MEASURING SENESCENCE IN HUMAN POPULATIONS

J. J. E. Koopman

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The cover shows *El Tiempo o Las Viejas* (*Time or The Old Women*) by Francisco José de Goya y Lucientes (1746–1828), who painted it between 1810 and 1812 at an age of around 65 years. By this time Goya had experienced several life-threatening illnesses and had become deaf. The back of the mirror reads „Que tal?" („How are you?"). By courtesy of Palais des Beaux-Arts de Lille, France. Reproduced with permission.

MEASURING SENESCENCE IN HUMAN POPULATIONS

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Temple of Apollo, Delphi, Greece

Εἴ τις δοκεῖ ἐγνωκέναι τι, οὔπω ἔγνω καθὼς δεῖ γνῶναι
I Corinthians 8:2

INDEX

1 GENERAL INTRODUCTION

Ageing and senescence

As humans grow older, the structures and functions of their bodies deteriorate. As a consequence, their risks of disability, disease, and death increase. It is an omen of the various confusions and controversies existing in the research on this process that the process itself has no universally accepted designation.

In line with renowned gerontologists, we distinguish between ageing and senescence.[1-5] Ageing refers to the mere passage of time. It encompasses all changes that occur in the body during time, whether their effects are detrimental, beneficial, or negligible. The progress of ageing is indicated by one's chronological age, which can be easily deduced from a birth registry. Senescence is part of ageing. It refers to the deterioration of the body's structures and functions and encompasses specifically the detrimental changes that appear with ageing. The progress of senescence is indicated by one's biological age, although it is still elusive how one's biological age can be precisely determined.[6-10]

Apart from senescence, ageing is accompanied by changes that are beneficial to the body's structures and functions. Such changes take place in a programmed order early in life as growth and development, are brought about by the body as regeneration when it repairs its damaged parts, for example during the healing of a fractured bone,[11] and can be effectuated by medical interventions, like the replacement of stem cells, which is called rejuvenation.[12] Ageing is also accompanied by changes that are, as far as we know, neither detrimental nor beneficial to the body. A classic example is the greying of hair. These changes are simply spoken of as age-related changes.[13]

Some researchers are accustomed to denote the senescence of cells in particular as senescence and to denote the senescence of individuals or populations as ageing.[14-18] However, as will be substantiated hereafter, there is no reason to fundamentally separate cellular senescence from senescence of individuals or populations.

Senescence at different levels

Senescence is generally attributed to an accumulation of random damage to the human body.[14,19-22] During life, the body is exposed to a wide variety of intrinsic stressors from within the body and extrinsic stressors from without the body. Examples of intrinsic stressors include DNA replication errors, spontaneous chemical reactions, and metabolic waste products. Examples of extrinsic stressors include pathogens, radiation, and mechanical forces. A stressor may at any moment damage the body's structure and function. In the case of sufficient damage, death may follow. In other cases, damage repair mechanisms, with which the body's cells and tissues are equipped, are activated. Some of the acquired damage can be repaired fully by these mechanisms, after which the body will have regenerated and recovered. Some damage can be repaired only partly or not at all, will irreversibly remain, and become apparent as dysfunction, disability, or disease. If a stressor affects the damage repair mechanisms, the accumulation of further damage is accelerated.

Alternative theories attribute senescence to other processes than the accumulation of random damage. A noteworthy theory, which is discussed in more detail in Chapter 5 of this thesis,[23] proposes that senescence may be the result of persistent developmental processes.[24,25] Nonetheless, these theories too acknowledge that the damaging effects of such processes are crucial in the causation of senescence.[23-26]

As schematically shown in Figure 1.1, the accumulation of damage occurs in the body at different levels of complexity. Stressors may damage the structure and function of a molecular component of a cell, an entire cell, an organ or tissue, or the body as a whole. Much research is devoted to the mechanisms of senescence at the molecular-cellular level.[17,27,28] Important roles have been attributed to DNA damage,[29-33] the effects of metabolic waste products such as reactive oxygen species,[34-37] the various forms of dysfunction that are observed in senescent cells,[27,38] the accumulation of senescent cells in tissues,[15,18,27,39] and chronic smouldering inflammation called inflammageing.[40]

The effects of damage acquired at lower levels seep through to higher levels of complexity in the body.[20,21,24,28,41] Molecular damage underlies cellular senescence.[17,27,38] Senescent cells secrete numerous signalling factors that induce senescence of other cells[17,27,38] and systemic symptoms of senescence, such as chronic smouldering inflammation.[42] Cellular senescence causes dysfunction and disease of tissues, such as impaired wound healing, cancer, and cardiovascular disease.[16,43-45] Molec-

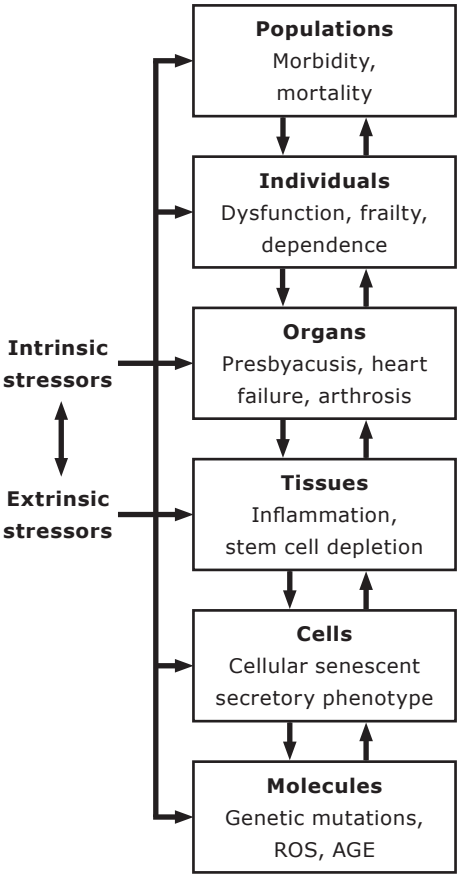

Figure 1.1 • Schematic overview of the accumulation of damage at different levels in the body during senescence. Intrinsic and extrinsic stressors, in interaction, damage the human body at different levels of complexity. The damage at the lower levels seeps through to the higher levels. The damage at the higher levels probably also drips down to the lower levels. ROS: reactive oxygen species. AGE: advanced glycation end products.

ular and cellular senescence are the basis of disorders characterised by similar clinical features of accelerated senescence.[46] The accumulation of damage in the body's structures and functions and the development of disabilities and diseases culminate in an increasing risk of death.[2,19,20,24,41,47-49]

Measuring senescence at the population level

As senescence manifests at different levels, it follows that it can be measured at different levels. For example, it can be measured at the molecular-cellular level through DNA lesions and cell cycle arrests, at the level of tissues and organs through inflammation and vascular calcification, and at the level of the body as a whole through instability and physical disability. In this thesis, we measure senescence in human populations. At the population level, senescence is defined as an increase in the risks of dysfunction, disease, and death with increasing chronological age.[2,19,47,50,51] A constant risk of death during ageing marks the absence of senescence.[52,53]

Knowledge of senescence can be obtained by studying molecules, cells, individuals, but also populations. These approaches are fundamentally different, but yield equally valuable and complementary insights. Observing and comparing populations with different characteristics is especially relevant in order to understand how the course of senescence can vary, what determinants account for this variation, and what preventive measures can ameliorate the senescence process.[54,55]

Aims of this thesis

In Part I of this thesis we investigate how a population's senescence can be measured through the increase in mortality with age. In Part II of this thesis we investigate how senescence can be measured through the increase in morbidity in a non-western population and thus be compared with the senescence process in western populations. A more detailed introduction to each research question is given at the opening of each part of this thesis.

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PAR

MEASURING SENESCENCE THROUGH MORTALITY

INTRODUCTION TO PART I

Age patterns of mortality

Humans die at any moment in their lives. As a result of senescence, the risk of death increases with chronological age.

The Panel on the next page shows three manners in which a population's senescence can be visualised with age patterns of mortality.[1,2] Firstly, the number of individuals that have survived from birth decreases with age until the entire population has died and this decrease quickens with age (Figure A in the Panel). Secondly, the number of individuals that have died is greater at higher than at lower ages (Figure B in the Panel). Thirdly, the mortality rate increases with age (Figure C in the Panel). As explained in the Panel, the age patterns of a population's survival, deaths, and mortality rate are directly related and can be derived from each other.

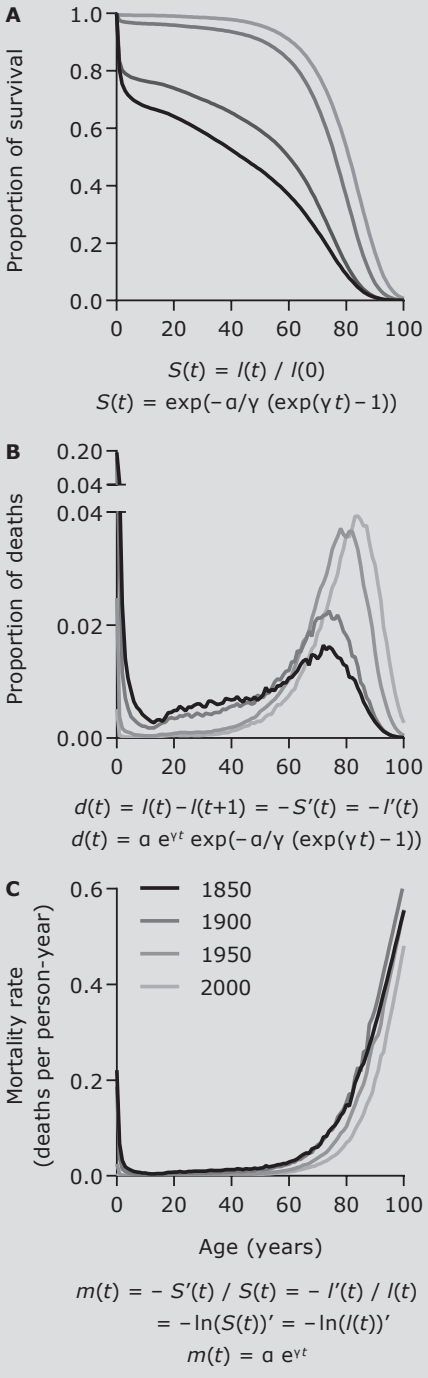
If senescence is the consequence of an accumulation of damage during life, it is plausible that senescence starts at conception. However, only from adolescence onward can senescence be discerned in age patterns of mortality. Early in life, the decline in survival slackens, the number of deaths decreases, and the mortality rate falls (Panel). Age patterns of mortality are the net result of senescence together with its opposing forces of growth, development, and regeneration. The balance between these processes determines whether an age pattern displays senescence. Early in life, growth, development, and regeneration dominate senescence and are of main influence on mortality.[3-6] During life, growth and development regress or derail and the body's capacity to regenerate diminishes.[7] When sufficient time has elapsed and enough damage has accumulated, a deterioration in the body's structures and functions becomes visible as an increase in the risk of death. This is schematically shown in Figure 2.1.

