimination metrics were reported by 3 studies.[12,13,78] The main outcome used was mortality (6 studies),[12,13,78,98,99,106] and 2 studies additionally assessed cause-specific mortality[78,106] while one additionally assessed incident cardiovascular disease and cancer.[99] Forrester 2018[94] measured health outcomes (a depression scale) 5 years before measurement of biomarkers for the biological age (Table 2.3.2), as the study treated biological age (which represented 'weathering' in this study) as a consequence of depression, health behaviours and socioeconomic circumstance. Kang's[117] method of validation, using a biological age developed only from metabolic syndrome biomarkers to compare cohorts stratified by presence of metabolic syndrome, was circular. All studies found that higher biological ages were associated with poorer health outcomes (except incident cancer[99]).

The Levine 2013[12] study found that discrimination for mortality was higher for biological ages constructed using KDM compared to those constructed with PCA and MLR (Table 2.3.2), and was also higher for younger and for broader age ranges. Two[12,78] of 3 studies[12,13,78] found that supplementing chronological age with biological ages significantly increased the AUC for mortality prediction.

HRs reported by the 6 studies[12,13,78,98,99,106] were adjusted for chronological age. These HRs were estimates of the absolute effect sizes of the biological ages on the risks of their respective incident health outcomes in the population, rather than their ability to discriminate between the relative levels of risk faced by individuals with different biological ages in the population. Four of these studies[12,13,99,106] reported HRs per year of biological age, allowing comparison of effect sizes across study populations, types of biological ages and health outcomes (Figure 2.3.2). Differences in HRs were generally larger between studies (which differed by cohort, age range, biomarkers and health outcomes) than between estimation methods,[12] biomarker choice or baseline date[99] (Figure 2.3.2). HRs (per year of biological age) for mortality were highest for the largest South Korean cohort and lowest for the smallest and oldest Canadian cohort (Figure 2.3.2). HRs in a US cohort study[99] were slightly above 1 (1.04–1.06) for incident cardiovascular disease and close to 1 (0.99–1.01) for incident cancer (Figure 2.3.2).

None of the included studies investigated whether the prediction of or associations with health outcomes were attributable to one or several constituent biomarkers of their biological ages. Age calibration and risk calibration were each checked in half the studies (Table A2.4). Both age and risk calibration were checked in only five studies[19,95,96,98,117] (Table A2.4).

Risk of bias assessment

Following the PROBAST framework, all of the included studies had a high risk of bias, 3 studies had a high degree of applicability and 7 studies had a low degree of applicability (Figure 2.3.3 and Table A2.5). The overall assessment of high risk of bias was mainly driven by the 'Analysis' category, as most studies did not evaluate model performance measures, appropriately handle missing data for participants, or account for overfitting and optimism in model performance (Table A2.5 and Figure A2.1). Aside from the 'Analysis' category, risk of bias was generally unclear in all other categories, particularly in relation to information on how the biomarkers were used in the

analyses (Domain 2 of the PROBAST checklist on predictors; Figure A2.1). Mitnitski 2017[13] was the only study that had low risk of bias in the remaining 3 assessment categories (the domains for participants, predictors and outcomes; Figure A2.1).

2.4 Discussion

This review identified several relevant and seminal studies in this relatively new subfield of ageing research, and highlighted areas of improvement in: diversity of research, reporting quality, consensus on definitions and characteristics, suitability as a clinical prediction tool, and reliance on chronological age. These studies provided overwhelming evidence that the biological ages were indicators or predictors of poorer health across different populations, health outcomes (except incident cancer; Figure 2.3.2) and estimation methods. However, insufficient information has been reported at present to enable their replication and implementation.

Level of diversity in biological age research

The studies had a multitude of research aims, spanning risk prediction, validation, comparison of estimation methods, trend analysis, biomarker selection and risk stratification, and several studies proposed clinical or public health applications (Table 2.3.1). In contrast, the concentration of research efforts on the US NHANES cohorts and the use of similar methods, biomarker panels and adverse health outcomes (particularly mortality outcomes), may lead to publication or research focus bias in this area in future. Since all reported biological ages were good indicators or predictors of poor health (except when incident cancer was used as the outcome; Figure 2.3.2), and none of these studies were preregistered, it is not clear if selective reporting was present. The concentration of research on a small number of datasets may be related to the nascence of the field and the complex analysis requirements of large-scale biobank and cohort studies.

One large-scale multi-population study was identified (which used a mixture of Canadian, South Korean, Russian and US cohorts with 2,000 –56,000 participants in each cohort; Table 2.3.2),[98] but the degree to which the hyperparameters of the estimation method (deep learning) are hardware- and population-specific is not clear, and is not helped by a finding of the study that the weightings of constituent biomarkers and the predictive power for mortality varied between the Canadian, South Korean and Russian derivation cohorts.[98] Cross-national differences in biological age strengthens the necessity of conducting biological age research on the target population for implementation.

Need for more detailed and accurate reporting of results

Studies that clearly reported or more robustly designed some aspects of their analysis were less rigorous in other ways, for example when describing the analysis cohort, dealing with missing values, describing biomarkers, stating assumptions of estimation methods, and assessing predictive power of biological ages. Biomarkers were frequently poorly described even though information on their identity and how they were used in the analysis was crucial. The need for further robust analysis is echoed by a scoping review of 'age' tools in the clinical trials context.[56] Studies generally did not report the information needed to assess the risk of bias in this review, even though this review was limited to relatively better validated studies.

The use of the same three estimation methods (largely unmodified, and with references to the original publications[51,52,36]) by most of the included studies, enabled clear reporting of the methods. However, for more complex methods, lack of open access to the computer code used makes replicating results more difficult, for example the KDM with chronological age as a 'biomarker'[51,52,98] and the deep neural network.[121]

Level of consensus on the definition of biological age and its characteristics

Studies varied in their biomarker selection procedures for their biological ages. For studies that selected biomarkers based on their correlation with chronological age, the strength of their association with health outcomes was via the use of chronological age as a proxy for the biological ageing process and was dependent on the characteristics of the population.[122] This selection procedure resulted in the choice of different biomarkers between studies, which together with differential availability of biomarker measurements in cohorts, made it difficult to identify the best performing panel of ageing biomarkers for biological age across populations. However, when permutations of constituent biomarkers were investigated in studies of homeostatic dysregulation (excluded during screening as the levels of dysregulation were not expressed in terms of an age), estimated levels of dysregulation from permutations of biomarker panels in US and Italian populations and showed that levels of dysregulation in these populations were relatively insensitive to the choice of biomarkers and population size.[123]

With respect to validation procedures, the differences in predictive power between cohorts and age ranges agreed with findings from a meta-epidemiological study of systematic reviews of prognostic models: study characteristics such as eligibility criteria, outcome definition, follow up duration, cohort size, and number of events affected the performance of prediction models.[122] However, there were too few included studies in this review and their study designs were too heterogeneous to evaluate the impact of these characteristics in depth. The outcome-specific differences in AUC in cohorts of different ages may be affected by the effect of mortality selection at older ages, in addition to age-specific differences in the discriminative abilities of the biological ages.

Readiness of biological age as a clinical risk prediction tool

The key research aims of these biological age studies, predicting or assessing health and classifying individuals by health status, matched the aims that were proposed for their constituent ageing biomarkers by these studies.[20] Despite proposing numerous clinical applications of biological ages (Table 2.3.1), considerations for implementation in clinical settings (such as external validation or

impact analysis[124]) were not discussed in these studies. Regardless, 2 of these studies[12,16] have provided the basis for the estimation of biological age as a secondary endpoint in a randomised trial of 25% caloric restriction in 220 adults, where participants in the treatment arm experienced significantly slower biological ageing.[33]

There was poor adherence to reporting guidelines (Section 2.3) and lack of information for risk of bias assessment, leading to the overall assessment of high risk of bias in all studies (Figure 2.3.3). These reporting standards were unlikely to be primary concerns for studies that had not specifically aimed to develop or validate biological ages for clinical risk prediction (Table 2.3.1). However, prediction features strongly in the aims of these studies, highlighting the importance of future adoption of clinical risk prediction reporting guidelines and validation of biological ages in a robust manner that considers discrimination and calibration,[125] both of which were infrequently reported in the studies (Table A2.4). Several of the risk of bias evaluation criteria in which these studies were lacking, relating to the evaluation of model performance and handling missing data (Section 2.3), are fundamental to many epidemiological studies. Moreover, biological ages in 2 of these studies[12,16] had already been used to inform a medical trial.

There were no clear or proposed benchmarks for assessing predictive power, especially since the included studies used different validation methods that produced results in different formats. It is also not clear how to benchmark the validation results if a single biological age was developed using models that combined data from vastly different populations.[98] Predictive power appeared to be higher in younger populations, based on comparisons between different age strata in the same cohort.[12,13,78] The suitability of the studied outcome in each population was difficult to assess, as number of events were poorly reported and the results were dependent on the biomarkers selected. The differences in AUCs between study cohorts were outcome-specific and differed by age range of the cohort. Only Mamoshina 2018[98] conducted extensive external validation in separate populations, as this study specifically aimed to assess predictive power of biological age in multiple populations.[19,95,96,98,117]

Unlike other risk prediction tools or health indices, biological age also needs to be calibrated to chronological age on average (age calibration), in addition to being calibrated to reflect differences in the risks of health outcomes on average (risk calibration) within the reference population.[125] This is particularly important due to the implicit use of chronological age as a benchmark for biological age by all of the estimation methods employed in these studies.

Reliance on the relationship between biological and chronological age

All included studies except for one[116] used estimation methods that assumed that biomarkers with the strongest relationship to chronological age contribute most to biological ageing. The exception was an external validation study of a heart age,[116] which did not explore the relationship between heart age and chronological age. This assumption was also applied through the selection of biomarkers with the strongest relationship to chronological age in the majority of these studies (Table A2.4). Moreover, after constructing biological ages from biomarkers, the relationship between biological and chronological age was not investigated except in one study,[13] despite the intrinsic link between the two ages in the estimation and interpretation of biological age. As estimation methods used in the 14 studies were based on the relationship between biomarker measurements and chronological age, it may be useful to estimate the proportion of a biological ageing effect represented by chronological age, and vice versa, in the analyses for this thesis.

Causal basis of biological age

Causal relationships between constituent biomarkers of biological ages and health outcomes can increase predictive power[126] and are critical to the effectiveness of interventions that involve the use of biological age. Guidelines for the impact analysis and implementation of clinical risk prediction tools recommended that causal effects should be demonstrated after the validation stage and prior to the implementation stage.[124] It would be ideal for biological ages to capture both causation and

prediction of health outcomes by biomarkers. None of these studies specifically aimed to investigate causal relationships, either through the use of epidemiological methods or assessment of criteria for causality. One study[96] that excluded individuals with prior disease to avoid confounding may have partially addressed reverse causality between biomarker levels and disease.

With respect to temporal associations, two studies reported that social, behavioural and health risk factors earlier in life influenced biological age.[94,97] The Forrester study[94] also framed biological age as a consequence of depression, highlighting the importance of considering non-biological determinants of biological age and reverse causality in the relationships between biomarkers and health conditions. Temporal associations between biological age and later life all-cause mortality, cause-specific mortality, cardiovascular events and cancer were also explored by several studies (Figure 2.3.2).

The impact of the choice of biomarkers used to estimate biological age was only briefly investigated in one study,[99] where a biological age constructed from commonly-measured clinical biomarkers was compared with another constructed from biomarkers linked to inflammation, and slight differences were seen in the effect sizes for the prediction of mortality, cardiovascular disease and cancer (Figure 2.3.2). In contrast, biomarker choice was investigated in greater detail in studies of other health indices for physiological dysregulation (which found that levels of physiological dysregulation were insensitive to choice of biomarkers)[123] and allostatic load (which found that its constituent immune and inflammatory, metabolic, cardiovascular and endocrine system biomarkers had different strengths of association with mortality).[81] Even though these health indices are not expressed in terms of an age, notwithstanding the slight differences in definitions, these health indices and biological ages summarise patterns of biomarkers that best indicate or predict poor health. Therefore the findings on how the choice of biomarker combinations affect these health indices could inform future biological age studies.

Review study limitations

A quantitative summary of all included studies was not feasible due to the lack of reported information and inaccurate information in some studies, even though this review was limited to validated studies of biological age in >550 adults to target more reliably conducted studies. In addition, the choice of biomarkers and validation methods were heterogeneous, and cross-national comparisons of ageing scores[98,123,127] showed mixed results on their stability across populations – on one hand, indices of physiological dysregulation predicted similar levels of mortality risk in US and Italian cohorts regardless of the biomarkers included,[123,127] whereas on the other hand, biological ages reported by an included study[98] emphasised different constituent biomarkers and predicted different levels of mortality risk in US and Canadian cohorts, depending on whether they were constructed from biomarker measurements of Canadian, South Korean or Russian cohorts.

The gap in larger-scale research outside of US and South Korean cohorts was highlighted in this review. Even though some cross-national validation of biological ages[16,98] has been conducted, results for these cohorts may not be as easily generalisable to the studied populations or other populations as studies conducted in the population(s) of interest that are validated intra-nationally.

The comprehensiveness of this review was enhanced by sensitivity analyses of the search strategy (Appendix 2.2) but restricted by the exclusion of risk prediction tools and health indices not expressed in terms of an age (Section 2.2 and Table A2.3). These tools and indices may nevertheless share many characteristics with biological age,[29] however, their inclusion would likely have made the evidence from this review more heterogeneous. At present, few studies have assessed changes in biological ages over time,[16,97] but when collecting repeated measures of biomarkers in cohort studies becomes more ubiquitous, reviewing studies of temporal changes in biological age (and accounting for differences in the reporting of these change scores) will build on the evidence base provided by studies of cross-sectional biological ages. The key study that was missed by the systematic search was also from the Levine group.[62] This study incorporated mortality risk into its estimated biological

ages, but it was not identified in the systematic search as it did not include any of the specified terms related to 'biological age' (Table A2.1) in its title or abstract, but instead used 'aging measure' and a term specific to the Levine group, 'phenotypic age'. More broadly, many research group- and company-specific terms such as 'GrimAge'[67] (an epigenetic biological age), 'Wii Fit Age'[128] (a fitness-related biological age measured by a video game console) and 'Vitality Age'[129] (a questionnaire-based biological age) have recently emerged, making the consolidation of evidence difficult and increasing the need for clearer definitions of a clinically relevant biological age.

2.5 Conclusion

Interest in biological age research and related research on frailty and multimorbidity is growing,[21,22] yet this review found insufficient evidence to permit a comprehensive assessment of the suitability of biological age as an indicator or predictor of health. Consensus at each analysis stage is difficult to achieve and is dependent on the context of the study (e.g. availability of biomarker measurements), but a preliminary basis for robust analysis is the use of large-scale datasets, clear processes for biomarker selection and biological age validation, and reporting standards for clinical prediction models. Existing reporting guidelines for clinical prediction models could be extended to provide a standard framework for biological age studies. The consensus around estimation methods was welcomed, although these methods emphasise biomarkers that have the strongest relationship with chronological age (as a proxy for biological ageing) rather than disease onset[120] or mortality,[62,64] suggesting a research gap in the estimation of biological ages that are more closely related to later life health outcomes. The few barriers to and quality controls for biological age research may impede wider implementation, despite the aspirations of many of these studies to use biological ages in clinical and public health settings. Implementation studies should be conducted on diverse populations or at least the populations of interest, as there were differences in the effects of biological age on health across populations. The growth of this research area and the recent maturity of modern, large-scale cohort studies provide opportunities for robust research and the consolidation of high quality evidence in future.

The studies identified in this review reflected the focus on epistemic rather than clinical or public health aims in estimating or validating a biological age within published literature (Section 1.3). Subsequently in this thesis, many of the biomarkers used in these studies were analysed in greater detail, enabled by the access to measurements for a large number of biomarkers in the UK Biobank (Chapter 3). Epidemiological approaches for limiting reverse causality were also applied (Chapter 3). The commonly-used estimation methods discussed in this review were adapted and combined (Chapter 4), and results on the most important biomarker constituents of biological ages and the internal validation of these ages as predictors of later life health outcomes are discussed in the remaining chapters of the thesis.

Chapter 2 tables and figures

Table 2.3.1: Research aims and proposed clinical or public health applications of the 15 included studies of biological ages

Study	Research aims	How research aims were addressed	Proposed clinical/public health applications
Forrester 2018[94]	Investigate weathering and the relationship between race and psychosocial factors and weathering in a cohort of middle-aged Black or White individuals	There is a large Black-White disparity in biological age and associations between psychosocial stress and weathering among Blacks	-
Levine 2018[97]	(1) Examine changes in biological age in the US between 1988-2010, (2) estimate the contribution of changes in smoking, obesity and medication use	(1) Period differences in biological age were estimated, (2) these differences were partially explained by age- and sex-specific changes in these factors	-
Mamoshina 2018[98]	Develop a deep learning-based biological age that predicts mortality in different populations	Predictive power variable across populations, and was higher for biological ages derived and validated on the same populations	Provide a measure for evaluating efficacy of therapeutic interventions for extending healthspan
Murabito 2018[99]	Test the association of biological age measures constructed from different types of biomarkers with mortality and age-related disease in a community-based sample, and assess if they make unique contributions to these outcomes	Biological ages constructed of different biomarkers were significantly associated with all outcomes except cancer, and provide complementary information in predicting mortality and disease	Inform interventions that delay aging and improve healthspan
Jee 2017[96]	(1) Obtain a set of biomarkers in a cohort representative of Korean females, (2) compare the three major prediction models, and (3) apply the models to people with diagnosed clinical risks to investigate model validity	(1) 8 biomarkers were selected, (2) KDM was more accurate (defined as calibrated to chronological age) than MLR and PCA, (3) PCA and KDM ages were significantly higher in groups of patients with impaired glucose tolerance or diabetes, further research on other age-related complications proposed	Differentiate patients with age-related health conditions in clinical settings
Kang 2017[117]	Propose and evaluate the clinical value of a novel Metabolic Syndrome biological age model	A novel Metabolic Syndrome age was proposed, but it is not clear how 'clinical value' was assessed	Evaluate and manage the health of individuals with Metabolic Syndrome, in various settings
Mitnitski 2017[13]	(1) Compare performance of biological age and frailty indices, (2) consider whether including chronological age sufficiently improved predictive power for mortality	(1) Biological age was more predictive of mortality than the separate but not combined frailty indices, (2) There was no advantage of including chronological age as a biomarker in biological age, if it was adjusted for in the prediction of mortality	-
Yoo 2017[106]	Assess validity of biological age in predicting mortality risk	Biological age could be used for predicting mortality, and predictive power varied by sex, chronological age and cause of death	Provide a more predictive index for mortality risk assessment procedures
Zhang 2017[19]	(1) Develop a more accurate biological age, (2) validate the biological age formula in a group of patients, (3) explore new ageing biomarkers in the nervous system	(1) Biological age was stated as more accurate (benchmark for accuracy was not given), (2) Biological age predicted the status of diseases, (3) Trail making test was included in the biological age formula	Promote clinical use of the developed biological age
Golab 2016[115]	(1) Present a method of classifying men based on declining or normal function in selected morphological features, (2) describe the relationship between biological ageing, physical activity and fitness	(1) Classification was carried out, based on 5 features that were correlated with chronological age, (2) there were significant and consistent differences in physical fitness by biological ages, but no significant relationship between biological age and physical activity	Convince older people to engage in regular physical exercise

Belsky 2015[16]	Detect accelerated ageing earlier in life	Accelerated ageing was detected for both cross sectional biological age and rate of ageing over time. People who aged more rapidly were less physically able, had greater cognitive decline, self-reported worse health and looked older	Provide a measure for studying early interventions to slow ageing in humans
Hollar 2015[116]	Investigate if people with and without disabilities differ in cardiovascular risk scores across 2000-2010, and the impact when disability is combined with Social Capital (having three or more friends)	Differences in biological age were found to be larger for mobility and vision disabilities but were not significant across other disabilities, except when heart age differentials were used in the 2001 cohort. The joint impact of disability and social capital remains unclear.	Rapidly assess patients' health and provide counselling in a clinical setting
Levine 2014[78]	Assess how well different biological ages predict all-cause and cause-specific mortality in a large, nationally representative US population	KDM predicted all-cause and cancer mortality better, while Framingham Risk Score predicted cardiovascular mortality better	-
Levine 2013[12]	Assess the validity and usefulness of different biological ages in predicting mortality	KDM was more valid and useful than others biological ages ('usefulness' was not defined), particularly when biomarkers were chosen by PCA	Identify individuals at increased risk of disability and disease, and develop preventative health interventions
Jee 2012[95]	(1) Identify biomarkers of biological age that are commonly tested, (2) develop true biological age prediction models through parsimonious stepwise procedures, (3) examine the validity of the models by comparing true biological age to chronological age for groups with a clinical risk of sarcopenia or obesity	(1) 14 routinely tested biomarkers were selected prior to analysis, (2) a manual stepwise procedure for selecting biomarkers was used ('true biological age' was not defined), (3) biological ages (alone and with respect to chronological ages) were higher for the at-risk groups	Rapidly screen and identify individuals in poor health for subsequent in-depth clinical diagnosis

Abbreviations of methods for estimating biological age – KDM: Klemera Doubal method; MLR: Multiple linear regression; PCA: Principal component analysis

Table 2.3.2: Key characteristics of the 15 included studies of biological ages

Study [and study type]	Sample size	Country (and dataset name)	Age range	Number of biomarkers	Methods used (and out-of-scope methods)	Prediction outcome (and out-of-scope outcomes)	Validation results (and respective method or outcome)
Forrester 2018[94] [Dev]	2694	US (CARDIA)	48-60 at baseline (but informed by data for ages 33-60)	Candidate: 7, selected: 7	KDM and KDM with multilevel model extension	Depression Scale (CES-D) (Alcohol consumption, tobacco use, socioeconomic status score, Experiences of Discrimination Scale, social participation score)	A 1-point increase in CES-D was associated with a 0.05-year [95% CI: -0.004, 0.10] difference in weathering (the biological age) 5 years later. Associations were stronger for Blacks than Whites
Levine 2018[97] [Dev]	21,575	US (NHANES III and IV)	20-79	Candidate: 8, selected: 8	KDM (and period difference in KDM age)	Examined relationships between biological age with smoker status and obesity (and relationships between period differences in biological age with these outcomes and medication use)	Biological ages were 0.54-3.74 times higher for combinations of unhealthier smoking status and weight
*Mamoshina 2018[98] [Dev, Val]	Derivation: 20,699 (Alberta Health), 65,760 (Gachon University), 55,920 (Invitro) Validation: 2,768 (NHANES), 20,699 or subset of the 20,699 (Alberta Health)	Canada (Alberta Health), USA (NHANES), South Korea (Gachon University), Russia (Invitro)	Derivation: 20+, validation: NI	Candidate: 19, selected: 19	Multilayer feed-forward neural network (and PCA, elastic net, random forest, partial least squares, gradient boosting machines)	Mortality (and chronological age)	Change in HR for individuals with age difference <-5 vs >-5: 24-29.2%, >5 vs <5: 32.6-185.8% (mortality)
Murabito 2018[99] [Dev]	2532 (clinical age), 3134 (inflammatory age)	US (Framingham Heart Study Offspring)	NI	Candidate: 14, selected: 14 (6 for clinical age and 9 for inflammatory age)	KDM for clinical age and inflammatory age, (and epigenetic age)	Mortality, incident cardiovascular events, incident cancer, grip strength, gait speed, MMSE score	Both ages were significantly associated with all incident outcomes [HR = 1.04-1.05], except cancer [HR = 1.01], where only inflammatory age was significant. Both ages were associated with gait speed and grip strength [p<0.05]. Inflammatory age was associated with MMSE score [p<0.001]
*Jee 2017[96] [Treated as Dev]	Derivation: 912 Validation: 425	Derivation: Korea (KNHANES waves 4 and 5 female participants), Validation: NI	Derivation: 30-80, Validation: NI	Candidate: 30 (mislabelled as 31), selected: 8	MLR, PCA, KDM	Compared means of chronological and biological ages in what appears as separate patient populations with impaired glucose tolerance or diabetes	PCA and KDM ages were significantly higher in both impaired glucose tolerance and diabetes groups compared to the reference group, while MLR ages were not significantly higher for either

Kang 2017[117] [Dev]	Development: 263,828 Validation: 188,886	Korea (several university health centres and community hospitals)	20+	Candidate: 8, (mislabelled as 6), selected: 5	PCA	Compared mean metabolic syndrome and chronological age differences in a separate population across 3 risk groups: normal, 1-2 risk variables, metabolic syndrome	Mean differences were significantly different between the 3 groups [p<0.001]
*Mitnitski 2017[13] [Dev]	1,013	Canada (CSHA)	65+	Candidate: 22, selected: 10	KDM (and frailty indices)	Mortality	AUC: 0.726 (for both versions of KDM)
*Yoo 2017[106] [Dev, Val]	Derivation: 469,754 Validation: 557,940	Derivation: South Korea (NI), Validation: South Korea (KMSMS)	Derivation: NI, Validation: 20-93	Candidate: 60, of which 15 were reported, selected: 15	PCA	Mortality: cancer and non-cancerous chronic disease, non-cancerous chronic disease, cancer	HR per year of biological age: 1.15 (chronic disease mortality), 1.25 (non-cancerous chronic disease mortality), 1.08 (cancer mortality)
Zhang 2017[19] [Dev, Val]	Derivation: 1,373 Validation: 266	China (Derivation: Cohort recruited from 5 cities, validation: patients recruited from a hospital)	Derivation: 19-93, validation: 20-85	Candidate: 85, of which 71 were reported (mislabelled as 74), selected: 5	PCA	Comparison with biological vs chronological ages of a separate hospitalised population with cardiovascular disease, nervous disorders, Type 2 diabetes, kidney, cancers or pulmonary diseases	Biological ages for hospitalised individuals were above the regression line of healthy individuals' biological ages against chronological age
Golab 2016[115] [Dev]	1400	Poland (male industrial workers in Krakow)	20-70	Candidate: 23, of which 5 were reported, selected: 5	Borkan and Norris 1980 method: averaging results from univariate linear regressions	5 Eurofit motor tests: Balance test, tapping, sit-and-reach, standing broad jump, handgrip test, step test, self-reported physical activity	Significant differences in motor test performance between biological age groups, particularly for men age 30-60. No significant relationship between biological age and physical activity
*Belsky 2015[16] [Val]	954	New Zealand (Dunedin)	38 (but followed up from 26-38)	Candidate: 18, of which 10 were reported, selected: 10	KDM (and Pace of Ageing)	Pace of Ageing, balance, grip strength, motor coordination, physical limitation, IQ, IQ decline, retinal imaging, self-rated health, facial ageing	Magnitude of correlation: 0.38 (Pace of Ageing), 0.13-0.22 (physical function), 0.09-0.17 (cognitive function), 0.17-0.2 (retinal imaging), 0.22 (self-rated health), 0.21 (facial ageing)
Hollar 2015[116] [Val]	52,195 across 5 cohorts (10,122-11,039 each)	US (NHANES)	20-85	Candidate: 3, 4 non-biomarker variables were also used, selected: 3	GCRP points-based heart age (and GCRP Cox model-based risk score)	Nine types of disability from clinical interview: hearing; vision; memory; physical, mental or emotional limitations; walking up ten steps; bending or kneeling; lifting or carrying; assistive devices; no disability	Heart age differentials were significantly different for the 2001 but not other cohorts across the 9 disabilities [$F_{8,2645} = 2.63$, $p = 0.01$]
*Levine 2014[78] [Val]	9,942	USA (NHANES III)	30+	Candidate: 10, selected: 10	KDM (and allostatic load, Framingham Risk Score)	All-cause mortality excluding HIV, violence or accidents, cardiovascular and cancer mortality in 10 years	AUC: 0.875 for all-cause mortality

*Levine 2013[12] [Dev]	9,389	USA (NHANES III)	30-75	Candidate: 21, selected: 10	MLR, PCA, KDM	Mortality excluding HIV, violence or accidents	AUC: 0.840 (PCA), 0.847-0.849 (MLR), 0.853-0.854 (KDM)
Jee 2012[95] [Treated as Dev]	Derivation: 2364 Validation: 2587	Derivation: Korea (Asan Medical Center patients), Validation: NI	Derivation: 30-85, Validation: NI	Candidate: 14, selected: 10	PCA	Compared biological and biological vs chronological ages of a subpopulation with and without increased risk of sarcopenia or BMI>27.5	Subgroups with increased risk of sarcopenia and with BMI>27.5 have significantly higher biological and biological vs chronological ages than those without those characteristics, except for men with vs without BMI>27.5

* One of the seven studies that appeared in a list of nine key studies identified prior to the systematic search. The process of refining the systematic search strategy was partly based on the ability of the strategy to detect these studies.
[Dev/Val]: Denotes whether study was a development and/or internal validation study (Dev), or an external validation study (Val), or both. Kang 2017[117] used split sample internal validation, while the format of validation for Jee 2012 and 2017[95,96] was unknown and were treated as development and/or internal validation studies.
Each row relates to one study and multiple biological ages or validation outcomes in the same study are listed in the same row.
Abbreviations:
NI: No information
CARDIA: Coronary Artery Risk in Young Adults study
CSHA: Canadian Study of Health and Aging
KMSMS: Korean Metabolic Syndrome Mortality Study
KNHANES: Korea National Health and Nutrition Examination Surveys
NHANES: National Health and Nutrition Examination Surveys
MLR: Multiple linear regression
PCA: Principal component analysis
KDM: Klemera Doubal method
GCRP: General Cardiovascular Risk Profile
HR: Hazard ratio
RR: Risk ratio
AUC: Area under the receiver operating characteristic curve

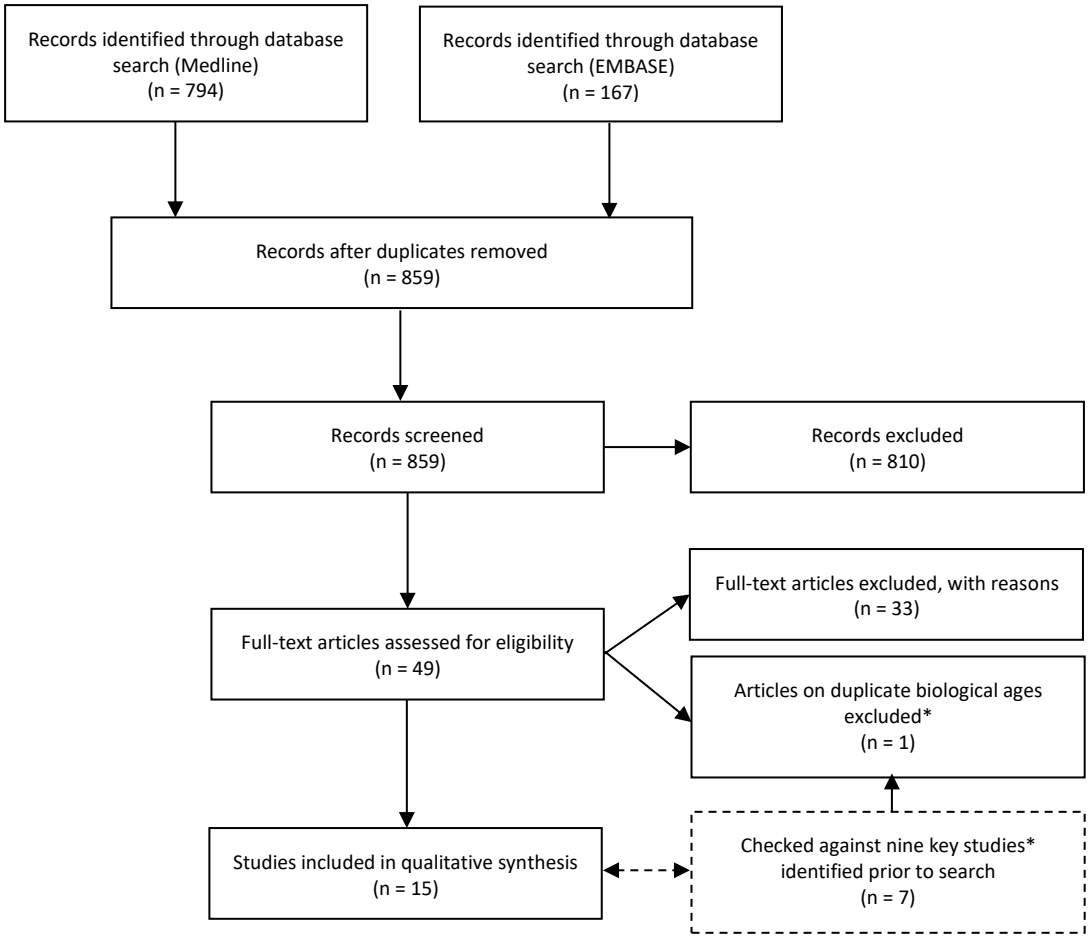
Table 2.3.3: List of candidate and selected biomarkers in each of the 15 included studies

Biomarker functional group and name / Study		Forrester 2018	Levine 2018	Mamoshina 2018	Murabito 2018 (Clinical age)	Murabito 2018 (Inflammatory age)	Jee 2017	Kang 2017	Mitnitski 2017	Yoo 2017	Zhang 2017	Golab 2016	Belsky 2015	Hollar 2015	Levine 2014	Levine 2013	Jee 2012	No. times candidate	No. times selected
Cardiovascular	Systolic blood pressure		Y		Y		Y	C	C	Y	C		Y	Y	Y	Y	C	12	8
	Diastolic blood pressure						C	C	Y	Y	C					C	Y	7	3
	Pulse pressure							C			Y							2	1
	Mean arterial blood pressure	Y						Y										2	2
	Heart rate						C				C					C		3	0
	High density lipoprotein cholesterol (HDL-C)	Y		Y			C	Y		Y	C			Y		C		8	5
	Low density lipoprotein cholesterol (LDL-C)			Y						Y	C							3	2
	Total cholesterol	Y	Y	Y	Y		Y				C		Y	Y	Y	Y		10	9
	Triglycerides			Y			C	Y		Y								4	3
	LP-PLA2 mass and activity					Y												1	1
	Echocardiographic measurements										Y							1	1
Respiratory	Forced expiratory volume in 1s (FEV1)	Y			Y		Y			Y			Y		Y	Y	Y	8	8
	Forced vital capacity (FVC)																C	1	0
	Ratio of FEV1 to FVC		Y															1	1
	Maximal oxygen uptake (VO2 Max)																Y	1	1
Musculoskeletal	BMI						C			Y	C	C					C	5	1
	Height										C							1	0
	Weight										C							1	0
	Body fat percent									Y							C	2	1
	Body muscle percent									Y								1	1
	Waist circumference						Y	Y		Y	C	Y					Y	6	5
	Hip circumference										C							1	0
	Waist-hip ratio	Y									C							2	1
	Pelvis-acromial index											Y						1	1
	Chest index											Y						1	1
	Total body water											Y						1	1
	Osteoprotegerin					Y												1	1
	Calcium			Y					Y									2	2
	Physical tests (incl. grip strength)																Y	1	1
Renal	Creatinine		Y	Y	Y		C		Y		C		Y		Y	Y		9	7
	Albumin		Y	Y					Y	Y	C		Y		Y	Y		8	7
	Urea nitrogen			Y			Y		Y	Y	C		Y		Y	Y		8	7
	Uric acid										C							1	0
	Creatinine in urine						Y											1	1
	Cystatin C										Y							1	1
	Inorganic phosphorus								Y									1	1
	Total protein			Y					Y		C							3	2
	Sodium			Y					C									2	1
	Potassium			Y					C									2	1
	Urinary waste products, haematuria and pH						C											1	0

Biomarker functional group and name / Study		Forrester 2018	Levine 2018	Mamoshina 2018	Murabito 2018 (Clinical age)	Murabito 2018 (Inflammatory age)	Jee 2017	Kang 2017	Mitnitski 2017	Yoo 2017	Zhang 2017	Golab 2016	Belsky 2015	Hollar 2015	Levine 2014	Levine 2013	Jee 2012	No. times candidate	No. times selected
Endocrine, metabolic and immune	C-reactive protein (CRP)	Y	Y		Y	Y					C		Y		Y	Y		7	6
	Glycated haemoglobin (HbA1c)		Y				C						Y		Y	Y		5	4
	Glucose	Y		Y														2	2
	Fasting blood sugar				Y		C	Y	C	Y	C							6	3
	Insulin						C											1	0
	Prealbumin										C							1	0
	Cytomegalovirus optical density												Y		Y	Y		3	3
	Thyroid-stimulating hormone (TSH)								Y									1	1
	Free thyroxine (free T4)								C									1	0
	Urine chrionic gonadotrophin								C									1	0
	Intercellular adhesion molecule-1					Y												1	1
	Interleukin-6 (IL6)					Y												1	1
	Monocyte chemoattractant protein-1 (MCP-1)					Y												1	1
	P-selectin					Y												1	1
	Tumor necrosis factor receptor II (TNFR2)					Y												1	1
Liver	Alkaline phosphatase		Y						Y				Y		Y	Y		5	5
	Alanine aminotransferase						C				C							2	0
	Aspartate aminotransferase						Y		C		C							3	1
	Gamma-glutamyl transpeptidase (GGT)									Y								1	1
	Bilirubin			Y			C				C							3	1
	Transferrin										C							1	0
Haematology	Haemoglobin			Y			C		Y		C					C		5	2
	RBC count			Y			C				C					C		4	1
	RBC distribution width										C							1	0
	Erythrocyte sedimentation rate									Y								1	1
	Haematocrit			Y			C				C					C		4	1
	Mean corpuscular haemoglobin measurements			Y							C							2	1
	Mean corpuscular volume			Y					C		C							3	1
	Ferritin						Y											1	1
	Folate and Vitamin B12								C									1	0
	WBC count (total and by cell type)						C		C		C					C		4	0
	Platelet count			Y							C					C		3	1
Cognitive	Trail making test										Y							1	1
	Language fluency test										C							1	0
	Drawing test										C							1	0
	Reaction time																Y	1	1
Candidate biomarkers in study		7	8	19	6	9	30	8	22	15	71	5	10	3	10	21	14		
Selected biomarkers in study		7	8	19	6	9	8	5	10	15	5	5	10	3	10	10	10		

C: Biomarker is in the candidate panel but not used to construct any biological age
Y: Biomarker is in the candidate panel and is used to construct a biological age
RBC: red blood cell, WBC: white blood cell
There are slight differences in the number of biomarkers displayed in this table and the candidate and selected biomarkers reported by the study, due to the grouping of several biomarkers in this table. All serum measurements instead of urine unless otherwise stated
Details on how biomarkers were selected prior to and during modelling are reported for each study in Table A2.4
Forrester 2018[94] used FEV1 / Height^2 instead of FEV1. Murabito 2018[99] used different biomarker panels for the clinical and inflammatory biological ages, these have been listed in separate columns

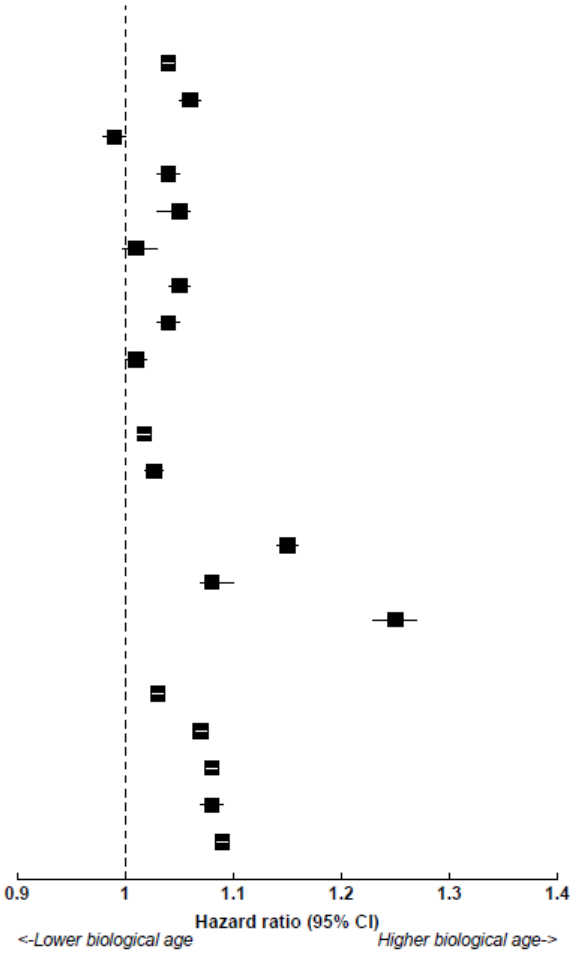
Figure 2.3.1: Flow diagram of systematic search results



* One of the nine key studies identified prior to the systematic search was the same article that used a duplicate biological age (Belsky 2018[57])

Figure 2.3.2: Hazard ratios per year of biological age for incident mortality or chronic disease, for 4 of the 15 included studies (all models adjusted for chronological age and sex)

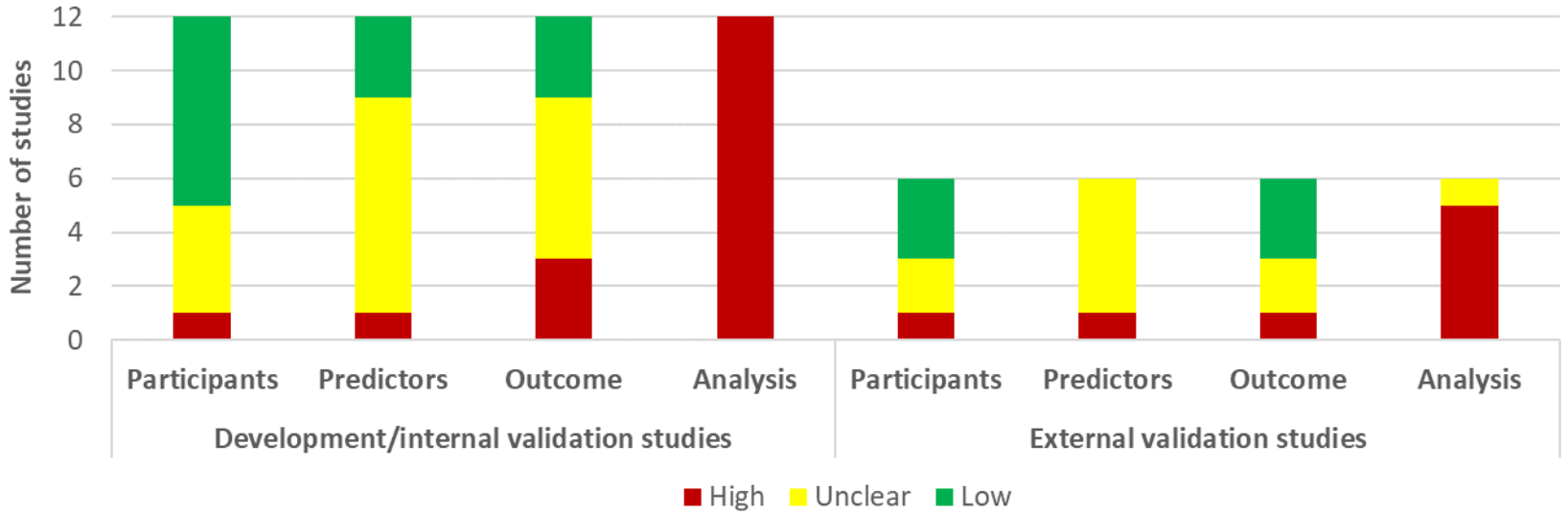
Biological age type	Outcome	Cohort size	Cohort age range	Hazard ratio (95% CI)
Murabito 2018, US (Framingham Offspring)				
KDM (clinical) at Exam 2	Mortality (all cause)^	3417	No information	1.04 (1.03, 1.04)
KDM (clinical) at Exam 2	Cardiovascular disease	3359	No information	1.06 (1.05, 1.07)
KDM (clinical) at Exam 2	Cancer	3367	No information	0.99 (0.98, 1.00)
KDM (clinical) at Exam 7	Mortality (all cause)	2532	No information	1.04 (1.03, 1.05)
KDM (clinical) at Exam 7	Cardiovascular disease	2400	No information	1.05 (1.03, 1.06)
KDM (clinical) at Exam 7	Cancer	2329	No information	1.01 (1.00, 1.03)
KDM (inflammatory) at Exam 7	Mortality (all cause)	3140	No information	1.05 (1.04, 1.06)
KDM (inflammatory) at Exam 7	Cardiovascular disease	2944	No information	1.04 (1.03, 1.05)
KDM (inflammatory) at Exam 7	Cancer	2859	No information	1.01 (1.00, 1.02)
Mitnitski 2017, Canada (CSHA)				
KDM (BA_E)	Mortality (all cause)	1013	65+	1.02 (1.01, 1.02)
KDM (BA_EC)	Mortality (all cause)	1013	65+	1.03 (1.02, 1.03)
Yoo 2017, South Korea (KMSMS)				
PCA	Mortality *	557940	20-93	1.15 (1.14, 1.16)
PCA	Cancer mortality	557940	20-93	1.08 (1.07, 1.10)
PCA	Non-cancer mortality *	557940	20-93	1.25 (1.23, 1.27)
Levine 2013, US (NHANES)				
PCA	Mortality ^**	9389	30-75	1.03 (1.02, 1.03)
MLR1	Mortality ^**	9389	30-75	1.07 (1.06, 1.07)
MLR2	Mortality ^**	9389	30-75	1.08 (1.07, 1.08)
KDM1	Mortality **	9389	30-75	1.08 (1.07, 1.09)
KDM2	Mortality ^**	9389	30-75	1.09 (1.08, 1.09)



^ Confidence intervals shifted by 0.005 to avoid confidence intervals equal to the mean
* Excludes deaths from HIV, violence and accidents
** Excludes deaths with ICD10 codes A00-B99, O30-O92, Q20-Q64, S00-T98 and V01-Y98

KDM: Klemera Doubal method; PCA: principal component analysis; MLR: multiple linear regression
CSHA: Canadian Study of Health and Aging; KMSMS: Korean Metabolic Syndrome Mortality Study;
NHANES: National Healthy and Nutrition Examination Surveys

Figure 2.3.3: PROBAST risk of bias assessment for the 15 included studies (consisting of 12 development/internal validation studies and 6 external validation studies)



PROBAST: Prediction model Risk Of Bias ASsessment Tool
The PROBAST checklist was downloaded from the PROBAST website[130] and referenced by the Moons et al article that provides an explanation of this checklist.[113]

Chapter 3: UK Biobank participant and biomarker characteristics

3.1 Introduction

This chapter outlines the UK Biobank data resource and the phenotyping procedures used, in order to conduct the biological age analyses described in later chapters. These procedures included: identifying participants who had sufficient data to form the study population, stratifying the population based on multiple measures of prior health, data cleaning of the biomarker measurements and dimensionally reducing these biomarker measurements. To analyse later life health outcomes for these participants, age-related chronic diseases were phenotyped and grouped into disease clusters, and mortality and frailty outcomes were also phenotyped.

3.2 Description of study population

The UK Biobank resource

The UK Biobank was established to allow in-depth research into the determinants of diseases of middle and old age.[131] It is a richly phenotyped resource with 0.5 million participants[131] that provides an unrivalled opportunity to investigate earlier stages of ageing in this thesis through biological, lifestyle and environmental factors that are easily measured at scale. Previous clinical biomarker-based studies of biological ageing were typically based on 100-10,000 participants with panels of fewer than 30 biomarkers.[20] Extensive phenotypic detail about the UK Biobank participants was collected and continues to be collected, including data from touchscreen questionnaires, physical measurements, blood and urine sample assays, "omics" data (e.g. genetic and metabolomic data), physical activity monitors, imaging and longitudinal follow-up for a wide range of health-related outcomes through data linkage.[131] Details on the recruitment, biomarker measurement and data linkage procedures used are available from the UK Biobank website.[103] Characteristics of the

participants, biomarker measurements and health outcomes used in analyses in this thesis are described within this chapter.

UK Biobank is an open-access data resource for bona fide researchers who wish to use it to conduct health-related research for the benefit of the public, and access procedures to the resource and returned datasets are also detailed on the UK Biobank website.[103] The research in this thesis used the UK Biobank resource through application number 8835 and was covered by the general ethical approval for UK Biobank studies from the NHS National Research Ethics Service on 17th June 2011 (Ref 11/NW/0382).

Study population at baseline assessment

UK Biobank participants attended baseline assessment in 2006-2010 at 22 assessment centres across England, Scotland and Wales.[131] A subset of ≈20,000 participants attended a repeat assessment in 2012-2013.[131] In this thesis, the data used was extracted in April 2019 for all participants who were aged 40-70 years at baseline (and who had not opted to have their data removed from the UK Biobank resource). Information available in the resource on sociodemographic characteristics, self-reported health behaviours, health ratings and medication use was collected from touchscreen questionnaires. Linkage to Hospital Episode Statistics (HES) provided information on secondary care outcomes both before and after baseline. Linkage to the Office for National Statistics (ONS) death registry provided date and cause of death for those participants whose death was recorded before data extraction.[131] Over 100 biomarkers were measured via physical measurement devices, blood assays and urine assays.[131] Sex, date of birth (recorded to the nearest month) and the date of assessment were available for all participants.

Of the 502,536 participants in the UK Biobank at April 2019, participants were excluded in all analyses presented in this thesis if they had no date of assessment or did not attend the verbal interview, had none of the blood count or biochemical biomarker measurements (i.e. they did not

have any blood test data), were younger than 40 or older than 70 years at baseline, or were missing their Index of Multiple Deprivation score. Index of Multiple Deprivation 2010 score was grouped into quintiles within the UK Biobank population in each country. After applying these exclusions, 480,019 participants remained in the study population (Figure 3.2.1). A further 36 participants were excluded from the disease risk-based biological age analyses in Chapters 6–7 as they withdrew from the UK Biobank between April 2019 and August 2020 (Figure 3.2.1), since this list of participants was released in August 2020 and these analyses were conducted after August 2020.

Participant follow up via electronic linkage

Included participants were followed up for a median of 8.7 years to the death record censoring date of 31 January 2018 for English and Welsh participants or 30 November 2016 for Scottish participants. HES records were available for a median follow up period of 8.0 years, until 31 March 2017 for English participants, 31 October 2016 for Scottish participants, and 29 February 2016 for Welsh participants.[103]

3.3 Population stratification based on prior health

The data from UK Biobank allowed for a substantial middle-aged and relatively healthy subpopulation to be identified, in order to assess the prognostic capability of biological ages for subsequent health and to reduce reverse causality from prior health or medication use affecting biomarker levels. A composite measure of prior health for stratification into 4 subpopulations was derived from self-reported characteristics at baseline interview and HES records, according to these definitions:

1. Healthy**:** No self-reported chronic disease medications, good self-reported health, steady/brisk walk speed, 0-2 HES episodes (periods of care in a hospital under a single consultant) prior to recruitment, never/ex smoker, no prior chronic disease or hip/wrist fracture

2. Some medications**:** 1-2 self-reported chronic disease medications, 0-2 HES episodes, no prior disease or hip/wrist fracture

3. Slightly unhealthy**:** Participants who do not fall into categories 1 or 2 but without prior disease or hip/wrist fracture

4. Poor health**:** Prior disease or hip/wrist fracture

Analyses in this thesis focused on the 1st (healthy) subpopulation. The categorisation of chronic disease medications (used to define subpopulation 2) is described in detail in Section 3.4 and Appendix 3.1, and the definition of prior disease (used in all subpopulation definitions) is described in Section 3.5. Prior hip and wrist fractures (used in all subpopulation definitions) were identified in participants using the ICD-10 codes S72 and S62 respectively. Data on HES episodes (used to define subpopulations 1 and 2) excluded episodes related to the delivery of children (ICD-10 codes O80-84). Participant characteristics of the healthy and poor health subpopulation are described in Section 3.9.

3.4 Categorisation of medications for chronic disease

Use of medications was self-reported by participants at the baseline assessment, and classified by a trained nurse during the verbal interview stage of the baseline assessment. In these analyses, medications were further classified as chronic disease-related, or not, using a data-driven and text-mining approach developed and applied to the 1366 medication names reported in the UK Biobank at April 2017 (details in Appendix 3.1). These medication names were mapped to British National Formulary (BNF) Chapters, Sections and Paragraphs as defined by BNF in December 2017.[132] A clinician reviewed the classification of the BNF Sections matched to the 787 types of medications (at BNF paragraph level) used by UK Biobank participants. This medication classification process is summarised in Figure A3.1, and the 50 BNF Sections that were classified as chronic disease-related and corresponding to these types of medications in this study are listed in Table 3.4.1.

A simple count of chronic disease medication types (BNF Paragraphs; Appendix 3.1) was carried out for each participant in the study, based on self-reported medication use at baseline. Counts of medication types have previously been found to be predictive of mortality.[133] No chronic disease medication use was reported by 51.6% of participants, while 39.7 reported using 1–2 types of medications and the remaining 8.7% reported using >2 types of medications at baseline (Table 3.4.2). The mean number of types of medications per participant for those reporting medication use was 1.72.

3.5 Phenotyping age-related chronic diseases

Definition of age-related chronic diseases

The diseases in scope for this thesis are age-related chronic diseases recorded as a primary or secondary diagnosis in HES. Prior disease identified before the baseline assessment was used as a criterion for population stratification (Section 3.3), while patterns of disease incidence after baseline were used in the estimation of disease risk-based body system ages (Chapter 4). Age-related chronic diseases selected for use in this study were based on the following criteria: (1) commonly treated by researchers as a chronic rather than acute disease, (2) clear increasing trend in incidence with chronological age, and (3) not defined solely by levels of a physical or biochemical biomarker measured in UK Biobank.

Selection of age-related diseases for this thesis

In total, 21 age-related chronic diseases were used in this thesis (Figure 3.5.1), consisting of 16 diseases used to define prior disease in the age-based biological age analysis (Chapter 5) and an additional 5 diseases used in the disease risk-based body system and biological age analysis (Chapters 6–7). The 16 diseases used in all analyses are: cardiac arrhythmia, chronic kidney disease, diabetes mellitus, heart failure, ischaemic heart disease, peripheral arterial disease, arthritis,

rheumatoid arthritis, osteoporosis, gout, dementia, stroke/transient ischaemic attack (TIA), chronic obstructive pulmonary disease, connective tissue disease, liver disease, and malignant cancer. The additional 5 diseases used in the disease risk-based analyses (Chapters 6–7) only are: peptic ulcer disease, gall bladder disease, diverticular disease, Parkinson's disease and motor neuron disease. Malignant cancer was only included in the prior disease and not incident disease definition.

The disease definitions used in this study were mainly based on a list of 58 chronic diseases and their respective ICD-10 codes published by the Tran et al study of chronic disease incidence in English adults.[134] This thesis used disease definitions for 17 of the diseases from the Tran et al list, after exclusions based on the principles described in Figure 3.5.1, and applied the ICD-10 codes to main or secondary diagnoses in the HES records linked to the UK Biobank. The choice of diseases in the Tran et al study[134] was based on a highly cited study of inequalities in multimorbidity prevalence in Scottish adults,[9] and Tran et al study published the ICD-10 codelists phenotyped by the authors for multiple chronic diseases across different body systems. The Tran et al[134] codelist partially relied on the CALIBER portal of codelists, which contains codelists for >300 health conditions contributed by different epidemiological studies.[135] Four other diseases not considered by Tran et al[134] but included in other studies of multiple diseases[9,10,136] were also considered in this study: Parkinson's disease, motor neuron disease, diverticular disease, and gall bladder disease. Irritable bowel disease, psoriasis and mood disorders were not considered even though they were included in these studies,[9,10,136] as these conditions were unlikely to result in hospital admissions.

The Tran et al study had grouped their list of 58 diseases into 7 disease clusters: cardiometabolic, mental health, respiratory, musculoskeletal, neurological, cancer and other diseases,[134] and the disease clusters reported in their study were used as a starting point for organising the selection or exclusion of individual diseases in this thesis. This phenotyping procedure had been refined over time based on clinical advice. For the more recent disease risk-based analyses in Chapter 6–7, the disease scope was extended to include 2 additional neurological diseases (motor neuron disease and Parkinson's disease) and 3 additional gut diseases (diverticular disease, gall bladder disease and

peptic ulcer disease). At the same time, the disease definitions in the disease risk-based analyses for 5 existing and additional diseases were also extended to include procedures in secondary care, recorded via OPCS codes in main or secondary diagnoses (Appendix 3.2). Both lists of additional ICD-10 codes and OPCS codes (Appendix 3.2) were reviewed by a clinician.

The following considerations were applied sequentially to the list of diseases (summarised in Figure 3.5.1) and were reviewed by a clinician:

1) Exclude the following disease clusters that are unrelated to ageing, for the following reasons:
    a. Diseases in the mental health cluster – the prevalence of mental health conditions in the UK population is not age-related[136] and poor mental health may not necessarily be seen as a chronic disease (as it could be a recurring short term condition). It is also likely to be selectively recorded and unlikely to be a primary diagnosis in secondary care.

    b. 'Other' cluster except connective tissue and liver disease – The 'Other' cluster was a diverse assortment of HIV/AIDS, peptic ulcer disease, connective tissue disease and liver disease. HIV/AIDS is an infectious disease and typically not included in ageing studies. Peptic ulcer disease was not related to any of the other disease clusters before other gut diseases were considered for inclusion in Chapters 6–7, and was not prevalent in hospital settings.[137] Connective tissue and liver disease were subsequently reassigned to more relevant clusters in Section 3.6.

2) All cancers (excluding for in-situ neoplasms and benign neoplasms) were treated as a single disease rather than multiple diseases belonging to a cancer cluster – cancer is commonly treated as a single disease in studies of multiple diseases.[9,10,136]

3) Exclude 3 diseases that are defined solely by levels of any biomarker measured in the UK Biobank:[104] hypertension (based on blood pressure measurements), hyperlipidaemia (based on

blood lipids) and obesity (based on BMI). The exclusion of these high-prevalence and low-severity diseases or conditions also avoided swamping the effects of more clinically relevant diseases with less clinically relevant conditions.

4) Exclude 4 diseases that more commonly occur during or before early adulthood: asthma,[138] epilepsy (which most commonly occurred in childhood and at ages over 75 years),[139] hemiplegia[140] and learning disability.[141] (Incidence rates for hemiplegia and learning disability were also unstable due to low numbers of events of <100 in the UK Biobank.)

The Tran et al codelists for musculoskeletal diseases[134] and hence the definition of musculoskeletal diseases in this thesis did not include hip or wrist fractures (ICD-10 code S72 and S62 respectively), which were part of the prior health definition that was used to stratify the participants in this thesis (Section 3.3). The numbers of hip or wrist fractures were not substantial in the UK Biobank, as only 0.4% of UK Biobank participants ever had hip or wrist fractures prior to baseline or during follow up.

Participants had prior disease if the first instance of the HES admission related to the disease occurred before the date of their baseline assessment, and had incident disease if the first instance occurred after baseline assessment and during follow up. The same set of in-scope diseases were used in analyses of prospective disease incidence, except that cancer was excluded from the group of prospective diseases for the following reasons: (1) cancer is difficult to analyse in risk factor studies as it is a collection of different diseases (with different biomarkers) that affect many body systems; (2) cancer incidence is not closely linked to physical and biochemical biomarkers measured in the UK Biobank; and (3) previous findings identified in the systematic review indicated that biological age was not clearly associated with incident cancer (Figure 2.3.2). The definition of prior cancer for this study was restricted to only malignant cancers, and therefore excluded in-situ neoplasms (ICD-10 codes D00-D09) and benign neoplasms (ICD-10 codes D10-D36).

3.6 Defining disease clusters

Objectives and criteria for clustering

The in-scope diseases identified in Section 3.5 were clustered for 2 main reasons: (1) to avoid low numbers of incident diagnoses per outcome for the less prevalent diseases, and (2) to reduce the complexity of the study design from 20 disease outcomes to a more manageable number of outcomes. The starting point for the clustering scheme was also the Tran et al[134] disease clusters, with the modifications described previously (Section 3.5). The criteria for clustering diseases, ranked by their importance in this thesis, were:

1. Concordant diseases ('representing parts of the same overall pathophysiologic risk profile and are more likely to be the focus of the same disease management plan')[142] were grouped together, and this scheme was reviewed by a clinician.
2. The need for a large enough number of events in each cluster in the healthy subpopulation. A power calculation was conducted to determine the feasibility of estimating body system ages given the number of events in the healthy subpopulation and the number of candidate biomarkers. Using the Riley et al[143] method for calculating sufficient sample sizes for developing prediction models, an indicative minimum number of events per biomarker was determined to be 20. This translates to 2000 events if 100 biomarkers were included, but 200 events would be sufficient if a subset of 10 biomarkers were included.
3. Incidence rates by chronological age for constituent diseases have broadly similar patterns.

Several changes were made to the clusters for this study, based on the criteria listed above:

1. The 'Cardiometabolic' cluster was split into 'Cardiovascular' and 'Metabolic', due to aetiological differences between cardiovascular and metabolic diseases and sufficiently large numbers of incident events. 'Cardiovascular' diseases were further split into 'Atherosclerotic' (ischaemic heart disease, peripheral arterial disease and stroke/TIA) and 'Cardiac' (arrhythmia and heart failure) for two reasons: (1) these diseases have different risk profiles and management

plans (clustering criterion 1), and (2) there were sufficient events (>2000 events in each group; criterion 2). Stroke/TIA was treated as a cardiovascular instead of neurological disease in this thesis.

2. Respiratory cancers were shifted from the 'Respiratory' to 'Cancers' group, as they appeared to be more commonly treated as cancers than respiratory diseases in the UK[9,134,136] (criterion 1).
3. Liver disease was moved to 'Metabolic' from the defunct 'Other' group, while gout was moved to 'Metabolic' from the 'Musculoskeletal' group (criterion 1).
4. The majority of connective tissue disease (from the defunct 'Other' group) appeared to be inflammatory, so it was moved to a new 'Inflammatory' cluster. Rheumatoid arthritis was also reclassified as 'Inflammatory' instead of 'Musculoskeletal' (criterion 1).

The analyses in this thesis focused on a healthy subpopulation in the UK Biobank, defined using the population stratification procedure described in Section 3.3. In the healthy subpopulation, incidence rates of all constituent diseases within clusters tended to have similar patterns across 5-year chronological age groups for both sexes, although the trends were unstable for individual diseases with small numbers of events (criterion 3; data not shown). When aggregated into disease clusters, increasing log-linear trends were observed in the crude incidence rates by 5-year chronological age groups (Figure 3.6.1).

Data-driven patterns of disease co-occurrence in UKB participants

As statistical clustering methods rather than clinical approaches to clustering diseases have often been used in previous studies of multiple chronic diseases,[144] a sensitivity analysis was conducted to explore if supplementary information on patterns of co-occurrence from a statistical clustering analysis could inform and improve the clustering procedure above.

An exploratory sensitivity analysis of data-driven patterns of disease co-occurrence in the UK Biobank participants was conducted as follows: eventual diagnoses (diagnoses prior to the baseline

date and during HES follow up) of the 21 in-scope diseases, stratified by subpopulation and sex, were analysed. The diseases were clustered by hierarchical clustering of diseases with average linkage and Yule Q similarity measure, as recommended by a review of statistical clustering methods in multimorbidity studies.[144] The resulting clusters are shown in Figure 3.6.2. The patterns of disease co-occurrence that were consistent across subpopulations and sexes were already aligned to clinical specialisms: cardiovascular, neurological, musculoskeletal and gut diseases (Figure 3.6.2). There did not appear to be other consistent patterns of co-occurrence within these 4 clusters, or among the diseases that were not in these 4 clusters (Figure 3.6.2). Therefore no supplementary information from this analysis was used in the disease clustering for this thesis.

Final list of disease clusters and their constituent diseases

The 20 in-scope incident diseases were grouped into 8 final clusters, with the following constituent diseases:

1. Atherosclerotic diseases: Ischaemic heart disease, peripheral arterial disease and stroke/TIA
2. Musculoskeletal diseases: Arthritis and osteoporosis
3. Gut diseases: Diverticular disease, gall bladder disease and peptic ulcer disease
4. Cardiac diseases: Cardiac arrhythmia and heart failure
5. Metabolic diseases: Diabetes mellitus, chronic kidney disease, liver disease and gout
6. Inflammatory diseases: Rheumatoid arthritis and connective tissue disease
7. Neurological diseases: Dementia, Parkinson's and motor neuron disease
8. Respiratory diseases: COPD

(Malignant cancer, described in Section 3.5, was used in the prior disease but not incident disease definition.)

The most prevalent (ever diagnosed) disease groups in the healthy subpopulation were musculoskeletal, followed by gut and atherosclerotic diseases (Figure 3.6.3). The majority of the

healthy participants had diseases in only one of the three most prevalent groups during follow up (Figure 3.6.3).

3.7 Defining general health outcomes

A key aim of this thesis is to investigate approaches for summarising biomarker measurements into an overall indicator of biological ageing (Section 1.4). Hence the predictive power of biological ages for general adverse health outcomes were investigated in this thesis: (1) mortality due to chronic disease and (2) incidence of age-related frailty, defined as the first admission to hospital for an age-related reason. These outcomes were phenotyped from death registry and HES records respectively, based on codelists and procedures published by previous studies.[12,78,106,145]

1. Mortality due to chronic disease:

   Mortality is the most objectively and most accurately recorded outcome available in UK Biobank. Based on ICD-10 coded causes of death, accidental deaths[12,78] and non-chronic disease deaths[106] were excluded from the outcome definition (participants who died of excluded causes were censored), following these previous studies' procedures. These deaths would not be much related to ageing processes and their relatively high prevalence at younger ages might mask ageing-related mortality effects. The exclusions were specified by ICD-10 Chapter: certain infectious and parasitic diseases (A00-B99), pregnancy, childbirth and the puerperium (O00-O99), congenital malformations, deformations and chromosomal abnormalities (Q00-Q99), injury, poisoning and certain other consequences of external causes (S00-T98) and external causes of morbidity and mortality (V01-Y98).

2. Age-related hospital frailty:

Frailty is a strong predictor for multimorbidity and mortality in the UK Biobank[146] and adverse health outcomes in many other populations.[147] It precedes mortality, and may be a general indicator of ageing earlier in life. However, not all components of frailty are age-related or are recorded in clinical care. Hospital admissions for age-related reasons may be symptoms of biological frailty.[5] Therefore, in this thesis these events were identified by selecting the admissions with a diagnosis of ICD-10 codes used in a frailty risk score for secondary care records[145] that were found to be age-related in UK Biobank participants by the process defined below. Not all components typically included in frailty assessment instruments are age-related or recorded in clinical care.[5] Therefore, for clarity, the term 'age-related frailty' will be used throughout the thesis.

The age-related frailty outcome in this study was constructed by combining frailty subtypes that were strongly associated with chronological age into a single time-to-event outcome: first ever frailty-related hospital admission observed in participants' HES records. These subtypes were chosen from the ICD-10 codes used in the hospital frailty risk score:

a. Incident cases in the UK Biobank at April 2017 were identified for each of 75 candidate frailty subtypes, defined as standalone 3-digit ICD-10 codes in HES main or secondary diagnosis records. Codes with >500 incident cases were considered individually; otherwise codes were grouped within the same ICD-10 block (Table 3.7.1). All codes with >500 incident cases were standalone codes within their ICD-10 blocks.
b. Hazard ratios per 10 years of baseline chronological age were then estimated for each candidate subtype as the outcome, with Cox models adjusted for smoking status, alcohol intake frequency and Townsend deprivation quintile.
c. Subtypes were included in the definition if this hazard ratio exceeded the threshold of 1.2. These subtypes included symptoms and conditions in neurological, renal, musculoskeletal, respiratory and cerebrovascular systems, and symptoms of physical disability (Table 3.7.1).

3.8 Biomarker selection and analysis

Biomarker data cleaning

As at April 2019, 110 physical and biochemical biomarkers were available in UK Biobank baseline data. Biomarkers were excluded from the panel in this thesis if (1) they were measured in <70% of the whole population, (2) if they were not measured on a continuous scale, or (3) if they measured the same biological trait (e.g. standardly-measured but not impedance device-measured weight was selected), leaving 74 biomarkers. Measurements of these biomarkers at baseline assessment were cleaned using the procedure described in Appendix 3.3.

A further 2 biomarkers, oestradiol and nucleic red blood cell count, were excluded due to poor reproducibility (intra-individual Pearson correlation coefficient for baseline and repeat measurements adjusted for baseline chronological age <0.1), leaving 72 biomarkers (Table 3.8.1 and Appendix 3.3). These 72 biomarkers were categorised by body system group (Table 3.8.1), based partially on the biomarker categorisation used by the review of biological age estimation methods.[20]

These 72 biomarkers included all of the biomarkers that were more commonly used by the 15 previous large-scale studies of biological age identified in the systematic review (Section 2.3) (defined as biomarkers that were in the candidate panels of 5 or more of the previous studies), except for fasting blood sugar (Table 3.8.2). The main differences in biomarker panels used in this thesis vs the 15 previous studies, which selected between 3 and 19 biomarkers each (Section 2.3), were the types of hormonal and cognitive function biomarkers with available measurements, and that renal biomarkers measured via urine tests were only available in the UK Biobank for this analysis (Table 3.8.2).

Statistical analysis of biomarker characteristics

Biomarker-age trends were assessed for linearity and for homogeneity between sexes and across prior health subpopulations. To estimate biomarker-age trends, linear regression was used to obtain least-square means and standard errors of standardised biomarker values by 2.5-year chronological age groups, separately by sex, adjusted for Index of Multiple Deprivation 2010 quintile, smoking status, alcohol consumption band and assessment centre. Trends for each biomarker were displayed on a common standardised scale for comparability (with original units included as a second scale), and visually assessed for linearity across age groups, as subsequent statistical methods assumed the linearity of biomarker-biomarker or biomarker-age relationships (Chapter 4).

To assess whether further stratification of the healthy subpopulation by smoker status was required, a sensitivity analysis of biomarker-age trends for healthy never vs ex smokers was carried out. All biomarkers were assessed, but with a focus on lung function as it appeared to have the strongest linear relationship with chronological age, and is adversely affected by smoking.[148] Figure 3.8.1 displays the trends for lung function biomarkers, which display the largest disparities by smoking status, and systolic blood pressure. The trends for the two lung function biomarkers were linear for each smoking status, with a slight convergence at older ages, and the trends for the remaining biomarkers appeared to be similar regardless of smoker status. Therefore further stratification was deemed not essential.

Pearson correlations were calculated for each biomarker and chronological age in the healthy subpopulation, and the correlation coefficients were ranked by magnitude. Many previous studies used biomarker-age correlations to pre-select biomarkers for inclusion in the biological age estimation process.[12,16,19,78,96] Pre-selection was not carried out in this study for two reasons: (1) to avoid selecting biomarkers that may be highly correlated with each other (due to their high correlation with chronological age), which is an undesirable property in subsequent estimation of biological ages, and (2) to allow methods for estimating biological age to perform their own biomarker selection or weighting process (described in Chapter 4).

Principal components of biomarkers

Principal Component Analysis (PCA) is one of the common methods to estimate biological ages (Section 2.3), but in the main analyses in this study, this method has solely been used to summarise the biomarkers into linearly independent biomarker principal components, which are linear combinations of the original biomarkers.[52] PCA was run on the full set of biomarkers after imputing missing biomarker values for the whole population (Appendix 3.3). The resulting biomarker principal components were ranked by their eigenvalues, representing the degree of variation in biomarker values that each principal component describes. Ranking principal components by their eigenvalues facilitated the selection of a smaller number of biomarker principal components that still represented the majority of variation in biomarker values (dimension reduction).

PCA was preferred to another common method for dimension reduction, factor analysis, which has also been used as an alternative method for estimating biological age.[149] PCA considers all of the variation in all biomarker values while factor analysis only considers the variation that the panel of biomarkers have in common, and ignores the remaining variance unique to a specific biomarker (which is a combination of the variation between individuals and measurement error).[150] It is possible that a specific biomarker measures an ageing trait that no other biomarker in the set has captured (e.g. bone density), therefore variation unique to one biomarker should be captured by the dimension reduction analysis instead of being excluded. In this thesis, biomarkers were selected to reflect a wide range of ageing processes (similar to the motivation of studies that consolidated diverse ageing biomarker panels[7,18,45,91]) instead of their shared variance in ageing processes, and biomarkers should not be excluded due to low shared variance with other biomarkers.

The use of sparse PCA for dimensionally reducing the biomarkers was also considered, but this method required additional input for the maximum number of biomarkers for each component instead of evaluating this number automatically. A review of sparse PCA methods also found that

these methods generally did not produce uncorrelated principal components, and required further mathematical corrections to be comparable with PCA.[151]

To aid clinical interpretation of these principal components, varimax rotation (which increases the contribution of biomarkers strongly loaded onto a principal component and decreases the contribution of those less strongly loaded, while preserving the independence of the biomarker principal components) was applied after PCA. For example, in a previous study, that combined 15 biomarkers into 3 factors by PCA, after varimax rotation, the factors were characterised as cognitive, physical, and eye and ear function.[42]

In this thesis, the rotated principal components were individually characterised based on the relative contributions of their constituent biomarkers, measured via rotated factor loadings. The rotated factor loadings and the eigenvalues of the principal components were similar when run on the healthy subpopulation and the whole population, thus only the results for the whole population were used in all subsequent analyses, for consistency in interpretation.

3.9 Cohort characteristics

The analyses in this thesis focused on the healthy subpopulation, to assess the prognostic capability of a biological age for subsequent health and to reduce reverse causality from prior health or medication use affecting biomarker levels. Of the 480,019 participants, 141,254 (29.4%) were in the healthy subpopulation (Table 3.9.1). Sociodemographic patterns and the proportion of participants healthy at baseline were similar between sexes.

The number of incident events for age-related frailty and mortality from chronic disease was summarised by prior health subpopulation and sex in Table 3.9.2. During a median follow up period of 8.7 years for mortality and 8.0 years for hospital admissions, 1.7% of healthy, and 3.9% of all participants died from chronic diseases; 16.0% of healthy and 23.1% of all participants who were

not admitted to hospital for age-related reasons (age-related frailty) prior to baseline had been admitted with diagnoses of these conditions during follow up (Table 3.9.2). In the healthy subpopulation, the pattern of crude incidence rates for mortality and frailty by 5-year chronological age groups broadly followed a log-linear trend (Figure 3.9.1).

The disease risk-based analysis excluded 36 participants who withdrew from the study between April 2019 and August 2020 from the whole population, and excluded 1637 participants initially in the healthy subpopulation who had prior age-related chronic diseases under the expanded disease definition from the updated healthy subpopulation. There were no substantial differences in participant characteristics among the (slightly fewer) participants included in the disease risk-based biological age analysis in Chapters 6–7 (data not shown) compared to those included in the age-based analysis (Table 3.9.1).

Incident disease diagnoses were only used in the disease risk-based analyses. The numbers of incident diagnoses for each disease and each disease cluster in the healthy subpopulation are summarised in Table 3.9.3. The most frequent incident disease cluster in this subpopulation (excluding cancer) was musculoskeletal diseases, followed by gut diseases, then atherosclerotic and cardiac diseases. The numbers of incident events in each disease cluster were higher than 2000 for all clusters apart from the neurological, respiratory and inflammatory clusters, which had 466-717 events each (Table 3.9.3). The number of total diagnoses across diseases was only slightly higher than the number across disease clusters (31,838 diagnoses across diseases vs 29,945 diagnoses across clusters, i.e. 6% higher; Table 3.9.3), suggesting that participants who had multiple incident diseases tended to have diseases in different rather than similar clusters. Since participants in the healthy subpopulation were non-smokers who had no prior disease at baseline assessment (Section 3.3), the incidence of respiratory diseases in the healthy subpopulation was much lower than in the rest of UK Biobank population (0.4% vs 2.1% of participants respectively had incident respiratory disease during follow up).

3.10 Biomarker characteristics

In the healthy subpopulation, the relationships of the means of most candidate biomarkers to chronological age were broadly linear or flat (Figure 3.10.1 and Figure 3.10.2). Several biomarkers displaying non-linear trends and differences by sex or by prior health are also highlighted in Figure 3.10.1. Lung function biomarkers, systolic blood pressure, cystatin C and reaction time had the steepest slopes with age and broadly linear relationships with age (Figure 3.10.1). When contrasted with the poor health subpopulation (who had prior disease, and whose characteristics are described in Tables 3.9.1 and 3.9.2), clear inverse U-shaped relationships with age were present for diastolic blood pressure, body mass index (BMI) and low density lipoprotein cholesterol (LDL-C) in the poor health subpopulation that were attenuated in the healthy subpopulation (Figure 3.10.1). Diastolic blood pressure peaked or plateaued at different ages and BMI displayed different trends in the healthy and poor health subpopulation (Figure 3.10.1). Biomarkers that displayed substantially different trends between sexes in the healthy subpopulation were heel bone density, LDL-C, calcium, alkaline phosphatase and phosphate (Figure 3.10.2).

Biomarkers most correlated with chronological age were FEV1/height, cystatin C, FVC/height, systolic blood pressure (for both sexes), sex hormone-binding globulin (for healthy men) and low density lipoprotein cholesterol (for healthy women, with magnitudes of Pearson correlation coefficients ≥ 0.295 (Table 3.10.1).

Many biomarker principal components had a single biomarker strongly loaded onto them and were easily characterised (Figure 3.10.3). Multiple biomarkers were strongly loaded onto the general and central adiposity (ranked by eigenvalue: 1 and 28), blood count (2, 5, 6, 8, 14 and 19), height (3), blood lipid (7 and 11), urinary salt (9), blood pressure (10), aminotransferase (12) and lung function (17) principal components (Figure 3.10.3). The first principal component, general adiposity, had an eigenvalue of 9.61, i.e. it described the variation in biomarker measurements that was equivalent to the variation described by almost 10 biomarkers on average. These biomarker principal components,

rather than the biomarker measurements, were taken forward to be used in the estimation of the biological ages (Chapter 4).

3.11 Conclusion

Through the investigations in this chapter, a healthy subpopulation of 141,254 out of 0.5 million UK Biobank participants was identified, in order to reduce reverse causality in analyses of the associations between biomarker levels and later life health. Measurements for 72 physical and biochemical biomarkers in all participants were condensed into biomarker principal components. Detailed phenotyping of the linked death registry and secondary care records enabled patterns of mortality from chronic disease, age-related frailty and 8 body system-specific groups of 20 diseases to also be analysed in this population. This UK Biobank population as well as the healthy subpopulation is generally larger and more deeply phenotyped than the previous studies of biological age reviewed in Chapter 2.

Chapter 3 tables and figures

Table 3.4.1: List of in-scope British National Formulary (BNF) Chapters and Sections related to chronic disease

BNF Chapter	BNF Section
Cardiovascular System	Anti-Arrhythmic Drugs
	Anticoagulants And Protamine
	Antifibrinolytic Drugs & Haemostatics
	Antiplatelet Drugs
	Beta-Adrenoceptor Blocking Drugs
	Diuretics
	Hypertension and Heart Failure
	Lipid-Regulating Drugs
	Local Sclerosants
	Nit,Calc Block & Other Antianginal Drugs
	Positive Inotropic Drugs
	Sympathomimetics
Central Nervous System	Analgesics
	Antidepressant Drugs
	Antiepileptic Drugs
	CNS Stimulants and drugs used for ADHD
	Dementia
	Drugs Used In Nausea And Vertigo
	Drugs Used In Park'ism/Related Disorders
	Drugs Used In Psychoses & Rel.Disorders
	Drugs Used In Substance Dependence
	Hypnotics And Anxiolytics
	Obesity
Endocrine System	Corticosteroids (Endocrine)
	Drugs Affecting Bone Metabolism
	Drugs Used In Diabetes
	Hypothalamic&Pituitary Hormones&Antioest
	Other Endocrine Drugs
	Thyroid And Antithyroid Drugs
Eye	Treatment Of Glaucoma
Gastro-Intestinal System	Antisecretory Drugs+Mucosal Protectants
	Antispasmod.&Other Drgs Alt.Gut Motility
	Chronic Bowel Disorders
	Drugs Affecting Intestinal Secretions
	Dyspep&Gastro-Oesophageal Reflux Disease
Infections	Antiviral Drugs
Malignant Disease & Immunosuppression	Cytotoxic Drugs
	Drugs Affecting The Immune Response
	Sex Hormones & Antag In Malig Disease
Musculoskeletal & Joint Diseases	Drugs Used In Neuromuscular Disorders
	Drugs Used In Rheumatic Diseases & Gout
	Soft-Tissue Disorders & Topical Pain Rel
Nutrition And Blood	Anaemias + Other Blood Disorders
	Metabolic Disorders
Respiratory System	Bronchodilators
	Corticosteroids (Respiratory)
	Cromoglycate,Rel,Leukotriene Antagonists
	Mucolytics
	Resp Stimulants & Pulmonary Surfactants
Skin	Preparations For Eczema And Psoriasis

Table 3.4.2: Summary statistics for chronic disease medication count at baseline in the UK Biobank

Chronic disease medication count	Persons (%)	Men (%)	Women (%)
None	51.6	52.5	50.8
1-2	39.7	37.7	41.4
>2	8.7	9.8	7.8

Table 3.7.1: Constituent ICD-10 codes for the age-related hospital admissions definition, ranked by hazard ratio of baseline age in the UK Biobank

No	ICD10 group	ICD-10 codes	Incident cases in UK Biobank	Hazard ratio for 10 years of age
1	Dementia	F00 F01 F03 G30	214	5.70
2	Parkinsons	G20	576	3.16
3	Chronic renal failure	N18	873	3.14
4	Osteoporosis without pathological fracture	M81	1454	2.66
5	Other disorders of fluid, electrolyte and acid-base balance	E87	554	2.29
6	Retention of urine	R33	1274	2.28
7	Transient cerebral ischaemic attacks and related syndromes	G45	508	2.27
8	Delirium	F05	54	2.27
9	Polyarthrosis	M15	913	2.19
10	Respiratory disease not infection	J69 J96	412	2.17
11	Cerebrovascular	I67 I69	794	2.11
12	Osteoporosis	M80	546	2.07
13	Cerebral Infarction	I63	574	2.07
14	Other hearing loss	H91	864	2.00
15	Other abnormal findings of blood chemistry	R79	1816	1.90
16	Renal failure	N17 N19	956	1.90
17	Neurodegenerative disease	G31	114	1.90
18	Problems related to social environment	Z60	839	1.86
19	Skin ulcer	L89 L97	308	1.83
20	Kidney urinary disorders	N28	876	1.82
21	Other arthrosis	M19	4403	1.80
22	Spinal stenosis (secondary code only)	M48	1038	1.73
23	Digestive disease	K26	1567	1.65
24	Pneumonia, organism unspecified	J18	1256	1.60
25	Blindness or low vision	H54	381	1.60
26	Dorsophathy	M41	379	1.59
27	Fall on same level from slipping, tripping and stumbling	W01	1597	1.59
28	Unspecified fall	W19	926	1.57
29	Hypotension	I95	717	1.51
30	Syncope and collapse	R55	1541	1.49
31	Metabolic disorder	E83 E86	1135	1.47
32	Cognition emotion behaviour symptoms	R40 R41 R44 R45 R47	1577	1.47
33	Symptoms and signs concerning food and fluid intake	R63	1405	1.45
34	Other external	Y84 Y95 Z22 Z50 Z73 Z74 Z75 Z93 Z99	3954	1.44
35	Hemiplegia	G81	381	1.43
36	Fall	W06 W18	850	1.39
37	Urinary system symptoms	R32	918	1.37
38	Unspecified acute lower respiratory infection	J22	998	1.36
39	Nervous and musculoskeletal symptoms	R26 R29	1000	1.35
40	Other bacterial agents as the cause of diseases classified to other chapters (secondary code)	B96	1051	1.33
41	Fall on and from stairs and steps	W10	639	1.32
42	Unspecified haematuria	R31	3447	1.31
43	Abnormalities of heart beat	R00	1939	1.30
44	Personal history of other diseases and conditions	Z87	6111	1.26
45	Skin infection	L08	627	1.26
46	Infection	A04 A41 B95	3444	1.25
47	Other anaemias	D64	2237	1.25
48	Dysphagia	R13	1554	1.25
49	Pancreatic disorder	E16	230	1.23
50	Abnormal results of function studies	R94	755	1.22
51	Other functional intestinal disorders	K59	1955	1.22
52	Gangrene	R02	137	1.22

This codelist excludes cancer or any form of neoplasms

P-values of hazard ratios for 10 years of age for each ICD-10 group were significant at the 10^{-3} level

Table 3.8.1: List of the 72 UK Biobank biomarkers selected for analysis, with percentage of missing data for each biomarker in the whole population

No.	Body system group	Biomarker description	% missing
1	**Cardiovascular:**	Diastolic blood pressure	0.1
2		Systolic blood pressure	0.1
3		Pulse rate	0.1
4		Apolipoprotein A	12.9
5		Apolipoprotein B	5
6		Lipoprotein (a)	7.6
7		High density lipoprotein cholesterol	12.7
8		Low density lipoprotein cholesterol	4.9
9		Triglycerides	4.7
10	**Clotting:**	Mean platelet volume	2.9
11		Platelet count	2.9
12		Platelet crit	2.9
13		Platelet distribution width	2.9
14	**Endocrine, metabolic**	Log C-Reactive Protein	4.8
15	**and immune:**	Blood glucose	12.8
16		HbA1c	5.3
17		Insulin-like growth factor 1	5.2
18		Sex hormone-binding globulin	13.4
19		Testosterone	5.6
20	**Liver:**	Albumin	12.7
21		Alanine aminotransferase	4.7
22		Aspartate aminotransferase	5.1
23		Direct bilirubin	7.5
24		Total bilirubin	5.1
25		Gamma Glutamyltransferase	4.7
26	**Musculoskeletal:**	Heel bone density	1.8
27		Body mass index*	0.4
28		Sitting height*	0.3
29		Standing height*	0.3
30		Hip circumference*	0.2
31		Waist circumference*	0.2
32		Waist-hip ratio*	0.2
33		Weight*	0.3
34		Body fat-free mass*	1.8
35		Body fat mass*	1.9
36		Body fat percentage*	1.8
37		Metabolic rate*	1.8
38		Hand grip strength/height*	0.4
39		Alkaline Phosphatase	4.7
40		Calcium	12.7
41		Rheumatoid factor	4.7
42		Vitamin D	8.5
43	**Nervous:**	Reaction time test	1.1
44		Pairs matching test	3.5
45	**Red blood cells:**	Haemoglobin concentration	2.9
46		HLS reticulocyte count	4.6
47		Immature reticulocyte fraction	4.6
48		Mean corpuscular volume	2.9
49		Mean reticulocyte volume	4.6
50		Mean spherical cell volume	4.6
51		Total red blood cell count	2.9
52		Red blood cell distribution width	2.9
53		Reticulocyte count	4.6
54		Mean corpuscular haemoglobin concentration	2.9

No.	Body system group	Biomarker description	% missing
55	**Renal:**	Urinary microalbumin	2.9
56		Urinary sodium	2.9
57		Urinary creatinine	2.7
58		Urinary potassium	2.9
59		Urea	4.8
60		Creatinine	4.7
61		Cystatin C	4.7
62		Phosphate	12.8
63		Total protein	12.8
64		Urate	4.8
65	**Respiratory:**	Forced expiratory volume in 1s/height*	8.9
66		Forced vital capacity/height*	8.9
67	**White blood cells:**	Eosinophil count	3.1
68		Lymphocyte count	3.1
69		Monocyte count	3.1
70		Neutrophil count	3.1
71		Basophil count	3.1
72		Total white blood cell count	2.9

* Values were standardised separately for men and women, due to large sex differences

All biochemical biomarkers were measured via blood assays unless labelled as 'urinary'

Table 3.8.2: List of biomarkers measured in the UK Biobank and used in this thesis vs the candidate and selected biomarkers in the 15 studies included in the systematic review

Biomarker functional group and name / Study		No. times candidate in the 15 previous studies	No. times selected in the 15 previous studies	Measured in UK Biobank and used in this thesis
Cardiovascular	Systolic blood pressure	12	8	Y
	Diastolic blood pressure	7	3	Y
	Pulse pressure	2	1	Y
	Mean arterial blood pressure	2	2	
	Heart rate	3	0	Y
	High density lipoprotein cholesterol (HDL-C)	8	5	Y
	Low density lipoprotein cholesterol (LDL-C)	3	2	Y
	Total cholesterol	10	9	C
	Triglycerides	4	3	Y
	LP-PLA2 mass and activity	1	1	
	Echocardiographic measurements	1	1	
	Apolipoprotein A	-	-	Y
	Apolipoprotein B	-	-	Y
	Lipoprotein (a)	-	-	Y
Respiratory	Forced expiratory volume in 1s (FEV1)	8	8	Y
	Forced vital capacity (FVC)	1	0	Y
	Ratio of FEV1 to FVC	1	1	C
	Maximal oxygen uptake (VO2 Max)	1	1	
Musculoskeletal	BMI	5	1	Y
	Height	1	0	Y
	Weight	1	0	Y
	Body fat percent	2	1	Y
	Body muscle percent	1	1	Y
	Waist circumference	6	5	Y
	Hip circumference	1	0	Y
	Waist-hip ratio	2	1	Y
	Pelvis-acromial index	1	1	
	Chest index	1	1	
	Total body water	1	1	
	Osteoprotegerin	1	1	
	Calcium	2	2	Y
	Physical tests (incl. grip strength)	1	1	Y
	Heel bone density	-	-	Y
	Rheumatoid factor	-	-	Y
	Vitamin D	-	-	Y
Renal	Creatinine	9	7	Y
	Albumin	8	7	Y
	Urea nitrogen	8	7	Y
	Uric acid	1	0	Y
	Urinary creatinine	1	1	Y
	Cystatin C	1	1	Y
	Inorganic phosphorus	1	1	
	Total protein	3	2	Y
	Sodium	2	1	Y
	Potassium	2	1	Y
	Urinary waste products, haematuria and pH	1	0	Y
	Phosphate	-	-	Y

Biomarker functional group and name / Study		No. times candidate in the 15 previous studies	No. times selected in the 15 previous studies	Measured in UK Biobank and used in this thesis
Endocrine, metabolic and immune	C-reactive protein (CRP)	7	6	Y
	Glycated haemoglobin (HbA1c)	5	4	Y
	Glucose	2	2	Y
	Fasting blood sugar	6	3	
	Insulin	1	0	
	Prealbumin	1	0	
	Cytomegalovirus optical density	3	3	
	Thyroid-stimulating hormone (TSH)	1	1	
	Free thyroxine (free T4)	1	0	
	Urine chrionic gonadotrophin	1	0	
	Intercellular adhesion molecule-1	1	1	
	Interleukin-6 (IL6)	1	1	
	Monocyte chemoattractant protein-1 (MCP-1)	1	1	
	P-selectin	1	1	
	Tumor necrosis factor receptor II (TNFR2)	1	1	
	Insulin-like growth factor-1	-	-	Y
	Sex hormone-binding globulin	-	-	Y
	Testosterone	-	-	Y
	Oestradiol	-	-	C
Liver	Alkaline phosphatase	5	5	Y
	Alanine aminotransferase	2	0	Y
	Aspartate aminotransferase	3	1	Y
	Gamma-glutamyl transpeptidase (GGT)	1	1	Y
	Bilirubin	3	1	Y
	Transferrin	1	0	
Haematology	Haemoglobin	5	2	Y
	Red blood cell count	4	1	Y
	Red blood cell distribution width	1	0	Y
	Erythrocyte sedimentation rate	1	1	
	Haematocrit	4	1	Y
	Mean corpuscular haemoglobin measurements	2	1	Y
	Mean corpuscular volume	3	1	Y
	Ferritin	1	1	
	Folate and Vitamin B12	1	0	
	White blood cell count (total and by cell type)	4	0	Y
	Platelet count	3	1	Y
	Platelet mean volume, crit and distribution width	-	-	Y
	Reticulocyte counts	-	-	Y
Cognitive	Trail making test	1	1	
	Language fluency test	1	0	
	Drawing test	1	0	
	Reaction time	1	1	Y
	Pairs matching test	-	-	Y

Y: Biomarker was in the candidate panel and was used in the biological age analyses
C: Biomarker was not included in the candidate panel due to double counting (total cholesterol and ratio of FEV1 to FVC) or low reproducibility (oestradiol)

The detailed list of biomarkers used in the 15 studies in the systematic review (categorised by the same functional groups) is in Table 2.3.3. There are slight differences in the number of biomarkers displayed in this table and the candidate and selected biomarkers reported by the study or UK Biobank, due to the grouping of several biomarkers in this table
All biochemical biomarkers were measured via blood assays unless labelled as 'urinary'

Table 3.9.1: Participant characteristics at baseline assessment in 2006-2010, for the healthy and poor health subpopulations and the whole UK Biobank population

	Healthy subpopulation			Poor health subpopulation			Whole population		
	Persons	**Men**	**Women**	**Persons**	**Men**	**Women**	**Persons**	**Men**	**Women**
Participants (n)	141,254	65,869	75,385	82,835	42,277	40,558	480,019	219,248	260,771
Person-years at risk (millions)	1.2	0.56	0.64	0.71	0.36	0.35	4.12	1.87	2.25
Deaths during follow up (%)	1.7	2.1	1.4	9.1	11.2	7.0	3.9	5.2	2.9
Median age at baseline (years)	56.0	55.7	56.4	62.2	62.5	61.8	58.3	58.8	58.0
Age band at baseline in years (%)									
40-44	12.1	13.9	10.5	3.9	3.9	3.9	10.2	10.4	10.1
45-49	15.9	16.5	15.4	6.5	6.2	6.8	13.1	12.7	13.4
50-54	17.8	17.1	18.4	10.5	9.7	11.3	15.1	14.4	15.8
55-59	19.4	18.6	20.1	16.8	15.7	17.9	18.1	17.5	18.6
60-64	21.9	21.0	22.6	30.2	30.0	30.4	24.3	24.3	24.3
65-70	12.9	12.9	13.0	32.1	34.5	29.6	19.2	20.8	17.9
Index of Multiple Deprivation quintile (%)									
Q1 (least deprived)	23.9	23.8	24.1	16.9	16.7	17.1	20.0	19.7	20.1
Q2	22.2	22.1	22.3	18.3	18.2	18.3	20.0	19.7	20.3
Q3	20.9	20.7	21.1	19.3	19.2	19.4	20.0	19.8	20.2
Q4	18.7	18.7	18.7	21.0	20.8	21.1	20.0	19.9	20.1
Q5 (most deprived)	14.3	14.7	13.9	24.6	25.1	24.0	20.0	20.9	19.3
Smoker status (%)									
Current	0.0	0.0	0.0	11.0	12.5	9.6	10.5	12.4	8.8
Previous	33.4	35.9	31.2	41.6	47.1	35.9	34.5	38.3	31.3
Never	66.6	64.1	68.8	46.6	39.7	53.7	54.5	48.8	59.4
No answer/missing	0.0	0.0	0.0	0.8	0.8	0.7	0.5	0.5	0.5
Alcohol consumption frequency (%)									
Never	5.5	4.5	6.5	11.5	8.8	14.2	8.0	6.3	9.5
Special occasions only	8.8	5.6	11.6	14.1	9.3	19.1	11.5	7.3	15.0
One to three times a month	10.5	8.6	12.3	10.8	9.1	12.6	11.1	8.9	13.0
Once or twice a week	27.3	27.2	27.4	24.5	25.5	23.5	25.8	25.9	25.7
Three or four times a week	26.8	29.5	24.5	19.8	23.1	16.4	23.1	26.1	20.5
Daily or almost daily	21.0	24.7	17.7	19.0	23.8	14.0	20.3	25.3	16.1
No answer/missing	0.0	0.0	0.0	0.3	0.3	0.3	0.2	0.2	0.2
Body mass index in kg/m^2 (%)									
<18.5	0.6	0.2	0.9	0.5	0.3	0.7	0.5	0.2	0.8
18.5-24.9	41.7	32.6	49.6	24.5	19.6	29.7	32.8	25.2	39.2
25-29.9	43.1	51.6	35.8	41.5	46.2	36.6	42.4	49.2	36.6
30-39.9	14.1	15.3	13.0	30.1	31.4	28.7	22.4	24.0	21.0
40+	0.5	0.3	0.7	3.4	2.5	4.3	1.9	1.3	2.4

Table 3.9.2: Number of events for each general health outcome in the healthy and poor health subpopulations and the whole UK Biobank population, by sex

	Persons	Men	Women
Healthy subpopulation			
Participants at baseline	141,254	65,869	75,385
Deaths from chronic disease	2,394	1,357	1,037
Prior age-related frailty	6,206	2,953	3,253
Incident age-related frailty	21,627	10,317	11,310
Poor health subpopulation			
Participants at baseline	82,835	42,277	40,558
Deaths from chronic disease	7,552	4,729	2,823
Prior age-related frailty	35,947	18,327	17,620
Incident age-related frailty	19,254	10,023	9,231
Whole population			
Participants at baseline	480,019	219,248	260,771
Deaths from chronic disease	18,799	11,362	7,437
Prior age-related frailty	74,811	35,401	39,410
Incident age-related frailty	93,716	43,700	50,016

Table 3.9.3: Final list of clustered diseases, with number of incident events (by disease and by cluster) in the UK Biobank healthy subpopulation