Although some gerontologists have drawn attention to the importance of early-life mortality for research on senescence,[4,5] we depart from the customary approach to leave out of consideration that mortality is high immediately after birth and decreases during childhood to its lowest levels in adolescence. Senescence is, in that manner, studied from adolescence onward, when mortality starts to increase with age. We will discuss the relevance of patterns of early-life mortality in Chapter 4 of this thesis.[8]

Modelling age patterns of mortality

Age patterns of mortality are commonly described using parametric models. Such models suit analytical purposes that are not or not well served by non-parametric models: they are capable of estimating age patterns of mortality continuously, thus for each specific age or age category, their estimated age patterns are precise and smooth, they can be used to assess the effects of measured variables on age patterns of mortality, and they enable the extrapolation and prediction of mortality beyond the observed ages and along alterations in these variables.[16,17]

A wide variety of parametric models exists that describe age patterns of mortal-



Panel • A population's senescence visualised with age patterns of mortality

Age patterns of mortality are shown for different years in the Netherlands.[14] The proportion of individuals that have survived from birth to a given age (A) and the proportion of individuals that have died at a given age (B) are shown relatively to the total number of individuals that have died at any age during life. The age pattern of deaths can be derived from the age pattern of survival, because the number of deaths at any age is the difference in the number of survivors between that age and the consecutive age, which equals the negative derivative function of the age pattern of survival. An age-specific mortality rate (C) can be interpreted as the rate at which individuals die at a given age. It describes the number of deaths per person-year, which is the number of deaths per period of time lived by the individuals in the population of that age. It can be derived from the age pattern of survival as the number of deaths relative to the number of survivors at any age, which equals the negative derivative function of the logarithmically transformed age pattern of survival.

Equations are given that describe the age patterns of survival S, deaths d, and mortality rate m non-parametrically[1] as dependent on the number of living individuals l at age t and according to the Gompertz model[9-13] as dependent on the model's parameters α and γ.

ity, among which the exponential model, gamma model, log-logistic model, Weibull model, and Gompertz model.[17] The Gompertz model is most commonly used in research on senescence.[11,13,18,19]

The British mathematician and actuary Benjamin Gompertz (1779–1865) was the first to observe that mortality rates increase exponentially with age in human populations and captured his observation mathematically as the model that was later named after him.[19,20] The Gompertz model describes the age pattern of a population's mortality rate as dependent on two parameters α and γ (see the Panel). The mortality rate equals α at age t = 0. An age in adolescence, around which mortality is lowest during life, is chosen as t = 0, because the Gompertz model cannot account for the decrease in early-life mortality. From this age onward, the mortality rate is modelled to increase exponentially from the minimal level α to the extent of γ.

The Gompertz model can be extended with a third parameter β that adds to the exponential increase in mortality in an age-independent manner. This model is known as the Gompertz-Makeham model.[9,11,13] As the parameter β is negligible in human populations, the Gompertz model is preferred over the Gompertz-Makeham model.[13,21,22]

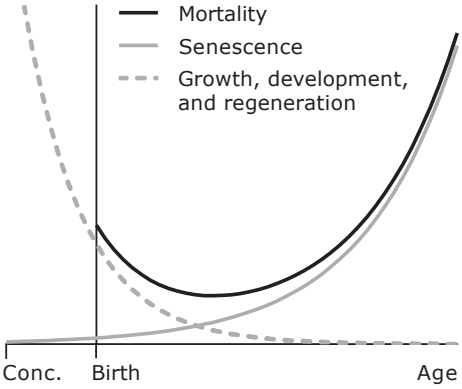


Figure 2.1 • Schematic illustration of the age pattern of mortality as a net result of senescence as well as growth, development, and regeneration. Similar schemes have been proposed by others.[15] Conc.: conception.

Measuring senescence through age patterns of mortality

A population's senescence can be measured through its age patterns of mortality in various manners. From the age pattern of survival, the mean, median, and maximal lifespan can be calculated. From the age pattern of deaths, the mean, median, and modal age at death can be calculated. The mean lifespan equals the mean age at death; both are more commonly referred to as the life expectancy.[23] These measures of senescence each represent a different aspect of a population's age pattern of survival or deaths and should be applied in a supplementary manner. However, as these measures summarise the varying levels of mortality throughout life in a single age-independent constant, they are little informative about its age pattern.[23,24]

Since senescence manifests in human populations as an increase in mortality rate with age, senescence is preferably and commonly measured through age patterns of mortality rates. This can be achieved by evaluating the age pattern of a population's

mortality rate, by graphically comparing the age patterns of different populations, and by calculating the age-specific differences of their mortality rates.[2,25-27]

The classical measure of a population's senescence rate

The Gompertz model is often preferred for modelling age patterns of mortality rates because it has the following advantage. When the Gompertz model is transformed logarithmically, the mortality rate increases linearly with age from adolescence onward. This linear increase is described by the model as: $\ln m(t) = \ln \alpha + \gamma t$ (compare with the Panel). The visual assessment of linear age patterns is much easier than that of exponential age patterns, as illustrated by Figure 2.2. Moreover, the linear increase in the mortality rate, thus the slope of the straight curve, is solely described by the model's parameter γ, as this parameter equals the derivative function of the aforementioned logarithmically transformed model. Differences in the slope of the straight curves are easily discernible on a logarithmic scale (Figure 2.2B).

The linear increase in the mortality rate on a logarithmic scale, thus the slope of the straight curve described by γ, is classically equated with the population's senescence rate. Both consciously and unconsciously this interpretation is widely used to interpret research on senescence.

From the linear increase in the mortality rate on a logarithmic scale, the period of time can be calculated during which a population's mortality rate doubles. This mortality rate doubling time (MRDT) is directly related to the slope of the straight curve described by γ: $\text{MRDT} = \ln 2 / \gamma$. The MRDT has been proposed as a more comprehensible variant of the slope of the straight curve as the measure of a population's senescence rate.[28]

Critique of the classical measure of a population's senescence rate

Although originally the Gompertz model is a purely empirical model, it has acquired the status of a law or dogma that is thought to apply universally to the age patterns of mortality rates of human populations.[19,21,29,30] Yet, the use of the linear increase in the mortality rate on a logarithmic scale, described by the model's parameter γ, as a measure of a population's senescence rate is a theoretical assumption that has never been empirically tested. Meanwhile, it has been objected that this measure of a population's senescence rate may be false.[31-35]

The critique concerns the logarithmic transformation of the Gompertz model, which is necessary for the model to adopt a linear age pattern.[35] This can be illustrated by the following observation.

At the end of the Second World War, civilians in Indonesia were caught as prisoners of war in a Japanese concentration camp. The conditions of life, which were similar for all prisoners, were harsh. They slept in crowded barracks on a small mattress, a couple of planks, or bare stones. Infectious diseases thrived. Hunger was commonplace with estimated average rations of

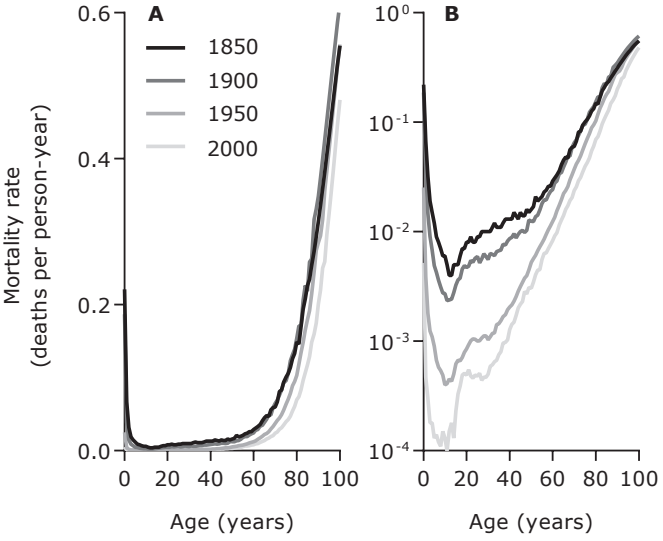
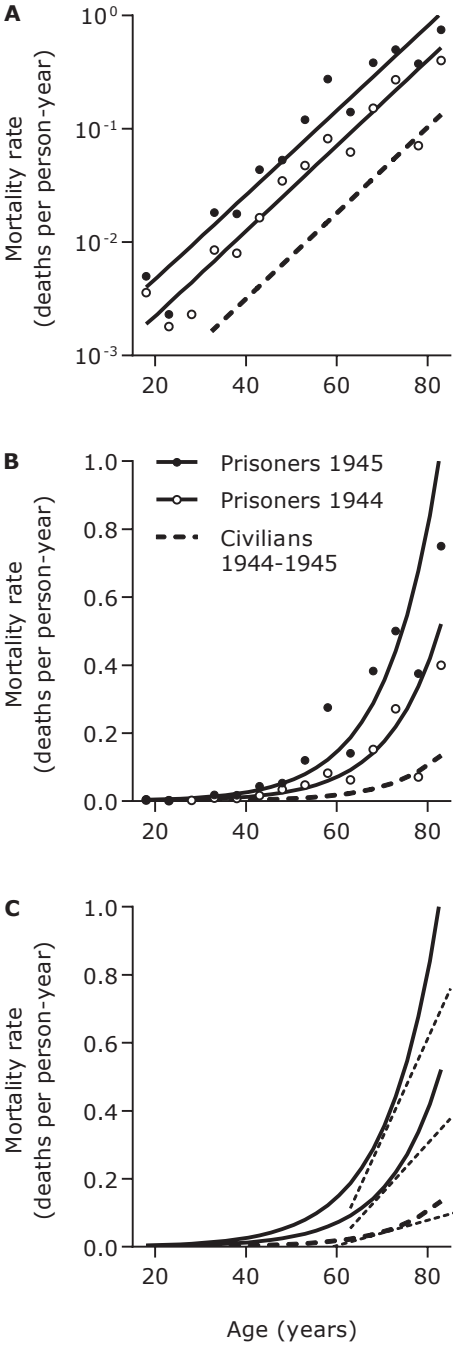


Figure 2.2 • Visualisation of mortality rates on an absolute scale and on a logarithmic scale. Identical mortality rates for different years in the Netherlands are shown on an absolute (A) and on a logarithmic scale (B). According to the Gompertz model, mortality rates increase exponentially with age on an absolute scale, but linearly with age on a logarithmic scale.

1250 kcal per day.[36] When the age pattern of their mortality rate is compared with that of Australian civilians in the same years on a logarithmic scale, the age patterns increase linearly with age and the increases of both populations are equal, as shown in Figure 2.3A. The parallel logarithmic age patterns are classically interpreted to demonstrate that the senescence rate of a human population is equal in adverse and affluent environments.[2,37,38]

However, as shown in Figure 2.3B, when the same age patterns of mortality rates are examined on an absolute scale, they increase exponentially and the difference in mortality rate between both populations grows with age. Interestingly, the comparison of these populations was originally made without any logarithmic transformation. The crude age patterns of the mortality rates were reported to diverge and interpreted as follows: „The harm done to the [prisoners of war] manifests itself in the increased death rate and can be put down to a process of rapid pathologic ageing. Of course the death rate is not the only indication of this process; there are many more, but the others are, although clearly visible, difficult to take down in figures. The premature greying of the hair, the excessive wrinkling of the skin, the difficulty in walking, the general hypotony of the musculature, the monotony in thought, the difficulty experienced in realising and analysing present problems, the predominance of past memories – all these and many more signs pointed in the same direction. But as mentioned before, the death rate as a symptom has two definite advantages: it is calculable and it is irreversible."[39]

It is generally ignored that logarithmic transformation renders an additive model into a multiplicative model. The increase in the mortality rates on a logarithmic scale, thus the slope of the straight curves



described by γ, reflects the factor by which the mortality rates are multiplied as the age increases by one year (Figure 2.3A). The increase in the mortality rates on an absolute scale reflects the factor that is added to the mortality rate as the age increases by one year (Figure 2.3B). In other words, when a linear age pattern of the mortality rate shifts upwards but remains parallel on a logarithmic scale, the difference in its crude exponential age pattern is unmasked on an absolute scale.[35]

For the mathematical assessment of a biological interaction, the use of additive models rather than multiplicative models is recommended.[40] From a multiplicative point of view, the equal increases in mortality rates with age across different environments suggests an absent interaction between the environment and the senescence process, but such an interaction becomes apparent by comparing their increases from an additive point of view.

Figure 2.3 • Age patterns of mortality rates of prisoners of war and civilians during the Second World War. The age patterns are shown on a logarithmic scale, as is classically done (A), and on an absolute scale (B). The dotted tangent lines indicate the rates at which the mortality rates increase on an absolute scale at the age of 70 years (C).[35] The data of the prisoners of war were derived from their original publication[36] and those of the civilians from the Human Mortality Database.[47] Figure A has also been presented by others.[2,37,38]

Following this line of reasoning, it has been proposed that a population's senescence rate should not be measured as the linear increase in its mortality rate on a logarithmic scale, but could be measured as the exponential increase in its mortality rate on an absolute scale. The latter increase is mathematically described by the derivative function of the exponential age pattern of the mortality rate. In Figure 2.3C, tangent lines at the age of 70 years illustrate the derivative functions and estimate the increases in the mortality rates at that age. According to the derivative function, the mortality rates increase more with age in the prisoners of war as compared with the civilians, indicating that the senescence rate is higher in an adverse environment.[35]

When the linear increases in the mortality rates on a logarithmic scale, described by γ, are interpreted as the senescence rates of both populations, their senescence rates would be not only invariant across different environments, but even unaffected by chronological age (Figure 2.3A). This would imply that the senescence rate of a 20-year-old civilian equals the senescence rate of an 80-year-old prisoner of war.[35] This classical interpretation runs counter to the fact that all molecular-cellular, physiological, and epidemiological markers of senescence rise or fall with age.[41-46] By contrast, when their senescence rates are measured as the increases in their mortality rates on an absolute scale by using the derivative function, the mortality rates increase more at higher ages, thus the senescence rates are higher at higher ages. This alternative conclusion is in line with the age-dependency of the markers of senescence.

Partitioning of intrinsic and extrinsic mortality

The classical interpretation of the Gompertz model's parameter γ as a measure of a population's senescence rate is based on the assumption that mortality is caused by two independent mechanisms. On one hand, intrinsic mortality would result from senescence unrelated to the environment. On the other hand, extrinsic mortality would result from environmental hazards unrelated to senescence. While the Gompertz model's parameter γ is thought to reflect intrinsic mortality, the parameter α is thought to reflect extrinsic mortality.[13,19,35] This classical line of thinking is supported by observations of stable senescence rates across different environments such as described above.

However, when the derivative function of the Gompertz model is used as a measure of a population's senescence rate, the increase in mortality rate with age is not solely described by γ, but dependent on both parameters. In addition, according to the derivative function, senescence rates vary across different environments. This raises the question whether intrinsic and extrinsic mortality are biological or merely mathematical phenomena.

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3 MEASURING SENESCENCE RATES USING THE GOMPERTZ MODEL

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Senescence rates in patients with end-stage renal disease: a critical appraisal of the Gompertz model.
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Background • The most frequently used model to describe the exponential increase in mortality rate with age is the Gompertz model. Logarithmically transformed, the model conforms to a straight curve, of which the slope is classically interpreted as a population's senescence rate. Earlier, it has been proposed that the derivative function of the Gompertz model is a superior measure of the senescence rate.

Methods • We tested both measures of the senescence rate in a population of patients with end-stage renal disease derived from the ERA-EDTA Registry. It is clinical dogma that patients on dialysis experience accelerated senescence, whereas those with a functional kidney transplant have mortality rates comparable to the general population. We calculated the age-specific mortality rates for European patients on dialysis (n = 274 221; follow-up = 594 767 person-years), for European patients with a functioning kidney transplant (n = 61 286; follow-up = 345 024 person-years), and for the general European population.

Results • We found a higher mortality rate, but a less steep slope of the logarithmic mortality curve in patients on dialysis compared with both patients with a functioning kidney transplant and the general population ($p < 0.001$). A classical interpretation of the Gompertz model would imply that the senescence rate in patients on dialysis is lower than in patients with a functioning transplant and lower than in the general population. In contrast, the derivative function of the Gompertz model yielded the highest senescence rate for patients on dialysis, whereas the senescence rate was similar in patients with a functioning transplant and the general population.

Conclusion • We conclude that a population's senescence rate is better measured by the derivative function of the Gompertz model than by the slope of the logarithmically transformed Gompertz model.

In 1825, Benjamin Gompertz observed that human mortality rates increase exponentially with age.[1] Since then, no other definition of senescence has gained so much common acceptance.[2] Mathematically, the model is known as the Gompertz model and has become the most frequently used model of senescence.[3,4] The model describes the mortality rate at a given age with parameters α and γ. When transformed logarithmically, the model conforms to a straight curve. The slope of this straight curve represents the increase in mortality rate on a logarithmic scale and is determined by parameter γ.

The Gompertz model fits mortality data very firmly, but it is purely empirical. Still, many investigators have tried to attribute biological properties to the estimated parameters. Based on mortality data from animal experiments[5-8] and historical

changes in human mortality patterns,[9-11] the slope of the logarithmically transformed mortality curve has classically been defined as the species-specific senescence rate.[2,12] However, based on theoretical considerations, the validity of this slope as a measure of the senescence rate has been criticised.[13-15] We have previously proposed to use the increase in mortality rate on an absolute scale instead, as described by the derivative function of the Gompertz model.[16]

This work aims to empirically test both the classical and the newly proposed measure of the senescence rate in patients with end-stage renal disease. For this, we have calculated the age-specific mortality rates of a very large population of European patients with end-stage renal disease, comprising both patients on dialysis and patients with a functioning kidney transplant, using the general European population as a reference. It has been widely recognised that patients on dialysis show accelerated senescence as compared with the general population.[17-20] Patients on dialysis have higher mortality rates.[21-23] Moreover, they suffer from age-related diseases with a higher frequency and more rapid progression, among which cardiovascular diseases,[22-26] the metabolic syndrome and insulin resistance,[27,28] cognitive impairment and dementia,[29] metabolic bone disease,[30] and dysfunction of the immune system.[31,32] Many of these patients are classified as frail.[33-35] Biologically, this clinically accelerated senescence is attributed to disturbed levels of metabolites and signalling molecules, such as urea, advanced glycosylation end products, homocysteine, and endothelin.[18,19,36-39] After transplantation, the age-related diseases in these patients improve and the age pattern of mortality of these patients converts toward the pattern of the general population.[25,40,41] Therefore, these populations provide an excellent opportunity to assess different measures of the senescence rate, as a valid measure of senescence rate should reflect these differences in senescence rates by attributing the highest senescence rate to patients on dialysis and a lower senescence rate to patients with a functioning kidney transplant and the general population.

Methods

Study population

The study population of patients with end-stage renal disease was derived from the Registry of the European Renal Association–European Dialysis and Transplant Association (ERA-EDTA Registry),[42] which records European patients who receive renal replacement therapy, either dialysis or kidney transplantation. Via national and regional registries, individual patient data were derived from Austria, the Flemish speaking region of Belgium, the French speaking region of Belgium, Denmark, Finland, Greece, Iceland, the Netherlands, Norway, Romania, Sweden, the United Kingdom, and from several regions in Italy and Spain. Data were gathered during a period beginning between 1985 and 2007, and ending at 1 January 2008 for four regions in Spain and Italy, and 1 January 2009 for the other regions and countries. For each individual patient, the following parameters were collected at baseline:

country or region of origin, date of birth, gender, primary cause of renal failure, and date and modality of first renal replacement therapy. Data on history of renal replacement therapy with dates and changes of modality and date were collected during follow-up. Primary renal diseases were classified according to the ERA-EDTA Registry's coding system.[42]

Mortality rates were calculated based on the follow-up data contributed by each individual patient, separated for follow-up on dialysis treatment and follow-up with a functioning kidney transplant. In case of the dialysis group, follow-up began six months after initiation of dialysis treatment, to account for acute treatment-related mortality,[43] and lasted until death, transplantation, recovery of renal function, loss to follow-up, or censoring at 1 January 2008 or 2009. In case of the patients with a functioning transplant, follow-up began six months after transplantation, to account for acute surgery-related mortality,[40,44] and lasted until death, transfer to dialysis due to transplant failure, loss to follow-up, or censoring at 1 January 2008 or 2009. For both treatment groups, per 5-year age group, the number of deaths was divided by the years of follow-up, yielding the age-specific mortality rates.