Cluster	Disease	Healthy men (diseases)	Healthy women (diseases)	Disease total	Cluster total
Cardiac	Cardiac arrhythmia	2,199	1,271	3,470	3,763
	Heart failure	426	245	671	
Atherosclerotic	Ischaemic heart disease	2,199	960	3,159	4,600
	Peripheral arterial disease	416	312	728	
	Stroke/TIA	648	440	1,088	
Musculoskeletal	Arthritis	4,158	5,552	9,710	10,481
	Osteoporosis	105	1,005	1,110	
Metabolic	Diabetes mellitus	822	496	1,318	2,841
	Chronic kidney disease	283	274	557	
	Liver disease	373	316	689	
	Gout	406	29	435	
Neurological	Dementia	122	103	225	466
	Parkinson	134	76	210	
	Motor neuron disease	34	29	63	
Respiratory	Chronic obstructive pulmonary disease	314	271	585	585
Inflammatory	Rheumatoid arthritis	114	232	346	717
	Connective tissue disease	236	460	696	
Gut	Diverticular disease	2,375	2,631	5,006	6,492
	Gall bladder disease	458	637	1,095	
	Peptic ulcer disease	363	314	677	
(*Not used)	Malignant cancers	5,023	5,259	10,282	10,282
Total incident events (excluding malignant cancers)		**16,185**	**15,653**	**31,838**	**29,945**
Total participants		**65,869**	**75,385**	**141,254**	

* Malignant cancer was included in the list of prior diseases but excluded from the list of incident diseases. The malignant cancer definition excludes in-situ and benign neoplasms, and non-melanoma skin cancer

Table 3.10.1: Pearson correlation coefficients of biomarkers with chronological age ranked by magnitude, in the healthy subpopulation, by sex

Healthy men

Rank	Biomarker	Pearson correlation coefficient
1	Forced expiratory volume in 1s/height	-0.377
2	Cystatin C	0.317
3	Sex hormone-binding globulin	0.315
4	Forced vital capacity/height	-0.313
5	Systolic blood pressure	0.295
6	Albumin	-0.283
7	Reaction time test	-0.281
8	Insulin-like growth factor 1	-0.259
9	Hand grip strength/height	-0.238
10	Metabolic rate	-0.224
11	Body fat-free mass	-0.214
12	HbA1c	0.208
13	Mean corpuscular volume	0.186
14	Sitting height	-0.177
15	Pairs matching test	-0.169
16	Waist-hip ratio	0.167
17	Mean spherical cell volume	0.166
18	Urea	0.159
19	Body fat percentage	0.151
20	Standing height	-0.148
21	Total protein	-0.146
22	Red blood cell distribution width	0.145
23	Alanine aminotransferase	-0.140
24	Apolipoprotein A	0.139
25	Mean reticulocyte volume	0.135
26	Monocyte count	0.126
27	Log C-Reactive Protein	0.125
28	Total red blood cell count	-0.125
29	Blood glucose	0.122
30	Calcium	-0.120
31	Urinary sodium	-0.119
32	Vitamin D	0.118
33	Urinary microalbumin	0.118
34	Platelet crit	-0.102
35	Neutrophil count	0.102
36	High density lipoprotein cholesterol	0.102
37	Weight	-0.093
38	Total white blood cell count	0.086
39	Apolipoprotein B	0.085
40	Urinary creatinine	-0.085
41	Diastolic blood pressure	0.083
42	Waist circumference	0.082
43	Low density lipoprotein cholesterol	0.079
44	Platelet count	-0.073
45	Reticulocyte count	-0.065
46	Body fat mass	0.057
47	Phosphate	-0.054
48	Alkaline Phosphatase	0.054
49	Basophil count	0.047
50	HLS reticulocyte count	-0.047
51	Heel bone density	-0.042
52	Mean corpuscular haemoglobin concentration	-0.041
53	Aspartate aminotransferase	-0.040
54	Pulse rate	0.039
55	Hip circumference	-0.038
56	Rheumatoid factor	0.036
57	Direct bilirubin	-0.035
58	Platelet distribution width	0.034
59	Mean platelet volume	-0.034
60	Haemoglobin concentration	-0.032
61	Creatinine	0.029

Healthy women

Rank	Biomarker	Pearson correlation coefficient
1	Forced expiratory volume in 1s/height	-0.441
2	Cystatin C	0.404
3	Forced vital capacity/height	-0.381
4	Systolic blood pressure	0.373
5	Low density lipoprotein cholesterol	0.359
6	HbA1c	0.342
7	Apolipoprotein B	0.336
8	Alkaline Phosphatase	0.328
9	Hand grip strength/height	-0.318
10	Urea	0.317
11	Insulin-like growth factor 1	-0.309
12	Reaction time test	-0.308
13	Triglycerides	0.235
14	Heel bone density	-0.233
15	Sitting height	-0.217
16	Aspartate aminotransferase	0.200
17	Urate	0.192
18	Haemoglobin concentration	0.185
19	Metabolic rate	-0.184
20	Blood glucose	0.182
21	Calcium	0.179
22	Body fat-free mass	-0.178
23	Standing height	-0.169
24	Pairs matching test	-0.159
25	Waist-hip ratio	0.153
26	Body fat percentage	0.151
27	Apolipoprotein A	0.148
28	Phosphate	0.144
29	Log C-Reactive Protein	0.138
30	Alanine aminotransferase	0.127
31	Total red blood cell count	0.127
32	Direct bilirubin	-0.127
33	Gamma Glutamyltransferase	0.126
34	Urinary sodium	-0.125
35	Urinary microalbumin	0.118
36	Waist circumference	0.108
37	Diastolic blood pressure	0.107
38	Testosterone	-0.103
39	High density lipoprotein cholesterol	0.102
40	Urinary creatinine	-0.092
41	Neutrophil count	-0.089
42	Vitamin D	0.087
43	Platelet crit	-0.084
44	Body fat mass	0.062
45	Mean platelet volume	-0.060
46	Mean spherical cell volume	0.060
47	Mean corpuscular volume	0.058
48	Sex hormone-binding globulin	-0.056
49	Lymphocyte count	0.055
50	Body mass index	0.054
51	Total white blood cell count	-0.054
52	Albumin	-0.053
53	Pulse rate	0.052
54	Lipoprotein (a)	0.049
55	Total protein	-0.043
56	Platelet count	-0.043
57	Total bilirubin	-0.040
58	Mean reticulocyte volume	0.039
59	Basophil count	-0.039
60	Creatinine	0.038
61	Platelet distribution width	0.035

62	Lymphocyte count	-0.026	62	Rheumatoid factor	0.031
63	Total bilirubin	-0.018	63	Weight	-0.023
64	Body mass index	-0.018	64	Monocyte count	0.021
65	Urinary potassium	0.013	65	Hip circumference	0.021
66	Testosterone	-0.010	66	Urinary potassium	0.013
67	Immature reticulocyte fraction	0.007	67	Eosinophil count	-0.012
68	Urate	0.006	68	Immature reticulocyte fraction	-0.009
69	Triglycerides	0.006	69	Reticulocyte count	0.007
70	Gamma Glutamyltransferase	-0.003	70	Red blood cell distribution width	0.007
71	Lipoprotein (a)	-0.002	71	Mean corpuscular haemoglobin concentration	0.006
72	Eosinophil count	0.001	72	HLS reticulocyte count	-0.002

Figure 3.2.1: Flowchart of selection of study population, before population stratification

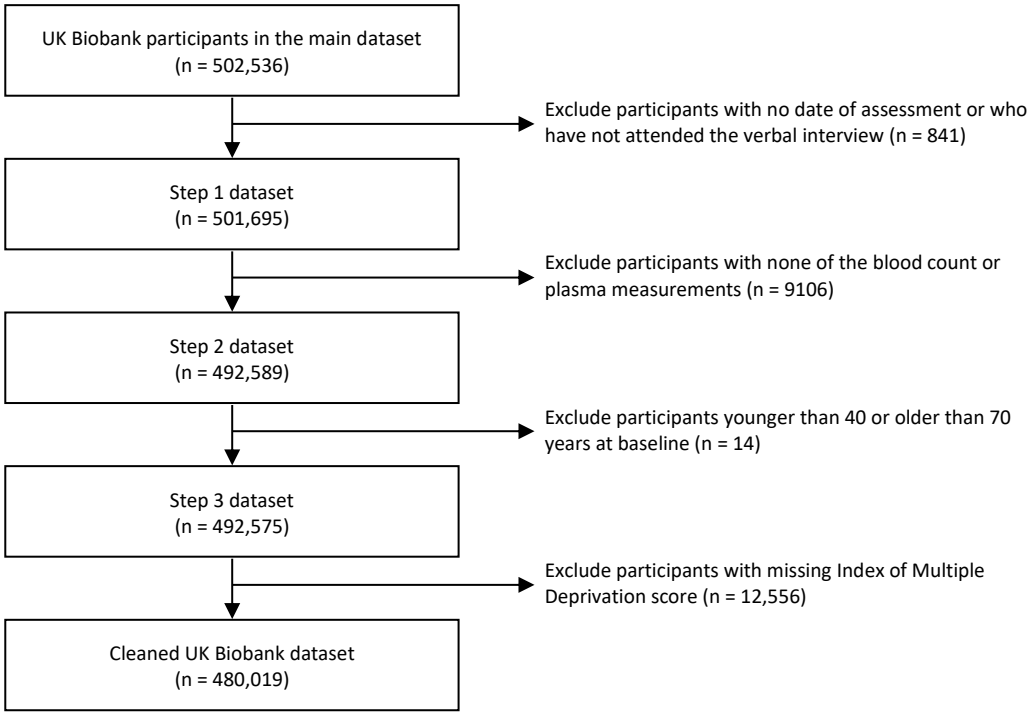
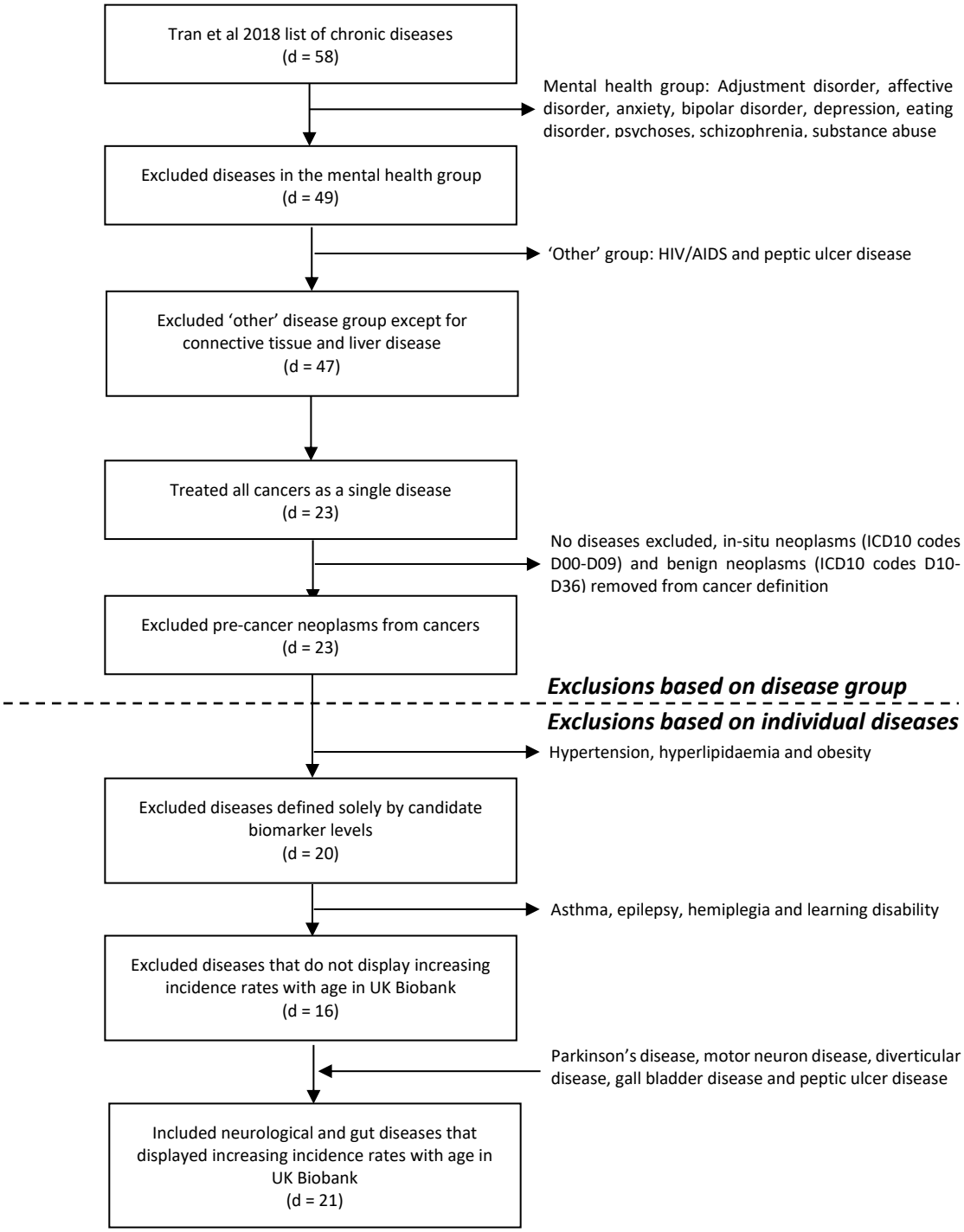


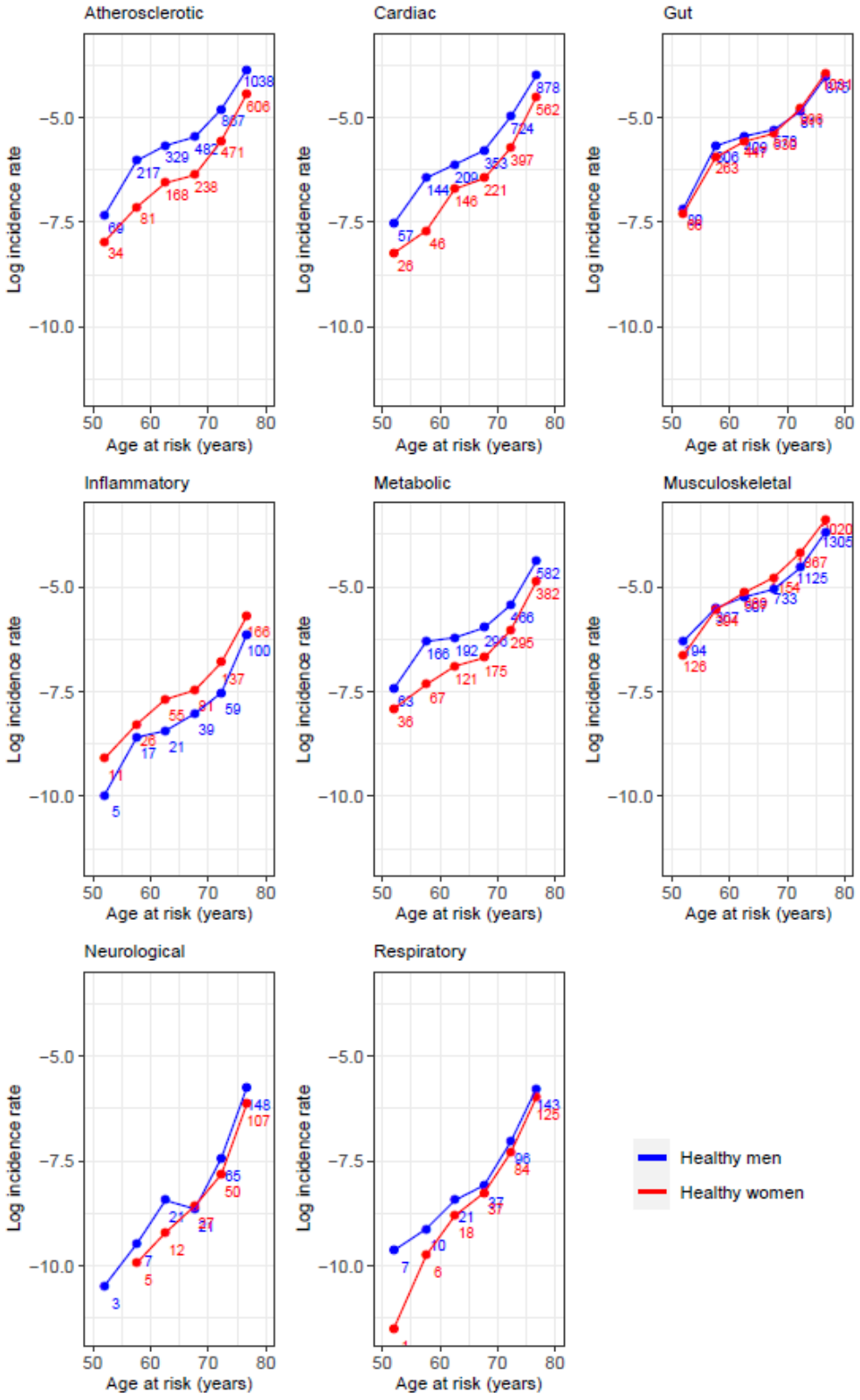
Figure 3.5.1: Flowchart of the age-related chronic disease selection process for this thesis



All steps in this selection process were reviewed by a clinician. The final list of diseases used in this thesis is: cardiac arrhythmia, chronic kidney disease, diabetes mellitus, heart failure, ischaemic heart disease, peripheral arterial disease, arthritis, rheumatoid arthritis, osteoporosis, gout, dementia, stroke/transient ischaemic attack, chronic obstructive pulmonary disease, connective tissue disease, liver disease, peptic ulcer disease, gall bladder disease, diverticular disease, Parkinson's disease, motor neuron disease and malignant cancer (21 diseases).

Figure 3.6.1: Log incidence rates of diseases in each disease group within the healthy subpopulation, by 5-year groups of (A) age at risk and (B) age at baseline

(A) By age at risk



(B) By age at baseline

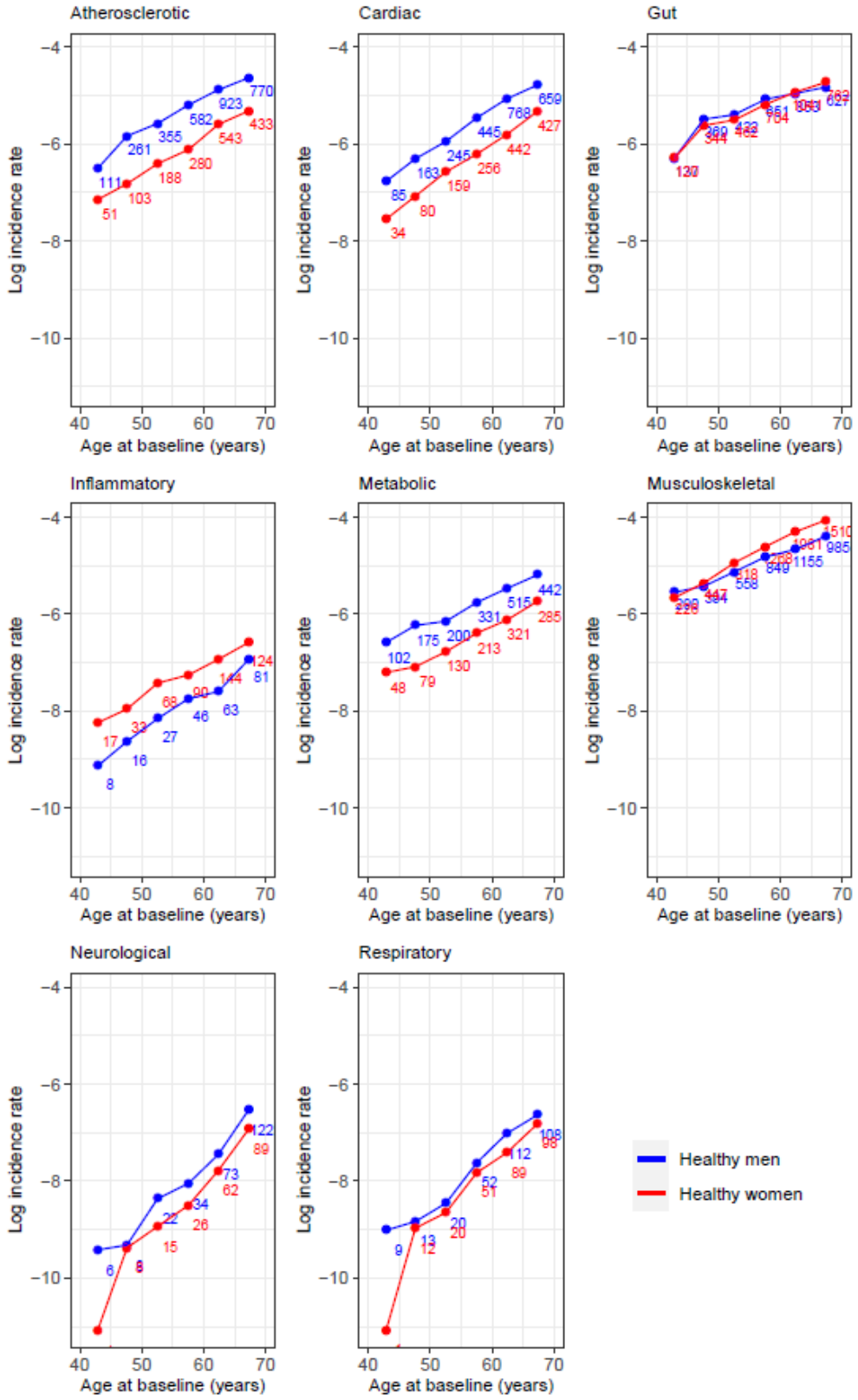
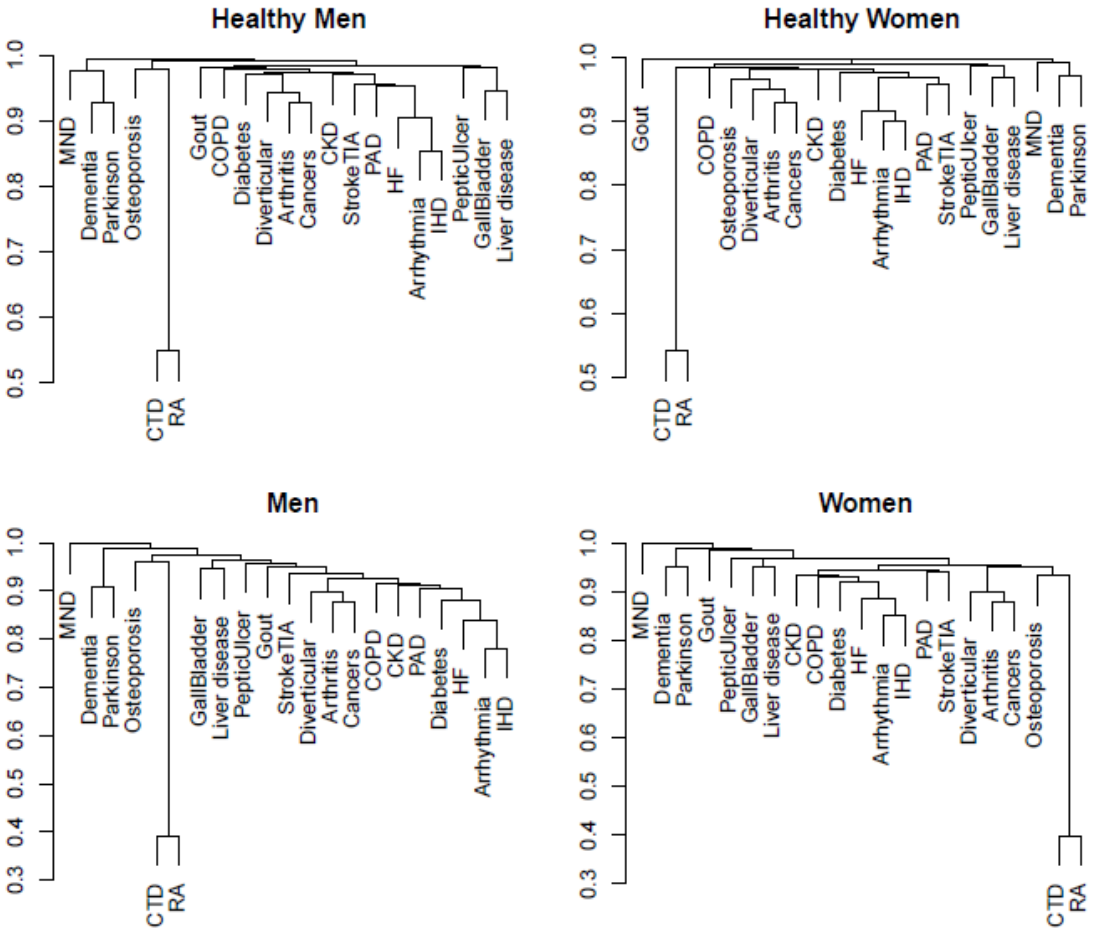


Figure 3.6.2: Dendrograms of patterns of disease co-occurrence for the 21 in-scope diseases for the healthy subpopulation vs whole population and men vs women



CTD: connective tissue disease, RA: rheumatoid arthritis, IHD: ischaemic heart disease, PAD: peripheral arterial disease, HF: heart failure; MND: motor neuron disease

These disease patterns were based on results from hierarchical clustering models. The y-axis denotes the level of similarity between the constituent diseases in a cluster. It ranges from 0 (all diseases are too dissimilar to form clusters) to 1 (all diseases are treated as similar enough to form one cluster)

Figure 3.6.3: Venn diagram of the numbers of participants with diseases diagnosed prior to baseline or during follow up in the three most prevalent groups, in the healthy subpopulation

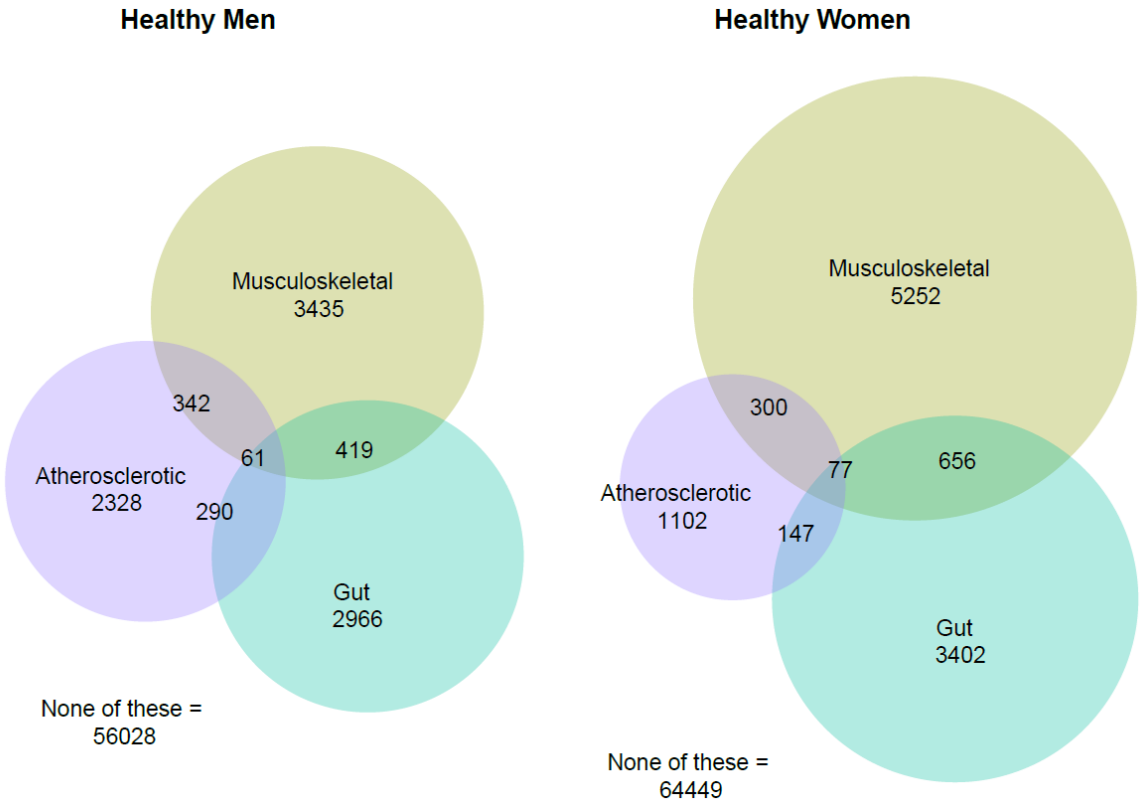


Figure 3.8.1: Assessment of the need for stratification of healthy never vs healthy ex smokers: biomarker-age trends for the lung function biomarkers and systolic blood pressure, by sex

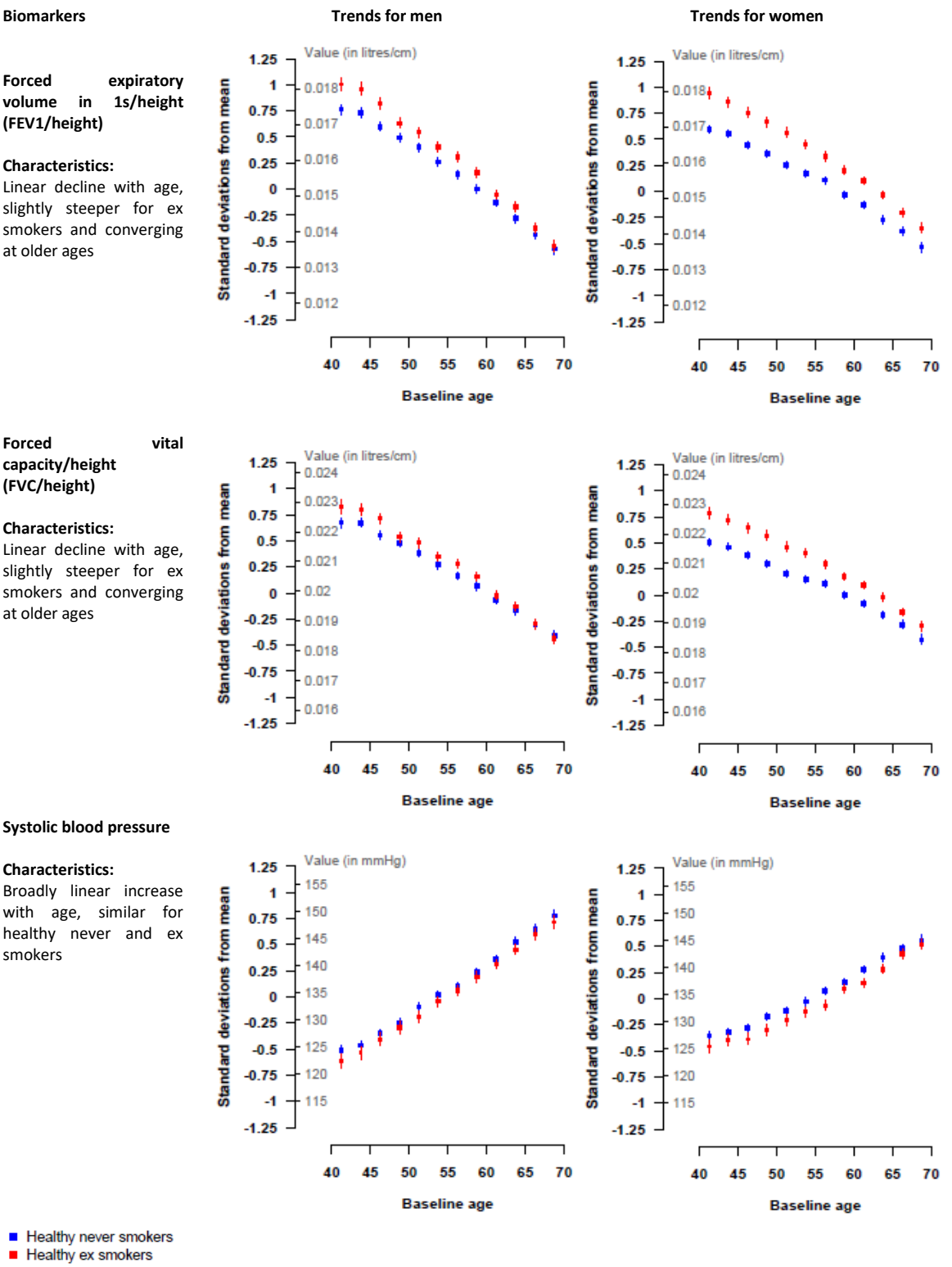
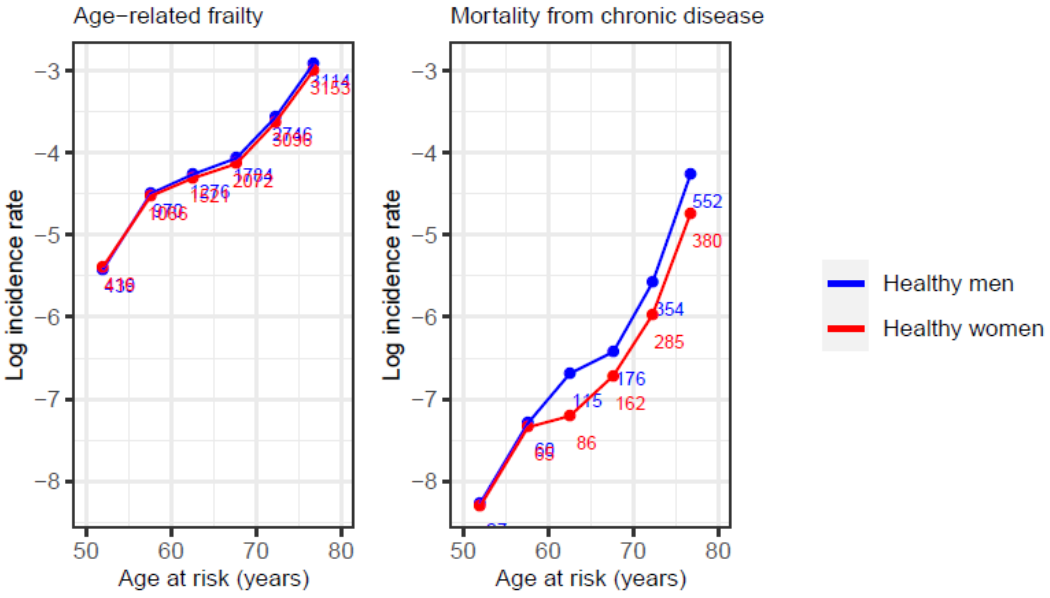


Figure 3.9.1: Log incidence rates of age-related frailty and mortality from chronic disease within the healthy subpopulation, by 5-year groups of chronological (A) age at risk and (B) age at baseline

(A) By age at risk



(B) By age at baseline

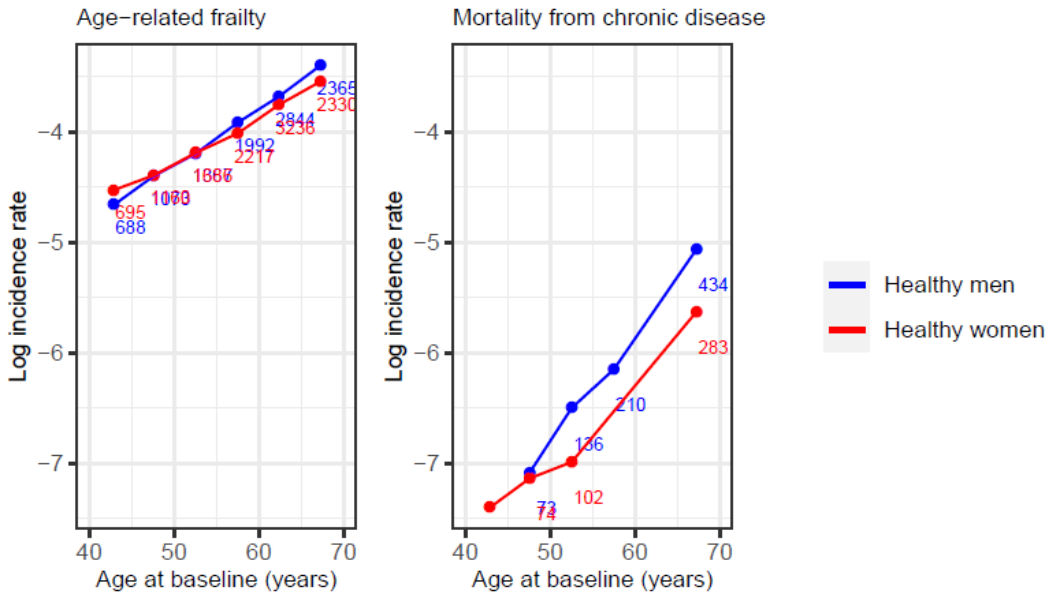
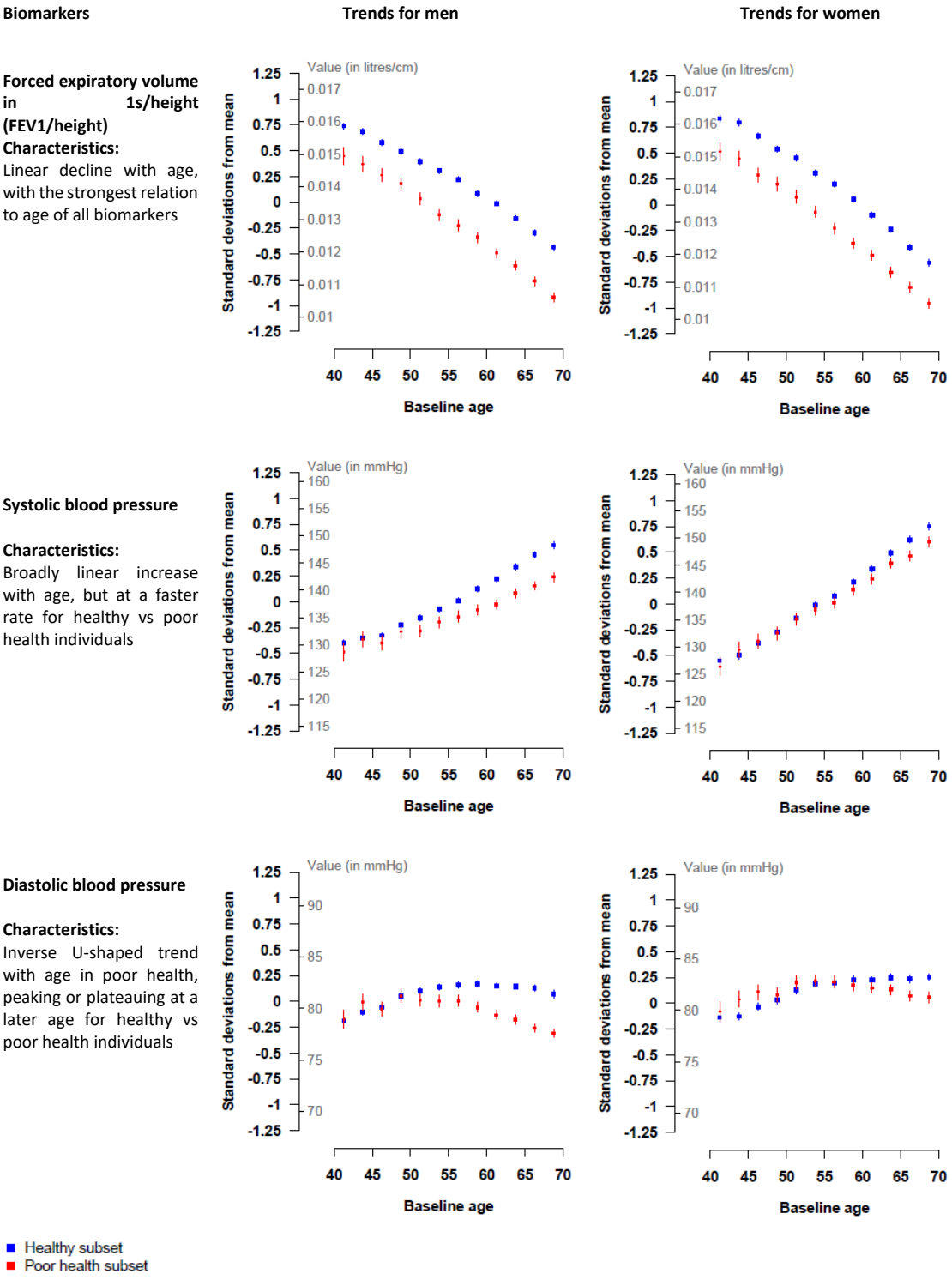
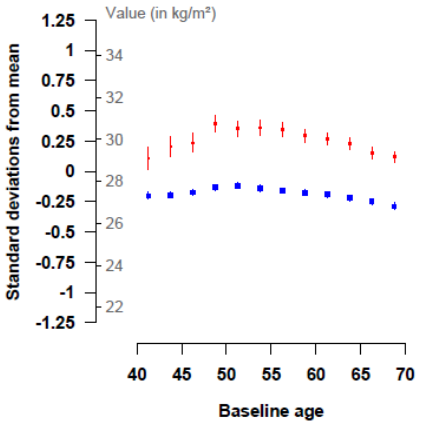
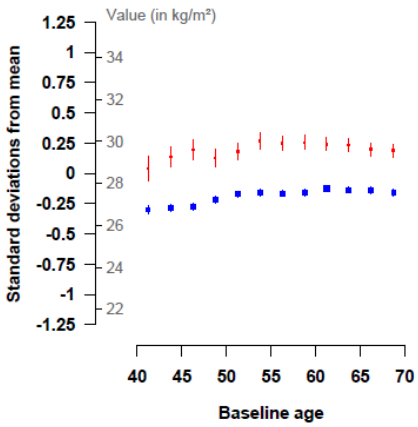
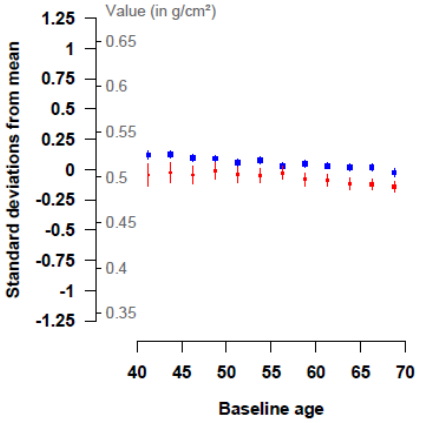
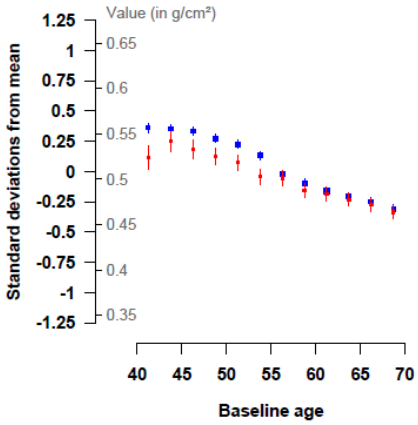
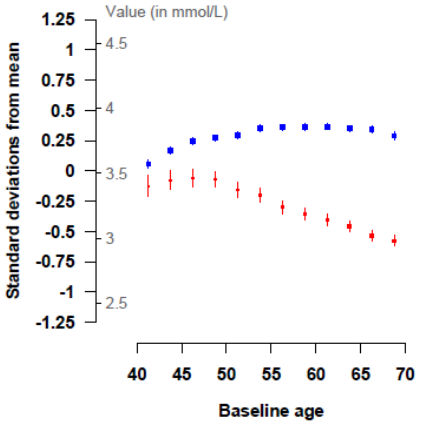
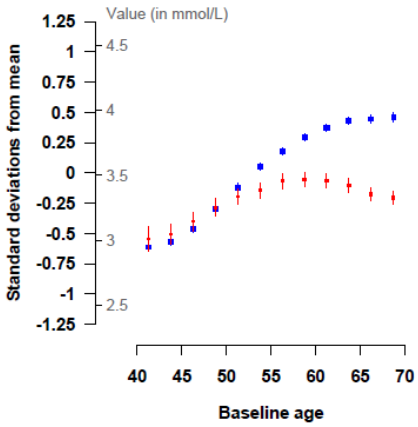


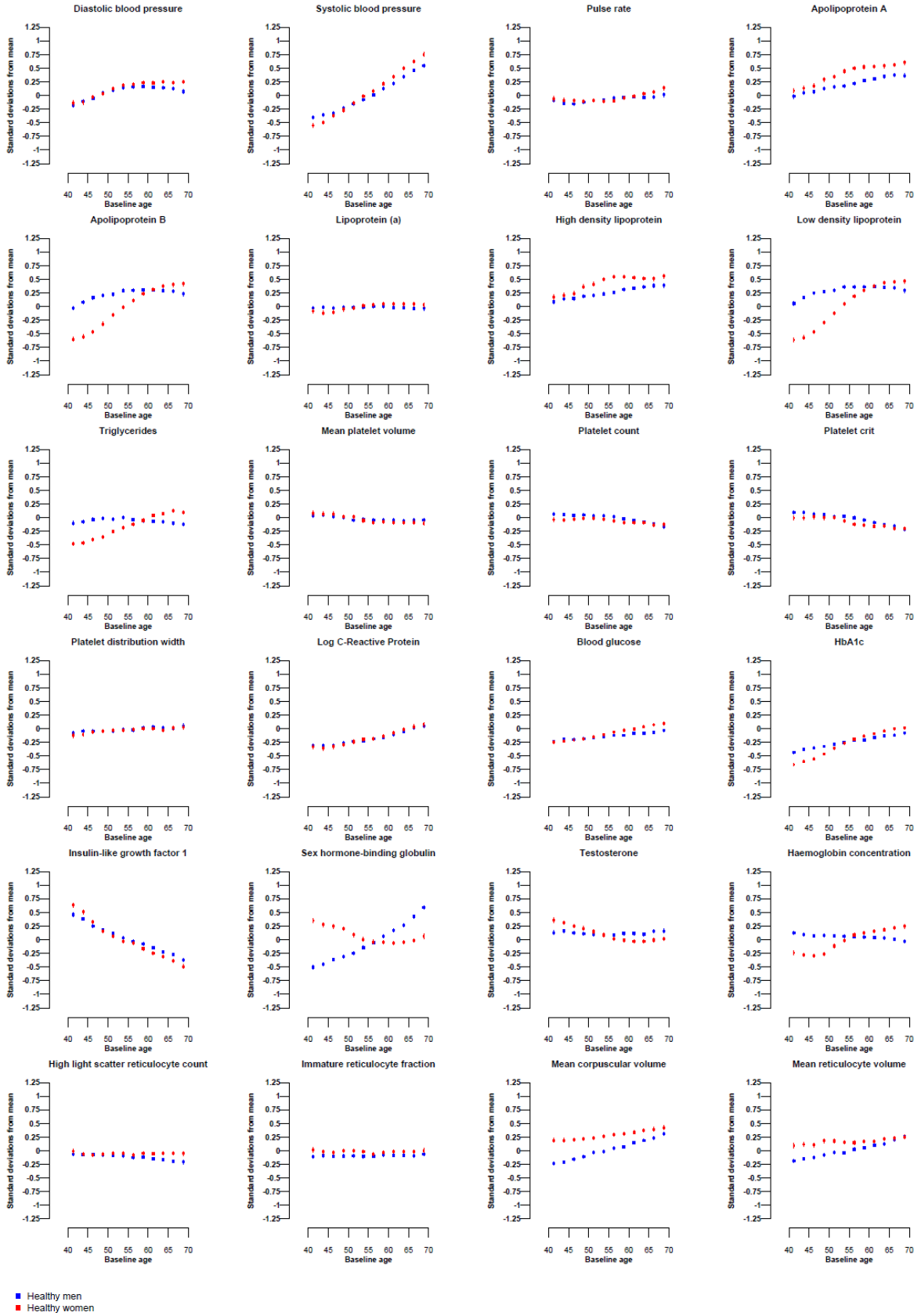
Figure 3.10.1: Biomarker-age trends (means and standard errors for each 2.5-year age group) for the healthy and poor health subpopulations and descriptions of their characteristics, for six selected biomarkers and by sex

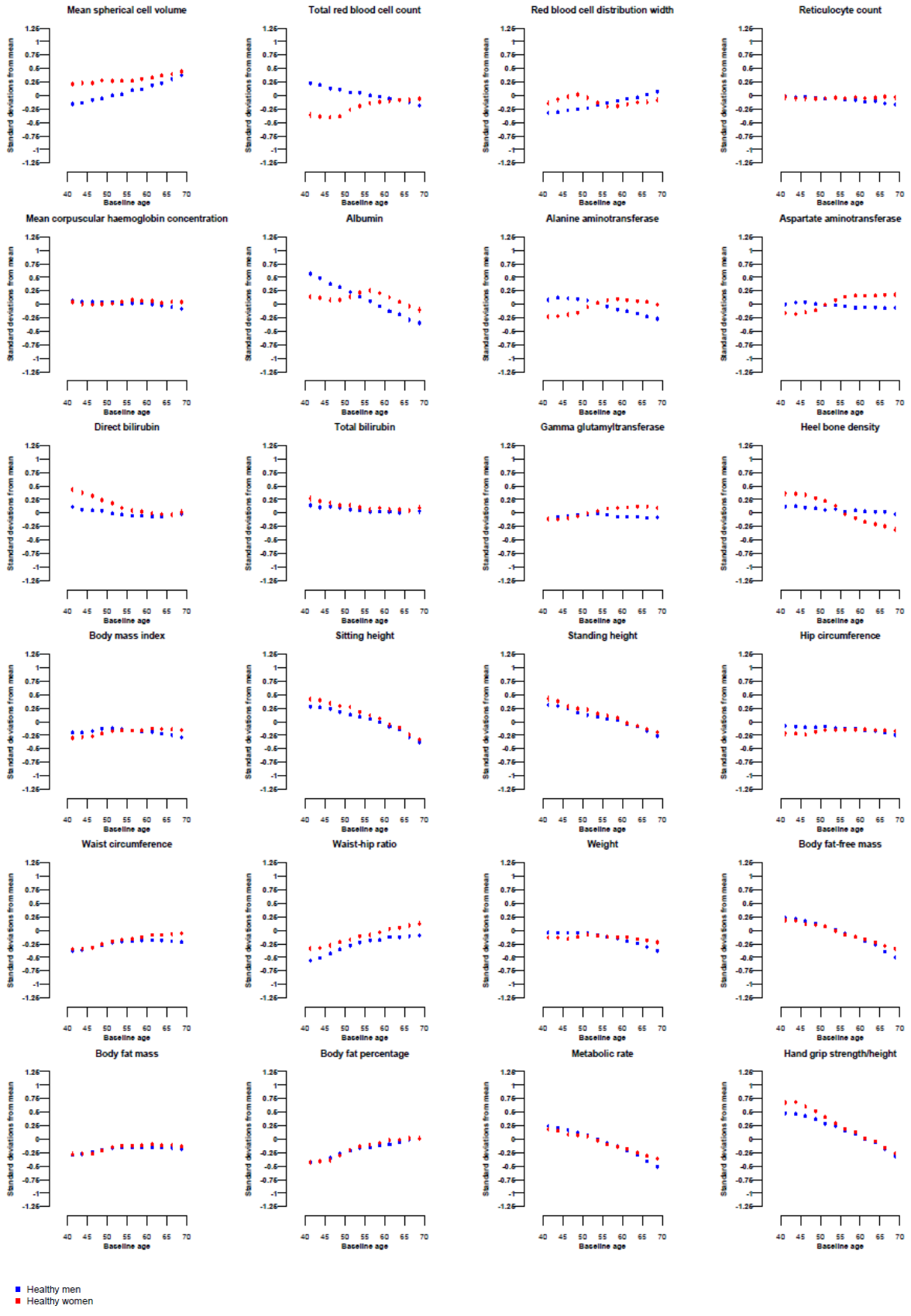


Biomarkers	Trends for men	Trends for women
Body mass index (BMI) **Characteristics:** Apparent differences in trends by prior health status: inverse U-shaped trend with age for poor health men, but broadly flat for healthy men		
Heel bone density **Characteristics:** No apparent difference in the level or trend of the biomarker with age between healthy and poor health individuals. Apparent differences in trends between men and women: a steeper decline with age is seen in women.		
Low density lipoprotein cholesterol **Characteristics:** Apparent differences in trends and turning points, by prior health status and sex: levels in poor health individuals decline, and diverge from those of healthy individuals, at younger ages for men compared to women.		

Healthy subset
Poor health subset

Figure 3.10.2: Biomarker-age trends for the 72 candidate biomarkers, for healthy men vs healthy women




Mean spherical cell volume
Total red blood cell count
Red blood cell distribution width
Reticulocyte count
Mean corpuscular haemoglobin concentration
Albumin
Alanine aminotransferase
Aspartate aminotransferase
Direct bilirubin
Total bilirubin
Gamma glutamyltransferase
Heel bone density
Body mass index
Sitting height
Standing height
Hip circumference
Waist circumference
Waist-hip ratio
Weight
Body fat-free mass
Body fat mass
Body fat percentage
Metabolic rate
Hand grip strength/height
Standard deviations from mean
Baseline age
Healthy men
Healthy women

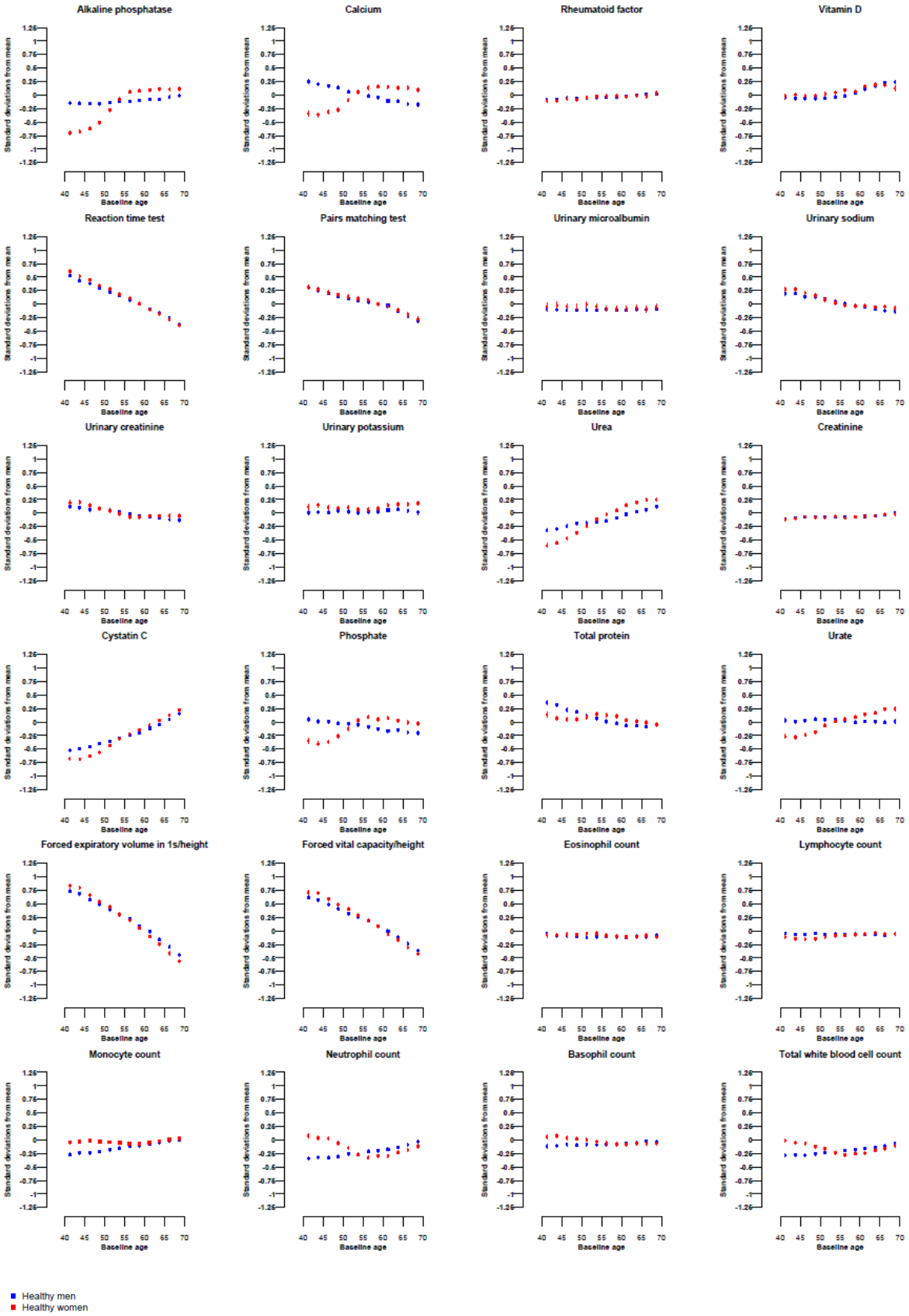
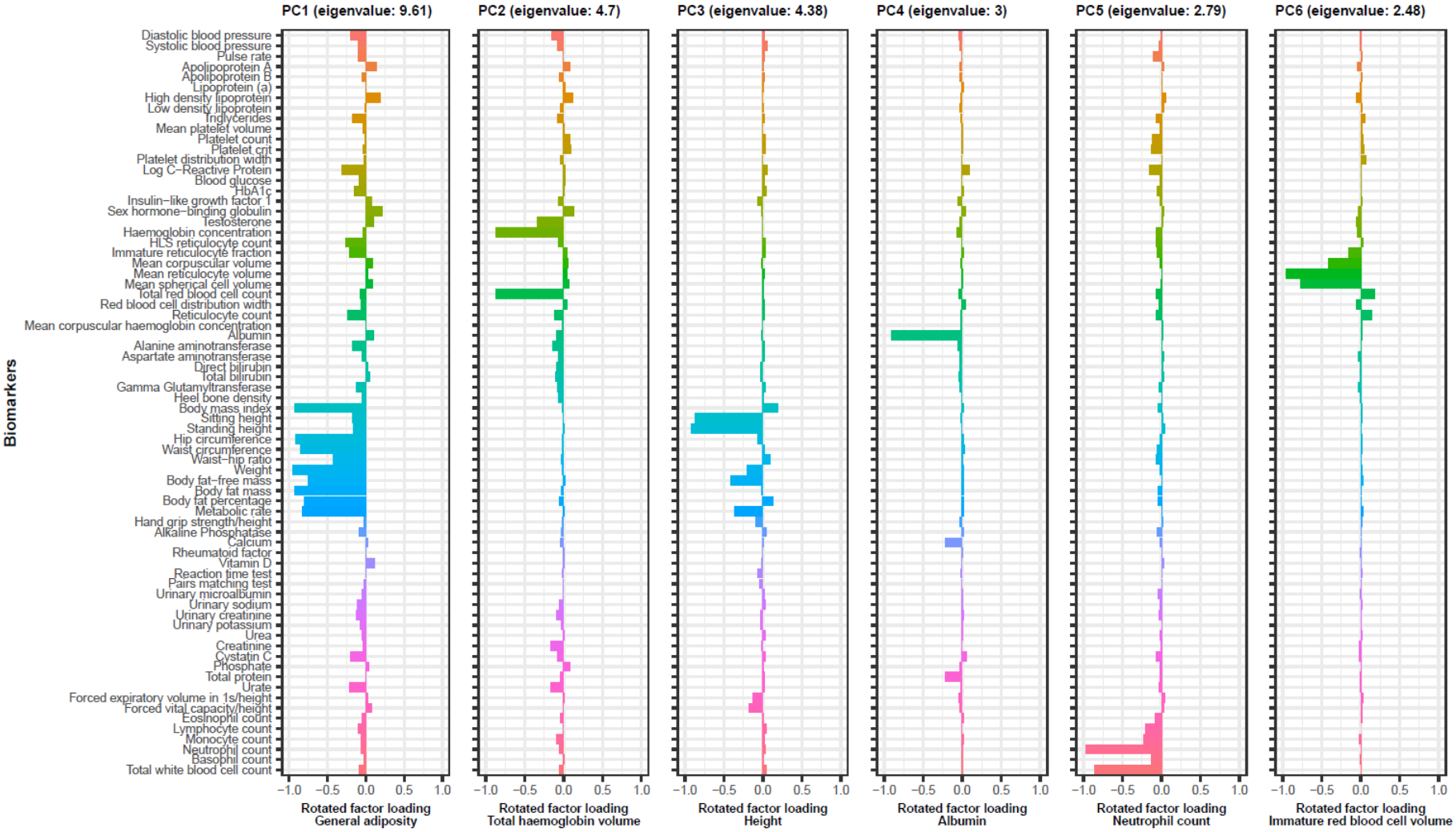
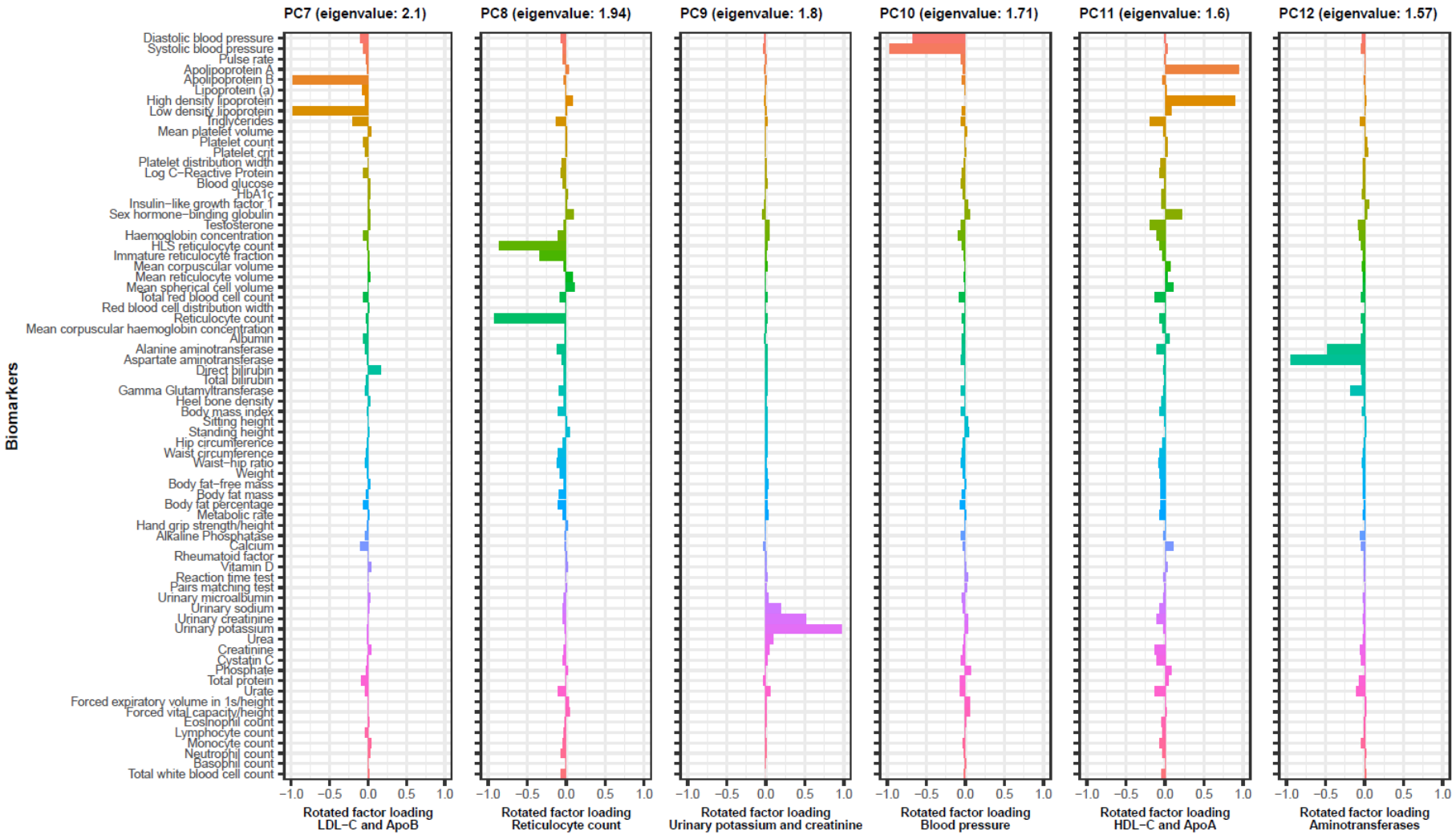
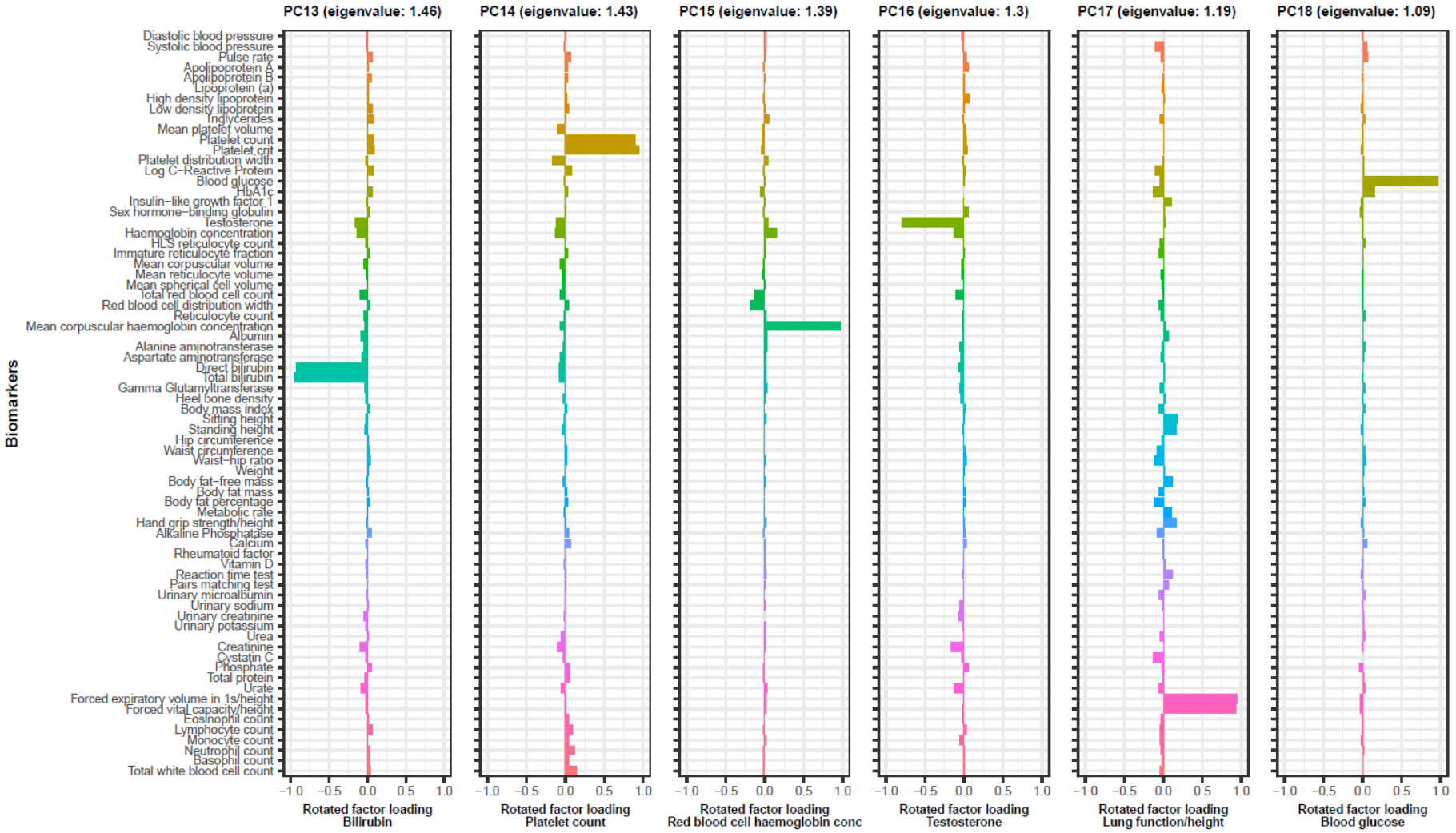
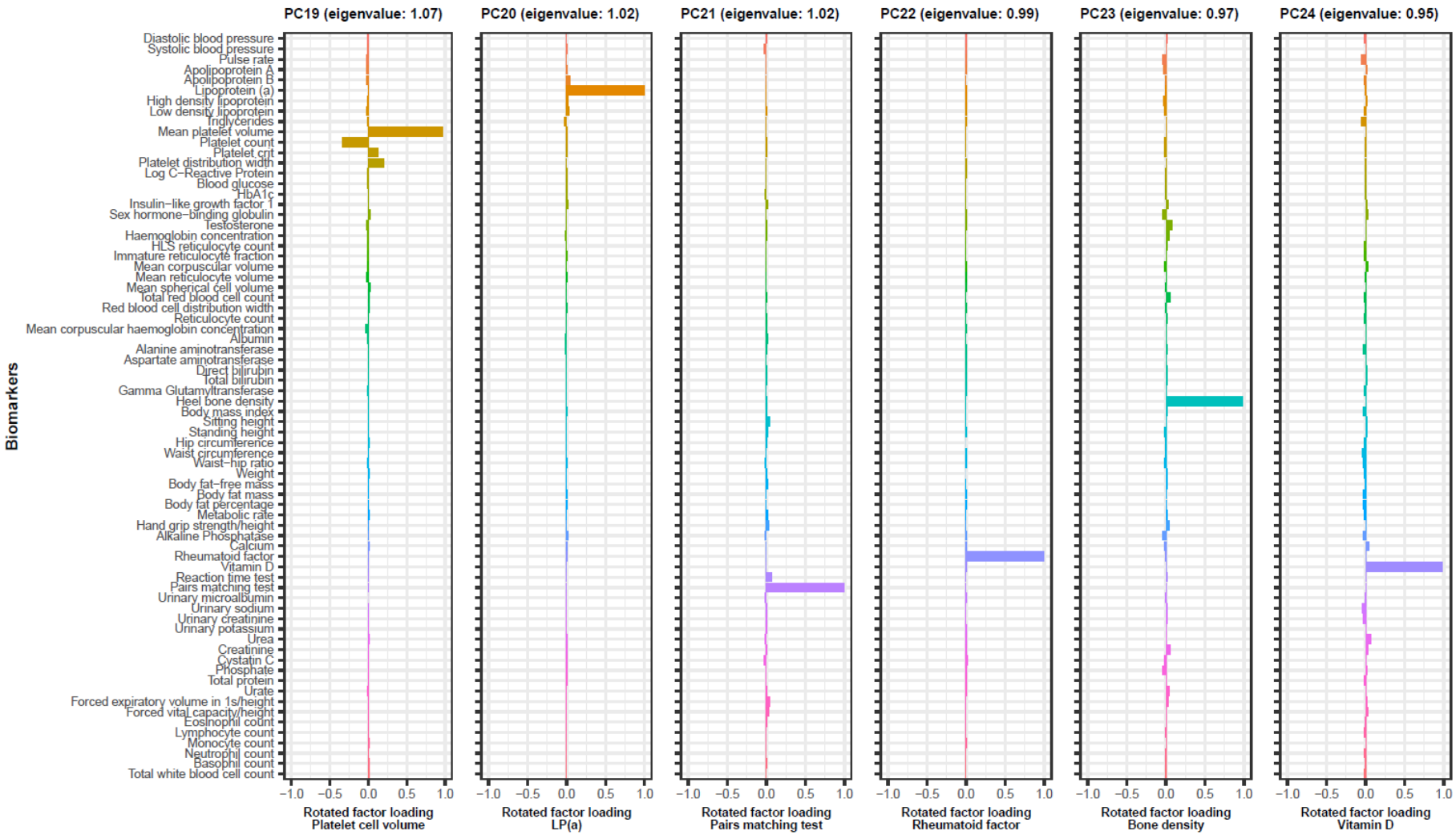

Alkaline phosphatase
Calcium
Rheumatoid factor
Vitamin D
Reaction time test
Pairs matching test
Urinary microalbumin
Urinary sodium
Urinary creatinine
Urinary potassium
Urea
Creatinine
Cystatin C
Phosphate
Total protein
Urate
Forced expiratory volume in 1s/height
Forced vital capacity/height
Eosinophil count
Lymphocyte count
Monocyte count
Neutrophil count
Basophil count
Total white blood cell count
Standard deviations from mean
Baseline age
Healthy men
Healthy women

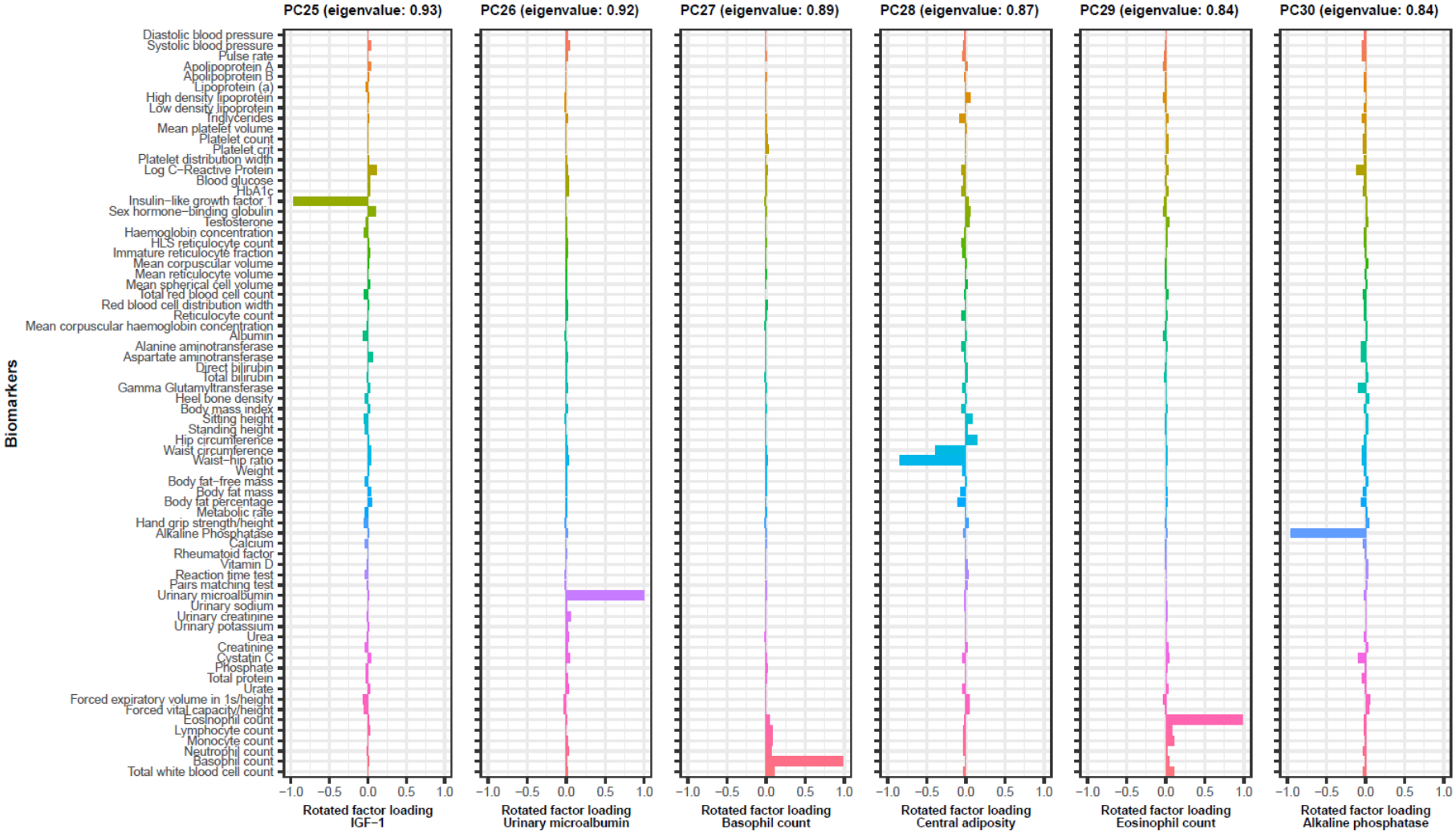
Figure 3.10.3: Characterisation of the first 54 biomarker principal components




PC7 (eigenvalue: 2.1)
PC8 (eigenvalue: 1.94)
PC9 (eigenvalue: 1.8)
PC10 (eigenvalue: 1.71)
PC11 (eigenvalue: 1.6)
PC12 (eigenvalue: 1.57)
Biomarkers
Diastolic blood pressure
Systolic blood pressure
Pulse rate
Apolipoprotein A
Apolipoprotein B
Lipoprotein (a)
High density lipoprotein
Low density lipoprotein
Triglycerides
Mean platelet volume
Platelet count
Platelet crit
Platelet distribution width
Log C-Reactive Protein
Blood glucose
HbA1c
Insulin-like growth factor 1
Sex hormone-binding globulin
Testosterone
Haemoglobin concentration
HLS reticulocyte count
Immature reticulocyte fraction
Mean corpuscular volume
Mean reticulocyte volume
Mean spherical cell volume
Total red blood cell count
Red blood cell distribution width
Reticulocyte count
Mean corpuscular haemoglobin concentration
Albumin
Alanine aminotransferase
Aspartate aminotransferase
Direct bilirubin
Total bilirubin
Gamma Glutamyltransferase
Heel bone density
Body mass index
Sitting height
Standing height
Hip circumference
Waist circumference
Waist-hip ratio
Weight
Body fat-free mass
Body fat mass
Body fat percentage
Metabolic rate
Hand grip strength/height
Alkaline Phosphatase
Calcium
Rheumatoid factor
Vitamin D
Reaction time test
Pairs matching test
Urinary microalbumin
Urinary sodium
Urinary creatinine
Urinary potassium
Urea
Creatinine
Cystatin C
Phosphate
Total protein
Urate
Forced expiratory volume in 1s/height
Forced vital capacity/height
Eosinophil count
Lymphocyte count
Monocyte count
Neutrophil count
Basophil count
Total white blood cell count
−1.0 −0.5 0.0 0.5 1.0
Rotated factor loading
LDL-C and ApoB
Rotated factor loading
Reticulocyte count
Rotated factor loading
Urinary potassium and creatinine
Rotated factor loading
Blood pressure
Rotated factor loading
HDL-C and ApoA
Rotated factor loading
Aminotransferases


PC13 (eigenvalue: 1.46)
PC14 (eigenvalue: 1.43)
PC15 (eigenvalue: 1.39)
PC16 (eigenvalue: 1.3)
PC17 (eigenvalue: 1.19)
PC18 (eigenvalue: 1.09)
Biomarkers
Diastolic blood pressure
Systolic blood pressure
Pulse rate
Apolipoprotein A
Apolipoprotein B
Lipoprotein (a)
High density lipoprotein
Low density lipoprotein
Triglycerides
Mean platelet volume
Platelet count
Platelet crit
Platelet distribution width
Log C-Reactive Protein
Blood glucose
HbA1c
Insulin-like growth factor 1
Sex hormone-binding globulin
Testosterone
Haemoglobin concentration
HLS reticulocyte count
Immature reticulocyte fraction
Mean corpuscular volume
Mean reticulocyte volume
Mean spherical cell volume
Total red blood cell count
Red blood cell distribution width
Reticulocyte count
Mean corpuscular haemoglobin concentration
Albumin
Alanine aminotransferase
Aspartate aminotransferase
Direct bilirubin
Total bilirubin
Gamma Glutamyltransferase
Heel bone density
Body mass index
Sitting height
Standing height
Hip circumference
Waist circumference
Waist-hip ratio
Weight
Body fat-free mass
Body fat mass
Body fat percentage
Metabolic rate
Hand grip strength/height
Alkaline Phosphatase
Calcium
Rheumatoid factor
Vitamin D
Reaction time test
Pairs matching test
Urinary microalbumin
Urinary sodium
Urinary creatinine
Urinary potassium
Urea
Creatinine
Cystatin C
Phosphate
Total protein
Urate
Forced expiratory volume in 1s/height
Forced vital capacity/height
Eosinophil count
Lymphocyte count
Monocyte count
Neutrophil count
Basophil count
Total white blood cell count
−1.0 −0.5 0.0 0.5 1.0
Rotated factor loading
Bilirubin
Rotated factor loading
Platelet count
Rotated factor loading
Red blood cell haemoglobin conc
Rotated factor loading
Testosterone
Rotated factor loading
Lung function/height
Rotated factor loading
Blood glucose


PC19 (eigenvalue: 1.07)
PC20 (eigenvalue: 1.02)
PC21 (eigenvalue: 1.02)
PC22 (eigenvalue: 0.99)
PC23 (eigenvalue: 0.97)
PC24 (eigenvalue: 0.95)
Biomarkers
Diastolic blood pressure
Systolic blood pressure
Pulse rate
Apolipoprotein A
Apolipoprotein B
Lipoprotein (a)
High density lipoprotein
Low density lipoprotein
Triglycerides
Mean platelet volume
Platelet count
Platelet crit
Platelet distribution width
Log C−Reactive Protein
Blood glucose
HbA1c
Insulin−like growth factor 1
Sex hormone−binding globulin
Testosterone
Haemoglobin concentration
HLS reticulocyte count
Immature reticulocyte fraction
Mean corpuscular volume
Mean reticulocyte volume
Mean spherical cell volume
Total red blood cell count
Red blood cell distribution width
Reticulocyte count
Mean corpuscular haemoglobin concentration
Albumin
Alanine aminotransferase
Aspartate aminotransferase
Direct bilirubin
Total bilirubin
Gamma Glutamyltransferase
Heel bone density
Body mass index
Sitting height
Standing height
Hip circumference
Waist circumference
Waist−hip ratio
Weight
Body fat−free mass
Body fat mass
Body fat percentage
Metabolic rate
Hand grip strength/height
Alkaline Phosphatase
Calcium
Rheumatoid factor
Vitamin D
Reaction time test
Pairs matching test
Urinary microalbumin
Urinary sodium
Urinary creatinine
Urinary potassium
Urea
Creatinine
Cystatin C
Phosphate
Total protein
Urate
Forced expiratory volume in 1s/height
Forced vital capacity/height
Eosinophil count
Lymphocyte count
Monocyte count
Neutrophil count
Basophil count
Total white blood cell count
−1.0 −0.5 0.0 0.5 1.0
Rotated factor loading
Platelet cell volume
Rotated factor loading
LP(a)
Rotated factor loading
Pairs matching test
Rotated factor loading
Rheumatoid factor
Rotated factor loading
Bone density
Rotated factor loading
Vitamin D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Systolic blood pressure
Pulse rate
Apolipoprotein A
Apolipoprotein B
Lipoprotein (a)
High density lipoprotein
Low density lipoprotein
Triglycerides
Mean platelet volume
Platelet count
Platelet crit
Platelet distribution width
Log C-Reactive Protein
Blood glucose
HbA1c
Insulin-like growth factor 1
Sex hormone-binding globulin
Testosterone
Haemoglobin concentration
HLS reticulocyte count
Immature reticulocyte fraction
Mean corpuscular volume
Mean reticulocyte volume
Mean spherical cell volume
Total red blood cell count
Red blood cell distribution width
Reticulocyte count
Mean corpuscular haemoglobin concentration
Albumin
Alanine aminotransferase
Aspartate aminotransferase
Direct bilirubin
Total bilirubin
Gamma Glutamyltransferase
Heel bone density
Body mass index
Sitting height
Standing height
Hip circumference
Waist circumference
Waist-hip ratio
Weight
Body fat-free mass
Body fat mass
Body fat percentage
Metabolic rate
Hand grip strength/height
Alkaline Phosphatase
Calcium
Rheumatoid factor
Vitamin D
Reaction time test
Pairs matching test
Urinary microalbumin
Urinary sodium
Urinary creatinine
Urinary potassium
Urea
Creatinine
Cystatin C
Phosphate
Total protein
Urate
Forced expiratory volume in 1s/height
Forced vital capacity/height
Eosinophil count
Lymphocyte count
Monocyte count
Neutrophil count
Basophil count
Total white blood cell count
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IGF−1
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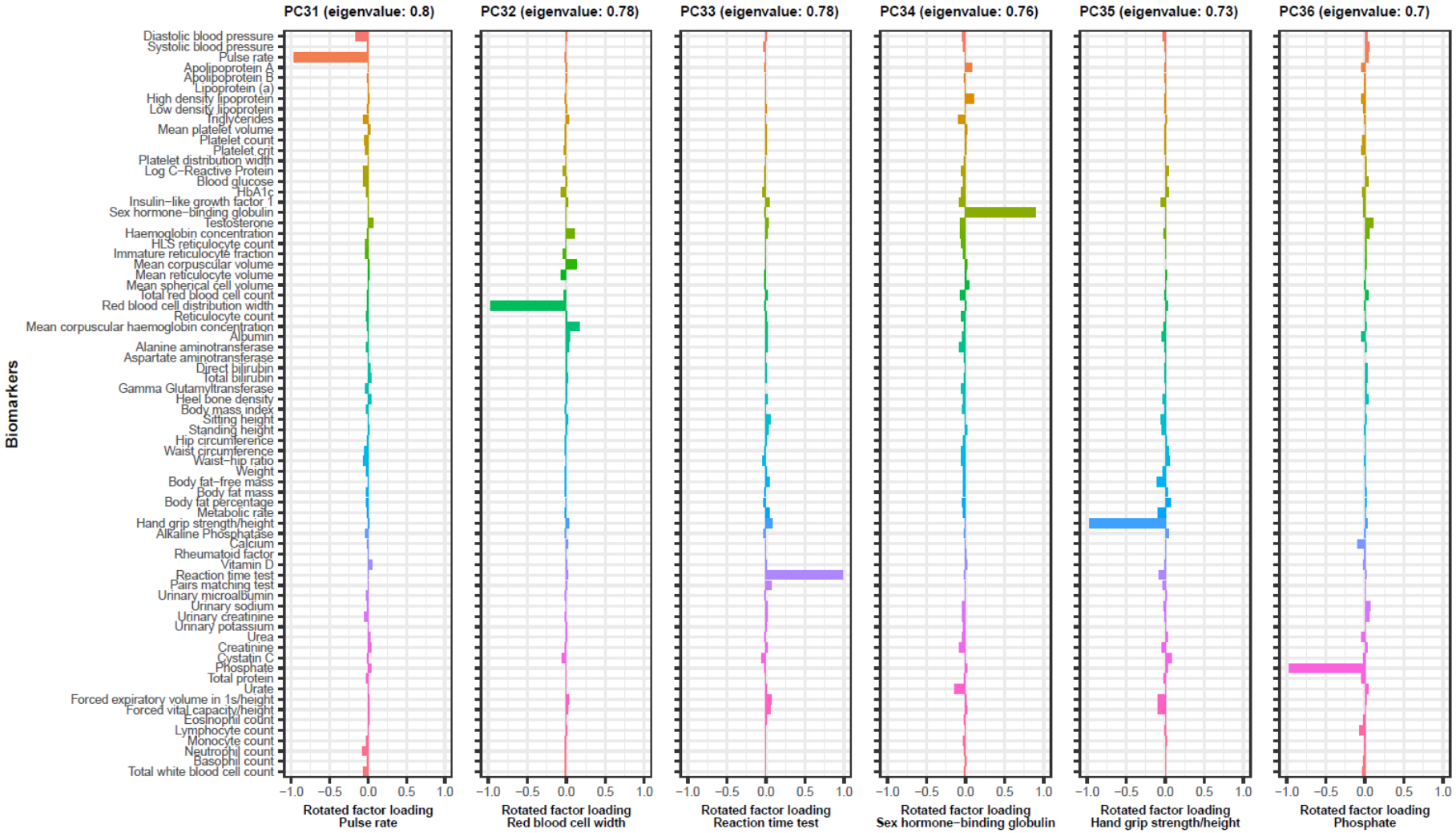
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Systolic blood pressure
Pulse rate
Apolipoprotein A
Apolipoprotein B
Lipoprotein (a)
High density lipoprotein
Low density lipoprotein
Triglycerides
Mean platelet volume
Platelet count
Platelet crit
Platelet distribution width
Log C-Reactive Protein
Blood glucose
HbA1c
Insulin-like growth factor 1
Sex hormone-binding globulin
Testosterone
Haemoglobin concentration
HLS reticulocyte count
Immature reticulocyte fraction
Mean corpuscular volume
Mean reticulocyte volume
Mean spherical cell volume
Total red blood cell count
Red blood cell distribution width
Reticulocyte count
Mean corpuscular haemoglobin concentration
Albumin
Alanine aminotransferase
Aspartate aminotransferase
Direct bilirubin
Total bilirubin
Gamma Glutamyltransferase
Heel bone density
Body mass index
Sitting height
Standing height
Hip circumference
Waist circumference
Waist-hip ratio
Weight
Body fat-free mass
Body fat mass
Body fat percentage
Metabolic rate
Hand grip strength/height
Alkaline Phosphatase
Calcium
Rheumatoid factor
Vitamin D
Reaction time test
Pairs matching test
Urinary microalbumin
Urinary sodium
Urinary creatinine
Urinary potassium
Urea
Creatinine
Cystatin C
Phosphate
Total protein
Urate
Forced expiratory volume in 1s/height
Forced vital capacity/height
Eosinophil count
Lymphocyte count
Monocyte count
Neutrophil count
Basophil count
Total white blood cell count
−1.0 −0.5 0.0 0.5 1.0
Rotated factor loading
Pulse rate
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Reaction time test
Rotated factor loading
Sex hormone-binding globulin
Rotated factor loading
Hand grip strength/height
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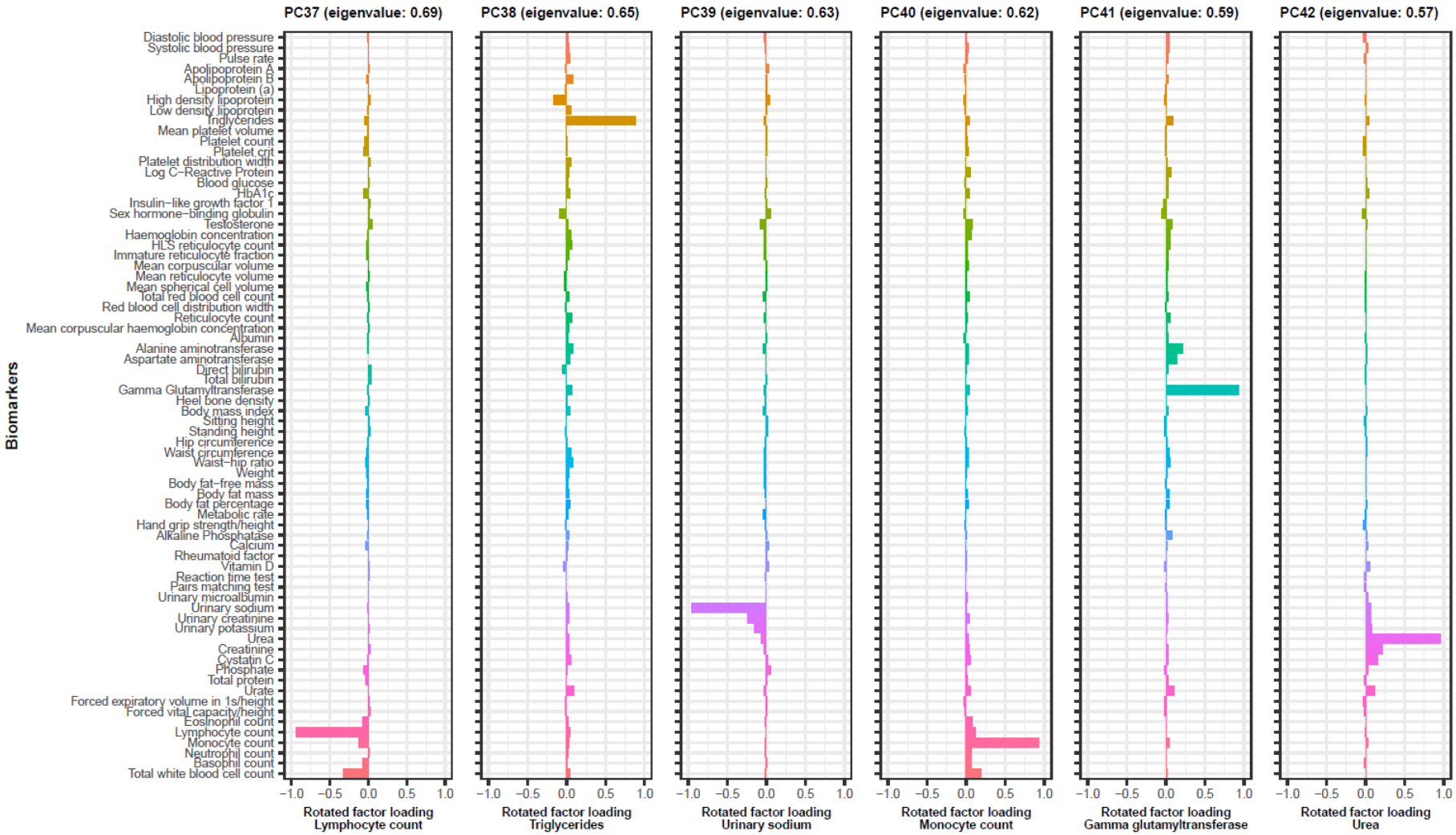
Phosphate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Systolic blood pressure
Pulse rate
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Apolipoprotein B
Lipoprotein (a)
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Low density lipoprotein
Triglycerides
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Platelet count
Platelet crit
Platelet distribution width
Log C-Reactive Protein
Blood glucose
HbA1c
Insulin-like growth factor 1
Sex hormone-binding globulin
Testosterone
Haemoglobin concentration
HLS reticulocyte count
Immature reticulocyte fraction
Mean corpuscular volume
Mean reticulocyte volume
Mean spherical cell volume
Total red blood cell count
Red blood cell distribution width
Reticulocyte count
Mean corpuscular haemoglobin concentration
Albumin
Alanine aminotransferase
Aspartate aminotransferase
Direct bilirubin
Total bilirubin
Gamma Glutamyltransferase
Heel bone density
Body mass index
Sitting height
Standing height
Hip circumference
Waist circumference
Waist-hip ratio
Weight
Body fat-free mass
Body fat mass
Body fat percentage
Metabolic rate
Hand grip strength/height
Alkaline Phosphatase
Calcium
Rheumatoid factor
Vitamin D
Reaction time test
Pairs matching test
Urinary microalbumin
Urinary sodium
Urinary creatinine
Urinary potassium
Urea
Creatinine
Cystatin C
Phosphate
Total protein
Urate
Forced expiratory volume in 1s/height
Forced vital capacity/height
Eosinophil count
Lymphocyte count
Monocyte count
Neutrophil count
Basophil count
Total white blood cell count
−1.0 −0.5 0.0 0.5 1.0
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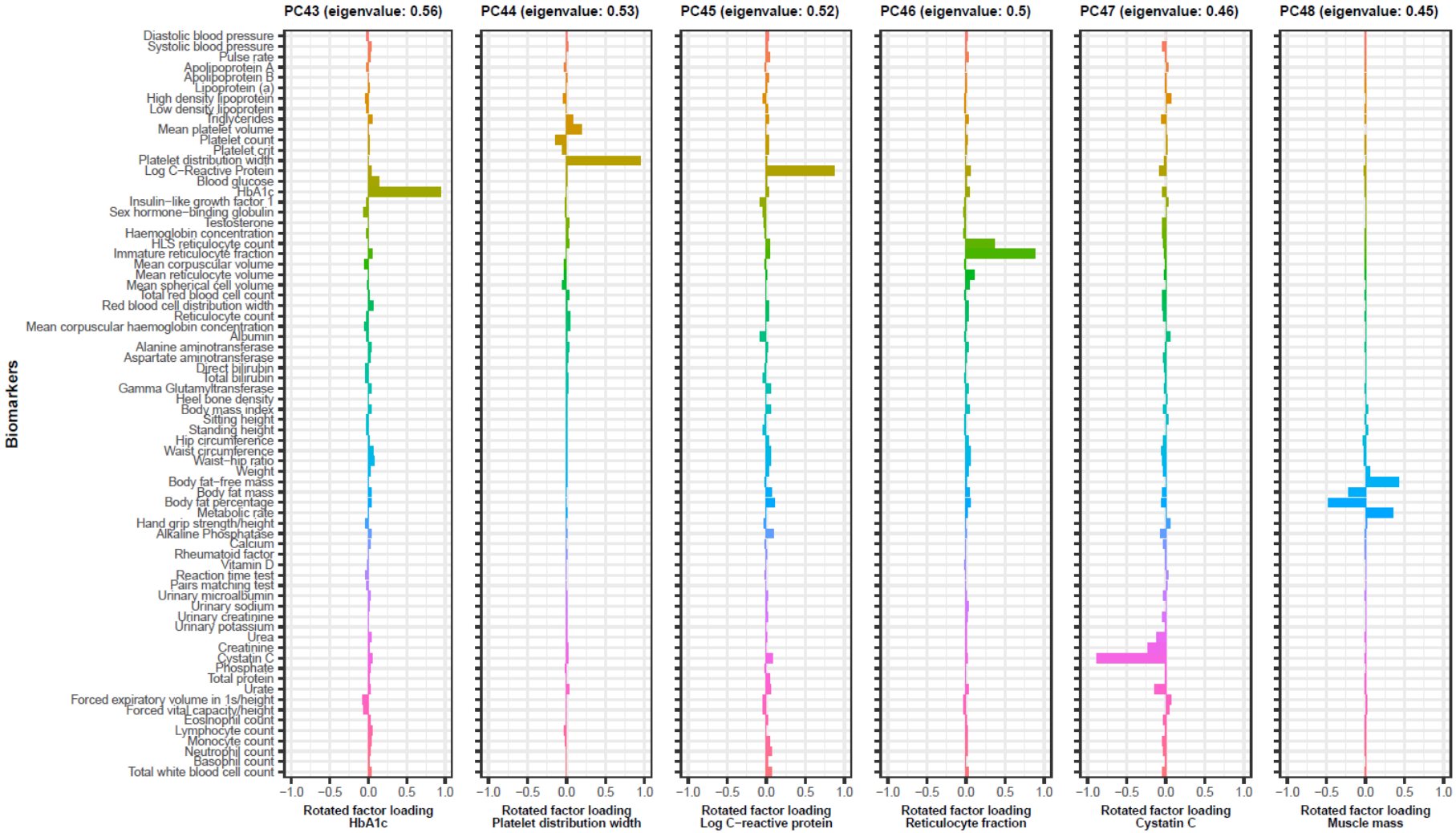
Urea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Systolic blood pressure
Pulse rate
Apolipoprotein A
Apolipoprotein B
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Platelet count
Platelet crit
Platelet distribution width
Log C−Reactive Protein
Blood glucose
HbA1c
Insulin−like growth factor 1
Sex hormone−binding globulin
Testosterone
Haemoglobin concentration
HLS reticulocyte count
Immature reticulocyte fraction
Mean corpuscular volume
Mean reticulocyte volume
Mean spherical cell volume
Total red blood cell count
Red blood cell distribution width
Reticulocyte count
Mean corpuscular haemoglobin concentration
Albumin
Alanine aminotransferase
Aspartate aminotransferase
Direct bilirubin
Total bilirubin
Gamma Glutamyltransferase
Heel bone density
Body mass index
Sitting height
Standing height
Hip circumference
Waist circumference
Waist−hip ratio
Weight
Body fat−free mass
Body fat mass
Body fat percentage
Metabolic rate
Hand grip strength/height
Alkaline Phosphatase
Calcium
Rheumatoid factor
Vitamin D
Reaction time test
Pairs matching test
Urinary microalbumin
Urinary sodium
Urinary creatinine
Urinary potassium
Urea
Creatinine
Cystatin C
Phosphate
Total protein
Urate
Forced expiratory volume in 1s/height
Forced vital capacity/height
Eosinophil count
Lymphocyte count
Monocyte count
Neutrophil count
Basophil count
Total white blood cell count
−1.0 −0.5 0.0 0.5 1.0
Rotated factor loading
HbA1c
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Rotated factor loading
Log C−reactive protein
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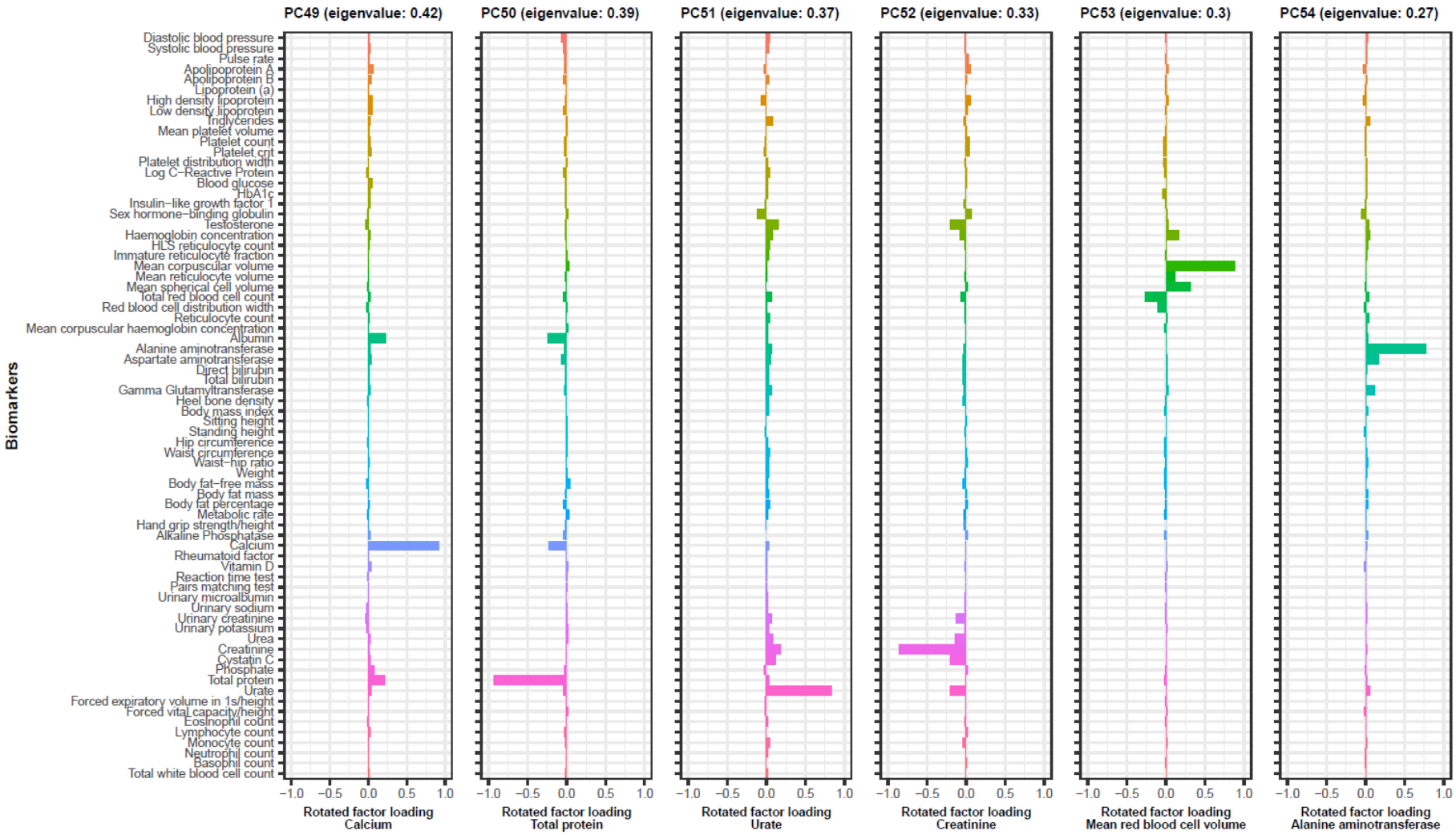
Reticulocyte fraction
Rotated factor loading
Cystatin C
Rotated factor loading
Muscle mass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Biomarkers
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Systolic blood pressure
Pulse rate
Apolipoprotein A
Apolipoprotein B
Lipoprotein (a)
High density lipoprotein
Low density lipoprotein
Triglycerides
Mean platelet volume
Platelet count
Platelet crit
Platelet distribution width
Log C−Reactive Protein
Blood glucose
HbA1c
Insulin−like growth factor 1
Sex hormone−binding globulin
Testosterone
Haemoglobin concentration
HLS reticulocyte count
Immature reticulocyte fraction
Mean corpuscular volume
Mean reticulocyte volume
Mean spherical cell volume
Total red blood cell count
Red blood cell distribution width
Reticulocyte count
Mean corpuscular haemoglobin concentration
Albumin
Alanine aminotransferase
Aspartate aminotransferase
Direct bilirubin
Total bilirubin
Gamma Glutamyltransferase
Heel bone density
Body mass index
Sitting height
Standing height
Hip circumference
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Weight
Body fat−free mass
Body fat mass
Body fat percentage
Metabolic rate
Hand grip strength/height
Alkaline Phosphatase
Calcium
Rheumatoid factor
Vitamin D
Reaction time test
Pairs matching test
Urinary microalbumin
Urinary sodium
Urinary creatinine
Urinary potassium
Urea
Creatinine
Cystatin C
Phosphate
Total protein
Urate
Forced expiratory volume in 1s/height
Forced vital capacity/height
Eosinophil count
Lymphocyte count
Monocyte count
Neutrophil count
Basophil count
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Rotated factor loading
Calcium
Rotated factor loading
Total protein
Rotated factor loading
Urate
Rotated factor loading
Creatinine
Rotated factor loading
Mean red blood cell volume
Rotated factor loading
Alanine aminotransferase

Chapter 4: Methods for estimating and validating biological ages

4.1 Introduction

The initial stage of this thesis was to investigate and implement reliable biological ages in the deeply-phenotyped UK Biobank resource. This chapter therefore investigates the statistical properties of established estimation methods that emphasised biomarkers that had the strongest relationships with chronological age (Section 2.4), and these methods were modified to produce more reliable age-based biological ages from a large number of candidate biomarkers. The subsequent stage of identifying key biomarker constituents of biological age utilised statistical methods for assessing biomarker importance in the estimated biological ages. Since this thesis also aimed to use biomarkers to supplement chronological age in the prediction of later life health outcomes, this chapter also investigates statistical approaches for selecting and emphasising biomarkers that had the strongest relationships with disease risk, building on previous research on mortality-based biological ages and body system ages (such as heart age and lung age; Section 1.2). Validation procedures to improve the reliability of these biological and body system ages (also identified in Section 2.4) are outlined.