Reference population

Mortality data of the general European population were available through the Human Mortality Database (HMD)[45] and Eurostat.[46] For the countries in our study, the population and death figures were retrieved from the HMD for each 5-year age category and for the years of data contribution. For Greece, Romania, and Spain, these mortality data were downloaded from Eurostat, as they were not available in the HMD. For the calculation of age-specific mortality rates of the general European population, per 5-year age groups and years of participation, the sum of all deaths was divided by the sum of all inhabitants of the participating countries.

Analyses

The application of the Gompertz model is limited to mortality data between the ages of approximately 20 and 80 to 90 years.[4] Moreover, after the age of 85 years, available mortality data were scarce. Follow-up after this age comprised 15 638 person-years (2.52%) and 8360 deaths (5.83%) for the patients on dialysis and 175 person-years (0.05%) and 25 deaths (0.26%) for the patients with a functioning kidney transplant. Therefore, data on patients below the age of 20 years and from the age of 85 years onward were excluded from this study.

The Gompertz curves were characterised by estimating the values of the parameters of the Gompertz model on the age-specific mortality data as well as the statistical significance of the differences in the model parameters between the treatment groups. The parameters α and γ are mathematically described by the Gompertz model as $m(t) = \alpha\, e^{\gamma t}$, where $m(t)$ is the mortality rate and t is the age in years. The calculations were performed by fitting the parametric proportional hazards Gompertz

model[47] on the individual patient data and by linear regression on the aggregated data of the general European population.

The classical senescence rates were given by γ, of which the values were derived by the aforementioned determination of the model parameters. In addition, according to the newly proposed method that we have described earlier,[16] the derivative function of the Gompertz model was applied to the mortality rates to estimate the senescence rates. For that, the values of the model parameters, determined as described above, were incorporated in the model's derivative function: $m'(t) = \alpha \gamma e^{\gamma t}$.

The management of the ERA-EDTA Registry database, the calculations of the age-group-specific mortality rates of the study population, and the linear regression analyses were carried out using PASW Statistics 17.0 (SPSS, Chicago, IL, USA). Linear regression was performed by the linear mixed model with the natural logarithms of the mortality rates as dependent variable, treatment group as factor, and age as covariate. All these calculations were repeated using Stata/SE 10.1 (StataCorp, College Station, TX, USA). The fitting of the Gompertz model was performed using Stata/SE 10.1.

Table 3.1 • General characteristics of the study population of patients with end-stage renal disease

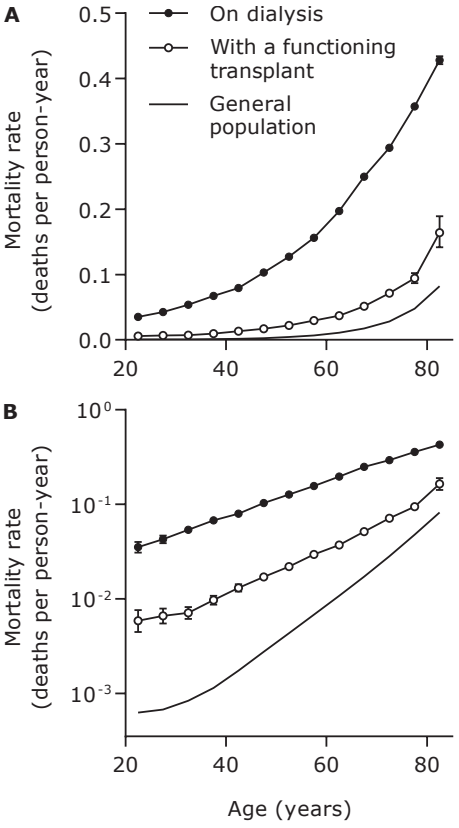
	All patients	Patients on dialysis	Patients with a functioning kidney transplant
By number of patients			
Total number of patients	290 510	274 221	61 286
Men, %	61.1	61.2	62.7
Age, median (iqr) years			
at first treatment	64.6 (52.0–73.3)	65.0 (52.7–73.5)	49.2 (38.3–58.6)
at death	71.0 (62.7–77.1)	71.1 (62.8–77.1)	60.5 (51.5–68.2)
Follow-up per patient, median (iqr) years	1.8 (0.3–4.7)	1.3 (0.2–3.1)	4.5 (1.4–8.7)
By years of follow-up			
Total years of follow-up, person-years (%)	942 458	594 767 (63.1)	345 024 (36.6)
Men, %	60.4	59.3	62.2

As patients could successively undergo dialysis treatment and kidney transplantation, patients can be represented in both the patient group on dialysis and the patient group with a functioning transplant. Iqr: interquartile range.

Results

Table 3.1 shows the basic characteristics of the study population, both presented as the total number of patients and by the number of years of follow-up. As starting dialysis treatment or receiving kidney transplantation occurred more than once in some patients, part of the population (n = 58 387 or 20.1%) contributed follow-up to both treatment modalities. The number of these consecutive treatment modalities ranged between 1 and 11 per patient.

Figure 3.1A shows the mortality rates per 5-year age groups for patients on dialysis, for patients with a functioning kidney transplant, and for the general population. In all groups, mortality rates increased exponentially with age from adolescence onward. For each age group, the mortality rate of the dialysis patients was the highest, whereas the mortality rate of patients with a functioning transplant was higher than that of the general population. After transformation of the mortality rates to a logarithmic scale, the mortality rates of all three groups conformed to straight curves from the age of 20 years and onward (Figure 3.1B). The r^2 values of these straight curves, indicating the fit of the Gompertz model, were 0.998 for patients on dialysis, 0.992 for patients with a functioning transplant, and 0.986 for the general population. Again, for each age group, the mortality rate of the patients on dialysis was the highest, the mortality rate of the group with a functioning transplant was intermediate, and the mortality rate of the general population was the lowest.



Age	20–39	40–59	60–79	80–84
—●—	54 801	170 078	330 071	45 107
—○—	85 643	173 004	96 474	1 133

Figure 3.1 • Age patterns of mortality rates of patients on dialysis, patients with a functioning kidney transplant, and the general population on a linear scale (A) and a logarithmic scale (B). Logarithmic transformation of the mortality rates yields straight curves, of which the slopes have classically been interpreted as senescence rates. For the mortality rates of the patients on dialysis and with a functioning transplant, the estimates are given with 95% confidence intervals. The follow-up in person-years for each treatment modality is shown in a table.

Table 3.2 • Quantitative description of the Gompertz model's parameters

	Patients on dialysis	Patients with a functioning kidney transplant	General population
ln α	-4.75 (-4.71 to -4.79)	-7.71 (-7.58 to -7.83)	9.55
$\alpha \times 10^{-2}$	0.86 (0.83 to 0.90)	0.04 (0.04 to 0.05)	0.01
$\gamma \times 10^{-2}$	4.29 (4.23 to 4.35)	6.70 (6.49 to 6.90)	8.50
MRDT	16.17 (15.95 to 16.40)	10.35 (10.05 to 10.68)	8.16

Estimated values of the Gompertz model's parameters for the mortality rates of patients on dialysis, patients with a functioning kidney transplant, and the general population. The mortality rate doubling times (MRDT) are derived from γ as MRDT = ln 2 / γ and are given in years.[48] The values of α were derived from those of ln α. The estimates are given with 95% confidence intervals. All estimates of the parameters were significantly different among the three groups and from zero ($p < 0.001$).

The quantitative description of the age patterns of the mortality rates by the Gompertz model's parameters is presented in Table 3.2. The intercept or basal mortality rate α was highest in patients on dialysis, intermediate in patients with a functioning kidney transplant, and lowest in the general population ($p < 0.001$). The slope γ of the straight curves was lowest in the patients on dialysis, intermediate in the patients with a functioning transplant, and highest in the general population ($p < 0.001$). The corresponding mortality rate doubling time was highest in the dialysis patients, intermediate in the patients with a functioning transplant, and lowest in the general population.

We performed various additional analyses. Stratification of the mortality rates of patients on renal replacement therapy by gender, primary renal disease, and country of origin yielded similar results. Stratification by calendar year, for which the data were divided into two periods from 1985 through 1996, and from 1997 through 2008, yielded similar results. Inclusion of only the first treatment period on dialysis or with a functioning transplant, did not affect the outcome. Furthermore, adjustment for duration of follow-up for different treatment modalities did not substantially influence the results (data not shown).

Next, we estimated the increase in the mortality rates on an absolute scale as described by the derivative function of the Gompertz model to determine the senescence rates for the various groups. The derivative function yielded estimates for the age-specific senescence rates as depicted in Figure 3.2. At every age, the senescence rate was highest in patients on dialysis as compared with the patients with a functioning kidney transplant and the general population. Contrary to a fixed senescence rate as determined by the Gompertz model's parameter γ, these senescence rates increased with age. This increase was greatest in patients on dialysis. Senescence rates estimated by the derivative function of the Gompertz model became similar to

those of the general population when the patients with end-stage renal disease had a functioning kidney transplant. These estimates do not preclude that age-specific mortality rates are higher in patients with a functioning transplant than in the general population for every age category.

Discussion

In this work, based on the commonly used Gompertz model, we have tested two measures of a population's senescence rate using a population of patients with end-stage renal disease as a model of accelerated senescence. The Gompertz model describes the mortality rate $m(t)$ at a given age t with parameters α and γ as:

$$m(t) = \alpha\, e^{\gamma t} \qquad (3.1)$$

The parameter α determines the mortality rate at the intercept, also referred to as the basal mortality rate, and is usually set at adolescence. The parameter γ determines the rate at which the mortality rate increases with age.[4,48] On a logarithmic scale, the mortality rates conform to a straight curve, which is described as:

$$\ln m(t) = \ln \alpha + \gamma\, t \qquad (3.2)$$

On the logarithmic scale, variation in α results in a parallel shift of the straight curve, whereas variation in γ results in a different slope. The slope of the curve has classically been regarded as an estimate of the senescence rate.[2,12] As an alternative estimate of the senescence rate, we have proposed to use the derivative function of the Gompertz model,[16] described as:

$$m'(t) = \alpha\, \gamma\, e^{\gamma t} \qquad (3.3)$$

Using the mortality data of a unique and unprecedented large population of patients with end-stage renal disease, both the classical measure of the senescence rate, based on the slope of the straight curve determined by γ, and the newly proposed measure of the senescence rate, estimated by the derivative function of the Gompertz model, were obtained. We showed that the mortality rates were highest and the slope of the straight curve was lowest in patients

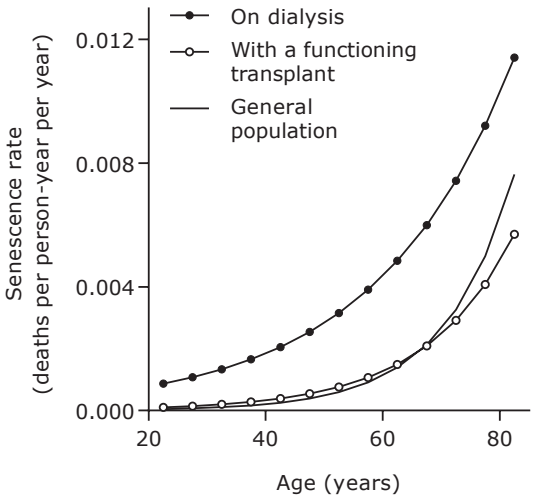


Figure 3.2 • Age patterns of senescence rates of patients on dialysis, patients with a functioning kidney transplant, and the general population. It is emphasised that, in contrast to the mortality rates in Figure 3.1, these curves depict senescence rates. According to the newly proposed method, the senescence rates were calculated using the derivative function of the Gompertz model. Values of the Gompertz model's parameters, as presented in Table 3.2, were incorporated into this function.

on dialysis. In patients with a functioning transplant, the mortality rates and the slope were intermediate. In the general population, the mortality rates were lowest, but the slope was highest compared with both patient groups. The classical interpretation of the parameters of the Gompertz model should lead to the conclusion that the senescence rate in patients on dialysis is lower than the senescence rates in patients with a functioning transplant and the general population. Moreover, successful kidney transplantation lowered the mortality rates, but would increase the senescence rate. This interpretation of the parameter estimates is in sharp contrast with the clinical notion that patients on dialysis experience an accelerated senescence, whereas after transplantation, their age pattern of the mortality rates shifts toward the pattern of the general population.

We have presented the derivative function of the Gompertz model as an alternative measure of the senescence rate. This measure yielded senescence rates that were highest in patients on dialysis compared with patients with a functioning transplant and the general population. The senescence rates in the patients with a functioning transplant and the general population were similar, although the mortality rates were higher in patients with a functional transplant than in the general population for every age category. Only at the highest ages, the senescence rate of patients with a functioning transplant slightly lagged behind that of the general population, probably due to preferential selection of healthy patients for transplantation. In contrast to the classical interpretation of parameter γ as a measure of the senescence rate, this result is consistent with the higher senescence rates observed in patients on dialysis compared with the general population and with the presumed return to normal mortality patterns after successful kidney transplantation.

From literature, it is well known that α and γ are inversely related.[10,49] Mathematical calculations show that this relationship between both parameters is necessary, called the Strehler-Mildvan correlation.[50] This correlation is in conflict with the classical interpretation of the Gompertz model. Accepting γ to represent the senescence rate and α to represent non-senescent mortality, both parameters should not be interdependent. This paradox is solved when using the derivative function as a measure for senescence rate: it relates both parameters to the senescence rate (Equation 3).

The interpretation of the Gompertz model has been subject to scrutiny of other scholars.[2,51,52] The critical difference between their analyses and ours is the logarithmic transformation. Until now, measures for the senescence rate have been relative, which is an immediate consequence of any logarithmic transformation. In contrast, the approach we have applied here is based on the non-transformed mortality rates, rendering a measure for the absolute senescence rate, as we have proposed in our earlier paper.[16]

It has to be noted that a distinction must be made between the clinical senescence rate on individual level and the demographic senescence rate on the population level.

The Gompertz model, based on aggregated mortality data, is used to estimate the senescence rate on the population level. Whether classical or novel, its interpretation provides the demographic senescence rate. Unlike implied by some studies, the individual senescence rate cannot be directly inferred from the demographically determined senescence rate.[50,53]

While Benjamin Gompertz was the first to introduce a mortality model, many alternative models have since been proposed that fit human mortality rates even better.[3,54-56] The quest here is not to arrive at the best statistical fit of the data, but to obtain parameters that can be estimated empirically and represent biological phenomena. The approach that is presented to estimate the senescence rate using the derivative function of the Gompertz model is such an attempt. This model is applicable to any model that fits mortality patterns. It is solely based on the definition of senescence as an increase in mortality rate with age and is independent of any biological interpretation of the model from which it is derived, as long as the model fits the mortality data. Other models may even be preferred over the Gompertz model, as the Gompertz model is limited to fit mortality data between adolescence and the age of 80 to 90 years.[4] Similarly, the approach presented here is applicable to the determination of senescence rates of non-human species, among which often used experimental animal models in which the effects of environmental factors and genotype could be assessed. It would, therefore, be worthwhile to test empirically the validity of this interpretation of the derivative function for alternative models and alternative species as well.

In conclusion, this study shows that empirical testing of parameter γ of the Gompertz curve as a measure of a population's senescence rate failed to identify the high senescence rate in patients with end-stage renal disease on dialysis and did not identify the improvement when these patients underwent kidney transplantation. In contrast, the recently proposed alternative measure of the senescence rate, determined by the derivative function of the Gompertz model, estimated the highest senescence rates for dialysis patients, and recognised the improved prognosis of patients with a functioning kidney transplant. Thus, we propose to use the derivative function of the Gompertz model to estimate the senescence rate.

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4

MEASURING SENESCENCE RATES WITHOUT USING THE GOMPERTZ MODEL

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Background • A population's senescence rate can be inferred from the rate at which its mortality rate increases with age. Such a senescence rate is generally estimated from parameters of a mathematical model fitted to the age pattern of the mortality rate. However, such models have limitations and underlying assumptions. Notably, they do not fit mortality rates at low and high ages.

Methods • We developed a novel method to directly calculate senescence rates from the increase in mortality rate without modelling the mortality rates. We applied the different methods to age-group-specific mortality data from the ERA-EDTA Registry, including patients with end-stage renal disease on dialysis, who are known to suffer from increased senescence rates (n = 302 455), and patients with a functioning kidney transplant (n = 74 490).

Results • From the age of 20 to 70 years, senescence rates were comparable when calculated with or without a model. However, when using non-modelled mortality rates, senescence rates were obtained at low and high ages that remained concealed when using modelled mortality rates. At low ages senescence rates were negative, while senescence rates declined at high ages.

Conclusion • Senescence rates can be calculated directly from non-modelled mortality rates, overcoming the disadvantages of an indirect estimation based on modelled mortality rates.

Across populations and species, mortality rates exhibit different age patterns.[1] Mortality rates can increase, be constant, or decrease with age. Demographers interpret increasing mortality rates at the population level as a manifestation of senescence at the organismal level. Likewise, they interpret constant or decreasing mortality rates as a manifestation of absent senescence.[2-5] Senescence is a result of manifold biological mechanisms that lead to an increasing vulnerability to death. Although biologists have made strong effort at explaining and measuring senescence, the nature of these biological mechanisms remains unclear and a reliable biomarker of senescence is lacking.[6,7] Moreover, it is continuously debated whether senescence and disease are distinct or related phenomena.[4,8]

The rate of mortality can be regarded as a speed function: it expresses the number of deaths per unit of time comparably with the speed of a car that is expressed as the number of driven metres per unit of time. It follows that an increase in mortality rate corresponds with an acceleration of mortality, while a decrease in mortality rate corresponds with a deceleration of mortality, similar to the acceleration or deceleration of the car.[9,10] As senescence is represented by an increase in mortality rate with age, the rate of senescence can be calculated from the acceleration of

mortality and is expressed as the increase in mortality rate per year of age.[9,11] As an advantage to both demographers and biologists, this approach requires neither any assumptions on the age pattern of accelerations and decelerations of mortality nor on the biological mechanisms underlying the process of senescence.

In this study, we describe and test a method to calculate the rate of senescence directly from non-modelled age-specific mortality rates. This method can be used at all ages and is free of biological assumptions. It is purely based on the definition of senescence as the increase in mortality rate with age and calculates the rate of senescence as the acceleration of mortality with age. We apply this method to mortality data of patients with end-stage renal disease who are either on dialysis therapy or have undergone kidney transplantation. Patients on dialysis therapy are biologically and clinically known to suffer from increased senescence rates.[12-16] Mortality rates and senescence rates in patients with a functioning transplant are lower than in those on dialysis[17] and approach those of the general population.[12]

Methods

Study population

Data were provided by the Registry of the European Renal Association–European Dialysis and Transplant Association (ERA-EDTA Registry), which records the treatment and survival history of European patients receiving renal replacement therapy, either dialysis or kidney transplantation.[18] Patients were included when renal replacement therapy was started during a period from 1985 through 2011. Follow-up ended on 1 January 2012. Individual patient data were available from 1985 for Austria, the French-speaking region of Belgium, Finland, Greece, Iceland, the Netherlands, Norway, and Scotland, from 1994 for the Flemish-speaking region of Belgium, from 1990 for Denmark, from 2006 for Romania, from 1991 for Sweden, and from 1997 for England, Wales, and Northern Ireland. In addition, individual patient data were available for several regions in France from 2008, for several regions in Italy, including data from 2007 for Abruzzo, Aosta Valley, Basilicata, Emilia-Romagna, Sardinia, Umbria, and Veneto, and from 1997 for Calabria, and for several regions in Spain, including data from 1985 for Andalusia, from 2002 for Aragon, from 1995 for Asturias, from 1992 for Basque Country, from 1985 for Catalonia, from 1994 for Cantabria, from 2003 for Castile-La Mancha, from 2002 for Castile and León, from 2005 for Extremadura, from 2007 for Galicia, and from 1992 for Valencia.

Mortality rates were calculated based on the follow-up data contributed by each individual patient, separated for follow-up during dialysis and follow-up with a functioning transplant. For patients on dialysis, follow-up started six months after initiation of dialysis therapy, to account for early treatment-related mortality, and lasted until death, transplantation, recovery of renal function, loss to follow-up, or censoring on 1 January 2012. For patients with a functioning transplant, follow-up started six months after transplantation, to ac-

count for acute surgery-related mortality, and lasted until death, transfer to dialysis due to transplant failure, loss to follow-up, or censoring at 1 January 2012. For both treatment groups, the age-specific mortality rates were derived by dividing the number of deaths by the years of follow-up per 5-year age group.

Reference population

As a reference, mortality rates were also calculated for the general European population. Numbers of deaths and population sizes were derived from Eurostat for the countries and regions included in this study.[19] For each 5-year age group, the number of deaths was divided by the population size, both summed for the countries and regions and the years during which the countries and regions contributed data. As data were mostly available up to the age of 100, we excluded mortality rates from that age onward.