Overall biological ages

Historical accounts of research on biological ageing and biological age show that biological age estimation was discussed from the 1950s onwards, mainly theoretically at first, and then more empirically as larger datasets became available for analysis.[25,152] Numerous methods for estimating an overall biological age from biomarkers have been proposed over this time:

1. Multiple Linear Regression (MLR), which appears to be first used in 1958,[20,47] where biological age is the weighted sum of individuals' biomarker measurements x_i, where *i = 1, …, n* for *n* candidate biomarkers, by their coefficients β_i ($\sum_{i=1}^{n} \beta_i x_i$), from the regression:

$$Chronological\ age \sim x_1 + x_2 + \cdots + x_n$$

2. Principal Component Analysis (PCA), proposed by Nakamura et al[52] in 1988, where biological age is the weighted sum of individuals' biomarker measurements x_i, by their factor loadings γ_i in the first principal component of the candidate biomarkers ($\sum_{i=1}^{n} \gamma_i x_i$), transformed into a T-score
3. Hochschild's method, proposed by Hochschild[118,153,154] in 1989, where biomarker-specific 'test ages' TA_i were first estimated by regressing individuals' biomarker measurements x_i on chronological age (CA) for each biomarker i, then standardised into STA_i:

$$x_i = m_i \times CA + \varepsilon_i$$

$$TA_i = -\frac{\varepsilon_i}{m_i} + \frac{1}{m_i} \times x_i$$

$$STA_i = \frac{TA_i/CA - \overline{TA_i/CA}}{\sigma(TA_i/CA)_i}$$

   Biological age is then the standardised weighted sum of STA_i, where the weights are their correlations with a composite score for 8 ecological and non-biological mortality risk factors ρ_i ($\sum_{i=1}^{n} \rho_i STA_i$).
4. Klemera Doubal Method (KDM), proposed by Klemera and Doubal[51] in 2006 (described in the next section)
5. Deep neural networks, proposed by employees of Insilico Medicine[98,155,156] in the last few years
6. Mortality-based biological ages, proposed by Ganna and Ingelsson[157] and Levine[62,66] in the last few years

The first four key methods were identified by Jia et al's review of biological age estimation methods in 2017.[20] The Hochschild method was very rarely used by later studies (only one such study was identified[42]), but the other three methods were frequently used by the studies identified in the Jia et al review.[20] Other ageing indices, e.g. frailty indices,[15] allostatic load[79,80] and physiological dysregulation,[80,84,85] are not expressed in terms of an age (Section 1.2) and therefore their estimation methods are less relevant. Statistical properties of biological age estimation were discussed as early

as 1988 (reliability, validity, generalisability of biomarkers; and accounting for time varying biomarker measurements, applying cross-validation, and considering the competing risks of multiple health outcomes in the biological age).[152,158]

Increasing availability of high-dimensional data in large numbers of individuals in the past decade has allowed application of newer estimation methods incorporating machine learning and deep learning techniques.[98,101,155] A review of deep learning methods for estimating biological age[53] was published in 2019 and described only one method for estimating a clinical biomarker-based biological age (deep neural networks),[98,155] which was also age-based and assessed in the systematic review (Chapter 2).

Previous studies have focused on three main stages of the biological age estimation process: statistical development of estimation methods, application of these methods to the population of interest, or application of the biological age equations proposed by previously published studies (normally for a set of biomarker coefficients) to a population of interest (Chapter 2). Apart from the pioneering studies identified above, most studies have belonged to the latter two categories.

The systematic review of large scale biomarker-based biological age studies (Chapter 2) found that almost all of the included studies used three methods that selected and weighted biomarkers based on their relationship with chronological age (age-based methods): KDM, MLR and PCA. The methods review by Jia et al[9] compared statistical properties and limitations of these three methods and listed more limitations for the MLR and PCA methods than for the KDM. The PCA method was described in Section 3.8 and used to derive biomarker principal components in this thesis. The Borkan and Norris method used by one included study in the systematic review (Section 2.3) was not listed above as a separate candidate method, as it was similar to the KDM except that it constrained the constituent markers to have equal weighting.[119] Unlike the other age-based methods, the Hochschild method required additional qualitative analysis of non-biological risk factors for mortality in the population,[42,118,153,154] such as life expectancy in the region of residence (Section 2.3).

Hence while it captured a wider range of health characteristics, it is not strictly just a biological age or an individualised indicator of health.

Another group of methods for deriving biological age not used in any of the included studies in the systematic review (Section 2.4) takes a prognostic approach, based on biomarkers' relation to the subsequent presence or absence of health outcomes (called "outcome-based methods"). Mortality tends to be the outcome used for estimating overall biological ages[62,157] or for validating age-based biological ages (Chapter 2.3). Ganna and Ingelsson indirectly estimated a biological age by predicting individuals' mortality rates from biological and non-biological risk factors in the UK Biobank,[14] then extrapolated the biological age ('Ubble age') from the chronological age that these predicted mortality rates corresponded to in national life tables.[64] This extrapolation method could have led to Ubble age being poorly calibrated to chronological age on average.[64] The Levine group proposed a similar 3-step approach to estimate their 'phenotypic age' (PhenoAge) from physical and biochemical biomarkers: (1) selecting the most important biomarkers to create a biomarker-based risk score, (2) estimating a mortality score from the selected biomarkers using a parametric survival model (Gompertz model) and (3) calibrating the mortality score to chronological age by equating characteristics of the earlier Gompertz model with those of a second Gompertz model that had chronological age as its only term, at a fixed duration of follow up.[62,66] The Levine group approach for selecting biomarkers was based on a common approach for deriving epigenetic ages from DNA methylation data,[36] elastic net (which is an optimal combination of the ridge and lasso penalisation methods for restricting the number of variables kept in the regression of high-dimensional data, to limit overfitting[159]), but adapted to predict a survival outcome (mortality during follow up). The third step in these approaches of transforming a risk score into biological age had been used in the estimation of several cardiovascular risk scores.[54] Spiegelhalter[64] proposed a similar but mathematically simpler approach to derive an individual's 'effective age' from a standard Cox model, with respect to a single biological or non-biological risk factor, but did not perform risk factor selection.

Body system ages

A biological age is most useful when it is related to both age and later life health outcomes such as multiple age-related diseases (Section 1.2). Using an age-based method to estimate biological age ensures its association with chronological age but not diseases, while using an outcome-based and specifically disease risk-based method ensures its association with diseases but not necessarily with chronological age. Therefore disease risk-based methods that could better capture the relationship between biomarkers and the onset of multiple age-related chronic diseases were also explored in this chapter.

Risk scores that are associated with body system-specific health conditions (not necessarily in later life) and that are expressed in terms of an age can generally be classified as body system ages (Section 1.2). Aggregation of multiple separate body system ages into an overall biological age also allows:

1. The assessment of the degree of commonality between the patterns of biomarkers that best predict each disease
2. Validation of overall biological ages as a common predictor for multiple diseases, using multiple-outcome statistical models
3. Enhanced health communication by aggregating an overall biological age from multiple body system ages and communicating biological and body system ages to individuals[76,77] (several of the objectives listed in Section 1.4).

In addition to the age-based and outcome-based types of estimation methods described above, two simpler types of methods have been used to estimate body system ages: (1) linearly interpolating the chronological age that corresponds to the same mean or median biomarker values for the population as the individual's biomarker measurement (value-based; only applies to a single candidate biomarker),[54] and (2) summing scores that were assigned to specific categories for each risk factor (points-based).[116,120] The value-based method is equivalent to the Klemera Doubal method applied

to a single biomarker, while scores used for the points-based method can be derived quantitatively[160] (using methods similar to the outcome-based method described above) then rounded to whole numbers, or qualitatively, as is done for health indices such as allostatic load[79] (Section 1.2).

Seven body system ages were identified in epidemiological literature: heart, lung, body shape, liver, pancreas, kidney and brain ages. Of these ages, three relevant ages were identified by a scoping review of systematic reviews and trials of ages:[56] heart age, lung age and 'body age'. A systematic review of vascular ages found 39 studies of vascular ages, which used outcome-based and value-based estimation methods.[54] These studies used a range of physical and biochemical cardiovascular biomarkers, as well as anthropometric biomarkers.[54] The only known published lung age[161] used a value-based method with forced vital capacity measurements reported in a 1979 study of healthy non-smoking adults as the only biomarker.[63] 'Body ages' used both age-based[75,115] and outcome-based methods[162] to summarise anthropometric biomarkers[75,115] or a combination of anthropometric and physical functional test biomarkers,[162] and therefore appeared to be more akin to body shape or functional capability ages rather than an overall biological age.

The MediAge research centre in South Korea developed 6 biomarker-based body system ages (cardiac, lung, liver, pancreas, kidney and body shape ages), by applying age-based methods to non-overlapping groups of pre-selected clinical biomarkers in each age.[74,75] This research centre illustrated an example of their commercially-available body system ages together with an overall biological age for a hypothetical individual.[77] Another commercial company, Bio-Age, illustrated a set of body system ages that overlapped with the MediAge body systems but which also included immune and joint ages.[76] In neither case, was it stated whether the overall biological age was related to or aggregated from the body system ages.

Kidney ages were also estimated in a US population using a value-based method applied to estimated glomerular filtration rate (eGFR) levels alone.[65] Brain ages were estimated in multiple studies using

age-based methods, from brain image biomarkers rather than physical or biochemical biomarkers.[38,163]

Validation of biological ages

The relevant validation and calibration approaches for biological ages were identified in the systematic review in Section 2.3, mainly from clinical risk prediction reporting guidelines.[112,164] The systematic review found that validation generally appeared to be poorly conducted and insufficiently reported in the included studies (Section 2.4), even though it only focused on studies that validated their biological age against at least one health outcome (Section 2.2). Due to the prevalence of age-based estimation methods, many studies simply checked their derived biological ages by comparing them with chronological age (Tables 2.3.2 and A2.4). The more similar biological age is to chronological age, the better our understanding of the biological processes that chronological age represents in epidemiological models, but perversely the less likely biological age is to supplement chronological age in predicting later life health (Section 1.4).

In this thesis, biological ages were estimated from biomarker principal components via two types of approaches: (1) treating chronological age as a proxy for the ageing process, using established age-based methods; and (2) deriving and aggregating 8 body system ages that predicted subsequent disease into an overall biological age by modifying and extending outcome-based methods. The estimated biological ages were also internally validated in the UK Biobank, following relevant clinical risk prediction reporting guidelines in the Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD) checklist.[164]

4.2 Estimating age-based biological ages

Biological ages were estimated in the healthy UK Biobank subpopulation separately for each sex, due to differences in biomarker-age trends by sex (Figures 3.10.1 and 3.10.2). Additionally, patterns

of mortality from chronic disease and prior and incident age-related frailty were different between sexes (Table 3.9.2). The statistical analysis for healthy participants of each sex was repeated on biomarkers corresponding to the 10 most important biomarker principal components in the biological ages, to evaluate whether or not a smaller and more practical panel would suffice. The analysis was also repeated on the whole UK Biobank population as a sensitivity analysis, and for comparisons with previous studies.

Main method: Klemera Doubal Method (KDM)

The main method of estimating age-based biological ages was the Klemera Doubal Method (KDM),[27] which was used by 8 of the 15 biological age studies identified in the systematic review (Table 2.3.2). This method assumes that constituent biomarker principal components are uncorrelated and is based on two principles: (1) biological age summarises the differences between individuals' actual biomarker principal component levels x_i, where *i = 1, ..., n* for *n* candidate biomarker principal components, and characteristic biomarker principal component levels for their chronological age; and (2) biomarker principal components with stronger linear relationships to chronological age contribute more to biological age.[51] Biological age is estimated by linearly regressing each included biomarker principal component x_i against chronological age, then taking the weighted sum of all the regression results, with the form:

$$Biological\ age \propto \sum_{i=1}^{\mathrm{n}} \left(\frac{k_j}{s_i^2}\right)(x_i - q_i)$$

where q_i = intercept, k_i = coefficient and s_i=standard error from the ith biomarker principal component-chronological age regression (illustrated graphically for a single biomarker in Figure 4.2.1). This approach therefore assigns larger weights to biomarkers that display larger changes with chronological age on aggregate (larger k_i) and biomarkers with smaller deviations from the linear relationship with chronological age (smaller s_i). Biomarker principal component-chronological age relationships that deviated more from the linear assumption were thus more prone to being down-

weighted (most of the biomarker-chronological age relationships were broadly linear or flat; Figure 3.10.2; however these relationships were not investigated in previous studies).

When estimating a KDM biological age, chronological age may or may not be treated as a constituent 'biomarker';[27] if it is not a constituent 'biomarker', the predictive value of the derived biological age for health outcomes can be compared with that of chronological age. If chronological age is treated as a biomarker, it is incorporated in the estimation procedure in a different manner from the other biomarkers.[27] Including chronological age as a biomarker has been controversial[13,42,50,52] and was discouraged by Nakamura as its inclusion would result in estimated biological ages that primarily predicted chronological age rather than summarised biomarker-measured ageing patterns.[50,52] A study that compared KDM biological ages estimated with and without chronological age as a constituent biomarker found that there was no advantage to using the former over the latter in prediction models that adjusted for chronological age.[13] To allow assessment of calculated biological ages both in isolation and jointly with chronological age, in this thesis, only the version of KDM without chronological age as a biomarker was used.

Since the KDM method does not involve biomarker selection and assumes that its constituent biomarkers are uncorrelated, in this thesis this method was applied to principal components of candidate biomarkers[25] rather than raw biomarker measurements. When the initial PCA step was used, it summarised the biomarkers into linearly independent biomarker principal components (Section 3.8), and reduced the interdependence between constituents of the biological age.

Sensitivity analysis estimation method

To compare the properties of biological ages estimated using a combined approach of KDM and PCA with those estimated using the remaining commonly-used method identified in the systematic review, MLR (Section 2.3), an alternative method, stepwise multiple linear regression, was applied to the same panel of biomarker principal components. The standard MLR method, where biological

age is the predicted value of chronological age regressed on all candidate biomarkers,[9,13] was extended with a stepwise procedure that iteratively selected biomarkers that best explained chronological age, in the presence of other selected biomarkers. Stepwise regression was chosen over other variable selection or shrinkage methods for linear regression,[159] as multiple testing could be accounted for easily through the specification of modified p-value thresholds for the selection procedure, in this case using the Bonferroni-corrected p-value at the 0.05 level (0.05 divided by the number of candidate biomarkers). The strict Bonferroni-correction used in this stepwise regression method provided a lower bound for the number of types of biomarkers to include in the biological ages. Biological ages were calculated by applying this method to biomarker principal components, to allow comparison with the KDM biological ages.

The stepwise regression method directly represents biological age as the linear combination of biomarkers that explains the most variation in chronological age. The resulting biological age is thus highly statistically dependent on chronological age, limiting its scope for prediction of health outcomes.

Benchmark mortality score

Previous studies have derived a mortality-based risk score[14] and biological age[62] using Cox proportional hazards models with variable selection, to determine the optimal pattern of variables that predicted mortality in the study population. As a benchmark for comparison with the calculated biological ages, a mortality score was constructed from the same biomarker panel as the age-based KDM, using a Cox lasso model, which was one of the main methods used in the estimation of the published mortality-based risk score[14] and biological age.[62]

Biomarker importance in age-based biological ages

To rank biomarker principal components by their relative importance in the biological ages, the proportion of variance in the biological ages that each component explained was estimated using the Fabbris/Genizi/Johnson method.[165] This method calculated the proportion of variance in the biological ages explained by each constituent biomarker in the presence of the other constituent biomarkers (R^2),[165] and was recommended by a review of relative importance estimation methods for linear regressions when there are large numbers of variables.[166]

4.3 Estimating disease risk-based body system ages

Similar to the age-based estimation process described in the previous section, body system ages and risk scores were estimated in the healthy subpopulation separately for each sex, based on the same panel of biomarker principal components. The body system age approach estimates the disease risks attributable to chronological age and biomarkers jointly, whereas the body system risk score approach estimates the disease risks attributable to biomarkers in the absence of chronological age. Therefore the body system age approach selected and emphasised biomarkers that provided complementary information to chronological age, to identify what the biomarkers add to chronological age, while the body system risk score approach selected and emphasised biomarkers that best predicted disease incidence in the absence of chronological age, to identify biomarkers that represent the chronological age effect and any additional biomarker effects on disease incidence.

Many of the biomarkers measured in UK Biobank had not been used by previous body system age studies (Table 3.8.2 and Section 4.1), hence candidate biomarkers were not pre-selected for each body system age or risk score. The outcomes used in estimating these body system ages and risk scores were the 8 clusters of 20 age-related chronic diseases, which were determined in Sections 3.5–3.6. The first incidences of a hospital admission related to each of the disease clusters during follow up were used as the time-to-event outcomes.

Body system ages

This thesis estimated body system ages based on biomarker patterns that best supplemented chronological age in predicting later life disease. The estimation method involved adapting and combining the biomarker selection approach proposed by the Levine group (PhenoAge)[62,66] and the 'effective age' approach of calibrating risk scores to chronological age in a Cox model proposed by Spiegelhalter[64] into a more parsimonious process. Both the PhenoAge and effective age approaches use mortality as the outcome and rely on the Gompertz and proportional hazard assumptions,[62,64,66] while the models for this analysis used the incidence of each of the 8 disease groups instead of mortality as the outcomes, hence they are specifically referred to as disease risk-based methods. Similar to the age-based biological age method described in Section 4.2, chronological age was not treated as a candidate 'biomarker' in this thesis, in contrast to the PhenoAge method,[66] in order to separate out the effects of chronological age and biomarker levels.

The adaptations in this thesis firstly involved combining and adapting the 3 steps in the PhenoAge approach into a single model, for 2 reasons: (1) to simplify the method and increase consistency by conducting biomarker selection and calibrating to chronological age in the same regression model, and (2) to avoid the requirement to manually specify a follow up duration where the risks of the health outcome described by biomarker measurements are equated with those described by chronological age. The lasso approach, which shrinks the regression coefficients (potentially to zero) by applying a penalty term based on the sum of absolute values of the coefficients,[159] was chosen from candidate biomarker selection methods for Cox models,[167] for the following reasons:

1. It is a commonly used penalisation approach that is implemented in published analytical software.[167]
2. It has been used to estimate a bodily age, brain age, from a large number of candidate biomarkers (brain images).[168]

3. It limits overfitting and performs biomarker selection. The other commonly used penalisation approaches, ridge and elastic net, do not automatically perform biomarker selection.
4. The PhenoAge study may have effectively used the lasso approach (which is a special case of the elastic net approach) as it did not report the use of a mixing parameter that is mandatory for the elastic net approach.[66]

Simultaneously, this method extended the Spiegelhalter approach from its single risk factor format[64] to accommodate multiple biomarkers, and to incorporate biomarker selection. This approach equates the hazard ratios (HRs) of chronological age and of a specified risk factor within a Cox mortality model, to get the relationship:

Difference in effective age from chronological age = log(HR of risk factor)/log(HR of age)

The estimation approach for this thesis used biomarker principal components and chronological age to jointly predict disease risk. For each body system age BSA_j and for the n biomarker principal component measurements $m_{1,j} \ldots m_{n,j}$, the hazard (or instantaneous risk of the outcome) h_1 for disease cluster incidence in the Cox lasso model had the form:

$$h_1 = \exp(\beta_{CA,j} CA + \sum_{i=1}^{n} \beta_{i,j} m_{i,j})$$

Where $\beta_{CA,j}$ and $\beta_{i,j}$ were determined by the Cox lasso regression

In the above equation, $\beta_{CA,j}$ or $\beta_{i,j}$ are also the log HR of chronological age at baseline (CA) or $m_{i,j}$ respectively, and any of $\beta_{1,j} \ldots \beta_{n,j}$ may be set to 0 due to lasso regularisation[66] (i.e. the biomarker may be excluded from the model). The timescale for this model was follow up duration. Chronological age assumed the role of a scaling factor ($\beta_{CA,j}$) in this equation, rather than a risk factor. This method therefore calibrated risk scores derived from the biomarker measurements

($\sum_{i=1}^{n} \beta_{i,j} m_{i,j}$) to the chronological age effect after cross-adjustment for biomarker principal components, rather than to a chronological age effect based on the unadjusted association of chronological age with disease. The functional form of chronological age in h_1 was not modified, as crude baseline age-specific incidence rates for all 8 disease clusters in this healthy UK Biobank subpopulation appeared to have a Gompertz shape, i.e. incidence rates were linear on a log scale (Figure 3.6.1).

Keeping h_1 constant, and equating a change of one year of chronological age to one year of biological age (applying the Spiegelhalter approach[64]):

$$BSA_j - CA = \delta_j = \frac{\sum_{i=1}^{n} \beta_{i,j} m_{i,j}}{\beta_{CA,j}}$$

$$BSA_j = CA + \delta_j = CA + \frac{\sum_{i=1}^{n} \beta_{i,j} m_{i,j}}{\beta_{CA,j}}$$

In order to focus on the component of the body system ages described by participants' biomarker levels over and above their chronological age, the body system age delta, δ_j, was the key result of interest in the analyses rather than the body system age itself.

After estimating the body system ages, the means and standard deviations of tertiles of each body system age delta were calculated, to analyse the spread of these body system ages around chronological age. To assess the similarity between body system biomarker patterns, Pearson correlations between each of the body system age deltas were calculated. Additionally, to assess the contribution of chronological ages to each body system age, pairwise Pearson correlations between chronological age and each of the body system ages were calculated.

Body system risk scores

Body system risk scores were also estimated to assess the patterns of biomarkers that best predict disease risk in the absence of adjustment for chronological age. A similar approach to the body system age estimation process was used, except chronological age at baseline was not included in the Cox model. Since this approach derived risk scores that were wholly constructed from biomarker measurements, it provided a more consistent comparison with the age-based biological ages estimated in Section 4.2 in terms of the extent to which biomarkers account for the effect of age. In this process, biomarkers most strongly related to chronological age (e.g. lung function and cystatin C; Section 3.10) could have been included in the body system risk scores as a proxy for the ageing effect on disease risk.

The body system risk scores S_j were therefore estimated using the same regression equation to the body system ages, but with the chronological age term omitted. For each body system risk score S_j and for biomarker principal component measurements $m_{1,j} \ldots m_{n,j}$, the hazard (or instantaneous risk of the outcome) h_2 for disease cluster incidence had the form:

$$h_2 = \exp(S_j) = \exp\left(\sum_{i=1}^{n} \beta_{i,j} m_{i,j}\right) \leftrightarrow S_j = \sum_{i=1}^{n} \beta_{i,j} m_{i,j}$$

where $\beta_{i,j}$ were determined by the Cox lasso regression

In order to explore the contributions of the biomarker-based body system risk scores and chronological age separately and jointly to disease risk, and to assess the goodness of fit of the proportional hazards assumption to biomarker levels, additional analysis was carried out using standard Cox models that included these risk scores and chronological ages as categorical rather than continuous exposures. The patterns in HRs were assessed for: (1) non-linearity in body system risk score-disease incidence or chronological age-disease incidence relationships, and (2) the relative strength of the relationships with body system risk scores vs chronological age. For each of the 8 disease groups, unadjusted Cox models were used to estimate HRs of disease incidence for quintiles of body system risk scores and quintiles of chronological age separately and jointly, with their respective disease group as the outcome. A sensitivity analysis was conducted with the first two

years of follow up omitted from the Cox model, to limit the effects of reverse causation of biomarker levels and disease risk.

Biomarker importance (measured via explained relative risk) in body system ages

The methods designed for calculating relative importance in linear regression models[166] were not directly applicable to survival models, as right censoring of follow up data was present.[169] Heller proposed a measure of explained relative risk (ERR) as a more appropriate method to assess biomarker importance in survival models.[169] This method is based on the minimisation of the entropy loss function of the Cox model,[169] and is independent of the follow up duration, an improvement from earlier relative risk methods.[170,171] This ERR method was used to calculate the relative risks for each biomarker in each Cox lasso model. Relative risks for each biomarker were then expressed as a percentage of the total risk explained by all biomarkers included in the Cox model, in a similar format to the biomarker importance for the age-based method (Section 4.2).

Sensitivity analyses for body system ages

Three types of sensitivity analyses were conducted on the 8 body system ages. Firstly, to obtain body system ages for the whole population, the body system age and risk score model coefficients estimated in the healthy subpopulation were applied to biomarker measurements in the whole UK Biobank population. This was because many participants who were not in the healthy subpopulation had prior age-related chronic diseases, therefore these participants would have been excluded from the outcome-based estimation process. Secondly, the body system ages were estimated on a reduced panel of the biomarker principal components that were found to be the top 10 most important across all 8 body system ages, in men and in women. Lastly, the stepwise selection method was reused and implemented in the Cox models in place of the lasso method, for continuity with the age-based sensitivity analysis and because correction for multiple testing could be explicitly specified (Section 4.2). The model structure was similar to the structure of the Cox lasso model described above.

4.4 Aggregating disease risk-based biological age

Since there were no known methods for aggregating multiple body system or biological ages, three candidate methods were explored. These methods are described in the order of increasing statistical and computational complexity: KDM (age-based), standard Cox models (outcome-based) and the main multi-state model method (MSM; outcome-based). The outcomes used in the estimation of the latter two methods were the 8 disease groups described in Section 3.6: atherosclerotic, musculoskeletal, gut, cardiac, metabolic, inflammatory, neurological and respiratory diseases. The multi-state model was chosen to be the main method as it had the best combination of statistical properties, and the other methods were used in sensitivity analyses.

Klemera Doubal method (KDM)

The KDM implemented in the age-based analysis (Section 4.2) was repurposed to aggregate the body system age deltas δ_i (instead of biomarkers) and chronological age into an overall biological age.

Standard Cox models with body system ages as exposures

Standard Cox models were used to estimate the hazards of mortality from chronic disease and age-related frailty separately. Chronological age and the 8 body system age deltas δ_i were treated as covariates in each model and the timescale was follow up duration. Similar to the Cox lasso method described in Section 4.3, the hazards h_3 had the form:

$$h_3 = \exp\left(\beta_{CA} CA + \sum_{j=1}^{8} \beta_j \delta_j\right)$$

$$= \exp\left\{\beta_{CA}\left(CA + \sum_{j=1}^{8} \frac{\beta_j}{\beta_{CA}} \delta_j\right)\right\}$$

Two versions of overall biological ages were estimated, one for mortality and the other for frailty, each having the form $CA + \sum_{j=1}^{8} \frac{\beta_j}{\beta_{CA}} \delta_j$. These are referred to as Cox mortality and Cox frailty ages respectively.

Main method: Multi-state model (MSM)

This aggregation method modelled longitudinal health trajectories across two health outcomes (mortality from chronic disease and age-related frailty) within a single regression framework. It estimated a single overall biological age, unlike the standard Cox method. Simultaneously, it takes into account the competing risks between the outcomes of interest: mortality from chronic disease and age-related frailty.

The model structure for this study consisted of 3 health states (non-frail, frail and died from chronic disease, or 'dead') and 3 permissible transitions between these states (non-frail to frail, non-frail to dead and frail to dead; Figure 4.4.1). This MSM was similar to jointly evaluating a collection of parametric versions of Cox models for age-related frailty and mortality from chronic disease, for each type of transition – participants who were in the non-frail state had not been admitted to hospital for an age-related frailty reason, while those in the frail state had such an admission, and participants who died from chronic disease from either of these states transited to the dead state. The frail to non-frail transition was not permissible as it was not detectable via hospital records. The weights for the constituent body system age deltas δ_j were constrained to be the same across transitions, in order to estimate a single aggregate biological age.

Similar to the Cox aggregation method described in the previous subsection, the hazards $h_{r,s}$ for each of the 3 permissible transitions from states *r* to *s* and each body system age delta δ_j had the form:

$$h_{r,s} = \exp\left(\beta_{CA,r,s} CA + \sum_{j=1}^{8} \beta_j \delta_j\right)$$

$$= \exp\left\{\beta_{CA,r,s}\left(CA + \sum_{j=1}^{8} \frac{\beta_j}{\beta_{CA,r,s}} \delta_j\right)\right\}$$

The MSM age was then $CA + \sum_{j=1}^{8} \frac{\beta_j}{\bar{\beta}_{CA}} \delta_j$, where $\bar{\beta}_{CA}$ represents the weighted average of the coefficients for chronological age $\beta_{CA,r,s}$, by the numbers of transitions for each transition $r \rightarrow s$ during follow up. The timescale for this model was chronological age at risk. Appendix 4.1 provides a detailed description of the model specification and assumptions.

After estimation of the MSM age, the relative weights of the constituent body system age deltas (represented by the coefficients $\frac{\beta_j}{\bar{\beta}_{CA}}$) were compared across body systems. The pattern of relative weights for the MSM age were also compared to weights for the disease risk-based KDM, Cox mortality and Cox frailty ages.

Sensitivity analyses where the Cox mortality and Cox frailty methods were applied directly to biomarker principal components instead of body system age deltas were also conducted, to assess whether the intermediate step of summarising biomarker principal components into body system age deltas placed a large constraint on biomarker patterns that predict mortality from chronic disease and age-related frailty.

Biomarker importance in disease risk-based biological ages

In order to assess the underlying pattern of biomarker principal components represented by the constituent body system age deltas, biomarker importance in the disease risk-based biological age delta was investigated. In the absence of a method for calculating explained relative risk in MSMs,

and to provide a more consistent comparison of biomarker importance between age-based and disease risk-based biological ages, biomarker importance was approximated by applying the same Fabbris/Genizi/Johnson method[165] used in the age-based analysis (Section 4.2). This method estimated the proportion of variance in the biological age explained by each biomarker principal component (Section 4.2), and was applied after linearly regressing the disease risk-based biological age delta on all biomarker principal components.

4.5 Validating biological ages

Since biological ages can be used as risk prediction scores, and the predictive power of bodily ages for health outcomes was investigated in this thesis, the TRIPOD guidelines[113] were followed. These guidelines recommended cross-validating the bodily ages, assessing the predictive powers of bodily ages for health outcomes and assessing the calibration of bodily ages to the risk of these health outcomes.[113] Additionally, since these bodily ages are expressed in terms of an age, the proportion of the overall biological and chronological age effect on frailty and mortality risk that was explained by each bodily age was also estimated, and the calibration of all bodily ages to chronological age was checked. These approaches are explained in further detail below.

Cross-validation of biological age estimation

For the age-based biological ages, cross-validation was carried out prior to biological age estimation, to check the stability of estimated biological ages and to identify the optimal number of biomarker principal components to include in the models. The standard 10-fold cross-validation approach of splitting the data into 10 folds, fitting the estimation model on $9/10^{ths}$ of the data and evaluating the model on the remaining $1/10^{th}$ for each fold, then averaging the prediction errors (the disparity between the estimated age-based biological ages and chronological ages) across folds, was used.

The optimal number of biomarker principal components to use in the analyses involved cross validation of the models for estimating biological age. The initial criterion for obtaining this number was the search for a single elbow point in a plot of prediction errors for biological age estimation models run with an increasing number of principal components, ordered by decreasing eigenvalue, where beyond the elbow point there were diminishing changes in prediction error by increasing the number of principal components included in the model. If no clear single elbow point in both subpopulations of healthy men and women were apparent, a second criterion of an eigenvalue threshold of >0.33 (⅓ of the average variation in biomarker measurements described by a single biomarker in the UK Biobank) was imposed, to avoid the inclusion of principal components that captured little biomarker variation in the population. The plot of prediction errors by numbers of principal components included in the age-based KDM biological age models in Figure 4.5.1 showed that there was no clear single elbow point in either subpopulation of healthy men or women, where beyond the elbow point there were diminishing changes in prediction error by increasing number of principal components. Hence the optimal number of principal components was determined to be 51, based on the predetermined eigenvalue threshold of >0.33.

The reported coefficients for the age-based biological ages were not cross-validated even though cross-validation had been carried out, as there was no known method for determining cross-validated coefficients for the KDM and stepwise regression methods. Cross-validation was also not applied to the stepwise Cox model used in sensitivity analyses for body system ages (Section 4.3) as there was also no known method for determining cross-validated coefficients for this approach.

The disease risk-based estimation methods used time-to-event rather than linear regression, hence specific cross-validation methods for survival models were required. For body system ages estimated using the Cox lasso method, cross-validated estimates of body system ages were obtained for the following reasons: (1) to avoid overfitting the models due to the large number of candidate biomarkers, (2) cross-validation was required to choose the optimal tuning parameters for biomarker selection and therefore the optimal biomarker weights, and (3) to avoid the circularity of using the

same health outcomes in the estimation and validation of body system ages. The number of candidate biomarker principal components to include in the disease risk-based models were the same as the number determined through the age-based analysis for consistency. The Verweij and van Houwelingen method[172] of cross validating the partial log likelihood was recommended as a method to address all three of these reasons.[173] This method avoided the possibility of numerical instability from standard cross validation approaches applied to Cox models if there was a small number of events.[174] The cross-validated body system age could also be treated as a covariate in a subsequent Cox model, along with other covariates (e.g. potential confounders) that were not used in the earlier penalised Cox model.[173] 10-fold cross validation was implemented for each of the body system age and risk score models, and the cross validated estimates were reported.

Prediction of adverse health outcomes

For both the mortality from chronic disease and age-related frailty outcomes defined in Section 3.7, Cox proportional hazards models were run on the same populations as those used in the bodily age estimation. For the age-based analyses, the biological age itself was used in the prediction models, while for the disease risk-based analyses, to isolate the biomarker effect and to avoid overlapping with chronological age, the bodily age deltas were used. These different entities were analysed using the same approaches and are therefore summarily referred to as 'bodily ages' in this subsection. For models that predicted age-related frailty, participants with prior events were excluded. These models were stratified by sex, and unadjusted models as well as models adjusted for Index of Multiple Deprivation 2010 (IMD) quintile, smoking status, alcohol consumption, assessment centre, and age combinations were run. Age combinations used were: (1) chronological age, (2) bodily age, and (3) both chronological and bodily age. The improvement in model fit by adding bodily age to the prediction model with chronological age was assessed using a likelihood ratio χ^2 test with 1 degree of freedom.

Predictive power was assessed using Harrell's C-index, a measure for survival models equivalent to area under the receiver operating characteristic curve, separately for the healthy subpopulation and for the whole population. The C-index and its standard errors were calculated using Kendall's tau.[175] As a sensitivity test, prediction of age-related frailty by the age-based biological age was compared with the benchmark mortality score (Section 4.2).

For age-based biological age, the proportion of variation in chronological age explained by biological age was estimated in terms of R^2 from univariate linear regressions. More importantly, for all bodily ages, the proportion of the combined age effect on either mortality or frailty risk that was explained by bodily age was estimated by comparing the log partial likelihoods of pairs of nested models (an extension of likelihood ratio tests) unadjusted for sociodemographic and health behavioural factors:

$$\frac{(l_{BA+CA} - l_{CA})}{(l_{BA+CA} - l_{base})}$$

where l_m: log-likelihood of model m, base: adjusted model without chronological or bodily age, BA: adjusted model with bodily age only and BA+CA: adjusted model with both bodily and chronological age

Similar ratios were taken to calculate the proportions explained by bodily age alone, chronological age alone and by both bodily and chronological age in predicting these health outcomes. The log-likelihood proportions above are equivalent to comparisons of the Nagelkerke pseudo-R^2 [176] of the same pairs of models, which are approximations of R^2 for Cox models.

Assessing calibration of biological ages

To assess the calibration of bodily ages with chronological age, the means and standard deviations for bodily ages (estimated for each participant at baseline) were plotted against baseline chronological age, for each 2.5-year chronological age band in the age range of 40–70. A perfectly calibrated biological age would have mean biological age equal to mean chronological age in each age band.

Bodily ages that were poorly calibrated to chronological age were further recalibrated using a modified version of the Dubina method.[177] The original Dubina recalibration method had been used by 3 studies identified in the systematic review that used the age-based PCA method of estimating biological ages[96,106,117] (Table A2.4). The original Dubina method involved linearly regressing the old bodily age (BA_{old}) on chronological age:

$$BA_{old} = \mathrm{b}_{CA}CA + c + \varepsilon$$

where $b_{CA}$is the coefficient (or slope) for chronological age, c is the intercept and ε is the residual term from the linear regression

In order to target a bodily age-chronological age slope of 1 (i.e. bodily ages equal to chronological ages on average, described by the identity line), an adjustment to the bodily age, z, was made, which used the mean chronological age in the population ($\overline{CA}$) as a pivot point in the original method:

$$BA_{new} = BA_{old} + z = BA_{old} + (CA - \overline{CA}) \times (1 - \mathrm{b}_{CA})$$

The calibration of bodily age to chronological ages was further improved when the pivot point was modified in the analysis for this thesis from $\overline{CA}$ to the chronological age at the point of intersection of the linear regression slope and the identity line, $\widetilde{CA}$:

$$\widetilde{CA} = \frac{c}{1 - \mathrm{b}_{CA}}$$

$$BA_{new} = BA_{old} + z = BA_{old} + (CA - \widetilde{CA}) \times (1 - \mathrm{b}_{CA})$$

This modified Dubina method had the effect of rescaling bodily ages by the absolute rather than the adjusted effect of chronological age, and resulted in bodily age deltas that are uncorrelated with chronological age. This method was equivalent to the recalibration methods that were applied to an epigenetic age[67] and a brain age.[163]

To assess the risk calibration of bodily ages, participants were stratified into 3 predicted risk groups based on the difference between their biological age (BA) and chronological age (CA):[13] (1) BA - CA < -5 years (biologically younger), (2) |BA – CA| < 5 years (biologically similar), and (3) BA - CA > 5 years (biologically older). For each biological age, sex and health outcome, Kaplan-Meier survival curves for the outcomes of mortality from chronic disease and age-related frailty, stratified by predicted risk group, were plotted and assessed for overlap. For body system ages, these survival curves were plotted for the incidence of their respective disease cluster instead. Log-rank tests were conducted to assess if the differences in survival without the outcome between these 3 risk groups was significant.

4.6 Summary and discussion

Summary of the biological age estimation and validation process

The processes described in this chapter for the two main estimation methods, age-based and disease risk-based methods, are summarised in Figure 4.6.1. Both methods resulted in an overall biological age, and the disease risk-based method also provided supplementary information in the form of its constituent body system-specific ages. Both age-based and disease risk-based methods were investigated in detail and implemented in this thesis, as:

- The age-based methods were more established, simpler to implement (particularly in situations where data on incident disease or health outcomes are absent) and allowed easier comparison with findings from previous studies.

- The disease risk-based methods satisfied a key aim of this study to investigate the pattern of biomarkers that best predict later life health outcomes. The large volume of data on a range of biomarkers and later life health outcomes permitted the use of these methods.

The key results from this series of analyses, detailed in Chapters 5–7, are: model coefficients for the estimation and aggregation of bodily ages from a candidate panel of 51 biomarker principal components, patterns of biomarker importance, correlations between body system ages and the proportion of the total age effect described by biological age in predicting later life health. The validation approaches used were: model goodness of fit, predictive power (discrimination), calibration to chronological age and calibration to risk of later life health outcomes.

Estimation, prediction and attribution

This thesis aimed to address multiple objectives of statistical models: explanation, prediction,[126,178] estimation and attribution (i.e. assigning biomarker importance).[178] The study design contained trade-offs between these objectives: the use of the healthy subpopulation limited reverse causality but worsened predictive power, while the emphasis on attributing biomarker importance in the biological ages limited the choice of suitable estimation models.

Many previous studies simply estimated biological ages and did not investigate the causal nature of their biological ages for poor health (Section 2.4). This analysis limited reverse causality by estimating biological age in the healthy subpopulation, and in the case of the outcome-based analyses, only capturing prognostic associations between biomarkers and later life health. This analysis is one of the first studies to critically assess biomarker importance in biological ages, for both age-based and outcome-based methods. Simply comparing biomarker coefficients in the estimation model[179] was not recommended by the review of variable importance methods.[166]

On the other hand, studies that validated biological age against a health outcome, be they indicative or prognostic studies, tended to focus on prediction-related aims (Section 2.3). The predictive power attributable to biological ages in the main analyses for this thesis was reduced by focusing on a healthier and younger subpopulation, and accounting for sociodemographic and health behavioural confounders. Hence the analyses in this thesis were repeated on the whole UK Biobank population to enable comparisons with other studies.

Strengths and limitations

All biological age validation methods identified through clinical prediction tool reporting guidelines and the systematic review (Section 2.3) were implemented in this analysis, hence this analysis was better validated than previous studies (Section 2.4). On the other hand, this meant that the validation approaches and approaches for attributing biomarker importance were not always available for some candidate estimation methods. These candidate methods were thus implemented as sensitivity analyses. Returning to the exemplary characteristics proposed in 1988 for biological age estimation methods,[158] cross validation has now been incorporated into this analysis, while the UK Biobank repeat assessment (described in Section 3.2) had insufficient biomarker data to support estimating biological age using time-varying biomarker measurements. The series of novel outcome-based approaches described in Sections 4.3-4.4 is the first known where estimation methods take into account multiple outcomes and competing risks.

The main estimation methods used in this thesis assumed that biomarkers with the strongest linear relation to the outcome of interest (chronological age and health outcomes) contribute most to biological age, but not all biomarkers strongly linked to ageing may have linear associations or strong associations with the outcome of interest. Klemera and Doubal noted that their method could be extended to smooth non-linear functional forms of biomarkers,[51] but insufficient mathematical proofs were provided for the full procedure. The candidate biomarkers may be down-weighted by any of the estimation methods used in this thesis, or excluded from the model by the stepwise

selection or lasso models. The lasso approach has better statistical properties than the stepwise approach when there are a large number of candidate biomarkers (e.g. reducing the prediction error of the model), although this is slightly ameliorated by the use of independent principal components and correction for multiple testing. All estimation methods in this thesis did not take into account interaction effects between biomarkers, and the materiality of these interaction effects could be checked using alternative statistical methods (Appendix 4.2).

There was insufficient data in UK Biobank to support methods that model time-varying biomarker trends, as only one repeat measurement for 20,000 participants was carried out approximately 5 years after baseline assessment.[131] Potential methods, e.g. landmark models and joint models, would more accurately model the effect of biomarker levels on the outcome of interest, but would pose more difficulties when used in prognosis as they require biomarker trajectories to be projected (e.g. it raises questions on whether it is fair to assume that biomarker trajectories in future follow the average trajectory that was measured in the past).

The methods for estimating body system ages required a sufficient number of disease events in order to be evaluated (Section 4.3), and they were more data-intensive than the age-based methods. Alternative methods for biological age estimation were considered (Appendix 4.2), and non-parametric methods that allowed for interaction effects between biomarkers, e.g. random forest survival and neural network models (Appendix 4.2), tend to be more data and computationally intensive, so there were trade-offs in the choice of models for these analyses.

As for any regression model, the methods for aggregating body system ages assumed that the constituent age deltas were not highly correlated. This assumption was checked by assessing the magnitudes of the Pearson correlation coefficients between each body system age delta. The multi-state model for aggregating body system age deltas had a relatively simple structure, differentiating only between 3 health states. Using more granular health states in the model could increase the predictive power of the resulting biological age, but could increase the potential for circularities in

deriving and aggregating body system ages based on similar health outcome definitions. Multi-state models with more complex structures (in terms of the number of allowable transitions between health states) would also be more computationally intensive, due to the usual approach for computing maximum likelihoods for this model.[180]

When biological ages were used to predict health outcomes, they were used in unadjusted models and models adjusted for sociodemographic and health behavioural factors, whereas when they were estimated, they were not adjusted by these factors. Focusing on a healthy subpopulation, where participants were more likely to have beneficial health behaviours and live in favourable environments for maintaining health, reduces the need to account for these non-biological risk factors. Many studies have shown that these non-biological factors are not just causally related to constituent biomarkers in the biological ages,[181] but are also potentially causally related to mortality and frailty via both biological and non-biological pathways.[182] Hence there is a risk of accounting for these risk factors more than once in the analytical process. One potential solution to avoid double counting, adding these non-biological risk factors to the estimation process, was not explored as it would result in 'biological ages' no longer being strictly biological, and increasing the difficulty of interpreting these biological ages due to ecological fallacy – e.g. if an individual moved house or reduced their alcohol consumption substantially, their estimated biological age would change immediately upon recalculation.

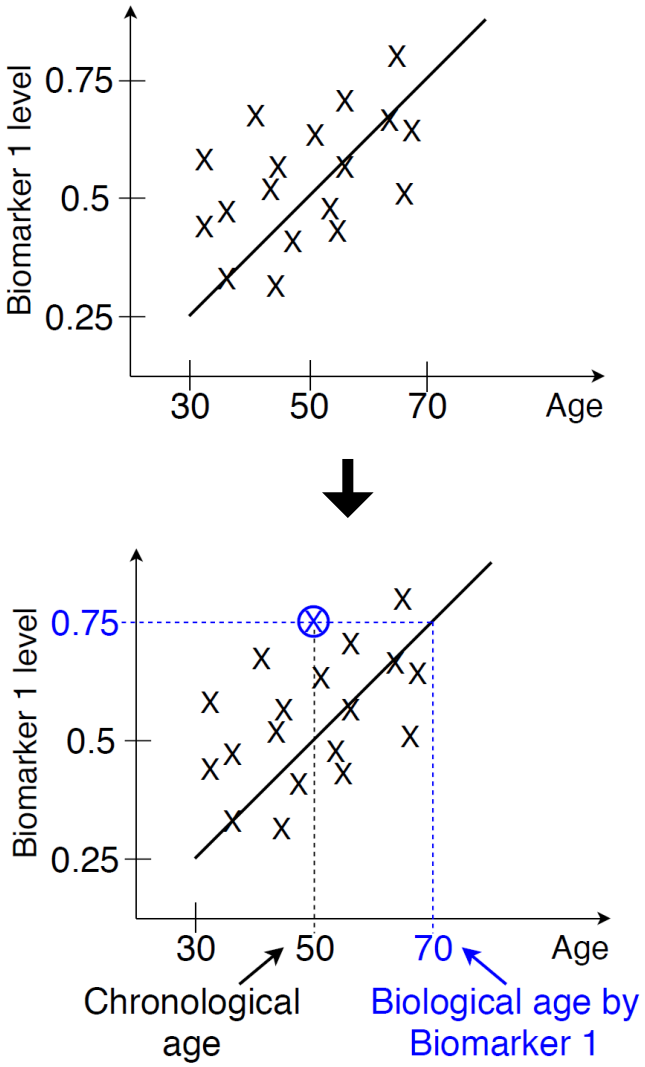
Conclusion

This chapter critically reviewed a wide range of methods used to estimate and validate biological ages, body system ages and biological ageing scores, to arrive at a statistically rigorous and consistent analytical process that was also clinically relevant. It also extended the framework of types of biological age estimation methods by contrasting age-based and disease risk-based methods, adding to the limited range of estimation methods that were not reliant on chronological age as a proxy for biological ageing. This thesis contains the first known description of methods to aggregate

body system ages into an overall biological age, allowing for the simultaneous and in-depth assessment of individuals' general and body system-specific health profiles.

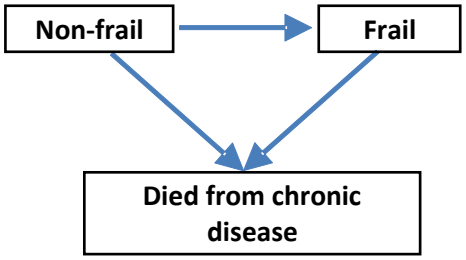
Chapter 4 tables and figures

Figure 4.2.1: Single biomarker illustration of the Klemera Doubal method

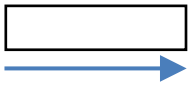


In this single biomarker illustration, the crosses represent the pattern of biomarker measurements in a population, and the diagonal regression line describes the average biomarker level at each age for this population. Using this line, a person aged 50 years with a biomarker level of 0.75 (circled in blue) is estimated to have a higher biological age of 70. The Klemera Doubal method aggregates these estimated biological ages for each biomarker across all constituent biomarkers, emphasising the biomarkers with the strongest linear relation to chronological age.

Figure 4.4.1: Structure of 3-state multi-state model used for the aggregation of body system ages



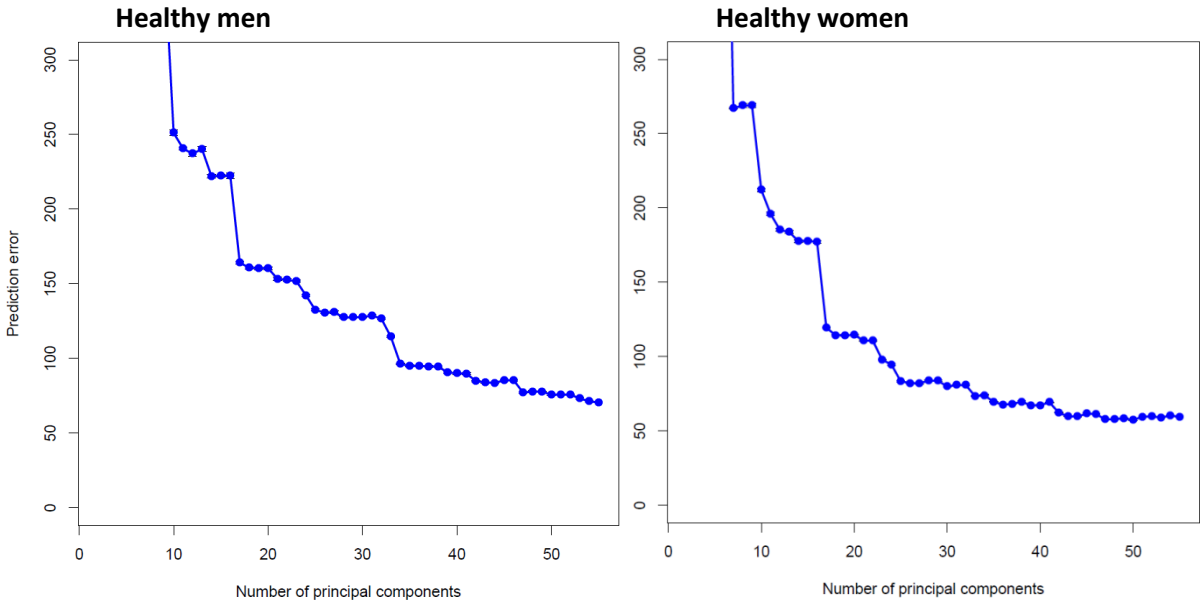
Key:



Non-overlapping health state. Individuals belong to exactly one health state at any given point in time

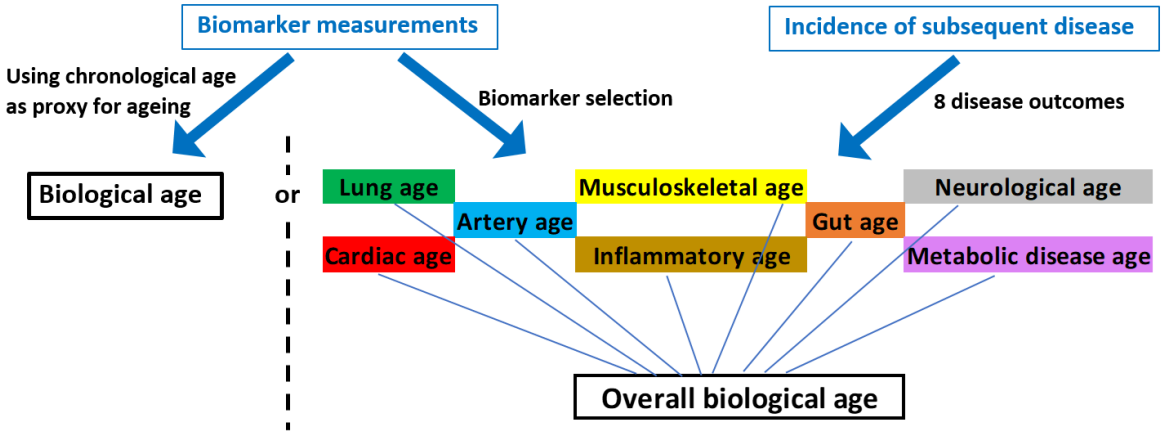
Allowable transitions of individuals between states

Figure 4.5.1: 10-fold cross validation prediction errors (with standard error bars) for each subset of principal components (to a maximum of 55) using the age-based Klemera Doubal method, for healthy men and healthy women



These plots display prediction errors (mean square errors of biological ages; y-axes) and their standard error bars, for each biological age constructed from the specified number of principal components (x-axes). They were used to search for an elbow point, where beyond the elbow point there were diminishing changes in prediction error by increasing number of principal components.

Figure 4.6.1: Summary of the biological age estimation process



The process on the left represents the age-based biological age analysis, while the process on the right represents the disease risk-based body system age and biological age analysis

Chapter 5: Age-based biological ages

5.1 Introduction

In previous chapters, the reviews of large scale, validated biological age studies (Chapter 2) and biological age estimation methods (Chapter 4) pinpointed 3 established methods for estimating biological age: multiple linear regression (MLR), principal component analysis (PCA) and Klemera Doubal method (KDM), all of which were based on the relationships of constituent biomarkers with chronological age. The details of applying these age-based methods to construct biological ages and validating the resulting ages were described in Chapter 4, and these age-based methods will also be contrasted with more novel outcome-based methods in subsequent chapters.

Few previous studies compared different methods for estimating biological age, but those that made comparisons generally applied the 3 established age-based methods, and favoured the KDM.[12,42,96,179] The KDM biological ages in these studies were found to be better calibrated to chronological ages and more indicative of individuals' health status in 2 studies of Korean populations,[42,96] while they were found to be more predictive of mortality in a US population.[12] KDM was favoured by the Singaporean study even though KDM biological ages were similarly predictive of mortality, incident and prevalent frailty to MLR and PCA biological ages.[179] Additionally, the Singaporean study compared KDM with several machine learning methods and found that the KDM biological age was more predictive of mortality and frailty than those estimated using machine learning methods in the same population.[179] When KDM biological age was compared with other indicators of ageing: homeostatic dysregulation, PhenoAge, and leukocyte telomere length, KDM biological age was also found to have the strongest correlation with indicators of physical and cognitive function that were not used to construct the biological age.[183] None of the other indicators of ageing in the latter comparison study were expressed in terms of an age, apart from PhenoAge, which is discussed in Chapters 4 and 6.

When Cho et al compared KDM biological ages estimated with and without prior PCA, they found that both types of biological ages were similarly indicative of individuals' health status.[42] Since many studies of KDM biological ages included chronological age as a constituent 'biomarker' of the biological age,[12,16,42,57,58,78,94,96,97,99,179,183] they reported results for the effects of the constituent biomarkers and chronological age jointly rather than separately. Comparisons of KDM biological age with vs without the inclusion of chronological age as a constituent 'biomarker' in a Canadian population showed that these two types of biological ages predicted mortality equally well when adjusted for chronological age and sex[13] (Section 4.2), but there was a slight difference in predictive power when adjusted for sex only (difference in C-index: 0.020).[13]

Only 2 previous studies that limited reverse causality between biomarkers and later life health were identified.[95,96] These 2 Korean studies of MLR, PCA and KDM biological ages restricted the analysis to healthier subpopulations that did not have prior health conditions.[95,96] The effect of restricting to a healthier subpopulation was not reported by either study.

This chapter describes findings from the age-based estimation and validation of biological ages in the healthy UK Biobank subpopulation, with the objectives of: (1) evaluating the performance of biological ages estimated using established age-based methods applied to biomarker principal components, (2) identifying the main biomarker determinants of the biological age, and (3) investigating the relationship between biological and chronological age in the prediction of mortality from chronic disease and age-related frailty.

5.2 Methods

For the main analyses in this thesis, biological ages were estimated for the healthy subpopulation, using the 51 biomarker principal components derived from the 72 candidate biomarkers (described in Sections 3.8 and 4.5). To estimate biological age, the Klemera Doubal method (KDM), which emphasised biomarkers most strongly related to chronological age, was applied to biomarker

principal components for men and women separately (Section 4.2). The stability of the estimated biological ages was checked via 10-fold cross-validation (Section 4.5).

Biomarker principal components were ranked by their importance, measured by the proportion of variance in the biomarker ages that they each explained (Section 4.2). The proportion of the overall biological and chronological age effect on the risks of mortality from chronic disease and age-related frailty that was explained by biological age was also estimated, by comparing the log-likelihoods from Cox models (Section 4.5). Further validation of biological ages (assessed in terms of their predictive power, their calibration to chronological age and their calibration to the risks of mortality and frailty) was conducted. These procedures used the analytical approaches described in Sections 4.2 and 4.5.

In separate sensitivity analyses, stepwise linear regression was used to estimate the biological ages, the biological ages were estimated using KDM applied to a reduced panel of the biomarkers that corresponded to the top 10 biomarker principal components in the main analysis separately for men and for women. Furthermore, the main analysis was repeated on the whole UK Biobank population (Section 4.2). Results for the sensitivity analysis using the stepwise regression method are detailed in Appendix 5.1. The Guidelines for Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD)[36] checklist in Appendix 5.2 summarises the reporting of these results and other aspects of this analysis. Subsequent sections in this chapter describe and discuss the results from these analyses.

5.3 Biomarker constituents of biological ages

The model coefficients for each biomarker principal component are summarised in Table 5.3.1. For biological ages calculated in the healthy subpopulation, reduced lung function featured most strongly (Figure 5.3.1), describing 12.4% (men) and 10.3% (women) of the variation in biological age (Table 5.3.2). Higher cystatin C, slower reaction time, lower insulin-like growth factor-1 (IGF-1), lower

hand grip strength, and higher blood pressure also featured strongly for both sexes; while lower albumin, higher sex hormone-binding globulin and lower muscle mass biomarkers featured strongly for men; and higher levels of alkaline phosphatase, LDL-C and apolipoprotein B and HbA1c for women. Multiple body systems were represented by these biomarkers: respiratory, renal, cardiovascular, musculoskeletal, endocrine, metabolic and immune, liver and nervous systems (Table 5.3.2 and 3.8.1).

There were 13 biomarkers for men and 12 for women that strongly loaded onto the top 10 biomarker components for each sex (Figure 5.3.1): forced expiratory volume in 1s/height, forced vital capacity/height, reaction time, IGF-1, cystatin C, hand grip strength/height, systolic and diastolic blood pressure in both sexes; albumin, sex hormone-binding globulin, fat-free mass, standing height and sitting height in men; and LDL-C, alkaline phosphatase, HbA1c and urea in women. These biomarkers formed the reduced panel for the sensitivity analysis discussed in the subsequent sections.

5.4 Biological and chronological age effect of health risks

Relationship between biological and chronological age

Biological ages described 44.0% and 51.3% of the variation in chronological age in healthy men and women respectively. More importantly, averaged across sexes, the biological ages described 66% and 63% of the overall biological and chronological age effect on mortality from chronic disease and age-related frailty, respectively in unadjusted analyses (Figure 5.4.1 and Table 5.4.1 (A)). Constructing the biological age from the reduced panel of biomarkers strongly loaded onto the most important 10 biomarker components noted in Section 5.3 (Figure 5.3.1 and Table 5.3.1) decreased the proportion explained by biomarkers to 53% and 50% for each respective outcome in men, but made little difference for women (Figure 5.4.1 and Table 5.4.1 (B)).

In the whole population, biological ages described substantially higher proportions of the overall biological and chronological age effect on these health outcomes. Averaged across sexes and using the full biomarker panel in unadjusted analyses, they described 86% and 80% for mortality from chronic disease and age-related frailty respectively (Table 5.4.1), ~20% higher than in the healthy subpopulation alone.

5.5 Validation results

Predictive power of biological ages

In the healthy subpopulation, adding biological ages to the unadjusted prediction models with chronological age statistically significantly improved model fit (p-values for likelihood ratio tests for mortality/frailty: $p<1\times10^{-10}$/$p<1\times10^{-10}$ in healthy men; $p<0.0025$/$p<1\times10^{-6}$ in healthy women). It also increased the C-indices for mortality/hospital admission only slightly (0.008/0.003 in men, 0.002/0.001 in women) in the healthy subpopulation but resulted in greater increases in the whole UK Biobank population (0.056/0.014 in men, 0.026/0.011 in women) (Table 5.5.1 (A)). C-indices for the prediction of age-related frailty were greater for biological age than for the benchmark mortality score (difference in C-indices: 0.111/0.084 in healthy men/women and 0.130/0.104 in all men/women; Table 5.5.1 (A)). The mortality score performed only slightly better than chance (equivalent to a C-index of 0.5; Table 5.5.1 (A)). Biological ages were less predictive of mortality and frailty than chronological age in the healthy subpopulation, but in the whole population, they had similar predictive power to the chronological ages for age-related frailty and were more predictive of mortality, based on C-indices from unadjusted prediction models (Table 5.5.1 (A)).

When the KDM biological age was constructed from the reduced biomarker panel, there was little change in its predictive power for these health outcomes in the healthy subpopulation (reduction in C-indices: 0.000–0.004; Table 5.5.1 (A)). In the whole population, the reduction was more

substantial for men (reduction in C-indices for mortality/frailty: 0.020/0.008) but not for women (reduction in C-indices for mortality/frailty: 0.002/0.002; Table 5.5.1 (A)).

Sociodemographic factors and health behaviours were also associated with higher biological ages (e.g. living in an area classified within the most vs least deprived Index of Multiple Deprivation 2010 (IMD) quintile conferred an extra 3.6/2.3 years of biological age for healthy men/women after adjusting for chronological age alone). However, adjustment for these factors did not substantially attenuate the differences in C-indices associated with adding biological age into the prediction models with chronological age (Table 5.5.1 (B)).

Calibration of biological ages

The small standard errors in the plot of cross-validated prediction errors in Figure 4.5.1 showed that biological ages estimated using KDM were relatively stable. The optimal number of biomarker principal components to include in the analysis was also determined to be 51 from the same cross-validation analysis (Section 4.5).

The KDM biological ages were well calibrated to chronological age, as they matched healthy participants' chronological ages on average (Figure 5.5.1). These KDM ages identified 17.1% of healthy participants who were ≥5 years biologically younger and 16.9% who were ≥5 years biologically older than their chronological age. Biologically older participants had the highest mortality and frailty rates, while biologically younger participants had the lowest rates, for both sexes (Figure 5.5.2). On aggregate, the differences in mortality and frailty rates between individuals who were biologically older, similar or younger than their chronological age were statistically significant based on log-rank tests ($p<0.0001$ for both sexes and outcomes, except for mortality in healthy women, where $p<0.018$).

5.6 Discussion

This analysis found that biological ages consisting of markers of impaired function in a range of organs accounted for a substantial proportion of the apparent effect of chronological age on mortality and frailty. The KDM produced a well-calibrated biological age that supplemented chronological age in predicting subsequent mortality from chronic disease and age-related frailty, but was not substantially more predictive than chronological age alone. In comparison, the stepwise regression method, an extension of the established MLR method (Chapter 4.2 and Appendix 5.1), produced biological ages that were similarly predictive to the KDM biological ages but were poorly calibrated and hence could not be recommended in this context.

Key biomarkers in biological ages and their relationships with chronological age

Lung, kidney, cognitive and liver function, IGF-1, hand grip strength and blood pressure biomarkers were key contributors to biological ages for both sexes, while sex hormone-binding globulin and muscle mass in men, and cardiovascular function and HbA1c in women were also important.

These top-ranking biomarkers in this UK population generally matched those in a Singaporean study[179] and slight differences by sex were seen in both populations. However, these lung and renal function biomarkers were not investigated in the study comparing Canadian, South Korean and Eastern European biological ages, which instead found that the top-ranking blood-based biomarkers varied by population and sex.[98] Studies of ageing biomarkers also found that lung and renal biomarkers were top-ranking determinants of functional decline[184] and variation in age-related traits.[185] The present study provides additional detail on the relative importance on ageing of biomarkers within body system groups, such as finding cystatin C to be more important than other renal biomarkers (creatinine and creatinine-based eGFR),[186] as previous studies each assessed only one of these biomarkers.[98,179,184,185]

Several key biomarkers in this study (blood pressure, blood lipids, height and lung function) have each been shown to be associated observationally (in prospective studies), and in some cases causally (in randomised trials and Mendelian randomisation studies), with a range of age-related chronic diseases (Table 5.6.1). Associations of other key biomarkers such as cystatin C and hand grip strength have been less extensively researched, and available studies focused on mortality and cardiovascular outcomes[186-190] (Table 5.6.1).

Lung function biomarkers, systolic blood pressure, cystatin C and reaction time had the strongest linear relationships with age (Table 3.10.1 and Figure 3.10.2), and therefore contributed substantially to variation in the KDM biological ages (Figure 5.3.1). However, biomarkers of age-related diseases that do not themselves have a strong relationship with chronological age may have been under-represented. For example, BMI has been causally linked to 30 diseases (including many age-related diseases),[191] but the general adiposity component was only 28th most important for men and 26th for women (Table 5.3.1). Likewise, lipid-related cardiovascular biomarkers, LDL-C and apolipoprotein B are causally linked to atherosclerotic cardiovascular disease in men and women,[192] but were only important in the KDM age for women and not men (Figure 5.3.1). On the other hand, blood pressure (10th most important for men and 8th for women; Figure 5.3.1) is well-established as a modifiable and causal risk factor of cardiovascular disease.[193]

Prediction in healthy versus unhealthier individuals

Knowledge of biological age is potentially more useful in apparently healthier individuals because in unhealthy individuals, their disease profile already provides diagnostic indicators of ageing.[22] Furthermore, the knowledge of risk of non-fatal outcomes is likely to provide a longer window for intervention and prevention than knowledge of mortality risk.

Biological ages were substantially better than the benchmark mortality score in predicting age-related frailty (Table 5.5.1), and in the whole UK Biobank, the improvement in predictive power for

mortality of biological age over chronological age after adjustment (Table 5.5.1 (B)) was comparable to or greater than the improvements reported by previous studies on US, Canadian and Singaporean populations, which showed improvements in C-indices of 0.014–0.041.[12,13,78,179] The predictive value of biological age was greater when the whole UK Biobank population (i.e. unhealthier individuals) were included in the estimation process (Table 5.5.1), likely reflecting an additional diagnostic element of these biomarkers in the less healthy individuals. Therefore, it is important to take into account the health and age profile of populations when comparing biological ages across different studies.[122] Other studies tended to analyse the whole population only – this analysis placed results from other studies in a clearer clinical context by demonstrating the variability of predictive power by prior health and by the inclusion or exclusion of chronological age (Table 5.5.1).

Clinical relevance of the findings

Comparison of an individual's biological age with their (unmodifiable) chronological age could provide a valuable means of communicating modifiable health risks, alongside their detailed biomarker profile.[76] A biological age could also augment a national prevention programme promoting clinical biomarker screening in a middle-aged population,[17] after causal factors underlying its constituents have been established. The most important biomarkers in biological age were measured via blood biochemistry measurements, spirometry and body size measurements, which can be administered routinely and simultaneously in clinical settings. For women, it may be suitable to measure just 12 key biomarkers (7 blood-based and 5 physical measurements) across 7 body systems to assess biological ageing, as relatively little explanatory and predictive value was compromised (Figure 5.4.1 and Table 5.5.1). Despite the successful use of clinical risk prediction tools such as 'heart age' and 'lung age' in clinical care,[64] there is little evidence as yet of implementation of an overall biological age. Previous studies of age-based biological ages proposed to use biological ages in drug development[40,98,106] and clinical care,[20,40,62] which are likely to be longer-term uses.

Strengths and limitations

The KDM permitted investigation of the relationship between biological and chronological ages with respect to predicting health outcomes (Section 5.4), and automatically calibrated biological ages to chronological age (Figure 5.5.1). In contrast to the stepwise regression method, it produced biological ages that were calibrated to chronological age (Figure 5.5.1 vs Figure A5.3) and mortality and frailty risk (Figure 5.5.2 vs Figure A5.4).

Unlike many other studies, this analysis did not target a biological age that replicated chronological age (by minimising its prediction error),[39,50,155] or include chronological age as a constituent 'biomarker' in the biological age.[12,16,42,57,58,78,94,96,97,99,179,183] Hence, it produced a biological age that was different from chronological age, and it could assess the degree to which constituent biomarkers accounted for the apparent effect of chronological age on mortality and frailty.

The investigation of key determinants of biological age were limited by the range of biomarkers available. The clinical biomarkers measured in the UK Biobank substantially overlapped with those used in previous large scale biological age studies (Table 3.8.2), however unlike the cohorts examined by recent studies of putative non-clinical ageing biomarkers,[18,46,58,100,194] the UK Biobank is not specifically a gerontological resource and has not yet obtained measurements of non-genetic molecular biomarkers. Comparisons of biological ages across cohort studies may also be limited by sociodemographic and health behavioural differences between the cohorts. Cohort effects in this population are difficult to disentangle, and may influence trends in body size. Hence, height (one of the top 15 most important biomarkers; Figure 5.3.1) may be acting as a proxy for cohort effects. Follow up data on UK Biobank participants was generally limited to hospital records, hence the analysis of later life sub-clinical functional and cognitive capability was restricted.

Biomarker trends with chronological age in the UK Biobank were not all completely linear (Figure 3.10.2), but a previous study showed that incorporating non-linearity and non-monotonicity (in

limited functional forms) only slightly improved the accuracy of its estimated biological ageing scores.[101] Moreover, biological ages estimated using KDM were found to be more predictive of mortality and frailty than biological ages estimated using machine learning methods in an older Singaporean population,[179] and the only clinical biomarker-based biological age[98] identified by a review of deep learning biological ageing scores[156] was also age-based but had not explored the improvement in predictive power from using a non-linear estimation method. The epidemiological reliability of these analyses was increased by focusing on a healthy subpopulation, using biomarker principal components, cross validation and adherence to clinical risk prediction reporting guidelines (Appendix 5.2).[164]

Even when biological age was estimated on the whole UK Biobank population, it may have captured a healthy volunteer effect due to the method used by UK Biobank for recruiting participants.[131] The calibration of biological ages to chronological ages on average should be reviewed if the biological ages are implemented in a different population. These biological ages apply to the chronological age range of the cohort (40-70 years) and can be extrapolated to ages outside of this range only after applying additional assumptions or conducting external validation.

Conclusions

An age-based biological age consisting of clinical biomarkers reflecting functionality of a range of organs accounted for a substantial proportion of the effect of age on mortality and frailty in the UK Biobank. It has the potential to be used and evaluated as a broader-based approach to risk identification and prevention than individual biomarkers. Of the most important biomarkers contributing to the KDM biological age, cardiometabolic biomarkers have well-studied causal associations with mortality and cardiovascular disease, but further research is needed to identify modifiable causal factors underlying all components, for the range of age-related diseases that constitute the outcomes of the ageing process. The subsequent chapters will bridge the gap between this broader-based approach and a more clinically-oriented approach that differentiates the extent of

ageing between body systems, and will discuss whether the more novel outcome-based approaches that take into account multiple age-related chronic diseases can improve the performance of biological ages in these respects.

Chapter 5 tables and figures

Table 5.3.1: Model coefficients for the age-based biological ages in the healthy subpopulation, by sex

Biomarker principal component number and description		Healthy men			Healthy women		
		q_j	k_j	s_j	q_j	k_j	s_j
PC1	General adiposity	55.224	0.004	11.865	56.673	-1.035	10.628
PC2	Total haemoglobin volume	56.276	1.035	11.814	58.763	-3.455	10.344
PC3	Height	56.007	3.638	10.626	56.429	3.049	10.038
PC4	Albumin	56.235	5.479	10.676	55.896	-0.074	10.896
PC5	Neutrophil count	55.806	-1.896	11.633	56.036	-0.543	10.875
PC6	Immature red blood cell volume	55.353	-2.078	11.565	55.880	0.151	10.894
PC7	LDL and ApoB	55.047	-0.746	11.824	55.293	-4.247	9.166
PC8	Reticulocyte count	55.226	0.055	11.865	56.530	-1.526	10.739
PC9	Urinary potassium and creatinine	55.248	-0.101	11.864	55.950	0.251	10.893
PC10	Blood pressure	54.700	-2.940	11.374	56.959	-3.768	9.829
PC11	HDL and ApoA	55.715	0.980	11.811	55.797	0.126	10.895
PC12	Aminotransferases	55.303	0.325	11.860	57.384	-4.235	10.206
PC13	Bilirubin	55.759	1.016	11.774	55.432	1.558	10.719
PC14	Platelet count	55.067	-0.343	11.858	55.798	0.319	10.888
PC15	Red blood cell haemoglobin concentration	55.442	-1.244	11.798	55.889	-0.048	10.896
PC16	Testosterone	54.896	-0.385	11.862	56.129	-0.345	10.896
PC17	Lung function/height	57.442	-5.247	9.508	57.285	-4.819	8.697
PC18	Blood glucose	55.467	2.362	11.694	56.254	3.519	10.508
PC19	Platelet cell volume	55.216	-0.181	11.864	55.929	-0.722	10.869
PC20	LP(a)	55.233	0.081	11.865	55.886	0.906	10.858
PC21	Pairs matching test	55.541	-3.107	11.464	56.001	-2.285	10.670
PC22	Rheumatoid factor	55.234	0.597	11.851	55.898	0.455	10.887
PC23	Bone density	55.485	-0.830	11.836	55.451	-2.792	10.587
PC24	Vitamin D	55.180	0.897	11.832	55.882	0.242	10.893
PC25	IGF-1	56.478	5.561	10.692	56.011	3.770	10.242
PC26	Urinary microalbumin	55.491	3.197	11.636	56.257	2.829	10.767
PC27	Basophil count	55.356	1.306	11.809	55.893	-0.153	10.895
PC28	Central adiposity	56.393	-4.109	11.274	56.605	-3.698	10.338
PC29	Eosinophil count	55.214	0.838	11.836	56.041	0.829	10.869
PC30	Alkaline phosphatase	55.614	-2.022	11.738	56.426	-5.813	9.215
PC31	Pulse rate	55.576	-1.408	11.783	55.926	-1.888	10.763
PC32	Red blood cell width	55.694	-3.190	11.552	55.904	-0.203	10.894
PC33	Reaction time test	56.417	-5.222	10.788	55.963	-4.210	10.109
PC34	Sex hormone-binding globulin	58.143	7.049	11.096	56.932	-2.355	10.600
PC35	Hand grip strength/height	56.156	5.354	10.716	56.783	5.022	9.845
PC36	Phosphate	54.881	1.169	11.806	55.469	-2.109	10.724
PC37	Lymphocyte count	55.340	-0.547	11.855	55.902	-2.055	10.722
PC38	Triglycerides	55.226	0.008	11.865	57.879	6.395	9.838
PC39	Urinary sodium	55.393	0.634	11.847	55.754	0.477	10.888
PC40	Monocyte count	54.981	2.936	11.544	56.276	1.301	10.841
PC41	Gamma glutamyltransferase	55.145	0.680	11.847	57.554	4.670	10.325
PC42	Urea	54.943	2.177	11.690	56.967	4.759	9.985
PC43	HbA1c	56.395	4.928	11.232	57.306	6.937	9.638
PC44	Platelet distribution width	55.217	0.071	11.865	55.964	0.583	10.882
PC45	Log C-reactive protein	56.097	4.298	11.264	56.620	4.315	10.172
PC46	Reticulocyte fraction	55.424	1.087	11.821	56.028	0.913	10.862
PC47	Cystatin C	55.163	-6.091	10.883	58.861	-7.437	9.137
PC48	Muscle mass	55.919	-6.416	10.722	56.018	-4.356	10.485
PC49	Calcium	55.023	-3.212	11.488	55.788	3.217	10.429
PC50	Total protein	55.330	2.042	11.695	55.903	-1.683	10.770
PC51	Urate	54.829	0.870	11.845	59.192	6.360	9.978

q_j = intercept, k_j = coefficient and s_j = square root of residual variance for the j^{th} biomarker component
These biological ages were estimated using the Klemera Doubal method

Table 5.3.2: Importances of the 51 biomarker principal components in the age-based biological ages for healthy men and healthy women

Healthy men

Rank	Biomarker principal component number and description	% of total R^2
1	PC17 Lung function/height	12.4
2	PC33 Reaction time test	6.9
3	PC25 IGF-1	6.7
4	PC47 Cystatin C	6.7
5	PC35 Hand grip strength/height	6.4
6	PC4 Albumin	6.3
7	PC34 Sex hormone-binding globulin	6.0
8	PC48 Muscle mass	5.9
9	PC3 Height	5.6
10	PC10 Blood pressure	3.5
11	PC43 HbA1c	3.5
12	PC28 Central adiposity	2.9
13	PC21 Pairs matching test	2.6
14	PC45 Log C-reactive protein	2.5
15	PC49 Calcium	2.1
16	PC6 Immature red blood cell volume	2.0
17	PC32 Red blood cell width	1.8
18	PC50 Total protein	1.7
19	PC40 Monocyte count	1.5
20	PC42 Urea	1.5
21	PC26 Urinary microalbumin	1.4
22	PC18 Blood glucose	1.2
23	PC5 Neutrophil count	1.1
24	PC11 HDL and ApoA	0.8
25	PC24 Vitamin D	0.6
26	PC30 Alkaline phosphatase	0.6
27	PC2 Total haemoglobin volume	0.6
28	PC1 General adiposity	0.5
29	PC39 Urinary sodium	0.5
30	PC7 LDL and ApoB	0.4
31	PC36 Phosphate	0.4
32	PC31 Pulse rate	0.4
33	PC13 Bilirubin	0.4
34	PC15 Red blood cell haemoglobin concentration	0.3
35	PC16 Testosterone	0.3
36	PC14 Platelet count	0.3
37	PC27 Basophil count	0.3
38	PC51 Urate	0.2
39	PC8 Reticulocyte count	0.2
40	PC23 Bone density	0.2
41	PC12 Aminotransferases	0.2
42	PC38 Triglycerides	0.2
43	PC46 Reticulocyte fraction	0.2
44	PC41 Gamma glutamyltransferase	0.1
45	PC22 Rheumatoid factor	0.1
46	PC29 Eosinophil count	0.1
47	PC37 Lymphocyte count	0.1
48	PC9 Urinary potassium and creatinine	0.1
49	PC44 Platelet distribution width	0.0
50	PC19 Platelet cell volume	0.0
51	PC20 LP(a)	0.0

Healthy women

Rank	Biomarker principal component number and description	% of total R^2
1	PC17 Lung function/height	10.3
2	PC47 Cystatin C	8.0
3	PC7 LDL and ApoB	7.0
4	PC30 Alkaline phosphatase	6.6
5	PC43 HbA1c	5.9
6	PC35 Hand grip strength/height	5.6
7	PC42 Urea	4.9
8	PC10 Blood pressure	4.9
9	PC33 Reaction time test	4.6
10	PC25 IGF-1	4.0
11	PC3 Height	3.8
12	PC38 Triglycerides	3.7
13	PC51 Urate	3.2
14	PC12 Aminotransferases	2.8
15	PC45 Log C-reactive protein	2.2
16	PC23 Bone density	2.2
17	PC2 Total haemoglobin volume	2.1
18	PC41 Gamma glutamyltransferase	1.9
19	PC18 Blood glucose	1.9
20	PC28 Central adiposity	1.8
21	PC48 Muscle mass	1.8
22	PC49 Calcium	1.7
23	PC21 Pairs matching test	1.3
24	PC36 Phosphate	1.0
25	PC34 Sex hormone-binding globulin	0.8
26	PC1 General adiposity	0.8
27	PC26 Urinary microalbumin	0.7
28	PC13 Bilirubin	0.7
29	PC37 Lymphocyte count	0.5
30	PC31 Pulse rate	0.4
31	PC8 Reticulocyte count	0.4
32	PC50 Total protein	0.3
33	PC11 HDL and ApoA	0.3
34	PC39 Urinary sodium	0.3
35	PC4 Albumin	0.2
36	PC20 LP(a)	0.2
37	PC16 Testosterone	0.2
38	PC24 Vitamin D	0.2
39	PC40 Monocyte count	0.1
40	PC19 Platelet cell volume	0.1
41	PC44 Platelet distribution width	0.1
42	PC9 Urinary potassium and creatinine	0.1
43	PC5 Neutrophil count	0.1
44	PC46 Reticulocyte fraction	0.1
45	PC14 Platelet count	0.1
46	PC29 Eosinophil count	0.1
47	PC32 Red blood cell width	0.1
48	PC22 Rheumatoid factor	0.1
49	PC6 Immature red blood cell volume	0.1
50	PC27 Basophil count	0.0
51	PC15 Red blood cell haemoglobin concentration	0.0

Table 5.4.1: Relative contribution (as a percentage of the total contribution of biological and chronological ages) of the age-based biological age and chronological age in explaining each health outcome, in (A) the main analysis and (B) when using the reduced biomarker panel, in the healthy subpopulation and whole UK Biobank population

(A) Main analysis

	Mortality from chronic disease (%)			Age-related frailty (%)		
	CA alone	CA and BA	BA alone	CA alone	CA and BA	BA alone
Men						
Healthy subpopulation	28.3	63.5	8.2	34.7	61.4	4.0
Whole population	8.3	56.6	35.1	20.8	64.5	14.7
Women						
Healthy subpopulation	39.0	58.3	2.7	39.6	58.7	1.7
Whole population	18.9	60.8	20.4	18.8	66.1	15.1

(B) Using the reduced biomarker panel

	Mortality from chronic disease (%)			Age-related frailty (%)		
	CA alone	CA and BA	BA alone	CA alone	CA and BA	BA alone
Men						
Healthy subset	47.0	49.9	3.1	50.0	48.2	1.9
Whole population	23.0	55.2	21.9	42.5	51.7	5.8
Women						
Healthy subpopulation	35.0	60.3	4.7	39.8	58.2	2.0
Whole population	20.4	61.3	18.3	21.2	65.1	13.6

CA: chronological age, BA: biological age

The reduced biomarker panel consisted of 13 biomarkers for men and 12 for women: forced expiratory volume in 1s/height, forced vital capacity/height, reaction time, IGF-1, cystatin C, hand grip strength/height, systolic and diastolic blood pressure in both sexes; albumin, sex hormone-binding globulin, fat-free mass, standing height and sitting height in men; and LDL-C, alkaline phosphatase, HbA1c and urea in women.

Table 5.5.1: Harrell's C-indices (with standard errors) for each health outcome, age-based biological age vs chronological age, mortality score and a reduced biomarker panel biological age

(A) Unadjusted analysis

Outcome and age predictor	Healthy subpopulation		Whole population	
	Men	Women	Men	Women
Mortality from chronic disease				
CA alone	0.712 (0.008)	0.667 (0.009)	0.686 (0.003)	0.670 (0.003)
BA alone	0.689 (0.008)	0.635 (0.009)	0.736 (0.003)	0.683 (0.003)
BA and CA	0.720 (0.008)	0.669 (0.009)	0.742 (0.003)	0.696 (0.003)
Improvement of BA and CA over CA	*0.008*	*0.002*	*0.056*	*0.026*
BA and CA (using reduced biomarker panel)	0.716 (0.008)	0.670 (0.009)	0.722 (0.003)	0.694 (0.003)
Change due to reduction in biomarkers	*-0.004*	*-0.001*	*-0.020*	*-0.002*
Age-related frailty				
CA alone	0.636 (0.003)	0.606 (0.003)	0.629 (0.001)	0.603 (0.001)
BA alone	0.615 (0.003)	0.586 (0.003)	0.630 (0.001)	0.605 (0.001)
BA and CA	0.639 (0.003)	0.608 (0.003)	0.643 (0.001)	0.614 (0.001)
Improvement of BA and CA over CA	*0.003*	*0.001*	*0.014*	*0.011*
Mortality score	0.504 (0.003)	0.503 (0.003)	0.500 (0.001)	0.501 (0.001)
Improvement of BA alone over mortality score	*0.111*	*0.084*	*0.130*	*0.104*
BA and CA (using reduced biomarker panel)	0.638 (0.003)	0.608 (0.003)	0.635 (0.001)	0.612 (0.001)
Change due to reduction in biomarkers	*-0.001*	*0.000*	*-0.008*	*-0.002*

(B) Adjusted for sociodemographic factors and health behaviours

Outcome and age predictor	Healthy subpopulation		Whole population	
	Men	Women	Men	Women
Mortality from chronic disease				
CA alone	0.724 (0.008)	0.688 (0.009)	0.725 (0.003)	0.699 (0.003)
BA alone	0.702 (0.008)	0.660 (0.009)	0.746 (0.003)	0.697 (0.003)
BA and CA	0.731 (0.008)	0.690 (0.009)	0.756 (0.003)	0.713 (0.003)
Improvement of BA and CA over CA	*0.007*	*0.002*	*0.031*	*0.014*
BA and CA (using reduced biomarker panel)	0.726 (0.008)	0.691 (0.009)	0.742 (0.003)	0.712 (0.003)
Change due to reduction in biomarkers	*-0.005*	*0.001*	*-0.014*	*-0.001*
Age-related frailty				
CA alone	0.660 (0.003)	0.633 (0.003)	0.677 (0.001)	0.656 (0.001)
BA alone	0.640 (0.003)	0.614 (0.003)	0.673 (0.001)	0.653 (0.001)
BA and CA	0.662 (0.003)	0.634 (0.003)	0.685 (0.001)	0.662 (0.001)
Improvement of BA and CA over CA	*0.002*	*0.001*	*0.008*	*0.006*
Mortality score	0.574 (0.003)	0.568 (0.003)	0.605 (0.001)	0.596 (0.001)
Improvement of BA over mortality score	*0.066*	*0.046*	*0.068*	*0.057*
BA and CA (using reduced biomarker panel)	0.661 (0.003)	0.634 (0.003)	0.680 (0.001)	0.662 (0.001)
Change due to reduction in biomarkers	*-0.001*	*0.000*	*-0.005*	*0.000*

CA: chronological age; BA: biological age

Analyses in (B) were adjusted for Index of Multiple Deprivation 2010 quintile, smoking status, alcohol consumption and assessment centre.
The reduced biomarker panel consisted of 13 biomarkers for men and 12 for women: forced expiratory volume in 1s/height, forced vital capacity/height, reaction time, IGF-1, cystatin C, hand grip strength/height, systolic and diastolic blood pressure in both sexes; albumin, sex hormone-binding globulin, fat-free mass, standing height and sitting height in men; and LDL-C, alkaline phosphatase, HbA1c and urea in women.

Table 5.6.1: Examples of associations in published studies between the top 10 biomarker principal components of biological age in the age-based biological ages and adverse health outcomes

Key biomarker principal component in the present study	**Evidence from meta-analyses of randomised trials**	**Evidence from Mendelian randomisation**	**Evidence from prospective studies**
Lung function/height	-	Respiratory and autoimmune diseases[28]	All-cause, circulatory disease, respiratory and cancer mortality[29]
Cystatin C	-	*(Not significantly associated with cardiovascular disease[23])*	Cardiovascular disease, mortality[21,22] and end-stage renal disease[22]
Reaction time test	-	-	Mortality[30]
IGF-1	Fracture risk[31]	*(Not significantly associated with Alzheimer's disease[32])*	Mortality and heart failure[33] and cognitive function[34]
Hand grip strength/height	-	Cardiovascular disease and mortality[24]	Mortality, cardiovascular disease, respiratory disease, cancer[25]
Blood pressure	Mortality and cardiovascular disease[26]	Type 2 diabetes,[35] Alzheimer's disease[36]	Vascular mortality[37]
Albumin *(men only)*	-	-	Coronary heart disease[38]
Sex hormone-binding globulin *(men only)*	-	Type 2 diabetes[39]	Type 2 diabetes[40]
Muscle mass *(men only)*	-	-	Cancer mortality,[41] physical disability[42]
Height *(men only)*	-	Cardiovascular disease, hip fracture, intervertebral disc disorder, vasculitis, gastro-oesophageal reflux disease and cancer[43]	Mortality from cardiovascular disease, liver disease, COPD, stomach and oral cancers, mental disorders[44]
LDL-C and ApoB *(women only)*	Major vascular disease, vascular and all-cause mortality[45]	Coronary heart disease[46]	Cardiovascular disease[47]
Alkaline phosphatase *(women only)*	-	Type 2 diabetes[48] *(Associations with IHD and T2D not robust after allowing for pleiotropy[49])*	Osteosarcoma[50] and cardiovascular disease[51]
HbA1c *(women only)*	*(Not significantly associated with cardiovascular disease[52])*	Coronary artery disease[53]	Mortality, cardiovascular disease, cancer, diabetes[54]
Urea *(women only)*	-	-	Coronary heart disease[55]

Figure 5.3.1: Importance of the top 15 biomarker principal components in the age-based biological ages for healthy men and healthy women.

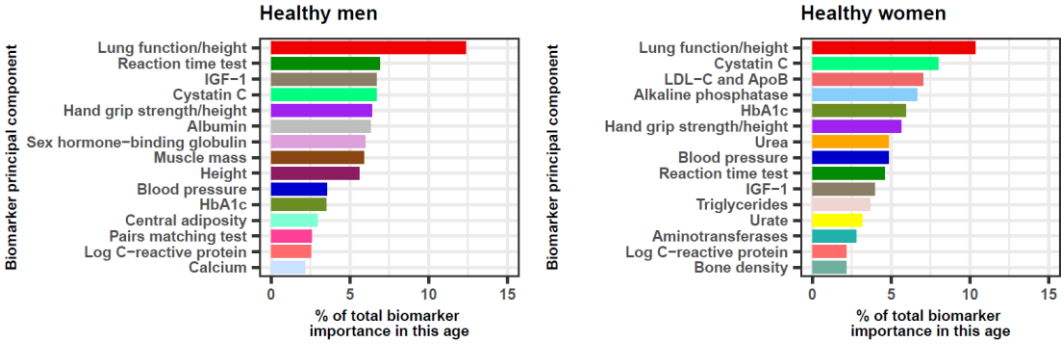
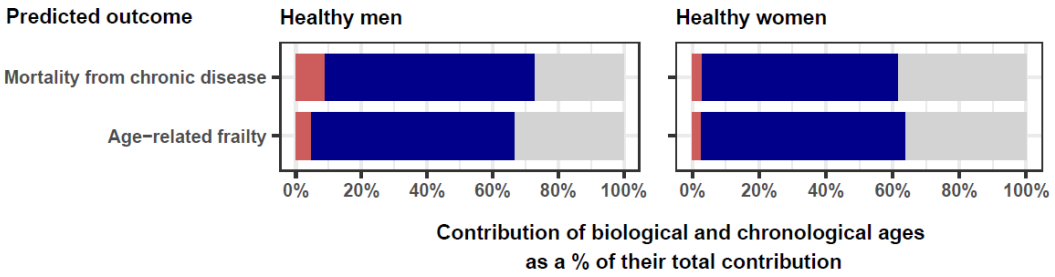
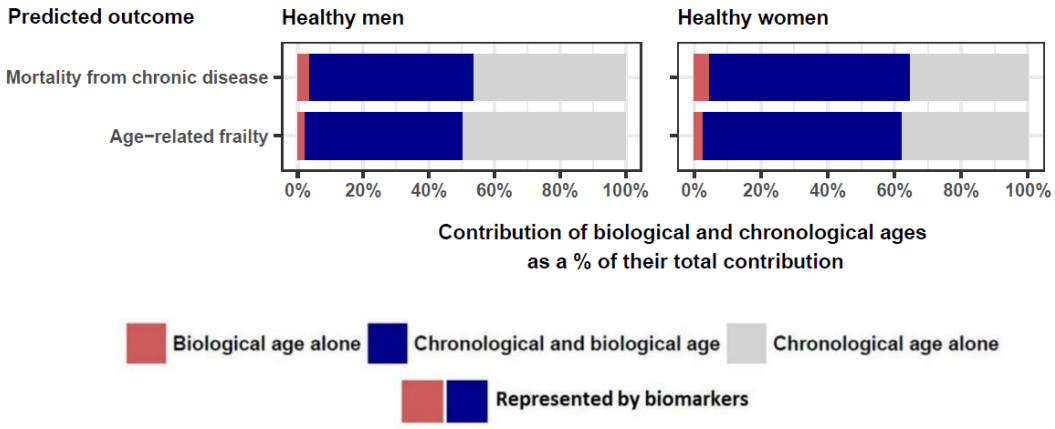


Figure 5.4.1: Relative contribution of age-based biological ages and chronological age in explaining each health outcome, in the (A) main analysis and when (B) using the reduced biomarker panel, for healthy men and women

(A) Main analysis

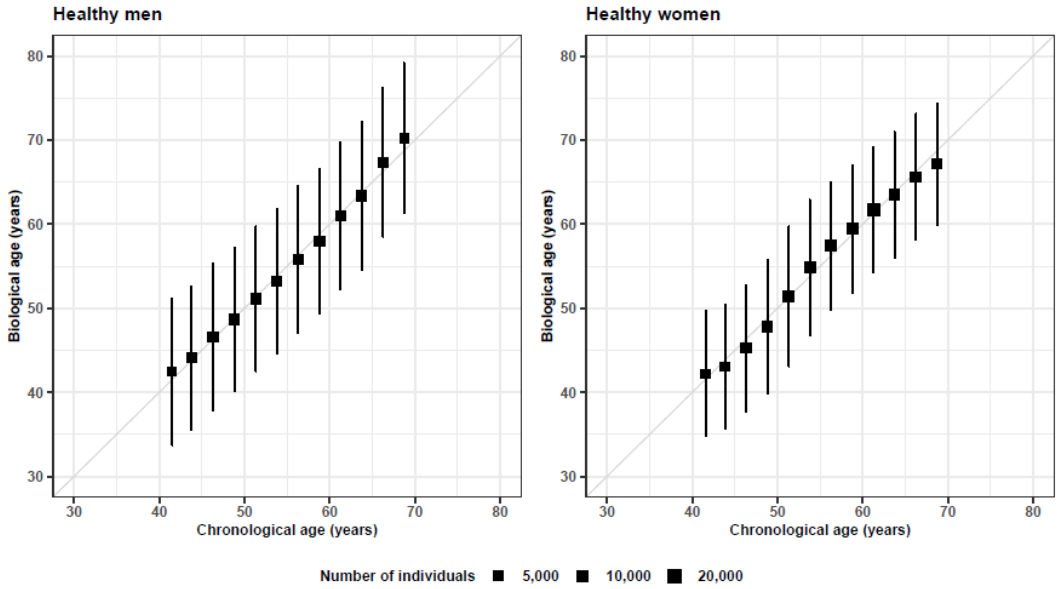


(B) Using the reduced biomarker panel



These percentages are also tabulated in Table 5.4.1

Figure 5.5.1: Means and standard deviations of age-based biological ages by 2.5-year chronological age groups, for healthy men and healthy women

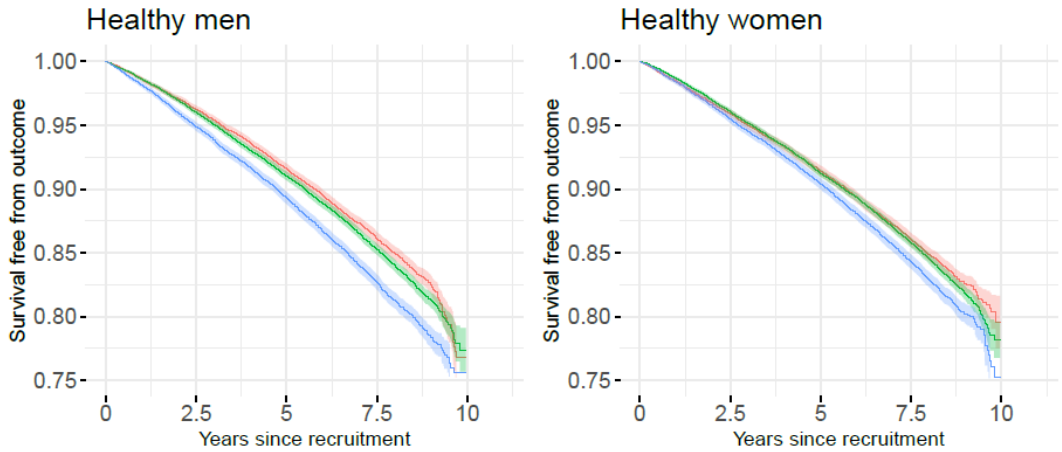


These plots indicate how well biological ages (y-axes) are calibrated to chronological age (x-axes), and the variability (indicated by ±1 standard deviation bars) of individuals' biological ages in each 2.5-year chronological age group.

Figure 5.5.2: Outcome-free survival of healthy men and healthy women for (A) mortality from chronic disease and (B) age-related frailty, according to whether their age-based biological age is younger, similar to or older than their chronological age

(A) Mortality from chronic disease

(B) Age-related frailty



Predicted risk group Kaplan-Meier curve and 95% confidence interval (shaded area):

BA - CA < -5 years (Biologically younger) | |BA – CA| < 5 years | BA - CA > 5 years (Biologically older)

Chapter 6: Disease risk-based body system ages and risk scores

6.1 Introduction

In the previous chapter, the age-based biological age was shown to identify biomarkers with the strongest relation to chronological age. This biological age was well calibrated to chronological age and identified markers of ageing across a range of body systems. However, it only slightly supplemented chronological age in predicting later life health. This chapter explores the construction of body system-specific ages and their companion risk scores from biomarkers of disease.

Limited research has been conducted on body system ages other than heart or vascular ages (Section 4.1).[54,56,116] The underlying risk scores for multi-biomarker (but not solely biomarker-based) heart ages were found to be prognostic for cardiovascular diseases in British primary care (from the QRISK group) and US Framingham Heart Study development cohorts.[195,196] The Framingham heart age was also found to be similarly predictive in diverse populations to standard Cox models that used the same information as these heart ages.[197]

The MediAge research centre has published the only known study of multiple body system ages formed by pre-selecting non-overlapping panels of biomarkers relevant to each body system and applying age-based estimation methods to South Korean clinical records (Section 4.1).[74,75] However, their study only assessed the relationships between each group of biomarkers and chronological age (Section 4.1), and did not assess (1) the relation of the body system ages to body system-specific disease outcomes, or (2) the relevance of the pre-selected biomarkers to each body system. In other studies, lung age and kidney age have been constructed from single biomarkers only (forced expiratory volume in 1s or forced vital capacity and estimated glomerular filtration rate [eGFR] respectively)[63,65] and there has been no research so far on how well they predicted later life disease, although definitions of these diseases are closely tied to these biomarker levels.

In contrast, there has been an abundance of research on disease risk scores, especially for cardiovascular disease.[69] The best known risk scores developed in British populations are QRISK scores for cardiovascular diseases[198] and the FRAX score for fractures.[199] Studies of disease risk scores and other epidemiological studies of biomarker-disease relationships tended to investigate only well-known biomarkers for those diseases, using hypothesis-driven rather than discovery-driven approaches. Hence there is limited information to assess the relative importance of a wide range of putative physical and biochemical biomarkers of different diseases.

This chapter describes findings from the analyses of 8 body system ages and risk scores estimated from biomarker measurements of UK Biobank participants, which represent the relative risks of body system-specific diseases either jointly with or independently from chronological age respectively. Body system ages were estimated as risk scores that were rescaled and expressed as an age to improve communication of disease risks to individuals (Chapter 1.4), using the estimation methods described in Section 4.3, while body system risk scores were estimated using a similar approach to body system ages and were not expressed as an age (Section 4.3). This chapter aimed to: (1) analyse the relationships between incidence of each disease group, its respective body system risk score and chronological age, (2) assess the most important biomarkers in each of the body system ages, compared with those for the respective body system risk scores, and (3) investigate the relationships between body system ages and chronological age in predicting later life disease.

6.2 Methods

The analysis for this chapter was conducted on the healthy subpopulation that was phenotyped in Section 3.3 and modified as described in Appendix 6.1 to exclude participants who could have been regarded as having screen-detected disease. This healthy subpopulation consisted of 54,948 men and 65,453 women after the exclusion (Table A6.1.1). The 8 body systems analysed in this thesis (and the disease groups that they represent) are artery (corresponding to atherosclerotic diseases),

musculoskeletal, gut, cardiac, metabolic disease, inflammatory, neurological and lung (corresponding to respiratory diseases).

Each of the 8 body system risk scores were estimated using the statistical approach described in Section 4.3: fitting a Cox lasso model to all candidate 51 biomarker principal components in the absence of chronological age, to select and emphasise the biomarkers most strongly related to incidence of the respective disease group (e.g. for cardiac age, the earliest hospital admission record with a diagnosis of cardiac arrhythmia or heart failure during follow up; Section 3.6). The risk score then consisted of the sum of the selected biomarker measurements weighted by their respective coefficients in the model.

Each of the 8 body system ages were estimated using a similar approach (Section 4.3): fitting a Cox lasso model to all 51 biomarker principal components in the presence of chronological age, and simultaneously rescaling the coefficients of the included biomarkers with the coefficient of chronological age in order to express the resulting risk score in terms of an age. All body system risk scores and ages were cross-validated (Section 4.5). Since initial analysis indicated that metabolic disease ages were poorly calibrated to chronological age on average, all 8 body system ages were recalibrated using the modified Dubina method described in Section 4.5 before further analysis and validation.

Firstly, the relationship of body system risk scores and chronological age to the disease risk were assessed in each of the 8 disease groups. Unadjusted Cox models were used to estimate hazard ratios of disease incidence for quintiles of each body system risk score and quintiles of chronological age separately (to isolate the contributions of biomarker levels and chronological age) and jointly (to assess their combined contribution), and with and without the first two years of follow up omitted to reduce the effect of reverse causation. Tertiles and Pearson correlations of the body system age deltas (the differences between body system ages and chronological age) in the healthy UK Biobank subpopulation were then summarised (Section 4.3). The importance of the biomarker principal

components in the 8 body system ages and risk scores based on their explained relative risk (Section 4.3) were compared.

The proportion of the overall age effect on the risks of each of the 8 disease groups that was explained by their respective body system age delta was assessed, and this analysis was repeated with body system risk scores in place of the age deltas. Further validation of body system ages (assessed in terms of their predictive power for disease incidence in their respective disease group, their calibration to chronological age and their calibration to the risks of their respective disease group) was conducted. These validation procedures were similar to those for the age-based biological ages (Sections 4.5 and 5.2). All the procedures described in this section used the analytical approaches described in Sections 4.3 and 4.5. Due to the large number of analyses in this chapter, supplementary tables and figures are reported in Appendix 6.2.

In separate sensitivity analyses, body system ages were estimated using a Cox stepwise regression (Section 4.3), and the body system ages were estimated using a Cox lasso model applied to a reduced panel of the 10 biomarker principal components that were most important across all 8 body system ages, separately for men and for women (Section 4.3). The main analysis was also repeated on the whole UK Biobank population. Results for the sensitivity analysis using the Cox stepwise regression method are detailed in Appendix 6.3. The Guidelines for Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD)[36] checklist in Appendix 6.4 summarises the reporting of these results and other aspects of this analysis. Subsequent sections in this chapter describe and discuss the results from these analyses.

6.3 Disease risk explained by body system risk scores and chronological ages

The biomarker principal component coefficients of the 8 risk scores are summarised in Table A6.2.1. Between 18 (in the inflammatory risk score for men) and 51 biomarker principal components (in the cardiac risk score for men) were selected for inclusion in these risk scores (Table A6.2.1).

For each of the 8 disease groups, hazard ratios of disease incidence for quintiles of body system risk scores (estimated independently from chronological age) and chronological age are summarised in Figure 6.3.1. The log hazard ratios increased broadly linearly with quintiles of chronological age and body system risk scores both when they were modelled separately and when they were cross-adjusted for both chronological age and body system risk scores (Figure 6.3.1 (A) and (B) respectively), except for the lowest body system risk score quintile for inflammatory diseases in both types of analyses.

The hazard ratios for the highest vs lowest quintiles of body system risk scores were also compared with those for chronological ages. In models that were not cross-adjusted, log hazard ratios for the highest vs lowest quintiles of body system risk scores were between 2.5 and 3.1 for metabolic and respiratory diseases in both sexes (Figure 6.3.1 (A)). In comparison, the hazard ratios for quintiles of chronological ages was much lower for metabolic disease (<1.5 for the highest vs lowest chronological age quintiles) and similar for respiratory disease (2.4 for healthy men and 2.9 for healthy women; Figure 6.3.1 (A)). Log hazard ratios for the highest vs lowest quintiles of body system risk scores for the remaining disease groups were between 1.4 –2.5 and were not substantially different from their respective ratios for highest vs lowest quintiles of chronological age (Figure 6.3.1 (A)). The patterns for adjusted models indicated that chronological age substantially attenuated the relationship between body system risk scores and disease risk, except for metabolic, inflammatory and respiratory diseases where its effect was less pronounced, and vice versa for body system risk scores and the relationship between chronological age and disease risk (Figure 6.3.1 (A) vs (B)).

When the first two years of follow up were omitted from the unadjusted models, the patterns of log hazard ratios across disease groups and sexes remained similar, but the hazard ratios for the highest body system risk score and chronological age quintiles were slightly attenuated (Figures 6.3.1 (A) vs A6.2.1).

6.4 Comparison of the eight body system ages

After estimating the body system ages, participants were ranked by body system age deltas and then split into tertiles of body system age deltas that represent people who were biologically younger, similar or older for each body system. The means and standard deviations of each tertile were summarised in Figure 6.4.1. The means for body system age deltas (apart from metabolic disease) were -17.9 to -1.8 years, 0.4 to 8.1 years and 7.1 to 51.7 years for biologically younger, similar or older participants respectively, while standard deviations (apart from metabolic disease) were between 1.1 and 8.2 years. Metabolic disease age displayed large differences from chronological age when split into tertiles and a large degree of variation within tertiles, with means of -28.9 to 131.8 years and standard deviations of 9.2 to 23.1 years respectively (Figure 6.4.1). The mean of 131.8 years for the biologically oldest tertile corresponded to metabolic disease ages of approximately 170–200 years, much greater than the maximum human lifespan of approximately 120 years.

Coefficients of the biomarker principal components for each body system age and each sex are displayed in Table A6.2.2. Between 14 (inflammatory age for men, and neurological and lung age for women) and 48 (artery age for men) biomarker principal components were included in these body system ages (Table A6.2.2).

Most body system age deltas were moderately positively correlated with each other (ρ=0.15–0.69 for pairs of body system age deltas excluding neurological age deltas, and ρ>0.3 for the majority of these pairs; Figure 6.4.2). The extent to which these correlations relate to common biomarker constituents of body system ages is investigated in Section 6.5. The exceptions were neurological age deltas for both sexes, which were slightly negatively correlated with all other body system age deltas for men (ρ=-0.27– -0.01) and slightly positively or negatively correlated for women (ρ=-0.37–0.19; Figure 6.4.2). Gut and metabolic disease age deltas had the highest correlation (ρ=0.69 for men and 0.66 for women; Figure 6.4.2). For healthy men, artery and gut, artery and inflammatory, gut

and lung age deltas had stronger correlations ($\rho \geq 0.5$), and for healthy women, artery and metabolic disease, cardiac and gut, cardiac and musculoskeletal, cardiac and metabolic disease, gut and musculoskeletal age deltas had stronger correlations (Figure 6.4.2).

All body system ages apart from metabolic age (ρ=0.335 for men and 0.232 for women) were highly positively correlated with chronological age, with $\rho \geq 0.772$ (Table 6.4.1). The most strongly positively correlated body system ages were neurological ages (ρ=0.927 for men and 0.951 for women).

6.5 Biomarker constituents of the body system ages and risk scores

Importance of the top 15 biomarker principal components in each body system age and risk score were ranked and displayed in Figures 6.5.1 (A) and (B) respectively. The biomarker importance represented the percentage that the biomarker explained of the total explained relative risk by all constituent biomarkers. The body system risk scores were estimated in the absence of chronological age while the body system ages were estimated in its presence (Section 6.2), hence differences in biomarker importance between the risk score and age for each body system were related to whether or not chronological age was adjusted for in the prediction model.

Most of the 8 body system ages had unique biomarkers that featured strongly, particularly lung function/height in lung age (biomarker importance: 40.7% for men and 43.8% for women) and HbA1c in metabolic age (45.1% for men and 36.9% for women; Figure 6.5.1 (A) and Table A6.2.3). The exception was neurological age, which consisted of a large range of similarly important biomarkers (Figure 6.5.1 (A)) even though its delta was poorly correlated with other body system age deltas (Figure 6.4.2). These biomarkers generally had directionally consistent associations across body system ages. The most commonly-featuring biomarkers across body system ages were general adiposity, lung function, blood pressure and hand grip strength/height.

Biomarker constituents of each body system age were generally different between body systems (Figure 6.5.1 (A)). The key similarities in biomarkers between body systems were adiposity biomarkers (which featured strongly in musculoskeletal, gut, cardiac and metabolic disease ages). Additionally, lung function featured in artery age (in addition to lung age; Figure 6.5.1 (A)). The most important biomarkers for healthy men vs women overlapped for artery, musculoskeletal, gut, metabolic disease and lung ages (Figure 6.5.1 (A)).

Due to the large number of differences, the 3 most important biomarker principal components are listed below by body system age and by sex (the directions relate to higher body system ages):

1. Artery age: higher blood pressure for both sexes, then higher LDL-C and ApoB and lower HDL-C and ApoA for men, and lower lung function/height and higher triglycerides for women
2. Musculoskeletal age: greater general adiposity for both sexes, then greater muscle mass and shorter height for men, and greater vitamin D levels and lower hand grip strength/height for women
3. Gut age: greater central adiposity for men but greater general adiposity for women, then greater general adiposity and higher reticulocyte count for men and greater central adiposity and lower hand grip strength/height for women
4. Cardiac age: taller height for men and greater general adiposity for women, then higher sex hormone-binding globulin and higher general adiposity for men, and taller height and poorer lung function/height for women
5. Metabolic disease age: higher HbA1c and greater general adiposity for both sexes, then higher reticulocyte count for men and higher cystatin C for women
6. Inflammatory age: higher rheumatoid factor for men and lower hand grip strength/height for women, then higher blood pressure for men and higher rheumatoid factor for women, then higher log C-reactive protein for both sexes

7. Neurological age: higher insulin growth factor-1 (IGF-1) for men and lower urate for women, then lower triglycerides for men and lower lung function/height for women, then lower scores for pairs matching test for both sexes
8. Lung age: lower lung function/height for both sexes, then lower albumin for men and higher basophil count for women, then higher immature red blood cell volume for both sexes

When this analysis was repeated on body system risk scores, similar patterns of top biomarkers featured in the respective body system risk scores, but biomarkers strongly associated with chronological age (such as lung function/height; Figures 3.10.2 and 5.3.1) were more highly weighted in the body system risk scores and biomarkers less strongly associated with chronological age (such as general and central adiposity; Section 5.6) were weighted more highly in the body system ages. The biomarker patterns for neurological age vs risk scores (Figures 6.5.1 (A) vs (B)) were substantially different. For the neurological ages, the top biomarker principal components were in IGF-1, triglycerides then pairs matching test for men and urate, lung function/height then pairs matching test in women, but for neurological risk scores, they were reaction time test, sex hormone-binding globulin then pairs matching test in men and lung function/height, blood pressure then cystatin C in women.

The estimation of the body system ages were then restricted to the key biomarkers across body systems. This analysis was carried out to assess if the patterns of biomarker importance would change substantially and if the moderate correlations between body system age deltas (Figure 6.4.2) would change substantially when body system ages were estimated from fewer biomarkers. The reduced biomarker panel of 14 key biomarker principal components consisted of the 10 most important biomarker principal components for men and 10 for women: general adiposity, lung function/height, blood pressure, HbA1c, height and rheumatoid factor in both sexes; central adiposity, albumin, muscle mass and reticulocyte count in men; and hand grip strength/height, vitamin D, log C-reactive protein and urate in women. There was little overall change in patterns of biomarker importance after restricting the estimation of these ages to this reduced panel of 14 biomarker principal components

(Figure A6.2.2), despite the slight increase in correlations between all body system age deltas (Figure 6.4.2 vs Figure A6.2.3). The most prominent changes were seen in neurological age for healthy men, where pairs matching test and IGF-1 were substituted with physical capability biomarkers (Figure A6.2.2).

6.6 Biological and chronological age effect on disease risks

To assess the relative contributions of biomarker levels and chronological age to the prediction of diseases for each body system, the proportion of the overall body system age delta and chronological age effect on disease risk (in unadjusted models) was assessed separately for each of the 8 disease groups (Table A6.2.4 (A) and Figure 6.6.1 (A)). There was substantial variation between diseases in the proportion of disease risk explained by body system age deltas alone, ranging from 24.4% for neurological age delta for healthy women to 81.0% for metabolic disease age delta for healthy women (bars in red; Figure 6.6.1 (A) and Table 6.2.4 (A)). In the healthy subpopulation and of all body system age deltas, metabolic disease age deltas for both sexes explained most of the overall age effect on the risk of their respective diseases (78.8% for men and 81.0% for women), followed by inflammatory age delta for women (63.9%) and lung age delta for men (53.4%; Table A6.2.4 (A)).

For disease groups apart from inflammatory and neurological diseases, a small negative proportion of the overall effect was explained by both their body system age delta and chronological age (-1.9–2.2%/-1.7–1.3% for healthy men/women across all 8 body systems; Table A6.2.4 (A)). These negative values were due to the pattern of log likelihoods underlying this calculation: the change in the log likelihood on adding each type of age or age delta (chronological age and the body system age delta) was greater when the other type of age was already in the model (Table 6.6.1). These proportions (including any negative proportions) still sum to 100% for each predicted outcome and subpopulation.

When body system age deltas were replaced with body system risk scores, the proportions of disease risk explained by body system risk scores alone were similar or slightly lower than the proportions explained by the age deltas alone (bars in red vs crosses; Figure 6.6.2) across all body systems (reductions in proportions: 0.1–8.5%; Tables A6.2.5 vs A6.2.4). The body system risk scores also substantially overlapped with chronological ages (bars in blue; Figure 6.6.2) such that the overall age effect was mostly represented by biomarker measurements (75.2–98.0%/77.8–99.2% for healthy men/women across all 8 body systems; Table A6.2.5). (Body system ages also captured most of the overall age effect as they are equivalent to the sum of body system age delta and chronological age.)

In sensitivity analyses where the estimation of the body system ages were restricted to the reduced panel of 14 biomarkers, there were slight reductions in the proportion explained by many body system age deltas, and more substantial reductions (of >10%) for artery and lung age deltas alone for both sexes and cardiac age for men (Figure 6.6.1 (A) vs (B) and Table A6.2.4 (A) vs (B)). For disease groups apart from inflammatory and neurological diseases, a small negative proportion of the overall effect was explained by both their body system age delta and chronological age in men for the same reason explained above (Table 6.6.1).

When body system ages derived based on the healthy subpopulation were applied to the whole population, greater proportions of the overall age effect were explained by body system age deltas alone for only gut, cardiac, metabolic and lung diseases for both sexes, and musculoskeletal and inflammatory diseases for women (changes of -9.9% to 16.7% across all 8 body systems; Table A6.2.4 (A)).

6.7 Validation results

Predictive power of body system ages

In the healthy subpopulation, adding each body system age delta to unadjusted prediction models with chronological age statistically significantly improved model fit (p-values for likelihood ratio tests across body systems and sexes were smaller than 1×10^{-11}; Table 6.6.1 (A)).

In unadjusted analyses, the predictive power of body system ages (including chronological age) for their respective disease group also varied by body system, with C-indices ranging from 0.654 (gut age for healthy men) to 0.813 (lung age for healthy men; Table 6.7.1 (A)). Metabolic disease (both sexes), inflammatory (for healthy women) and lung (for healthy men) age deltas alone were better than chronological age alone at predicting their respective diseases (Table 6.7.1 (A)). Across the 8 body systems, these C-indices were slightly higher for healthy men than for women (Table 6.7.1 (A)). When chronological age was supplemented with each body system age delta, the increase in C-indices in unadjusted models was largest for metabolic disease age deltas (healthy men: 0.128; healthy women: 0.130), followed by lung age (healthy men: 0.086; healthy women: 0.063) and inflammatory age (healthy men: 0.050; healthy women: 0.078; Table 6.7.1 (A)). These increases were only slightly attenuated in models adjusted for Index of Multiple Deprivation 2010 quintile, smoking status, alcohol consumption and UK Biobank assessment centre (Table 6.7.1 (A) vs (B)).

The predictive power was generally but not consistently higher when body system ages derived on the healthy subpopulation were applied to the whole population than when applied to the healthy population, for both adjusted and unadjusted analyses (Table 6.7.1). In unadjusted analyses, the increases in C-indices were slightly larger in the whole population for gut, cardiac, metabolic disease and lung age for men, and for all body system ages apart from neurological age for women (Table 6.7.1 (A)). In unadjusted analyses, C-indices for models with chronological age only were generally lower in the whole population compared to the healthy subpopulation (with the exceptions of models for artery, cardiac and metabolic diseases for women, where there were slight increases; Table 6.7.1 (A)), while in adjusted analyses, these C-indices were generally higher in the whole population (with the exceptions of inflammatory and neurological diseases for men and musculoskeletal diseases for women; Table 6.7.1 (B)).

Calibration of biological ages

The body system ages were well calibrated to chronological age after recalibrating to chronological age using the modified Dubina method (described in Section 4.5), as they matched healthy participants' chronological ages on average (Figure 6.7.1 (A)). Before recalibration, means of the metabolic disease ages by chronological age groups increased with chronological age but were too low at younger chronological age groups, and the standard deviations for each group were larger than those for other body system ages, while means of lung ages were also slightly lower than chronological ages at younger chronological age groups (Figure 6.7.1 (B)). The other 6 body system ages were general well calibrated to chronological age (Figure 6.7.1 (B)). The recalibrated metabolic disease ages matched chronological ages for each chronological age group on average, but the standard deviations for each group remained large (>20 years of body system age for all chronological age bands; Figure 6.7.1 (A)).

Biologically older participants had the highest disease incidence rates, while biologically younger participants had the lowest rates, for both sexes in the healthy subpopulation (Figure 6.7.2). The differences in disease incidence rates between individuals who were biologically older, similar or younger than their chronological age were highly statistically significant based on log-rank tests ($p<1\times10^{-14}$ for both sexes and all disease groups).

6.8 Discussion

The 8 body system ages estimated via the novel Cox lasso method linked biomarker patterns in each body system age directly to the prognosis of later life diseases in their respective body system (Section 4.3). This analysis allowed a systematic investigation of the relationship between a wide range of prognostic physical and biochemical biomarkers and age-related diseases, with and without the effect of chronological age. Body system age deltas (represented by biomarkers of diseases) were

moderately correlated with each other except for neurological age (ρ>0.3 for the majority of these pairs; Figure 6.4.2), despite substantial overlaps in the most important biomarkers only between body system ages such as gut, cardiac and metabolic disease ages (Section 6.5). On aggregate, the limited overlap across the constituent biomarkers of the 8 body system ages suggests that the patterns of biomarkers that are generally highly predictive of diseases across a range of body systems (Table 6.7.1) display a common ageing effect across body systems that is only partially attributable to overlapping biomarkers.

Body system risk scores vs body system ages

The similarity in biomarker importance patterns between most body system ages and risk scores (estimated in the presence vs absence of chronological age respectively) indicate that the biomarker patterns shown in Figure 6.5.1 best predict disease onset both independently and in relation to chronological age. The exception was neurological ages vs body system risk scores, which did not have a single strongly-featuring biomarker constituent, and where epidemiological research has tended to focus on biomarkers other than the commonly-measured physical or biochemical biomarkers,[200,201] except for pairs matching tests, which is a putative biomarker of early dementia.[202] The differences between the top biomarker constituents of neurological ages vs risk scores may be due to lung function and blood pressure being a substitute for chronological age in predicting neurological disease in the neurological risk score but not the neurological age, due to the strong linear relationship between these biomarkers and chronological age (Sections 3.10 and 5.3).

Including chronological age as a covariate generally attenuated the effect of body system risk scores on disease incidence, and had a substantial effect on disease incidence independently of body system risk scores and other characteristics of the population (Section 6.3 and Table 6.7.1 (B)). Furthermore, all body system age deltas alone (which represented biomarker patterns that complemented chronological age in predicting disease risk; Section 4.3) contributed similarly or slightly more than body system risk scores alone to the overall age effect in predicting their respective disease group

(Figure 6.6.2). Hence the combination of biomarker levels (represented by body system age deltas) and chronological ages into body system ages was most predictive of disease risk. In addition, the main estimation method for body system ages resulted in body system ages that were well calibrated to chronological age after recalibration was applied (Figure 6.7.1). In contrast, body system ages estimated using the sensitivity analysis stepwise regression method were less well calibrated to chronological age and could not be cross-validated (Appendix A6.3).

Key biomarkers in body system ages and risk scores

The patterns of biomarker importance highlighted both (1) key predictors of each disease and (2) the commonality of biomarkers between diseases. In terms of (1), biomarkers that featured strongest for each body system age and body system risk score (Figure 6.5.1) broadly match medical knowledge of biomarkers of disease (Table 6.8.1), with a few exceptions where there has been little research (e.g. associations between serum albumin and respiratory disease, and biochemical markers of neurological disease). However, a larger number of biomarker principal components (14 to 48; Table A6.2.2) were selected for inclusion in the body system ages than the numbers of biomarkers typically included in previous studies of disease risk scores. Evaluation of the added benefit of using larger panels of biomarkers to estimate risk scores or body system ages should be explored, taking into account the costs and complexities of measuring a larger number or range of biomarkers in a population. In artery age, established cardiovascular risk factors (blood pressure and blood lipids) featured strongest in both sexes, but lung function also featured strongly in women (Figure 6.5.1), even though all healthy participants reported being non-smokers at baseline. For gut ages, the sex differences in importance of central vs general adiposity were corroborated by two single-sex observational studies for diverticular disease,[203,204] which is the most common constituent of gut diseases in UK Biobank (Figure 3.9.3).

With regards to commonality of biomarkers (the second group of findings), general adiposity biomarkers featured strongly in musculoskeletal, gut and metabolic ages for both sexes and cardiac

ages for women (Figure 6.5.1). Cardiometabolic biomarkers that are on the causal pathway from obesity, such as blood pressure and blood lipids,[205] featured strongly in artery ages for both sexes (Figure 6.5.1). Otherwise, there appeared to be little overlap in the most important biomarkers between body system ages (Figure 6.5.1). The moderately positive correlations between most body system age deltas (Figure 6.4.2) despite the limited biomarker overlap suggest that a common ageing effect across body systems on an individual level can be detected through these biomarkers prior to disease onset. This common ageing effect was described by biomarkers that supplemented chronological age in predicting disease risk independently of the effect described by chronological age (Section 4.3 and Figure 6.6.1). These findings highlight the importance of elucidating the extent to which this cross-body system ageing effect is due to the different biomarkers describing interdependence among underlying biological ageing mechanisms in these participants.

There were no known studies of physical and biochemical biomarker-based risk scores or ages for neurological diseases that corroborate the slightly negative correlation of neurological age deltas with several of the other age deltas (Figure 6.4.2). Previous research on multiple aspects of brain ageing identified through brain image biomarkers in the UK Biobank had found strong associations between these brain ageing scores and almost all of the physical and several of the blood count biomarkers measured in the UK Biobank.[206] This brain ageing study did not investigate associations with biochemical biomarkers, nor did it assess the relative importance of the reported associations. These warrant further investigation into the epidemiological relationships between physical and biochemical biomarkers and incidence of neurological disease, and investigation into the relative importance of commonly measured physical and biochemical vs putative neurological biomarkers measured in other ways.

Even though artery and metabolic disease age deltas were one of the most highly correlated pairs of body system age deltas (Figure 6.4.2), the overlap between their constituent biomarkers (Figure 6.5.1) was not substantial enough to account for all the correlation. Biological associations between different cardiometabolic biomarkers, potentially along the causal pathway from obesity (e.g. insulin

secretion and action, energy metabolism, lipid biology or adipogenesis[205]), may have contributed to these correlations. Other similar pairings included artery and inflammatory, and gut and lung age deltas for healthy men, and cardiac and musculoskeletal age deltas for healthy women. This suggests that some of the common ageing patterns (in terms of elevation of disease risk) were described by different biomarkers in this healthier UK Biobank subpopulation.

Musculoskeletal age was most strongly determined by greater general adiposity, however higher vitamin D levels and higher muscle mass also contributed to this age for both sexes (Section 6.5). The direction of the association was unexpected for vitamin D and muscle mass, but this phenomenon could be related to people engaged in more intensive sport or physically strenuous jobs having a higher risk of musculoskeletal disease (which consisted mainly of osteoarthritis and also included osteoporosis in the healthy UK Biobank participants; Table 3.9.3) in a middle aged population. There is little evidence to support or refute this association, and a study of clinical records for women in UK found mixed patterns of association between 7 types of indoor and outdoor physical activities and risk of fracture in 7 different locations.[207] Despite the high incidence of musculoskeletal disease in this UK Biobank population (Table 3.9.3), recorded incidence of hip or wrist fractures was rare (only 0.4% in the whole UK Biobank population had a hospital admission with hip or wrist fracture during follow up; Section 3.5). Published epidemiological studies have only reported associations of low vitamin D with cancer mortality,[208,209] respiratory mortality[208,209] and multiple sclerosis,[210] and have not found significant associations with musculoskeletal conditions, apart from a meta-analysis that found significant associations between vitamin D levels and fracture risk in observational studies but not vitamin D supplementation and fracture risk in randomised controlled trials[211] (Table 6.8.1).

Predictive power of body system ages

In the healthy subpopulation, the proportion of disease risk explained by body system age deltas alone, which supplemented the chronological age effect on disease risk, varied substantially between

disease groups (24.4% of the overall age effect for neurological age delta for women to 81.0% for metabolic disease age delta for women; Table A6.2.4 (A)). These proportions were consistently higher than the proportions of mortality and frailty risk explained by the age-based biological age alone (1.7–8.2% of the overall age effect; Table 5.4.1). The proportions for body system age deltas alone were larger or similar to the proportions for body system risk scores alone (by 0.1–8.5%; Tables A6.2.4 vs A6.2.5), which consisted of biomarkers that best predicted disease risk rather than biomarkers that best supplemented chronological age in predicting disease risk, therefore body system ages (the sum of body system age deltas and chronological age) were at least as informative as their respective risk scores.

The large increases in C-indices for body system ages over chronological age alone for metabolic and respiratory diseases (Section 6.7) could be attributable to HbA1c and cystatin C featuring strongly in the metabolic disease age, and lung function biomarkers featuring strongly in the lung age (Section 6.5). The levels of these biomarkers are also key determinants of clinical diagnoses for diabetes, chronic kidney disease and chronic obstructive pulmonary disease respectively,[212-214] even though participants who could have been regarded as having screen-detected disease based on their biomarker levels were excluded (Appendix 6.1), and having biomarker levels above the National Institute for Health and Care Excellence (NICE) guideline thresholds do not automatically trigger hospital admissions. Since neurological age was most strongly positively correlated with chronological age (Table 6.4.1), chronological age contributed more to the prediction of neurological diseases than the constituent biomarkers of neurological age (Sections 6.6–6.7). Therefore the physical and biochemical biomarkers used in this analysis may not be adequate for predicting later life neurological diseases (predominantly dementia and Parkinson's disease; Table 3.9.3) in these relatively healthy participants.

Metabolic disease, inflammatory and lung age deltas alone predicted their respective disease outcome substantially better than chronological age alone (Section 6.6) and substantially supplemented chronological age in predicting their respective diseases (Figure 6.6.1 and Table

6.7.1). For these body systems, the relative effects of their respective risk scores on disease incidence were also larger than the effects of chronological age (Section 6.3).

Prediction in healthier vs unhealthier individuals

In contrast to findings that the age-based biological age supplemented chronological age in the prediction of mortality and frailty to a greater extent in the whole UK Biobank population vs the healthy subpopulation (Section 5.5), the body system ages were not consistently more predictive of their respective disease outcome for those who did not have the disease at baseline in the whole population, compared to the healthy subpopulation (Table 6.7.1). Additionally, body system age deltas did not consistently explain a larger proportion of the overall age effect on disease risk in the whole population vs the healthy subpopulation (Table A6.4.2). Hence the additional prognostic or diagnostic capability of the biomarker patterns across the 8 body systems above chronological age in generally unhealthier vs healthier individuals appeared to vary by body system. The relatively higher predictive power of artery and neurological age (Figure 6.6.1 and Table 6.7.1) for their respective diseases in a healthier subpopulation vs the whole population suggests the potential for early detection of disease, although this difference in predictive power was minimal (Section 6.7). Additionally, further investigation is required to assess the extent to which chronological age is a proxy for risk factors not included in these analyses for the whole population, e.g. type and severity of prior diseases.

C-indices for fully adjusted models were also reported by other studies of risk scores underlying heart ages, and were 0.842 and 0.828 for men and women respectively in a British population aged 30-84 years[196] and between 0.63–0.83 for the US Framingham risk score when applied to several Caucasian cohorts.[197] The absolute values of C-indices from this analysis for artery and cardiac ages (including chronological age) ranged between 0.686–0.726 for unadjusted analyses and 0.704–0.747 in adjusted analyses for the whole UK Biobank population (Table 6.7.1), however whether the differences in C-indices between studies relate to differences in demographic profiles of the cohorts,

inclusion of biological and non-biological risk factors in the risk scores or any other factors is not clear. Nevertheless, the prognostic capability of these body system ages for disease in a healthier population earlier in life is useful as it permits a greater window for intervention.

Strengths and limitations

Developing and applying the method of estimating body system ages to the deeply phenotyped UK Biobank allowed many insights into body system-specific and cross-body system ageing patterns. The broadly log-linear relationship between hazard ratios and quintiles of body system risk scores and chronological age (Section 6.3) indicated that the Cox models used to estimate body system ages were generally a good fit to the biomarker and chronological age profiles of these participants.

However, this analysis also uncovered several limitations of the methods. Firstly, the large deviations in metabolic disease age from chronological age in the population (Figures 6.4.1 and 6.7.1 (A)), despite the recalibration to chronological age and the exclusion of participants who had HbA1c or cystatin C levels that would meet the thresholds of a diabetes or chronic kidney disease diagnosis but were undiagnosed (Appendix 6.1), demonstrates that expressing and communicating disease risk in terms of body system ages is not as useful in situations where disease risk is much more strongly determined by biomarker levels than by chronological age (Appendix 6.1). In this specific situation, communicating HbA1c levels separately as a measure of diabetes risk may be much more informative than communicating metabolic disease ages, while cystatin C levels could be communicated either in terms of eGFR or as a standalone kidney age.[65]

The negative proportion of the overall age effect explained by the majority of the body system ages (Figure 6.6.1) resulted from the inability of these proportions to represent the interactive effects of these body system age deltas and chronological ages. According to the underlying log-likelihoods of these models (Table 6.6.1), these interactive effects arising from including both types of age (chronological age and the body system age delta) in the model was greater than the sum of effects

for each type of age. This phenomenon was beneficial and realistic, but difficult to interpret due to the negative log likelihood values. Further research is required to synthesise an improved representation of these proportions.

The stability and calibration of these body system ages to chronological age depended on the number of incident cases of the disease for each sex, which themselves depend on other demographic and health behavioural characteristics of the subpopulation. For example, neurological age may have been more strongly determined by chronological age than the in-scope biomarkers (Table 6.4.1) for one or more reasons: there were fewer neurological diseases in the population since few UK Biobank participants had been followed up beyond the age of 80 years at the time of writing, and few neurological biomarkers were available in the UK Biobank for this analysis. Key biomarkers that had been investigated in research on neurological diseases, such as amyloid and tau proteins in cerebrospinal fluid, and inflammatory and antioxidant blood biomarkers such as interleukin 6, vitamins A, C and E and Coenzyme Q10,[215] were not measured in the UK Biobank. Apolipoprotein E genotype and biomarkers derived from brain imaging were also key biomarkers used in neurological research[200,215] that were available in the UK Biobank upon request,[103] and could be used in future research to supplement the biomarker panel used in this analysis.

The analyses in this chapter required the additional assessment of body system risk scores, even though the key results in this chapter were the 8 body system ages, to address multiple analytical objectives. Firstly, focusing on body system risk scores permitted analysis of the contribution of biomarker measurements to the prediction of later life health outcomes in a similar way to the analysis of age-based biological age in Chapter 5, and found that these biomarker patterns contributed substantially more to the prediction of later life health risks than age-based biological ages. Secondly, comparing body system age deltas with their respective risk scores showed that patterns of biomarkers that best complement chronological age in the prediction of disease risk were similar to those that best predict disease risk in the absence of chronological age for all body systems apart from neurological age. Lastly, assessing body system ages and their companion risk scores

permitted the limitations of each estimation method and the unusual distribution of metabolic disease ages in the population to be explored.

Conclusions

This chapter provided an in-depth and body system-specific investigation of biomarkers of ageing, both independently of and in relation to chronological age. It highlighted avenues for further epidemiological and biological research on within- and cross-body system biomarker-disease associations. The 8 body system ages discussed in this chapter made explicit the close links between biomarkers and later life diseases for all 8 disease groups apart from neurological diseases (without the use of chronological age as a proxy for later life health) and provided a framework for interpreting composite body system ages and risk scores in relation to chronological age. These body system ages also have the potential to be aggregated into an improved overall biological age, which is reported and discussed in Chapter 7.

Chapter 6 tables and figures

Table 6.4.1: Pearson correlation coefficients between each body system age and chronological age, for healthy men and healthy women

	Body system ages	
Body system	**Healthy men**	**Healthy women**
Artery	0.783	0.724
Musculoskeletal	0.801	0.885
Gut	0.824	0.804
Cardiac	0.874	0.880
Metabolic disease	0.335	0.232
Inflammatory	0.822	0.537
Neurological	0.927	0.951
Lung	0.565	0.757

The correlation coefficients between each body system age delta and chronological age are very close to zero, as expected from the modified Dubina recalibration procedure described in Section 4.5.

Table 6.6.1: Log likelihoods for models with and without body system age deltas and chronological age for their respective disease outcome (with χ^2 p-values for the addition of body system age deltas to a model with chronological age), in the (A) main analysis and (B) when using the reduced biomarker panel, for healthy men and women

(A) Main analysis

	CA alone-Base	BA alone-Base	CA and BA - Base	CA and BA - CA alone	CA and BA - BA alone	χ^2 p-value (adding BA to CA)
Healthy men						
*Artery**	416	200	619	203	419	3.97 x 10^-90
*Musculoskeletal**	354	258	624	269	366	3.57 X 10^-119
*Gut**	229	110	343	114	233	1.06 X 10^-51
*Cardiac**	426	138	567	141	430	2.74 X 10^-63
*Metabolic disease**	150	551	708	558	157	1.31 X 10^-244
Inflammatory	43	36	78	35	42	5.57 X 10^-17
Neurological	109	50	155	47	105	5.08 X 10^-22
*Lung**	55	62	118	63	56	3.88 X 10^-29
Healthy women						
*Artery**	215	117	334	119	218	1.08 X 10^-53
*Musculoskeletal**	704	214	933	229	719	1.27 X 10^-101
*Gut**	284	157	447	164	290	4.05 X 10^-73
*Cardiac**	234	77	315	82	239	1.72 X 10^-37
*Metabolic disease**	89	371	465	376	93	1.23 X 10^-165
Inflammatory	41	73	115	73	41	1.03 X 10^-33
Neurological	78	27	103	25	77	1.26 X 10^-12
*Lung**	34	32	67	33	35	4.61 X 10^-16

(B) Using the reduced biomarker panel

	CA alone-Base	BA alone-Base	CA and BA - Base	CA and BA - CA alone	CA and BA - BA alone	χ^2 p-value (adding BA to CA)
Healthy men						
*Artery**	416	106	525	108	419	4.12 X 10^-49
*Musculoskeletal**	354	192	555	200	363	3.40 X 10^-89
*Gut**	229	82	315	86	232	2.38 X 10^-39
*Cardiac**	426	66	500	74	434	7.55 X 10^-34
*Metabolic disease**	150	437	600	450	164	6.75 X 10^-198
Inflammatory	43	28	71	27	42	1.69 X 10^-13
Neurological	109	12	121	12	108	8.26 X 10^-7
*Lung**	55	38	94	40	56	6.04 X 10^-19
Healthy women						
*Artery**	215	69	285	69	216	5.97 X 10^-32
*Musculoskeletal**	704	177	892	188	715	1.19 X 10^-83
*Gut**	284	129	420	136	291	2.66 X 10^-61
*Cardiac**	234	46	283	49	237	3.27 X 10^-23
*Metabolic disease**	89	272	372	284	101	1.55 X 10^-125
Inflammatory	41	49	90	49	41	4.65 X 10^-23
Neurological	78	24	100	22	76	2.50 X 10^-11
*Lung**	34	16	50	16	34	1.94 X 10^-08

CA: chronological age, BA: body system age delta

* These body system ages had negative % of the overall age effect explained by chronological age and their age delta (Table A6.2.4)

These log-likelihoods were used in the calculation of the relative contribution of body system age deltas and chronological age in explaining their respective disease outcome (Table A6.2.4 and Figure 6.6.1)

The reduced biomarker panel consisted of the top 10 biomarker principal components for men and 10 for women: general adiposity, lung function/height, blood pressure, HbA1c, height and rheumatoid factor in both sexes; central adiposity, albumin, muscle mass and reticulocyte count in men; and hand grip strength/height, vitamin D, C-reactive protein and urate in women

Table 6.7.1: Harrell's C-indices for each of the 8 body system age deltas and chronological age in (A) unadjusted and (B) adjusted prediction models for their respective disease group outcomes

(A) Unadjusted analysis

Outcome and age predictor	Healthy subpopulation		Whole population	
	Men	Women	Men	Women
CA alone				
Artery	0.667 (0.006)	0.665 (0.008)	0.663 (0.002)	0.666 (0.003)
Musculoskeletal	0.631 (0.005)	0.650 (0.004)	0.626 (0.002)	0.639 (0.002)
Gut	0.627 (0.006)	0.629 (0.005)	0.627 (0.002)	0.622 (0.002)
Cardiac	0.692 (0.007)	0.682 (0.009)	0.687 (0.002)	0.696 (0.003)
Metabolic disease	0.644 (0.009)	0.634 (0.011)	0.633 (0.002)	0.634 (0.003)
Inflammatory	0.686 (0.023)	0.636 (0.015)	0.663 (0.007)	0.630 (0.004)
Neurological	0.779 (0.020)	0.771 (0.023)	0.752 (0.007)	0.769 (0.008)
Lung	0.727 (0.024)	0.695 (0.025)	0.688 (0.004)	0.676 (0.004)
BA alone				
Artery	0.614 (0.006)	0.614 (0.008)	0.632 (0.002)	0.683 (0.003)
Musculoskeletal	0.611 (0.005)	0.584 (0.004)	0.607 (0.002)	0.621 (0.002)
Gut	0.587 (0.006)	0.595 (0.005)	0.616 (0.002)	0.628 (0.002)
Cardiac	0.606 (0.007)	0.601 (0.009)	0.671 (0.002)	0.675 (0.003)
Metabolic disease	0.737 (0.009)	0.733 (0.011)	0.806 (0.002)	0.829 (0.003)
Inflammatory	0.671 (0.023)	0.662 (0.015)	0.675 (0.007)	0.716 (0.004)
Neurological	0.678 (0.020)	0.667 (0.023)	0.650 (0.007)	0.710 (0.008)
Lung	0.733 (0.024)	0.689 (0.025)	0.815 (0.004)	0.782 (0.004)
BA and CA				
Artery	0.701 (0.006)	0.697 (0.008)	0.686 (0.002)	0.700 (0.003)
Musculoskeletal	0.670 (0.005)	0.671 (0.004)	0.663 (0.002)	0.673 (0.002)
Gut	0.654 (0.006)	0.659 (0.005)	0.657 (0.002)	0.661 (0.002)
Cardiac	0.716 (0.007)	0.704 (0.009)	0.721 (0.002)	0.726 (0.003)
Metabolic disease	0.772 (0.009)	0.763 (0.011)	0.807 (0.002)	0.829 (0.003)
Inflammatory	0.736 (0.023)	0.713 (0.015)	0.712 (0.007)	0.729 (0.004)
Neurological	0.805 (0.020)	0.800 (0.023)	0.777 (0.007)	0.790 (0.008)
Lung	0.813 (0.024)	0.758 (0.025)	0.817 (0.004)	0.787 (0.004)
Improvement of BA and CA over CA				
Artery	*0.034*	*0.032*	*0.023*	*0.034*
Musculoskeletal	*0.039*	*0.021*	*0.036*	*0.034*
Gut	*0.027*	*0.030*	*0.030*	*0.039*
Cardiac	*0.024*	*0.022*	*0.034*	*0.030*
Metabolic disease	*0.128*	*0.130*	*0.174*	*0.195*
Inflammatory	*0.050*	*0.078*	*0.049*	*0.099*
Neurological	*0.026*	*0.030*	*0.025*	*0.020*
Lung	*0.086*	*0.063*	*0.129*	*0.111*

(B) Adjusted for sociodemographic factors and health behaviours

Outcome and age predictor	Healthy subpopulation		Whole population	
	Men	Women	Men	Women
CA alone				
Artery	0.683 (0.006)	0.689 (0.008)	0.689 (0.002)	0.709 (0.003)
Musculoskeletal	0.657 (0.005)	0.672 (0.004)	0.656 (0.002)	0.669 (0.002)
Gut	0.660 (0.006)	0.659 (0.005)	0.670 (0.002)	0.667 (0.002)
Cardiac	0.711 (0.007)	0.701 (0.009)	0.713 (0.002)	0.724 (0.003)
Metabolic disease	0.688 (0.009)	0.679 (0.011)	0.694 (0.002)	0.705 (0.003)
Inflammatory	0.728 (0.023)	0.671 (0.015)	0.706 (0.007)	0.682 (0.004)
Neurological	0.801 (0.020)	0.794 (0.023)	0.780 (0.007)	0.797 (0.008)
Lung	0.797 (0.024)	0.769 (0.025)	0.828 (0.004)	0.834 (0.004)
BA alone				
Artery	0.641 (0.006)	0.649 (0.008)	0.655 (0.002)	0.713 (0.003)
Musculoskeletal	0.640 (0.005)	0.613 (0.004)	0.637 (0.002)	0.647 (0.002)
Gut	0.628 (0.006)	0.631 (0.005)	0.656 (0.002)	0.666 (0.002)
Cardiac	0.638 (0.007)	0.630 (0.009)	0.698 (0.002)	0.701 (0.003)
Metabolic disease	0.758 (0.009)	0.755 (0.011)	0.825 (0.002)	0.846 (0.003)
Inflammatory	0.721 (0.023)	0.689 (0.015)	0.714 (0.007)	0.743 (0.004)
Neurological	0.716 (0.020)	0.720 (0.023)	0.700 (0.007)	0.741 (0.008)
Lung	0.795 (0.024)	0.754 (0.025)	0.863 (0.004)	0.856 (0.004)
BA and CA				
Artery	0.712 (0.006)	0.716 (0.008)	0.704 (0.002)	0.731 (0.003)
Musculoskeletal	0.688 (0.005)	0.687 (0.004)	0.682 (0.002)	0.693 (0.002)
Gut	0.679 (0.006)	0.682 (0.005)	0.689 (0.002)	0.695 (0.002)
Cardiac	0.730 (0.007)	0.720 (0.009)	0.740 (0.002)	0.747 (0.003)
Metabolic disease	0.789 (0.009)	0.781 (0.011)	0.827 (0.002)	0.846 (0.003)
Inflammatory	0.769 (0.023)	0.736 (0.015)	0.742 (0.007)	0.755 (0.004)
Neurological	0.823 (0.020)	0.821 (0.023)	0.800 (0.007)	0.812 (0.008)
Lung	0.843 (0.024)	0.800 (0.025)	0.869 (0.004)	0.864 (0.004)
Improvement of BA and CA over CA				
Artery	*0.029*	*0.027*	*0.016*	*0.022*
Musculoskeletal	*0.030*	*0.015*	*0.026*	*0.024*
Gut	*0.019*	*0.023*	*0.019*	*0.028*
Cardiac	*0.019*	*0.019*	*0.027*	*0.023*
Metabolic disease	*0.101*	*0.102*	*0.133*	*0.141*
Inflammatory	*0.041*	*0.065*	*0.036*	*0.073*
Neurological	*0.023*	*0.027*	*0.020*	*0.015*
Lung	*0.046*	*0.031*	*0.041*	*0.030*

CA: chronological age; BA: body system age delta
Analyses in (B) were adjusted for Index of Multiple Deprivation 2010 quintile, smoking status, alcohol consumption and assessment centre

Table 6.8.1: Examples of associations in published studies between the top 10 biomarker principal components of the body system ages for each sex, and adverse health outcomes

Key biomarker principal component	**Relevant body system ages^**	**Evidence from meta-analyses of randomised trials**	**Evidence from Mendelian randomisation**	**Evidence from prospective studies**
General adiposity	Musculoskeletal, gut, metabolic disease, cardiac (women only)	Osteoarthritis[216]	Cardiometabolic diseases[191,217] including chronic kidney disease,[218] inflammatory, musculoskeletal, neurological and respiratory diseases,[191 219] *(Not significantly associated with stroke[217] or Alzheimer's disease[220])*	Cardiovascular diseases,[221] diverticular diseases,[222] chronic kidney disease and albuminuria[219] , and dementia[223]
Lung function/height*	Neurological, artery (women only for both)	-	Respiratory and autoimmune diseases[28]	All-cause, circulatory disease, respiratory and cancer mortality[29]
Blood pressure*	Artery	Mortality and cardiovascular disease[26]	Type 2 diabetes,[35] Alzheimer's disease[36]	Vascular mortality[37]
HbA1c*	Metabolic disease	*(Not significantly associated with cardiovascular disease[52])*	Coronary artery disease[53]	Mortality, cardiovascular disease, cancer, diabetes[54]
Height*	Cardiac, musculoskeletal (women only)	-	Cardiovascular disease, hip fracture, intervertebral disc disorder, vasculitis, gastro-oesophageal reflux disease and cancer[43]	Mortality from cardiovascular disease, liver disease, COPD, stomach and oral cancers, mental disorders[44]
Rheumatoid factor	Inflammatory	-	-	Rheumatoid arthritis,[224] all cause and cancer mortality[225] *(Not significant for cardiovascular mortality[225])*
C-reactive protein *(women only)*	Inflammatory	-	Type 2 diabetes[226] *(Not significantly associated with coronary artery disease, inflammatory bowel disease, Crohn disease, psoriatic arthritis, knee osteoarthritis after adjusting for heterogeneity[227] or ischaemic stroke[228])*	Coronary heart disease, ischaemic stroke, vascular mortality, non-vascular mortality,[229] osteoporotic fracture[230] and invasive ovarian cancer[231]
Hand grip strength/ height* *(women only)*	Musculoskeletal (women only), inflammatory (men only)	-	Cardiovascular disease and mortality[24]	Mortality, cardiovascular disease, respiratory disease, cancer[25]
Central adiposity *(men only)*	Gut	-	Type 2 diabetes, coronary heart disease[232] and chronic kidney disease,[218] *(Not significantly associated with Alzheimer's disease[220])*	Cardiovascular diseases,[221 220]
Reticulocyte count *(men only)*	Metabolic disease (men only)	-	Asthma, coeliac disease, diabetes, coronary heart disease[233] *(Not significantly associated with inflammatory bowel disease, multiple sclerosis, rheumatoid arthritis, chronic kidney disease or Alzheimer's disease[233])*	Cardiovascular and all cause mortality[234] *(Not significantly associated with prostate cancer[235])*
Vitamin D *(women only)*	Musculoskeletal	*Cancer mortality[209] (Not significantly associated with all cause, cardiovascular or non-cancer non-cardiovascular mortality[209], fractures[211] or falls[236])*	*Multiple sclerosis[210] (Not significantly associated with vascular disease,[237] diabetes, ischaemic heart disease, non-vertebral fracture[238] or cancer[239])*	Cancer and respiratory mortality[208] and fractures[211] *(Weak association with cardiovascular mortality[208 211])*
Muscle mass* *(men only)*	Musculoskeletal	-	-	Cancer mortality,[41] physical disability[42]

Urate* *(women only)*	Neurological (women only)	End-stage renal disease[240] *(Not significant for or mortality[240])*	Gout and inflammatory polyarthropathies[241] *(Not significantly associated with chronic kidney disease[242], unclear association with coronary heart disease[243])*	Hypertension, chronic kidney disease[240] and cardiovascular mortality[244]
Albumin* *(men only)*	Lung (men only)	Mixed results for mortality in trials of critically ill participants[245]	*(Not significantly associated with atrial fibrillation[246])*	Coronary heart disease, myocardial infarction, venous thromboembolism, cancer mortality, fracture,[247] atrial fibrillation, [246] ischemic heart disease, myocardial infarction and stroke[248] *(Not significantly associated with diabetes* [247])

All biochemical biomarkers listed above were measured via blood tests

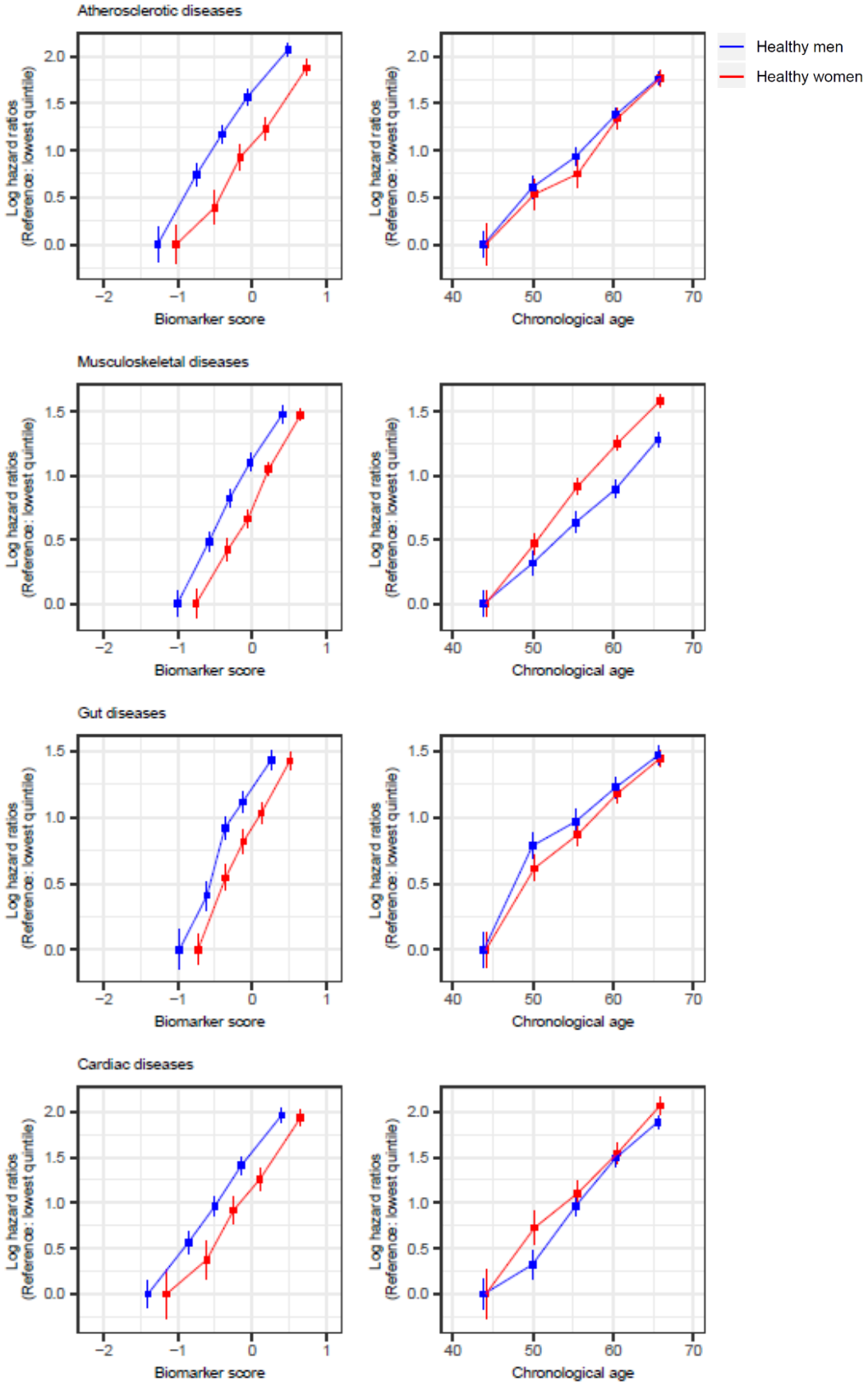
* These biomarkers were also key biomarkers in the age-based Klemera Doubal biological age (Figure 5.6.1)

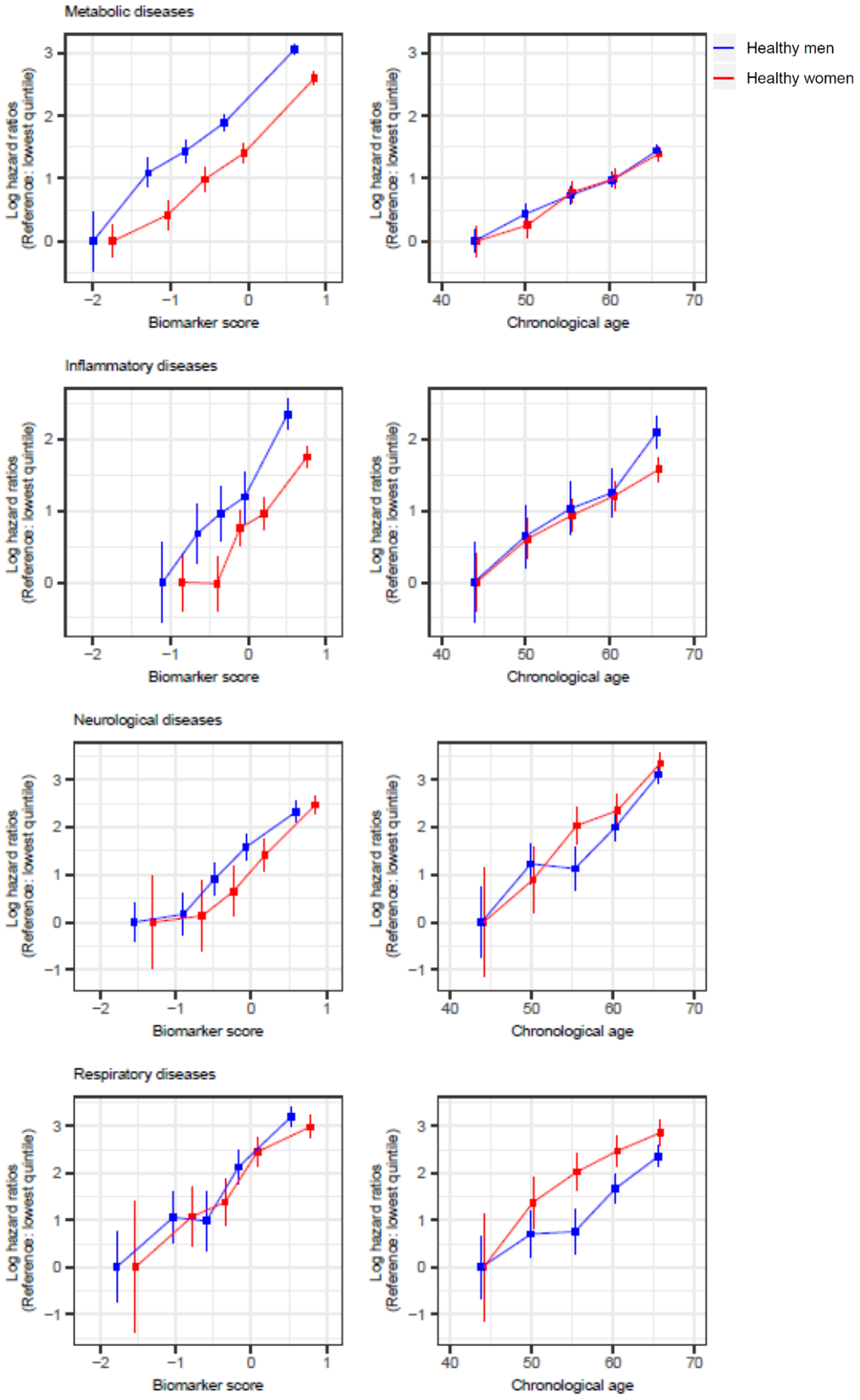
Key age-based biological age biomarkers that did not feature strongly in the body system ages: reaction time test, sex hormone-binding globulin, LDL-C and ApoB, insulin growth factor-1, alkaline phosphatase and urea

^ Relevant body system ages are are the top 10 most important ages for each sex

Figure 6.3.1: Hazard ratios for quintiles of chronological age and body system risk scores for each of the eight diseases groups, when (A) modelled separately and (B) modelled jointly

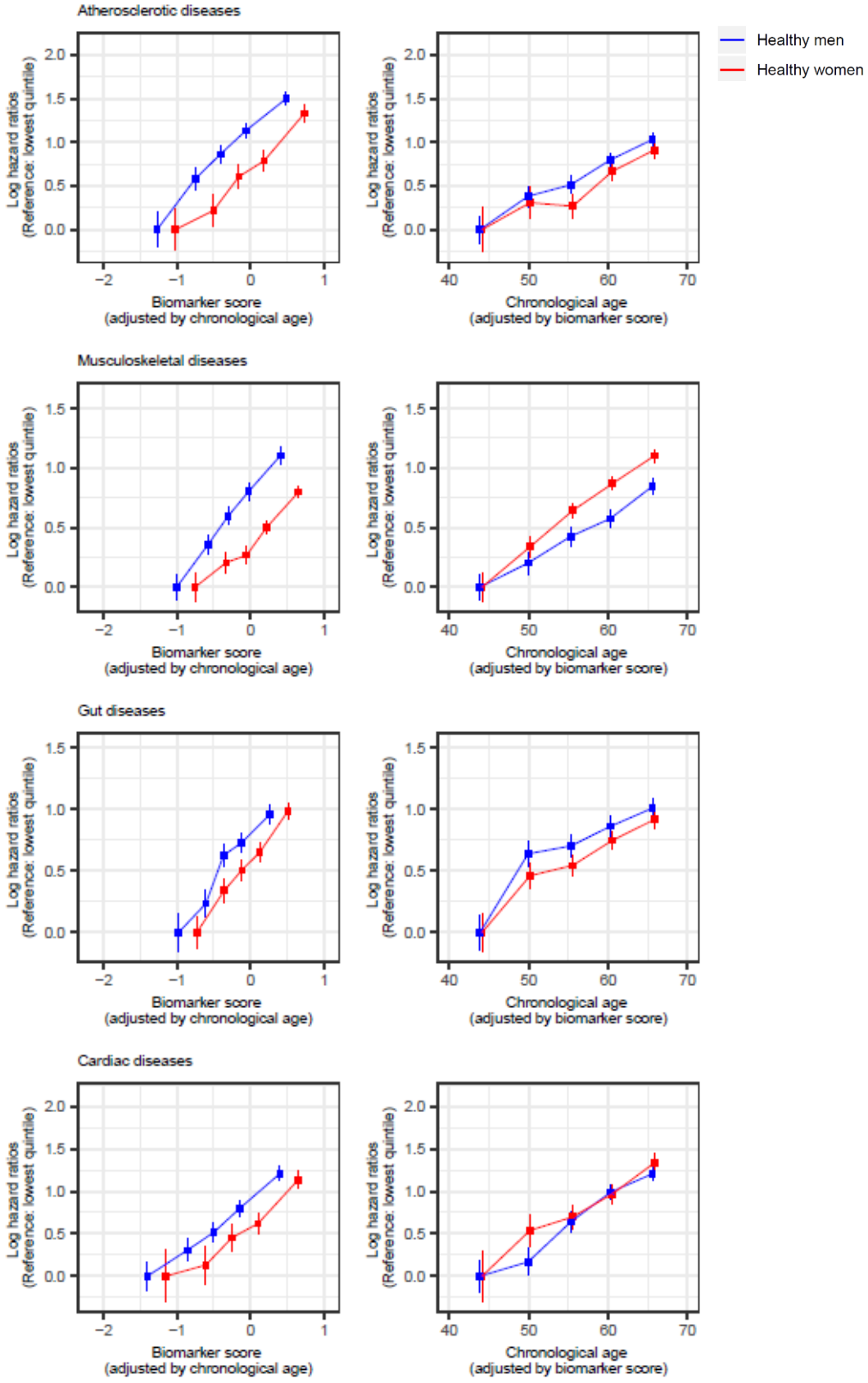
(A) Chronological age and body system risk scores modelled separately

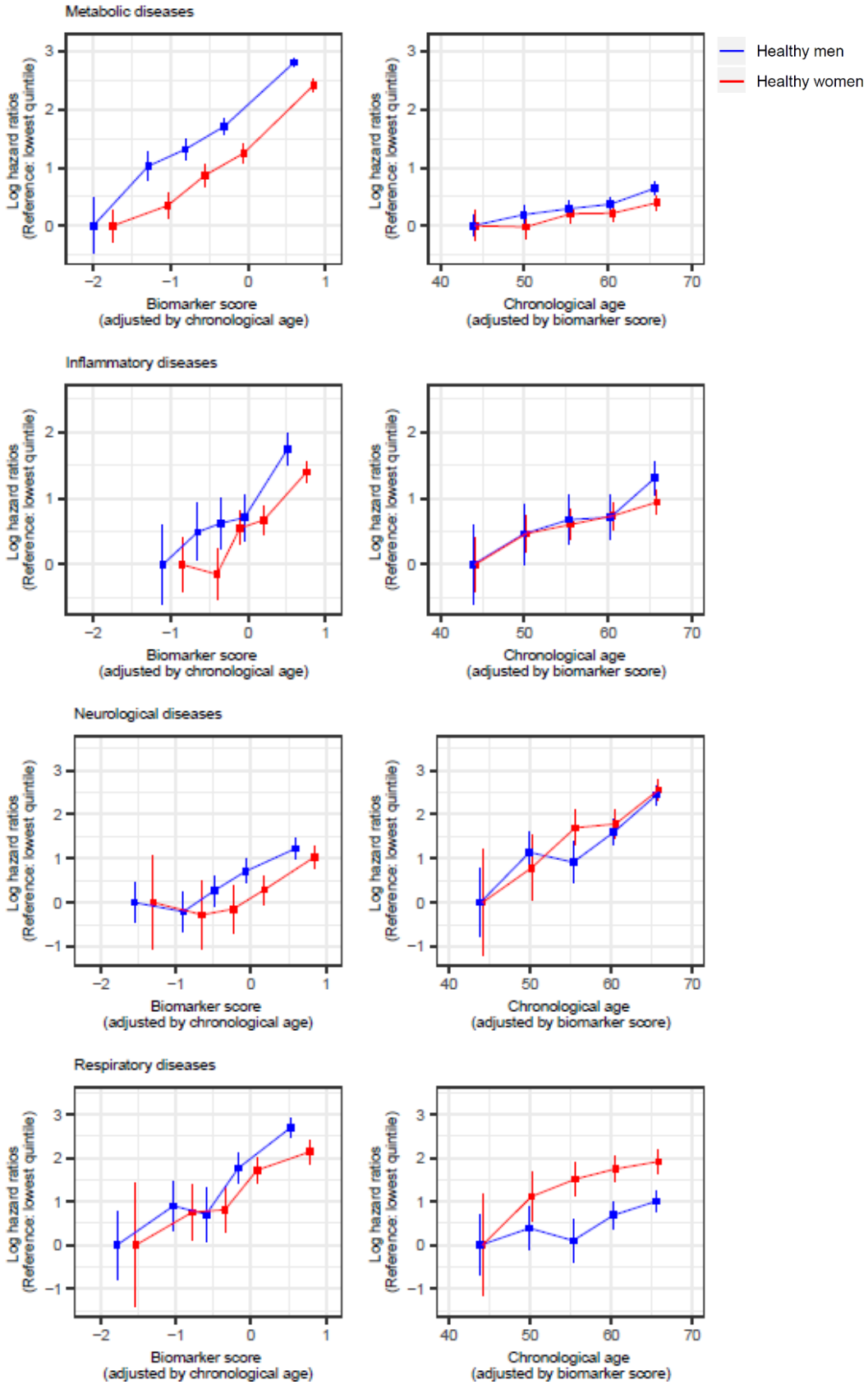




The error bars displayed are floating absolute risk 95% confidence intervals

(B) Chronological age and body system risk scores modelled jointly

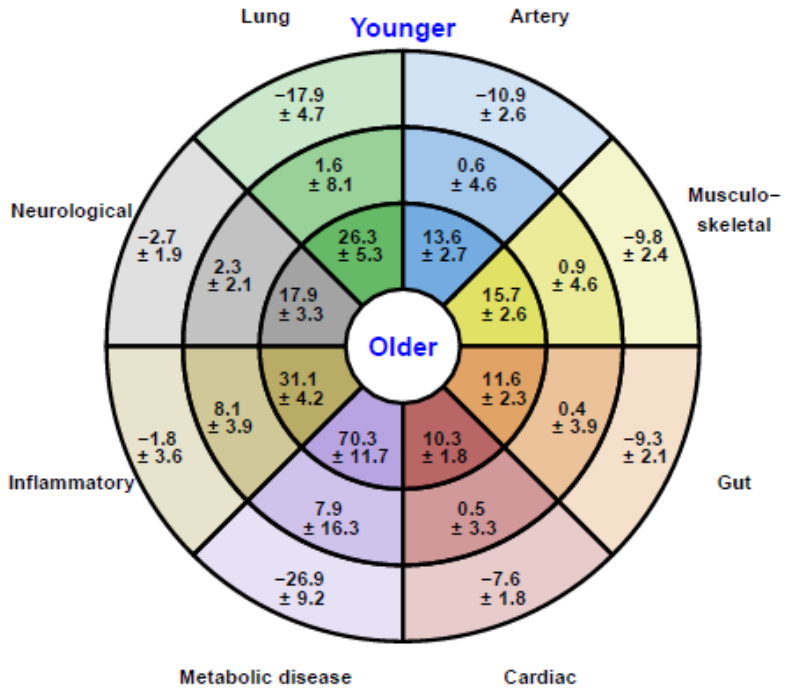




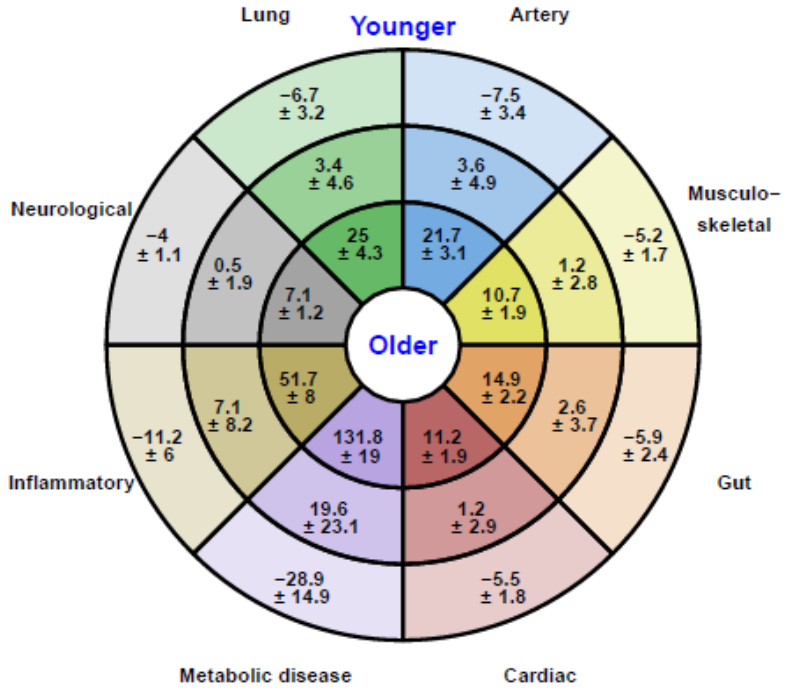
The error bars displayed are floating absolute risk 95% confidence intervals

Figure 6.4.1: Means (±1 standard deviation) of differences between each body system age and chronological age in years, for tertiles of healthy men and healthy women (separately for each body system age)

Healthy men

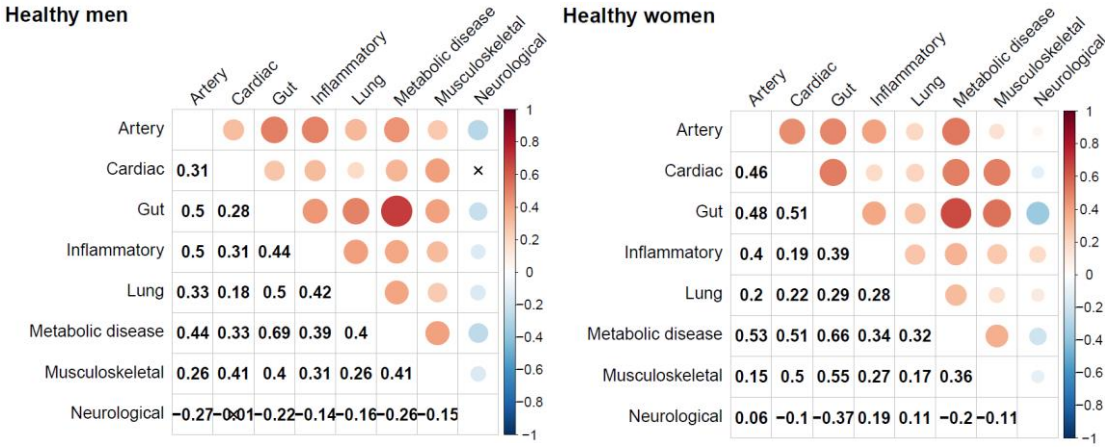


Healthy women



Outer circle: biologically youngest third, inner circle: biologically oldest third
Mean ± 1 standard deviation of chronological ages for biologically youngest, similar and oldest tertiles were 45.4 ± 2.8, 55.4 ± 2.9, 64.4 ± 2.7 for healthy men and 45.9 ± 2.8, 55.8 ± 3, 64.5 ± 2.6 years for healthy women respectively
Each segment represents an equal number of participants, regardless of the area of the segment
These body system ages have been recalibrated to chronological age

Figure 6.4.2: Pearson correlations between body system age deltas, for healthy men and women

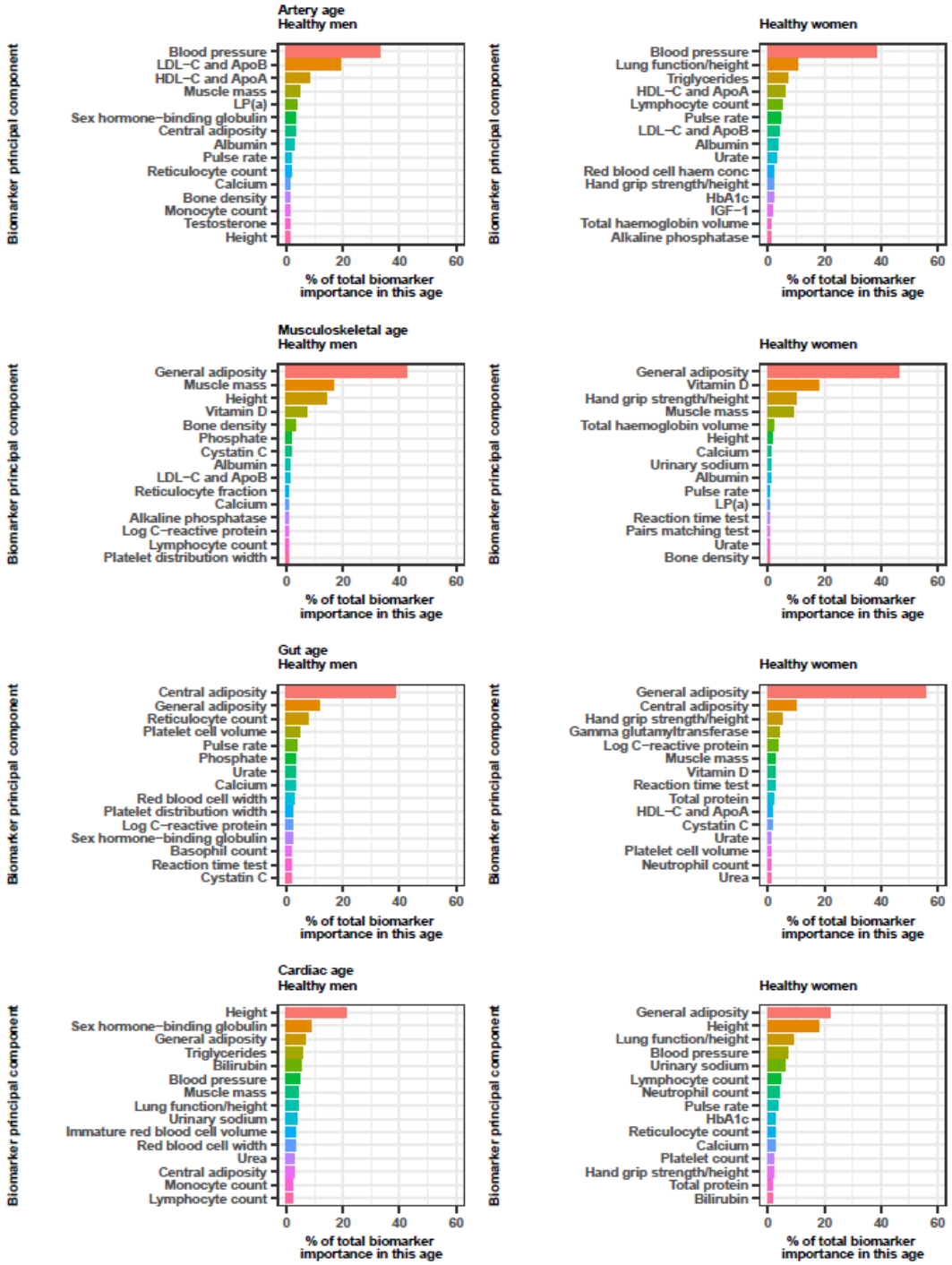


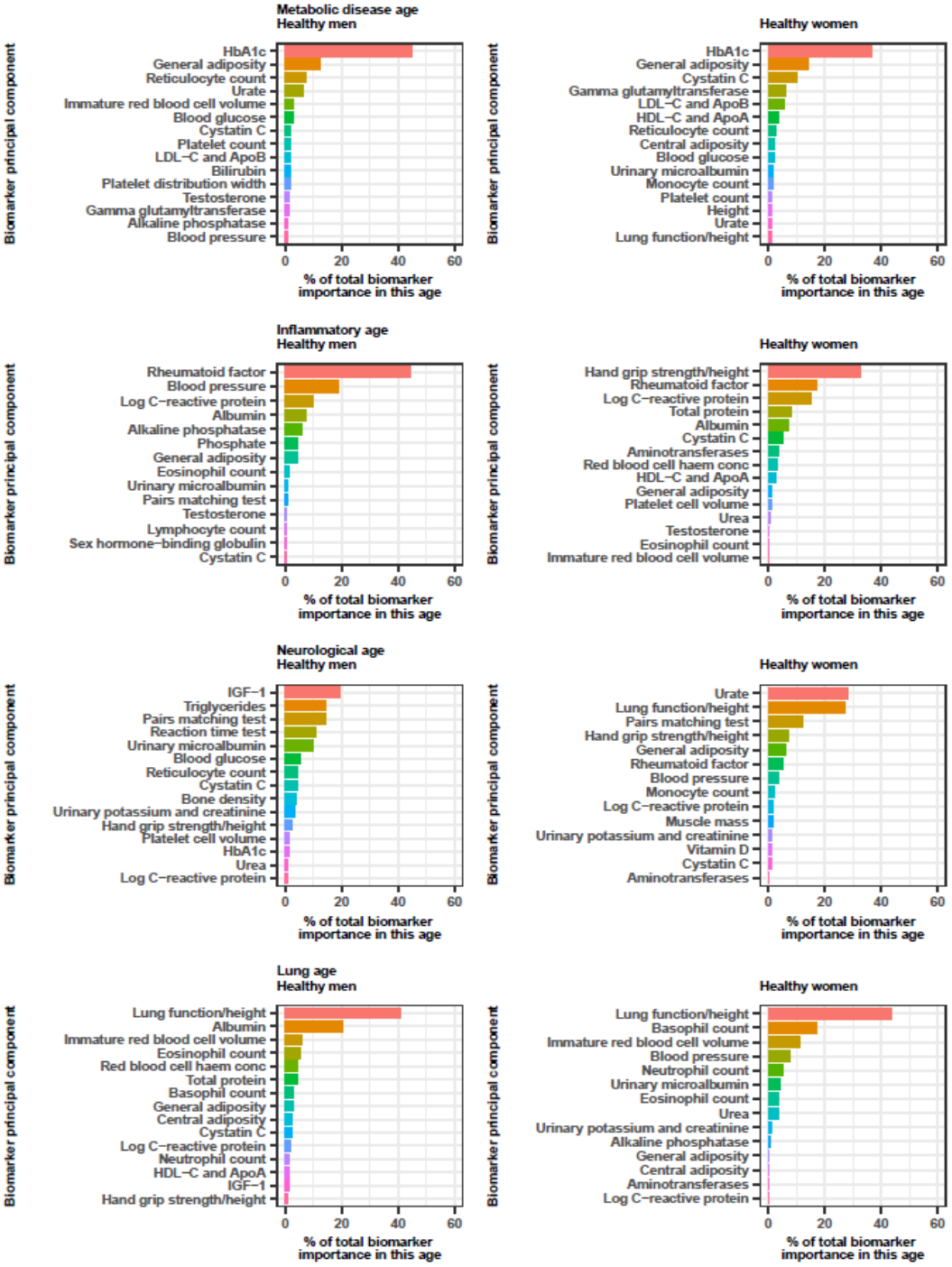
Circle sizes correspond to the magnitude of the pairwise correlation
Correlation coefficients that are crossed out are not statistically significant at the 95% level

The reduced biomarker panel consisted of the top 10 biomarker principal components for men and top 10 for women: general adiposity, lung function/height, blood pressure, HbA1c, height and rheumatoid factor in both sexes; central adiposity, albumin, muscle mass and reticulocyte count in men; and hand grip strength/height, vitamin D, C-reactive protein and urate in women

Figure 6.5.1: Importance of the top 15 biomarker principal components (% of explained relative risk by biomarker principal components) in each (A) body system age and (B) disease risk score, for healthy men and healthy women

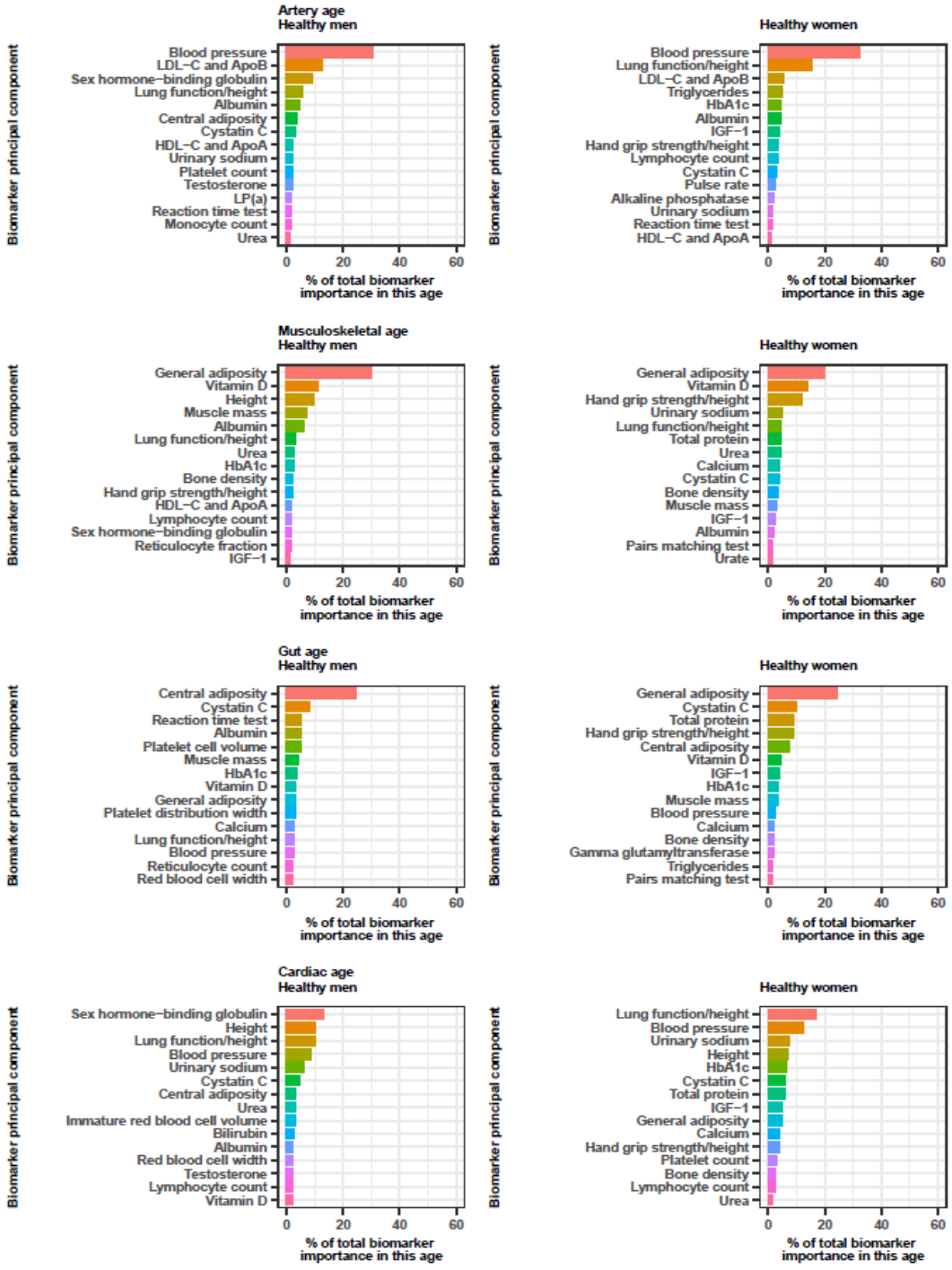
(A) Body system ages

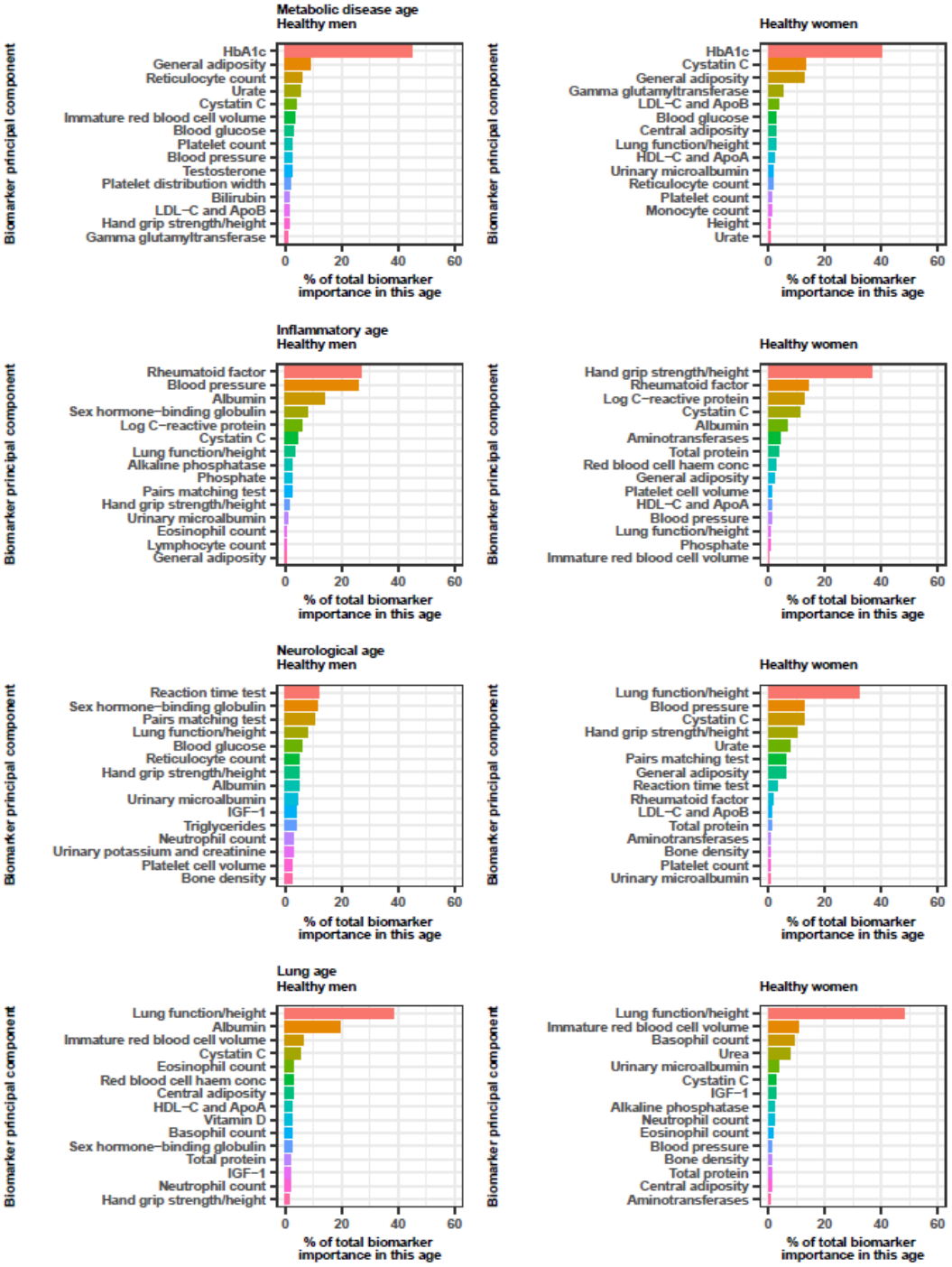




Red blood cell haem conc: red blood cell haemoglobin concentration

(B) Body system risk scores

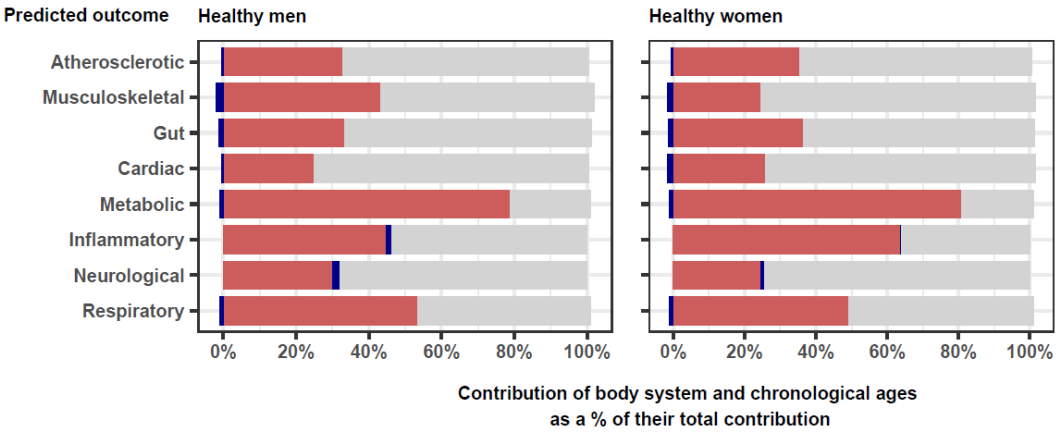




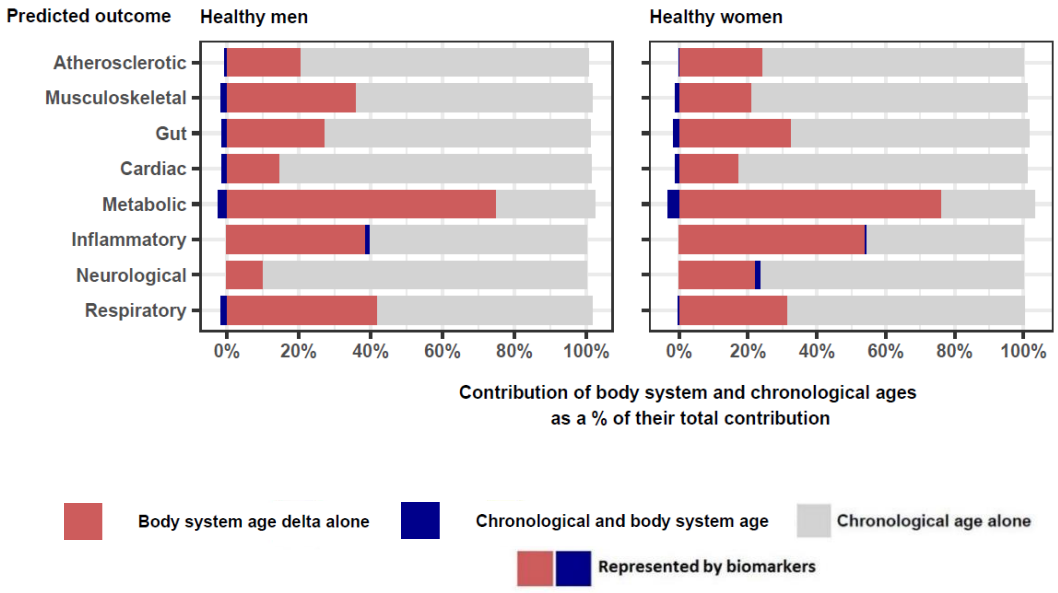
Red blood cell haem conc: red blood cell haemoglobin concentration

Figure 6.6.1: Relative contribution of body system age deltas and chronological age in explaining their respective disease outcome, in unadjusted models for the (A) main analysis and (B) when using the reduced biomarker panel, for healthy men and women

(A) Main analysis



(B) Using the reduced biomarker panel

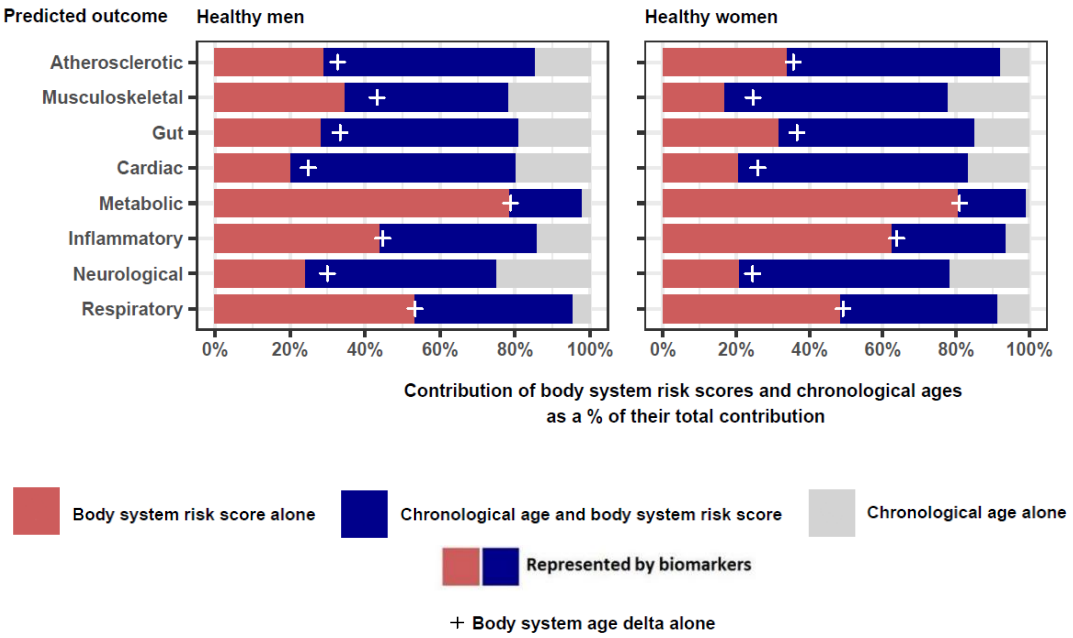


These percentages are also tabulated in Table A6.2.4

Small negative percentage values for the chronological and body system age effect were due to the pattern of log likelihoods underlying this calculation: the change in the log likelihood on adding each type of age or age delta was greater when the other type of age or age delta was already in the model. The percentages (including any negative percentages) still sum to 100% for each predicted outcome and subpopulation.