Analyses

To compare the use of non-modelled mortality rates with the use of modelled mortality rates, the mortality rates were modelled with the Gompertz model and senescence rates were estimated as previously described (Chapter 3 of this thesis).[12] The Gompertz model is mathematically described as $m(t) = \alpha\, e^{\gamma t}$, where $m(t)$ is the mortality rate at age t in years and α and γ are the model's parameters. The minimal mortality rate at $t = 0$ is determined by α, while the subsequent exponential increase in mortality rate with age is determined by γ. On a logarithmic scale, the model conforms to a straight line, which is described by $\ln m(t) = \ln \alpha + \gamma t$. The slope of this line is determined by γ, describes the acceleration of mortality on the logarithmic scale, and estimates the relative senescence rate. The derivative function of the Gompertz model describes the acceleration of mortality on an absolute scale, estimates the absolute senescence rate, and is mathematically described by $m'(t) = \alpha\, \gamma\, e^{\gamma t}$. Considering the applicability of the model, mortality data were included for the ages of 20 to 85 years.[12,20]

The method proposed here calculates the absolute senescence rate directly from non-modelled mortality rates on an absolute scale. The method is based on the mathematical definition of a derivative function.[21] In general, for a given function $y = f(x)$, the derivative function is $f'(x) = dy / dx$, where d denotes an infinitesimal change in y or x. In the case of mortality rate m calculated for age t, the notations y and x are replaced: $m'(t) = dm / dt$. When we take d as small as possible, dt corresponds to the difference in age between two age groups and dm equals the difference in mortality rate between both age groups. Using this method, we calculate the rate at which the mortality rate changes, thus the acceleration of mortality, between two age groups on average. Applied, we calculated the senescence rate of an age group as the mortality rate of the following age group minus the mortality rate of the age group of interest, divided by the difference in age between both age groups, the latter constantly being 5 years because of the use of 5-year age groups.

We excluded mortality rates from the analyses when the number of person-years was less than 200 per 5-year age group. Patients on dialysis aged 100 years and older were excluded, corresponding to less than 0.01% of follow-up and 0.01% of deaths. Patients with a functioning transplant aged 90 years and older were excluded, corresponding to 0.02% of follow-up and 0.05% of deaths. Due to the nature of this method, senescence rates could not be calculated for the highest age group. It was not necessary to exclude mortality data for the lowest age groups.

The calculations of the age-specific mortality rates were performed using SPSS Statistics 20 (IBM, Armonk, NY, USA). The Gompertz model was fitted to the mortality data using Stata/SE 12.1 (StataCorp, College Station, TX, USA), as previously described.[12]

Results

Table 4.1 provides the general characteristics of the study population of patients with end-stage renal disease on dialysis or with a functioning kidney transplant. As patients could successively undergo dialysis treatment and kidney transplantation, some patients contributed follow-up to both treatment groups; this was the case for 59 781 patients (18.8%) and 554 809 years of follow-up (41.5%). The maximum number of different treatment periods per patient was 13.

In Figure 4.1, the different methods to infer senescence rates from mortality rates are compared. For comparison with the method proposed here, we replicated the estimations of senescence rates from mortality data that were modelled by the Gompertz model, as described previously (Chapter 3 of this thesis).[12] Figures 4.1A and 4.1B show the modelled mortality rates against age on a logarithmic scale and the relative senescence rates estimated from the model's parameters. According to this method, senescence rates were constant with age, lowest in patients on dialysis, intermediate in patients with a functioning transplant, and highest in the general population. Figures 4.1C and 4.1D show the modelled mortality rates against age on an absolute scale and the absolute senescence rates estimated from the model's parameters. According to this method, senescence rates increased with age, were highest in patients on dialysis, intermediate in patients with a functioning transplant, and lowest in the general population.

Figure 4.1E shows the crude non-modelled mortality rates against age on an absolute scale. For both patients on dialysis and patients with a functioning transplant, the exponential increase in mortality rates from the age of 20 to at least 70 years provided visual justification for the application of the Gompertz model. However, in both groups, at lower and higher ages mortality rates deviated from the exponential increase and the Gompertz model was not applicable. Before the age of 20 years, mortality rates decreased with age. From the age of 70 to 90 years onward, the exponential increase in mortality rates levelled off. At all ages, mortality rates were highest in patients on dialysis and lowest in the general population.

Table 4.1 • General characteristics of the study population of patients with end-stage renal disease

	All patients	Patients on dialysis	Patients with a functioning kidney transplant
By number of patients			
Total number of patients	317 168	302 455	74 490
Men, %	61.5	61.5	62.7
Age, median (iqr) years			
at first treatment	64.1 (51.0–73.7)	65.0 (52.4–74.1)	48.6 (36.9–58.6)
at death	72.2 (63.5–78.8)	73.1 (64.8–79.3)	63.5 (54.7–70.6)
Follow-up per patient, median (iqr) years	2.7 (1.1–5.6)	2.0 (0.9–3.8)	5.4 (2.3–9.8)
By years of follow-up			
Total years of follow-up, person-years (%)	1 337 832	841 109 (62.9)	496 723 (37.1)
Men, %	60.6	59.8	62.0

As patients could successively undergo dialysis treatment and kidney transplantation, patients can be represented in both the patient group on dialysis and the patient group with a functioning transplant. Iqr: interquartile range.

Figure 4.1F shows the absolute senescence rates as calculated directly from the non-modelled mortality rates by the method proposed here. In contrast with the results obtained when using modelled mortality rates, senescence rates could be calculated for all ages. From the age of 20 to approximately 70 years, the absolute senescence rates were comparable to those determined with the derivative function of the Gompertz model (Figure 4.1D). Using this direct calculation from non-modelled mortality rates, senescence rates were negative below the age of 5 years and increased thereafter in the general population. In patients with a functioning transplant, senescence rates were negative below the age of 20 years, then increased until the age of 75 years, after which they decreased to a similar level as in the general population. In patients on dialysis, senescence rates were negative below the age of 15 years and were more pronounced than in patients with a functioning transplant and the general population. Above this age, their senescence rates increased until the age of 90 years, after which they decreased to a lower level than in the general population.

Discussion

The aim of this study is to describe and empirically test a method for calculating the rate of senescence directly from non-modelled mortality rates. This method strictly follows the definition of senescence as the increase in mortality rate with age.[2-5] In line with this definition, the method calculates the senescence rate as the acceleration of mortality with age, similar to the calculation of a derivative function. We validated our method by applying it to mortality data of patients with end-stage renal disease on dialysis, who are known to suffer from increased senescence rates, and of patients with a functioning kidney transplant, who have senescence rates that approach those of the general population. As an immediate advantage, this method yielded senescence rates for low and high ages that remained unrevealed when using modelled mortality rates.

We compared the outcomes of different methods to determine senescence rates from mortality rates. Classically, mortality rates are modelled by the Gompertz model and subsequently presented on a logarithmic scale, rendering easily comparable straight lines of which the slopes are determined by a single parameter of the Gompertz model (Figure 4.1A). Senescence rates are derived from the increase in these lines and are thus fixed with age (Figure 4.1B).[2,22-24] Due to the logarithmic scale, this method estimates the senescence rate as the relative acceleration of mortality with age. However, it has been theoretically objected that the estimation of the senescence rate from the relative acceleration of mortality with age is false[25-27] and that the senescence rate should be defined instead as the absolute acceleration of mortality with age (Chapter 2 of this thesis).[11] According to this view, we have proposed to model mortality rates on an absolute scale (Figure 4.1C). Earlier, we have demonstrated that senescence rates can be adequately derived from the absolute acceleration of mortality with age, using the derivative function of the Gompertz model (Figure 4.1D; Chapter 3 of this thesis).[12] Contrary to the relative senescence rates, but in line with biological and clinical knowledge, the absolute senescence rates were highest in patients on dialysis and lowest in the general population. The principle of using the derivative function can be applied to any model of the age pattern of a mortality rate.

Modelled mortality data are not always preferably used over crude mortality data. The ability of mathematical models to describe mortality is limited to a specific age range. The Gompertz model does not fit mortality rates at the lowest and highest ages.[20,28] This is explained by the fact that these models necessarily mould mortality data into a prescribed pattern. The Gompertz model assumes mortality rates to increase exponentially with age.[28] The mortality rates at the lowest and highest ages in patients on dialysis and patients with a functioning transplant deviated from an exponential increase with age, which became only apparent when assessing non-modelled mortality rates (Figure 4.1E). Models as the Gompertz model do not account for such deviating age patterns of mortality rates that may be valuable