The reduced biomarker panel consisted of the top 10 biomarker principal components for men and top 10 for women: general adiposity, lung function/height, blood pressure, HbA1c, height and rheumatoid factor in both sexes; central adiposity, albumin, muscle mass and reticulocyte count in men; and hand grip strength/height, vitamin D, C-reactive protein and urate in women

Figure 6.6.2: Comparison of the relative contributions of body system risk scores and chronological age (represented by bars) vs body system age deltas alone ('+') in explaining their respective disease outcome, in unadjusted models for healthy men and women

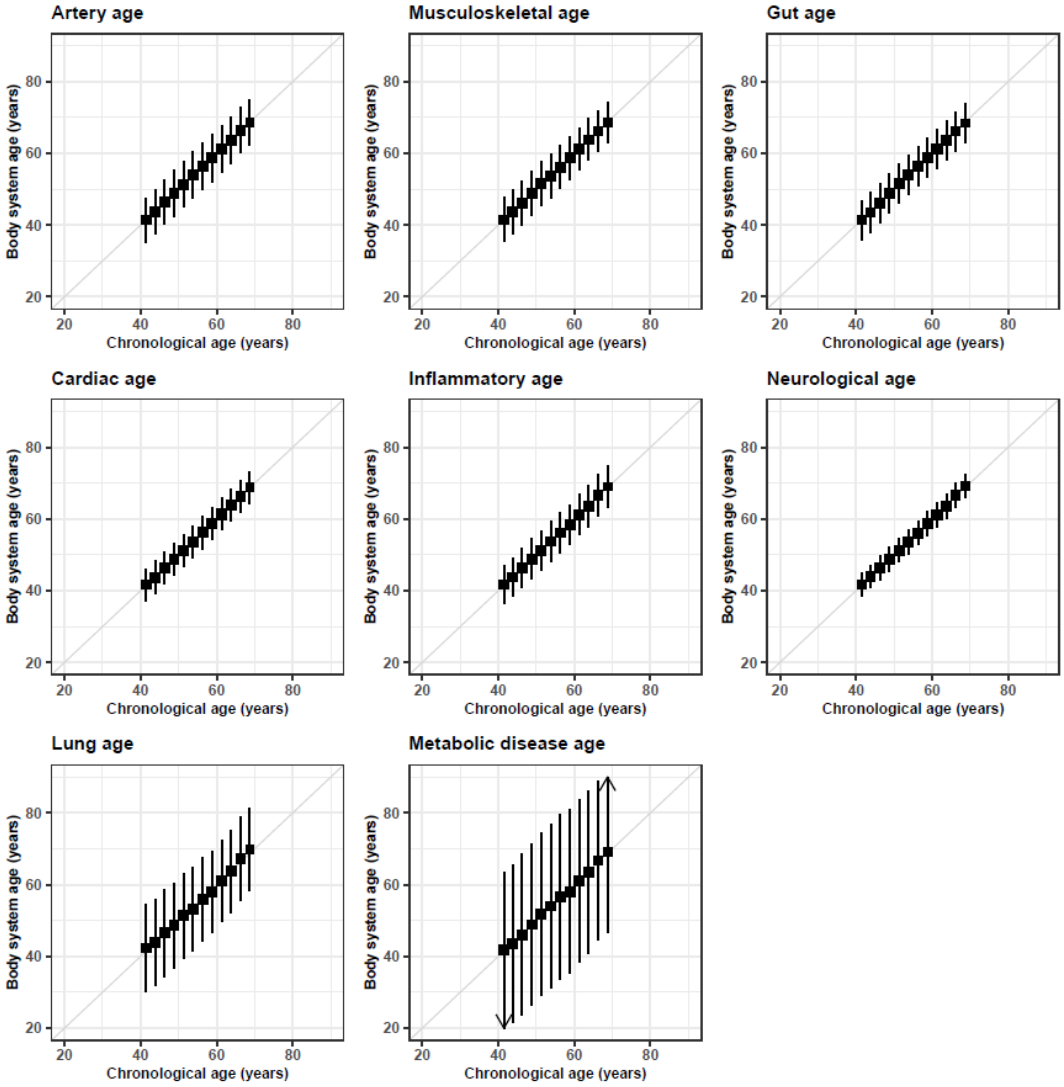


The percentages for body system age deltas alone were from separate analyses of body system age deltas vs chronological age, and the total contribution of age deltas and chronological age vs total contribution of risk scores and chronological age were the same These percentages are also tabulated in Tables A6.2.4 and A6.2.5

Figure 6.7.1: Means and standard deviations of body system ages by 2.5-year chronological age groups, for healthy men and healthy women, in the (A) main analysis (after recalibration) and (B) intermediate analysis (before recalibration)

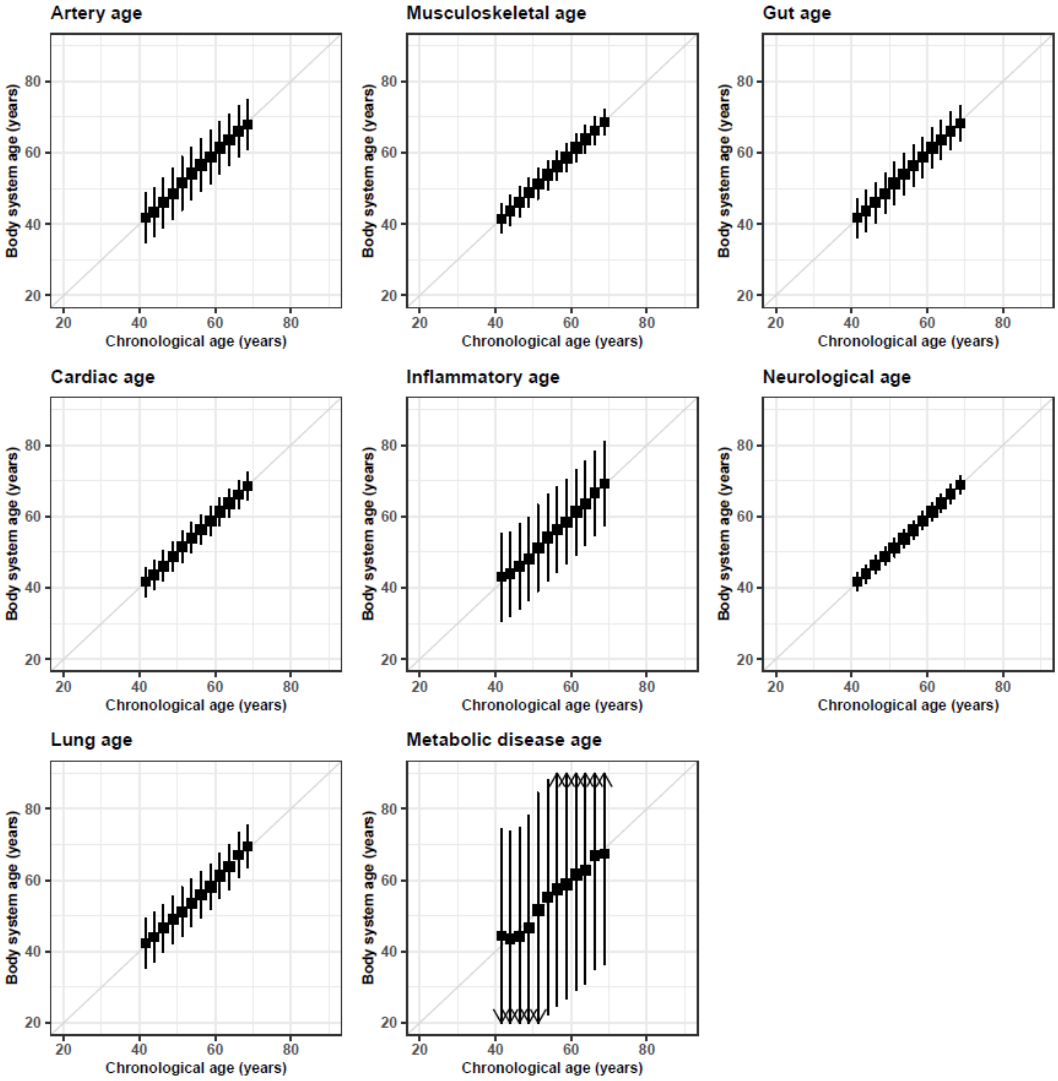
(A) Main analysis (after recalibration)

Healthy men



Metabolic disease ages were plotted on the same scales as the other body system ages
Recalibration to chronological age was applied using the modified Dubina method (Section 4.5)

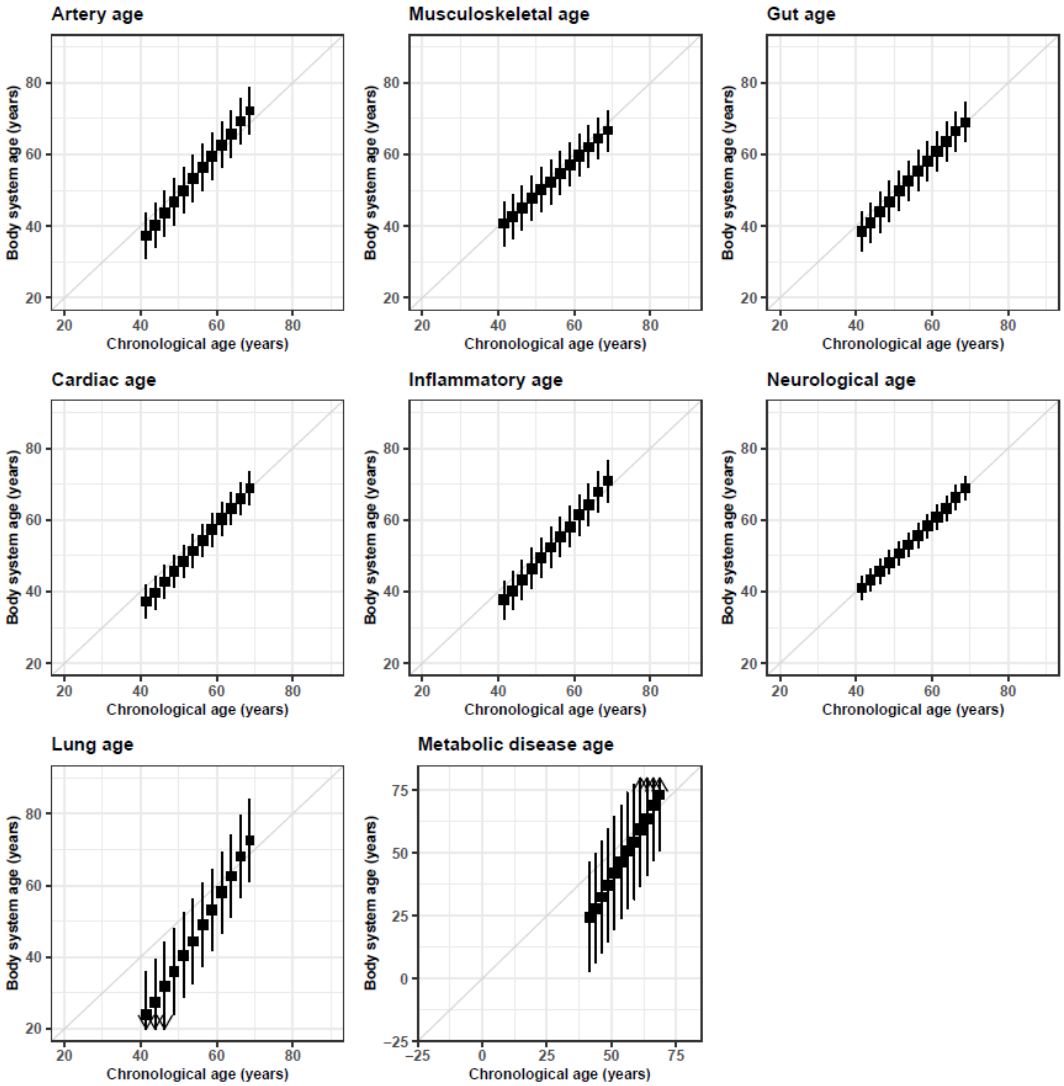
Healthy women



Metabolic disease ages were plotted on the same scales as the other body system ages
Recalibration to chronological age was applied using the modified Dubina method (Section 4.5)

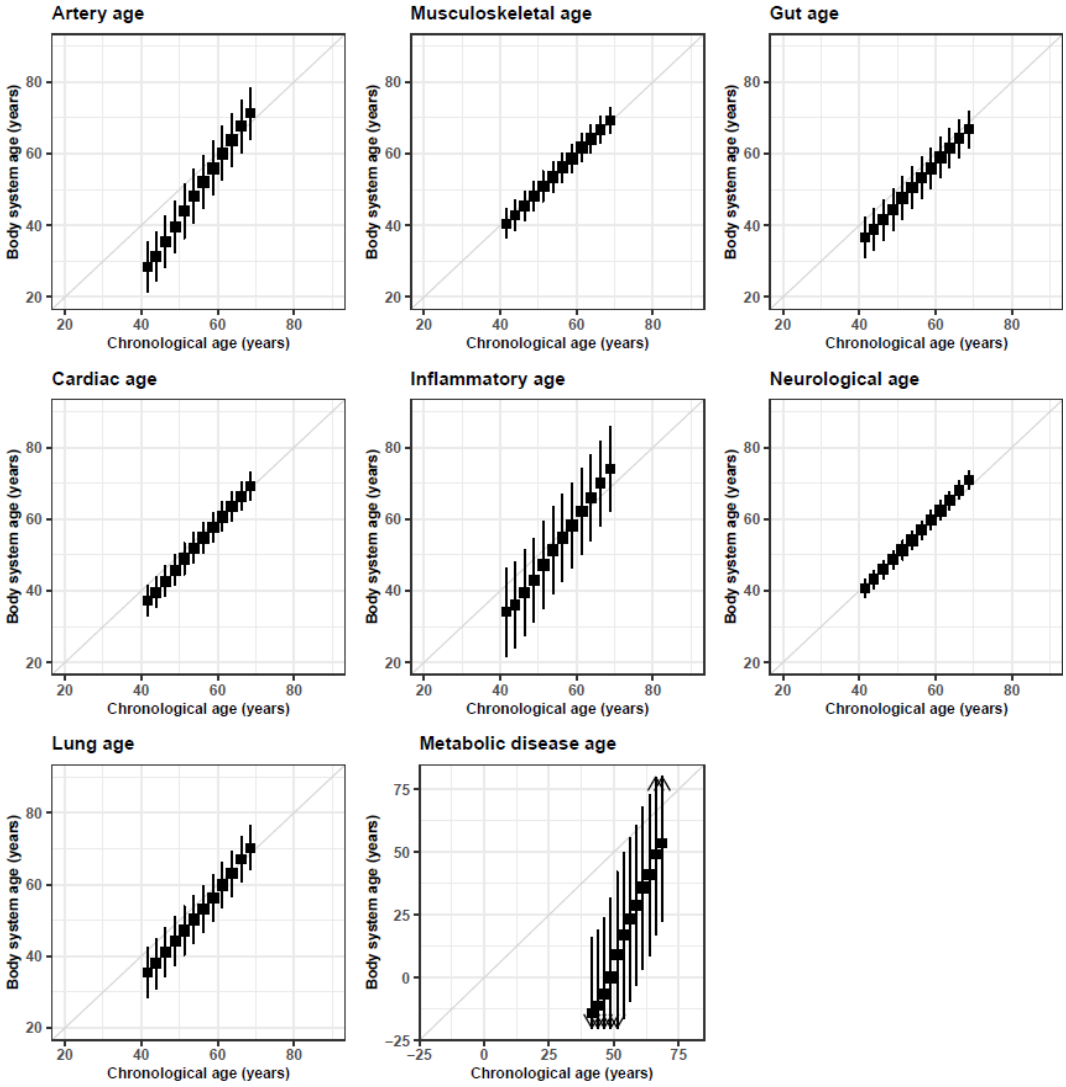
(B) Intermediate analysis (before recalibration)

Healthy men



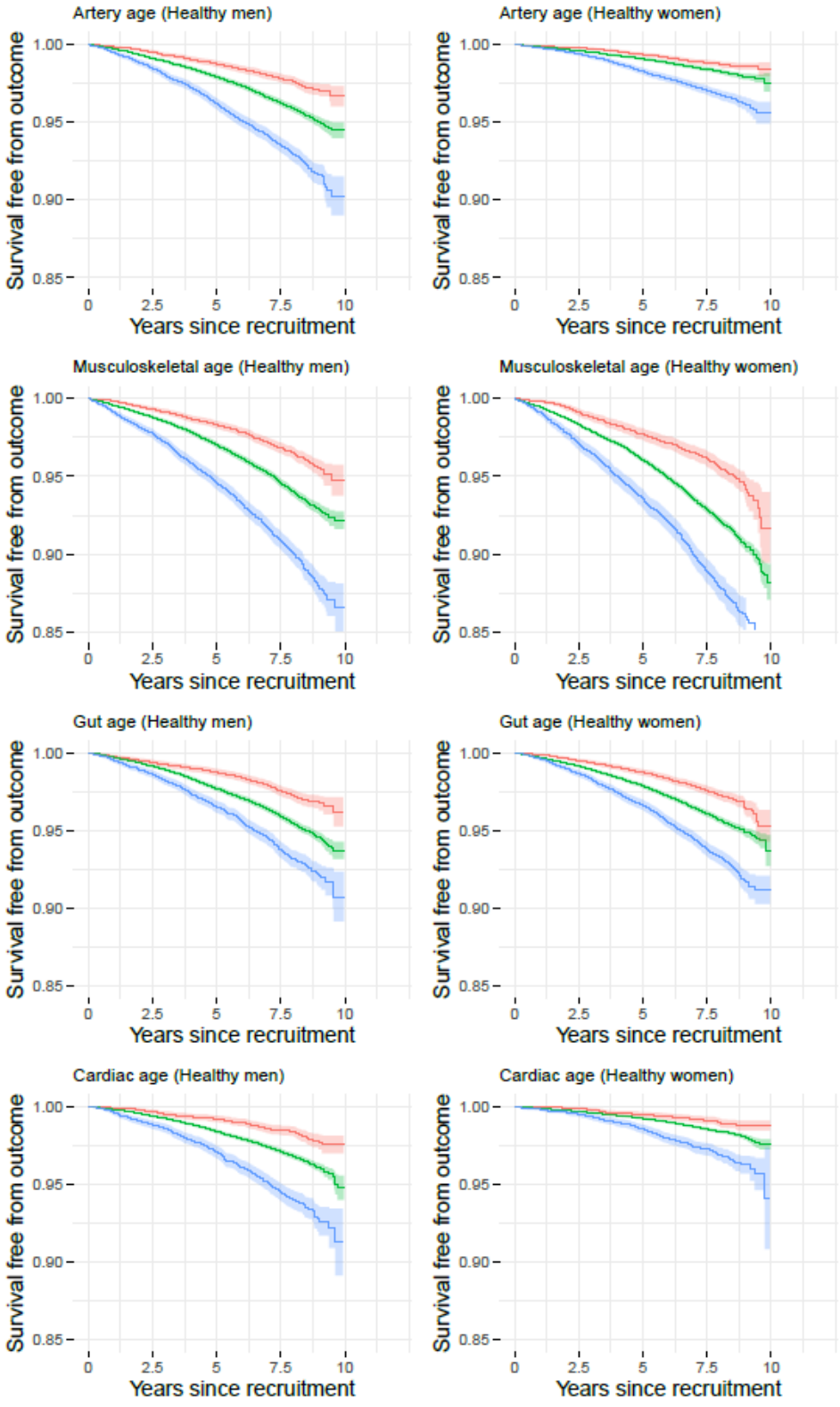
Metabolic disease ages were plotted on an extended scale for the y-axis (body system age (years)) in order to display their full range

Healthy women



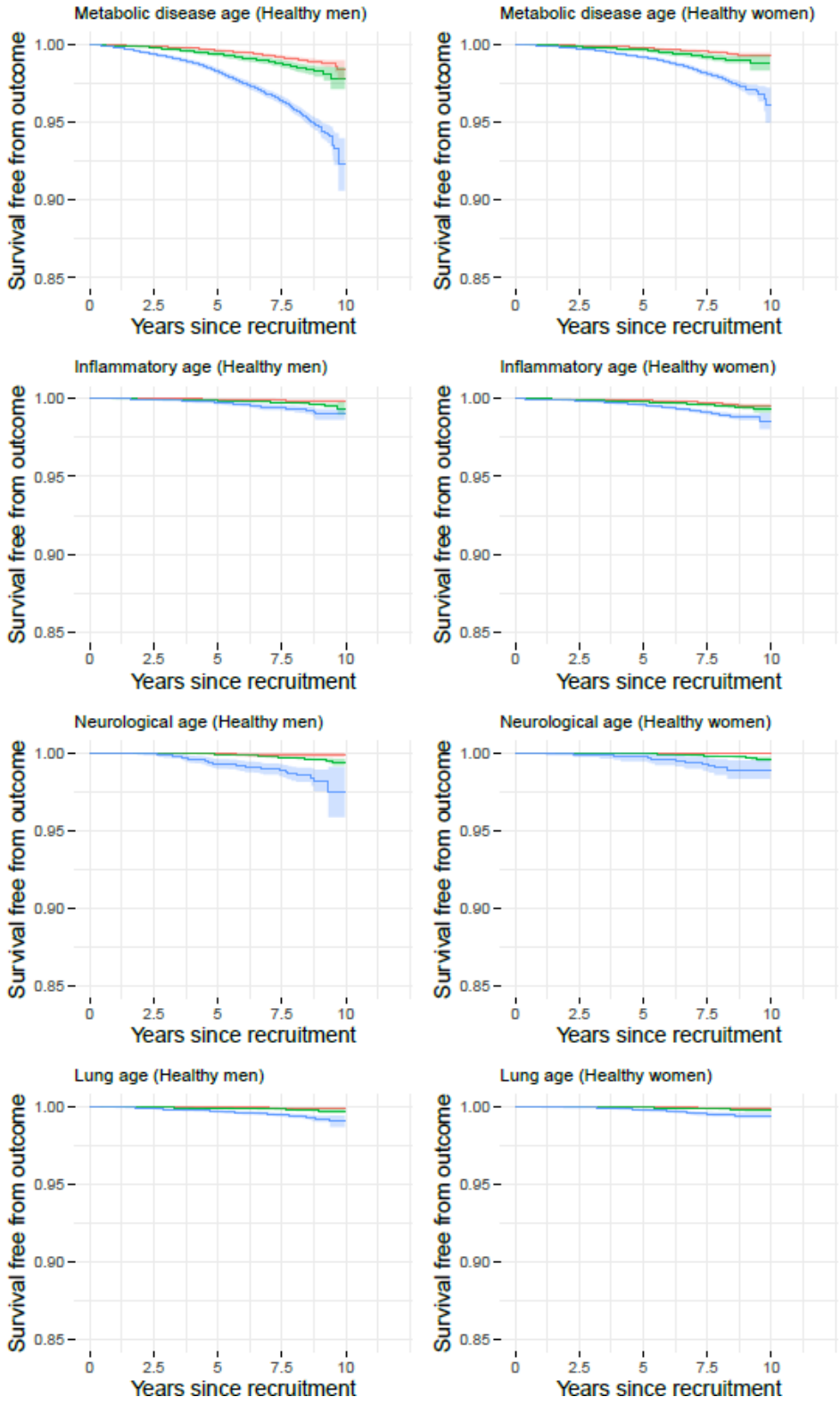
Metabolic disease ages were plotted on an extended scale for the y-axis (body system age (years)) in order to display their full range

Figure 6.7.2: Outcome-free survival of healthy men and healthy women free from their respective disease outcome, according to whether their body system age is younger, similar to or older than their chronological age



Predicted risk group Kaplan-Meier curve and 95% confidence interval (shaded area):

BA - CA < -5 years (Biologically younger) | |BA – CA| < 5 years | BA - CA > 5 years (Biologically older)



Predicted risk group Kaplan-Meier curve and 95% confidence interval (shaded area):

BA - CA < -5 years (Biologically younger)

|BA – CA| < 5 years

BA - CA > 5 years (Biologically older)

Chapter 7: Aggregation of body system ages into disease risk-based biological ages

7.1 Introduction

This chapter considers the aggregation of the 8 body system ages (Chapter 6) into an overall disease risk-based biological age. The 8 body system ages estimated in the previous chapter allowed the prognosis of multiple age-related chronic diseases via physical and biochemical biomarkers to be investigated in detail, but it remained to summarise these prognostic biomarkers into a single, succinct indicator of health risks in a similar format to the age-based biological age discussed in Chapter 5.

The only apparent examples of aggregating body system ages into biological age were those advertised by commercial South Korean companies (MediAge[77] and Bio-Age;[76] Section 4.1), but their methods for doing so are not publicly available. Other previous multi-system biological ages based on health outcomes did not aggregate separate body system ages, and used only mortality as the prediction outcome.[39,62] Liu et al investigated the link between their mortality-based biological age (PhenoAge) estimated from blood biomarkers in a representative US population and diseases by additionally assessing its predictive power for 7 causes of death (rather than just overall mortality) and its association with counts of prior disease.[62] Their study showed that their biological age was highly predictive of all-cause mortality both in an unadjusted model (C-index: 0.879), and when stratified by age, ethnicity, education, prior disease count and health behaviours.[62] It was also significantly associated with mortality from heart disease, cancer, chronic lower respiratory disease, diabetes, influenza/pneumonia and nephritis/nephrosis separately, but not mortality from cerebrovascular disease (numbers of deaths from each cause ranged from 15–227).[62] Another study of mortality-based biological ages in a similar US population did not report its biomarker constituents, but proposed that their biological age was a marker of frailty based on its associations with self-reported health and lifestyle characteristics.[39]

Apart from mortality-based risk scores, a study of multiple ageing scores estimated from one panel of physical and blood count biomarker data from UK Biobank, which captured differential ageing patterns described by the biomarkers, classified these scores based on their associations with a range of prior diseases and health conditions.[101] The 5 estimated ageing scores, ranked by highest to lowest chronological age-related variation explained, were blood pressure, cognitive, heart conditions, lung, and blood and bone rates of ageing.[101] The relative weights of these ageing scores and the extent of overlap between constituent biomarkers of each score were not quantified.

These studies reflect a paucity of research on summarising multiple ageing scores into a single, general purpose biological age that could track or represent a measureable, overall ageing process prior to death. Historically, there has been great interest in biological ageing research and biological age studies in uncovering a singular ageing process or reflecting a singular measure of physiological function, regardless of the multiplicity of putative biological ageing mechanisms and ageing biomarkers across body systems (Section 1.1). From an individual perspective, a single biological age is also more readily comparable to a single chronological age for an overview of general health (Section 1.1). It is also unclear whether mortality-based risk scores capture signs of ageing early in life prior to disease onset.

This chapter therefore describes findings from the aggregation of body system ages into an overall disease risk-based biological age, with the objectives of: (1) identifying the body system ages and biomarkers that contribute most to an overall disease risk-based biological age, (2) contrasting the body system ages and biomarkers that were most strongly related to chronological age vs mortality from chronic disease vs age-related frailty (defined as the first admission to hospital for an age-related reason, based on a previously-published hospital frailty score; Section 3.7), and (3) investigating the relationship between the disease risk-based biological age and chronological age in the prediction of mortality from chronic disease and age-related frailty.

7.2 Methods

Four types of methods were considered for aggregating the 8 body system ages estimated and assessed in Chapter 6 into an overall disease risk-based biological age:

1. A multi-state model with both mortality from chronic disease and age-related frailty as the outcomes (MSM age)
2. A standard Cox model with mortality from chronic disease as the outcome (Cox mortality age)
3. A standard Cox model with age-related frailty as the outcome (Cox frailty age)
4. The Klemera Doubal method (KDM age), which emphasised body system ages with the strongest relation to chronological age

The MSM method was chosen to be the main method as it combined the prediction of both of the general health outcomes analysed in this thesis, mortality from chronic disease and age-related frailty, in a single statistical approach (Section 4.4). The remaining 3 methods were used in sensitivity analyses to compare the effects of emphasising biomarkers that most strongly related to: the singular outcomes of mortality from chronic disease (Cox mortality age), age-related frailty (Cox frailty age) and chronological age (KDM age). To perform the aggregation, each of these models estimated relative weights for the 8 body system age deltas by regressing health outcomes (for MSM, Cox mortality and Cox frailty ages) or chronological age (for KDM age) on these age deltas, then calculated the weighted sum of these 8 body system age deltas to construct the overall biological age. The parameterisations of each of these models are described and discussed in Section 4.4, with additional details on the MSM provided in Appendix 4.1. All disease risk-based biological ages in this chapter refer to MSM ages unless otherwise stated. The analysis was conducted on the healthy subpopulation (as phenotyped in Section 3.3 and modified in Appendix 6.1), and was repeated on the whole UK Biobank population.

Coefficients for constituent body system ages in the biological ages aggregated by the main method and each of the 3 sensitivity analysis methods were reweighted so that their absolute values summed to 1, and detailed model results for each of these methods are in Appendix 7.1. Results for the main disease risk-based biological ages (the relative weights of the constituent body system ages, the importance of the biomarker principal components) were assessed, and compared with those for the other 3 methods.

Further validation of the main disease risk-based biological ages was conducted by assessing (1) the proportion of the overall age effect on the risks of mortality from chronic disease and age-related frailty explained by the biological age deltas (the differences between biological and chronological age), assessed in terms of their predictive power, and (2) calibration to chronological age and calibration to the risks of the mortality and frailty outcomes. These validation procedures were similar to those used for the age-based and body system ages (Sections 5.2 and 6.2, with statistical details provided in Section 4.5), except that biological age deltas, instead of biological ages, were analysed to separate the contributions of the constituent biomarkers from chronological age in the predictions. Since one of the 8 body system ages, metabolic disease age, was found to display substantial variation within 2.5-year chronological age groups in the previous assessment of calibration for individual body system ages (Figure 6.7.1), a sensitivity analysis that excluded metabolic age from the aggregated biological ages was conducted.

7.3 Biomarker and body system age constituents of aggregated biological age

The relative weights of each body system age delta in the 4 types of biological ages for the healthy subpopulation are summarised in Figure 7.3.1 and Table A7.5, and were compared between aggregation methods. These weights were derived from the model coefficients (Tables A7.1, A7.3 and A7.4). In the MSM age, the constituent body system age deltas with the largest weights were gut, cardiac and neurological ages (similarly weighted) for men, and musculoskeletal, then neurological ages for women. In the Cox mortality age, those with the largest weights were

neurological, gut then inflammatory ages for men, and cardiac, then gut and lung ages (both negatively weighted) for women. The weights in the Cox frailty age were similar to those for the MSM age, but cardiac ages were more prominent for men and less prominent for women, while inflammatory ages were less prominent for men. The relative weights for the MSM age were a combination of the weights for the Cox mortality and frailty ages, with much greater similarity with the Cox frailty weights as there were many more transitions to the frail than the dead state during follow up (Table A7.2). However, in the KDM age, cardiac, artery, inflammatory and then lung ages had the largest weights for men, and neurological, artery and then cardiac ages had the highest weights for women.

Metabolic disease age had the smallest weights in all of the aggregated biological ages (Figure 7.3.1 (A) and Table A7.5 (A)). Therefore the exclusion of metabolic disease age from the aggregation of the biological ages resulted in very small changes in the weights for the body system ages, except for gut and neurological ages in the Cox mortality and Cox frailty aggregation methods (changes in weights for gut and neurological age deltas respectively: -0.062–0.031 and -0.018–0.045; Table A7.5).

Based on the relative weights of the body system ages and their constituent biomarker principal components in the MSM ages, the relative weights of the constituent biomarker principal components were elicited (Table 7.3.1) and the top 15 most important biomarker principal components for each sex were calculated and are displayed in Figure 7.3.2. Higher general adiposity, lower central adiposity, higher log C-reactive protein (CRP), poorer lung function/height and then higher blood pressure featured strongest for healthy men; while lower hand grip strength/height, higher general adiposity, poorer lung function/height, higher log CRP and then higher vitamin D featured strongest for healthy women (Figure 7.3.2). These top 15 biomarkers were broadly similar between sexes, with central adiposity featuring strongly in healthy men and hand grip strength/height featuring strongly in healthy women being the key exceptions (Figure 7.3.2).

7.4 Biological and chronological age effect of mortality and frailty risks

The proportions of the overall biological and chronological age effects on mortality and frailty risk are displayed in Table 7.4.1 and Figure 7.4.1. The proportions of mortality risk explained by biological age deltas alone were 17.4% for healthy men and 10.7% for healthy women, while those of frailty risk were 11.2% for healthy men and 16.6% for healthy women (Table 7.4.1 (A) and Figure 7.4.1 (A)). The proportions of mortality or frailty risk explained by both biological age delta and chronological age were either zero or slightly negative (0.0–-0.4%), and this phenomenon was also seen in several body system ages where the interactive effects arising from having both types of age (chronological age and the bodily age delta) in the model was greater than the sum of effects for each type of age (Section 6.8).

When biological ages instead of biological age deltas were used in the analysis, the proportion of the overall age effect described by both biological and chronological age overlapped substantially (mortality risk: 81.9%/88.9%, frailty risk: 88.8%/83.3% for men/women respectively; Table 7.4.1 (B) and Figure 7.4.1 (B)). When the disease risk-based biological ages derived based on the healthy subpopulation were applied to the whole population, greater proportions of the overall age effect were explained by disease risk-based biological age deltas alone (mortality risk: 36.7%/26.9%, frailty risk: 25.0%/37.6% for men/women respectively; Table 7.4.1).

7.5 Validation results

Predictive power of biological ages

In the healthy subpopulation, adding biological ages to the unadjusted prediction models with chronological age statistically significantly improved model fit (p-values for likelihood ratio tests for mortality/frailty: $p<1\times10^{-27}$/$p<1\times10^{-51}$ in healthy men; $p<1\times10^{-7}$/$p<1\times10^{-53}$ in healthy women). It also increased the C-indices for mortality/frailty slightly (increases in C-indices: 0.018/0.008 from

0.707/0.634 in men, 0.008/0.008 from 0.663/0.602 in women; Table 7.5.1 (A)) in the healthy subpopulation. Greater increases were seen when biological ages derived on the healthy subpopulation were used in the whole UK Biobank population (0.042/0.018 from 0.686/0.629 in men, 0.024/0.025 from 0.670/0.603 in women) (Table 7.5.1 (A)). These increases were attenuated in models adjusted for Index of Multiple Deprivation 2010 quintile, smoking status, alcohol consumption and UK Biobank assessment centre (Table 7.5.1 (A) vs (B)). In unadjusted analyses, C-indices for prediction models with chronological age alone were generally lower in the whole population compared to the healthy subpopulation (Table 7.5.1 (A)), while in adjusted analyses, these differences in C-indices across populations were slightly attenuated (Table 7.5.1 (B)).

Calibration of biological ages

For the multi-state model, the additional parametric assumptions imposed on the shape of the baseline hazard and the coefficients of the constituent body system ages (Appendix 4.1) were assessed via the goodness of fit of modelled vs observed state prevalences across follow up durations of up to 9 years in the healthy subpopulation, for each of the 3 states (Figure 7.5.1). The observed state prevalence was close to the modelled state prevalence for both sexes at all follow up durations in all 3 states, after allowing for differential follow up in the population (Figure 7.5.1). With respect to the multi-state and the 2 Cox models, it was previously observed that the incidence patterns by chronological age for all relevant health outcomes (the 8 disease groups, mortality from chronic disease and age-related frailty) were broadly log-linear (Figures 3.6.1 and 3.9.1) and therefore the parameterisation of chronological age as a linear term in these models (Section 4.4) was suitable.

The disease risk-based biological ages aggregated via the MSM method were well calibrated to chronological age, as they matched healthy participants' chronological ages on average (Figure 7.5.2). Additionally, biological ages aggregated by the other 3 methods were also well calibrated to chronological age, and their standard deviations from chronological age (within each 2.5-year

chronological age group of healthy men and women) were larger than or similar to those for the MSM-aggregated age (Figure A7.3).

Biologically older participants (those with biological ages >5 years older than their chronological ages) had the highest mortality and frailty rates, while biologically younger (those with biological ages >5 years younger than their chronological ages) participants had the lowest rates, for both sexes (Figure 7.5.3). The observed time since baseline to 1% having the mortality outcome for biologically older, similar and younger participants was approximately 2, 5 and 10 years for healthy men and 4, 7 and 9 years for healthy women, while time to 10% having the frailty outcome was approximately 3, 6 and 9 years for healthy men and 4, 6 and 8 years for healthy women respectively. The differences in these rates between individuals who were biologically older, similar or younger than their chronological age were highly statistically significant based on log-rank tests ($p<1\times10^{-12}$ for all sexes and outcomes).

7.6 Discussion

The novel method of aggregating 8 body system ages into an overall disease risk-based biological age in this thesis summarised biomarker patterns that predicted later life diseases, frailty and mortality into a single measure of health. The analysis of disease risk-based biological ages also enabled the following key findings: (1) a biological age whose constituent biomarkers were prognostic of diseases across multiple body systems (which was aggregated using the main MSM method) supplemented chronological age in predicting subsequent mortality from chronic disease and age-related frailty, (2) biomarker and body system determinants of mortality and frailty risk in this cohort were in part sex-specific, and (3) patterns of biomarkers and body system ages that predicted mortality, predicted frailty and changed more substantially with chronological age (when comparing biological ages estimated from the different aggregation methods) were not the same.

Risk communication using disease risk-based biological and body system ages

To illustrate how these body system and biological ages could be communicated, radar charts (which Nakamura first proposed as a method to visualise individuals' biological age and its constituents[249]) were plotted to display the mean disease risk-based biological age and body system age deltas, for each tertile of healthy men or women ranked by their disease risk-based biological age deltas (Figure 7.6.1). Large differences between the biologically youngest and oldest tertiles for metabolic disease ages were seen in this illustration (55 years for men and 70 years for women; Figure 7.6.1(A)), yet these differences contributed very little to the aggregated biological age (differences in the means of each biological age tertile when metabolic disease ages were included vs excluded were <0.1 years; Figure 7.6.1 (A) vs (B)). Therefore excluding metabolic disease age from the illustration (Figure 7.6.1 (A) vs (B)) and instead communicating HbA1c levels and cystatin C levels (either in terms of an estimated glomerular filtration rate [eGFR] or as a standalone kidney age[65] for cystatin C levels), as discussed in Section 6.8, may improve the communication of these risks. In either case, the mean disease risk-based biological ages for these healthy men and women deviated by approximately -4, 1 and 8 years from their mean chronological ages for the biologically younger, similar and older tertiles respectively (Figure 7.6.1). Neurological age did not vary substantially between tertiles (Figure 7.6.1), as it was not substantially correlated with the other body system ages ($|\rho|<0.3$; Figure 6.4.2) that made up the large majority of the overall biological age (Figure 7.3.1), and neurological age was very highly correlated with chronological age ($\rho>0.92$; Table 6.4.1).

Biomarker and body system age constituents of disease risk-based biological ages

The most important biomarkers for the main disease risk-based biological age were similar to those in several body system ages, but did not span the full range of the most important biomarkers for each of the 8 body system ages (Figures 7.3.2 vs 6.5.1 (A)). For instance, of the biomarkers featuring strongly in the overall biological age, general adiposity featured strongly in musculoskeletal, gut and metabolic ages, and lung function/height featured strongly in lung age for both sexes and artery,

cardiac, neurological and lung ages for women (Figures 7.3.2 vs 6.5.1 (A)). Whereas HbA1c did not feature strongly in the disease risk-based biological age but featured most strongly in the metabolic disease age (Figure 7.3.2 vs 6.5.1 (A)), despite being a key determinant of clinical diagnoses for diabetes.[213] In addition, rheumatoid factor did not feature strongly in the disease risk-based biological age but featured most strongly in the inflammatory age for healthy men (Figure 7.3.2 vs 6.5.1 (A)). Vitamin D was the 5th most important biomarker in healthy women (where its association with biological age was positive, Section 7.3 and Figure 7.3.2) and only strongly featured in musculoskeletal age in women, even though its associations with respiratory mortality[208,209] and multiple sclerosis[210] had been reported in previous studies while associations with musculoskeletal diseases was unclear (Section 6.8).

These biomarker and body system age patterns highlight the variation in strength of associations between body system-specific ageing patterns and more general health outcomes such as mortality or frailty, which has not been investigated in mortality-based biological age or ageing score studies[62,72,73] so far. This variation is likely to be better captured by the biological age estimation approach used in the analyses for this chapter than cumulative deficits approaches used by indices such as the frailty index and allostatic load, which tend to assign equal weights to constituent biomarkers or risk factors (Section 1.2).

Several previous studies reported the key biomarker constituents of mortality-based risk scores,[72,73] but there was little overlap in their candidate biomarker panels. A critical issue in this area is that the set of biomarkers used in biological ages vary between studies, making direct comparisons difficult. A study of mortality scores in a US population utilised a different set of biomarkers from these analyses, and found that creatinine, then HbA1c, then red blood cell distribution width were the most important biomarkers,[72] while another study that used the physical biomarker measurements and questionnaire responses in the UK Biobank found that the relative importance of the physical biomarkers in mortality scores varied by estimation method (although forced expiratory volume in 1s featured strongly across all methods) and were generally less important than sociodemographic

factors, health behavioural factors and prior disease.[73] The sensitivity analyses of mortality-based biological ages in this thesis likewise found that coefficients in Cox mortality scores were different between the two estimation methods used (estimated from body system age deltas vs estimated directly from biomarkers), but the largest coefficients in both methods included central adiposity, blood pressure and reaction time test in healthy men; and hand grip strength/height, height, blood pressure and muscle mass in healthy women (Table A7.6 and Figure A7.2).

The relative weights of the body system age delta constituents of the disease risk-based biological age also provided insights into the relative importance of body systems and biomarkers in the prognosis of age-related health outcomes. The largest weights were assigned to gut, cardiac and neurological age deltas for men, and musculoskeletal, then neurological age deltas for women, and this pattern of relative weights was much more similar to patterns for the Cox frailty compared to Cox mortality ages (Figure 7.3.1). There were relatively larger weights on body system age deltas related to frailty than mortality in women in this healthy subpopulation (particularly musculoskeletal age delta), as they had a higher frailty incidence rate compared to men (Table A7.2), and musculoskeletal diseases and frailty may be closely linked as frailty indices generally emphasise the detection of decline in physical function in individuals. Neurological age deltas featured strongly even though the incidence of neurological disease in this subpopulation was low (Table 3.9.3), and many key biomarkers for neurological diseases used in biological research tend to be measured via cerebrospinal fluid, brain images or genotyping[215] and so were not included in this thesis. Therefore the physical and biochemical biomarker constituents of neurological ages in this analysis (which were generally different from those of other body system ages; Figure 6.5.1) may also capture ageing effects linked to biomarkers or risk factors not investigated in this thesis.

The very low weighting assigned to metabolic disease age in the disease risk-based biological age (Figure 7.3.1 (A)) may have been due to both the large population variation of metabolic disease ages (Section 6.3) and a poor link between metabolic disease and mortality from chronic disease or age-related frailty. The poor link is suggested by coefficients of the Cox lasso mortality and frailty

ages (Table A7.3), and diabetes (the most common metabolic disease in this population; Table 3.9.3) being relatively less lethal than other diseases (diabetes accounted for 10% global prevalence in adults vs 4% of global deaths in people aged <70 years[250]). Metabolic disease ages therefore contributed little to the disease risk-based biological ages (i.e. they were less related to mortality or frailty in later life than other body system ages), despite being highly predictive of metabolic diseases (Table 6.7.1).

Lung ages also contributed little to the disease risk-based biological ages (Figure 7.3.1), despite lung function/height being the 3rd or 4th most important biomarker component in the disease risk-based biological age deltas (Figure 7.3.2) and being highly predictive of respiratory disease (Table 6.7.1). Lung function/height was also an important biomarker in artery, cardiac and neurological ages in healthy women (Figure 6.5.1), therefore it would have contributed to the overall disease risk-based biological age via these other body system ages as well as via lung age. Since lung function/height also had the strongest linear relation to chronological age in this population (Section 3.10), it was possible that lung function/height contributed to several of these bodily ages as a proxy for an ageing effect not captured by any of the 51 biomarker principal components.

The differences in weights of body system ages by prediction outcome (i.e. in biological ages estimated from the main vs alternative aggregation methods) could be related to differential patterns of causes of death or frailty. The types of diseases that are most prevalent, or had high incidence in this middle-aged population e.g. musculoskeletal and gut diseases (Table 3.9.3), are not necessarily the most lethal. Dementia and ischaemic heart disease are current leading causes of death in the UK.[251] Even though dementia is lethal, it is prevalent at older ages in the UK in addition to less lethal diseases such as diabetes and chronic kidney disease.[136] Additionally, patterns of causes of death vary over time and by sex and age groups.[251] Although frailty may be partially defined by prior disease in many research studies and clinical indices and has been shown to be associated with numerous diseases (such as cardiovascular and catabolic diseases),[5] research on the relative strengths of these disease associations is limited. The main MSM-aggregated age had similar weightings to

the Cox frailty age as many more participants became frail than died during follow up in this population, particularly for women (Table A7.2), otherwise the weightings in the MSM age shared similarities with the Cox mortality age (Section 7.3). Hence larger weightings were given to body system ages that best described frailty risk (particularly for women where musculoskeletal age featured very strongly; Figure 7.3.1), and these results are likely to be population-specific and dependent on the age profile of the reference population.

Prediction in healthy versus unhealthier individuals

Similar to the age-based biological age, knowledge of the disease risk-based biological age is potentially more useful in apparently healthier individuals because in unhealthy individuals, their disease and frailty already provide diagnostic indicators of ageing[22] (Section 5.6). The disease risk-based biological ages supplemented chronological age in predicting both mortality from chronic disease and age-related frailty in the healthy UK Biobank subpopulation (increases in C-indices≥0.008 in the healthy subpopulation and ≥0.018 in the whole population; Table 7.5.1). Additionally, the biomarker constituents of the disease risk-based biological age (represented by the biological age deltas) accounted for a substantial proportion of the biological and chronological age effect on predicting these outcomes (>10% of the overall biological and chronological age effects; Figure 7.4.1). The predictive value of biological age was greater in the whole UK Biobank population (i.e. unhealthier individuals; Table 7.5.1), likely reflecting an additional diagnostic element of these biomarkers in the less healthy individuals.

Strengths and limitations

This thesis utilised the rich biomarker and linked hospital data in the UK Biobank to conduct a novel biostatistical analysis that aggregated the 8 body system ages into an overall disease risk-based age. This multi-level approach enabled the risks of age-related chronic diseases and general health outcomes to be summarised coherently into a single age-specific multi-system risk score. The

resulting disease risk-based biological ages were well calibrated to chronological age and to mortality and frailty risk in the healthy subpopulation (Figures 7.5.2–7.5.3).

The aggregation methods used in this analysis apart from KDM relied on the capability of detecting mortality from chronic disease through death registry records and age-related frailty through hospital admission records. Hence the limitations of detecting frailty only through these records (using the codelist in Table 3.7.1) would have affected the weights of the body system ages, and may contribute to the relatively lower predictive power of both age-based and disease risk-based biological ages for age-related frailty (Table 7.5.1). Using a graduated scale for hospital admissions related to frailty, e.g. by extending the multi-state model to contain separate states for counts of hospital admission episodes or different reasons for admission, would make overall biological age more sensitive to the severity of these hospital admissions.

In the UK, death recording is generally treated as accurate and complete as there is a legal requirement to register a death within 5 days.[252] However, the larger standard errors in the coefficients when mortality was used as the only prediction outcome (Appendix 7.1) were related to smaller numbers of deaths for this middle aged population. Further investigation of these biological ages with longer follow up of this cohort well beyond the ages of 80 years may prove interesting, when this data becomes available over the next decade. Supplementing the prediction of mortality with additional information from frailty-related transitions in the MSM reduced the standard errors of the model parameters (Tables A7.3 vs A7.1), as there were far more frailty-related transitions than mortality-related transitions in this cohort (Table A7.2).

Since this analysis focused on the healthy subpopulation and on biomarkers rather than clinical diagnoses as determinants of biological ageing, the potential for increasing the predictive power of biological ages in a less healthy population by supplementing them with information on multiple diseases (either as additional outcomes or time-varying covariates) was not investigated, but may be substantial.[62,73] This investigation may require methodological development of high-dimensional

models that can incorporate the 8 groups of the 20 age-related chronic diseases identified in Section 3.5.

Conclusions

This investigation of disease risk-based biological ages contrasted key biomarkers and body system determinants of later life health and found that the biomarker constituents accounted for a substantial proportion of the biological and chronological age effect on mortality and frailty in the UK Biobank. The disease risk-based biological age has the potential to be communicated in conjunction with its constituent body system ages as indicators of overall and body system-specific health. It is also amenable to the incorporation of additional information on biomarkers and health outcomes for future refinements.

Chapter 7 tables and figures

Table 7.3.1: Biomarker principal component coefficients for the disease risk-based biological ages, for healthy men and women

Biomarker principal component		Healthy men	Healthy women
PC1	General adiposity	-0.033	-0.037
PC2	Total haemoglobin volume	0.006	0.031
PC3	Height	-0.006	0.008
PC4	Albumin	0.053	0.038
PC5	Neutrophil count	-0.013	-0.007
PC6	Immature red blood cell volume	-0.021	-0.012
PC7	LDL and ApoB	-0.006	0.003
PC8	Reticulocyte count	-0.004	-0.013
PC9	Urinary potassium and creatinine	0.020	-0.008
PC10	Blood pressure	-0.040	-0.015
PC11	HDL and ApoA	-0.007	-0.014
PC12	Aminotransferases	0.010	-0.017
PC13	Bilirubin	-0.020	-0.004
PC14	Platelet count	-0.008	-0.003
PC15	Red blood cell haemoglobin concentration	-0.007	-0.023
PC16	Testosterone	0.020	0.019
PC17	Lung function/height	-0.036	-0.054
PC18	Blood glucose	0.022	-0.006
PC19	Platelet cell volume	-0.023	-0.012
PC20	LP(a)	0.010	-0.013
PC21	Pairs matching test	-0.037	-0.035
PC22	Rheumatoid factor	0.019	0.029
PC23	Bone density	-0.005	-0.015
PC24	Vitamin D	0.025	0.059
PC25	IGF-1	-0.040	0.006
PC26	Urinary microalbumin	0.041	0.010
PC27	Basophil count	0.020	0.012
PC28	Central adiposity	-0.060	0
PC29	Eosinophil count	0.014	0.014
PC30	Alkaline phosphatase	-0.019	-0.003
PC31	Pulse rate	-0.006	0.028
PC32	Red blood cell width	-0.028	0
PC33	Reaction time test	-0.039	0.010
PC34	Sex hormone-binding globulin	0.034	0.009
PC35	Hand grip strength/height	0.030	0.110
PC36	Phosphate	-0.028	-0.013
PC37	Lymphocyte count	0.024	0.013
PC38	Triglycerides	-0.055	0.006
PC39	Urinary sodium	0.013	0.028
PC40	Monocyte count	0.018	-0.010
PC41	Gamma glutamyltransferase	0.002	0.009
PC42	Urea	0.005	0.014
PC43	HbA1c	-0.003	0.004
PC44	Platelet distribution width	0.006	0
PC45	Log C-reactive protein	0.020	0.019
PC46	Reticulocyte fraction	-0.014	-0.011
PC47	Cystatin C	0.005	-0.035
PC48	Muscle mass	0.048	0.087
PC49	Calcium	0.023	0.022
PC50	Total protein	-0.008	-0.005
PC51	Urate	0.021	-0.061

The aggregation method used in this model is the main multi-state model method

Table 7.4.1: Contributions of disease risk-based biological age and chronological age as a percentage of their total contribution to the explanation of each health outcome, when comparing (A) biological age delta vs chronological age or (B) biological age vs chronological age, for healthy men and women

(A) Biological age delta and chronological age

	Mortality from chronic disease (%)			Age-related frailty (%)		
	CA alone	CA and BA	BA alone	CA alone	CA and BA	BA alone
Men						
Healthy subset	82.6	-0.1	17.4	89.2	-0.4	11.2
Whole population	28.8	34.5	36.7	38.2	36.8	25.0
Women						
Healthy subset	89.3	0.0	10.7	83.7	-0.3	16.6
Whole population	34.0	39.1	26.9	22.1	40.3	37.6

CA: chronological age, BA: Disease risk-based biological age delta

(B) Biological age and chronological age

	Mortality from chronic disease (%)			Age-related frailty (%)		
	CA alone	CA and BA	BA alone	CA alone	CA and BA	BA alone
Men						
Healthy subset	0.6	81.9	17.4	0.0	88.8	11.2
Whole population	7.2	56.1	36.7	3.8	71.2	25.0
Women						
Healthy subset	0.4	88.9	10.7	0.1	83.3	16.6
Whole population	2.0	71.1	26.9	8.7	53.7	37.6

CA: chronological age, BA: Disease risk-based biological age (biological age delta + chronological age)

These percentages were derived from comparing the log-likelihoods of prediction models that included different combinations of ages (Section 4.5)
The large reduction in percentages for the prediction model with CA alone for (B) vs (A) was due to the inclusion of CA in the biological ages in (B), hence BA and CA substantially overlapped in (B)

Table 7.5.1: Harrell's C-indices (with standard errors) for each health outcome, disease risk-based biological age delta vs chronological age

(A) Unadjusted analysis

Outcome and age predictor	Healthy subpopulation		Whole population	
	Men	Women	Men	Women
Mortality from chronic disease				
CA alone	0.707 (0.009)	0.663 (0.010)	0.686 (0.003)	0.670 (0.003)
BA alone	0.597 (0.009)	0.557 (0.010)	0.695 (0.003)	0.658 (0.003)
BA and CA	0.725 (0.009)	0.671 (0.010)	0.728 (0.003)	0.694 (0.003)
Improvement of BA and CA over CA	*0.018*	*0.008*	*0.042*	*0.024*
Age-related frailty				
CA alone	0.634 (0.003)	0.602 (0.003)	0.629 (0.001)	0.603 (0.001)
BA alone	0.549 (0.003)	0.544 (0.003)	0.616 (0.001)	0.612 (0.001)
BA and CA	0.642 (0.003)	0.611 (0.003)	0.647 (0.001)	0.627 (0.001)
Improvement of BA and CA over CA	*0.008*	*0.008*	*0.018*	*0.025*

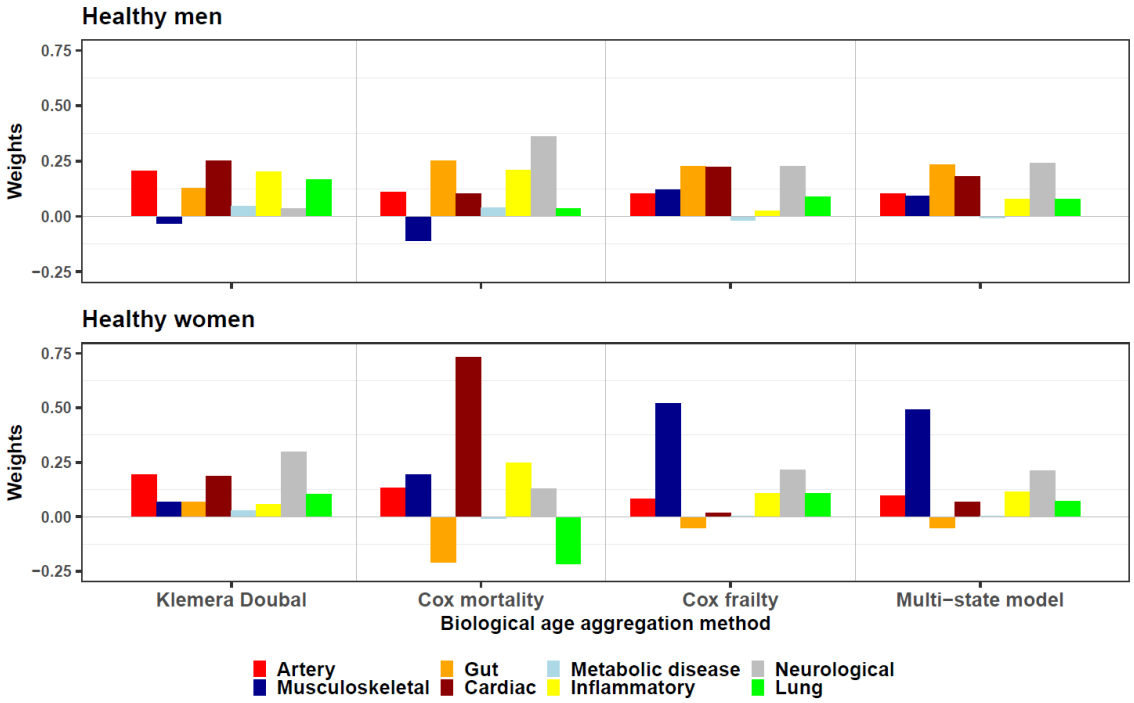
(B) Adjusted for sociodemographic factors and health behaviours

Outcome and age predictor	Healthy subpopulation		Whole population	
	Men	Women	Men	Women
Mortality from chronic disease				
CA alone	0.721 (0.009)	0.687 (0.010)	0.724 (0.003)	0.699 (0.003)
BA alone	0.626 (0.009)	0.606 (0.010)	0.717 (0.003)	0.681 (0.003)
BA and CA	0.735 (0.009)	0.693 (0.010)	0.751 (0.003)	0.716 (0.003)
Improvement of BA and CA over CA	*0.014*	*0.007*	*0.026*	*0.017*
Age-related frailty				
CA alone	0.659 (0.003)	0.631 (0.003)	0.677 (0.001)	0.656 (0.001)
BA alone	0.588 (0.003)	0.581 (0.003)	0.659 (0.001)	0.654 (0.001)
BA and CA	0.664 (0.003)	0.636 (0.003)	0.688 (0.001)	0.670 (0.001)
Improvement of BA and CA over CA	*0.005*	*0.005*	*0.011*	*0.014*

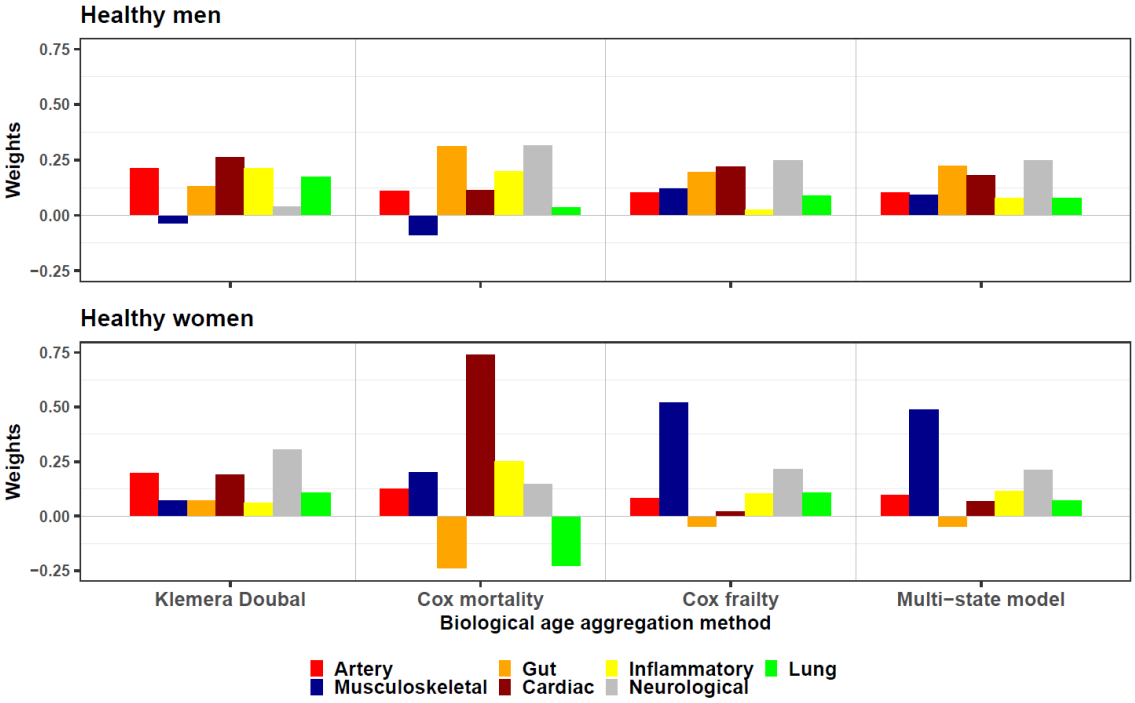
CA: chronological age; BA: Multi-state model-aggregated biological age delta
Analyses in (B) were adjusted for Index of Multiple Deprivation 2010 quintile, smoking status, alcohol consumption and assessment centre

Figure 7.3.1: Weights of constituent body system age deltas for biological ages aggregated by 4 different methods, in the (A) main analysis and (B) excluding metabolic disease ages from the aggregation, for healthy men and women

(A) Main analysis



(B) Excluding metabolic disease age



Weights: Coefficients of the body system age deltas in the model, rescaled to sum to 1

Figure 7.3.2: Importance of the top 15 biomarker principal components in the disease risk-based biological age delta, for healthy men and healthy women

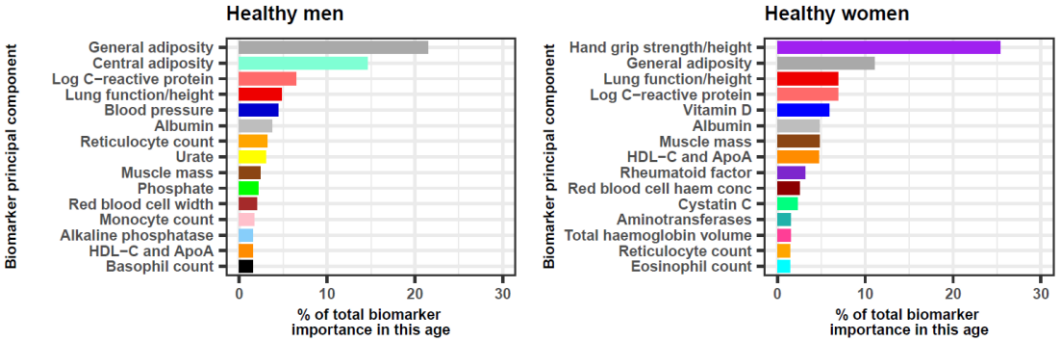
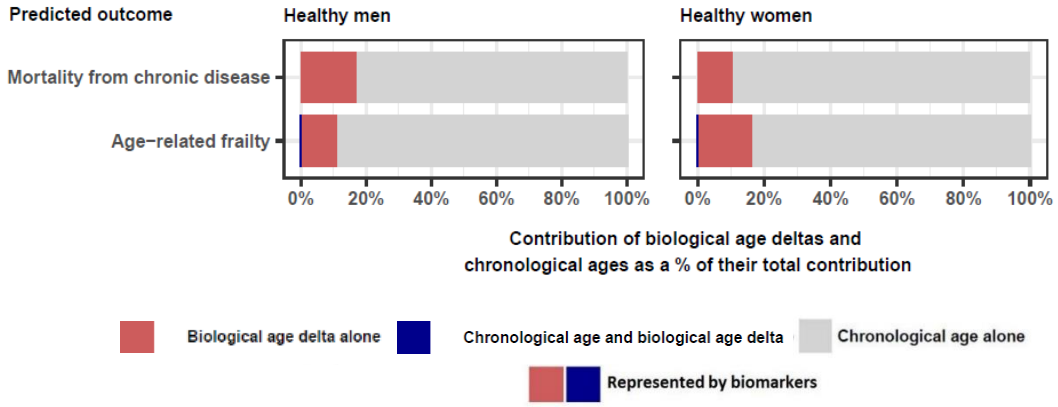
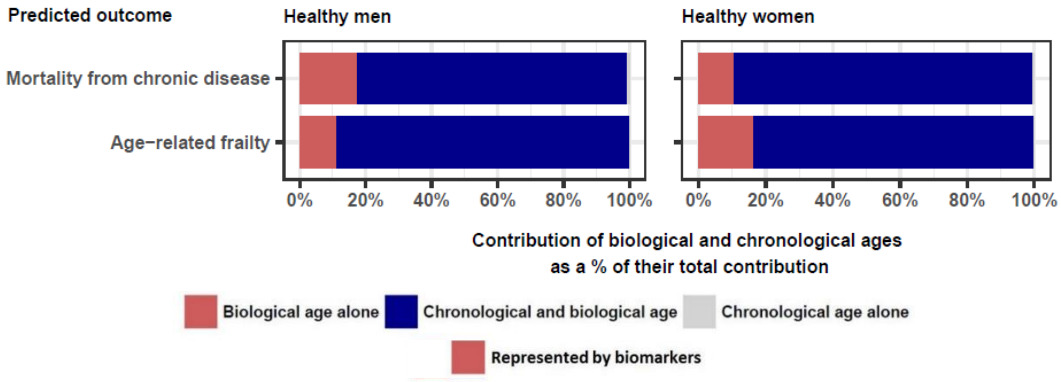


Figure 7.4.1: Relative contribution of the aggregated disease risk-based biological age and chronological age in explaining each general health outcome, when comparing (A) biological age delta and chronological age, or (B) biological age and chronological age, for healthy men and women

(A) Biological age delta and chronological age



(B) Biological age and chronological age

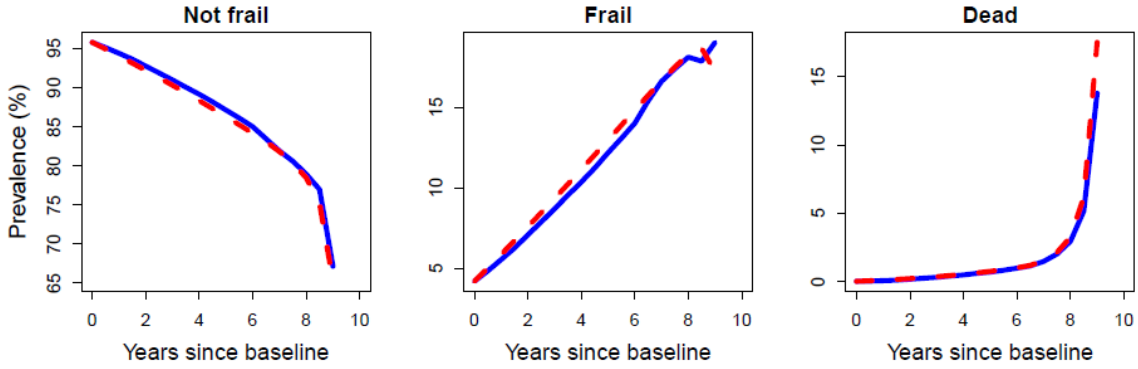


These percentages are also tabulated in Table 7.4.1

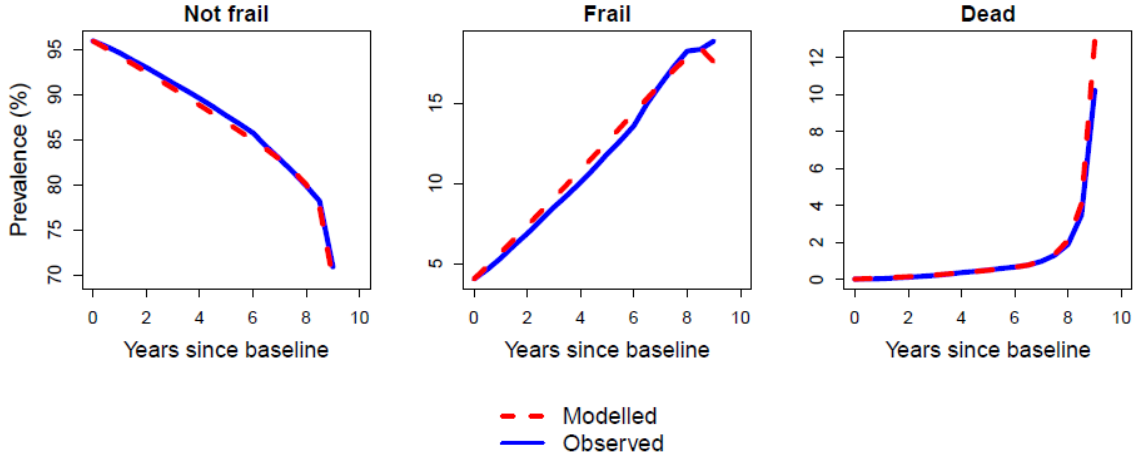
In both (A) and (B), the proportion of the overall age effect described by biological age or its delta alone (the bars in red) are the same

Figure 7.5.1: Goodness of fit of multi-state model state prevalence, for each state and for healthy men and women

Healthy men



Healthy women



Median [interquartile range] follow up duration for healthy men: 7.98 [7.29–8.57] years; healthy women: 7.99 [7.32–8.56] years

State prevalences were plotted for 0 to 9 years of follow up since baseline, and account for loss to follow up in the alive states. Since the majority of participants were followed up for 7.3–8.6 years, the prevalence in the 'Dead' state increased substantially during this period, when participants were rapidly lost to follow up.

Figure 7.5.2: Means and standard deviations of disease risk-based biological ages aggregated using the multi-state model method, by 2.5-year chronological age groups, for healthy men and healthy women

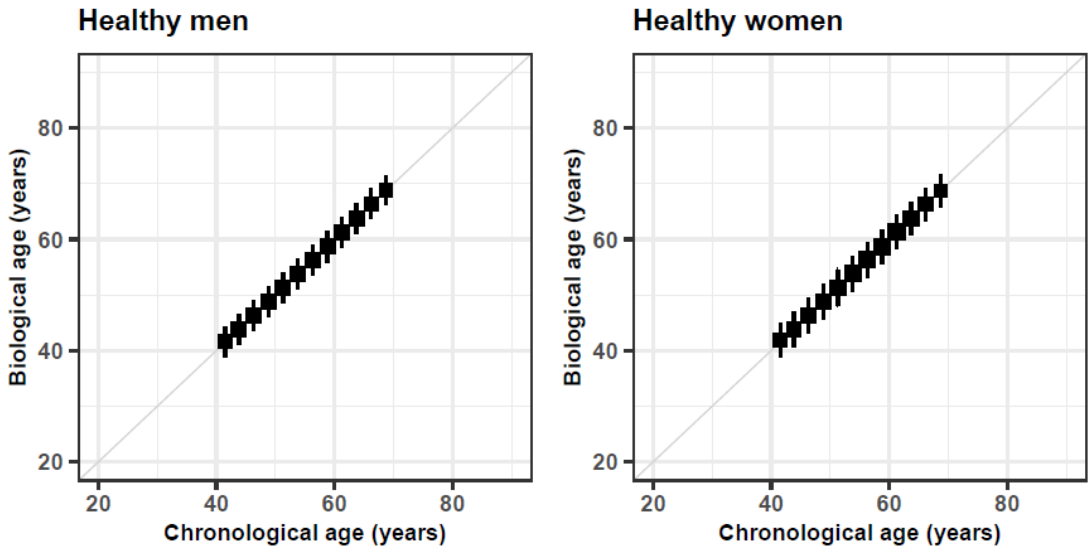
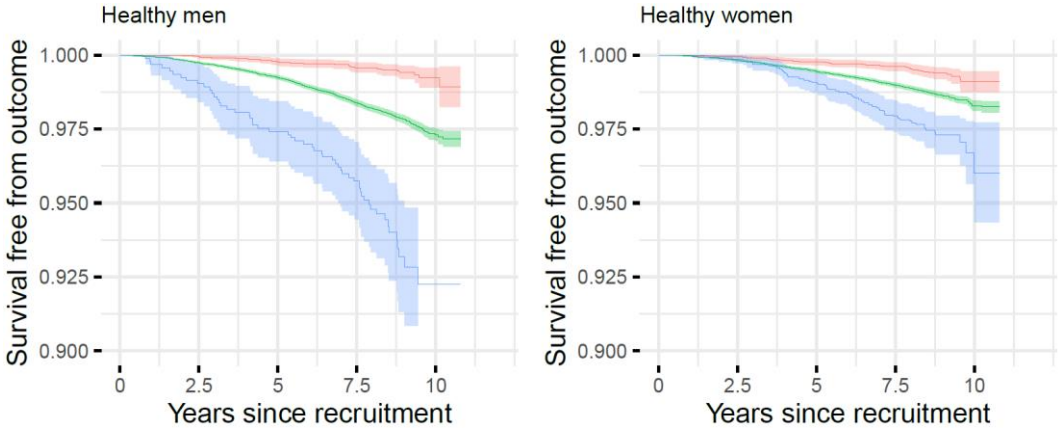


Figure 7.5.3: Outcome-free survival of healthy men and healthy women for (A) mortality from chronic disease and (B) age-related frailty, according to whether their disease risk-based biological age is younger, similar to or older than their chronological age

(A) Mortality from chronic disease



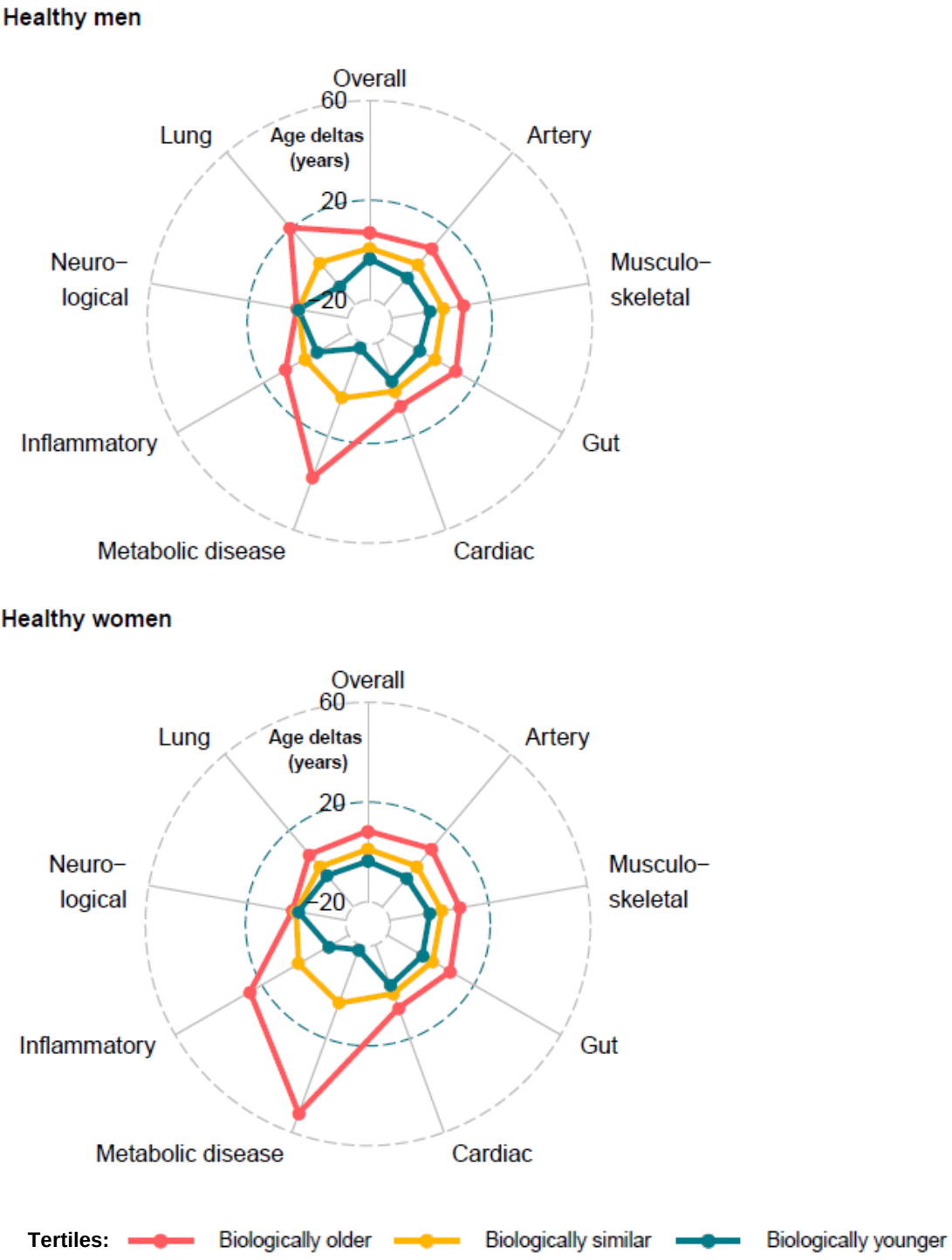
(B) Age-related frailty

Predicted risk group Kaplan-Meier curve and 95% confidence interval (shaded area):

BA - CA < -5 years (Biologically younger) | |BA – CA| < 5 years | BA - CA > 5 years (Biologically older)

Figure 7.6.1: Mean disease risk-based biological age and body system age deltas, in the (A) main analysis and (B) excluding metabolic disease ages from the aggregation, for tertiles of healthy men and women based on their biological age deltas

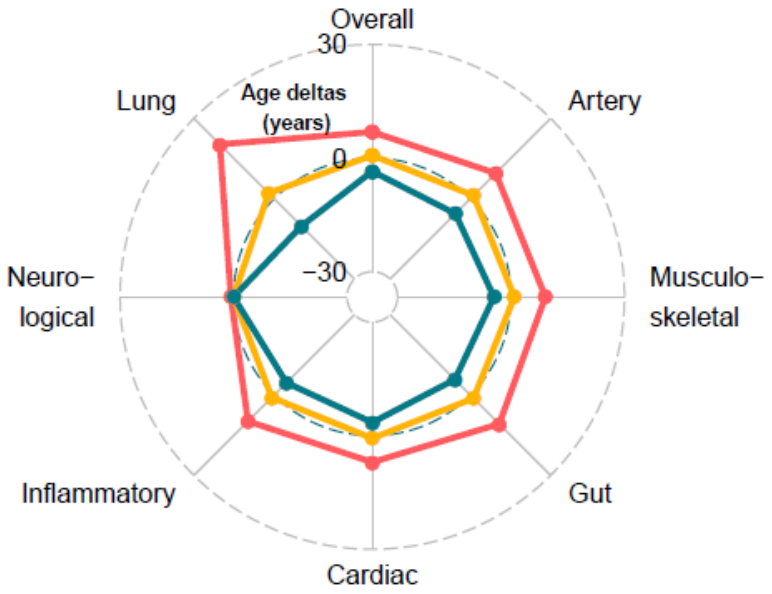
(A) Main analysis



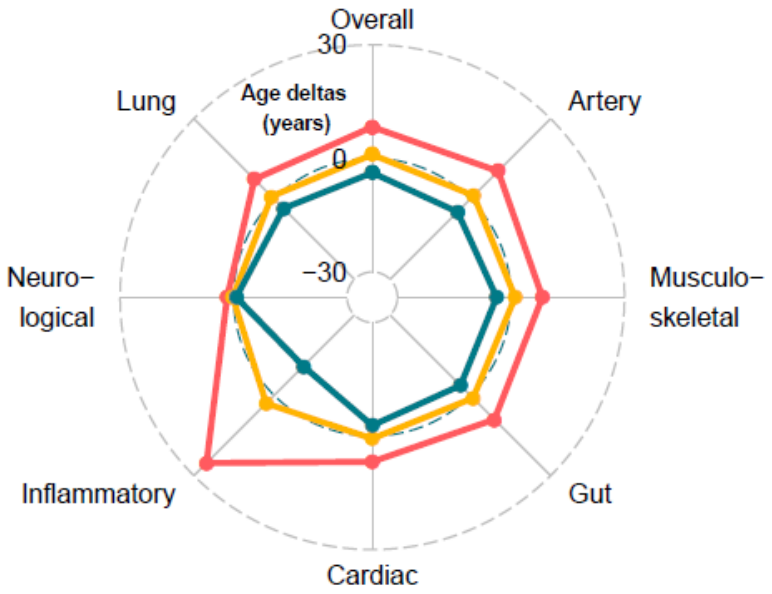
The mean disease risk-based biological age deltas for the biologically younger, similar and older tertiles were -3.5, 0.7 and 6.9 years for healthy men and -3.7, 1.1 and 8.4 years for healthy women respectively. The mean chronological ages were 54.7, 54.7 and 53.7 years for healthy men and 55.2, 55.5 and 54.2 years for healthy women respectively.

(B) Excluding metabolic disease age

Healthy men



Healthy women



The mean disease risk-based biological age deltas for the biologically younger, similar and older tertiles were -3.5, 0.7 and 6.9 years for healthy men and -3.8, 1.0 and 8.3 years for healthy women respectively. The mean chronological ages were 54.7, 54.7 and 53.8 years for healthy men and 55.2, 55.5 and 54.4 years for healthy women respectively.

Chapter 8: Discussion

8.1 Overview of findings from biological and body system ages

This thesis used established methods to estimate an age-based biological age in a healthy UK Biobank subpopulation (Chapter 5). A novel method where 8 body system ages that predicted disease risk (Chapter 6) were aggregated into an overall biological age (Chapter 7) was also investigated, to improve on the results obtained in the age-based biological age analysis. This section compares results from the age-based and disease risk-based biological ages, focusing on their key biomarker and body system age constituents, their predictive power for later life health outcomes and their relationship with chronological age.

Key biomarker and body system age constituents of biological ages

The UK Biobank resource collected measurements of many biomarkers spanning a wide range of functional groups (Table 3.8.1). This thesis used 72 biomarkers dimensionally reduced into 51 biomarker principal components (Section 4.5), in comparison to the 3–19 biomarkers selected by 15 previous large-scale studies of biological age identified in the systematic review (Chapter 2). The methods selected most or all of these 51 biomarker principal components, and emphasised biomarkers with the strongest relation to chronological age (for the age-based biological age) or biomarkers that best predicted risk of later life disease, mortality and frailty (for the disease risk-based biological age). However, relatively little explanatory and predictive value was compromised when the age-based biological age for women was estimated using just 12 key biomarkers (Section 5.6).

Biomarkers that featured strongest in the age-based biological ages and disease risk-based biological age deltas (which represented the biomarker constituents of the disease risk-based biological age) overlapped substantially (Figure 8.1.1 (A) vs (B)). Of the top 15 biomarkers in each biological age,

lung function/height, blood pressure, hand grip strength/height, cystatin C, muscle mass and log C-reactive protein generally overlapped in both men and women, while albumin overlapped in men and aminotransferases overlapped in women (Figure 8.1.1 (A) vs (B)). These biomarkers reflected both the chronological age-related and disease-related aspects of biological ageing. Additionally, lung function, hand grip strength, muscle mass and log C-reactive protein are putative biomarkers of frailty.[253,254] Lung function/height and cystatin C featured most strongly in the age-based biological age for both sexes, but less strongly in the disease risk-based biological age, suggesting that despite their close relationship with chronological age, they were not as prognostic as the adiposity and hand grip strength/height biomarkers for mortality and frailty (Figure 8.1.1 (A) vs (B)).

Body size measurements (general adiposity, central adiposity and muscle mass) strongly predicted multiple diseases (Figure 6.5.1) and most strongly featured in the disease risk-based biological ages (except for central adiposity in women; Figure 8.1.1 (B)), yet did not feature strongly in the age-based biological ages (Figure 8.1.1 (A)). Unpicking the effects of adiposity may require more complex epidemiological analysis, as previous studies have demonstrated J-shaped associations between BMI and several disease-specific causes of death,[255,256] but it has been difficult to isolate the effects of reverse causality on these associations. Adiposity biomarkers and their epidemiological associations with a wide range of diseases have been well-studied (Table 6.8.1), and they may be modifiable through several health behaviours such as diet and exercise. Higher log C-reactive protein (log CRP) for both sexes and higher vitamin D for women featured more strongly in the disease risk-based than age-based biological ages (Figure 8.1.1 (B)), and several studies had found evidence for associations of each of higher CRP and lower vitamin D levels with a range of diseases (Table 6.8.1).

Sex differences in the most important biomarkers were more pronounced in the disease risk-based than the age-based biological age (Figure 8.1.1 (B) vs (A)). This may have been a consequence of both differences in incidence patterns of disease by body system (reflected in the relative weights of body system ages in the disease risk-based biological age; Figure 7.3.1) and any differences in incidence patterns of frailty and mortality during follow up (Figure 3.9.1). In the disease risk-based

biological age, central adiposity and blood pressure featured strongly in healthy men only, while hand grip strength/height and vitamin D featured strongly in healthy women only (Figure 8.1.1 (B)).

There was moderate correlation between pairs of body system age deltas, with the exception of neurological age (Figure 6.4.2), as the biomarkers in this age did not appear to predict neurological diseases well (Section 6.8). This moderate correlation despite the limited biomarker overlap between body system ages suggest that a common ageing effect across body systems can be detected through these biomarkers prior to disease onset (Section 6.8). All body system ages (apart from metabolic disease age and lung age) also contributed substantially to the prediction of mortality and frailty (Figure 7.3.1).

Limited research on biomarker importance in biological age had been conducted in previous studies (Chapters 2, 5 and 7), and studies that assessed biomarker importance in their ageing scores found that the patterns of biomarkers varied substantially by population[98] or by estimation method.[73] The results in this thesis relate specifically to the UK Biobank population and external validation is required to assess if these patterns of biomarkers are more generalisable. Research on associations between the most important biomarkers of the bodily ages estimated in this thesis and cardiovascular diseases was relatively more abundant than research on other chronic diseases (Tables 5.6.1 and 6.8.1) even though neither artery nor cardiac ages were assigned relatively large weights in the disease risk-based biological age (Figure 7.3.1), suggesting a mismatch between research focus and body systems most closely related to mortality and frailty. The incidence of cardiovascular disease in this healthy subpopulation during follow up was also relatively high (there were 4600 atherosclerotic and 3763 cardiac incident cases compared to 10,481 musculoskeletal incident cases and 585–6,492 incident cases for the other 5 disease groups; Table 3.9.3). However, traditional cardiovascular biomarkers (blood pressure and blood lipids) that featured most strongly in the artery ages (Figure 6.5.1) were not most important in either type of biological age even though several of these biomarkers featured in the top 10 (Figure 8.1.1). Apart from adiposity biomarkers (common biomarkers for numerous body system ages) and lung function/height (which strongly featured in

artery and lung ages), key biomarkers of the disease risk-based biological ages featured strongly only in single body system ages (Figures 6.5.1 and 8.1.1). Even though many of the most important biomarkers in each body system age were established biomarkers for their respective disease groups, there was limited research on the causal associations between these biomarkers and diseases relevant to several of these body systems (Section 6.8).

Biological and chronological age effect of mortality and frailty risks

The age-based biological age explained a substantial proportion of the chronological age effect on later life health, while the disease risk-based biological age supplemented the chronological age effect with the effect of biomarkers that best predicted a range of age-related chronic diseases (Figure 8.1.2), thereby elucidating the effect of chronological age on the risks of these outcomes. The age-based approach differentiates individuals by the levels of biomarkers normally associated with chronological ageing, regardless of their association with disease, while the disease risk-based approach differentiates individuals by whether they have more hazardous levels of biomarkers associated with diseases, regardless of whether these biomarkers are ones that change with chronological age.

Compared to the age-based biological ages alone, the proportions of biological and chronological age effects explained by the disease risk-based biological ages alone in the healthy subpopulation were higher by >7% (proportion of the overall biological and chronological age effect: 11–17% vs 2–8% for the disease risk-based vs age-based biological age alone; Tables 5.4.1 and 7.4.1), particularly in the prediction of frailty risk for healthy women, where the proportion was 14.9% higher (bars in red; Figure 8.1.2 (A) vs (B) and Tables 5.4.1 and 7.4.1). The substantial improvement in explaining this non-fatal frailty outcome potentially offers a greater scope for intervening earlier in life to improve health. Age-based biological age alone was not as predictive as disease risk-based biological age alone for age-related chronic diseases, contributing little (<20%) to the overall biological and chronological age effect for atherosclerotic, musculoskeletal, gut, cardiac,

inflammatory and neurological disease groups (bars in red; Figure 8.1.3 (A) vs (B)). In comparison, the 8 body system ages that constituted the disease risk-based biological age each contributed much more substantially to the prediction of their respective disease outcome than either type of biological age (proportions explained by the body system age deltas alone, represented by crosses, ranged from 24–81%; Figure 8.1.3 (A) vs (B) vs (C)), while their respective body system risk scores alone contributed almost as much to these predictions (Figure 8.1.3 (C)).

Predictive power of biological ages

In the updated healthy subpopulation (Appendix 6.1), the disease risk-based biological age generally supplemented chronological age slightly more than the age-based biological ages in the predictions of mortality from chronic disease and age-related frailty (the increases in C-indices in adjusted analyses were 0.005–0.014 vs 0.001–0.007 when disease risk-based vs age-based biological ages were used to supplement chronological age; Figure 8.1.4). In earlier analyses, each of the 8 body system ages also supplemented chronological age substantially more than the age-based biological ages in the predictions of their respective disease group (Table 6.7.1). Chronological age itself was a strong predictor for mortality from chronic disease and age-related frailty (Figure 8.1.4), highlighting the need to investigate the biological processes tracked by chronological age.

Both chronological age on its own and combined with biological age were more predictive of mortality and frailty for healthy men than healthy women (Figure 8.1.4), and in earlier analyses body system ages were also generally slightly more predictive for their respective disease groups in healthy men than women (Table 6.7.1). These sex differences warrant further investigation in conjunction with population-level demographic patterns (women tend to live longer but also live longer in ill health than men; the 'gender paradox'[257]), particularly as previous biological age studies did not report predictive power of their biological ages by sex (Section 2.3)

In the whole population, the disease risk-based biological ages supplemented chronological age slightly more than the age-based biological ages in the prediction of age-related frailty (increases in C-indices in adjusted analyses: 0.011/0.014 vs 0.008/0.006 for disease risk-based vs age-based biological ages for all men/women; Figure 8.1.4), but did not supplement it as much in the prediction of mortality from chronic disease in men (0.026/0.017 vs 0.031/0.014 for disease risk-based vs age-based biological ages for all men/women; Figure 8.1.4). This was partially due to the loss in predictive power from only estimating the disease risk-based biological ages in the healthy subpopulation (as many of the other participants already had the outcome of interest at baseline assessment; Section 4.4). Even though this thesis focused on the prognostic rather than diagnostic capabilities of biomarkers, this finding suggests that the age-based approach (used by many previous biological age studies; Section 4.1) was more suited to characterising the diagnostic element of these biomarkers (for disease) in less healthy individuals than the disease risk-based approach, in which case a biological age that captured prior disease information would further improve its diagnostic capabilities. The patterns of constituent biomarkers in biological ages may also differ by the health profile of the derivation cohort. The increases in C-indices for mortality prediction using age-based biological age were comparable to or greater than the improvements reported by previous studies on older US, Canadian and Singaporean populations[12,13,78,179] (Section 5.6).

Both types of biological ages were well calibrated to chronological age (Figures 5.5.1, 6.7.1 and 7.5.2), and since metabolic disease and lung ages were initially poorly calibrated, all 8 body system ages were recalibrated to chronological age (Figures 6.7.1). The large variation in metabolic disease ages that remained after recalibration suggested that communicating its main constituents, HbA1c or cystatin C levels (with respect to risks of diabetes and chronic kidney disease), may be more informative than metabolic disease ages (Sections 6.7–6.8). Alternatively, a single biomarker-based 'kidney age' (median chronological ages in a US population corresponding to estimated glomerular filtration rate (eGFR) thresholds for each stage of chronic kidney disease) has been proposed as an alternative approach for communicating chronic kidney disease risk.[65]

Conclusion

Both the age-based and disease risk-based biological ages summarised markers of impaired function across a range of organs, which accounted for a substantial proportion of the apparent effect of chronological age and supplemented chronological age in the prediction of later life outcomes respectively. The disease risk-based biological age improved on the age-based chronological age in several respects: (1) its biomarker constituents were directly linked to the prognosis of multiple age-related chronic diseases, mortality from chronic disease and age-related frailty rather than indirectly through the use of chronological age as a proxy for health outcomes, (2) it provided consistent summary of the disease risks through the 8 body system ages, (3) it described a substantially larger proportion of the overall age effect on mortality and frailty risk, and (4) it was slightly more predictive of these 2 health outcomes in a healthy subpopulation and more predictive of frailty in the whole population. Each of the 8 constituent body system ages in the disease risk-based biological age also summarised the biomarker patterns that broadly match medical knowledge of biomarkers of their respective disease groups apart from neurological diseases (where research mainly focused on cerebrospinal fluid, brain image and Apolipoprotein E genotype biomarkers; Section 6.8). There was a common ageing effect described by moderate correlations between these biomarker patterns (apart from the patterns for neurological diseases), yet there was little biomarker overlap between body system ages apart from general adiposity biomarkers.

However, compared to the disease risk-based biological age, the age-based biological age was relatively easier to estimate and did not require substantial additional incidence data on health outcomes. The age-based biological age also supplemented chronological age in predicting mortality in the whole UK Biobank population more than the disease risk-based biological age.

The disease risk-based biological age, along with the 8 constituent body system ages, is thus the recommended approach as it provided the most information on later life health risks and was more predictive of these risks than the age-based biological age. Nevertheless, the age-based biological

age may be more useful in situations where there is limited data on disease incidence, a simpler estimation procedure is preferred, or comparison with previous studies is desired.

8.2 Recommendations for future research

The previous section highlighted the advantages that the disease risk-based approach for estimating body system and biological ages conferred over the established age-based biological age approach. Therefore, greater use of disease risk-based approaches in research applications, for estimating biological ages and for assessing the prognostic value of risk prediction tools or health indices is recommended. Subsequently, disease risk-based approaches may be more clinically applicable as clinical practice tends to be organised by body systems, and they are more relevant to the early detection of poor health in contrast to end-of-life measures such as mortality-based biological ages.

This thesis highlighted several biomarker-disease relationships with known causal associations (Tables 5.6.1 and 6.8.1), and also recommends further epidemiological and biological research on causal mechanisms of biological ageing where evidence is limited (particularly those related to non-cardiovascular diseases; Tables 5.6.1 and 6.8.1). In addition, research could be prioritised for common biomarkers that featured more strongly across the 8 disease groups (Section 6.5). Epidemiological approaches such as Mendelian Randomisation could be used to elucidate causal associations between each biomarker and biological ages, however the large number of constituent biomarkers necessitates multiple analyses. Potential interactive effects between constituent biomarkers (e.g. body mass index susceptibility loci were found to overlap with loci for other body size, blood lipid and blood pressure loci[205]) also suggest the need for implementing multivariate and dimension reduction extensions to these approaches. A recent genome-wide association study has identified causal associations of cardiovascular and inflammatory genetic instruments with physical and biochemical biomarker-based biological ages.[68]

A tailored framework for critically assessing biological or bodily ages, which builds on existing frameworks for reporting clinical prediction tools,[164] is also recommended (Section 2.5). This framework should take into account the additional complexities of expressing biological risk scores in terms of ages and assessing the association of bodily ages with health outcomes in the presence and the absence of chronological age.

The key limitation of this and most other biological age studies was that biological ages were estimated from biomarkers measured at a single time point and could not capture changes in biomarker profiles in the same individuals over time. Therefore, it is important to investigate trajectories of biological ageing from repeated measurements of biomarkers in longitudinal studies (such as previous research that investigated changes in biological ages in New Zealand and US cohorts over 2–3 timepoints[16,97]), but without substantial trade-offs in cohort size and analytical rigour. Potential methods for analysing these trajectories were discussed in Section 4.6. Data requirements and methods for evaluating these longitudinal estimates, e.g. assessing the association of changes in biological age with changes in health outcomes over the same time period, would also need to be investigated.

In this analysis, only linked hospital admission data were available for the phenotyping of disease onset and age-related frailty. The effect of sex and other sociodemographic differences in missed hospital diagnoses on the predictive power of these biological ages should be investigated, e.g. when linked primary care data that record disease screening and diagnosis becomes available in the UK Biobank.[103] Further research is also required to assess the ability of the relatively new health record-based frailty indices to detect biological frailty.[20]

The putative links between underlying poor health (detected through a bodily age) and susceptibility to debilitating age-related acute illnesses such as COVID-19 and more generally severe flu and pneumonia, should also be investigated further. The recently-published COVID age[258] summarised the cumulative effect of poor general health on severe COVID-19 outcomes that was reported by a

study of multiple risk factors of COVID-19 in 17 million English adults.[259] Another study found links via inflammatory pathways between a mortality-based biological age derived in a US population (PhenoAge) and COVID-19 hospitalisations or deaths in the UK Biobank.[260] In the UK, hospital admissions of older adults diagnosed with a range of chronic and infectious diseases normally surge in winter, and many of these people subsequently die from pneumonia.[261]

Previous research found that several types of biological ageing scores were associated or correlated with early life socioeconomic factors.[81,93,94,262] Research on putative socioeconomic and environmental determinants of biological ages will be carried out in the department (Nuffield Department of Population Health), using the age-based biological ages for UK Biobank participants discussed in Chapter 5 as the outcome.

Another avenue for future research is the supplementation of the panel of physical and biochemical biomarkers with other putative biomarkers of ageing. This research would be particularly useful if these additional biomarkers are easily measured in clinical practice or in the community, modifiable, cost-effective, and if they supplement the predictive power of the currently-used biomarkers and chronological age for later life health outcomes. Candidate biomarkers across a wide range of modalities that were also measured in the UK Biobank could be investigated in the nearer future: genetic, metabolomic, brain image, heart image and activity sensor data.[103] Preliminary analysis of approximately 70,000 of these UK Biobank participants with sufficient activity sensor measurements showed that a summary activity sensor biomarker, proportion of time spent doing moderate or vigorous activity, featured more strongly than the 8 body system ages in a prediction model for age-related frailty (similar to the prediction model described in Section 4.5). Studies of epigenetic (or DNA methylation) ages[57,66,67,263] and telomere length as a biomarker of ageing[18,37,57] have proliferated in recent years, although research on the associations of epigenetic ages or telomere length with age-related health outcomes is inconclusive.[18,37,263] These biomarkers are not currently widely measured in clinical practice and cohort studies, but may be more widely measured in future due to the growth

in research interest. Proteomic[264] and multi-omic[58,100] biomarkers have also been used to estimate biological ages in relatively smaller cohorts.

Before implementing biological age-related interventions, the reliability of the estimated bodily ages should be assessed via external validation,[124] preferably in a representative population[164] or the target population. Previous research found that UK Biobank participants displayed similar associations between several health risk factors and mortality to representative UK cohorts,[265] but there is currently no evidence for other health outcomes. External validation of these bodily ages in other countries may also increase its generalisability, and is particularly important for the novel disease risk-based approach as no empirical biological age studies using similar methods have been published (Sections 4.1 and 6.1). Few other cohort studies used in other large scale biological age studies have numbers of participants and ranges of biomarkers similar to those for the UK Biobank (Chapter 2), therefore the viability of external validation in other large population biobanks[266] or ageing cohort studies[267] based on these cohort characteristics should be explored initially.

In addition to exploring alternative methods for estimating bodily ages, which can capture non-linearity and interaction effects in biomarker measurements but are more data and computationally intensive (Appendix 4.2 and Section 4.6), quantifying the extent to which common ageing patterns across body systems were described by overlapping or different biomarkers (Section 6.5), could also be explored. Potential approaches such as mathematical decomposition methods for high dimensional biomarker data and simulation studies of biomarker profiles may require methodological development.

8.3 Considerations for biological age estimation and implementation

Many biological age studies have aimed to improve our biological and statistical understanding of ageing processes, while several studies have proposed potential applications in public health, without in-depth considerations of research translation (Sections 1.3 and 2.4). This section reflects on the

potential translation of bodily ages from simply an epistemic tool for research and health promotion, into implementation in health systems and society, and the issues that may arise.

Biological age as a research tool

Biological ages are currently predominantly used in research settings, but increasingly in commercial health provision and promotion.[76,77,88,98,100,102,268] The use of biological age in healthcare requires a degree of causal associations between constituent biomarkers and ageing to be demonstrated, yet biological age is mostly developed within observational studies and predicated on correlation rather than causation (the Jia et al estimation methods review[20] and the systematic review of biological age studies in Chapter 2 found many more age-based than risk-based biological ages). Research studies that endeavour to establish causality and to predict health outcomes are generally designed differently[126] (Section 4.6). Despite the strong biological focus of ageing research (Section 1.1), mechanisms of ageing may not be purely biological[81,93,94] and biological ages may inadvertently capture these non-biological mechanisms. For example, if an individual has dementia (reflected by cognitive biomarker constituents), they may forget to take prescribed medications that are preventive for other diseases.

The large variety of biological ages and ageing scores were described and delineated in Section 1.2, but the public and the broader research community may be confused by the multiple conceptualisations of biological ages. Additionally, apart from geriatricians and primary care practitioners, medical professionals tend to work within specialties. Traditionally, research funding was targeted towards research on specific diseases, but there has been a recent surge in research interest in multimorbidity[6] and frailty.[5] However as discussed in Sections 1.2, 5.6 and 7.6, frailty is not well measured in hospital records, and as it is also conceptualised in multiple ways,[5] the degree of overlap between frailty and ageing is not well defined.

Implementation considerations

The implementation of biological age may be part of a wider move to digitalise healthcare provision.[17] Health providers need to ensure they have consent to collect and use all of this data in a trustworthy manner, and be clear about who can access this data.[269] If biological age is intended for use as a monitoring tool for overall health risks (Section 1.3), provisions should be made to collect biomarker information from individuals at multiple time points, and at suitable time intervals. The initial estimation and validation of biological ages should address the numerous considerations described in Chapters 2–4 and detailed in guidelines for reporting clinical risk prediction tools,[164] e.g. reliability of biomarker measurements, predictive power for health outcomes, calibration and external validation. The choice of biomarkers to measure should consider their relative predictive power for health outcomes (Chapters 6–7) and trade-offs between potential screening costs and reductions in predictive power of the biological age when a reduced panel is used (Section 5.6).

Interventions involving biological age include the disclosure of biological age to individuals, and any medical or policy decision making or health promotion that is based on the knowledge of biological age, with the potential and measureable target of either slowing or reversing individuals' rate of biological ageing. One type of potentially cost effective preventative intervention could be the promotion of health behaviours that improve overall physiological health, such as exercise.[270] Another type of intervention, caloric restriction, was found to have a beneficial effect on biological age in a small trial,[33] however the longer term effects of caloric restriction are not yet known and there may be safety concerns over its promotion. No clear existing link between biological age and health interventions or behaviours has been made yet (unlike links between heart ages and controlling blood lipid levels,[54] or lung ages and quitting smoking[56]). We have yet to identify whether the use of risk scores or bodily ages in health systems confers net benefits, as health economic evaluations are rare for clinical risk prediction tools in general[271] and appear to be non-existent for bodily ages. The optimal frequency of measuring the constituent biomarkers to track changes in health status is not clear, except for blood pressure.[272] This frequency may be biomarker-specific,

and is dependent on fluctuations due to environmental factors or biological rhythms and the capability of the assay procedure to detect age-related changes in biomarker levels.

Individuals who find out their biological ages from websites, published research or direct-to-consumer testing, may prompt them to visit their doctors more or less often than recommended. If the quality of biomarker measurement or biological age estimation is low, there may be a large number of false positives (or its continuous equivalent), which is an issue that has emerged in direct-to-consumer genetic tests[273] and artificial intelligence-based clinical risk prediction tools.[274] Large-scale biomarker measurement or screening may burden health systems, both in terms of infrastructure costs and additional clinical consultations.[275] These potential scenarios of overtesting and 'biomarkup' may be encouraged for private profits, and non-specific biomarkers (such as biological age, which is itself a composite biomarker; Section 1.1) may be especially profitable.[275] Currently, there is no specific legislation or regulation related to the use of biological age or other clinical risk prediction tools, and the legal basis for permitting age change to biological age or emotional age[276] does not exist yet.

Health communication

A key and relatively achievable public health aim of biological age is its use as a means of health communication (Section 1.3). Despite biological age being an attractive concept, and the synthesis of a novel approach for jointly illustrating biological age and body system ages in this thesis, we cannot assume all individuals want to know their biological age. In fact, those who seek to know their biological ages may be individuals who use them for positive reinforcement of their salutary health behaviours,[268] and are less likely to be individuals at higher risk of poor health, which this research sought to identify.

Age can be a very sensitive subject, and there is a huge diversity in perspectives and experiences of ageing across cultures and societies[277] – even chronological age is counted in different ways.[278] A

modifiable biological age can give people hope that they can improve their health, in contrast to chronological age, which implies a constant rate of decline in health, or risk scores based on non-modifiable biomarkers such as genetic markers.[279] Yet the multiplicity of biological ages (Section 1.2) and the variation in their predictive power for later life health (Section 2.4) may also increase scepticism in the public in biological ages, even for the most predictive ages.

If healthcare professionals are to be tasked with providing information on biological ages to patients, information asymmetries may arise from the different levels of expertise, e.g. disagreement over treatment options due to different interpretations of the underlying biomarker information. Healthcare professionals may also inherit the challenge of communicating the uncertainties inherent in the estimation of biological ages and the measurement of the underlying biomarkers.

Health inequality

The reduction in health inequality is one of the UK government's key healthcare targets[17] Health inequality is traditionally measured via health outcomes, such as mortality and disease incidence rates,[280] rather than health determinants such as biomarkers or behaviours. However, in different contexts, biological age could be a consequence, a measure or a cause of health inequalities:

1. Consequence of inequality: Indicators of socioeconomic inequality (e.g. education level, income and neighbourhood deprivation) tend to reflect environmental exposures earlier in life or over a substantial period of time, and previous research has found that measures of socioeconomic inequality (including those in earlier life) and biological age are strongly associated.[93,94]

2. Measure of inequality: Studies of health inequalities typically identify inequalities by comparing the health of specific groups of people and observing differences or gradients in levels of health, in contrast to a null hypothesis that health is randomly distributed across

these groups.[280] Similar to other risk scores, biological ages indicate differences in health and could be used as a means of risk stratification to detect individuals at higher risk of poor health. The extent to which risk stratification or profiling is perceived as just or unjust (e.g. Dworkin's 'brute luck' vs 'option luck')[281] may depend on the apparent extent to which the constituent biomarkers are fixed throughout life or modifiable. Biological age may be partially determined by health behaviours such as smoking, which are generally perceived as personal choices but are not wholly conscious choices, as they are affected by policy decisions and by social and environmental factors.[280]

3. Cause of inequality: Using biological age to differentiate groups of people in place of chronological age would reduce chronological age discrimination, but could promote a different kind of ageism that is more health-based and potentially endemic, such as discrimination based on outward appearance. Ageism is possibly already inherent in ageing research,[282] and this may have affected priorities of research into biological ageing.

Health resource constraints and existing resource allocation procedures may limit attempts to improve fairness. Those who pay for private biomarker tests and those who are at higher risk of specific diseases are more likely to have biomarker measurements. The biological ageing process itself may affect the likelihood of biomarker measurement, e.g. spirometry measurements of lung function cannot be conducted in people with poor respiratory health, introducing selection bias into the estimation of bodily ages, particularly in older populations. Even algorithmic methods for dealing with missing biomarker measurements may introduce bias or propagate biases inherent in the data.[283]

The choice of intervention or policy design alone may introduce inequalities. In the absence of due consideration for the target population, biological age-based interventions may disproportionately benefit those who need less care by default: those who are healthier, more affluent, more digitally literate or more willing to share their medical records. Minority groups within a population may be doubly disadvantaged from predictions that do not adjust for prejudices inherent in the underlying

data, and less accurate predictions of their risks due to having less data collected on them. As both types of biological ages in this analysis were less predictive of later life health in women than men for mortality and frailty outcomes, coupled with the 'gender paradox'[257] (Section 8.1), these biological ages may not be as useful as a prognostic tool for a larger proportion of women's compared to men's lives.

8.4 Conclusions

This thesis systematically investigated the use of a wide range of physical and biochemical biomarkers and their associations with a wide range of later life health outcomes that were phenotyped from hospital and death registry data.

Firstly, the systematic review of biological age studies found that biological ages were indicators or predictors of poorer health, but relatively few large-scale studies of biological age had been conducted and had validated their biological ages against health outcomes. Fewer followed good practice for estimating and reporting their biological ages.

The best performing established age-based methods of estimating biological age were combined and extended to analyse a large number of candidate biomarkers and to improve its reliability. This approach utilised chronological age as a (non-biological) proxy for biological age, but nevertheless resulted in an age-based biological age that slightly supplemented chronological age in the prediction of mortality and frailty. The key contributing biomarkers (including lung function and cystatin C) had the strongest relationships with chronological age.

A novel disease risk-based method was also developed to inspect biomarker and body system-specific patterns of ageing more closely, by summarising biomarker patterns into 8 body system ages and aggregating them into an overall biological age. This newer biological age had much greater prognostic power for the key age-related chronic diseases, and its biomarker constituents explained

a substantially larger proportion of the overall biological and chronological age effects on mortality and frailty. The key biomarkers of its constituent body system ages varied by body system (matching established risk factors for the relevant disease groups, where previous research had been conducted), yet these biomarkers appeared to describe a common ageing effect through moderate correlations between body system ages. These key biomarkers were generally different from those that had the strongest relationships with chronological age. General adiposity was a shared risk factor across the body systems and therefore featured strongest in the disease risk-based biological age (followed by central adiposity in men and hand grip strength in women). All body system ages (apart from metabolic disease age and lung age) also contributed substantially to the prediction of mortality and frailty in the overall biological age.

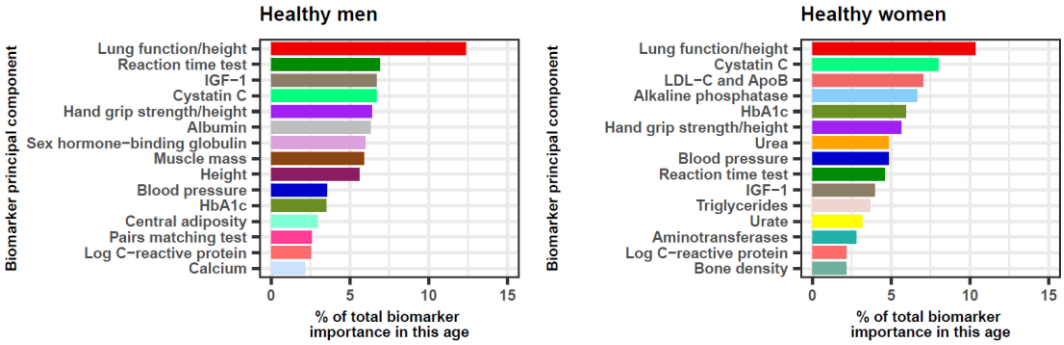
Both types of biological ages improved our understanding of the biomarker-disease relationships that were represented by chronological age and slightly supplemented chronological age in predicting later life health outcomes (more so for the disease risk-based biological age), providing a case for considering the use of reliable biological ages in conjunction with, but not in place of, chronological age. Before biological ages are used more widely in research and clinical practice, the challenges described in the previous section should be addressed, and the added benefit of implementing a biological age over the current use of chronological age and ageing biomarkers should be investigated.

In time, the reliance on chronological age as a proxy for measuring health may lessen if novel biomarkers of ageing and medical discoveries improve our understanding of mechanisms of ageing or the prediction of later life health outcomes. This thesis identified several areas of priority for future biomarker and biological age research and for methodological improvements for estimating and validating bodily ages. Research on biological age is constantly growing and the increased demand for digital and personalised measures of health risks may spur us on to improving our understanding, critical appraisal and utilisation of biological ages.

Chapter 8 tables and figures

Figure 8.1.1: Importance of the top 15 biomarker principal components in the age-based biological age vs disease risk-based biological age deltas, for healthy men and healthy women

(A) Age-based biological ages



(B) Disease risk-based biological age deltas

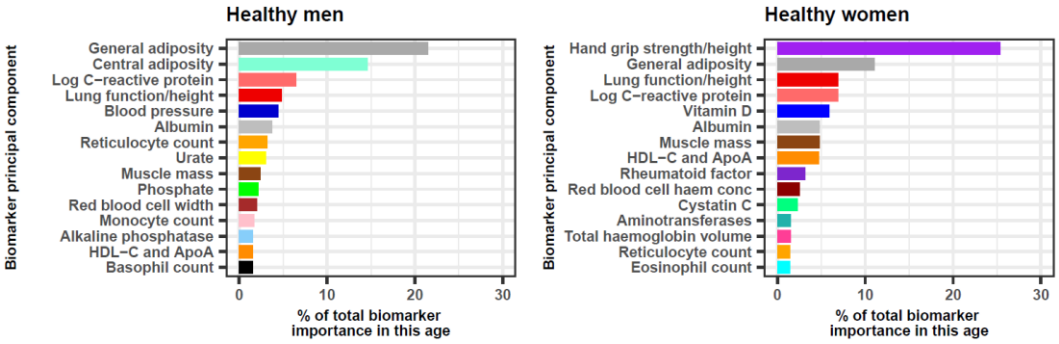
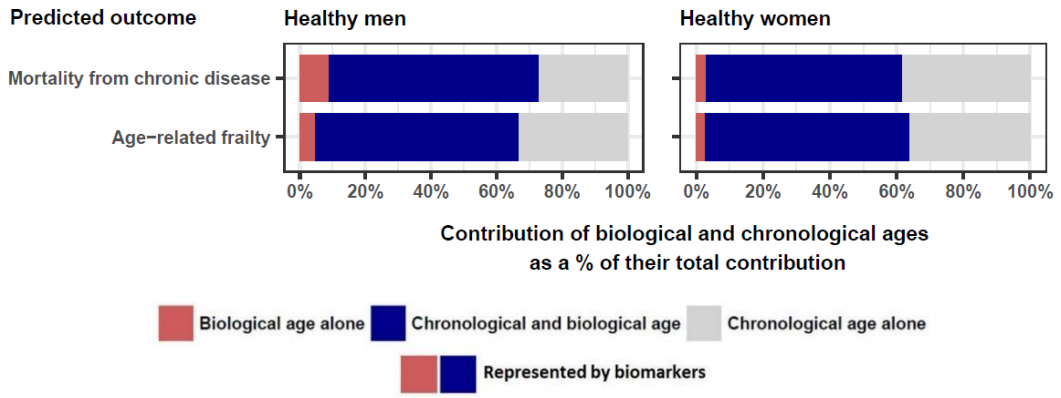
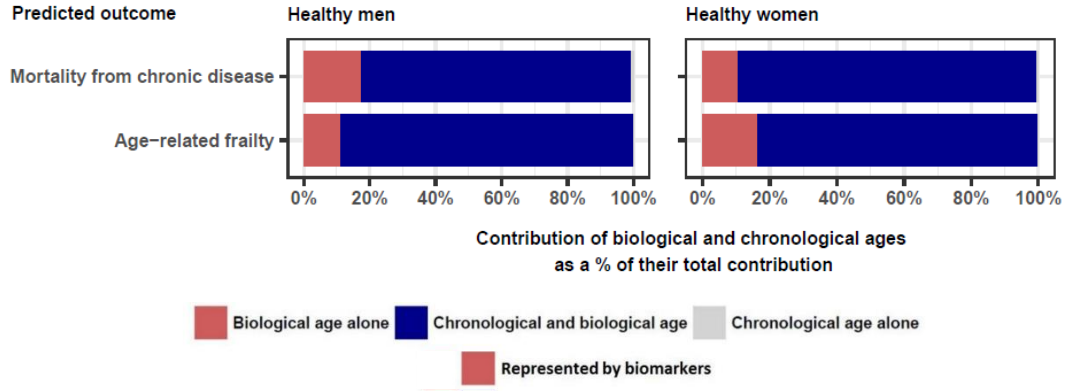


Figure 8.1.2: Relative contribution of biological ages and chronological age in explaining each health outcome, in the (A) age-based biological age and (B) disease risk-based biological age, for healthy men and women

(A) Age-based biological age



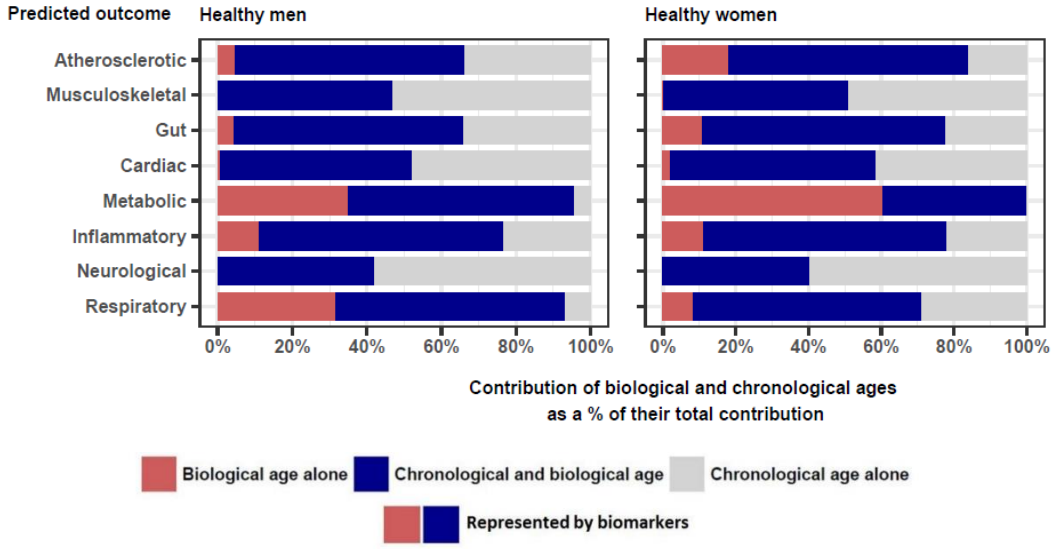
(B) Disease risk-based biological age



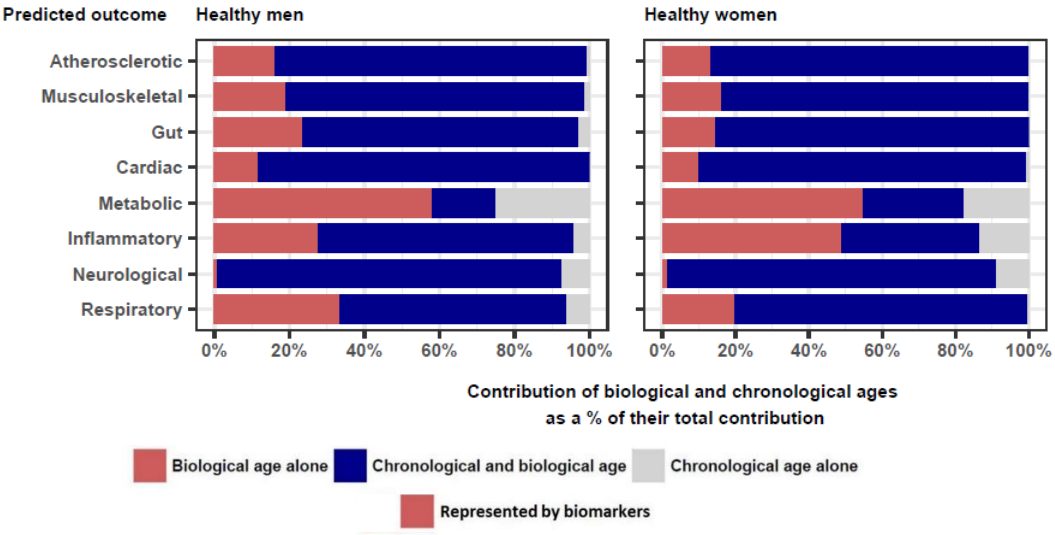
In (B), the disease risk-based biological age used is equivalent to the sum of disease risk-based biological age delta (linear combination of biomarker measurements) and chronological age. If disease risk-based biological age delta was used in place of its biological age, the biological age delta alone (the bars in red) contributed the same proportion to the overall age effect but the biological age delta and chronological age (the bars in blue) contributed almost nothing to the overall age effect (Figure 7.4.1).

Figure 8.1.3: Relative contribution of (A) age-based biological age vs (B) disease risk-based biological age vs (C) body system risk scores and age deltas and chronological age in explaining each disease group outcome, for healthy men and women

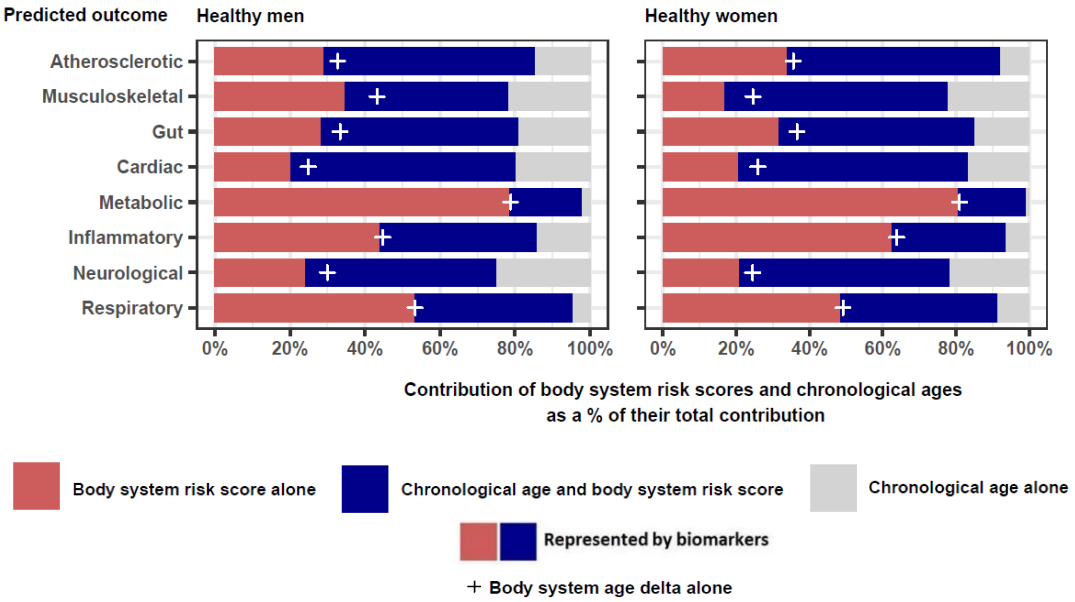
(A) Age-based biological age



(B) Disease risk-based biological age



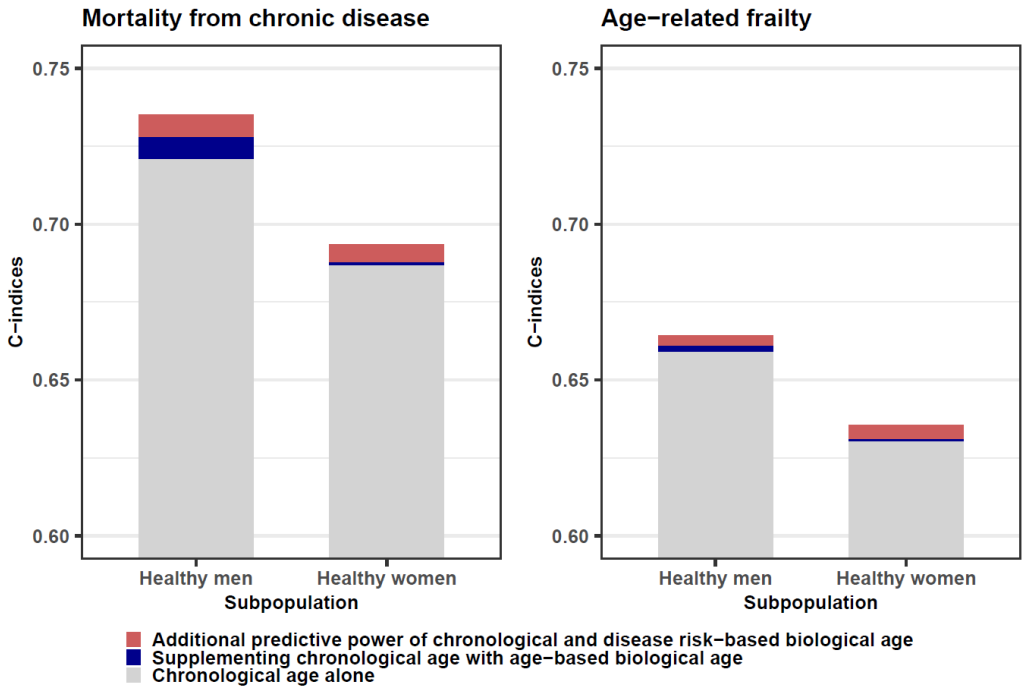
(C) Body system risk scores and age deltas



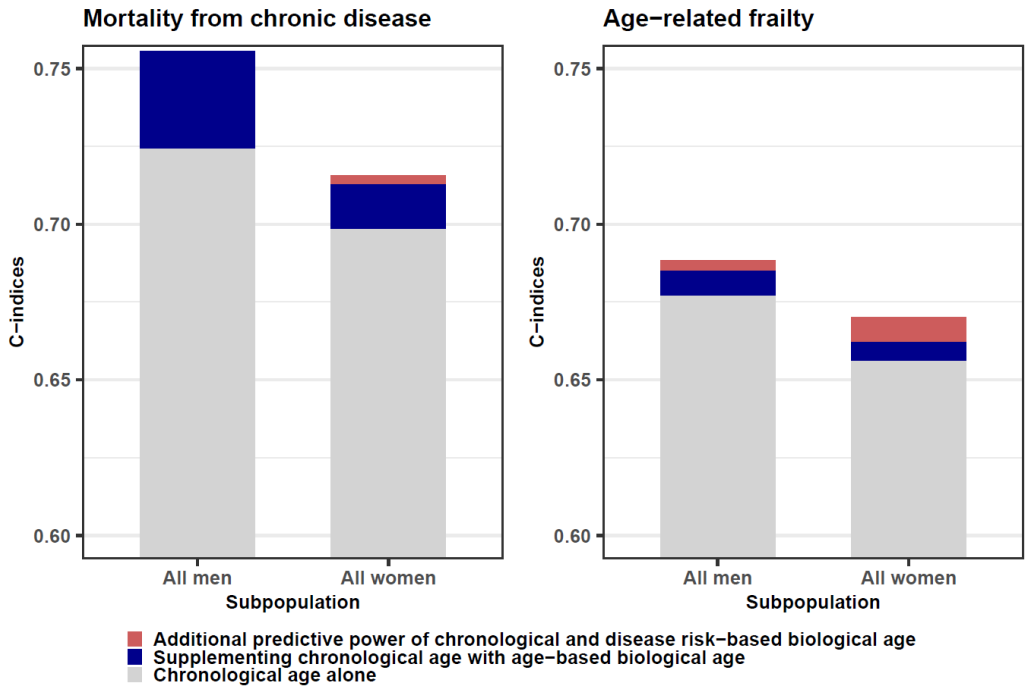
The body system ages used in each prediction in (C) were the respective body system age for the disease group of interest

Figure 8.1.4: Harrell's C-indices for the prediction of mortality from chronic disease and age-related frailty in adjusted analyses, for combinations of age-based biological age, disease risk-based biological age and chronological age, in the (A) healthy subpopulation and (B) whole population

(A) Healthy subpopulation



(B) Whole population



Analyses were adjusted for Index of Multiple Deprivation 2010 quintile, smoking status, alcohol consumption and assessment centre
The healthy subpopulation in (A) excluded participants who could have been regarded as having screen-detected diabetes, chronic kidney disease or chronic obstructive pulmonary disease (Appendix 6.1)
For mortality from chronic disease in all men (B), disease risk-based biological ages did not supplement chronological age as much as age-based biological ages (increase in C-index was 0.005 lower for disease risk-based vs age-based biological age)

Appendices

Appendix 2.1: Systematic review protocol registered on PROSPERO

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PROSPERO
International prospective register of systematic reviews

Systematic review of large-scale studies of biological ageing in humans

Mei Sum Chan, Jong-Wook Ban, Matthew Arnold, Rafael Perera, Sarah Parish

Citation

Mei Sum Chan, Jong-Wook Ban, Matthew Arnold, Rafael Perera, Sarah Parish. Systematic review of large-scale studies of biological ageing in humans. PROSPERO 2019 CRD42019079241
Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42019079241

Review question

Aim: To consolidate evidence on the utility and reliability of biological ages constructed from clinical biomarkers, in the public health context.

Questions to be addressed:

1. Create an overall mapping of different datasets, study designs, constituent variables and methods used, to compare the merits of the estimated biological ages.
2. Summarise studies' aims of estimating biological ages and assess how they achieved their aims. This may give contextual information on the choice of study design and methods, and on the applicability of a biological age.

Searches

The Ovid MEDLINE and Ovid EMBASE databases will be searched for articles published since 1 January 2007, with no language restriction. Any additional articles identified from an initial pragmatic search and meeting all search criteria will be included.
Search terms will include:
(bio* age or bio* ag?ing).mp.
and
(estimat* or measure* or calculat* or evaluat* or valid* or diagnost* or predict* or scor*).mp.
and
(biomarker* or biological marker* or (bio* adj1 factor*) or multiple marker* or parameter* or biological variable* or variable*).mp.

Search strategy

Types of study to be included

Large prospectively collected observational cohort studies

Condition or domain being studied

The measure being studied is biological age, which is a composite health risk score estimated from measurements of multiple clinical biomarkers, and can be used as a predictor or indicator of general health. Biological age is the primary outcome, while the constituent biomarkers are the exposure.

Participants/population

1. Human subjects only (alive at baseline).
2. Large cohort study of adults – adequate sample size of ?550 and inclusive of ages 30+.
3. Exclude studies where the cohort is selected based on the presence of specific health conditions at baseline.

Intervention(s), exposure(s)

The exposures are the constituent clinical biomarkers of each biological age. Clinical biomarkers are defined

as non-genetic and non-epigenetic predictors of individuals' health status, excluding predictors directly related to the presence or absence of any health outcome (for example prior disease, medication and treatment), health behaviours, socioeconomic factors, environmental factors, sex and chronological age. Biological ages are intended to be reflective of a general rather than an organ- or disease-specific ageing process, and should capture more than one specific body system or the interaction between two body systems. Correspondingly, eligible biological ages in studies should include at least 3 biomarkers, to capture a wide enough range of biomarkers across domains (Lara et al, BMC Med 2015) or physiological systems (Burkle et al, Mech Ageing Dev 2015).

Comparator(s)/control
Either the biological ageing score or its validation model should have controlled for or stratified by sex and age.

Main outcome(s)
At least one biological ageing score, which is continuous or scalar and has been expressed in terms of an age.

* Measures of effect

As above.

Additional outcome(s)
The incidence or prevalence of at least one health outcome, including mortality. There is no restriction on health outcomes in scope, although they are intended to represent clinical manifestations of the ageing process and should be used in the validation for at least one biological ageing score (primary outcome). The method of validation will not be pre-specified.

* Measures of effect

Not applicable.

Data extraction (selection and coding)
Selecting studies: Search results will be screened and data will be extracted independently for the selected studies by two reviewers, with a third reviewer adjudicating any discrepancies.
Data to be extracted for the selected studies: aims, how and whether these aims were achieved, sample size, age range, number of incident events for secondary outcomes, candidate biomarkers, biomarkers selected for inclusion in biological ages, methods used to assess primary and secondary outcomes, type of secondary outcomes assessed and validation results for each primary outcome using secondary outcomes.

Risk of bias (quality) assessment
Presence of pre-registered study protocols, and independence or overlap of study populations will be assessed. Since this review primarily aims to consolidate evidence on the utility and reliability of biological ages rather than summarise effect sizes, overlapping studies will be noted but included as separate studies. If effect sizes can be summarised, the analysis will take into account any overlaps. One of several potential applications of biological age is as a clinical risk prediction tool. For this type of application, risk of bias will be assessed for the selected studies using the recently-published PROBAST checklist for risk of bias of prediction modelling studies, or a relevant subset of the checklist. It will inform the narrative synthesis.

Strategy for data synthesis
A preliminary pragmatic literature search showed heterogeneity in the aims, biomarker panels, methods, outcomes and results format. Data will be summarised in tabular form and further quantitative summary of the results will be attempted for studies that are comparable. A narrative synthesis will be done to summarise and qualitatively assess the extracted data and risk of bias.

Analysis of subgroups or subsets
None planned

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Type and method of review
Methodology, Narrative synthesis, Prognostic, Systematic review

Anticipated or actual start date
01 July 2018

Anticipated completion date
31 December 2019

Funding sources/sponsors
Nuffield Department of Population Health, University of Oxford

Conflicts of interest
None known

Language
English

Country
England

Stage of review
Review Ongoing

Subject index terms status
Subject indexing assigned by CRD

Subject index terms
Aging; Humans; Research

Date of registration in PROSPERO
13 March 2019

Date of first submission
27 February 2019

Stage of review at time of this submission

Stage	Started	Completed
Preliminary searches	Yes	Yes
Piloting of the study selection process	Yes	Yes
Formal screening of search results against eligibility criteria	Yes	Yes
Data extraction	No	No
Risk of bias (quality) assessment	No	No
Data analysis	No	No

Revision note

The PROBAST checklist for risk of bias of prediction modelling studies was recently published, and it is the only known tool for this purpose. Risk of bias assessment has not commenced.

The record owner confirms that the information they have supplied for this submission is accurate and complete and they understand that deliberate provision of inaccurate information or omission of data may be construed as scientific misconduct.

The record owner confirms that they will update the status of the review when it is completed and will add publication details in due course.

Versions

13 March 2019
21 June 2019

Appendix 2.2: Further details of the systematic review procedure

Prior to the systematic database search, 9 key studies were identified from a review of biological age estimation methods[20] and initial database searches: Belsky 2015,[16] Belsky 2018,[57] Jee 2017,[96] Levine 2013,[12] Levine 2014,[78] Liu 2018,[62] Mamoshina 2018,[98] Mitnitski 2017[13] and Yoo 2017.[106] The process of refining the systematic search strategy was partly based on the ability of the strategy to detect these studies.

Full details of the search terms for the Ovid Medline and EMBASE databases are in Table A2.1. The final search was run on the databases on 25 February 2019.

Determining minimum sample size for study inclusion:

A power calculation was conducted to determine the minimum sample size for study inclusion, using the G*Power software (version 3.1.9.4).[284] The power calculation was designed to detect a small effect size, conventionally defined as Cohen's f^2=0.02,[285] when fitting a linear multivariable regression of biological age with the minimum number of biomarkers specified in the inclusion criteria (3 biomarkers; Section 1.2). This is similar to detecting an increase of 0.02 in the proportion of variance explained by the biomarkers in the assumed regression. An F-test was used and the input parameters specified were power level $(1-\beta)$ of 0.8 and a one-sided probability level (α) of 0.05. This calculation assumed that the statistical power properties of eligible biological ages are equivalent to a biological age estimated from a single multivariable linear regression, and that a small effect size was more appropriate to counterbalance larger numbers of constituent biomarkers used than the minimum specified. Based on these parameters and assumptions, the power calculation found that the minimum sample size was 550 people, at a critical F statistic of 2.62.

Sensitivity checks on the search strategy:

1. Selection of search terms for ‘biological ageing’ (Step 1, Table A2.1):

After including the MeSH term ‘Aging’ in the search strategy, a rapid title and abstract screening showed that it did not add any relevant articles. To increase specificity, the MeSH term was thus omitted and additional keywords were considered for inclusion, in order to make the search more comprehensive. Relaxing the search term to allow for adjacency of one or two words, ‘((bio* or physiol*) adj2 (age or ag?ing))’, was considered but a rapid screening of titles and abstracts showed that it did not add any relevant articles. To increase specificity, adjacency was not used.

2. Alternative search terms for ‘biological ageing’ (Step 2, Table A2.1):

The list of alternative terminology was picked up from references in the Jia 2017 methods review[20] and iterative initial searches. An article on bodily ages[64] listed three other terms that have been used in the literature: ‘effective age’, ‘vital age’ and ‘healthy age’. A search was carried out on these three alternative terms only, along with the method and outcome components, and a rapid screening of titles and abstracts showed that the results were irrelevant except when they overlapped with the main search strategy results. These alternative terms were thus excluded.

3. Selection of publication date restriction (Step 12, Table A2.1):

The Jia 2017 methods review[20] and the set of key articles identified showed that the majority of relevant articles had been published since 2010, and older studies tended to have fewer participants, fewer biomarkers in the panel, or both, and would likely be excluded from this review. Restricting to more recent years also increases specificity. The cut-off was set as 1 January 2007, to reflect that large cohort studies are a recent development. A recent systematic review of biological age biomarkers had a cut-off date of 2010,[18] but the earlier date was chosen for this review to increase sensitivity.

The inclusion criteria used in screening the search results is detailed in the systematic review protocol (Appendix 2.1), and was designed based on the PICO (Population/problem, Intervention/exposure, Comparator, Outcome) framework (Table A2.2).

Table A2.1: Search terms in Ovid Medline and EMBASE databases

Search step	Medline search terms	EMBASE search terms, where different from Medline
1	(bio* age or bio* ag?ing).mp.	
2	(frail* index or age index or ag?ing index or ag?ing clock or age clock or age score or ag?ing score or physiologic* dysreg* or physiologic* deterior* or physiol* age or physiol* ag?ing or pace of ag* or allostatic load or cumulative biological risk or healthspan*).mp.	
3	1 or 2	
4	(estimat* or measure* or calculat* or evaluat* or valid* or diagnost* or predict* or scor*).mp.	
5	Biomarkers/	Biological markers/
6	(biomarker* or biological marker* or (bio* adj1 factor*) or multiple marker* or parameter* or biological variable* or variable*).mp.	
7	5 or 6	
8	3 and 4 and 7	
9	8 not (cost-effect* or cost effect* or health economic* or mouse* or mice* or elegans* or worm* or drosophil* or zebra* or rodent* or rat or rats or macaqu* or monkey* or gorilla* or fly or flies or bee or bees or bird* or fish or whale* or snail*).mp.	
10	9 not (exp animals/ not humans.sh.)	9 not (animal* or nonhuman* or non-human*).mp.
11	10 not (*CpG*/ or *DNA*/ or *RNA*/ or methyl* or fibroblast* or histolog* or in vitro).mp.	
12	11 and ("2007" or "2008" or "2009" or 201*).dp.	
13		12 not conference*.pt.

Table A2.2: Study screening criteria, and their respective PICO (Population/problem, Intervention/exposure, Comparator, Outcome) framework component

Number	Screening criteria	Component of PICO framework
1	Identification and evaluation of studies of biological ages that are indicative or predictive of poor health	Problem
2	Large cohort study of older people, with sample size >550 and inclusive of ages 30+	Population
3	Cohort selection is not based on specific adverse health outcomes	Population
4	Includes at least 3 clinical biomarkers	Exposure
5	The biological age can be used to differentiate health profiles of individuals and is expressed in terms of an age	Comparator
6	At least one biological age has been validated against at least one health outcome	Outcomes

Table A2.3: Articles excluded after full text screening, with reasons for exclusion

Number	Study	Reason(s) for exclusion
1	Arbeev et al (2016)	Not expressed in terms of an age (Mahalanobis distance)
2	Arbeev et al (2018)	Not expressed in terms of an age (Mahalanobis distance)
3	Bae et al (2013a)	Not validated against an outcome other than chronological age
4	Bae et al (2013b)	Not validated against an outcome other than chronological age
5	Bai et al (2010)	Not validated against an outcome other than chronological age
6	Bai et al (2011) (Full text in Chinese)	Biological age for this study was calculated in the Bai et al (2010) study (also excluded). Not validated against an outcome other than chronological age
7	Bai (2018)	Not validated against an outcome other than chronological age (Analysis is the same as in Bai et al 2010)
8	Barber et al (2016a)	Not expressed in terms of an age (cumulative biological risk score)
9	Barber et al (2016b)	Not expressed in terms of an age (cumulative biological risk score)
10	Beekman et al (2016)	Not expressed in terms of an age (Framingham Risk Score)
11	Bello and Dumancas (2017)	Not expressed in terms of an age (random forest survival approach)
12	Bello et al (2018)	Not expressed in terms of an age (gradient boosting approach)
13	Blokh and Stambler (2015)	Not expressed in terms of an age (information theoretical approach), sample sizes for each of the datasets used had less than the minimum required
14	Blokh and Stambler (2017)	Not expressed in terms of an age (information theoretical approach)
15	Burkle et al (2015)	Not an observational cohort study
16	Chiappa et al (2013)	Biological age was only developed from 1 biomarker (FEV1)
17	Cohen et al (2013)	Sample size was less than the minimum required and not expressed in terms of an age (Mahalanobis distance)
18	Cohen et al (2014)	Not expressed in terms of an age (Mahalanobis distance)
19	Cohen et al (2015)	Not expressed in terms of an age (Mahalanobis distance)
20	Cornman et al (2017)	Not expressed in terms of an age (Fried frailty phenotype and a cumulative deficit index for physiological dysregulation)
21	Fedintsev et al (2017)	Sample size less than the minimum required
22	Finkel et al (2017)	Not validated against a health outcome
23	Giampieri et al (2015)	Not an observational cohort study
24	Guryeva et al (2016)	Age range was younger than the minimum required, not expressed in terms of an age ('coefficient of ageing rate')
25	Guryeva et al (2018)	Abstract showed that the same method as Guryeva et al (2016) was used to construct the biological age, thus it would not be expressed in terms of an age ('coefficient of ageing rate'), so full text was not requested
26	Karrasch et al (2018)	Sample size less than the minimum required
27	Li et al (2015)	Not expressed in terms of an age (Mahalanobis distance)
28	Lin et al (2017)	Not validated against a health outcome, although the gene expression score derived from the biological age (but not expressed as an age) was validated against a health outcome
29	Lixie et al (2015)	Not expressed in terms of an age (physiological ageing score) and not validated against an outcome other than chronological age
30	Lucicesare et al (2009)	Not expressed in terms of an age (Rockwood frailty index and Conselice Study of Brain Aging Score)
31	Milot et al (2014)	Not expressed in terms of an age (Mahalanobis distance)
32	Sanders et al (2018)	Not expressed in terms of an age (Biomarker Index and Physiologic Index)
33	Sebastiani et al (2016)	Not expressed in terms of an age (hierarchical clustering into biomarker signatures)

Table A2.4: Detailed cohort characteristics, biomarker selection criteria and validation methods for the 15 included studies of biological ages

Study [and study type]	Cohort characteristics	Baseline and follow up dates (if follow up used)	Number of events if applicable (cohort size) used in validation	Whether % missing per biomarker reported and how missingness handled	How biomarkers were selected prior to and during modelling	Calibrated to chronological age	Validation method used including risk calibration
Forrester 2018[94] [Dev]	Derivation: Longitudinal cohort with repeated measures of biomarkers over 15 years Internal validation: Cross-section of the same cohort	Baseline: 2015-2016, Retrospective follow up: outcomes measured 5 years before biomarkers at 2010-2011	Non-event outcome (all 2694 in dataset)	NA (complete case study)	Prior: general knowledge of association with ageing, availability in cohort, represent several biological systems, significant association with CA	No	Effect size of prediction: linear regression of BA-CA on continuous outcomes and t-tests/ANOVA for dichotomous and categorical outcomes
Levine 2018[97] [Dev]	Repeated cross-sectional cohort, age 20-79	Baseline: 1988-1994, 2007-2010	Non-event outcome, NI	NA (complete case study)	Prior: used in Levine 2013	No	Effect size of association: Assessed association of BA and period differences in BAs with smoker status, obesity and medication use
Mamoshina 2018[98] [Dev, Val]	Derivation: Age 20+ External validation: Multiple longitudinal cohorts, individuals who had mortality follow up data	Baseline: NI Follow up: NI	Alberta: 340 (20,699) NHANES: 873 (2768)	In the Alberta cohort, 4 biomarkers not completely measured were imputed via regression. Missingness: 31% (calcium), 36% (total bilirubin), 66% (total protein), 73% (urea)	NI	Yes	Effect size of prediction: Cox models Risk calibration: Compared effect sizes for groups with BA-CA of more or less than -5 or 5 years
Murabito 2018[99] [Dev]	Longitudinal cohorts	Baseline: 1979-1983, 1998-2001, 2005-2008, Follow up: to 2014	238-718 (2231-3140) for mortality, 163-413 (2065-2944) for cardiovascular events, 136-510 (1906-2859) for cancer	NA (complete case study)	Prior: representative of diverse biological systems (and for clinical age, availability in cohort)	No	Effect size of prediction: Cox models
Jee 2017[96] [Treated as Dev]	Derivation: Cross-section of longitudinal cohort, female, age 30-80, no diagnosed medical conditions, biomarker values within clinically normal range Validation: individuals with impaired glucose tolerance or diabetes	Baseline: 2009-2011 Validation: NI	Non-event outcome (350 individuals with impaired glucose tolerance and 75 individuals with diabetes)	NA (appears to be complete case study)	Prior: correlation with CA >0.15, not clear if selected based on level of missingness, During: for PCA age, biomarkers that loaded onto the first PC	Yes (Dubina adjustment, Bland-Altman plots and Cohen's D)	Risk calibration: Compared increases in means of CA and BA for the validation cohort above those for the derivation cohort

Kang 2017[117] [Dev]	Derivation and internal validation: cross-sectional cohort, received routine health check-ups, age 20+	Derivation: Baseline: 2014-2015 Validation: Baseline: 2012-2013	Non-event outcome (all 188,886 in validation dataset)	NA (complete case study)	Prior: correlation (not clear if correlated with CA or other biomarkers, NI on threshold) During: biomarkers that loaded onto the first PC (all biomarkers)	Yes (Dubina adjustment)	Risk calibration: Compared mean metabolic syndrome age and CA differentials using ANOVA across 3 groups: normal, those with 1-2 risk variables and those with metabolic syndrome
Mitnitski 2017[13] [Dev]	Longitudinal cohort, age 65+, no missing data for the FI-LAB	Baseline 1991-1992, Follow up: 6 years from baseline	NI	NA (complete case study)	Prior: significant correlation with CA	Yes	Effect size of prediction: Cox models Discrimination: AUC
Yoo 2017[106] [Dev, Val]	Derivation: Longitudinal cohort, age 20-93 External validation: NI	Derivation: Baseline: 1993-2004 Follow up: to 2011 (but model run on data for 2002-2009) Validation: NI	13,106 (557,940)	NI, imputed population averages of the biomarkers measured in the relevant age group and sex	Prior: level of missingness, correlation with other biomarkers <0.7 During: maximises biological age R^2	Yes (Dubina adjustment)	Effect size of prediction: Cox models Risk calibration: Kaplan-Meier plots of BA-CA groups
Zhang 2017[19] [Dev, Val]	Derivation: Cross-sectional study, age 19-93, healthy at recruitment, with no anomalous biomarker levels or missing self-reported lifestyle information at baseline External validation: hospital patients with common diseases, age 20-85	NI for derivation and validation	Non-event outcome (all 266 in validation dataset)	NI	Prior: correlation with CA >0.4 with p<0.05, correlation with other biomarkers <0.65 with p<0.05 During: biomarkers that loaded onto the first PC (all biomarkers)	Yes	Risk calibration: Scatter plot of validation cohort BA against CA between healthy and ill patients with regression line of derivation results
Golab 2016[115] [Dev]	Cross-sectional cohort of men in industrial jobs in a city, age 20-70	Baseline: 2001-2002	Non-event outcome, NI	NA (appears to be complete case study)	Prior: significant and linear association with CA	No	Risk calibration: Compared results for the 5 outcomes across 3 Z-score groups calculated from BA-CA differences
Belsky 2015[16] [Val]	Retrospective observational cohort, born in 1972-1973 and who survived to age 38 (baseline age)	Baseline: 2010-2011 Retrospective follow up: 1998-2011	Non-event outcome, NI	NA (appears to be complete case study)	Prior: used in Levine 2013	No	Effect size of association: Standardised beta coefficients from linear regressions adjusted for sex (similar to Pearson correlation coefficients)
Hollar 2015[116] [Val]	5 cross-sectional cohorts, age 20-85. For disability outcome, those who do not have both a sensory disability and mobility/assistive devices. For	Baseline: Two years per cohort over 2001-2010	Non-event outcome, NI	NI	Prior: used in the GCRP Heart Age model	No	Risk calibration: Compared differences in heart age-CA for those with and those without the outcome (using ANOVA and Kruskal-Wallis)

	social capital outcome, age 40+ and in the 4 cohorts 2000-2008						
Levine 2014[78] [Val]	Longitudinal cohort, age 30+, non=-institutionalised	Baseline: 1988-1994 Follow up: to 2006	1076 (9942)	NA (complete case study)	Prior: used in Levine 2013	No	Effect size of prediction: Cox models Discrimination: AUC
Levine 2013[12] [Dev]	Longitudinal cohort, age 30-75, no missing data on at least 1 biomarker	Baseline: 1988-1994 Follow up: to 2006	1843 (9389)	NA (complete case study)	Prior: correlation with CA >0.1 During: additional biological ages estimated from the subset of biomarkers that loaded onto the first PC	Yes for PCA and MLR ages (appears to be the Dubina adjustment), no for KDM ages	Effect size of prediction: Cox models Discrimination: AUC
Jee 2012[95] [Treated as Dev]	Derivation: Cross-sectional cohort, age 30-85, received routine clinical health exams, clinically normal ranges of biomarkers, no prior cancer or medication for hypertension, diabetes, dyslipidaemia or thyroid dysfunction Validation: NI	Baseline: 2004-2007	Non-event outcome (all 2587 participants validated with respect to sarcopenia or 3162 participants validated with respect to obesity)	NA (appears to be complete case study for derivation and validation datasets)	Prior: correlation with CA >0.15 and high correlation with other biomarkers	Yes (Dubina adjustment, regression slope)	Risk calibration: Compared increases in means of CA and BA in individuals with risk of sarcopenia or BMI>27.5 above those for healthy individuals

[Dev/Val]: Denotes whether study was a development and/or internal validation study (Dev), or an external validation study (Val), or both. Kang 2017[117] used split sample internal validation, while the format of validation for Jee 2012 and 2017[95,96] was unknown (no further information was provided by the corresponding author) and they were treated as development and/or internal validation studies
Each row relates to one study and multiple biological ages or validation outcomes in the same study are listed in the same row
Abbreviations:
NI: No information
BA: Biological age
CA: Chronological age
PC, PCA: Principal component, Principal component analysis
GCRP: General Cardiovascular Risk Profile
Dubina adjustment: The Dubina method of adjusting biological ages,[177] which reduces systematic differences between biological and chronological ages in a population
AUC: Area under the receiver operating characteristic curve

Table A2.5: Summary of risk of bias and applicability in each of the 15 included studies, assessed using the PROBAST checklist

Study		Risk of bias (ROB)				Applicability			Overall	
		Participants	Predictors	Outcome	Analysis	Participants	Predictors	Outcome	ROB	Applicability
1	Forrester 2018 [Dev]	+	?	-	-	+	+	-	-	-
2	Levine 2018 [Dev]	+	?	?	-	+	?	-	-	-
3	Mamoshina 2018 [Dev]	?	?	?	-	?	?	?	-	?
	Mamoshina 2018 [Val]	?	?	?	-	?	?	?		
4	Murabito 2018 [Dev]	+	?	+	-	+	+	+	-	+
5	Jee 2017 [Treated as Dev]	?	?	?	-	?	?	?	-	?
6	Kang 2017 [Dev]	+	-	-	-	+	-	-	-	-
7	Mitnitski 2017 [Dev]	+	+	+	-	?	+	+	-	+
8	Yoo 2017 [Dev]	?	?	?	-	+	?	?	-	+
	Yoo 2017 [Val]	+	?	+	-	+	?	+		
9	Zhang 2017 [Dev]	-	+	?	-	+	+	?	-	-
	Zhang 2017 [Val]	-	-	?	?	?	-	-		
10	Golab 2016 [Dev]	+	?	?	-	+	+	?	-	?
11	Belsky 2015 [Val]	+	?	-	-	-	?	-	-	-
12	Hollar 2015 [Val]	?	?	+	-	+	?	-	-	-
13	Levine 2014 [Val]	+	?	+	-	+	?	+	-	?
14	Levine 2013 [Dev]	+	?	+	-	+	?	+	-	?
15	Jee 2012 [Treated as Dev]	?	+	-	-	?	+	-	-	-

Risk of bias judgement:

Low	Unclear	High
+	?	-

PROBAST: Prediction model Risk Of Bias ASsessment Tool
The PROBAST checklist was downloaded from the PROBAST website[130] and referenced by the Moons et al article that provides an explanation of this checklist.[113]

Table A2.6: PRISMA checklist for this systematic review

Section/topic	#	Checklist item	Reported in section
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Chapter title
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	NA
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	2.1
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	2.1
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	2.2
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Appendix 2.2
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	2.2
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Appendix 2.2
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	2.2
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	2.2
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Tables 2.3.1-3, A2.4
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	2.2
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	2.3
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	NA

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	2.2
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Figure 2.3.2
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Figure 2.3.1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Tables 2.3.1-3, A2.4
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Figure 2.3.3, Table A2.5
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Figure 2.3.2
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	NA
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	2.3
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Figure 2.3.2
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	2.4
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	2.4
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	2.4-2.5
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	Acknowledgements

Appendix 2.3: Original CHARMS and PROBAST checklists

Table A2.7: Original CHARMS checklist

Domain	Key items	Reported on page #
SOURCE OF DATA	Source of data (e.g., cohort, case-control, randomized trial participants, or registry data)	
PARTICIPANTS	Participant eligibility and recruitment method (e.g., consecutive participants, location, number of centers, setting, inclusion and exclusion criteria)	
	Participant description	
	Details of treatments received, if relevant	
	Study dates	
OUTCOME(S) TO BE PREDICTED	Definition and method for measurement of outcome	
	Was the same outcome definition (and method for measurement) used in all patients?	
	Type of outcome (e.g., single or combined endpoints)	
	Was the outcome assessed without knowledge of the candidate predictors (i.e., blinded)?	
	Were candidate predictors part of the outcome (e.g., in panel or consensus diagnosis)?	
	Time of outcome occurrence or summary of duration of follow-up	
CANDIDATE PREDICTORS (OR INDEX TESTS)	Number and type of predictors (e.g., demographics, patient history, physical examination, additional testing, disease characteristics)	
	Definition and method for measurement of candidate predictors	
	Timing of predictor measurement (e.g., at patient presentation, at diagnosis, at treatment initiation)	
	Were predictors assessed blinded for outcome, and for each other (if relevant)?	
	Handling of predictors in the modelling (e.g., continuous, linear, non-linear transformations or categorised)	
SAMPLE SIZE	Number of participants and number of outcomes/events	
	Number of outcomes/events in relation to the number of candidate predictors (Events Per Variable)	
MISSING DATA	Number of participants with any missing value (include predictors and outcomes)	
	Number of participants with missing data for each predictor	
	Handling of missing data (e.g., complete-case analysis, imputation, or other methods)	
MODEL DEVELOPMENT	Modelling method (e.g., logistic, survival, neural network, or machine learning techniques)	
	Modelling assumptions satisfied	
	Method for selection of predictors **for inclusion** in multivariable modelling (e.g., all candidate predictors, pre-selection based on unadjusted association with the outcome)	
	Method for selection of predictors **during multivariable modelling** (e.g., full model approach, backward or forward selection) and criteria used (e.g., p-value, Akaike Information Criterion)	
	Shrinkage of predictor weights or regression coefficients (e.g., no shrinkage, uniform shrinkage, penalized estimation)	
MODEL PERFORMANCE	Calibration (calibration plot, calibration slope, Hosmer-Lemeshow test) and Discrimination (C-statistic, D-statistic, log-rank) measures with confidence intervals	
	Classification measures (e.g., sensitivity, specificity, predictive values, net reclassification improvement) and whether a-priori cut points were used	

MODEL EVALUATION	Method used for testing model performance: development dataset only (random split of data, resampling methods e.g. bootstrap or cross-validation, none) or separate external validation (e.g. temporal, geographical, different setting, different investigators)	
	In case of poor validation, whether model was adjusted or updated (e.g., intercept recalibrated, predictor effects adjusted, or new predictors added)	
RESULTS	Final and other multivariable models (e.g., basic, extended, simplified) presented, including predictor weights or regression coefficients, intercept, baseline survival, model performance measures (with standard errors or confidence intervals)	
	Any alternative presentation of the final prediction models, e.g., sum score, nomogram, score chart, predictions for specific risk subgroups with performance	
	Comparison of the distribution of predictors (including missing data) for development and validation datasets	
INTERPRETATION AND DISCUSSION	Interpretation of presented models (confirmatory, i.e., model useful for practice versus exploratory, i.e., more research needed)	
	Comparison with other studies, discussion of generalizability, strengths and limitations.	

CHARMS: Checklist for critical Appraisal and data extraction for systematic Reviews of prediction Modelling Studies
The CHARMS checklist is in Supplementary Text S1 of the Moons et al article that provides an explanation of this checklist.[112] Modified versions of this checklist were used in this systematic review (Tables 2.3.2, 2.3.3 and A2.4)

Figure A2.1: Original PROBAST checklist (version 15/05/2019)

PROBAST
(Prediction model study Risk Of Bias Assessment Tool)

Published in Annals of Internal Medicine (freely available):

1. PROBAST: A Tool to Assess the Risk of Bias and Applicability of Prediction Model Studies
2. PROBAST: A Tool to Assess Risk of Bias and Applicability of Prediction Model Studies: Explanation and Elaboration

What does PROBAST assess?
PROBAST assesses both the *risk of bias* and *concerns regarding applicability* of a study that evaluates (develops, validates or updates) a multivariable diagnostic or prognostic prediction model. It is designed to assess primary studies included in a systematic review.

Bias occurs if systematic flaws or limitations in the design, conduct or analysis of a primary study distort the results. For the purpose of prediction modelling studies, we have defined *risk of bias* to occur when shortcomings in the study design, conduct or analysis lead to systematically distorted estimates of a model's predictive performance or to an inadequate model to address the research question. Model predictive performance is typically evaluated using calibration, discrimination and sometimes classification measures, and these are likely inaccurately estimated in studies with high risk of bias. *Applicability* refers to the extent to which the prediction model from the primary study matches your systematic review question, for example in terms of the participants, predictors or outcome of interest.

A primary study may include the development and/or validation or update of more than one prediction model. A PROBAST assessment should be completed for each distinct model that is developed, validated or updated (extended) for making individualised predictions. Where a publication assesses multiple prediction models, only complete a PROBAST assessment for those models that meet the inclusion criteria for your systematic review. Please note that subsequent use of the term "model" includes derivatives of models, such as simplified risk scores, nomograms, or recalibrations of models.

PROBAST is not designed for all multivariable diagnostic or prognostic studies. For example, studies using multivariable models to identify predictors associated with an outcome but not attempting to develop a model for making individualised predictions are not covered by PROBAST.

PROBAST includes four steps.

Step	Task	When to complete
1	Specify your systematic review question(s)	Once per systematic review
2	Classify the type of prediction model evaluation	Once for each model of interest in each publication being assessed, for each relevant outcome
3	Assess risk of bias and applicability	Once for each development and validation of each distinct prediction model in a publication
4	Overall judgment	Once for each development and validation of each distinct prediction model in a publication

If this is your first time using PROBAST, we strongly recommend reading the detailed explanation and elaboration (E&E, see link above) paper and to check the examples on www.probast.org

Step 1: Specify your systematic review question

State your systematic review question to facilitate the assessment of the applicability of the evaluated models to your question. *The following table should be completed once per systematic review.*

Criteria	Specify your systematic review question
Intended use of model:	
Participants *including selection criteria and setting:*	
Predictors *(used in prediction modelling), including types of predictors (e.g. history, clinical examination, biochemical markers, imaging tests), time of measurement, specific measurement issues (e.g., any requirements/ prohibitions for specialized equipment):*	
Outcome to be predicted:	

Step 2: Classify the type of prediction model evaluation

Use the following table to classify the evaluation as model development, model validation or model update, or combination. Different signalling questions apply for different types of prediction model evaluation. If the evaluation does not fit one of these classifications then PROBAST should not be used.

Classify the evaluation based on its aim			
Type of prediction study	**PROBAST boxes to complete**	**Tick as appropriate**	**Definition for type of prediction model study**
Development only	Development		Prediction model development without external validation. These studies may include internal validation methods, such as bootstrapping and cross-validation techniques.
Development and validation	Development and validation		Prediction model development combined with external validation in other participants in the same article.
Validation only	Validation		External validation of existing (previously developed) model in other participants.

This table should be completed once for each publication being assessed and for each relevant outcome in your review.

Publication reference	
Models of interest	
Outcome of interest	

Step 3: Assess risk of bias and applicability

PROBAST is structured as four key domains. Each domain is judged for risk of bias (low, high or unclear) and includes signalling questions to help make judgements. Signalling questions are rated as yes (Y), probably yes (PY), probably no (PN), no (N) or no information (NI). All signalling questions are phrased so that "yes" indicates absence of bias. Any signalling question rated as "no" or "probably no" flags the potential for bias; you will need to use your judgement to determine whether the domain should be rated as "high", "low" or "unclear" risk of bias. The guidance document contains further instructions and examples on rating signalling questions and risk of bias for each domain.

The first three domains are also rated for concerns regarding applicability (low/ high/ unclear) to your review question defined above.
Complete all domains separately for each evaluation of a distinct model. Shaded boxes indicate where signalling questions do not apply and should not be answered.

DOMAIN 1: Participants			
A. Risk of Bias			
Describe the sources of data and criteria for participant selection:			
		Dev	Val
1.1 Were appropriate data sources used, e.g. cohort, RCT or nested case-control study data?			
1.2 Were all inclusions and exclusions of participants appropriate?			
Risk of bias introduced by selection of participants	**RISK:** *(low/ high/ unclear)*		
Rationale of bias rating:			
B. Applicability			
Describe included participants, setting and dates:			
Concern that the included participants and setting do not match the review question	**CONCERN:** *(low/ high/ unclear)*		
Rationale of applicability rating:			

DOMAIN 2: Predictors			
A. Risk of Bias			
List and describe predictors included in the final model, e.g. definition and timing of assessment:			
		Dev	Val
2.1 Were predictors defined and assessed in a similar way for all participants?			
2.2 Were predictor assessments made without knowledge of outcome data?			
2.3 Are all predictors available at the time the model is intended to be used?			
Risk of bias introduced by predictors or their assessment	**RISK:** *(low/ high/ unclear)*		
Rationale of bias rating:			
B. Applicability			
Concern that the definition, assessment or timing of predictors in the model do not match the review question	**CONCERN:** *(low/ high/ unclear)*		
Rationale of applicability rating:			

DOMAIN 3: Outcome		
A. Risk of Bias		
Describe the outcome, how it was defined and determined, and the time interval between predictor assessment and outcome determination:		
	Dev	Val
3.1 Was the outcome determined appropriately?		
3.2 Was a pre-specified or standard outcome definition used?		
3.3 Were predictors excluded from the outcome definition?		
3.4 Was the outcome defined and determined in a similar way for all participants?		
3.5 Was the outcome determined without knowledge of predictor information?		

3.6 Was the time interval between predictor assessment and outcome determination appropriate?			
Risk of bias introduced by the outcome or its determination	**RISK:** *(low/ high/ unclear)*		
Rationale of bias rating:			
B. Applicability			
At what time point was the outcome determined: *If a composite outcome was used, describe the relative frequency/distribution of each contributing outcome:*			
Concern that the outcome, its definition, timing or determination do not match the review question	**CONCERN:** *(low/ high/ unclear)*		
Rationale of applicability rating:			

DOMAIN 4: Analysis			
Risk of Bias			
Describe numbers of participants, number of candidate predictors, outcome events and events per candidate predictor:			
Describe how the model was developed (for example in regards to modelling technique (e.g. survival or logistic modelling), predictor selection, and risk group definition):			
Describe whether and how the model was validated, either internally (e.g. bootstrapping, cross validation, random split sample) or externally (e.g. temporal validation, geographical validation, different setting, different type of participants):			
Describe the performance measures of the model, e.g. (re)calibration, discrimination, (re)classification, net benefit, and whether they were adjusted for optimism:			
Describe any participants who were excluded from the analysis:			
Describe missing data on predictors and outcomes as well as methods used for missing data:			
		Dev	Val
4.1 Were there a reasonable number of participants with the outcome?			
4.2 Were continuous and categorical predictors handled appropriately?			
4.3 Were all enrolled participants included in the analysis?			
4.4 Were participants with missing data handled appropriately?			
4.5 Was selection of predictors based on univariable analysis avoided?			
4.6 Were complexities in the data (e.g. censoring, competing risks, sampling of controls) accounted for appropriately?			
4.7 Were relevant model performance measures evaluated appropriately?			
4.8 Were model overfitting and optimism in model performance accounted for?			
4.9 Do predictors and their assigned weights in the final model correspond to the results from multivariable analysis?			
Risk of bias introduced by the analysis	**RISK:** *(low/ high/ unclear)*		
Rationale of bias rating:			

Step 4: Overall assessment

Use the following tables to reach overall judgements about risk of bias and concerns regarding applicability of the prediction model evaluation (development and/or validation) across all assessed domains.

Complete for each evaluation of a distinct model.

Reaching an overall judgement about risk of bias of the prediction model evaluation	
Low risk of bias	If all domains were rated low risk of bias.

	If a prediction model was developed without any external validation, and it was rated as low risk of bias for all domains, consider downgrading to **high risk of bias**. Such a model can only be considered as low risk of bias, if the development was based on a very large data set and included some form of internal validation.
High risk of bias	If at least one domain is judged to be at **high risk of bias**.
Unclear risk of bias	If an unclear risk of bias was noted in at least one domain and it was low risk for all other domains.

Reaching an overall judgement about applicability of the prediction model evaluation	
Low concerns regarding applicability	If low concerns regarding applicability for all domains, the prediction model evaluation is judged to have **low concerns regarding applicability**.
High concerns regarding applicability	If high concerns regarding applicability for at least one domain, the prediction model evaluation is judged to have **high concerns regarding applicability**.
Unclear concerns regarding applicability	If unclear concerns (but no "high concern") regarding applicability for at least one domain, the prediction model evaluation is judged to have **unclear concerns regarding applicability** overall.

Overall judgement about risk of bias and applicability of the prediction model evaluation		
Overall judgement of risk of bias	**RISK:** *(low/ high/ unclear)*	
Summary of sources of potential bias:		
Overall judgement of applicability	**CONCERN:** *(low/ high/ unclear)*	
Summary of applicability concerns:		

PROBAST: Prediction model Risk Of Bias ASsessment Tool
The PROBAST checklist was downloaded from the PROBAST website[130] and referenced by the Moons et al article that provides an explanation of this checklist.[113] Summary results from this checklist were reported in this systematic review (Figure 2.3.3 and Table A2.5)

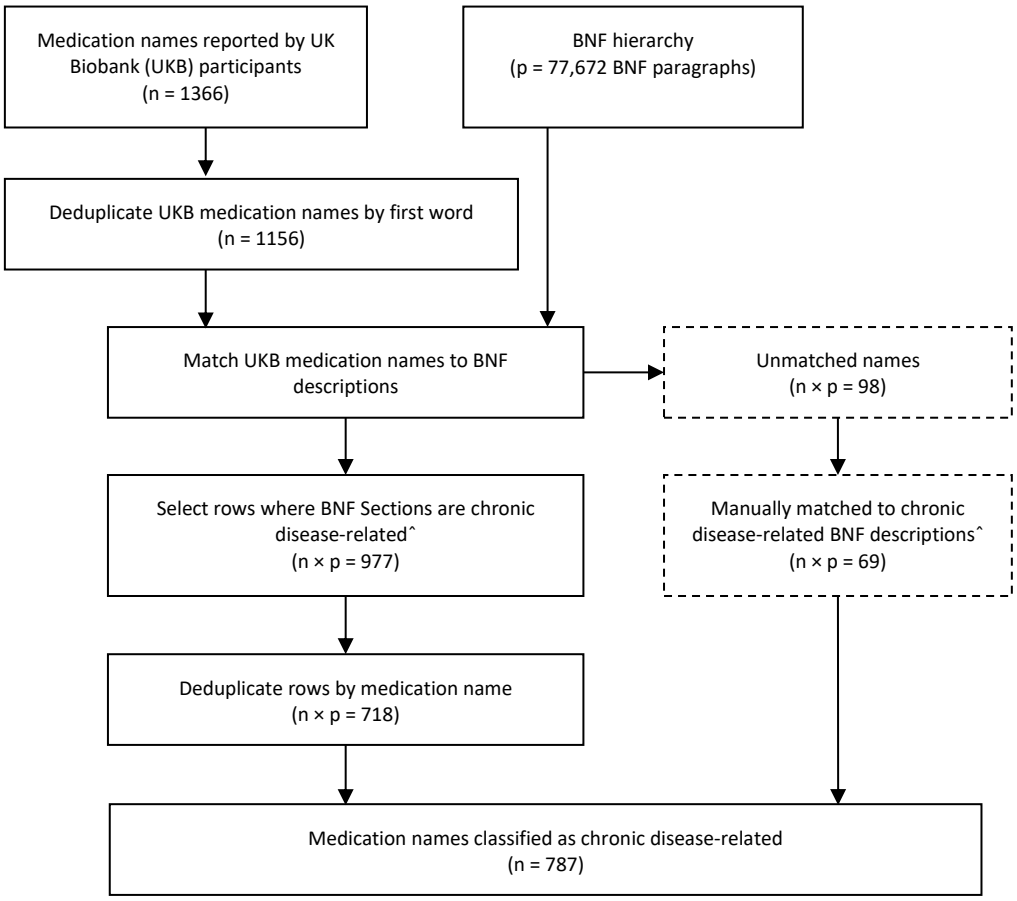
Appendix 3.1: Medication classification using British National Formulary (BNF) codes

UK Biobank medication names were categorised in order to stratify participants by prior health, in part by the counts of chronic disease-related medications that they reported using (Section 3.3). Medication names were self-reported and recorded during the nurse-led interview at baseline assessment.[103]

The current classification procedure is based on British National formulary (BNF) hierarchical mapping of medication names that had 6 levels (from top to bottom: Chapter, Section, Paragraph, Product, Presentation, Substance).[132] It was adapted from a previously published approach for deriving the number of prescribed medications per individual based on unique BNF paragraphs.[133] The study that proposed this approach found that this medication count was highly predictive of future medical consultations and mortality.[133]

The classification process for this thesis used the procedures described in the flowchart in Figure A3.1, to classify the 1366 medication names reported by UK Biobank participants into 787 types of medications at BNF Paragraph level. These medication names belonged to the BNF Chapters and Sections listed in Table 3.4.1. The steps that required manual selection or classification of medication names were reviewed by a clinician (Figure A3.1).

Figure A3.1: Flowchart of the classification process for UK Biobank medication names



^ Classification in this step was reviewed by a clinician

Appendix 3.2: ICD-10 and OPCS codelists for phenotyped diseases

Codelists for the 21 in scope age-related chronic diseases for this thesis (Section 3.6) are listed in this appendix. The Tran et al[134] ICD-10 codelist was used for 16 of the 20 age-related chronic diseases in this study, with no modifications. These diseases are: arthritis, cardiac arrhythmia, chronic kidney disease, chronic obstructive pulmonary disease, connective tissue disease, dementia, diabetes mellitus, gout, heart failure, ischaemic heart disease, liver disease, osteoporosis, peptic ulcer disease, peripheral arterial disease, rheumatoid arthritis and stroke/TIA. ICD-10 codes for malignant cancer were identified in the Tran et al[134] codelist, and were also used to identify participants with prior malignant cancer when stratifying the participants (Section 3.3).

For the disease-based analyses in Chapters 6–7, the codes used to phenotype the remaining 4 diseases (diverticular disease, gall bladder disease, motor neuron disease and Parkinson's disease) are listed in Table A3.1. Procedural OPCS codes that were used to extend the disease definitions (Section 3.5) for cardiac arrhythmia, ischaemic heart disease, peripheral arterial disease, arthritis and gall bladder disease are listed in Table A3.2.

Table A3.1: List of ICD-10 codes for diseases not captured in the Tran et al 2018 codelist

Disease	ICD-10 code	Description	Disease group
Parkinson's	G20	Parkinson disease	Neurological
Motor neuron disease	G122	Motor neuron disease	Neurological
Diverticular disease	K57	Diverticular disease of intestine	Gut
Gall bladder disease	K800	Calculus of gallbladder with acute cholecystitis	Gut
Gall bladder disease	K801	Calculus of gallbladder with other cholecystitis	Gut
Gall bladder disease	K810	Acute cholecystitis	Gut
Gall bladder disease	K811	Chronic cholecystitis	Gut
Gall bladder disease	K818	Other cholecystitis	Gut
Gall bladder disease	K819	Cholecystitis, unspecified	Gut
Gall bladder disease	K820	Obstruction of gallbladder	Gut
Gall bladder disease	K821	Hydrops of gallbladder	Gut
Gall bladder disease	K822	Perforation of gallbladder	Gut
Gall bladder disease	K823	Fistula of gallbladder	Gut
Gall bladder disease	K824	Cholesterolosis of gallbladder	Gut
Gall bladder disease	K828	Other specified diseases of gallbladder	Gut
Gall bladder disease	K829	Disease of gallbladder, unspecified	Gut

Table A3.2: List of OPCS codes used in disease definitions

Disease	OPCS code	Description	Disease group
Cardiac arrhythmia	K576	Percutaneous transluminal ablation of ventricular wall NEC	Cardiac
Cardiac arrhythmia	K641	Percutaneous radiofrequency ablation of epicardium	Cardiac
Cardiac arrhythmia	X503	Advanced cardiac pulmonary resuscitation	Cardiac
Cardiac arrhythmia	X504	External ventricular defibrillation	Cardiac
Cardiac arrhythmia	X508	Other specified external resuscitation	Cardiac
Cardiac arrhythmia	X509	Unspecified external resuscitation	Cardiac
Cardiac arrhythmia	K223	Exclusion of left atrial appendage NEC	Cardiac
Cardiac arrhythmia	K571	Percutaneous transluminal ablation of atrioventricular node	Cardiac
Cardiac arrhythmia	K621	Percutaneous transluminal ablation of pulmonary vein to left atrium conducting system	Cardiac
Cardiac arrhythmia	K622	Percutaneous transluminal ablation of atrial wall for atrial flutter	Cardiac
Cardiac arrhythmia	K623	Percutaneous transluminal ablation of conducting system of heart atrial flutter NEC	Cardiac
Cardiac arrhythmia	K624	Percutaneous transluminal internal cardioversion NEC	Cardiac
Cardiac arrhythmia	X501	Direct current cardioversion	Cardiac
Cardiac arrhythmia	X502	External cardioversion NEC	Cardiac
Ischaemic heart disease	K401	Saphenous vein graft replacement of one coronary artery	Atherosclerotic
Ischaemic heart disease	K402	Saphenous vein graft replacement of two coronary arteries	Atherosclerotic
Ischaemic heart disease	K403	Saphenous vein graft replacement of three coronary arteries	Atherosclerotic
Ischaemic heart disease	K404	Saphenous vein graft replacement of four or more coronary arteries	Atherosclerotic
Ischaemic heart disease	K408	Other specified saphenous vein graft replacement of coronary artery	Atherosclerotic
Ischaemic heart disease	K409	Unspecified saphenous vein graft replacement of coronary artery	Atherosclerotic
Ischaemic heart disease	K411	Autograft replacement of one coronary artery NEC	Atherosclerotic
Ischaemic heart disease	K412	Autograft replacement of two coronary arteries NEC	Atherosclerotic
Ischaemic heart disease	K413	Autograft replacement of three coronary arteries NEC	Atherosclerotic
Ischaemic heart disease	K414	Autograft replacement of four or more coronary arteries NEC	Atherosclerotic
Ischaemic heart disease	K418	Other specified other autograft replacement of coronary artery	Atherosclerotic
Ischaemic heart disease	K419	Unspecified other autograft replacement of coronary artery	Atherosclerotic
Ischaemic heart disease	K421	Allograft replacement of one coronary artery	Atherosclerotic
Ischaemic heart disease	K422	Allograft replacement of two coronary arteries	Atherosclerotic
Ischaemic heart disease	K423	Allograft replacement of three coronary arteries	Atherosclerotic
Ischaemic heart disease	K424	Allograft replacement of four or more coronary arteries	Atherosclerotic
Ischaemic heart disease	K428	Other specified allograft replacement of coronary artery	Atherosclerotic
Ischaemic heart disease	K429	Unspecified allograft replacement of coronary artery	Atherosclerotic
Ischaemic heart disease	K431	Prosthetic replacement of one coronary artery	Atherosclerotic
Ischaemic heart disease	K432	Prosthetic replacement of two coronary arteries	Atherosclerotic
Ischaemic heart disease	K433	Prosthetic replacement of three coronary arteries	Atherosclerotic
Ischaemic heart disease	K434	Prosthetic replacement of four or more coronary arteries	Atherosclerotic
Ischaemic heart disease	K438	Other specified prosthetic replacement of coronary artery	Atherosclerotic
Ischaemic heart disease	K439	Unspecified prosthetic replacement of coronary artery	Atherosclerotic
Ischaemic heart disease	K441	Replacement of coronary arteries using multiple methods	Atherosclerotic
Ischaemic heart disease	K442	Revision of replacement of coronary artery	Atherosclerotic
Ischaemic heart disease	K448	Other specified other replacement of coronary artery	Atherosclerotic
Ischaemic heart disease	K449	Unspecified other replacement of coronary artery	Atherosclerotic
Ischaemic heart disease	K451	Double anastomosis of mammary arteries to coronary arteries	Atherosclerotic
Ischaemic heart disease	K452	Double anastomosis of thoracic arteries to coronary arteries NEC	Atherosclerotic
Ischaemic heart disease	K453	Anastomosis of mammary artery to left anterior descending coronary artery	Atherosclerotic
Ischaemic heart disease	K454	Anastomosis of mammary artery to coronary artery NEC	Atherosclerotic
Ischaemic heart disease	K455	Anastomosis of thoracic artery to coronary artery NEC	Atherosclerotic
Ischaemic heart disease	K456	Revision of connection of thoracic artery to coronary artery	Atherosclerotic
Ischaemic heart disease	K458	Other specified connection of thoracic artery to coronary artery	Atherosclerotic
Ischaemic heart disease	K459	Unspecified connection of thoracic artery to coronary artery	Atherosclerotic
Ischaemic heart disease	K461	Double implantation of mammary arteries into heart	Atherosclerotic
Ischaemic heart disease	K462	Double implantation of thoracic arteries into heart NEC	Atherosclerotic
Ischaemic heart disease	K463	Implantation of mammary artery into heart NEC	Atherosclerotic
Ischaemic heart disease	K464	Implantation of thoracic artery into heart NEC	Atherosclerotic
Ischaemic heart disease	K465	Revision of implantation of thoracic artery into heart	Atherosclerotic

Ischaemic heart disease	K468	Other specified other bypass of coronary artery	Atherosclerotic
Ischaemic heart disease	K469	Unspecified other bypass of coronary artery	Atherosclerotic
Ischaemic heart disease	K471	Endarterectomy of coronary artery	Atherosclerotic
Ischaemic heart disease	K491	Percutaneous transluminal balloon angioplasty of one coronary artery	Atherosclerotic
Ischaemic heart disease	K492	Percutaneous transluminal balloon angioplasty of multiple coronary arteries	Atherosclerotic
Ischaemic heart disease	K493	Percutaneous transluminal balloon angioplasty of bypass graft of coronary artery	Atherosclerotic
Ischaemic heart disease	K494	Percutaneous transluminal cutting balloon angioplasty of coronary artery	Atherosclerotic
Ischaemic heart disease	K498	Other specified transluminal balloon angioplasty of coronary artery	Atherosclerotic
Ischaemic heart disease	K499	Unspecified transluminal balloon angioplasty of coronary artery	Atherosclerotic
Ischaemic heart disease	K501	Percutaneous transluminal laser coronary angioplasty	Atherosclerotic
Ischaemic heart disease	K502	Percutaneous transluminal coronary thrombolysis using streptokinase	Atherosclerotic
Ischaemic heart disease	K503	Percutaneous transluminal injection of therapeutic substance into coronary artery NEC	Atherosclerotic
Ischaemic heart disease	K504	Percutaneous transluminal atherectomy of coronary artery	Atherosclerotic
Ischaemic heart disease	K508	Other specified other therapeutic transluminal operations on coronary artery	Atherosclerotic
Ischaemic heart disease	K509	Unspecified other therapeutic transluminal operations on coronary artery	Atherosclerotic
Ischaemic heart disease	K751	Percutaneous transluminal balloon angioplasty and insertion of 1-2 drug-eluting stents into coronary artery	Atherosclerotic
Ischaemic heart disease	K752	Percutaneous transluminal balloon angioplasty and insertion of 3 or more drug-eluting stents into coronary artery	Atherosclerotic
Ischaemic heart disease	K753	Percutaneous transluminal balloon angioplasty and insertion of 1-2 stents into coronary artery	Atherosclerotic
Ischaemic heart disease	K754	Percutaneous transluminal balloon angioplasty and insertion of 3 or more stents into coronary artery NEC	Atherosclerotic
Ischaemic heart disease	K758	Other specified percutaneous transluminal balloon angioplasty and insertion of stent into coronary artery	Atherosclerotic
Ischaemic heart disease	K759	Unspecified percutaneous transluminal balloon angioplasty and insertion of stent into coronary artery	Atherosclerotic
Ischaemic heart disease	L161	Emergency bypass of aorta by anastomosis of axillary artery to femoral artery	Atherosclerotic
Ischaemic heart disease	L162	Bypass of aorta by anastomosis of axillary artery to femoral artery NEC	Atherosclerotic
Ischaemic heart disease	L168	Other specified extra-anatomic bypass of aorta	Atherosclerotic
Ischaemic heart disease	L169	Unspecified extra-anatomic bypass of aorta	Atherosclerotic
Ischaemic heart disease	L181	Emergency replacement of aneurysmal segment of ascending aorta by anastomosis of aorta to aorta	Atherosclerotic
Ischaemic heart disease	L182	Emergency replacement of aneurysmal segment of thoracic aorta by anastomosis of aorta to aorta NEC	Atherosclerotic
Ischaemic heart disease	L183	Emergency replacement of aneurysmal segment of suprarenal abdominal aorta by anastomosis of aorta to aorta	Atherosclerotic
Ischaemic heart disease	L184	Emergency replacement of aneurysmal segment of infrarenal abdominal aorta by anastomosis of aorta to aorta	Atherosclerotic
Ischaemic heart disease	L185	Emergency replacement of aneurysmal segment of abdominal aorta by anastomosis of aorta to aorta NEC	Atherosclerotic
Ischaemic heart disease	L186	Emergency replacement of aneurysmal bifurcation of aorta by anastomosis of aorta to iliac artery	Atherosclerotic
Ischaemic heart disease	L188	Other specified emergency replacement of aneurysmal segment of aorta	Atherosclerotic
Ischaemic heart disease	L189	Unspecified emergency replacement of aneurysmal segment of aorta	Atherosclerotic
Ischaemic heart disease	L191	Replacement of aneurysmal segment of ascending aorta by anastomosis of aorta to aorta NEC	Atherosclerotic
Ischaemic heart disease	L192	Replacement of aneurysmal segment of thoracic aorta by anastomosis of aorta to aorta NEC	Atherosclerotic
Ischaemic heart disease	L193	Replacement of aneurysmal segment of suprarenal abdominal aorta by anastomosis of aorta to aorta NEC	Atherosclerotic
Ischaemic heart disease	L194	Replacement of aneurysmal segment of infrarenal abdominal aorta by anastomosis of aorta to aorta NEC	Atherosclerotic
Ischaemic heart disease	L195	Replacement of aneurysmal segment of abdominal aorta by anastomosis of aorta to aorta NEC	Atherosclerotic
Ischaemic heart disease	L196	Replacement of aneurysmal bifurcation of aorta by anastomosis of aorta to iliac artery NEC	Atherosclerotic
Ischaemic heart disease	L198	Other specified other replacement of aneurysmal segment of aorta	Atherosclerotic
Ischaemic heart disease	L199	Unspecified other replacement of aneurysmal segment of aorta	Atherosclerotic
Ischaemic heart disease	L201	Emergency bypass of segment of ascending aorta by anastomosis of aorta to aorta NEC	Atherosclerotic
Ischaemic heart disease	L202	Emergency bypass of segment of thoracic aorta by anastomosis of aorta to aorta NEC	Atherosclerotic

Ischaemic heart disease	L203	Emergency bypass of segment of suprarenal abdominal aorta by anastomosis of aorta to aorta NEC	Atherosclerotic
Ischaemic heart disease	L204	Emergency bypass of segment of infrarenal abdominal aorta by anastomosis of aorta to aorta NEC	Atherosclerotic
Ischaemic heart disease	L205	Emergency bypass of segment of abdominal aorta by anastomosis of aorta to aorta NEC	Atherosclerotic
Ischaemic heart disease	L206	Emergency bypass of bifurcation of aorta by anastomosis of aorta to iliac artery NEC	Atherosclerotic
Ischaemic heart disease	L208	Other specified other emergency bypass of segment of aorta	Atherosclerotic
Ischaemic heart disease	L209	Unspecified other emergency bypass of segment of aorta	Atherosclerotic
Ischaemic heart disease	L211	Bypass of segment of ascending aorta by anastomosis of aorta to aorta NEC	Atherosclerotic
Ischaemic heart disease	L212	Bypass of segment of thoracic aorta by anastomosis of aorta to aorta NEC	Atherosclerotic
Ischaemic heart disease	L213	Bypass of segment of suprarenal abdominal aorta by anastomosis of aorta to aorta NEC	Atherosclerotic
Ischaemic heart disease	L214	Bypass of segment of infrarenal abdominal aorta by anastomosis of aorta to aorta NEC	Atherosclerotic
Ischaemic heart disease	L215	Bypass of segment of abdominal aorta by anastomosis of aorta to aorta NEC	Atherosclerotic
Ischaemic heart disease	L216	Bypass of bifurcation of aorta by anastomosis of aorta to iliac artery NEC	Atherosclerotic
Ischaemic heart disease	L218	Other specified other bypass of segment of aorta	Atherosclerotic
Ischaemic heart disease	L219	Unspecified other bypass of segment of aorta	Atherosclerotic
Ischaemic heart disease	L251	Endarterectomy of aorta and patch repair of aorta	Atherosclerotic
Ischaemic heart disease	L252	Endarterectomy of aorta NEC	Atherosclerotic
Ischaemic heart disease	L253	Open embolectomy of bifurcation of aorta	Atherosclerotic
Ischaemic heart disease	L254	Operations on aneurysm of aorta NEC	Atherosclerotic
Ischaemic heart disease	L481	Emergency replacement of aneurysmal common iliac artery by anastomosis of aorta to common iliac artery	Atherosclerotic
Ischaemic heart disease	L482	Emergency replacement of aneurysmal iliac artery by anastomosis of aorta to external iliac artery	Atherosclerotic
Ischaemic heart disease	L483	Emergency replacement of aneurysmal artery of leg by anastomosis of aorta to common femoral artery	Atherosclerotic
Ischaemic heart disease	L484	Emergency replacement of aneurysmal artery of leg by anastomosis of aorta to superficial femoral artery	Atherosclerotic
Ischaemic heart disease	L491	Replacement of aneurysmal common iliac artery by anastomosis of aorta to common iliac artery NEC	Atherosclerotic
Ischaemic heart disease	L492	Replacement of aneurysmal iliac artery by anastomosis of aorta to external iliac artery NEC	Atherosclerotic
Ischaemic heart disease	L493	Replacement of aneurysmal artery of leg by anastomosis of aorta to common femoral artery NEC	Atherosclerotic
Ischaemic heart disease	L494	Replacement of aneurysmal artery of leg by anastomosis of aorta to superficial femoral artery NEC	Atherosclerotic
Ischaemic heart disease	L501	Emergency bypass of common iliac artery by anastomosis of aorta to common iliac artery NEC	Atherosclerotic
Ischaemic heart disease	L502	Emergency bypass of iliac artery by anastomosis of aorta to external iliac artery NEC	Atherosclerotic
Ischaemic heart disease	L503	Emergency bypass of artery of leg by anastomosis of aorta to common femoral artery NEC	Atherosclerotic
Ischaemic heart disease	L504	Emergency bypass of artery of leg by anastomosis of aorta to deep femoral artery NEC	Atherosclerotic
Ischaemic heart disease	L511	Bypass of common iliac artery by anastomosis of aorta to common iliac artery NEC	Atherosclerotic
Ischaemic heart disease	L512	Bypass of iliac artery by anastomosis of aorta to external iliac artery NEC	Atherosclerotic
Ischaemic heart disease	L513	Bypass of artery of leg by anastomosis of aorta to common femoral artery NEC	Atherosclerotic
Ischaemic heart disease	L514	Bypass of artery of leg by anastomosis of aorta to deep femoral artery NEC	Atherosclerotic
Ischaemic heart disease	L261	Percutaneous transluminal balloon angioplasty of aorta	Atherosclerotic
Ischaemic heart disease	L262	Percutaneous transluminal angioplasty of aorta NEC	Atherosclerotic
Ischaemic heart disease	L263	Percutaneous transluminal embolectomy of bifurcation of aorta	Atherosclerotic
Ischaemic heart disease	L265	Percutaneous transluminal insertion of stent into aorta	Atherosclerotic
Ischaemic heart disease	L266	Transluminal aortic stent graft with fenestration NEC	Atherosclerotic
Ischaemic heart disease	L267	Transluminal aortic branched stent graft NEC	Atherosclerotic
Ischaemic heart disease	L271	Endovascular insertion of stent graft for infrarenal abdominal aortic aneurysm	Atherosclerotic
Ischaemic heart disease	L272	Endovascular insertion of stent graft for suprarenal aortic aneurysm	Atherosclerotic
Ischaemic heart disease	L273	Endovascular insertion of stent graft for thoracic aortic aneurysm	Atherosclerotic
Ischaemic heart disease	L274	Endovascular insertion of stent graft for aortic dissection in any position	Atherosclerotic
Ischaemic heart disease	L275	Endovascular insertion of stent graft for aortic aneurysm of bifurcation NEC	Atherosclerotic