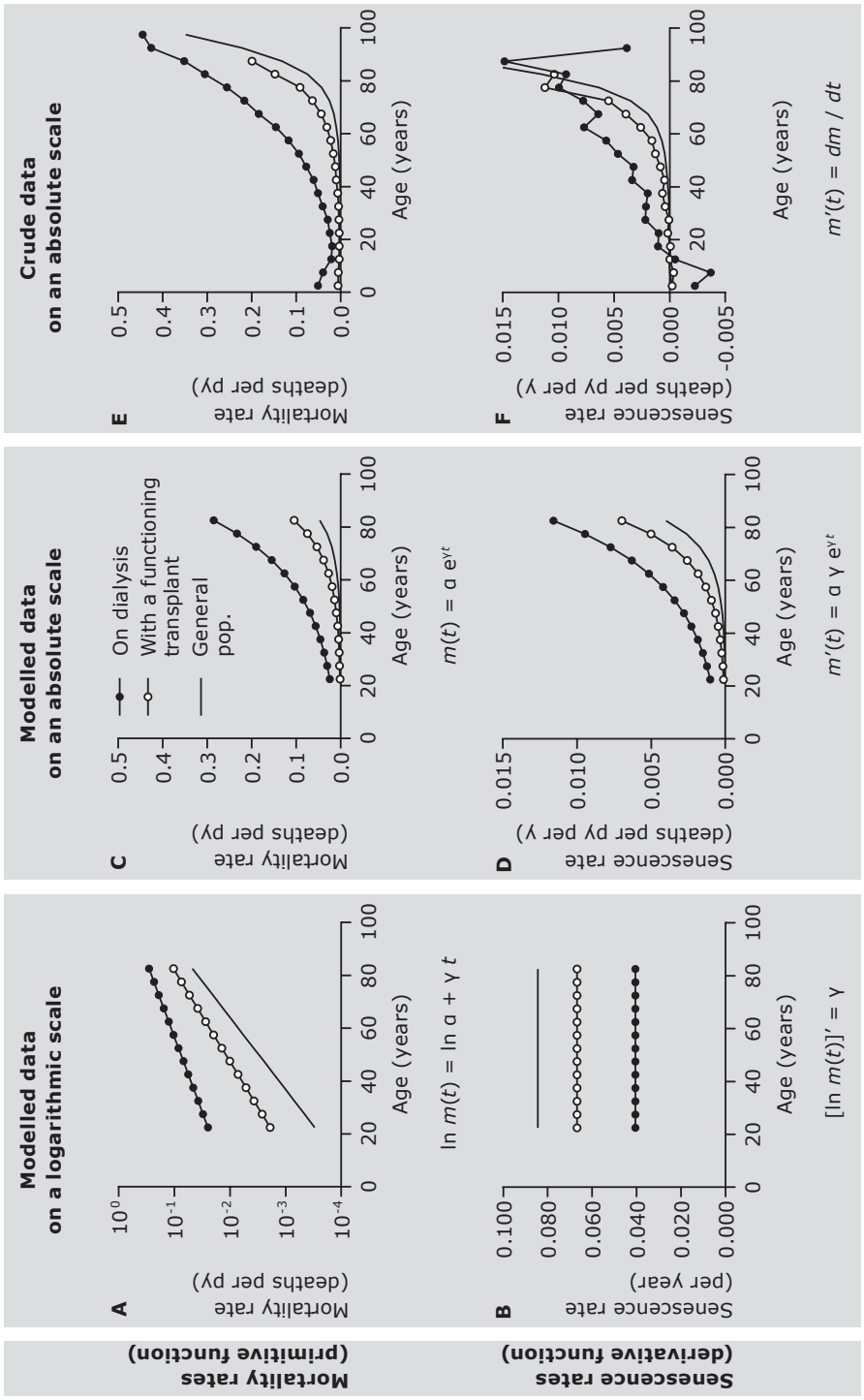

Figure 4.1 • A comparison of different methods to infer senescence rates from mortality rates. This overview shows different methods that use age-specific mortality rates (A, C, E) to calculate senescence rates (B, D, F), including the classical method (A, B), a method as proposed earlier by us (C, D), and a method presented in this article (E, F). The methods were applied to patients with end-stage renal disease on dialysis therapy and with a functioning kidney transplant and to the general population, as explained in the text. Py: person-year. Y: year.

to measure, as will be discussed hereafter. Moreover, these models, which are by themselves of only mathematical nature, are mistakenly interpreted biologically.[28-30] Apart from the Gompertz model, the Weibull model and the logistic model are often used without any of them superiorly fitting age patterns of mortality. The choice of a model is consequently based on biological assumptions explaining the age pattern that is imposed by the model.[28,31] As we have shown previously (Chapter 3 of this thesis),[12] such a biological meaning is generally attributed to the mathematical parameters of the Gompertz model, but has no empirical foundation and is biologically invalid.[11,25-27] An alternative method has been proposed to calculate senescence rates from non-modelled mortality rates, but only on a logarithmic scale, thus rendering relative senescence rates.[32,33]

Here we extend our earlier analyses by applying a method that circumvents the need to first model mortality rates for the calculation of senescence rates on an absolute scale (Figures 4.1E and 4.1F). From the age of 20 to approximately 70 years, the senescence rates calculated by this method were similar to those estimated by the derivative function of the Gompertz model. However, this method additionally offers the possibility to calculate senescence rates for low and high ages that are unaccounted for by the Gompertz model.

At low ages, the method proposed here disclosed negative senescence rates. Senescence rates were more negative in patients on dialysis than in patients with a functioning transplant and in the general population. Negative senescence rates at low ages have not been frequently addressed for humans, as changes in mortality are mainly studied from adolescence onward. For several non-human species it has been recognised that mortality rates decline during life, a finding which has been termed negative senescence.[34] At the beginning of human life, negative senescence is universally observed as a decline in mortality rate caused by processes that are distinct from senescence. It may be the result of a reduction in children's vulnerability during development and growth or of a reduction in a population's vulnerability due to the early death of the frailest children and the selective survival of healthier children.[35,36] In the cases of dialysis therapy[37] and kidney transplantation,[38] development and growth are impaired in children, although the deficits are partly compensated at a later age. The negative senescence rates in both groups more likely arise from a sharp decline in mortality rate due to early death of the frailest patients and the selective survival of healthier patients. Particularly in the lowest patients, mortality rates are high due to the underlying renal disease, congenital disorders that are associated with pædiatric renal disease, and the complications of dialysis therapy or transplantation.[39-41] The more negative senescence rates in children on dialysis as compared with children with a functioning transplant and the general population can be explained as they display higher mortality rates at the lowest ages with a subsequent sharper decline in mortality and improved growth. In addition, their negative senescence rates may be more pronounced due to a stronger effect of se-

lective survival of the less frail patients: as those selected for transplantation are relatively healthier,[40] the survival probabilities of children admitted to dialysis vary more than those of children undergoing kidney transplantation and in the general population. Negative senescence early in life has received little attention of gerontologists, but interacts closely with senescence later in life.[36] It would be interesting to include the age at which negative senescence rates turn into positive rates in studies on senescence. Comparable turning points in the age pattern of senescence rates have been studied, but only at higher ages.[42]

At high ages, the method proposed here uncovered declining senescence rates that are not accounted for by many models. The phenomenon of levelling mortality rates, and thus declining senescence rates, at high ages has been described in different populations.[2,43] Heterogeneity in survival patterns due to individual differences in frailty is thought to bring about levelling mortality rates.[44] In selected homogeneous populations, mortality rates continue to increase exponentially up to the highest ages.[45] The decline in senescence rates in patients on dialysis and patients with a functioning transplant can be explained by the selective survival to the higher ages of the relatively healthier patients and by the selection of relatively healthier patients to undergo these therapies at higher ages. That the senescence rates in patients with a functioning transplant declined at lower ages compared with patients on dialysis suggests that these selective processes are stronger in this group, probably because of the reluctance to perform kidney transplantation in older patients. Senescence rates in the general population did not decrease, probably because heterogeneity effects emerge at higher ages in large populations with low senescence rates.[2,43] The method proposed here can be applied to further study declines in senescence rates in populations at the highest ages.

This study investigated methods that determine senescence rates at the population level. The senescence rate of an individual cannot be inferred directly from the senescence rate of the population to which he belongs.[26] Still, the increased senescence rates in patients on dialysis can be attributed to biological and clinical mechanisms that have been observed and promote senescence in the individual patients.[8,46] In patients with end-stage renal disease as well as in older people in the general population, podocytes lining the epithelium of glomeruli show signs of damage and dysfunction, resulting in glomerulosclerosis and reduced glomerular filtration.[47] Renal dysfunction causes accumulation of mineral and uræmic toxins, oxidative stress, and systemic inflammation, which are normally seen at high ages. These processes promote cellular senescence through DNA damage, mitochondrial dysfunction, and telomere shortening. Widespread cellular injury and dysfunction lead to increased risks and rates of atherosclerosis, cardiac disease, cancer, immune deficiency, cognitive impairment, sarcopenia, and osteoporosis. These disorders, together with dialysis therapy itself, again induce further hæmodynamic and immunological disturbances and loss of renal function. Eventually, these disorders lead to increased

mortality rates.[12-16,47-50] The method proposed here can be applied to compare the senescence rates of different populations, to identify populations with increased or decreased senescence rates, and to evaluate the effects of interventions on the senescence rate.

The method presented here has several advantages. As it does not use modelled mortality rates, it can be applied to any mortality data, at all ages, and independent of species, geographic origin, calendar period, and birth cohort. As it does not mould the mortality rates into a prescribed age pattern, it closely follows the crude mortality data, does not discard any mortality data, and is very sensitive to changes in the senescence rate. Particularly those patterns of mortality that are not predicted by models may be informative about the process of senescence, as illustrated earlier for low and high ages. Finally, as this method is free of assumptions about the age pattern of mortality or the biological determinants underlying these patterns, it can only be interpreted based on its mathematical meaning. It measures the acceleration of mortality with age and thereby, given the definition of senescence as the increase in mortality rate with age, describes the senescence rate.

The use of non-modelled mortality data for the calculation of the senescence rate also has disadvantages. First, substantial amounts of age-specific crude data are required to prevent high variability in the estimates due to measurement error. Although large cohorts were available in this study, the senescence curves that were calculated with the proposed method show more variability than those calculated with the Gompertz model. We excluded age groups with few observations; each of these comprised less than 25 person-years of follow-up. Each age group included in the study comprised more than 200 person-years of follow-up. It is difficult to determine a level above which variability is unacceptably high. We cannot distinguish whether variability has arisen from measurement error or real age-related effects, which are both suppressed by modelling. Much of the variability depends on the width of the age groups for which the mortality rates have been calculated. This width can be adjusted in this method to reduce the effect of data variability. This reduction in variability will probably render the senescence rates more similar to those calculated from modelled mortality data. Furthermore, this method can be extended with techniques to smoothen the senescence curves, but that again introduces modelling based on statistical or biological assumptions. Second, the senescence rate can only be determined for those age groups with available mortality data. Without a model of the age pattern of mortality, extrapolation to other ages is not possible.

In conclusion, this study shows how the absolute rate of senescence can be calculated directly from age-specific non-modelled mortality rates. The methodology is simple, sensitive to changes in mortality with age, free of limitations in its applicability, and does not require any biological interpretation of mathematical models.

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INTRINSIC AND EXTRINSIC MORTALITY REUNITED

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Intrinsic and extrinsic mortality are often separated in order to understand and measure senescence. Intrinsic mortality is assumed to be a result of senescence and to increase with age, whereas extrinsic mortality is assumed to be a result of environmental hazards and be constant with age. However, allegedly intrinsic and extrinsic mortality have an exponentially increasing age pattern in common. Theories of senescence assert that a combination of intrinsic and extrinsic stressors underlies the increasing risk of death. Epidemiological and biological data support that the control of intrinsic as well as extrinsic stressors can alleviate the senescence process. We argue that senescence and death can be better explained by the interaction of intrinsic and extrinsic stressors than by classifying mortality itself as being either intrinsic or extrinsic. Recognition of the tight interaction between intrinsic and extrinsic stressors in the causation of senescence leads to the recognition that senescence is not intractable, but malleable through the environment.

To understand and measure how senescence leads to an increase in the rate of mortality, many clinicians and scholars separate intrinsic and extrinsic mortality. Intrinsic mortality is envisioned as the result of processes of physical and functional degradation originating within the human body. As these processes arise with increasing age, intrinsic mortality would represent senescence. Extrinsic mortality is seen as the result of hazards from the environment. As the human body is exposed to these hazards uniformly across ages, extrinsic mortality would not represent senescence and would be constant during life.[1-3] While intrinsic and extrinsic mortality are often separated implicitly, their separation influences biomedical research, clinical practice, and public health.

Different classifications of intrinsic and extrinsic mortality have been proposed, without any having received general acceptance. Meanwhile, the assumption that causes of death originate in either the intrinsic senescence process or the extrinsic environment has never been formally tested. Here, we compare the age patterns of typical examples of allegedly intrinsic and extrinsic mortality, discuss theories of the intrinsic and extrinsic causes of senescence, and summarise epidemiological and biological data on intrinsic and extrinsic causes of senescence. We argue that the partitioning between intrinsic and extrinsic mortality is not meaningful.