Ischaemic heart disease	L276	Endovascular insertion of stent graft for aorto-uniiliac aneurysm	Atherosclerotic
Ischaemic heart disease	L278	Other specified transluminal insertion of stent graft for aneurysmal segment of aorta	Atherosclerotic
Ischaemic heart disease	L279	Unspecified transluminal insertion of stent graft for aneurysmal segment of aorta	Atherosclerotic
Ischaemic heart disease	L281	Endovascular insertion of stent for infrarenal abdominal aortic aneurysm	Atherosclerotic
Ischaemic heart disease	L282	Endovascular insertion of stent for suprarenal aortic aneurysm	Atherosclerotic
Ischaemic heart disease	L283	Endovascular insertion of stent for thoracic aortic aneurysm	Atherosclerotic
Ischaemic heart disease	L284	Endovascular insertion of stent for aortic dissection in any position	Atherosclerotic
Ischaemic heart disease	L285	Endovascular insertion of stent for aortic aneurysm of bifurcation NEC	Atherosclerotic
Ischaemic heart disease	L286	Endovascular insertion of stent for aorto-uniiliac aneurysm	Atherosclerotic
Ischaemic heart disease	L288	Other specified transluminal operations on aneurysmal segment of aorta	Atherosclerotic
Ischaemic heart disease	L289	Unspecified transluminal operations on aneurysmal segment of aorta	Atherosclerotic
Ischaemic heart disease	L291	Replacement of carotid artery using graft	Atherosclerotic
Ischaemic heart disease	L292	Intracranial bypass to carotid artery NEC	Atherosclerotic
Ischaemic heart disease	L293	Bypass to carotid artery NEC	Atherosclerotic
Ischaemic heart disease	L294	Endarterectomy of carotid artery and patch repair of carotid artery	Atherosclerotic
Ischaemic heart disease	L295	Endarterectomy of carotid artery NEC	Atherosclerotic
Ischaemic heart disease	L296	High-flow interposition extracranial to intracranial bypass from external carotid artery to middle cerebral artery	Atherosclerotic
Ischaemic heart disease	L297	Bypass of carotid artery by anastomosis of superficial temporal artery to middle cerebral artery	Atherosclerotic
Ischaemic heart disease	L303	Open embolectomy of carotid artery	Atherosclerotic
Ischaemic heart disease	L304	Operations on aneurysm of carotid artery	Atherosclerotic
Ischaemic heart disease	L309	Unspecified other open operations on carotid artery	Atherosclerotic
Ischaemic heart disease	L331	Excision of aneurysm of cerebral artery	Atherosclerotic
Ischaemic heart disease	L334	Obliteration of aneurysm of cerebral artery NEC	Atherosclerotic
Ischaemic heart disease	L311	Percutaneous transluminal angioplasty of carotid artery	Atherosclerotic
Ischaemic heart disease	L313	Endovascular repair of carotid artery	Atherosclerotic
Ischaemic heart disease	L314	Percutaneous transluminal insertion of stent into carotid artery	Atherosclerotic
Ischaemic heart disease	L318	Other specified transluminal operations on carotid artery	Atherosclerotic
Ischaemic heart disease	L319	Unspecified transluminal operations on carotid artery	Atherosclerotic
Ischaemic heart disease	L353	Percutaneous transluminal insertion of stent into cerebral artery	Atherosclerotic
Peripheral arterial disease	L485	Emergency replacement of aneurysmal iliac artery by anastomosis of iliac artery to iliac artery	Atherosclerotic
Peripheral arterial disease	L486	Emergency replacement of aneurysmal artery of leg by anastomosis of iliac artery to femoral artery	Atherosclerotic
Peripheral arterial disease	L488	Other specified emergency replacement of aneurysmal iliac artery	Atherosclerotic
Peripheral arterial disease	L489	Unspecified emergency replacement of aneurysmal iliac artery	Atherosclerotic
Peripheral arterial disease	L495	Replacement of aneurysmal iliac artery by anastomosis of iliac artery to iliac artery NEC	Atherosclerotic
Peripheral arterial disease	L496	Replacement of aneurysmal artery of leg by anastomosis of iliac artery to femoral artery NEC	Atherosclerotic
Peripheral arterial disease	L498	Other specified other replacement of aneurysmal iliac artery	Atherosclerotic
Peripheral arterial disease	L499	Unspecified other replacement of aneurysmal iliac artery	Atherosclerotic
Peripheral arterial disease	L505	Emergency bypass of iliac artery by anastomosis of iliac artery to iliac artery NEC	Atherosclerotic
Peripheral arterial disease	L506	Emergency bypass of artery of leg by anastomosis of iliac artery to femoral artery NEC	Atherosclerotic
Peripheral arterial disease	L508	Other specified other emergency bypass of iliac artery	Atherosclerotic
Peripheral arterial disease	L509	Unspecified other emergency bypass of iliac artery	Atherosclerotic
Peripheral arterial disease	L515	Bypass of iliac artery by anastomosis of iliac artery to iliac artery NEC	Atherosclerotic
Peripheral arterial disease	L516	Bypass of artery of leg by anastomosis of iliac artery to femoral artery NEC	Atherosclerotic
Peripheral arterial disease	L518	Other specified other bypass of iliac artery	Atherosclerotic
Peripheral arterial disease	L519	Unspecified other bypass of iliac artery	Atherosclerotic
Peripheral arterial disease	L521	Endarterectomy of iliac artery and patch repair of iliac artery	Atherosclerotic

Peripheral arterial disease	L522	Endarterectomy of iliac artery NEC	Atherosclerotic
Peripheral arterial disease	L533	Operations on aneurysm of iliac artery NEC	Atherosclerotic
Peripheral arterial disease	L561	Emergency replacement of aneurysmal femoral artery by anastomosis of femoral artery to femoral artery	Atherosclerotic
Peripheral arterial disease	L562	Emergency replacement of aneurysmal femoral artery by anastomosis of femoral artery to popliteal artery using prosthesis	Atherosclerotic
Peripheral arterial disease	L563	Emergency replacement of aneurysmal femoral artery by anastomosis of femoral artery to popliteal artery using vein graft	Atherosclerotic
Peripheral arterial disease	L564	Emergency replacement of aneurysmal femoral artery by anastomosis of femoral artery to tibial artery using prosthesis	Atherosclerotic
Peripheral arterial disease	L565	Emergency replacement of aneurysmal femoral artery by anastomosis of femoral artery to tibial artery using vein graft	Atherosclerotic
Peripheral arterial disease	L566	Emergency replacement of aneurysmal femoral artery by anastomosis of femoral artery to peroneal artery using prosthesis	Atherosclerotic
Peripheral arterial disease	L567	Emergency replacement of aneurysmal femoral artery by anastomosis of femoral artery to peroneal artery using vein graft	Atherosclerotic
Peripheral arterial disease	L568	Other specified emergency replacement of aneurysmal femoral artery	Atherosclerotic
Peripheral arterial disease	L569	Unspecified emergency replacement of aneurysmal femoral artery	Atherosclerotic
Peripheral arterial disease	L571	Replacement of aneurysmal femoral artery by anastomosis of femoral artery to femoral artery NEC	Atherosclerotic
Peripheral arterial disease	L572	Replacement of aneurysmal femoral artery by anastomosis of femoral artery to popliteal artery using prosthesis NEC	Atherosclerotic
Peripheral arterial disease	L573	Replacement of aneurysmal femoral artery by anastomosis of femoral artery to popliteal artery using vein graft NEC	Atherosclerotic
Peripheral arterial disease	L574	Replacement of aneurysmal femoral artery by anastomosis of femoral artery to tibial artery using prosthesis NEC	Atherosclerotic
Peripheral arterial disease	L575	Replacement of aneurysmal femoral artery by anastomosis of femoral artery to tibial artery using vein graft NEC	Atherosclerotic
Peripheral arterial disease	L576	Replacement of aneurysmal femoral artery by anastomosis of femoral artery to peroneal artery using prosthesis NEC	Atherosclerotic
Peripheral arterial disease	L577	Replacement of aneurysmal femoral artery by anastomosis of femoral artery to peroneal artery using vein graft NEC	Atherosclerotic
Peripheral arterial disease	L578	Other specified other replacement of aneurysmal femoral artery	Atherosclerotic
Peripheral arterial disease	L579	Unspecified other replacement of aneurysmal femoral artery	Atherosclerotic
Peripheral arterial disease	L581	Emergency bypass of femoral artery by anastomosis of femoral artery to femoral artery NEC	Atherosclerotic
Peripheral arterial disease	L582	Emergency bypass of femoral artery by anastomosis of femoral artery to popliteal artery using prosthesis NEC	Atherosclerotic
Peripheral arterial disease	L583	Emergency bypass of femoral artery by anastomosis of femoral artery to popliteal artery using vein graft NEC	Atherosclerotic
Peripheral arterial disease	L584	Emergency bypass of femoral artery by anastomosis of femoral artery to tibial artery using prosthesis NEC	Atherosclerotic
Peripheral arterial disease	L585	Emergency bypass of femoral artery by anastomosis of femoral artery to tibial artery using vein graft NEC	Atherosclerotic
Peripheral arterial disease	L586	Emergency bypass of femoral artery by anastomosis of femoral artery to peroneal artery using prosthesis NEC	Atherosclerotic
Peripheral arterial disease	L587	Emergency bypass of femoral artery by anastomosis of femoral artery to peroneal artery using vein graft NEC	Atherosclerotic
Peripheral arterial disease	L588	Other specified other emergency bypass of femoral artery	Atherosclerotic
Peripheral arterial disease	L589	Unspecified other emergency bypass of femoral artery	Atherosclerotic
Peripheral arterial disease	L591	Bypass of femoral artery by anastomosis of femoral artery to femoral artery NEC	Atherosclerotic
Peripheral arterial disease	L592	Bypass of femoral artery by anastomosis of femoral artery to popliteal artery using prosthesis NEC	Atherosclerotic
Peripheral arterial disease	L593	Bypass of femoral artery by anastomosis of femoral artery to popliteal artery using vein graft NEC	Atherosclerotic
Peripheral arterial disease	L594	Bypass of femoral artery by anastomosis of femoral artery to tibial artery using prosthesis NEC	Atherosclerotic
Peripheral arterial disease	L595	Bypass of femoral artery by anastomosis of femoral artery to tibial artery using vein graft NEC	Atherosclerotic
Peripheral arterial disease	L596	Bypass of femoral artery by anastomosis of femoral artery to peroneal artery using prosthesis NEC	Atherosclerotic

Peripheral arterial disease	L597	Bypass of femoral artery by anastomosis of femoral artery to peroneal artery using vein graft NEC	Atherosclerotic
Peripheral arterial disease	L598	Other specified other bypass of femoral artery	Atherosclerotic
Peripheral arterial disease	L599	Unspecified other bypass of femoral artery	Atherosclerotic
Peripheral arterial disease	L601	Endarterectomy of femoral artery and patch repair of femoral artery	Atherosclerotic
Peripheral arterial disease	L602	Endarterectomy of femoral artery NEC	Atherosclerotic
Peripheral arterial disease	L603	Profundoplasty of femoral artery and patch repair of deep femoral artery	Atherosclerotic
Peripheral arterial disease	L604	Profundoplasty of femoral artery NEC	Atherosclerotic
Peripheral arterial disease	L624	Operations on aneurysm of femoral artery NEC	Atherosclerotic
Peripheral arterial disease	L541	Percutaneous transluminal angioplasty of iliac artery	Atherosclerotic
Peripheral arterial disease	L542	Percutaneous transluminal embolectomy of iliac artery	Atherosclerotic
Peripheral arterial disease	L544	Percutaneous transluminal insertion of stent into iliac artery	Atherosclerotic
Peripheral arterial disease	L548	Other specified transluminal operations on iliac artery	Atherosclerotic
Peripheral arterial disease	L549	Unspecified transluminal operations on iliac artery	Atherosclerotic
Peripheral arterial disease	L631	Percutaneous transluminal angioplasty of femoral artery	Atherosclerotic
Peripheral arterial disease	L632	Percutaneous transluminal embolectomy of femoral artery	Atherosclerotic
Peripheral arterial disease	L635	Percutaneous transluminal insertion of stent into femoral artery	Atherosclerotic
Peripheral arterial disease	L623	Ligation of aneurysm of popliteal artery	Atherosclerotic
Arthritis	W37	Total prosthetic replacement of hip joint using cement (Clean)	Musculoskeletal
Arthritis	W371	Primary total prosthetic replacement of hip joint using cement	Musculoskeletal
Arthritis	W372	Conversion to total prosthetic replacement of hip joint using cement	Musculoskeletal
Arthritis	W373	Revision of total prosthetic replacement of hip joint using cement	Musculoskeletal
Arthritis	W374	Revision of one component of total prosthetic replacement of hip joint using cement.	Musculoskeletal
Arthritis	W378	Other specified total prosthetic replacement of hip joint using cement	Musculoskeletal
Arthritis	W379	Unspecified total prosthetic replacement of hip joint using cement	Musculoskeletal
Arthritis	W370	Conversion from previous cemented total prosthetic replacement of hip joint	Musculoskeletal
Arthritis	W38	Total prosthetic replacement of hip joint not using cement (Clean)	Musculoskeletal
Arthritis	W381	Primary total prosthetic replacement of hip joint not using cement	Musculoskeletal
Arthritis	W382	Conversion to total prosthetic replacement of hip joint not using cement	Musculoskeletal
Arthritis	W383	Revision of total prosthetic replacement of hip joint not using cement	Musculoskeletal
Arthritis	W384	Revision of one component of total prosthetic replacement of hip joint not using cement	Musculoskeletal
Arthritis	W388	Other specified total prosthetic replacement of hip joint not using cement	Musculoskeletal
Arthritis	W389	Unspecified total prosthetic replacement of hip joint not using cement	Musculoskeletal
Arthritis	W380	Conversion from previous uncemented total prosthetic replacement of hip joint	Musculoskeletal
Arthritis	W39	Other total prosthetic replacement of hip joint (Clean)	Musculoskeletal
Arthritis	W391	Primary total prosthetic replacement of hip joint not elsewhere classified	Musculoskeletal
Arthritis	W392	Conversion to total prosthetic replacement of hip joint not elsewhere classified	Musculoskeletal
Arthritis	W393	Revision of total prosthetic replacement of hip joint not elsewhere classified	Musculoskeletal
Arthritis	W395	Revision of one component of total prosthetic replacement of hip joint not elsewhere classified	Musculoskeletal
Arthritis	W398	Other specified other total prosthetic replacement of hip joint	Musculoskeletal
Arthritis	W399	Unspecified other total prosthetic replacement of hip joint	Musculoskeletal
Arthritis	W390	Conversion from previous total prosthetic replacement of hip joint not elsewhere classified	Musculoskeletal
Arthritis	W93	Hybrid prosthetic replacement of hip joint using cemented acetabular component (Clean)	Musculoskeletal

Arthritis	W931	Primary hybrid prosthetic replacement of hip joint using cemented acetabular component	Musculoskeletal
Arthritis	W932	Conversion to hybrid prosthetic replacement of hip joint using cemented acetabular component	Musculoskeletal
Arthritis	W933	Revision of hybrid prosthetic replacement of hp joint using cemented acetabular component	Musculoskeletal
Arthritis	W938	Other specified	Musculoskeletal
Arthritis	W939	Unspecified	Musculoskeletal
Arthritis	W930	Conversion from previous hybrid prosthetic replacement of hip joint using cemented acetabular component.	Musculoskeletal
Arthritis	W94	Hybrid prosthetic replacement of hip joint using cemented femoral component (Clean)	Musculoskeletal
Arthritis	W941	Primary hybrid prosthetic replacement of hip joint using cemented femoral component	Musculoskeletal
Arthritis	W942	Conversion to hybrid prosthetic replacement of hip joint using cemented femoral component.	Musculoskeletal
Arthritis	W943	Revision of hybrid prosthetic replacement of hip joint using cemented femoral component.	Musculoskeletal
Arthritis	W948	Other specified	Musculoskeletal
Arthritis	W949	Unspecified	Musculoskeletal
Arthritis	W940	Conversion from previous hybrid prosthetic replacement of hip joint using cemented femoral component.	Musculoskeletal
Arthritis	W95	Hybrid prosthetic replacement of hip joint using cement (Clean)	Musculoskeletal
Arthritis	W951	Primary hybrid prosthetic replacement of hip joint using cement NEC	Musculoskeletal
Arthritis	W952	Conversion of hybrid prosthetic replacement of hip joint using cement NEC	Musculoskeletal
Arthritis	W953	Revision of hybrid prosthetic replacement of hip joint using cement NEC	Musculoskeletal
Arthritis	W954	Attention to hybrid prosthetic replacement of hip joint using cement NEC	Musculoskeletal
Arthritis	W958	Other specified	Musculoskeletal
Arthritis	W959	Unspecified	Musculoskeletal
Arthritis	W950	Conversion from previous hybrid prosthetic replacement of hip joint using cement NEC	Musculoskeletal
Arthritis	W40	Total prosthetic replacement of knee joint using cement (Clean)	Musculoskeletal
Arthritis	W401	Primary total prosthetic replacement of knee joint using cement	Musculoskeletal
Arthritis	W402	Conversion to total prosthetic replacement of knee joint using cement	Musculoskeletal
Arthritis	W403	Revision of total prosthetic replacement of knee joint using cement	Musculoskeletal
Arthritis	W404	Revision of one component of total prosthetic replacement of knee joint using cement	Musculoskeletal
Arthritis	W408	Other specified total prosthetic replacement of knee joint using cement	Musculoskeletal
Arthritis	W409	Unspecified total prosthetic replacement of knee joint using cement	Musculoskeletal
Arthritis	W400	Conversion from previous cemented total prosthetic replacement of knee joint	Musculoskeletal
Arthritis	W41	Total prosthetic replacement of knee joint not using cement (Clean)	Musculoskeletal
Arthritis	W411	Primary total prosthetic replacement of knee joint not using cement	Musculoskeletal
Arthritis	W412	Conversion to total prosthetic replacement of knee joint not using cement	Musculoskeletal
Arthritis	W413	Revision of total prosthetic replacement of knee joint not using cement	Musculoskeletal
Arthritis	W414	Revision of one component of total prosthetic replacement of knee joint not using cement.	Musculoskeletal
Arthritis	W418	Other specified total prosthetic replacement of knee joint not using cement	Musculoskeletal
Arthritis	W419	Unspecified total prosthetic replacement of knee joint not using cement	Musculoskeletal
Arthritis	W410	Conversion from previous uncemented total prosthetic replacement of knee joint	Musculoskeletal
Arthritis	W42	Other total prosthetic replacement of knee joint (Clean)	Musculoskeletal
Arthritis	W421	Primary total prosthetic replacement of knee joint not elsewhere classified	Musculoskeletal
Arthritis	W422	Conversion to total prosthetic replacement of knee joint not elsewhere classified	Musculoskeletal
Arthritis	W423	Revision of total prosthetic replacement of knee joint not elsewhere classified	Musculoskeletal
Arthritis	W428	Other specified	Musculoskeletal
Arthritis	W429	Unspecified	Musculoskeletal
Arthritis	W420	Conversion from previous total prosthetic replacement of knee joint not elsewhere classified	Musculoskeletal
Arthritis	W425	Revision of one component of total prosthetic replacement of knee joint NEC	Musculoskeletal
Arthritis	W52	Prosthetic replacement of articulation of other bone using cement (Clean)	Musculoskeletal
Arthritis	W521	Primary prosthetic replacement of articulation of bone using cement not elsewhere classified	Musculoskeletal

Arthritis	W522	Conversions to prosthetic replacement of articulation of bone using cement not elsewhere classified	Musculoskeletal
Arthritis	W523	Revision of prosthetic replacement of articulation of bone using cement not elsewhere classified	Musculoskeletal
Arthritis	W528	Other specified	Musculoskeletal
Arthritis	W529	Unspecified	Musculoskeletal
Arthritis	W520	Conversion from previous cemented prosthetic replacement of articulation of bone not elsewhere classified	Musculoskeletal
Arthritis	W53	Prosthetic replacement of articulation of other bone not using cement (Clean)	Musculoskeletal
Arthritis	W531	Primary prosthetic replacement of articulation of bone not using cement not elsewhere classified	Musculoskeletal
Arthritis	W532	Conversions to prosthetic replacement of articulation of bone not using cement not elsewhere classified	Musculoskeletal
Arthritis	W533	Revision of prosthetic replacement of articulation of bone not using cement not elsewhere classified	Musculoskeletal
Arthritis	W538	Other specified	Musculoskeletal
Arthritis	W539	Unspecified	Musculoskeletal
Arthritis	W530	Conversion from previous uncemented prosthetic replacement of articulation of bone not elsewhere classified	Musculoskeletal
Arthritis	W54	Other prosthetic replacement of other bone (Clean)	Musculoskeletal
Arthritis	W541	Primary prosthetic replacement of articulation of bone not elsewhere classified	Musculoskeletal
Arthritis	W542	Conversion to prosthetic replacement of articulation of bone not elsewhere classified	Musculoskeletal
Arthritis	W543	Revision of prosthetic replacement of articulation of bone not elsewhere classified	Musculoskeletal
Arthritis	W548	Other specified	Musculoskeletal
Arthritis	W549	Unspecified	Musculoskeletal
Arthritis	W540	Conversion from previous prosthetic replacement of articulation of bone not elsewhere classified	Musculoskeletal
Arthritis	W58	Other reconstruction of joint (Clean)	Musculoskeletal
Arthritis	W581	Primary resurfacing arthroplasty of joint	Musculoskeletal
Arthritis	W582	Revision of resurfacing arthroplasty of joint	Musculoskeletal
Arthritis	W588	Other specified other reconstruction of joint	Musculoskeletal
Arthritis	W589	Unspecified other reconstruction of joint	Musculoskeletal
Arthritis	W580	Conversion from previous resurfacing arthroplasty of joint	Musculoskeletal
Gall bladder disease	J18	Total or Partial Cholecystectomy	Gut
Gall bladder disease	J38	Endoscopic incision of sphincter of Oddi (Includes: Endoscopic retrograde incision of sphincter of Oddi, Papilla of Vater)	Gut
Gall bladder disease	J39	Other therapeutic endoscopic operations on ampulla of Vater (Includes: Therapeutic endoscopic retrograde operations on ampulla of Vater, Papilla of Vater, Sphincter of Oddi)	Gut
Gall bladder disease	J413	Endoscopic retrograde lithotripsy of calculus of bile duct	Gut
Gall bladder disease	J423	Endoscopic retrograde removal of calculus from pancreatic duct	Gut
Gall bladder disease	J492	Percutaneous removal of calculus from bile duct along T tube track (Includes: Percutaneous removal of calculus from bile duct along T tube track using image control)	Gut

Appendix 3.3: Biomarker data cleaning procedure

The UK Biobank collected 110 physical and biochemical biomarkers. In this thesis, biomarkers were excluded from the panel in this thesis if (1) they were measured in <70% of the whole population, (2) if they were not measured on a continuous scale, or (3) if they measured the same biological trait (e.g. standardly-measured but not impedance device-measured weight was selected), leaving 74 biomarkers (Section 3.8). Special investigations were carried out for the lung function biomarkers, as they had the highest levels of missingness yet they were derived from up to three raw measurements that could be used to supplement missing data for these biomarkers.

'Best' measures for the included lung function biomarkers, forced expiratory volume in 1 second (FEV1) and forced vital capacity (FVC), were defined by UK Biobank[103] and available for 72% of men and 70% of women in this analysis (Table A3.3). Up to 3 raw readings for each biomarker were also available. Both lung function biomarkers had the highest proportions of data missing within the biomarker panel, if only best measures were considered (Table A3.3). The raw readings provided two types of lower-quality measurements: (1) Remainder with 'accept' flags, created by taking the means of up to 3 non-missing readings flagged as 'accept' (available in an additional 4% of men and 4% of women); and subsequently (2) all remaining readings, created by taking the non-missing means of up to 3 available readings (measured in an additional 14% of men and 18% of women; Table A3.3). Best measure, type (1) and type (2) baseline measurements were moderately or highly correlated with the type (1) repeated measures (Pearson correlation coefficients >0.585; Table A3.3). Therefore, it is likely that these lower quality readings are more highly correlated with usual values than any general imputed value would be. The best measures were thus supplemented with these two types of measurements, reducing the missingness in the hybrid FEV1 and FVC measures to 10% and 8% for men and women respectively (Table A3.3). The use of additional readings has also been advocated by others.[286]

FEV1 and FVC, in addition to hand grip strength, were each divided by standing height.[287] This was done to ensure that measurements for these derived biomarkers were not strongly determined by body size.

In this thesis, the biomarker measurement quality threshold was set at an intra-individual Pearson correlation coefficient adjusted by baseline age of more than 0.1, in the UK Biobank repeat assessment subset of 9447 men and 9888 women (Table A3.4). Of the 74 biomarkers, a further 2 biomarkers, oestradiol and nucleic red blood cell count, were excluded due to poor reproducibility, leaving 72 biomarkers (Table 3.8.1 and Table A3.4).

Before releasing the biochemical biomarker data to researchers, the UK Biobank had processed the biomarker data to minimise error related to sample collection, processing, retrieval and assay measurement.[288] In this thesis, the candidate 72 physical and biochemical biomarker measurements were cleaned by this procedure:

1. Urinary and blood plasma biomarkers, flagged as below or above the assay reportable range[289] were replaced with the respective limit of the range.
2. All biomarkers, values were standardised by subtracting their overall mean and dividing by their standard deviation. Body size biomarkers exhibited sex differences, therefore they were standardised separately within each sex.
3. Standardised values outside ±4 were treated as outliers, set to missing, and were subsequently imputed in step 4. An alternative threshold of ±10 was investigated as a sensitivity analysis, which resulted in the treatment of 0.28% fewer biomarker values as outliers in total compared to the main method. The percentages of missing data for each biomarker are listed in Table 3.8.1.
4. Imputation was carried out by replacing all missing values with the overall medians within 5-year groups of baseline chronological age (separately by sex for body size biomarkers), similar to a procedure based on imputing overall means.[106] This was preferred to assigning a non-central biomarker value through multiple imputation[98] and complete case analysis.[12,13,78,96] Relative to

chronological age, multiple imputation would skew individuals' biological ages in the direction that is indicated by the available biomarkers for each individual, and the degree of statistical inference applied to the missing biomarkers within the imputation procedure and the resulting uncertainty in biological age estimation are not communicated through their biological age. The use of complete cases based on biomarker measurements introduces bias into the estimation of biological ages that is difficult to quantify.

Table A3.3: Missingness and Pearson correlations of best measure and supplemented lung function baseline and repeated measurements

Men:

	Forced expiratory volume in 1s (FEV1)				Forced vital capacity (FVC)			
	Participants at baseline		Participants with 'accept' flagged repeat assessment		Participants at baseline		Participants with 'accept' flagged repeat assessment	
	Number	% total	Correlation	Number	Number	% total	Correlation	Number
Best measure	158,140	72%	0.722	5888	158,140	72%	0.729	5888
Remainder with 'accept' flags	9,113	4%	0.753	140	9,113	4%	0.828	140
All remaining readings	31,125	14%	0.654	664	31,125	14%	0.664	664
All readings	198,378	90%	0.720	6692	198,378	90%	0.729	6692
No data	*20,870*	*10%*			*20,870*	*10%*		

Women:

	Forced expiratory volume in 1s (FEV1)				Forced vital capacity (FVC)			
	Participants at baseline		Participants with 'accept' flagged repeat assessment		Participants at baseline		Participants with 'accept' flagged repeat assessment	
	Number	% total	Correlation	Number	Number	% total	Correlation	Number
Best measure	183,323	70%	0.723	5978	183,323	70%	0.743	5978
Remainder with 'accept' flags	9,710	4%	0.762	127	9,710	4%	0.778	127
All remaining readings	46,423	18%	0.585	747	46,423	18%	0.614	747
All readings	239,456	92%	0.709	6852	239,456	92%	0.730	6852
No data	*21,315*	*8%*			*21,315*	*8%*		

Best measure lung function measurements were defined by UK Biobank.[2] Two types of lung function measurements were used to supplement best measure lung function: (1) remainder with 'accept' flags and (2) all remaining readings.

Table A3.4: Repeated measures Pearson correlation coefficients adjusted for baseline age for each of the 74 candidate biomarkers, by sex

Biomarker name	Men	Women
Diastolic blood pressure	0.603	0.664
Systolic blood pressure	0.609	0.656
Pulse rate	0.652	0.636
Apolipoprotein A	0.753	0.716
Apolipoprotein B	0.628	0.686
Lipoprotein (a)	0.973	0.968
High density lipoprotein cholesterol	0.815	0.807
Low density lipoprotein cholesterol	0.614	0.664
Triglycerides	0.570	0.620
Mean platelet volume	0.836	0.838
Platelet count	0.725	0.746
Platelet crit	0.701	0.707
Platelet distribution width	0.649	0.640
Log C-Reactive Protein	0.543	0.656
Blood glucose	0.447	0.364
HbA1c	0.771	0.720
Insulin-like growth factor 1	0.770	0.744
Sex hormone-binding globulin	0.832	0.745
Testosterone	0.641	Not considered
Oestradiol*	Not considered	0.094
Haemoglobin concentration	0.635	0.594
HLS reticulocyte count	0.551	0.588
Immature reticulocyte fraction	0.430	0.405
Mean corpuscular volume	0.748	0.716
Mean reticulocyte volume	0.546	0.506
Mean spherical cell volume	0.706	0.691
Total red blood cell count	0.732	0.718
Red blood cell distribution width	0.566	0.480
Reticulocyte count	0.326	0.311
Mean corpuscular haemoglobin concentration	0.251	0.198
Nucleic red blood cell count*	0.013	0.003
Albumin	0.453	0.465
Alanine aminotransferase	0.482	0.326
Aspartate aminotransferase	0.445	0.329
Direct bilirubin	0.699	0.708
Total bilirubin	0.764	0.740
Gamma Glutamyltransferase	0.682	0.615
Heel bone density	0.689	0.713
Body mass index	0.931	0.925
Sitting height	0.796	0.799
Standing height	0.986	0.984
Hip circumference	0.805	0.839
Waist circumference	0.823	0.829
Waist-hip ratio	0.664	0.653
Weight	0.944	0.930
Body fat-free mass	0.945	0.914
Body fat mass	0.902	0.906
Body fat percentage	0.859	0.869
Metabolic rate	0.949	0.928
Hand grip strength/height	0.615	0.515
Alkaline Phosphatase	0.743	0.689
Calcium	0.371	0.406
Rheumatoid factor	0.755	0.856
Vitamin D	0.558	0.547
Reaction time test	0.518	0.494
Pairs matching test	0.233	0.206
Urinary microalbumin	0.400	0.357
Urinary sodium	0.325	0.296
Urinary creatinine	0.267	0.288
Urinary potassium	0.232	0.251
Urea	0.573	0.542
Creatinine	0.653	0.671
Cystatin C	0.759	0.802
Phosphate	0.409	0.393
Total protein	0.494	0.492
Urate	0.719	0.780

Forced expiratory volume in 1s/height	0.503	0.502
Forced vital capacity/height	0.655	0.649
Eosinophil count	0.619	0.603
Lymphocyte count	0.859	0.646
Monocyte count	0.420	0.341
Neutrophil count	0.539	0.528
Basophil count	0.143	0.112
Total white blood cell count	0.787	0.593

* Biomarkers with correlation coefficients of <0.1

Both baseline and repeated measurements for these biomarkers were available for 2657-9444 men and 2213-9873 women

The 36 excluded biomarkers are: pulse pressure; total cholesterol; peak expiratory flow (spirometry); heel bone density measurements represented as: Broadband ultrasound attenuation, quantitative ultrasound index, speed of sound through heel; impedance device-measured weight; total mass, fat mass, fat free mass and fat percentage for: trunk, left leg, right leg, left arm, right arm; Metabolic Equivalent Task (MET) minutes per week for moderate activity; MET minutes per week for vigorous activity; mean corpuscular haemoglobin; haematocrit percentage; visual acuity; hearing test; numeric memory test; fluid intelligence test; and prospective memory test.

Appendix 4.1: Description of multi-state model for biological age estimation

This appendix provides further details on the structure of the multi-state model used to aggregate body system ages, and the statistical assumptions that are implicit in this model. The multi-state model specified for this analysis is a progressive, time-inhomogeneous, piecewise exponential Markov multi-state model of age-related frailty and mortality from chronic disease, with 3 permissible states and constrained to 3 permissible transitions (Figure 4.4.1). The descriptions that follow are elaborated in more statistical detail in the van den Hout book on multi-state models,[290] and in the documentation for the R package that was used.[180]

1. Progressive: no backward transitions to a previously occupied state were allowed. Therefore once a participant who has been admitted to hospital for any reason that constitutes age-related frailty, they do not return to the 'non-frail' state. Any subsequent improvement in health is generally not detectable via hospital records and the prevalence of frailty has been shown to increase substantially with age in several populations.[5] Participants who have died from chronic disease were not permitted to transit back to an alive state.

2. Time-inhomogeneous: the rate of disease accumulation depends on the duration of follow up through the dependence of duration on chronological age, as chronological age is used as the timescale in this model.

3. Piecewise exponential survival function (or piecewise constant hazard): This approach is used to approximate a Gompertz relationship between chronological age at risk (which is a linear term in the model) and the risk of moving between states. Secular trends of incidence rates for age-related frailty and mortality from chronic disease show that this Gompertz relationship is broadly satisfied (Figure 3.9.1).

4. For the ‘frail’ state, first hospital admission for age-related frailty (Table 3.7.1) instead of frailty onset or onset of impaired function as the outcome of interest: age of first relevant hospital admission or death (rounded down to nearest month) is used to define the age at entry into the states, instead of making inferences about the unmeasured, latent age of frailty onset that require the use of interval censoring in the model.[10] In the context of this thesis, either approach is appropriate, and the use of the first hospital admission was preferred as using frailty onset introduces the use of additional assumptions in the model and thus additional uncertainty to the parameter estimates.

5. Permissible health states and transitions: The permissible health states and transitions described in Figure 4.4.1 were defined a priori to reflect the research question and the health profile of the population, and were constrained by computational limits and ease of clinical interpretation of results. As the healthy subpopulation was studied, the timing of the first hospital admission in relation to age-related frailty was more clinically relevant than subsequent admissions. Therefore to maintain a parsimonious model structure, the number of states for these subsequent frail states and the transitions between them were minimised. Participants who died of non-chronic causes during follow up were censored and did not transit to the dead state.

6. Constraints on covariates: Coefficients for chronological age were allowed to vary across all permitted transitions, to reflect differential patterns of frailty incidence and mortality by chronological age (differences in secular trends were observed in Figure 3.9.1). Coefficients for the body system ages, the remaining covariates, were constrained to be the same across transitions, in order to obtain a single set of coefficients for these ages and therefore a single overall biological age, instead of multiple overall biological ages that best describe each transition.

Appendix 4.2: Alternative methods for biological age estimation

Future research on biological age estimation could explore non-linear and computationally intensive methods further, to check the materiality of the linear assumption used in the main methods described in Sections 4.2–4.4. Key non-linear methods that may be suitable for future research are random forest survival models, Cox multiple fractional polynomial models and Cox models with splines (i.e. piecewise polynomial forms of covariates).[291] The random forest survival model is an extension of the standard Cox model that allowed the modelling of non-parametric forms of biomarkers and interaction effects between the biomarkers.[292] Additionally, it selects the most important biomarkers for predicting the outcome of interest.[72] Separately, the Cox model has also been extended to incorporate fractional polynomials[293] or splines[291] of biomarker measurements, to model functional forms of biomarkers more accurately. Software packages for standard versions of these non-linear models are available,[291,292,294] but functionality for variable selection and multiple survival outcomes may not be available for these methods, and metrics such as biomarker importance would not be easily calculable without further statistical research.

Additionally, the two-step disease risk-based approach used in this thesis could be condensed into a single step multi-state model (MSM) that simultaneously performs biomarker selection. Existing software does not yet incorporate all of the features required for this model: the software used in the MSM in this analysis[180] constrains the biomarker coefficients to be the same across transitions but does not perform automatic biomarker selection, and vice versa for a penalised fusion lasso MSM.[295]

Other alternative estimation methods were not considered in this thesis for the following reasons:

1. Mahalanobis distance (age-based):[85] Not expressed in terms of an age, and treats above- and below-average biomarker measurements as having the same effects on health.
2. Frailty or ageing indices such as the laboratory-measured frailty index (FI-LAB)[13] and allostatic load (points-based):[79] Not expressed in terms of an age, and the biomarkers tend to be equally weighted or weighted based on a qualitative assessment of biomarker importance.

3. Neural networks (age-based):[98,155,179] Computationally intensive and does not allow interpretation of the biomarker values.
4. Machine learning methods such as nearest neighbours, support vector machines and decision trees (age-based):[155,179] Computationally intensive and does not allow interpretation of the biomarker values. Outcome-based methods that are extensions of these methods (e.g. random forest survival models, which build on decision tree methods) are preferred as they relate to the prediction of health outcomes rather than the prediction of chronological age.
5. Equating risks via life tables (outcome-based): Life table data is generally only available if all-cause mortality is used as the outcome. This method relies on the assumption that the age-specific mortality patterns in the life table population and the population of interest are the same, and there was little follow up data for ages above 80 years in the UK Biobank. The Gompertz relation between mortality risk and chronological age that was assumed in the Cox models and multi-state model in this thesis in Sections 4.2–4.4 is evident in populations worldwide.[296]
6. Non-linear regression with latent parameters and neural networks (age-based):[101] Mathematically complex, does not allow interpretation of the biomarker values, and the multiple biological ageing scores that were constructed[101] were not expressed in terms of ages.

Appendix 4.3: Software and R packages used in statistical analysis

The statistical analysis for the chronological age-based biological ages was run in R version 3.3.3, while the analysis for the disease risk-based biological ages was run in R version 3.6.1. Most plots were produced using the ggplot2 R package.[297] In this study, custom R code was produced for part of the estimation of age-based and disease risk-based biological ages (Sections 4.2–4.4) and for part of the validation procedure (Section 4.5), where no existing R functions or packages were available. This code includes R functions for principal component analysis with rotation, stepwise linear regression (with cross validation), the Klemera Doubal method (for separate reference and target populations and with cross validation) and the penalised Cox models. This code is available upon request.

Section 4.2: Estimating age-based biological age

- Principal component analysis with rotation: own extension of the 'prcomp' function in R
- Stepwise linear regression: 'stepAIC' R package[298]
- Klemera Doubal: 'kdm' R function (own implementation of formulae in the Klemera and Doubal paper[51])

Section 4.3: Estimating disease risk-based body system ages and risk scores

- Calculation of floating absolute risk 95% confidence intervals for hazard ratios from Cox models: 'epi' R package[299]
- Stepwise Cox regression: 'stepAIC' R package[298]
- Cox lasso regression: 'glmnet' R package[167]
- Body system age estimation: 'bsage' R function (own implementation, incorporating the stepwise and lasso Cox regressions)

Section 4.4: Aggregating disease risk-based biological age

- Klemera Doubal: ‘kdm’ R function described above
- Multi-state model: ‘msm’ R package,[180] with own extension to extract model output to estimate biological age
- Multi-state model goodness of fit: own extension of R code to plot observed vs modelled state-specific prevalence (described in the book on multi-state survival models,[290] original code provided courtesy of the author), to account for differential follow up durations

Section 4.5: Validating biological ages

- Relative importance for linear regressions: ‘relaimpo’ R package[300]
- Explained relative risk: own modification of the ‘coxphERR’ function in the ‘clinfun’ R package[301] to accommodate output from cross-validated rather than standard Cox lasso models
- C-index for survival models: ‘survConcordance’ function in R
- Cox models and likelihood metrics for chronological and biological age combinations: ‘predoutcome’ R function (own implementation utilising the ‘coxph’ function in R)

Appendix 5.1: Results from sensitivity analyses of age-based biological ages

Introduction

This appendix serves as a counterpart to the analysis results for the age-based Klemera Doubal (KDM) biological ages described in Chapter 5. It details the analysis for the sensitivity analysis using the stepwise regression method (described in Chapter 4.2), which was also applied to the same set of biomarker principal components as the KDM to estimate an alternative set of biological ages. The same validation methods were used for the stepwise regression and KDM biological ages (Section 4.5).

Stepwise regression age cannot be combined with chronological age in a prediction model, because it was directly constructed by regressing its constituent biomarkers against chronological age. Hence, the proportion of the age effect described by stepwise regression age could not be elucidated.

Biomarker constituents of biological ages

The stepwise regression method included most biomarker principal components in the estimation of the biological ages (Table A5.1). For healthy men, red blood cell haemoglobin concentration, lipoprotein(a) (LP(a)) and basophil count were excluded; while for healthy women, height, bilirubin, LP(a), pulse rate, red blood cell width and lymphocyte count were excluded (Table A5.1).

Reduced lung function featured most strongly in the stepwise regression ages, while higher cystatin C, slower reaction time, lower IGF-1, lower hand grip strength, and higher blood pressure also featured strongly for both sexes (Figure A5.1 and Table A5.2). The importance of biomarker principal components in the stepwise regression ages and the sex differences broadly matched those for KDM biological ages (Section 5.3).

Validation results

In the healthy subpopulation, KDM and stepwise regression biological ages were similarly predictive of mortality and frailty in unadjusted and adjusted models, in the absence of chronological age (Table A5.3). C-indices were slightly higher for KDM compared to stepwise regression ages in the whole population (improvements in C-indices for mortality and frailty respectively from using KDM instead of stepwise regression: 0.029 and 0.009 in men, 0.018 and 0.008 in women in unadjusted analyses; reducing to 0.016 and 0.007 in men, 0.009 and 0.004 in women in adjusted analyses; Table A5.3).

The plots of prediction errors for stepwise regression biological ages vs chronological ages by number of principal components included in the models were similar to those for KDM biological ages (Figures A5.2 and 4.5.1). Therefore the same number of biomarker principal components (51 components) determined in the main analysis (Chapter 5.4) were used.

The stepwise regression age was too high at younger chronological ages and too low at older chronological ages (Figure A5.3). Further rescaling to calibrate stepwise regression age to chronological age was not relevant for the assessment of its constituents and its relative predictive power, but would be important for implementation in a clinical setting. Additionally, the predicted risk groups based on stepwise regression biological ages clearly differentiated risk of each health outcome after 4 years from baseline, but in the reverse direction, with biologically younger participants having higher mortality and frailty risk than biologically older participants (Figure A5.4).

Discussion

The stepwise regression found that most of the 51 biomarker principal components significantly related to chronological age and therefore contributed to the biological age. The weights assigned to

biomarker principal components in the stepwise regression biological ages, and thus the importance of these components and the predictive power for health outcomes, were broadly similar to those for the KDM biological age. In contrast to KDM, the stepwise regression method resulted in a poorly-calibrated biological age in the UK Biobank despite its frequent use in biological ageing studies[5,9,12] (where its risk calibration was not investigated), and could not be recommended for application in this context.

Table A5.1: Model coefficients for the stepwise regression biological ages in the healthy subpopulation, by sex

Biomarker principal component number and description		Healthy men	Healthy women
		Coefficients	
(Intercept)		59.015	55.942
PC1	General adiposity	0.189	0.146
PC2	Total haemoglobin volume	0.491	-0.264
PC3	Height	-0.288	-
PC4	Albumin	1.017	0.410
PC5	Neutrophil count	-0.423	0.225
PC6	Immature red blood cell volume	-0.398	-0.312
PC7	LDL and ApoB	-0.455	-0.720
PC8	Reticulocyte count	0.156	0.087
PC9	Urinary potassium and creatinine	0.332	0.203
PC10	Blood pressure	-1.255	-0.988
PC11	HDL and ApoA	0.570	0.513
PC12	Aminotransferases	0.397	-0.182
PC13	Bilirubin	-0.134	-
PC14	Platelet count	-0.520	-0.314
PC15	Red blood cell haemoglobin concentration	-	0.084
PC16	Testosterone	0.687	1.556
PC17	Lung function/height	-1.103	-1.030
PC18	Blood glucose	0.401	0.486
PC19	Platelet cell volume	-0.432	-0.247
PC20	LP(a)	-	-
PC21	Pairs matching test	-0.520	-0.379
PC22	Rheumatoid factor	0.154	0.066
PC23	Bone density	-0.128	-0.825
PC24	Vitamin D	0.682	0.512
PC25	IGF-1	0.822	1.218
PC26	Urinary microalbumin	0.312	0.617
PC27	Basophil count	-	-0.111
PC28	Central adiposity	-0.860	-0.176
PC29	Eosinophil count	-0.186	-0.250
PC30	Alkaline phosphatase	-0.106	-0.790
PC31	Pulse rate	0.119	-
PC32	Red blood cell width	-0.422	-
PC33	Reaction time test	-1.042	-0.918
PC34	Sex hormone-binding globulin	2.661	-0.075
PC35	Hand grip strength/height	0.430	0.679
PC36	Phosphate	0.180	-0.283
PC37	Lymphocyte count	0.395	-
PC38	Triglycerides	0.416	0.441
PC39	Urinary sodium	1.056	1.067
PC40	Monocyte count	0.472	0.187
PC41	Gamma glutamyltransferase	-0.113	-0.265
PC42	Urea	0.801	1.110
PC43	HbA1c	0.918	1.382
PC44	Platelet distribution width	0.328	0.116
PC45	Log C-reactive protein	-0.199	-0.289
PC46	Reticulocyte fraction	-0.187	-0.212
PC47	Cystatin C	-1.774	-1.483
PC48	Muscle mass	-1.182	-0.519
PC49	Calcium	0.139	0.660
PC50	Total protein	0.757	1.189
PC51	Urate	-0.398	-0.490

Table A5.2: Importances of the 51 biomarker principal components in the stepwise regression biological ages for healthy men and healthy women

Healthy men

Rank	Biomarker principal component	% of total R^2
1	Lung function/height	10.6
2	Sex hormone-binding globulin	9.9
3	Cystatin C	9.1
4	Blood pressure	7.1
5	Reaction time test	6.5
6	Albumin	5.3
7	IGF-1	4.9
8	Muscle mass	4.3
9	Hand grip strength/height	3.4
10	HbA1c	3.1
11	Urinary sodium	3.0
12	Urea	2.8
13	Central adiposity	2.6
14	Total protein	2.4
15	Pairs matching test	2.2
16	HDL-C and ApoA	2.0
17	Immature red blood cell volume	2.0
18	Vitamin D	2.0
19	Platelet count	1.3
20	Red blood cell width	1.3
21	Monocyte count	1.2
22	Blood glucose	1.2
23	Height	1.1
24	LDL-C and ApoB	1.1
25	Neutrophil count	1.1
26	Total haemoglobin volume	1.0
27	Urinary microalbumin	0.9
28	Calcium	0.8
29	Aminotransferases	0.7
30	Testosterone	0.6
31	General adiposity	0.6
32	Log C-reactive protein	0.5
33	Reticulocyte count	0.5
34	Triglycerides	0.4
35	Lymphocyte count	0.3
36	Urate	0.3
37	Platelet cell volume	0.3
38	Platelet distribution width	0.3
39	Phosphate	0.3
40	Urinary potassium and creatinine	0.3
41	Alkaline phosphatase	0.2
42	Bone density	0.2
43	Gamma glutamyltransferase	0.2
44	Pulse rate	0.2
45	Reticulocyte fraction	0.1
46	Rheumatoid factor	0.1
47	Eosinophil count	0.1
48	Bilirubin	0.1
49	LP(a)	0.0
50	Basophil count	0.0
51	Red blood cell haemoglobin concentration	0.0

Healthy women

Rank	Biomarker principal component	% of total R^2
1	Lung function/height	11.6
2	Cystatin C	8.3
3	IGF-1	7.1
4	Blood pressure	6.8
5	HbA1c	6.6
6	LDL-C and ApoB	6.6
7	Urea	6.4
8	Reaction time test	6.0
9	Hand grip strength/height	5.2
10	Alkaline phosphatase	5.0
11	Bone density	3.9
12	Urinary sodium	2.4
13	Total protein	2.0
14	HDL-C and ApoA	1.9
15	Blood glucose	1.9
16	Triglycerides	1.9
17	Calcium	1.7
18	Pairs matching test	1.4
19	Muscle mass	1.3
20	Total haemoglobin volume	1.3
21	Aminotransferases	1.2
22	Phosphate	1.1
23	Vitamin D	1.0
24	Urinary microalbumin	1.0
25	Albumin	0.7
26	Platelet count	0.7
27	Central adiposity	0.6
28	Urate	0.6
29	Neutrophil count	0.5
30	Immature red blood cell volume	0.5
31	General adiposity	0.4
32	Log C-reactive protein	0.4
33	Testosterone	0.3
34	Platelet cell volume	0.3
35	Gamma glutamyltransferase	0.3
36	Sex hormone-binding globulin	0.3
37	Urinary potassium and creatinine	0.2
38	Reticulocyte count	0.2
39	Eosinophil count	0.1
40	Reticulocyte fraction	0.1
41	Basophil count	0.1
42	Monocyte count	0.1
43	Platelet distribution width	0.1
44	Red blood cell haemoglobin concentration	0.1
45	Rheumatoid factor	0.1
46	Red blood cell width	0.0
47	LP(a)	0.0
48	Pulse rate	0.0
49	Lymphocyte count	0.0
50	Bilirubin	0.0
51	Height	0.0

Table A5.3: Harrell's C-indices (with standard errors) for each health outcome, Klemera Doubal biological age vs stepwise regression biological age

(A) Unadjusted analysis

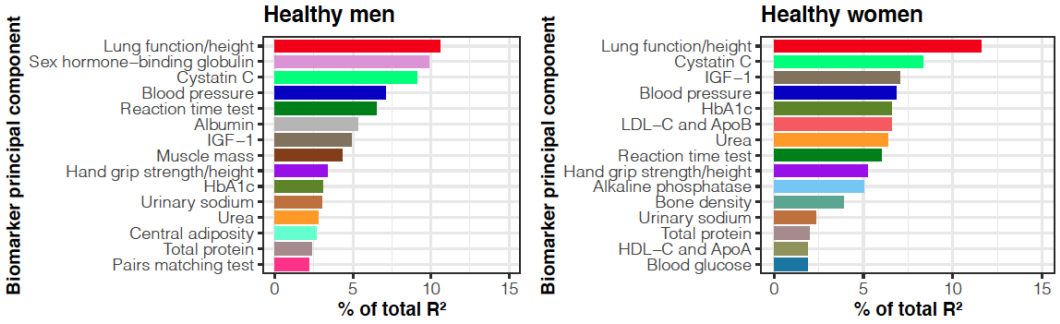
Outcome and age predictor	Healthy subpopulation		Whole population	
	Men	Women	Men	Women
Mortality from chronic disease				
CA alone	0.712 (0.008)	0.667 (0.009)	0.686 (0.003)	0.670 (0.003)
Klemera Doubal BA alone	0.689 (0.008)	0.635 (0.009)	0.736 (0.003)	0.683 (0.003)
Stepwise regression BA alone	0.686 (0.008)	0.640 (0.009)	0.707 (0.003)	0.665 (0.0034)
Improvement from using Klemera Doubal instead of stepwise regression	*-0.003*	*-0.005*	*0.029*	*0.018*
Age-related frailty				
CA alone	0.636 (0.003)	0.606 (0.003)	0.629 (0.001)	0.603 (0.001)
Klemera Doubal BA alone	0.615 (0.003)	0.586 (0.003)	0.630 (0.001)	0.605 (0.001)
Stepwise regression BA alone	0.616 (0.003)	0.591 (0.003)	0.621 (0.001)	0.597 (0.001)
Improvement from using Klemera Doubal instead of stepwise regression	*-0.001*	*-0.005*	*0.009*	*0.008*

(B) Adjusted for sociodemographic factors and health behaviours

Outcome and age predictor	Healthy subpopulation		Whole population	
	Men	Women	Men	Women
Mortality from chronic disease				
CA alone	0.724 (0.008)	0.688 (0.009)	0.725 (0.003)	0.699 (0.003)
Klemera Doubal BA alone	0.702 (0.008)	0.660 (0.009)	0.746 (0.003)	0.697 (0.003)
Stepwise regression BA alone	0.699 (0.008)	0.665 (0.009)	0.730 (0.003)	0.688 (0.003)
Improvement from using Klemera Doubal instead of stepwise regression	*-0.003*	*-0.005*	*0.016*	*0.009*
Age-related frailty				
CA alone	0.660 (0.003)	0.633 (0.003)	0.677 (0.001)	0.656 (0.001)
Klemera Doubal BA alone	0.640 (0.003)	0.614 (0.003)	0.673 (0.001)	0.653 (0.001)
Stepwise regression BA alone	0.642 (0.003)	0.619 (0.003)	0.666 (0.001)	0.649 (0.001)
Improvement from using Klemera Doubal instead of stepwise regression	*-0.002*	*-0.005*	*0.007*	*0.004*

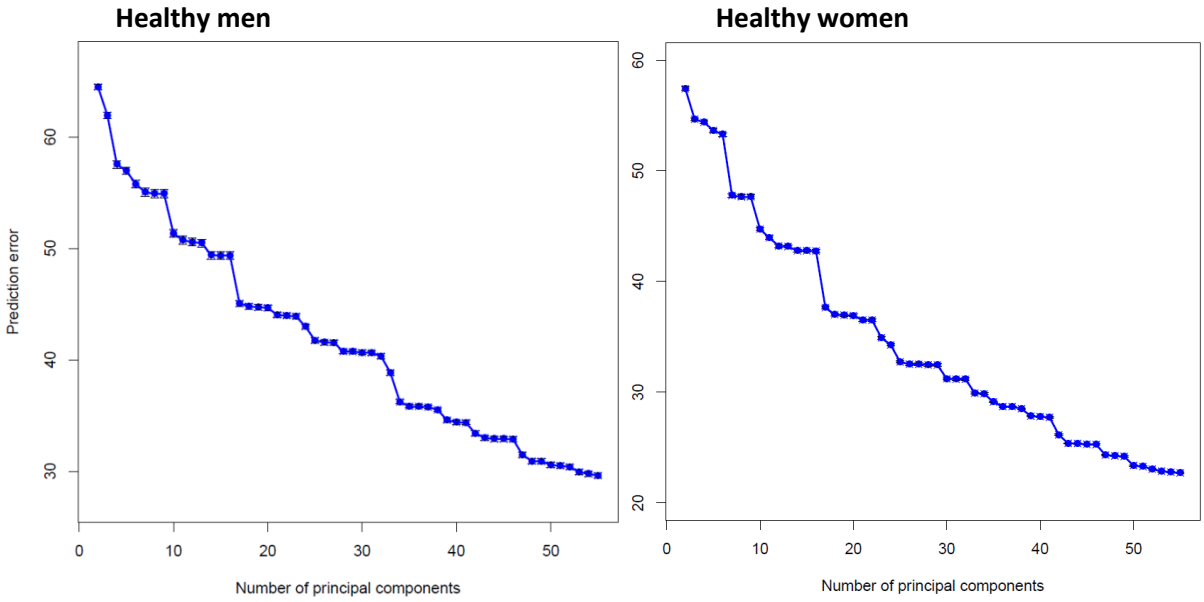
CA: chronological age; BA: biological age
Analyses in (B) were adjusted for Index of Multiple Deprivation 2010 quintile, smoking status, alcohol consumption and assessment centre

Figure A5.1: Importance of the top 15 biomarker principal components in the stepwise regression biological ages for healthy men and healthy women.



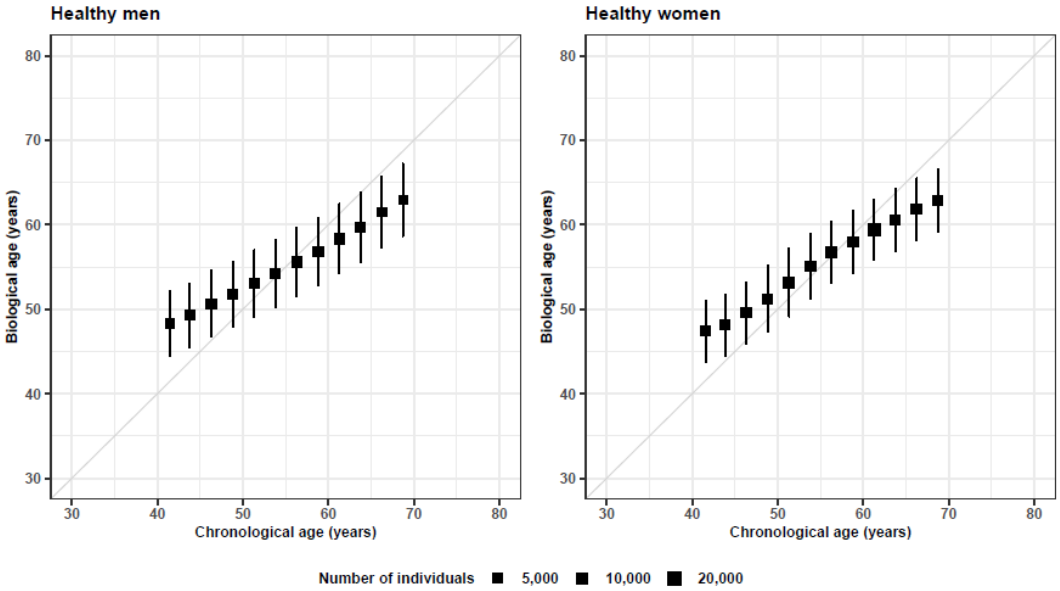
The percentage of R^2 denotes the percentage of variation in the biological age explained by each biomarker

Figure A5.2: 10-fold cross validation prediction errors (with standard error bars) for each subset of principal components (to a maximum of 55) using stepwise regression biological ages for healthy men and healthy women



These plots display prediction errors (mean square errors of biological ages; y-axes) and their standard error bars, for each biological age constructed from the specified number of principal components (x-axes). They were used to search for an elbow point, where beyond the elbow point there were diminishing changes in prediction error by increasing number of principal components.

Figure A5.3: Means and standard deviations of stepwise regression biological ages by 2.5-year chronological age groups, for healthy men and healthy women

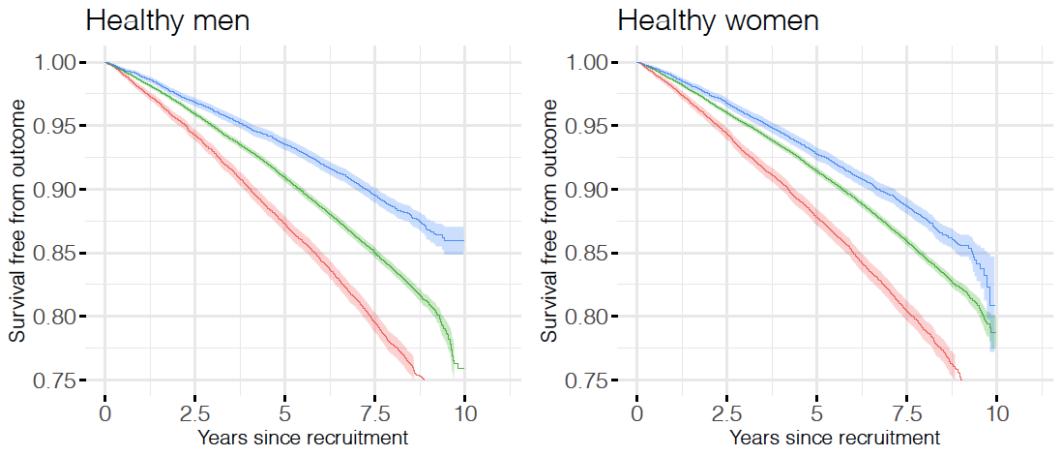


These plots indicate how well biological ages (y-axes) are calibrated to chronological age (x-axes), and the variability (indicated by ±1 standard deviation bars) of individuals' biological ages in each 2.5-year chronological age group.

Figure A5.4: Outcome-free survival of healthy men and healthy women for (A) mortality from chronic disease and (B) age-related frailty, according to whether their stepwise regression biological age is younger, similar to or older than their chronological age

(A) Mortality from chronic disease

(B) Age-related frailty



Predicted risk group Kaplan-Meier curve and 95% confidence interval (shaded area):
BA - CA < -5 years (Biologically younger)
|BA – CA| < 5 years
BA - CA > 5 years (Biologically older)

Appendix 5.2: Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD) checklist for the age-based biological age analysis

Section/Topic	Item*		Checklist Item	Section/Item
Title and abstract				
Title	1	D;V	Identify the study as developing and/or validating a multivariable prediction model, the target population, and the outcome to be predicted.	Section 4.2 (not in title)
Abstract	2	D;V	Provide a summary of objectives, study design, setting, participants, sample size, predictors, outcome, statistical analysis, results, and conclusions.	NA
Introduction				
Background and objectives	3a	D;V	Explain the medical context (including whether diagnostic or prognostic) and rationale for developing or validating the multivariable prediction model, including references to existing models.	Sections 1.3–1.4
	3b	D;V	Specify the objectives, including whether the study describes the development or validation of the model or both.	Section 1.4
Methods				
Source of data	4a	D;V	Describe the study design or source of data (e.g., randomized trial, cohort, or registry data), separately for the development and validation data sets, if applicable.	Section 3.2
	4b	D;V	Specify the key study dates, including start of accrual; end of accrual; and, if applicable, end of follow-up.	Section 3.2
Participants	5a	D;V	Specify key elements of the study setting (e.g., primary care, secondary care, general population) including number and location of centres.	Sections 3.2–3.3 and 3.9
	5b	D;V	Describe eligibility criteria for participants.	Sections 3.2–3.3
	5c	D;V	Give details of treatments received, if relevant.	NA
Outcome	6a	D;V	Clearly define the outcome that is predicted by the prediction model, including how and when assessed.	Section 3.7
	6b	D;V	Report any actions to blind assessment of the outcome to be predicted.	NA
Predictors	7a	D;V	Clearly define all predictors used in developing or validating the multivariable prediction model, including how and when they were measured.	Section 3.8 and 3.10
	7b	D;V	Report any actions to blind assessment of predictors for the outcome and other predictors.	NA
Sample size	8	D;V	Explain how the study size was arrived at.	Sections 3.2–3.3
Missing data	9	D;V	Describe how missing data were handled (e.g., complete-case analysis, single imputation, multiple imputation) with details of any imputation method.	Appendix 3.3
Statistical analysis methods	10a	D	Describe how predictors were handled in the analyses.	Sections 3.8 and 4.2
	10b	D	Specify type of model, all model-building procedures (including any predictor selection), and method for internal validation.	Sections 4.2 and 4.5
	10c	V	For validation, describe how the predictions were calculated.	Section 4.5
	10d	D;V	Specify all measures used to assess model performance and, if relevant, to compare multiple models.	Sections 4.2 and 4.5
	10e	V	Describe any model updating (e.g., recalibration) arising from the validation, if done.	NA
Risk groups	11	D;V	Provide details on how risk groups were created, if done.	NA
Development vs. validation	12	V	For validation, identify any differences from the development data in setting, eligibility criteria, outcome, and predictors.	NA

Section/Topic	Item*		Checklist Item	Section/Item
Results				
Participants	13a	D;V	Describe the flow of participants through the study, including the number of participants with and without the outcome and, if applicable, a summary of the follow-up time. A diagram may be helpful.	Section 3.9
	13b	D;V	Describe the characteristics of the participants (basic demographics, clinical features, available predictors), including the number of participants with missing data for predictors and outcome.	Section 3.9 and Appendix 3.3
	13c	V	For validation, show a comparison with the development data of the distribution of important variables (demographics, predictors and outcome).	NA
Model development	14a	D	Specify the number of participants and outcome events in each analysis.	Section 3.9
	14b	D	If done, report the unadjusted association between each candidate predictor and outcome.	NA (adjusted association in Figure 3.10.2)
Model specification	15a	D	Present the full prediction model to allow predictions for individuals (i.e., all regression coefficients, and model intercept or baseline survival at a given time point).	Table 5.3.1
	15b	D	Explain how to use the prediction model.	Section 4.2
Model performance	16	D;V	Report performance measures (with CIs) for the prediction model.	Table 5.5.1
Model-updating	17	V	If done, report the results from any model updating (i.e., model specification, model performance).	NA
Discussion				
Limitations	18	D;V	Discuss any limitations of the study (such as nonrepresentative sample, few events per predictor, missing data).	Section 5.6
Interpretation	19a	V	For validation, discuss the results with reference to performance in the development data, and any other validation data.	NA
	19b	D;V	Give an overall interpretation of the results, considering objectives, limitations, results from similar studies, and other relevant evidence.	Section 5.6
Implications	20	D;V	Discuss the potential clinical use of the model and implications for future research.	Section 5.6
Other information				
Supplementary information	21	D;V	Provide information about the availability of supplementary resources, such as study protocol, Web calculator, and data sets.	Section 3.2
Funding	22	D;V	Give the source of funding and the role of the funders for the present study.	Acknowledgements

This analysis is a development and internal validation study according to the TRIPOD guidelines[164]
* Items relevant to model development are denoted by 'D', items relating to model validation are denoted by 'V'

Appendix 6.1: Exclusion of participants who could have been regarded as having diseases based on biomarker measurements

While assessing the distribution of body system ages in healthy UK Biobank participants (described in Section 4.3 and displayed in Section 6.4), metabolic disease age displayed large differences from chronological age and a large degree of variation within metabolic disease age tertiles (Figure 6.4.1).

Since HbAlc is a key determinant of clinical diagnosis of diabetes, the most common metabolic disease in the UK Biobank (Figure 3.9.3), the pattern of HbA1c principal component and raw HbA1c levels were analysed by metabolic disease age and by chronological age (Figure A6.1.1). Both HbA1c principal component and raw HbA1c levels increased substantially with increasing metabolic disease age, however the levels appeared relatively flat with a large degree of variation across chronological ages (Figure A6.1.1).

Therefore, the subpopulation of healthy men and women in this analysis was modified to exclude those who could have been regarded as having diabetes based on their baseline HbA1c measurements. All results reported in Chapter 6 pertain to the healthy subpopulation of 54,948 men and 65,453 women after the exclusion of these participants (15.7% of healthy men and 12.1% of healthy women were excluded; Table A6.1.1). Participants who could have been regarded as having screen-detected disease for two other diseases: chronic kidney disease (CKD, stages 3 and above) or chronic obstructive pulmonary disease (COPD), based their baseline cystatin C levels and forced expiratory volume in 1s/forced vital capacity (FEV1/FVC) ratios respectively, were also excluded for consistency.

Few men and women were excluded for having HbA1c and cystatin C levels above the threshold for diabetes (HbA1c>48mmol/mol) and CKD (estimated glomerular filtration rate based on cystatin C using CKD-EPI equations[302]<60mg/L) respectively based on the National Institute for Health and Care Excellence (NICE) guidelines.[212,213] The proportion of healthy participants with detectable

diabetes: 0.78% and 0.38%; CKD: 1.16% and 1.40%; for men and women respectively, but 14.06% of men and 10.57% of women were excluded for having FEV1/FVC ratios under the threshold for COPD (FEV1/FVC ratios<0.7; Table A6.1.1) recommended by NICE guidelines.[214] Very few of these participants had both exceeded the threshold and reported the disease at baseline for any of the 3 diseases (0.18% and 0.11% of all healthy men and women respectively; Table A6.1.1).

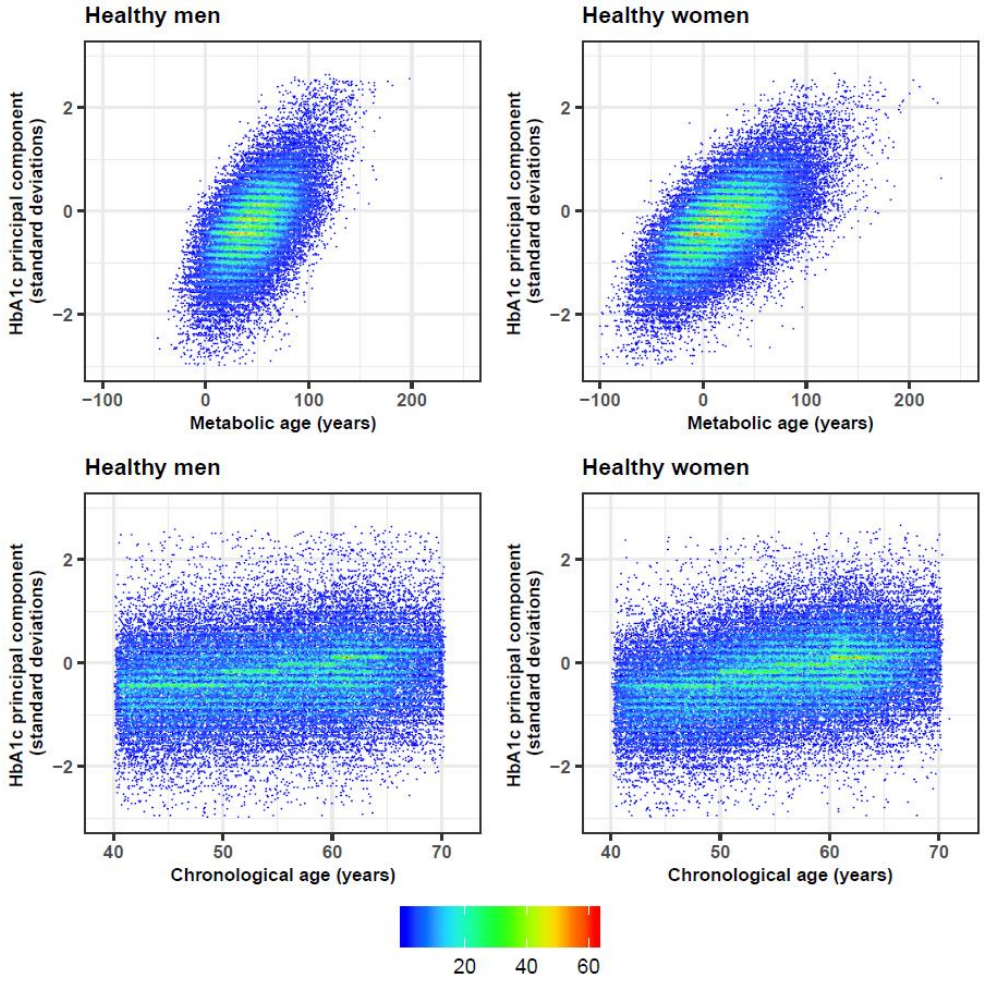
Table A6.1.1: Proportion of healthy men and women who could have been regarded as having screen-detected diabetes, chronic kidney disease (stages 3 and above) or chronic obstructive pulmonary disease based on their baseline biomarker measurements

Disease name	Threshold used	Threshold exceeded		Threshold exceeded and self reported the disease at baseline	
		Healthy men	Healthy women	Healthy men	Healthy women
Diabetes	HbA1c above 48 mmol/mol	0.78%	0.38%	0.16%	0.08%
CKD	eGFRCystatin (using CKD-EPI equations)<60mg/L	1.16%	1.40%	0.00%	0.01%
COPD	FEV1/FVC ratio <0.7	14.06%	10.57%	0.02%	0.03%
Any of the 3 diseases	See above thresholds	15.65%	12.11%	0.18%	0.11%

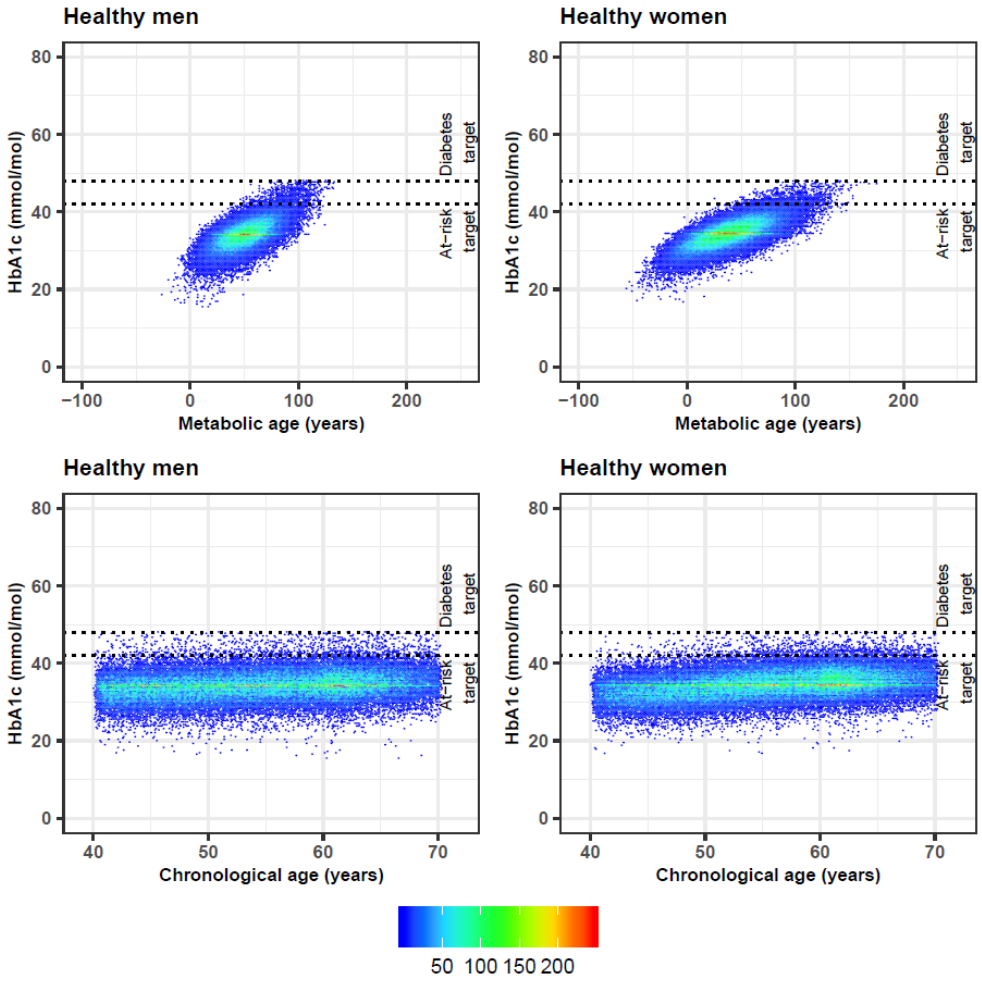
CKD: chronic kidney disease (stages 3 and above); COPD: chronic obstructive pulmonary disease
The thresholds used for these 3 diseases were based on NICE guidelines[212-214] and on CKD-EPI reference equations for calculating estimated glomerular filtration rate (eGFR) from cystatin C[302]
There were 54,948 healthy men and 65,453 healthy women in the subpopulation after excluding participants with any of the 3 diseases (based on the thresholds above)

Figure A6.1.1: Population densities by metabolic disease age compared with chronological age, across (A) HbA1c principal component and (B) raw HbA1c levels, in healthy men and healthy women

(A) By HbA1c principal component levels



(B) By raw HbA1c levels



These plots excluded participants who could have been regarded as having screen-detected diabetes, chronic kidney disease (stages 3 and above) or chronic obstructive pulmonary disease based on their baseline biomarker measurements. Prior to these exclusions, there was a very small number of participants above the HbA1c threshold of 48 mmol/mol in (B)
In (A), not all of the population is displayed in these plots; although >99.7% of the population (corresponding to the middle 6 standard deviations) is displayed
The biomarker that most strongly loaded onto the HbA1c principal component was HbA1c (factor loading: 0.9; Table 3.10.1)

Appendix 6.2: Supplementary tables and figures for analyses of body system ages and risk scores

This appendix contains the supplementary tables and figures for the analysis of body system ages and risk scores in Chapter 6. These tables and figures contain detailed results that support the main analyses in the chapter, in Sections 6.3–6.7.

Table A6.2.1: Model coefficients for each body system risk score in the healthy subpopulation, by sex

Healthy men

Biomarker principal component		Artery	Musc	Gut	Cardiac	Metab	Inflam	Neuro	Lung
PC1	General adiposity	0	-0.115	-0.024	-0.023	-0.094	-0.017	0.008	-0.038
PC2	Total haemoglobin volume	0	0.053	0.018	0.068	0	0	0.049	0
PC3	Height	0.015	0.107	0	-0.131	0.033	0	0.020	0
PC4	Albumin	0.126	0.129	0.077	0.100	0.048	0.207	0.164	0.352
PC5	Neutrophil count	-0.009	-0.010	-0.014	-0.067	-0.040	0	-0.098	-0.078
PC6	Immature red blood cell volume	-0.029	-0.040	-0.035	-0.084	-0.104	0	0	-0.147
PC7	LDL and ApoB	-0.145	0.017	0	-0.022	0.065	0	0	0
PC8	Reticulocyte count	-0.029	-0.014	-0.037	-0.034	-0.131	0	0.120	0
PC9	Urinary potassium and creatinine	0.044	0.020	0	0.032	0	0	0.101	0
PC10	Blood pressure	-0.255	-0.043	-0.044	-0.150	-0.098	-0.227	-0.06	0
PC11	HDL and ApoA	-0.071	0.060	0	0.051	-0.015	0	0	0.103
PC12	Aminotransferases	0.055	0.020	0.034	0.019	0	0	0.092	0
PC13	Bilirubin	-0.015	-0.028	-0.019	-0.066	-0.064	0	-0.059	0
PC14	Platelet count	-0.066	-0.043	-0.023	-0.066	-0.102	0	-0.069	-0.004
PC15	Red blood cell haemoglobin concentration	-0.001	0.018	-0.023	0.057	0.045	0	0	-0.129
PC16	Testosterone	0.111	0.024	0	0.130	0.161	0	0.142	0
PC17	Lung function/height	-0.099	-0.069	-0.039	-0.144	-0.056	-0.073	-0.150	-0.349
PC18	Blood glucose	-0.024	0.014	0	0.035	0.149	0	0.201	0
PC19	Platelet cell volume	-0.025	-0.053	-0.070	-0.004	-0.003	0	-0.112	0
PC20	LP(a)	0.076	-0.004	0	0.040	0	0	-0.043	-0.001
PC21	Pairs matching test	-0.008	-0.011	-0.028	-0.036	-0.025	-0.080	-0.221	0
PC22	Rheumatoid factor	0	0	0.001	0.039	0	0.211	0	0
PC23	Bone density	0.040	0.076	0.002	0.027	0.048	0	-0.109	-0.045
PC24	Vitamin D	0.052	0.166	0.058	0.086	-0.001	0	0.052	0.117
PC25	IGF-1	0.049	0.063	0.021	0.044	0.081	0.017	-0.148	0.109
PC26	Urinary microalbumin	0.046	0.003	0.024	0.026	0.058	0.070	0.150	0.025
PC27	Basophil count	0.035	0.005	0.039	0.017	0.065	0	0	0.127
PC28	Central adiposity	-0.117	-0.013	-0.174	-0.121	-0.071	0	-0.119	-0.137
PC29	Eosinophil count	0	0	-0.021	0.015	0	0.041	-0.050	0.129
PC30	Alkaline phosphatase	-0.045	-0.061	-0.020	-0.021	-0.083	-0.099	0	0
PC31	Pulse rate	0.063	0.023	-0.040	-0.007	-0.016	0	0.027	-0.047
PC32	Red blood cell width	-0.060	-0.050	-0.056	-0.107	0	0	-0.003	-0.005
PC33	Reaction time test	-0.074	-0.027	-0.077	-0.073	-0.077	-0.022	-0.247	0
PC34	Sex hormone-binding globulin	0.271	0.103	0.032	0.356	0.120	0.243	0.388	0.183
PC35	Hand grip strength/height	0.054	0.074	0.049	0.043	0.086	0.063	0.162	0.098
PC36	Phosphate	-0.005	-0.053	-0.033	-0.023	-0.025	-0.080	0	0
PC37	Lymphocyte count	0.043	0.068	0.044	0.096	0.077	0.045	0	0
PC38	Triglycerides	0.018	0.022	0.004	-0.066	0.050	0	-0.139	0
PC39	Urinary sodium	0.078	0.043	0.028	0.145	0.019	0	0.076	0
PC40	Monocyte count	0.073	0.027	0.040	0.087	0.015	0	0	0.005
PC41	Gamma glutamyltransferase	-0.028	0.008	0.007	-0.018	0.077	0	0	0
PC42	Urea	0.070	0.086	0.022	0.117	0.030	0	0	0
PC43	HbA1c	0.077	0.109	0.083	0.118	0.684	0	0	0.076
PC44	Platelet distribution width	0.008	-0.021	0.055	0.037	0.099	0	0	0
PC45	Log C-reactive protein	0.008	0.039	0.026	-0.013	0	0.146	-0.083	0.091
PC46	Reticulocyte fraction	-0.036	-0.064	-0.007	-0.069	-0.056	-0.005	-0.006	0
PC47	Cystatin C	-0.135	-0.015	-0.127	-0.177	-0.204	-0.156	0	-0.239
PC48	Muscle mass	0.040	0.169	-0.081	0.016	0	0	0	-0.095
PC49	Calcium	0.053	0.058	0.058	0.051	0.047	0	0.010	0
PC50	Total protein	0.019	0.011	0.040	0.075	0	0	0	-0.105
PC51	Urate	-0.013	0	0.033	0.029	0.219	0	0	0
Number of biomarker principal components selected		47	48	44	51	41	18	33	26

Healthy women

Biomarker principal component		Artery	Musc	Gut	Cardiac	Metab	Inflam	Neuro	Lung
PC1	General adiposity	0	-0.083	-0.067	-0.048	-0.107	0.035	0.065	0
PC2	Total haemoglobin volume	0.033	0.028	-0.023	0	0.014	0	0	0
PC3	Height	0	0.038	0.019	-0.099	0.051	0	0	0
PC4	Albumin	0.104	0.065	0.016	0.016	0.030	0.147	0.003	0
PC5	Neutrophil count	0	0.019	-0.008	-0.043	0	0	0	-0.068
PC6	Immature red blood cell volume	0.010	-0.038	-0.019	-0.002	0	-0.026	0	-0.148
PC7	LDL and ApoB	-0.079	-0.022	-0.017	-0.031	0.102	0	-0.049	0
PC8	Reticulocyte count	0	-0.021	0	-0.031	-0.081	0	0.006	0
PC9	Urinary potassium and creatinine	0	0	0	0.005	-0.008	0	-0.029	0.040
PC10	Blood pressure	-0.209	-0.038	-0.041	-0.144	-0.047	-0.043	-0.168	0.057
PC11	HDL and ApoA	-0.039	0.015	0	0.012	-0.081	-0.041	0	0
PC12	Aminotransferases	-0.034	-0.009	0	0.017	-0.014	-0.117	-0.064	-0.053
PC13	Bilirubin	-0.028	-0.005	0	-0.037	-0.039	0	0	0
PC14	Platelet count	-0.032	-0.015	0	-0.068	-0.065	0	-0.040	0
PC15	Red blood cell haemoglobin concentration	-0.043	-0.013	0.007	-0.030	0	-0.086	0	0
PC16	Testosterone	0.032	0.077	0	0	-0.149	0.188	0	-0.020
PC17	Lung function/height	-0.140	-0.073	-0.023	-0.163	-0.086	-0.030	-0.257	-0.307
PC18	Blood glucose	-0.015	0.030	0.037	0.026	0.141	0	0	0.040
PC19	Platelet cell volume	-0.035	-0.028	-0.036	-0.034	0	-0.055	0	0
PC20	LP(a)	0.022	-0.034	-0.006	-0.021	0	0	0	-0.003
PC21	Pairs matching test	0	-0.058	-0.044	-0.035	-0.005	0	-0.151	-0.047
PC22	Rheumatoid factor	-0.027	0.006	0	0	0.032	0.164	0.076	0
PC23	Bone density	-0.005	-0.090	-0.048	-0.091	0	-0.006	-0.062	-0.077
PC24	Vitamin D	0	0.167	0.069	0.011	0	0.016	0	0
PC25	IGF-1	0.099	0.074	0.065	0.118	0.010	0	0.011	0.096
PC26	Urinary microalbumin	0.050	0.045	0.005	0.053	0.134	0	0.067	0.145
PC27	Basophil count	0	0	0.018	0.013	0.029	0	0	0.173
PC28	Central adiposity	-0.018	-0.019	-0.095	0	-0.122	0	0	-0.063
PC29	Eosinophil count	0.020	-0.006	0	0.025	0.054	0.011	-0.020	0.084
PC30	Alkaline phosphatase	-0.065	-0.024	-0.033	-0.013	0.006	0	0	-0.090
PC31	Pulse rate	0.086	0.030	0.009	0.067	0	0	0	0
PC32	Red blood cell width	0	0.003	-0.005	0	0	0	0	0
PC33	Reaction time test	-0.065	-0.020	0	-0.045	0	0	-0.106	0
PC34	Sex hormone-binding globulin	0	0.019	0.006	0.031	0.046	0	0	0
PC35	Hand grip strength/height	0.095	0.162	0.103	0.110	0.022	0.343	0.205	0.020
PC36	Phosphate	-0.047	-0.032	-0.036	-0.059	-0.051	-0.042	0	0
PC37	Lymphocyte count	0.093	0.003	0	0.086	-0.002	0	0	0
PC38	Triglycerides	0.140	0.008	0.058	0	0.042	0	0	0
PC39	Urinary sodium	0.071	0.111	0.045	0.162	0	0	0	0
PC40	Monocyte count	0	0	0	0.036	-0.094	0	-0.044	0
PC41	Gamma glutamyltransferase	0.018	0.010	0.057	0.028	0.200	0	0	0
PC42	Urea	0.035	0.100	0.005	0.073	0.060	0	0	0.179
PC43	HbA1c	0.139	0.070	0.088	0.189	0.640	0	0	0
PC44	Platelet distribution width	0	0.005	0	0	0.016	0	0	0
PC45	Log C-reactive protein	0	0.007	0.041	0.014	0.051	0.207	-0.045	0.012
PC46	Reticulocyte fraction	-0.045	-0.043	-0.026	0	-0.025	0	-0.016	0
PC47	Cystatin C	-0.113	-0.118	-0.136	-0.173	-0.349	-0.236	-0.285	-0.136
PC48	Muscle mass	0	0.116	-0.090	0	0.048	0	0.008	0
PC49	Calcium	0.036	0.090	0.047	0.105	0.070	0	0	0.045
PC50	Total protein	0.021	0.097	0.099	0.130	-0.018	-0.103	0.066	0.065
PC51	Urate	0.064	-0.081	0.023	-0.011	0.103	0	-0.244	0
Number of biomarker principal components selected		37	48	38	42	40	19	24	23

Musc: Musculoskeletal, Metab: metabolic disease, Inflam: inflammatory, Neuro: neurological

Table A6.2.2: Model coefficients for each body system age in the healthy subpopulation, by sex

Healthy men

Biomarker principal component		Artery	Musc	Gut	Cardiac	Metab	Inflam	Neuro	Lung
PC1	General adiposity	-0.015	-0.128	-0.030	-0.039	-0.106	-0.034	0	-0.043
PC2	Total haemoglobin volume	-0.013	0.027	0	0.028	0	0	0	0
PC3	Height	0.029	0.121	0	-0.110	0.041	0	0	0
PC4	Albumin	0.066	0.059	0.025	0.022	0.021	0.111	0	0.291
PC5	Neutrophil count	0.003	0.007	0	-0.034	-0.030	0	-0.014	-0.058
PC6	Immature red blood cell volume	-0.004	-0.014	-0.009	-0.050	-0.092	0	0	-0.116
PC7	LDL and ApoB	-0.127	0.039	0.009	0	0.078	0	0.006	0
PC8	Reticulocyte count	-0.037	-0.017	-0.041	-0.032	-0.141	0	0.068	0
PC9	Urinary potassium and creatinine	0.027	0.004	0	0.003	0	0	0.067	0
PC10	Blood pressure	-0.190	0.021	0	-0.065	-0.062	-0.144	0	0
PC11	HDL and ApoA	-0.098	0.027	-0.020	0.012	-0.037	0	-0.009	0.057
PC12	Aminotransferases	0.028	0	0.010	-0.002	-0.008	0	0.021	0
PC13	Bilirubin	-0.008	-0.018	-0.009	-0.054	-0.063	0	-0.025	0
PC14	Platelet count	-0.034	-0.006	0	-0.022	-0.086	0	0	0
PC15	Red blood cell haemoglobin concentration	-0.006	0.011	-0.024	0.044	0.044	0	0	-0.126
PC16	Testosterone	0.063	-0.002	-0.005	0.071	0.131	-0.044	0.026	0
PC17	Lung function/height	-0.031	0	0	-0.054	-0.015	0	0	-0.291
PC18	Blood glucose	-0.049	-0.002	0	0.004	0.140	0	0.115	0
PC19	Platelet cell volume	-0.004	-0.026	-0.042	0.014	0	0	-0.052	0
PC20	LP(a)	0.079	0	0	0.035	0	0	-0.019	0
PC21	Pairs matching test	0.011	0.015	0	0	-0.008	-0.036	-0.155	0.001
PC22	Rheumatoid factor	-0.007	-0.005	0	0.024	-0.003	0.202	0	0
PC23	Bone density	0.046	0.083	0.004	0.024	0.053	0	-0.085	-0.030
PC24	Vitamin D	0.007	0.124	0.015	0.032	-0.037	0	0	0.047
PC25	IGF-1	0.005	0.014	0	0	0.059	0	-0.195	0.071
PC26	Urinary microalbumin	0.036	-0.011	0.004	0	0.058	0.053	0.140	0
PC27	Basophil count	0.029	0	0.029	0.007	0.064	0	0	0.117
PC28	Central adiposity	-0.074	0.023	-0.140	-0.065	-0.049	0	-0.013	-0.101
PC29	Eosinophil count	0.001	0.004	-0.008	0.016	0	0.046	-0.007	0.135
PC30	Alkaline phosphatase	-0.037	-0.051	-0.010	-0.003	-0.085	-0.110	0	0
PC31	Pulse rate	0.055	0.020	-0.039	-0.008	-0.023	0	0	-0.038
PC32	Red blood cell width	-0.038	-0.023	-0.038	-0.072	0	0	0	0
PC33	Reaction time test	-0.019	0.026	-0.027	0	-0.046	0	-0.142	0.003
PC34	Sex hormone-binding globulin	0.117	-0.035	-0.049	0.170	0.022	0.033	0.001	0.040
PC35	Hand grip strength/height	0.023	0.036	0.018	0	0.069	0	0.068	0.057
PC36	Phosphate	-0.016	-0.063	-0.037	-0.032	-0.032	-0.079	0	0
PC37	Lymphocyte count	0.022	0.044	0.020	0.059	0.068	0.027	0	0
PC38	Triglycerides	0	0	0	-0.086	0.037	0	-0.164	0
PC39	Urinary sodium	0.019	-0.008	0	0.063	0	0	0	0
PC40	Monocyte count	0.047	0	0.013	0.058	0.003	0	0	0
PC41	Gamma glutamyltransferase	-0.014	0.022	0.010	0	0.089	0	0	0
PC42	Urea	0.023	0.036	0	0.063	0.001	0	-0.049	0
PC43	HbA1c	0.015	0.037	0.020	0.031	0.657	0	-0.066	0.008
PC44	Platelet distribution width	0	-0.038	0.031	0.017	0.093	0	0	0
PC45	Log C-reactive protein	0.016	0.049	0.034	0	0	0.140	-0.052	0.097
PC46	Reticulocyte fraction	-0.028	-0.048	0	-0.042	-0.059	0	0	0.006
PC47	Cystatin C	-0.036	0.081	-0.035	-0.049	-0.142	-0.024	0.121	-0.125
PC48	Muscle mass	0.105	0.240	-0.021	0.091	0.028	0	0.021	-0.025
PC49	Calcium	0.051	0.048	0.038	0.023	0.049	0	0	0
PC50	Total protein	-0.002	-0.016	0	0.017	-0.015	0	0	-0.126
PC51	Urate	0	0.014	0.047	0.053	0.232	0	0	0
Coefficient for chronological age		0.060	0.060	0.061	0.048	0.077	0.036	0.065	0.127
Number of biomarker principal components selected		48	45	34	43	43	14	26	24

Healthy women

Biomarker principal component		Artery	Musc	Gut	Cardiac	Metab	Inflam	Neuro	Lung
PC1	General adiposity	0	-0.092	-0.077	-0.065	-0.114	0.024	0.032	-0.013
PC2	Total haemoglobin volume	0.040	0.049	0	0.017	0.030	0.014	0	0
PC3	Height	0	0.031	0.012	-0.101	0.057	0	0	0
PC4	Albumin	0.071	0.034	0	0	0.029	0.131	0	0
PC5	Neutrophil count	0	0.001	-0.019	-0.054	-0.005	0	0	-0.078
PC6	Immature red blood cell volume	0.024	-0.010	0	0	0	-0.013	0	-0.109
PC7	LDL and ApoB	-0.049	0.015	0	0	0.126	0	0	0
PC8	Reticulocyte count	-0.001	-0.020	0	-0.043	-0.096	0	0	0
PC9	Urinary potassium and creatinine	0	-0.008	0	0	-0.021	0	-0.032	0.042
PC10	Blood pressure	-0.170	0.018	0	-0.070	-0.028	0	-0.044	0.095
PC11	HDL and ApoA	-0.063	-0.004	-0.022	0	-0.105	-0.058	0	0
PC12	Aminotransferases	-0.031	0	0	0.029	-0.013	-0.097	-0.011	-0.029
PC13	Bilirubin	-0.021	0	0	-0.033	-0.046	0	0	0
PC14	Platelet count	-0.006	0	0.011	-0.036	-0.064	0.005	0	0
PC15	Red blood cell haemoglobin concentration	-0.048	-0.014	0	-0.032	0	-0.085	0	0
PC16	Testosterone	0	0	0	0	-0.220	0.174	0	0
PC17	Lung function/height	-0.086	0	0.013	-0.076	-0.057	0	-0.115	-0.213
PC18	Blood glucose	-0.038	0	0.003	0	0.132	-0.020	0	0
PC19	Platelet cell volume	-0.020	-0.011	-0.025	-0.013	0	-0.044	0	0
PC20	LP(a)	0.022	-0.029	-0.004	-0.021	0	0	0	0
PC21	Pairs matching test	0	-0.029	-0.019	-0.003	0	0	-0.103	0
PC22	Rheumatoid factor	-0.026	0	0	0	0.037	0.161	0.064	0
PC23	Bone density	0	-0.027	-0.002	-0.026	0.009	0	0	0
PC24	Vitamin D	0	0.140	0.041	0	0	0	-0.035	0
PC25	IGF-1	0.043	0	0	0.033	0	0	0	0
PC26	Urinary microalbumin	0.013	0	-0.005	0	0.131	0	0	0.123
PC27	Basophil count	0	0	0.023	0.018	0.037	0	0	0.169
PC28	Central adiposity	-0.012	-0.001	-0.083	0	-0.119	0	0	-0.028
PC29	Eosinophil count	0.032	0	0.002	0.042	0.068	0.019	0	0.086
PC30	Alkaline phosphatase	-0.036	0.006	0	0	0.032	0	0	-0.036
PC31	Pulse rate	0.082	0.032	0.006	0.067	-0.007	0	0	0
PC32	Red blood cell width	0	0	-0.003	0	0	0	0	0
PC33	Reaction time test	-0.02	0.029	0.042	0	0	0	0	0
PC34	Sex hormone-binding globulin	0	0.016	0.005	0.024	0.059	0	0	0
PC35	Hand grip strength/height	0.051	0.111	0.060	0.050	0.008	0.287	0.084	0
PC36	Phosphate	-0.030	-0.013	-0.020	-0.040	-0.054	-0.017	0	0
PC37	Lymphocyte count	0.083	0	0	0.077	-0.009	0	0	0
PC38	Triglycerides	0.122	-0.004	0.029	-0.027	0.036	-0.003	0	0
PC39	Urinary sodium	0.023	0.040	0	0.091	-0.024	0	0	0
PC40	Monocyte count	0	-0.001	0	0.018	-0.117	0	-0.050	0
PC41	Gamma glutamyltransferase	0.030	0.015	0.063	0.033	0.213	0	0	0
PC42	Urea	0	0.022	-0.025	0	0.041	-0.040	0	0.087
PC43	HbA1c	0.067	-0.017	0	0.078	0.620	0	0	0
PC44	Platelet distribution width	0	0	0	0	0.017	0	0	0
PC45	Log C-reactive protein	0	0.012	0.050	0.021	0.059	0.197	-0.044	0.021
PC46	Reticulocyte fraction	-0.023	-0.019	-0.008	0.006	-0.038	0	0	0
PC47	Cystatin C	-0.034	-0.009	-0.039	-0.056	-0.310	-0.147	-0.041	0
PC48	Muscle mass	0	0.143	-0.060	0.033	0.070	0	0.053	0
PC49	Calcium	0.009	0.037	0.009	0.053	0.064	0	0	0
PC50	Total protein	0	0.018	0.035	0.043	-0.054	-0.133	0	0
PC51	Urate	0.090	-0.040	0.039	0	0.113	0	-0.227	0
Coefficient for chronological age		0.053	0.049	0.067	0.052	0.076	0.025	0.038	0.115
Number of biomarker principal components selected		34	37	32	34	42	20	14	14

Musc: Musculoskeletal, Metab: metabolic disease, Inflam: inflammatory, Neuro: neurological

Table A6.2.3: Relative importance of the biomarker principal components (% of explained relative risk by biomarker principal components) in each body system age, for healthy men and healthy women

Healthy men

Biomarker principal component		Artery	Musc	Gut	Cardiac	Metab	Inflam	Neuro	Lung
PC1	General adiposity	0.9	42.7	11.9	6.9	12.3	4.3	0	2.8
PC2	Total haemoglobin volume	0.1	0.4	0	0.8	0	0	0	0
PC3	Height	1.2	14.1	0	21.4	0.7	0	0	0
PC4	Albumin	2.6	1.4	1.4	0.3	0.1	7.3	0	20.5
PC5	Neutrophil count	0	0	0	1.4	0.3	0	0.2	1.4
PC6	Immature red blood cell volume	0	0.1	0.3	3.5	2.7	0	0	6.1
PC7	LDL and ApoB	19.4	1.2	0.4	0	2.0	0	0	0
PC8	Reticulocyte count	1.7	0.3	7.7	1.6	7.4	0	4.6	0
PC9	Urinary potassium and creatinine	0.6	0	0	0	0	0	3.5	0
PC10	Blood pressure	33.3	0.3	0	4.7	1.0	18.8	0	0
PC11	HDL and ApoA	8.3	0.4	1.2	0.2	0.3	0	0.1	1.2
PC12	Aminotransferases	0.6	0	0.3	0	0	0	0.3	0
PC13	Bilirubin	0.1	0.3	0.4	5.2	1.7	0	0.8	0
PC14	Platelet count	1.1	0	0	0.6	2.0	0	0	0
PC15	Red blood cell haemoglobin concentration	0	0.1	1.5	1.7	0.4	0	0	4.5
PC16	Testosterone	1.3	0	0	2	1.6	0.6	0.2	0
PC17	Lung function/height	1.1	0	0	4.3	0.1	0	0	40.7
PC18	Blood glucose	1.1	0	0	0	2.7	0	5.3	0
PC19	Platelet cell volume	0	0.3	4.6	0.2	0	0	1.6	0
PC20	LP(a)	3.9	0	0	0.9	0	0	0.2	0
PC21	Pairs matching test	0.1	0.1	0	0	0	0.8	14.4	0
PC22	Rheumatoid factor	0	0	0	0.5	0	44.4	0	0
PC23	Bone density	1.5	3.2	0	0.5	0.6	0	4.1	0.3
PC24	Vitamin D	0	7.0	0.6	0.9	0.3	0	0	0.6
PC25	IGF-1	0	0.1	0	0	0.6	0	19.2	1.2
PC26	Urinary microalbumin	0.5	0	0	0	0.4	1.1	9.7	0
PC27	Basophil count	0.5	0	1.6	0	0.6	0	0	3.1
PC28	Central adiposity	3.0	0.2	38.8	2.8	0.4	0	0.1	2.3
PC29	Eosinophil count	0	0	0.2	0.2	0	1.4	0	5.2
PC30	Alkaline phosphatase	0.7	0.9	0.2	0	1.1	6.1	0	0
PC31	Pulse rate	2.0	0.2	3.6	0	0.1	0	0	0.4
PC32	Red blood cell width	0.7	0.2	2.6	3.2	0	0	0	0
PC33	Reaction time test	0.2	0.3	1.5	0	0.4	0	10.8	0
PC34	Sex hormone-binding globulin	3.3	0.2	2.1	8.7	0	0.3	0	0.2
PC35	Hand grip strength/height	0.3	0.5	0.8	0	0.8	0	2.4	0.8
PC36	Phosphate	0.2	1.9	3.5	0.9	0.2	4.3	0	0
PC37	Lymphocyte count	0.3	0.7	0.8	2.4	0.7	0.4	0	0
PC38	Triglycerides	0	0	0	6.0	0.3	0	14.4	0
PC39	Urinary sodium	0.3	0	0	3.6	0	0	0	0
PC40	Monocyte count	1.3	0	0.4	2.4	0	0	0	0
PC41	Gamma glutamyltransferase	0.1	0.2	0.2	0	1.6	0	0	0
PC42	Urea	0.3	0.5	0	2.8	0	0	1.2	0
PC43	HbA1c	0.1	0.3	0.5	0.4	45.1	0	1.3	0
PC44	Platelet distribution width	0	0.7	2.3	0.2	1.7	0	0	0
PC45	Log C-reactive protein	0.1	0.8	2.2	0	0	10.0	1.1	2.0
PC46	Reticulocyte fraction	0.5	1.0	0	1.3	0.6	0	0	0
PC47	Cystatin C	0.4	1.5	1.5	1.1	2.1	0.2	4.4	2.3
PC48	Muscle mass	4.7	16.8	0.7	4.4	0.1	0	0.2	0.1
PC49	Calcium	1.5	0.9	3.1	0.4	0.4	0	0	0
PC50	Total protein	0	0.1	0	0.2	0	0	0	4.3
PC51	Urate	0	0.1	3.3	1.5	6.7	0	0	0

Healthy women

Biomarker principal component		Artery	Musc	Gut	Cardiac	Metab	Inflam	Neuro	Lung
PC1	General adiposity	0	46.6	55.6	21.8	14.3	1.4	6.5	0.5
PC2	Total haemoglobin volume	1.3	2.3	0	0.2	0.2	0.1	0	0
PC3	Height	0	1.8	0.4	17.8	1.2	0	0	0
PC4	Albumin	3.7	1.0	0	0	0.1	7.0	0	0
PC5	Neutrophil count	0	0	1.0	4.3	0	0	0	5.5
PC6	Immature red blood cell volume	0.8	0.2	0	0	0	0.1	0	11.1
PC7	LDL and ApoB	3.9	0.4	0	0	5.7	0	0	0
PC8	Reticulocyte count	0	0.6	0	2.6	2.7	0	0	0
PC9	Urinary potassium and creatinine	0	0.1	0	0	0.1	0	1.4	1.2
PC10	Blood pressure	38.6	0.5	0	7.0	0.2	0	3.7	7.7
PC11	HDL and ApoA	6.3	0	1.5	0	3.9	3.0	0	0
PC12	Aminotransferases	0.7	0	0	0.6	0	3.7	0.1	0.4
PC13	Bilirubin	0.6	0	0	1.6	0.7	0	0	0
PC14	Platelet count	0	0	0.4	2.1	1.3	0	0	0
PC15	Red blood cell haemoglobin concentration	1.9	0.2	0	0.9	0	3.4	0	0
PC16	Testosterone	0	0	0	0	0.2	0.3	0	0
PC17	Lung function/height	10.7	0	0.5	9.1	1.1	0	27.4	43.8
PC18	Blood glucose	0.8	0	0	0	2.3	0.1	0	0
PC19	Platelet cell volume	0.4	0.1	1.2	0.2	0	1.0	0	0
PC20	LP(a)	0.4	0.8	0	0.4	0	0	0	0
PC21	Pairs matching test	0	0.7	0.5	0	0	0	12.2	0
PC22	Rheumatoid factor	0.5	0	0	0	0.3	17.5	5.4	0
PC23	Bone density	0	0.6	0	0.5	0	0	0	0
PC24	Vitamin D	0	18.2	2.7	0	0	0	1.3	0
PC25	IGF-1	1.5	0	0	1.0	0	0	0	0
PC26	Urinary microalbumin	0	0	0	0	1.8	0	0	4.4
PC27	Basophil count	0	0	0.8	0.3	0.3	0	0	17.1
PC28	Central adiposity	0.1	0	10.1	0	2.4	0	0	0.4
PC29	Eosinophil count	0.7	0	0	1.4	0.8	0.1	0	3.6
PC30	Alkaline phosphatase	1.1	0	0	0	0.2	0	0	0.7
PC31	Pulse rate	4.5	0.8	0.1	3.3	0	0	0	0
PC32	Red blood cell width	0	0	0	0	0	0	0	0
PC33	Reaction time test	0.3	0.8	2.7	0	0	0	0	0
PC34	Sex hormone-binding globulin	0	0.3	0	0.6	0.8	0	0	0
PC35	Hand grip strength/height	1.9	10.2	5.2	2.0	0	33.0	7.3	0
PC36	Phosphate	0.6	0.1	0.6	1.2	0.5	0.1	0	0
PC37	Lymphocyte count	5.0	0	0	4.7	0	0	0	0
PC38	Triglycerides	6.9	0	0.8	0.3	0.1	0	0	0
PC39	Urinary sodium	0.3	1.2	0	5.9	0.1	0	0	0
PC40	Monocyte count	0	0	0	0.2	1.8	0	2.1	0
PC41	Gamma glutamyltransferase	0.4	0.1	3.9	0.6	6.1	0	0	0
PC42	Urea	0	0.4	0.8	0	0.3	0.6	0	3.5
PC43	HbA1c	1.9	0.1	0	2.8	36.9	0	0	0
PC44	Platelet distribution width	0	0	0	0	0.1	0	0	0
PC45	Log C-reactive protein	0	0.1	3.5	0.3	0.5	15	1.9	0.2
PC46	Reticulocyte fraction	0.4	0.3	0.1	0	0.2	0	0	0
PC47	Cystatin C	0.5	0	1.4	1.6	10.1	5.5	1.1	0
PC48	Muscle mass	0	9.2	2.8	0.5	0.4	0	1.6	0
PC49	Calcium	0.1	1.3	0.1	2.5	0.8	0	0	0
PC50	Total protein	0	0.3	2.0	1.7	0.5	8	0	0
PC51	Urate	3.1	0.7	1.2	0	1.1	0	28.0	0

Musc: Musculoskeletal, Metab: metabolic disease, Inflam: inflammatory, Neuro: neurological

Table A6.2.4: Relative contribution of body system age deltas and chronological age (as percentages of the overall age effect) in explaining their respective disease outcome, in unadjusted models for the (A) main analysis and (B) when using the reduced biomarker panel

(A) Main analysis

	CA alone	CA and BA	BA alone
Healthy men			
Artery	67.7	-0.4	32.7
Musculoskeletal	58.7	-1.9	43.2
Gut	67.9	-1.3	33.4
Cardiac	75.7	-0.6	24.9
Metabolic disease	22.1	-0.9	78.8
Inflammatory	53.7	1.6	44.7
Neurological	67.8	2.2	30.0
Lung	47.6	-1.0	53.4
Healthy women			
Artery	65.1	-0.7	35.6
Musculoskeletal	77.1	-1.7	24.6
Gut	64.9	-1.5	36.6
Cardiac	75.7	-1.7	26.0
Metabolic disease	20.1	-1.1	81.0
Inflammatory	35.9	0.2	63.9
Neurological	74.3	1.3	24.4
Lung	51.9	-1.0	49.1
All men			
Artery	49.2	-0.4	32.7
Musculoskeletal	57.7	-1.9	43.2
Gut	45.7	-1.3	33.4
Cardiac	39.2	-0.6	24.9
Metabolic disease	3.0	-0.9	78.8
Inflammatory	33.4	1.6	44.7
Neurological	71.5	2.2	30.0
Lung	3.4	-1.0	53.4
All women			
Artery	17.6	-0.7	35.6
Musculoskeletal	52.4	-1.7	24.6
Gut	40.2	-1.5	36.6
Cardiac	40.7	-1.7	26.0
Metabolic disease	1.1	-1.1	81.0
Inflammatory	9.1	0.2	63.9
Neurological	52.9	1.3	24.4
Lung	6.7	-1.0	49.1

(B) Using the reduced biomarker panel

	CA alone	CA and BA	BA alone
Healthy men			
Artery	79.8	-0.5	20.7
Musculoskeletal	65.5	-1.6	36.1
Gut	73.9	-1.2	27.4
Cardiac	86.8	-1.5	14.7
Metabolic disease	27.3	-2.3	75.0
Inflammatory	60.2	1.3	38.5
Neurological	89.8	0.2	10.1
Lung	59.8	-1.7	41.9
Healthy women			
Artery	75.7	0.0	24.3
Musculoskeletal	80.2	-1.3	21.1
Gut	69.2	-1.7	32.5
Cardiac	83.8	-1.2	17.4
Metabolic disease	27.1	-3.3	76.2
Inflammatory	45.5	0.3	54.1
Neurological	76.2	1.6	22.2
Lung	68.8	-0.4	31.6

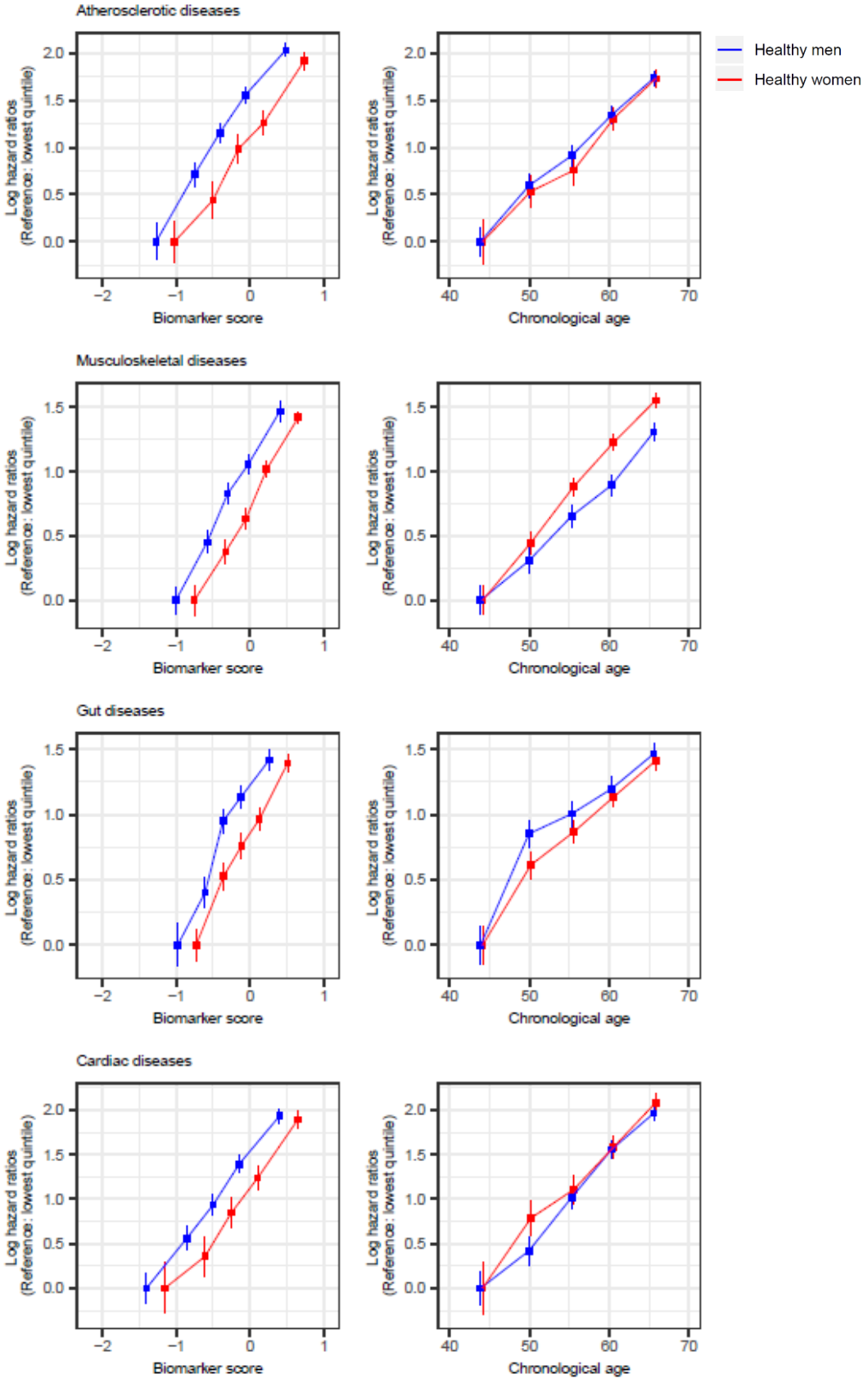
CA: chronological age, BA: body system age delta
The reduced biomarker panel consisted of 10 biomarker principal components for men and 10 for women: general adiposity, lung function/height, blood pressure, HbA1c, height and rheumatoid factor in both sexes; central adiposity, albumin, muscle mass and reticulocyte count in men; and hand grip strength/height, vitamin D, C-reactive protein and urate in women

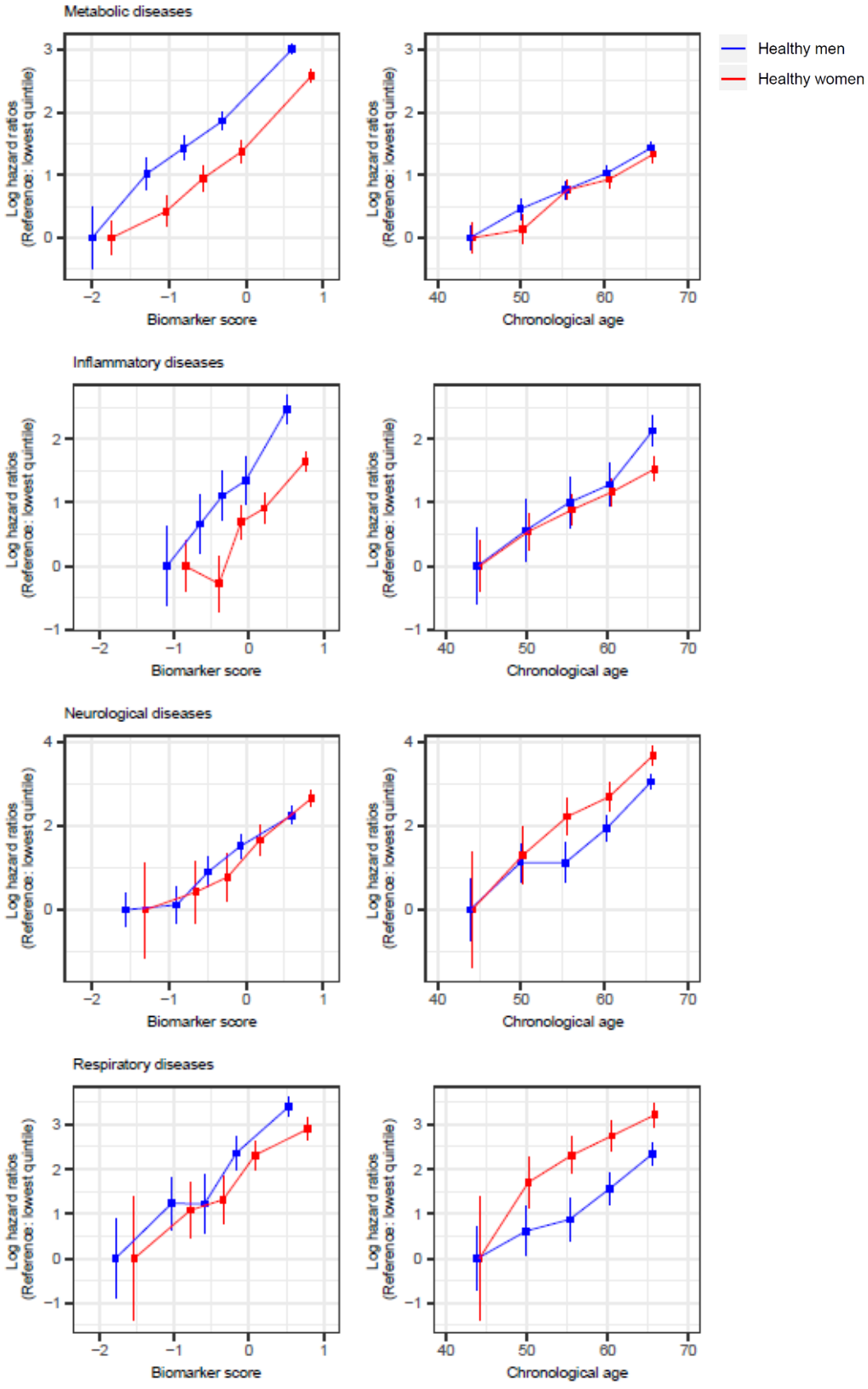
Table A6.2.5: Relative contribution of body system risk scores and chronological age (as percentages of the overall age effect) in explaining their respective disease outcome, in unadjusted models for healthy men and women

	CA alone	CA and risk score	Risk score alone
Healthy men			
Artery	14.5	56.5	29.0
Musculoskeletal	21.6	43.7	34.7
Gut	18.9	52.7	28.4
Cardiac	19.8	59.9	20.3
Metabolic disease	2.0	19.5	78.5
Inflammatory	14.2	41.9	43.9
Neurological	24.8	51.0	24.2
Lung	4.7	42.0	53.3
Healthy women			
Artery	7.8	58.4	33.9
Musculoskeletal	22.2	61.0	16.8
Gut	14.8	53.6	31.6
Cardiac	16.6	62.7	20.7
Metabolic disease	0.9	18.5	80.7
Inflammatory	6.3	31.1	62.6
Neurological	21.5	57.6	20.8
Lung	8.7	42.9	48.4

CA: chronological age

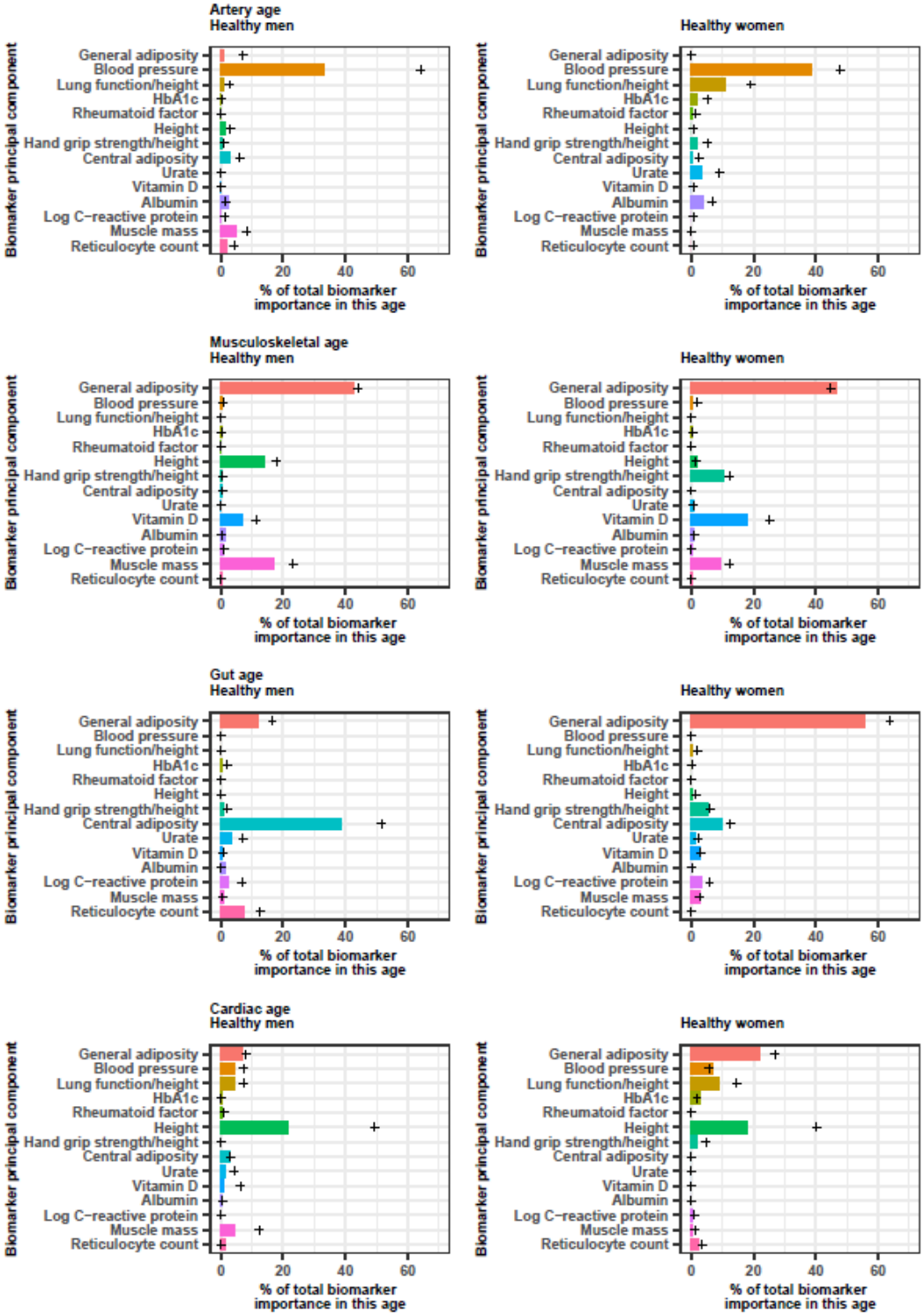
Figure A6.2.1: Hazard ratios for quintiles of chronological age and body system risk scores modelled separately for each of the eight disease groups, with the first two years of follow up omitted

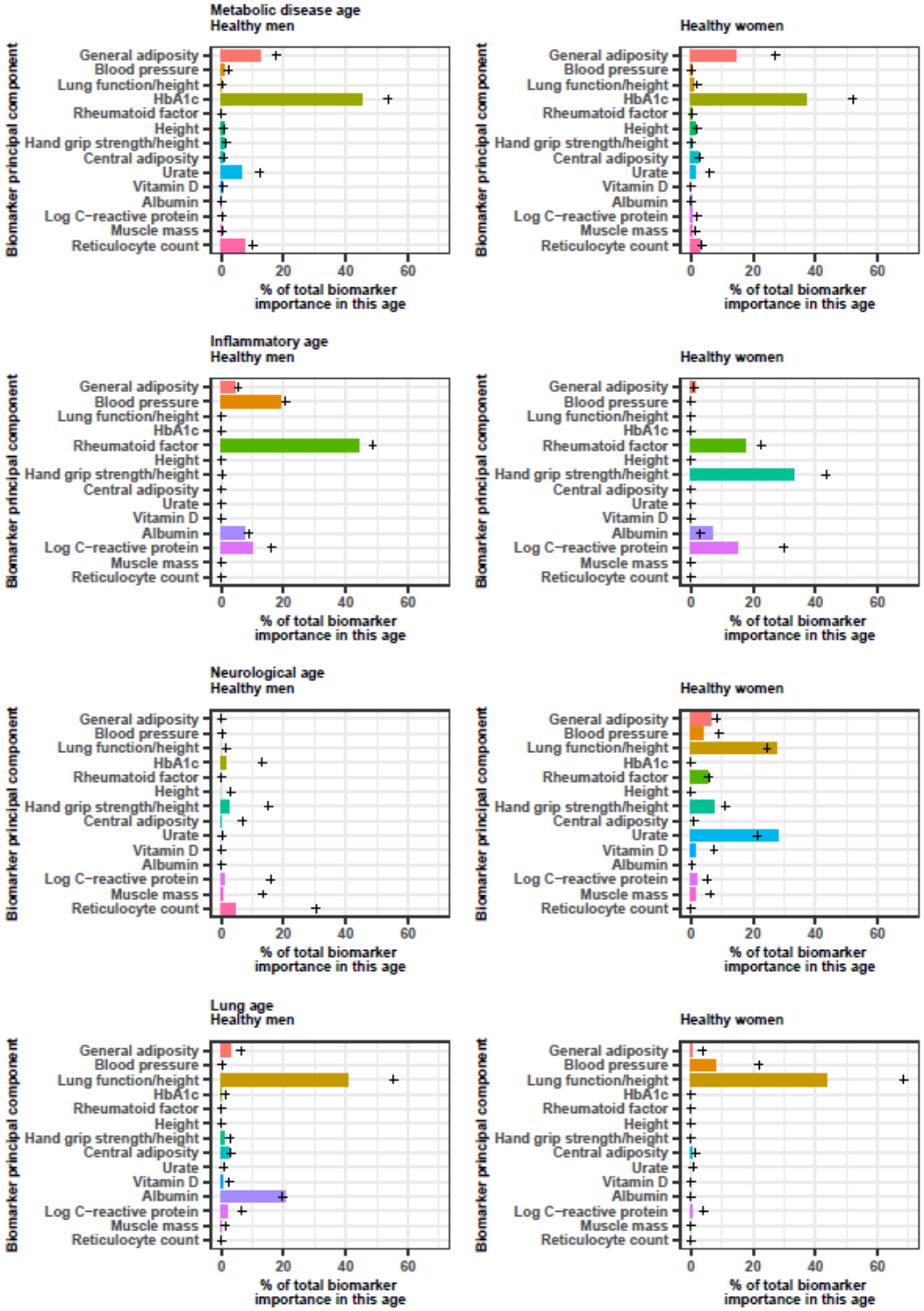




The error bars displayed are floating absolute risk 95% confidence intervals

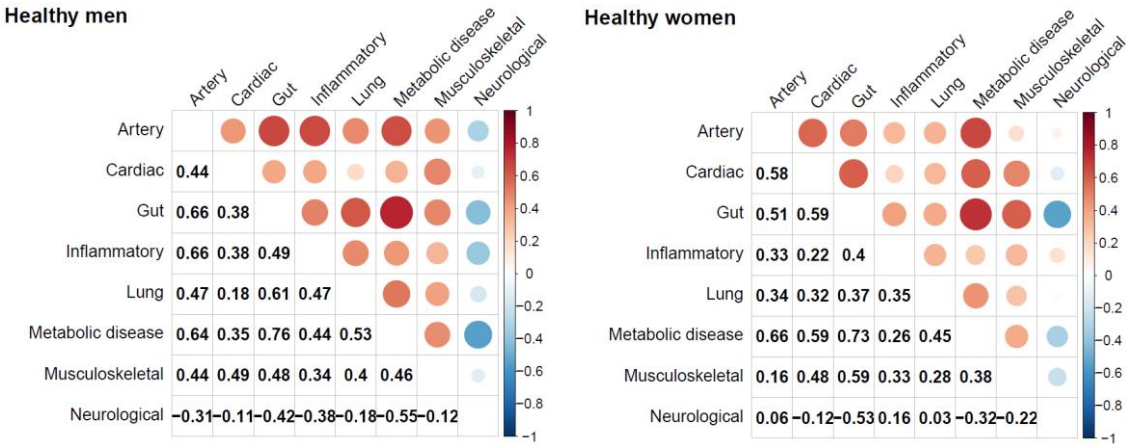
Figure A6.2.2: Importance of the top 14 biomarker principal components (% of explained relative risk by biomarker principal components) across the eight body system ages, compared to importance for body system risk scores (marked with '+') estimated from a reduced panel of these biomarker principal components only, for healthy men and healthy women





This biomarker panel consisted of the top 10 biomarker principal components for men and 10 for women: general adiposity, lung function/height, blood pressure, HbA1c, height and rheumatoid factor in both sexes; central adiposity, albumin, muscle mass and reticulocyte count in men; and hand grip strength/height, vitamin D, C-reactive protein and urate in women

Figure A6.2.3: Pearson correlations between body system age deltas estimated from the reduced biomarker panel of 14 biomarker principal components, for healthy men and women



Circle sizes correspond to the magnitude of the pairwise correlation
All correlation coefficients are statistically significant at the 95% level

The reduced biomarker panel consisted of the top 10 biomarker principal components for men and top 10 for women: general adiposity, lung function/height, blood pressure, HbA1c, height and rheumatoid factor in both sexes; central adiposity, albumin, muscle mass and reticulocyte count in men; and hand grip strength/height, vitamin D, C-reactive protein and urate in women

Appendix 6.3: Results from sensitivity analyses of body system ages

Introduction

This appendix serves as a counterpart to the analysis results for the body system ages estimated using the Cox lasso method, which were reported in Chapter 6. It details the results for the sensitivity analysis using the Cox stepwise regression method (described in Section 4.3), which was also applied to the same set of biomarker principal components as the Cox lasso method to estimate an alternative set of biological ages. The same validation methods were used for the stepwise regression and lasso biological ages (Section 4.5).

Since there was no known method for estimating a cross validated stepwise regression age, these ages could not be used in a prediction model without introducing circularity. Hence, this appendix only reports results for the biomarker principal components selected and weighted by the stepwise regression method, and the calibration of the stepwise regression ages to chronological age, for comparison with results for the main Cox lasso method.

Biomarker constituents of biological ages

The stepwise regression method included half or less of the 51 biomarker principal components in the estimation of the biological ages (Table A6.3.1). For healthy men, 10-30 biomarker principal components were included in each age; while for healthy women, 11-27 were included (Table A6.3.1). These included principal components were generally subsets of those selected by the lasso method (Tables A6.2.2 and A6.3.1).

Those biomarkers that featured most strongly in the lasso body system ages (Section 6.5) also featured most strongly in the stepwise regression ages, with few differences in the rankings of the most important biomarkers in each age between estimation methods (Figures 6.5.1 (A) vs A6.3.1).

The percentages of the most important biomarker principal components in the Cox stepwise regression ages and the sex differences broadly matched those for the Cox lasso ages (Figures 6.5.1 (A) vs A6.3.1), except lung function/height in lung ages, which despite remaining the top biomarker, had its importance reduced by 16% for healthy men and 20% for healthy women. Other substantial reductions in biomarker importance for the top biomarkers when the stepwise regression method was used were seen in gut, inflammatory and neurological ages (Figures 6.5.1 (A) vs A6.3.1).

Validation results

Each of the Cox stepwise body system ages had slightly worse calibration with chronological age, compared to the lasso ages (Figures A6.3.2 vs 6.7.1 (A)). The stepwise regression age was too high at younger chronological ages and too low at older chronological ages (Figure A6.3.2). The wider range covered by stepwise body system ages on average compared to the chronological age range in the UK Biobank contrasts with the narrower range covered by age-based stepwise biological ages on average (Figures A6.3.2 vs A5.3).

Discussion

For all body system ages, the Cox stepwise regression selected similar but fewer biomarker principal components than the Cox lasso method, potentially due to the application of the strict Bonferroni correction. The Cox stepwise regression method also resulted in less well-calibrated body system ages compared to the Cox lasso method, potentially due in part to its selection of fewer biomarkers. However, the narrower stepwise regression age range commonly seen in age-based biological ages in previous studies[20] and in this thesis (Figure A5.3) was not replicated. The use of fewer biomarker measurements may be preferred in clinical practice, however, due to the absence of an approach for cross validated Cox stepwise regression ages, further methodological development is required and the Cox stepwise regression method could not be recommended for application in this context at present.

Table A6.3.1: Model coefficients for each body system age (using the Cox stepwise regression method) in the healthy subpopulation, by sex

Healthy men

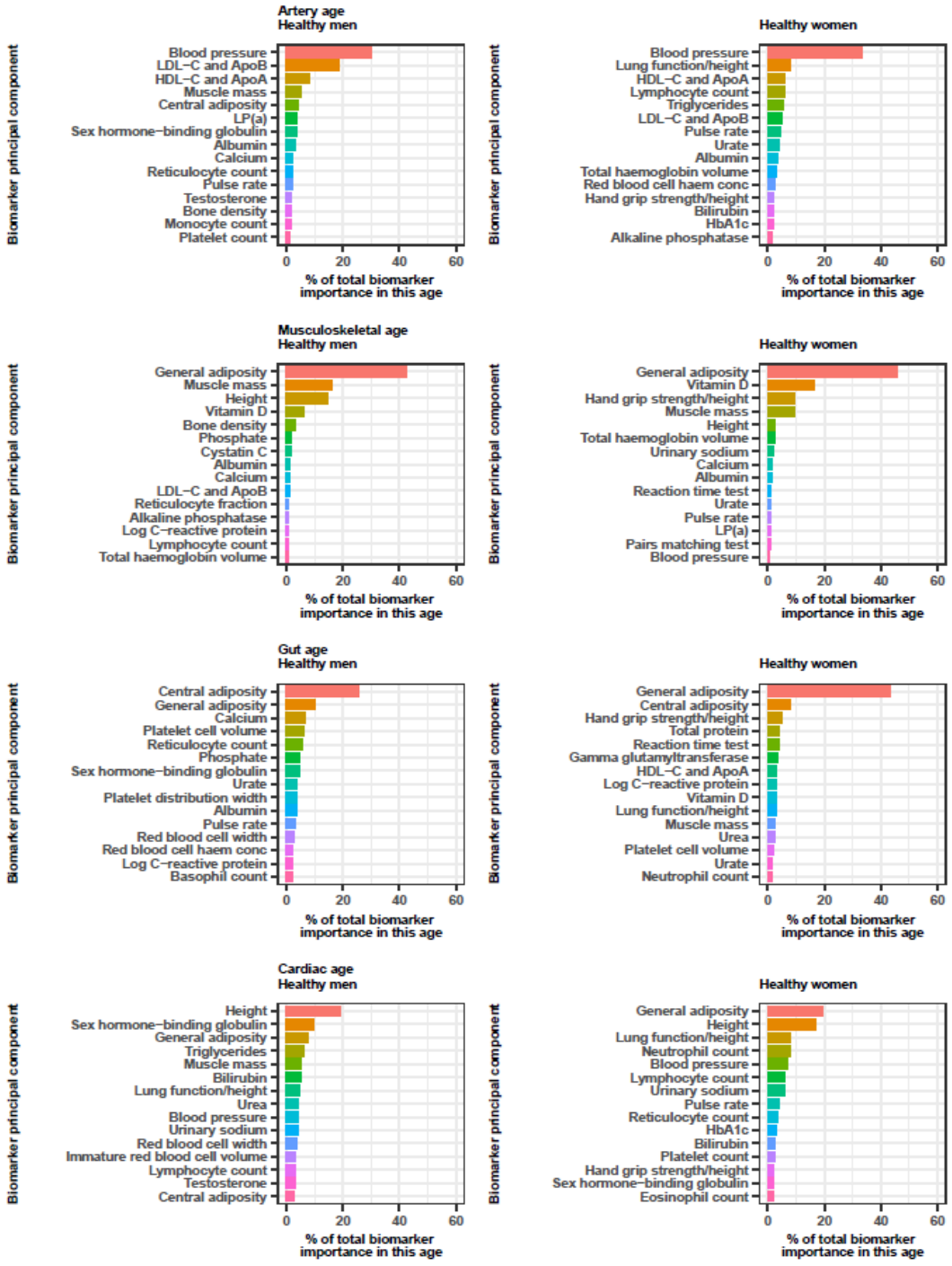
Biomarker principal component		Artery	Musc	Gut	Cardiac	Metab	Inflam	Neuro	Lung
PC1	General adiposity	0	-0.139	-0.035	-0.045	-0.118	-0.082	0	-0.092
PC2	Total haemoglobin volume	0	0.039	0	0	0	0	0	0
PC3	Height	0.027	0.134	0	-0.115	0.052	0	0	0
PC4	Albumin	0.077	0.063	0.050	0	0	0.191	0	0.418
PC5	Neutrophil count	0	0	0	-0.037	-0.043	0	0	0
PC6	Immature red blood cell volume	0	0	0	-0.054	-0.108	0	0	-0.150
PC7	LDL and ApoB	-0.132	0.041	0.028	0	0.086	0	0	0
PC8	Reticulocyte count	-0.047	-0.023	-0.045	0	-0.156	0	0.124	0
PC9	Urinary potassium and creatinine	0.031	0	0	0	0	0	0.142	0
PC10	Blood pressure	-0.193	0.030	0	-0.067	-0.070	-0.215	0	0.111
PC11	HDL and ApoA	-0.105	0.033	-0.031	0	-0.060	0	-0.123	0.149
PC12	Aminotransferases	0.036	0	0	0	0	0	0	0
PC13	Bilirubin	0	-0.026	0	-0.058	-0.064	0	0	0
PC14	Platelet count	-0.041	0	0	0	-0.092	0	0	0
PC15	Red blood cell haemoglobin concentration	0	0	-0.039	0.054	0.058	0	0	-0.206
PC16	Testosterone	0.081	0	-0.053	0.098	0.144	-0.234	0.153	0
PC17	Lung function/height	-0.037	0	0	-0.065	0	0	0	-0.337
PC18	Blood glucose	-0.054	0	0	0	0.142	0	0.185	0
PC19	Platelet cell volume	0	-0.030	-0.062	0	0	0	-0.119	0
PC20	LP(a)	0.085	0	0	0.045	0	0	0	0
PC21	Pairs matching test	0	0	0	0	0	0	-0.190	0
PC22	Rheumatoid factor	0	0	0	0	0	0.218	0	0
PC23	Bone density	0.053	0.090	0	0	0.061	0	-0.145	0
PC24	Vitamin D	0	0.128	0	0.040	-0.057	0	0	0.152
PC25	IGF-1	0	0	0	0	0.071	0	-0.285	0.137
PC26	Urinary microalbumin	0.040	0	0	0	0.060	0	0.156	0
PC27	Basophil count	0	0	0.043	0	0.074	0	0	0.176
PC28	Central adiposity	-0.093	0.037	-0.143	-0.074	0	0	0	-0.150
PC29	Eosinophil count	0	0	0	0	0	0.138	0	0.186
PC30	Alkaline phosphatase	-0.042	-0.055	0	0	-0.096	-0.186	0	0
PC31	Pulse rate	0.064	0	-0.047	0	0	0	0	-0.146
PC32	Red blood cell width	-0.050	0	-0.048	-0.086	0	0	0	0
PC33	Reaction time test	0	0.036	-0.036	0	-0.053	0	-0.178	0.143
PC34	Sex hormone-binding globulin	0.135	0	-0.093	0.198	0	0	0	0
PC35	Hand grip strength/height	0	0.042	0	0	0.079	0	0.124	0.133
PC36	Phosphate	0	-0.067	-0.055	-0.044	-0.046	-0.220	0	0
PC37	Lymphocyte count	0	0.052	0.039	0.076	0.090	0.181	0	0
PC38	Triglycerides	0	0	0	-0.097	0	0	-0.292	0
PC39	Urinary sodium	0	0	0	0.074	0	0	0	0
PC40	Monocyte count	0.055	0	0	0.070	0	0	0	0
PC41	Gamma glutamyltransferase	0	0.028	0	0	0.099	0	0	0
PC42	Urea	0	0.043	0	0.083	0	0	-0.131	0
PC43	HbA1c	0	0.046	0	0	0.676	0	-0.205	0
PC44	Platelet distribution width	0	-0.041	0.049	0.039	0.101	0	0	0
PC45	Log C-reactive protein	0	0.055	0.045	0	0	0.179	-0.137	0
PC46	Reticulocyte fraction	-0.039	-0.054	0	0	-0.087	0	0	0
PC47	Cystatin C	-0.050	0.094	0	0	-0.148	0	0.218	-0.198
PC48	Muscle mass	0.116	0.254	-0.04	0.109	0	0	0	0
PC49	Calcium	0.070	0.062	0.07	0	0.062	0	0	0
PC50	Total protein	0	0	0	0	0	0	0	-0.276
PC51	Urate	0	0	0.062	0.075	0.243	0	0	0
Coefficient for chronological age		0.062	0.062	0.052	0.080	0.038	0.078	0.148	0.067
Number of biomarker principal components selected		25	27	21	23	30	10	17	17

Healthy women

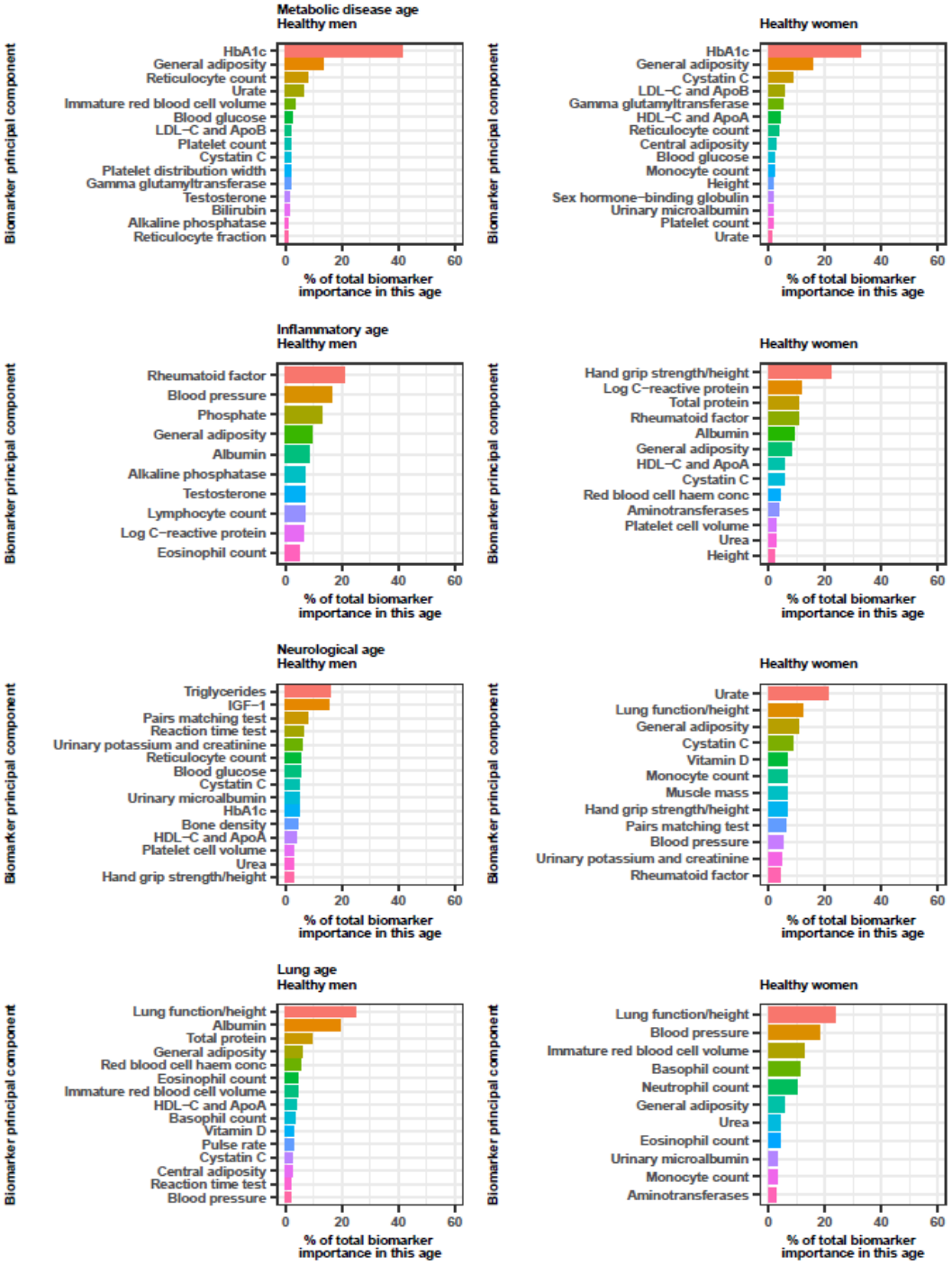
Biomarker principal component		Artery	Musc	Gut	Cardiac	Metab	Inflam	Neuro	Lung
PC1	General adiposity	0	-0.102	-0.084	-0.074	-0.130	0.082	0.089	-0.073
PC2	Total haemoglobin volume	0.073	0.056	0	0.053	0.073	0	0	0
PC3	Height	0	0.043	0.025	-0.118	0.077	-0.068	0	0
PC4	Albumin	0.087	0.049	0	0	0	0.211	0	0
PC5	Neutrophil count	0	0	-0.031	-0.088	0	0	0	-0.180
PC6	Immature red blood cell volume	0.037	0	0	0	0	0	0	-0.201
PC7	LDL and ApoB	-0.066	0.020	0	0	0.138	0	0	0
PC8	Reticulocyte count	0	0	0	-0.061	-0.122	0	0	0
PC9	Urinary potassium and creatinine	0	0	0	0	0	0	-0.123	0
PC10	Blood pressure	-0.191	0.026	0	-0.085	0	0	-0.109	0.256
PC11	HDL and ApoA	-0.076	0	-0.039	0	-0.122	-0.114	0	0
PC12	Aminotransferases	-0.054	0	0	0	0	-0.135	0	-0.127
PC13	Bilirubin	-0.048	0	0	-0.05	-0.049	0	0	0
PC14	Platelet count	0	0	0	-0.048	-0.076	0	0	0
PC15	Red blood cell haemoglobin concentration	-0.066	-0.021	0	-0.046	0	-0.134	0	0
PC16	Testosterone	0	0	0	0	0	0	0	0
PC17	Lung function/height	-0.091	0	0.039	-0.086	-0.059	0	-0.163	-0.270
PC18	Blood glucose	-0.064	0	0	0	0.141	0	0	0
PC19	Platelet cell volume	0	0	-0.041	0	0	-0.097	0	0
PC20	LP(a)	0	-0.036	0	0	0	0	0	0
PC21	Pairs matching test	0	-0.035	-0.033	0	0	0	-0.154	0
PC22	Rheumatoid factor	-0.049	0	0	0	0.049	0.173	0.110	0
PC23	Bone density	0	-0.034	0	0	0	0	0	0
PC24	Vitamin D	0	0.147	0.053	0	0	0	-0.169	0
PC25	IGF-1	0.05	0	0	0	0	0	0	0
PC26	Urinary microalbumin	0	0	0	0	0.134	0	0	0.165
PC27	Basophil count	0	0	0.034	0	0.054	0	0	0.233
PC28	Central adiposity	0	0	-0.092	0	-0.134	0	0	0
PC29	Eosinophil count	0.053	0	0	0.061	0.082	0	0	0.161
PC30	Alkaline phosphatase	-0.055	0	0	0	0	0	0	0
PC31	Pulse rate	0.100	0.039	0	0.089	0	0	0	0
PC32	Red blood cell width	0	0	0	0	0	0	0	0
PC33	Reaction time test	0	0.040	0.063	0	0	0	0	0
PC34	Sex hormone-binding globulin	0	0	0.029	0.054	0.093	0	0	0
PC35	Hand grip strength/height	0.068	0.118	0.073	0.065	0	0.325	0.169	0
PC36	Phosphate	-0.054	0	-0.039	-0.055	-0.069	0	0	0
PC37	Lymphocyte count	0.112	0	0	0.105	0	0	0	0
PC38	Triglycerides	0.134	0	0	0	0	0	0	0
PC39	Urinary sodium	0	0.055	0	0.109	0	0	0	0
PC40	Monocyte count	0	0	0	0	-0.136	0	-0.193	-0.161
PC41	Gamma glutamyltransferase	0	0	0.074	0	0.216	0	0	0
PC42	Urea	0	0.029	-0.052	0	0	-0.113	0	0.168
PC43	HbA1c	0.084	-0.040	0	0.102	0.638	0	0	0
PC44	Platelet distribution width	0	0	0	0	0	0	0	0
PC45	Log C-reactive protein	0	0	0.058	0	0.072	0.240	0	0
PC46	Reticulocyte fraction	-0.044	0	0	0	-0.073	0	0	0
PC47	Cystatin C	0	0	-0.047	-0.067	-0.317	-0.210	-0.242	0
PC48	Muscle mass	0	0.160	-0.073	0	0.100	0	0.232	0
PC49	Calcium	0	0.047	0.038	0	0.077	0	0	0
PC50	Total protein	0	0	0.064	0	-0.065	-0.214	0	0
PC51	Urate	0.123	-0.054	0.059	0	0.135	0	-0.420	0
Coefficient for chronological age		0.062	0.052	0.069	0.055	0.079	0.030	0.046	0.124
Number of biomarker principal components selected		23	20	22	19	27	13	12	11

Musc: Musculoskeletal, Metab: metabolic disease, Inflam: inflammatory, Neuro: neurological

Figure A6.3.1: Importance of the top 15 biomarker principal components (% of explained relative risk by biomarker principal components) in each Cox stepwise regression body system age, for



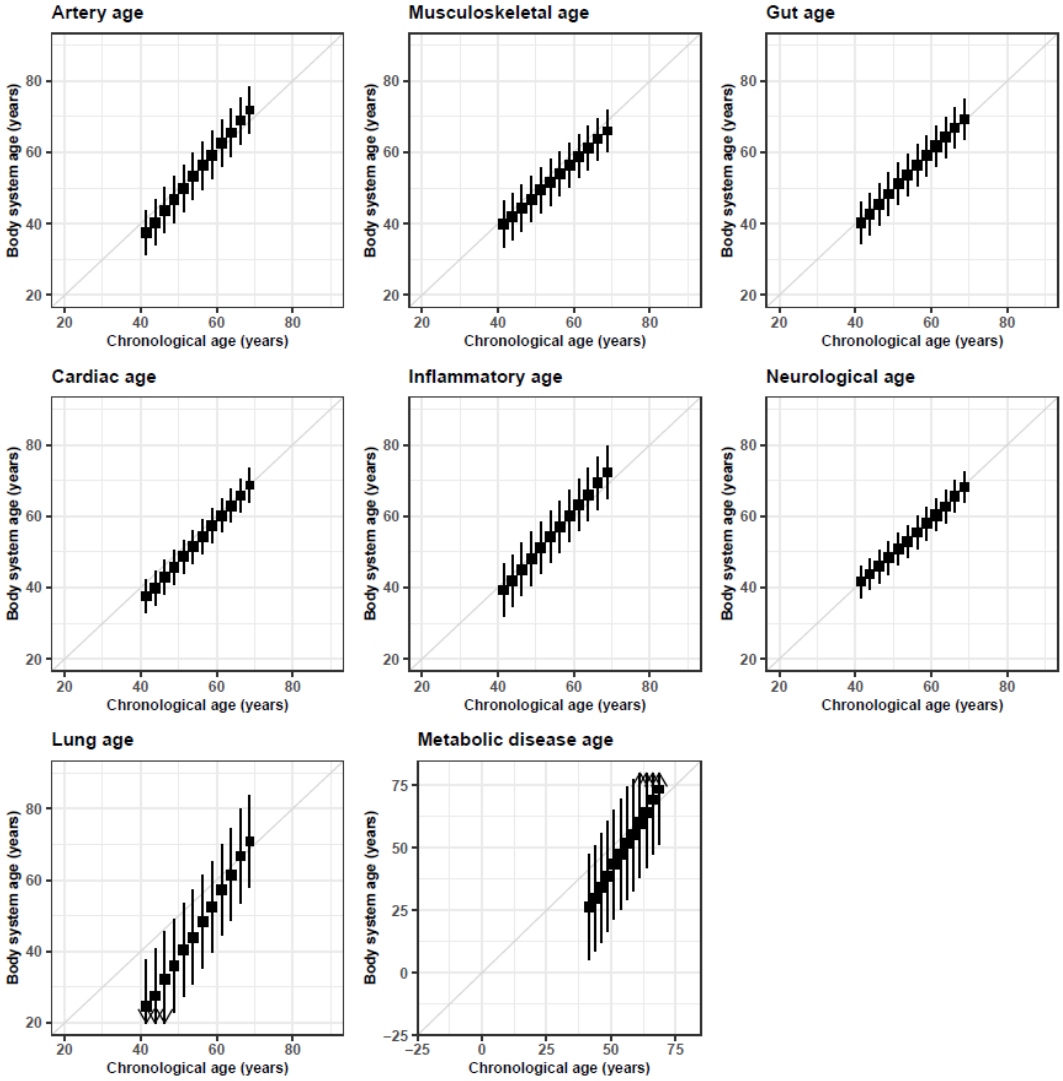
healthy men and healthy women



Fewer than 15 biomarkers were included in: inflammatory ages for men and women, neurological age for women and lung age for women

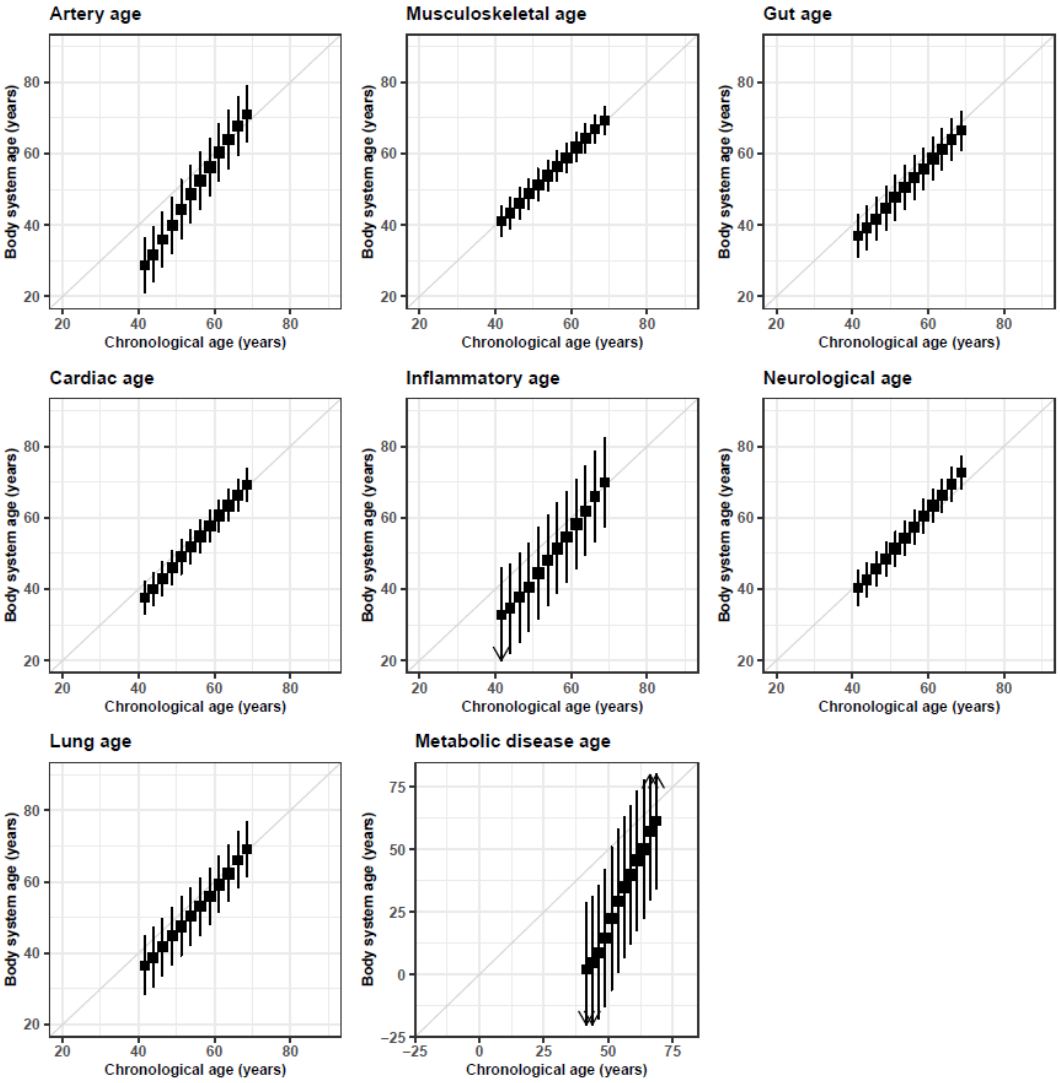
Figure A6.3.2: Means and standard deviations of Cox stepwise regression body system ages by 2.5-year chronological age groups, for healthy men and healthy women

Healthy men



Metabolic ages were plotted on an extended scale for the y-axis (body system age (years)) in order to display their full range

Healthy women



Metabolic ages were plotted on an extended scale for the y-axis (body system age (years)) in order to display their full range

Appendix 6.4: Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD) checklist for the body system age and risk score analysis

Section/Topic	Item*		Checklist Item	Section/Item
Title and abstract				
Title	1	D;V	Identify the study as developing and/or validating a multivariable prediction model, the target population, and the outcome to be predicted.	Section 4.3 (not in title)
Abstract	2	D;V	Provide a summary of objectives, study design, setting, participants, sample size, predictors, outcome, statistical analysis, results, and conclusions.	NA
Introduction				
Background and objectives	3a	D;V	Explain the medical context (including whether diagnostic or prognostic) and rationale for developing or validating the multivariable prediction model, including references to existing models.	Sections 1.3–1.4
	3b	D;V	Specify the objectives, including whether the study describes the development or validation of the model or both.	Section 1.4
Methods				
Source of data	4a	D;V	Describe the study design or source of data (e.g., randomized trial, cohort, or registry data), separately for the development and validation data sets, if applicable.	Section 3.2
	4b	D;V	Specify the key study dates, including start of accrual; end of accrual; and, if applicable, end of follow-up.	Section 3.2
Participants	5a	D;V	Specify key elements of the study setting (e.g., primary care, secondary care, general population) including number and location of centres.	Sections 3.2–3.3 and 3.9
	5b	D;V	Describe eligibility criteria for participants.	Sections 3.2–3.3
	5c	D;V	Give details of treatments received, if relevant.	NA
Outcome	6a	D;V	Clearly define the outcome that is predicted by the prediction model, including how and when assessed.	Section 3.5–3.7
	6b	D;V	Report any actions to blind assessment of the outcome to be predicted.	NA
Predictors	7a	D;V	Clearly define all predictors used in developing or validating the multivariable prediction model, including how and when they were measured.	Section 3.8 and 3.10
	7b	D;V	Report any actions to blind assessment of predictors for the outcome and other predictors.	NA
Sample size	8	D;V	Explain how the study size was arrived at.	Sections 3.2–3.3
Missing data	9	D;V	Describe how missing data were handled (e.g., complete-case analysis, single imputation, multiple imputation) with details of any imputation method.	Appendix 3.3
Statistical analysis methods	10a	D	Describe how predictors were handled in the analyses.	Sections 3.8 and 4.3
	10b	D	Specify type of model, all model-building procedures (including any predictor selection), and method for internal validation.	Sections 4.3 and 4.5
	10c	V	For validation, describe how the predictions were calculated.	Section 4.5
	10d	D;V	Specify all measures used to assess model performance and, if relevant, to compare multiple models.	Sections 4.3 and 4.5
	10e	V	Describe any model updating (e.g., recalibration) arising from the validation, if done.	Section 4.5 and 6.2
Risk groups	11	D;V	Provide details on how risk groups were created, if done.	Section 4.3
Development vs. validation	12	V	For validation, identify any differences from the development data in setting, eligibility criteria, outcome, and predictors.	NA

Section/Topic	Item*		Checklist Item	Section/Item
Results				
Participants	13a	D;V	Describe the flow of participants through the study, including the number of participants with and without the outcome and, if applicable, a summary of the follow-up time. A diagram may be helpful.	Section 3.9
	13b	D;V	Describe the characteristics of the participants (basic demographics, clinical features, available predictors), including the number of participants with missing data for predictors and outcome.	Section 3.9 and Appendix 3.3
	13c	V	For validation, show a comparison with the development data of the distribution of important variables (demographics, predictors and outcome).	NA
Model development	14a	D	Specify the number of participants and outcome events in each analysis.	Section 3.9
	14b	D	If done, report the unadjusted association between each candidate predictor and outcome.	NA
Model specification	15a	D	Present the full prediction model to allow predictions for individuals (i.e., all regression coefficients, and model intercept or baseline survival at a given time point).	Tables A6.2.1–A6.2.2
	15b	D	Explain how to use the prediction model.	Section 4.3
Model performance	16	D;V	Report performance measures (with CIs) for the prediction model.	Tables 6.7.1–6.7.2
Model-updating	17	V	If done, report the results from any model updating (i.e., model specification, model performance).	NA
Discussion				
Limitations	18	D;V	Discuss any limitations of the study (such as nonrepresentative sample, few events per predictor, missing data).	Section 6.8
Interpretation	19a	V	For validation, discuss the results with reference to performance in the development data, and any other validation data.	NA
	19b	D;V	Give an overall interpretation of the results, considering objectives, limitations, results from similar studies, and other relevant evidence.	Section 6.8
Implications	20	D;V	Discuss the potential clinical use of the model and implications for future research.	Section 6.8
Other information				
Supplementary information	21	D;V	Provide information about the availability of supplementary resources, such as study protocol, Web calculator, and data sets.	Section 3.2
Funding	22	D;V	Give the source of funding and the role of the funders for the present study.	Acknowledgements

This analysis is a development and internal validation study according to the TRIPOD guidelines[164]
* Items relevant to model development are denoted by 'D', items relating to model validation are denoted by 'V'

Appendix 7.1: Supplementary results for the aggregation of body system ages into an overall disease risk-based biological age

Introduction

The aggregation of body system ages into an overall biological age involved preliminary comparisons of biological ages aggregated using 4 methods: multi-state model (MSM age; the main method), Cox model with mortality from chronic disease as the outcome (Cox mortality age), Cox model with age-related frailty as the outcome (Cox frailty age), and the Klemera Doubal method (KDM age) (Section 7.2). Biological ages aggregated by the main MSM method are assessed and the comparisons between methods are summarised in Chapter 7, while further details of the results for these candidate biological ages and the calibrations of each of these candidate ages to chronological age are summarised in this appendix.

Biomarker and body system age constituents of biological ages

The model coefficients for the MSM age for the healthy subpopulation are summarised in Table A7.1, while the numbers of transitions between each of the 3 health states in the MSM during follow up are listed in table A7.2. The model coefficients for the remaining 3 aggregation methods are summarised in Tables A7.3–A7.4. Coefficient values greater than 0 indicate that higher body system age deltas contributed to higher aggregated biological ages, and vice versa for coefficients below 0. As the model structures differed between these 4 methods and the coefficients were not directly comparable, the coefficients for body system ages in each model were reweighted to sum to 1 (Table A7.5) in order to provide a basis for comparison across aggregation methods in Section 7.3. Additionally, since the overall disease risk-based biological ages were linear combinations of body system ages (Tables A7.1, A7.3 and A7.4), which in turn are linear combinations of biomarker principal components (Table A6.2.2), the effective biomarker principal component coefficients for the overall biological ages could be calculated and are summarised in Table 7.3.1 for the MSM age

and Table A7.6 for all 4 aggregation methods. The top 15 biomarker principal components, in terms of the magnitudes of the coefficients in the MSM, were displayed in Figure A7.1. These coefficients for the top 15 principal components varied between aggregation methods but were generally in the same direction across aggregation methods and sexes (Figure A7.1). The coefficients for the MSM and Cox frailty aggregation models were similar and had similar magnitudes of standard errors, while the standard errors for the Cox mortality model coefficients were larger (Tables A7.1 and A7.3).

In Table A7.6, the coefficients for biological ages aggregated via Cox mortality and frailty models applied to body system ages in the healthy subpopulation were also compared to those for Cox lasso models for the same 2 outcomes directly applied to biomarker principal components. The coefficients for these 2 sets of Cox models for the same 15 biomarker principal components were also displayed, in Figure A7.2. These coefficients were broadly similar across the type of Cox model and within health outcomes. The key differences for the direct method were: for mortality, muscle mass and central adiposity were given larger weights for men, while reaction time was given a larger weight and general adiposity, blood pressure and albumin smaller weights for women; for frailty, lung function/height, general adiposity, triglycerides and insulin-like growth factor 1 were given smaller weights for men, while muscle mass, lung function/height, general adiposity, rheumatoid factor and urate were given smaller weights for women (Figure A7.2).

Calibration of biological ages to chronological age

The calibration of biological ages aggregated by all 4 methods to chronological age were assessed, and they were well calibrated for all methods (Figure A7.3). Standard deviations of biological ages for each chronological age group were largest for the KDM-aggregated age (≈15 years), relatively small for the Cox mortality and frailty ages and smallest for the MSM-aggregated age (≈3 years for Cox mortality, Cox frailty and MSM ages; 95% confidence intervals displayed in Figure A7.3).

Discussion

The additional results in this appendix enabled further investigation of the patterns of body system ages and biomarker principal components that were most strongly associated with chronological age (KDM) or most predictive of mortality or frailty (Cox models) or most predictive of both mortality and frailty (MSM method). Since the MSM method combined the prediction of both outcomes in a single statistical approach, this thesis focused on the use of the MSM aggregation method to estimate disease risk-based biological age, however these alternative biological ages are also well-calibrated to chronological age and may be of interest in single-outcome or other research contexts.

Table A7.1: Model terms (means and standard errors for coefficients and intercepts) for the disease risk-based multi-state model-aggregated biological ages in the healthy subpopulation, by sex

	Healthy men	Healthy women
Coefficients for body system age deltas		
Artery	0.006 (0.002)	0.005 (0.002)
Musculoskeletal	0.005 (0.002)	0.024 (0.003)
Gut	0.013 (0.003)	-0.002 (0.003)
Cardiac	0.010 (0.003)	0.003 (0.003)
Metabolic disease	0.000 (0.001)	0.000 (0.000)
Inflammatory	0.004 (0.002)	0.005 (0.001)
Neurological	0.013 (0.003)	0.010 (0.005)
Lung	0.004 (0.001)	0.003 (0.002)
Coefficients for chronological age at risk		
Non-frail -> frail transition	0.062 (0.001)	0.051 (0.001)
Non-frail -> dead transition	0.088 (0.009)	0.088 (0.012)
Frail -> dead transition	0.084 (0.006)	0.056 (0.006)
Intercept		
Non-frail -> frail transition	-5.058 (0.032)	-4.897 (0.031)
Non-frail -> dead transition	-9.235 (0.216)	-9.910 (0.285)
Frail -> dead transition	-6.365 (0.159)	-6.006 (0.161)

Hazard ratios for the coefficients can be obtained by taking the exponential of these values
The coefficients for the body system age deltas were constrained to be the same across transition types, while those for chronological age at risk were not constrained

Table A7.2: Numbers of transitions between states and non-transiting individuals in the multi-state models for healthy men and for healthy women

Types of transitions or states	Healthy men	Healthy women
Transitions		
Non-frail -> frail	8278	9409
Non-frail -> dead	226	144
Frail -> dead	640	518
Non-transitions		
Non-frail	44,129	53,243
Frail	15,075	16,575

Transitions from the frail state to non-frail state and from the dead (from chronic disease) state to any state were not permitted in the model structure

Table A7.3: Model coefficients (means and standard errors) for the Cox-aggregated biological ages using mortality from chronic disease or (B) age-related frailty as the outcome of interest, in the healthy subpopulation, by sex

Body system age delta / outcome	Mortality from chronic disease		Age-related frailty	
	Healthy men	Healthy women	Healthy men	Healthy women
Artery	0.012 (0.006)	0.007 (0.006)	0.006 (0.002)	0.004 (0.002)
Musculoskeletal	-0.011 (0.006)	0.010 (0.012)	0.007 (0.002)	0.026 (0.003)
Gut	0.027 (0.008)	-0.011 (0.011)	0.013 (0.003)	-0.003 (0.003)
Cardiac	0.011 (0.008)	0.040 (0.011)	0.012 (0.003)	0.001 (0.003)
Metabolic disease	0.004 (0.002)	0.000 (0.002)	-0.001 (0.001)	0.000 (0.000)
Inflammatory	0.022 (0.006)	0.013 (0.003)	0.001 (0.002)	0.005 (0.001)
Neurological	0.038 (0.009)	0.007 (0.017)	0.013 (0.004)	0.011 (0.005)
Lung	0.004 (0.003)	-0.012 (0.006)	0.005 (0.001)	0.005 (0.002)

Hazard ratios for these coefficients can be obtained by taking the exponential of these values

Table A7.4: Model coefficients for the Klemera Doubal-aggregated biological ages in the healthy subpopulation, by sex

Body system age delta	Healthy men			Healthy women		
	q_j	k_j	s_j	q_j	k_j	s_j
Artery	-15.699	0.280	6.348	-39.093	0.616	7.256
Musculoskeletal	0.802	-0.040	5.977	-3.868	0.066	4.005
Gut	-8.299	0.130	5.493	-10.614	0.133	5.638
Cardiac	-11.458	0.169	4.455	-12.361	0.191	4.111
Metabolic disease	-50.305	0.795	22.510	-127.325	1.656	31.960
Inflammatory	-12.750	0.211	5.537	-29.706	0.501	11.955
Neurological	-1.217	0.013	3.246	-5.582	0.112	2.489
Lung	-50.580	0.777	11.689	-17.938	0.271	6.566

q_j = intercept, k_j = coefficient and s_j = square root of residual variance for the j^{th} body system age constituent

Table A7.5: Relative weights of constituent body system ages in the overall biological ages for the healthy subpopulation, by aggregation method and by sex, in the (A) main analysis and (B) excluding metabolic disease ages from the aggregation, for healthy men and women

(A) Main analysis

Body system age delta / aggregation method	Healthy men				Healthy women			
	Multi-state	Cox (mortality)	Cox (frailty)	Klemera Doubal	Multi-state	Cox (mortality)	Cox (frailty)	Klemera Doubal
Artery	0.103	0.109	0.104	0.204	0.096	0.131	0.081	0.193
Musculoskeletal	0.093	-0.108	0.121	-0.032	0.491	0.192	0.522	0.068
Gut	0.232	0.250	0.227	0.126	-0.051	-0.209	-0.051	0.069
Cardiac	0.182	0.102	0.222	0.251	0.066	0.733	0.017	0.186
Metabolic disease	-0.005	0.038	-0.016	0.046	0.001	-0.009	0.002	0.027
Inflammatory	0.077	0.210	0.026	0.202	0.113	0.247	0.105	0.058
Neurological	0.240	0.361	0.228	0.037	0.212	0.130	0.216	0.297
Lung	0.077	0.037	0.088	0.167	0.072	-0.214	0.107	0.103

(B) Excluding metabolic disease age

Body system age delta / aggregation method	Healthy men				Healthy women			
	Multi-state	Cox (mortality)	Cox (frailty)	Klemera Doubal	Multi-state	Cox (mortality)	Cox (frailty)	Klemera Doubal
Artery	0.103	0.110	0.104	0.214	0.097	0.126	0.082	0.198
Musculoskeletal	0.092	-0.087	0.119	-0.034	0.489	0.199	0.519	0.070
Gut	0.223	0.312	0.196	0.132	-0.048	-0.237	-0.047	0.071
Cardiac	0.181	0.115	0.220	0.263	0.067	0.741	0.020	0.191
Metabolic disease	-	-	-	-	-	-	-	-
Inflammatory	0.077	0.198	0.024	0.212	0.113	0.252	0.105	0.059
Neurological	0.246	0.316	0.246	0.039	0.210	0.145	0.213	0.305
Lung	0.077	0.037	0.090	0.175	0.072	-0.226	0.107	0.106

Weights: Coefficients of the body system age deltas in the model, rescaled to sum to 1

Table A7.6: Biomarker principal component coefficients for each biological age aggregation method, by sex

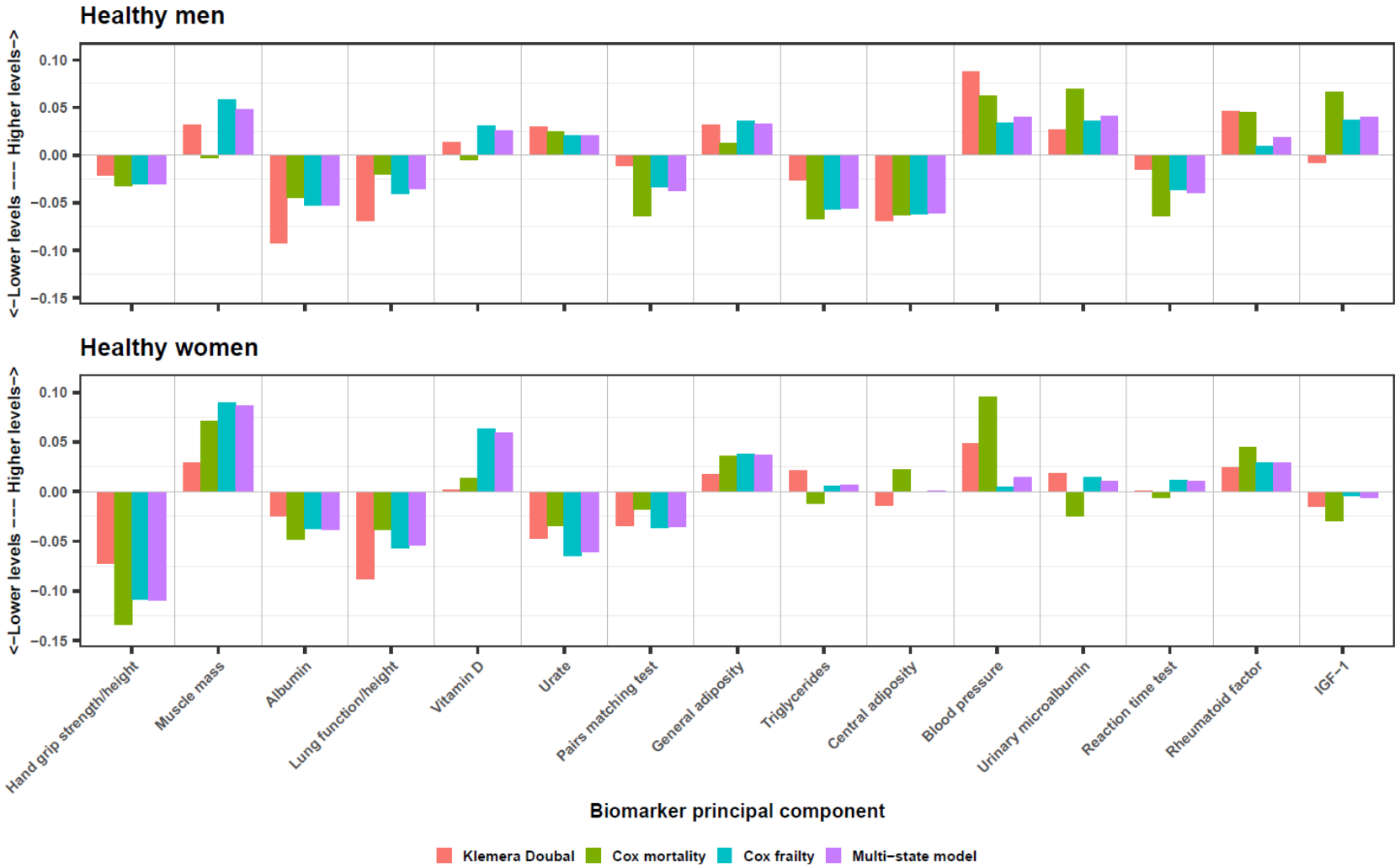
Healthy men

Biomarker principal component		Aggregated from body system ages				Estimated directly from biomarker principal components	
		Klemera Doubal	Cox (mortality)	Cox (frailty)	Multi-state	Mortality	Frailty
PC1	General adiposity	-0.031	-0.012	-0.036	-0.033	-0.012	-0.016
PC2	Total haemoglobin volume	0.004	-0.001	0.008	0.006	0.092	0.028
PC3	Height	-0.024	-0.020	-0.007	-0.006	0	0.003
PC4	Albumin	0.092	0.044	0.053	0.053	0.053	0.045
PC5	Neutrophil count	-0.020	-0.012	-0.014	-0.013	-0.018	-0.005
PC6	Immature red blood cell volume	-0.038	-0.014	-0.024	-0.021	0	0
PC7	LDL and ApoB	-0.022	-0.010	-0.006	-0.006	0	0.002
PC8	Reticulocyte count	-0.024	0.003	-0.005	-0.004	0	-0.011
PC9	Urinary potassium and creatinine	0.008	0.027	0.019	0.020	0	-0.006
PC10	Blood pressure	-0.087	-0.062	-0.034	-0.040	-0.094	-0.017
PC11	HDL and ApoA	-0.013	-0.020	-0.005	-0.007	-0.041	-0.022
PC12	Aminotransferases	0.007	0.013	0.010	0.010	0	0
PC13	Bilirubin	-0.019	-0.018	-0.022	-0.020	-0.030	-0.021
PC14	Platelet count	-0.016	-0.009	-0.008	-0.008	0	0
PC15	Red blood cell haemoglobin concentration	-0.013	-0.006	-0.007	-0.007	-0.023	-0.016
PC16	Testosterone	0.028	0.018	0.024	0.020	-0.014	-0.005
PC17	Lung function/height	-0.069	-0.020	-0.041	-0.036	0	-0.015
PC18	Blood glucose	0.002	0.042	0.019	0.022	0.080	0
PC19	Platelet cell volume	-0.004	-0.026	-0.022	-0.023	-0.022	-0.007
PC20	LP(a)	0.024	0.005	0.012	0.010	0.023	0
PC21	Pairs matching test	-0.011	-0.064	-0.033	-0.037	-0.005	-0.006
PC22	Rheumatoid factor	0.046	0.045	0.009	0.019	0.052	0
PC23	Bone density	0.008	-0.030	-0.002	-0.005	-0.035	0
PC24	Vitamin D	0.014	-0.005	0.031	0.025	-0.007	0.018
PC25	IGF-1	0.008	-0.066	-0.037	-0.040	0	0
PC26	Urinary microalbumin	0.027	0.070	0.036	0.041	0.044	0.039
PC27	Basophil count	0.034	0.018	0.020	0.020	0.011	0.017
PC28	Central adiposity	-0.069	-0.062	-0.062	-0.060	-0.131	-0.059
PC29	Eosinophil count	0.035	0.012	0.014	0.014	0	0
PC30	Alkaline phosphatase	-0.034	-0.028	-0.014	-0.019	-0.058	-0.042
PC31	Pulse rate	-0.004	-0.009	-0.005	-0.006	-0.082	-0.005
PC32	Red blood cell width	-0.030	-0.019	-0.031	-0.028	-0.056	-0.067
PC33	Reaction time test	-0.015	-0.064	-0.036	-0.039	-0.067	-0.016
PC34	Sex hormone-binding globulin	0.076	0.031	0.039	0.034	0.117	0.072
PC35	Hand grip strength/height	0.021	0.033	0.030	0.030	0.062	0.020
PC36	Phosphate	-0.031	-0.025	-0.026	-0.028	0.006	-0.011
PC37	Lymphocyte count	0.029	0.017	0.025	0.024	0	0.012
PC38	Triglycerides	-0.026	-0.067	-0.057	-0.055	-0.001	-0.014
PC39	Urinary sodium	0.020	0.009	0.015	0.013	-0.039	0
PC40	Monocyte count	0.026	0.014	0.021	0.018	0.112	0.025
PC41	Gamma glutamyltransferase	0.002	0.002	0.002	0.002	0.047	0
PC42	Urea	0.017	-0.013	0.010	0.005	0	0.022
PC43	HbA1c	0.041	0.007	-0.007	-0.003	0.026	0
PC44	Platelet distribution width	0.014	0.017	0.005	0.006	0.040	0.001
PC45	Log C-reactive protein	0.048	0.019	0.016	0.020	0.062	0.027
PC46	Reticulocyte fraction	-0.016	-0.004	-0.017	-0.014	0	0
PC47	Cystatin C	-0.054	0.002	0.006	0.005	-0.036	-0.028
PC48	Muscle mass	0.032	-0.003	0.058	0.048	0.060	0.039
PC49	Calcium	0.022	0.014	0.024	0.023	0	0.027
PC50	Total protein	-0.017	-0.002	-0.009	-0.008	-0.015	-0.002
PC51	Urate	0.029	0.024	0.020	0.021	0	0
Coefficient for chronological age		-	-	-	-	0.075	0.049

Healthy women

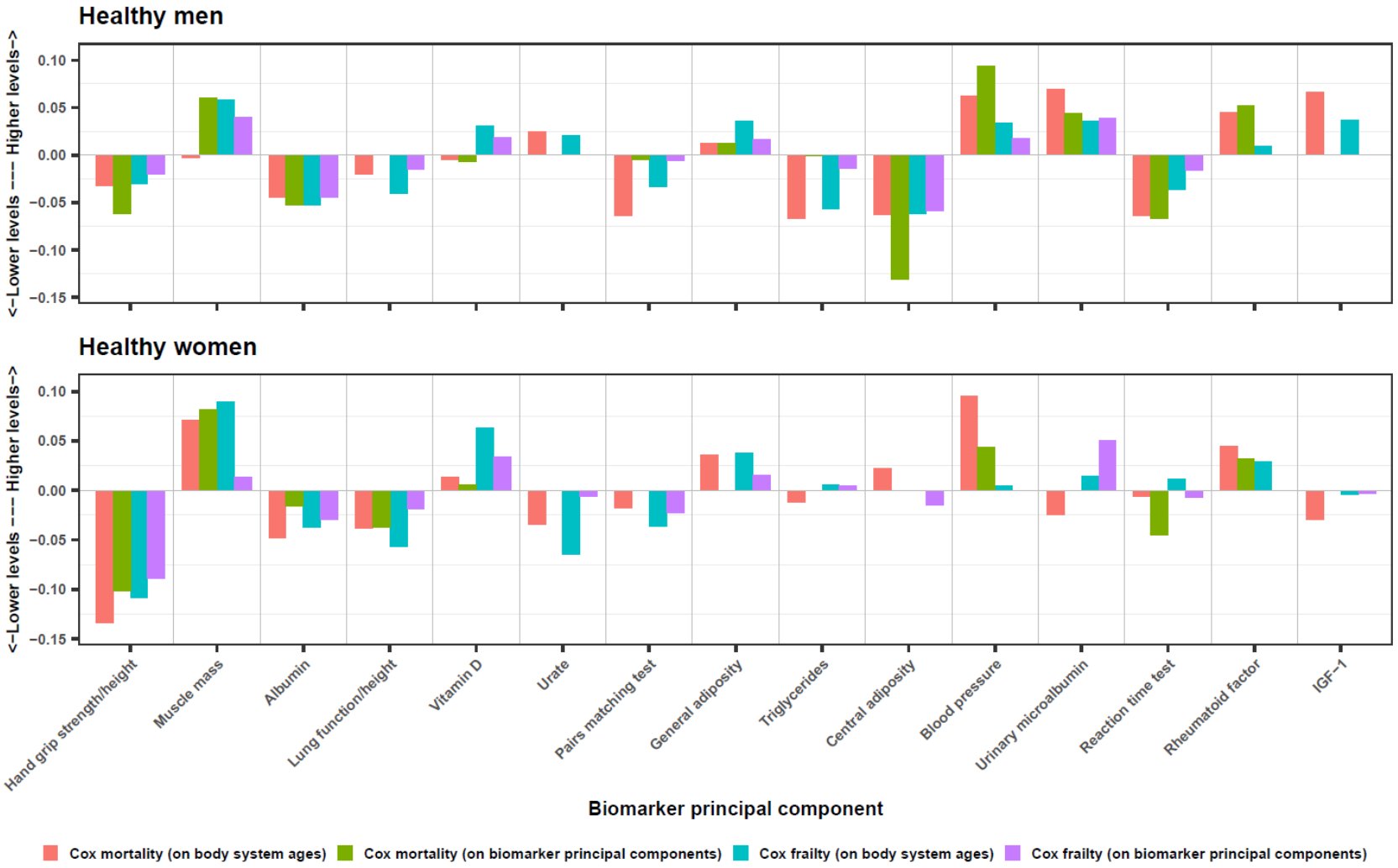
		Aggregated from body system ages				Estimated directly from biomarker principal components	
Biomarker principal component		**Klemera Doubal**	**Cox (mortality)**	**Cox (frailty)**	**Multi-state**	**Mortality**	**Frailty**
PC1	General adiposity	-0.017	-0.036	-0.037	-0.037	0	-0.015
PC2	Total haemoglobin volume	0.016	0.030	0.031	0.031	0.025	0.057
PC3	Height	-0.014	-0.071	0.014	0.008	-0.085	0
PC4	Albumin	0.024	0.048	0.037	0.038	0.015	0.029
PC5	Neutrophil count	-0.019	-0.018	-0.008	-0.007	0	0
PC6	Immature red blood cell volume	-0.008	0.021	-0.017	-0.012	0	0
PC7	LDL and ApoB	-0.005	-0.005	0.004	0.003	-0.005	0.009
PC8	Reticulocyte count	-0.012	-0.035	-0.011	-0.013	-0.024	0
PC9	Urinary potassium and creatinine	-0.006	-0.015	-0.007	-0.008	0	0
PC10	Blood pressure	-0.048	-0.095	-0.005	-0.015	-0.043	0
PC11	HDL and ApoA	-0.020	-0.018	-0.013	-0.014	-0.059	-0.041
PC12	Aminotransferases	-0.013	-0.002	-0.018	-0.017	-0.060	-0.016
PC13	Bilirubin	-0.011	-0.026	-0.003	-0.004	-0.038	0
PC14	Platelet count	-0.009	-0.028	-0.001	-0.003	0.031	0.009
PC15	Red blood cell haemoglobin concentration	-0.021	-0.053	-0.021	-0.023	-0.041	-0.007
PC16	Testosterone	0.004	0.045	0.018	0.019	0	-0.041
PC17	Lung function/height	-0.088	-0.038	-0.057	-0.054	-0.037	-0.019
PC18	Blood glucose	-0.005	-0.012	-0.005	-0.006	0.027	-0.012
PC19	Platelet cell volume	-0.011	-0.020	-0.011	-0.012	-0.043	0
PC20	LP(a)	-0.002	-0.017	-0.013	-0.013	0.050	-0.003
PC21	Pairs matching test	-0.034	-0.017	-0.036	-0.035	0	-0.023
PC22	Rheumatoid factor	0.024	0.045	0.029	0.029	0.032	0
PC23	Bone density	-0.007	-0.024	-0.015	-0.015	0	-0.055
PC24	Vitamin D	0.002	0.014	0.063	0.059	0.006	0.034
PC25	IGF-1	0.015	0.030	0.004	0.006	0	0.004
PC26	Urinary microalbumin	0.018	-0.025	0.015	0.010	0	0.051
PC27	Basophil count	0.023	-0.028	0.017	0.012	-0.022	0.010
PC28	Central adiposity	-0.014	0.023	-0.001	0	0	-0.015
PC29	Eosinophil count	0.026	0.020	0.015	0.014	-0.034	0.039
PC30	Alkaline phosphatase	-0.009	0.004	-0.004	-0.003	0	0.001
PC31	Pulse rate	0.031	0.065	0.024	0.028	-0.021	0
PC32	Red blood cell width	0	0.001	0	0	-0.054	-0.014
PC33	Reaction time test	0.001	-0.006	0.011	0.010	-0.045	-0.008
PC34	Sex hormone-binding globulin	0.008	0.019	0.009	0.009	0.051	0.032
PC35	Hand grip strength/height	0.073	0.134	0.108	0.110	0.102	0.089
PC36	Phosphate	-0.018	-0.035	-0.011	-0.013	0	0
PC37	Lymphocyte count	0.030	0.067	0.008	0.013	0.001	0.024
PC38	Triglycerides	0.021	-0.012	0.006	0.006	0	0.005
PC39	Urinary sodium	0.023	0.077	0.024	0.028	0.005	0.008
PC40	Monocyte count	-0.015	0.007	-0.011	-0.010	0	-0.007
PC41	Gamma glutamyltransferase	0.023	0.016	0.008	0.009	-0.023	0.032
PC42	Urea	0.008	-0.019	0.018	0.014	-0.007	-0.001
PC43	HbA1c	0.043	0.057	-0.001	0.004	0	0
PC44	Platelet distribution width	0	0	0	0	0.066	0
PC45	Log C-reactive protein	0.010	0.045	0.018	0.019	0.073	0.009
PC46	Reticulocyte fraction	-0.006	-0.001	-0.012	-0.011	0.019	0.005
PC47	Cystatin C	-0.049	-0.078	-0.031	-0.035	-0.028	-0.035
PC48	Muscle mass	0.029	0.071	0.090	0.087	0.082	0.014
PC49	Calcium	0.016	0.044	0.021	0.022	0.003	0.015
PC50	Total protein	0.003	-0.005	-0.006	-0.005	0	0
PC51	Urate	-0.047	-0.035	-0.064	-0.061	0	-0.006
Coefficient for chronological age		-	-	-	-	0.067	0.040

Figure A7.1: Comparison of coefficients for biomarker principal components across disease risk-based biological age aggregation methods, for healthy men and healthy women



The 15 biomarker principal components displayed have the largest absolute coefficients (from left to right) in the multi-state model-aggregated biological ages, across sexes
E.g. lower hand grip strength/height most strongly featured in the biological ages, followed by higher muscle mass, in terms of the total variation of the biomarker levels in the population

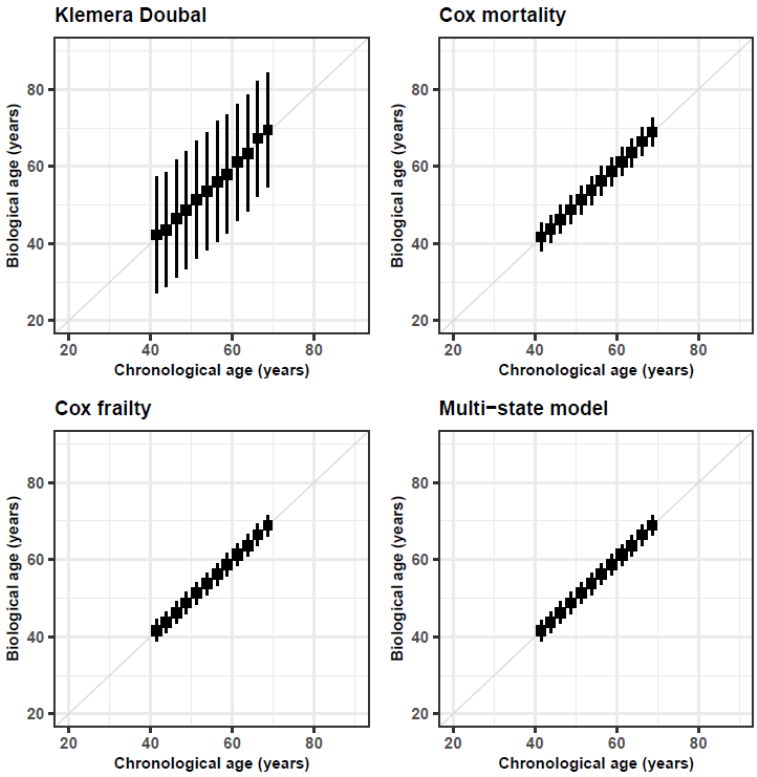
Figure A7.2: Comparison of coefficients for biomarker principal components for biological ages aggregated via Cox models applied to body system ages vs Cox lasso models directly applied to biomarker principal components, for healthy men and healthy women



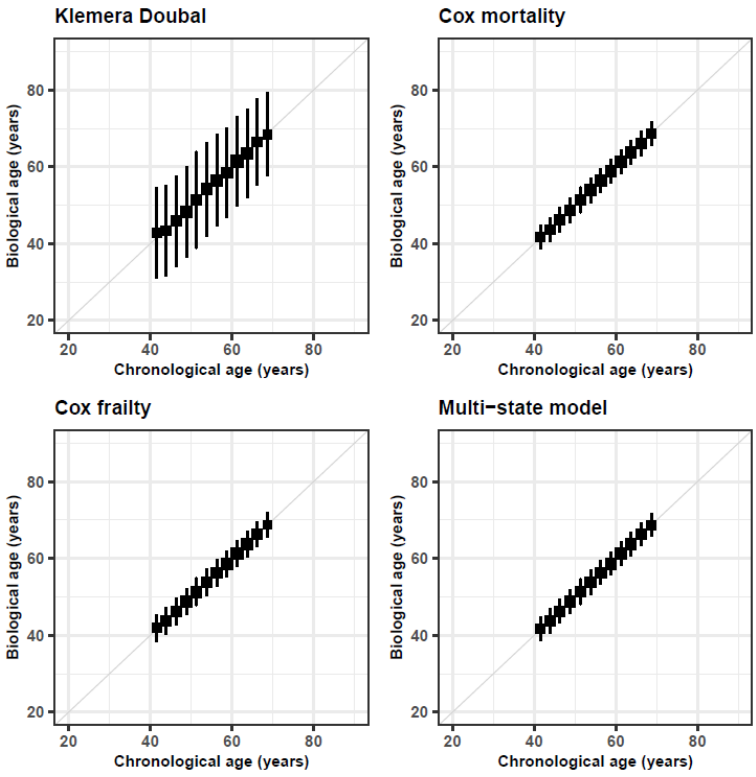
The 15 biomarker principal components displayed have the largest absolute coefficients (from left to right) in the multi-state model-aggregated biological ages, across sexes
E.g. lower hand grip strength/height most strongly featured in the biological ages, followed by higher muscle mass, in terms of the total variation of the biomarker levels in the population

Figure A7.3: Means and standard deviations of disease risk-based biological ages aggregated using 4 different methods, by 2.5-year chronological age groups, for healthy men and healthy women

Healthy men



Healthy women



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