Intrinsic and extrinsic mortality have exponentially increasing age patterns

Figure 5.1 shows the traditional and alternative investigations of the age patterns of allegedly intrinsic and extrinsic mortality. As a traditional investigation, the numbers of intrinsic and extrinsic deaths as proportions of the total number of deaths are

plotted against age (Figure 5.1A). The proportions give the impression that intrinsic mortality concentrates at higher ages, while extrinsic mortality concentrates at lower ages. However, since they do not reflect any differences in the total number of deaths, the proportions of either intrinsic or extrinsic deaths cannot be compared directly between ages. By contrast, when the absolute numbers of intrinsic and extrinsic deaths are plotted against age, the numbers of either intrinsic or extrinsic deaths can be compared between ages and both intrinsic and extrinsic mortality concentrate at the highest ages (Figure 5.1B). As another traditional investigation, rates of intrinsic and extrinsic mortality are plotted against age on a logarithmic scale

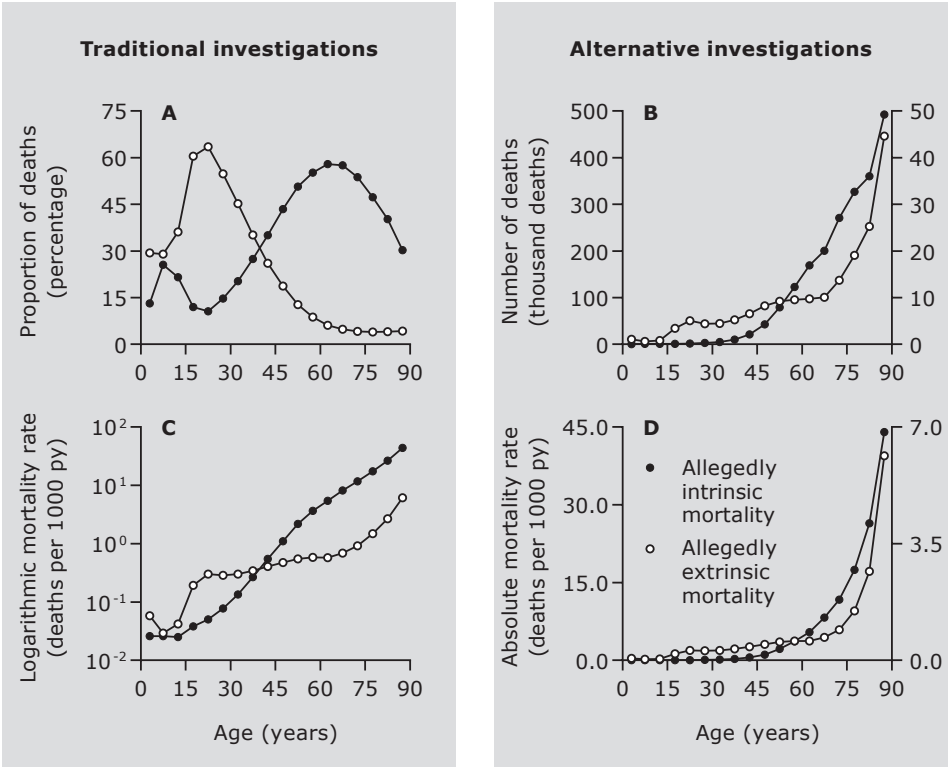

Figure 5.1 • Traditional and alternative investigations of the age patterns of allegedly intrinsic mortality due to senescence and extrinsic mortality due to the environment. Intrinsic mortality includes death due to ischæmic heart disease (ICD-10 codes I20–I25), diabetes mellitus (E10–E14), and cancer (C00–C97). Extrinsic mortality includes death due to infectious diseases (A00–B99) and due to so-called external causes (V01–Y98), among which accidents and natural disasters. Data have been derived from the European Detailed Mortality Database of the World Health Organization for 31 European countries and Israel in 2009 or 2010.[43] In panels B and D, allegedly intrinsic mortality is plotted on the left axis and allegedly extrinsic mortality on the right axis.

(Figure 5.1C). The logarithmic rates give the impression that intrinsic mortality increases continuously, while extrinsic mortality is almost constant with age. However, on a logarithmic scale, multiplicative changes are revealed while absolute changes are concealed. We have previously illustrated that interpretations of multiplicative changes in mortality rates with age do not necessarily apply to the absolute changes in the same mortality rates (Chapter 2 of this thesis).[4] Plotting allegedly intrinsic and extrinsic mortality on an absolute scale reveals an exponential increase with age for both (Figure 5.1D). Whereas the traditional investigations seem to support a distinction between the age patterns of intrinsic and extrinsic mortality, the alternative investigations reveal that both intrinsic and extrinsic mortality have exponentially increasing age patterns.

In Figure 5.2, the age patterns of mortality rates are shown on an absolute scale for typical examples of allegedly intrinsic and extrinsic causes of death.[1,2] Minor differences between the age patterns can be observed. Mortality rates due to cancer start increasing at earlier ages. Mortality rates due to accidents are slightly elevated throughout adulthood. Mortality rates due to natural disasters are elevated from the age of 50 to 60 years and decrease slightly from the age of 60 to 75 years. However, the rates of intrinsic and extrinsic mortality share the common feature of an exponential increase with the highest values at the highest ages.

The age patterns of incidence rates for the same typical examples of allegedly intrinsic and extrinsic disorders can also be compared. Although minor differences can be observed, the incidence rates of intrinsic and extrinsic disorders share the common feature of an exponential increase to a maximum at the highest ages, as shown in Figure 5.3.

Intrinsic and extrinsic stressors interact in the causation of senescence

The human body is exposed to intrinsic stressors originating within and extrinsic stressors originating outside the body. During life, the repetitive exposure to these stressors leads to an accumulation of permanent damage, which leads to dysfunction, disease, and ultimately death.[5] The various damages that have been acquired up to a certain age increase the body's vulnerability to be damaged by intrinsic and extrinsic stressors at subsequent ages. As senescence amounts to the increasing risks of disease and death, senescence is a consequence of the accumulation of damages from intrinsic as well as extrinsic sources.[5,6] For example, senescence is partly attributed to damage to the DNA, which is induced by intrinsic stressors such as spontaneous chemical reactions, replication errors, and metabolic waste products, but also by extrinsic stressors such as radiation and viruses. Damage impairs the DNA's repair function, decreases its resistance to further damage caused by intrinsic and extrinsic stressors, and increases the risks of disease and death.[7]

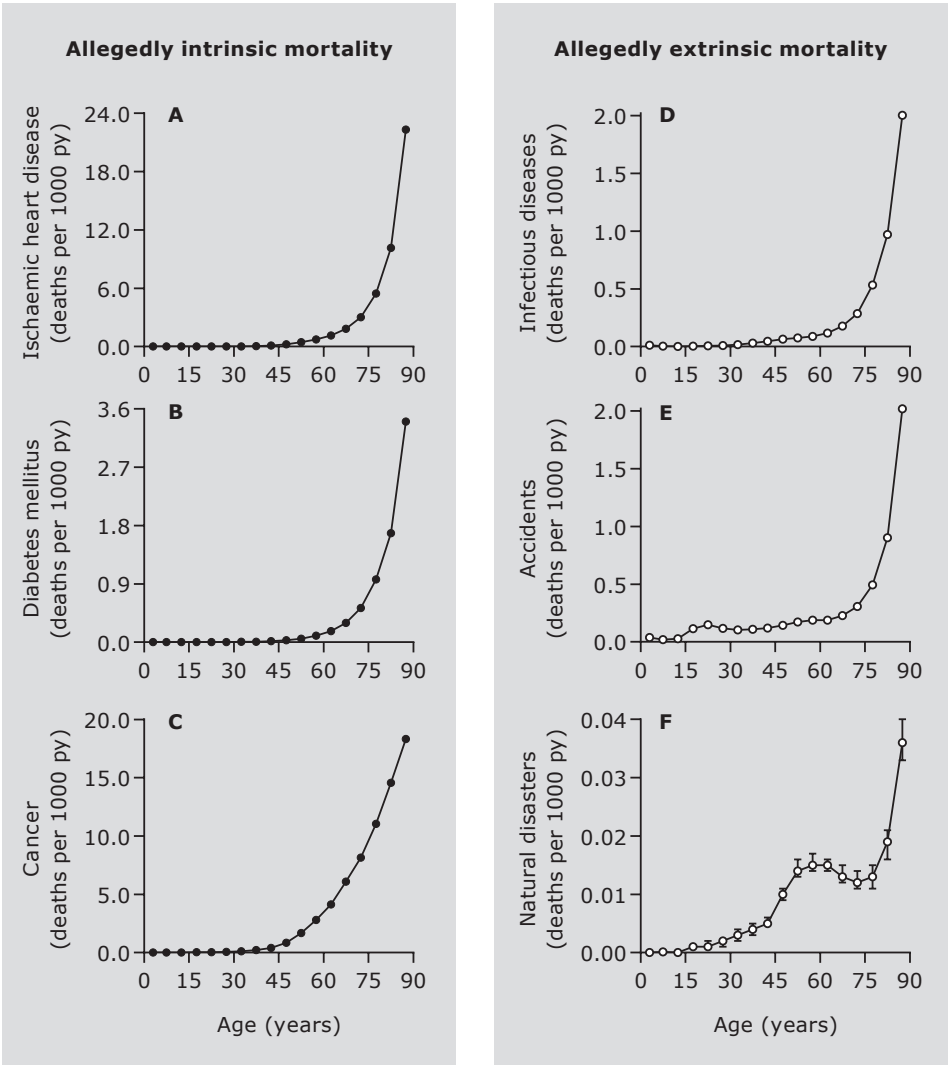

Figure 5.2 • Age patterns of mortality rates for typical examples of allegedly intrinsic mortality due to senescence and extrinsic mortality due to the environment. As typical examples of intrinsic mortality, death due to ischæmic heart disease (ICD-10 codes I20–I25), diabetes mellitus (E10–E14), and cancer (C00–C97) have been included. As typical examples of extrinsic mortality, death due to infectious diseases (A00–B99), accidents such as transport accidents, falls, drowning, and exposure to mechanical forces (V01–X29), and natural disasters such as excessive heat or cold, lightning, earthquakes, storms, and floods (X30–X39) have been included. Data have been derived from the European Detailed Mortality Database of the World Health Organization for 31 European countries and Israel in 2009 or 2010.[43] Mortality rates are given as numbers of deaths per 1000 person-years (py) with 95% confidence intervals.

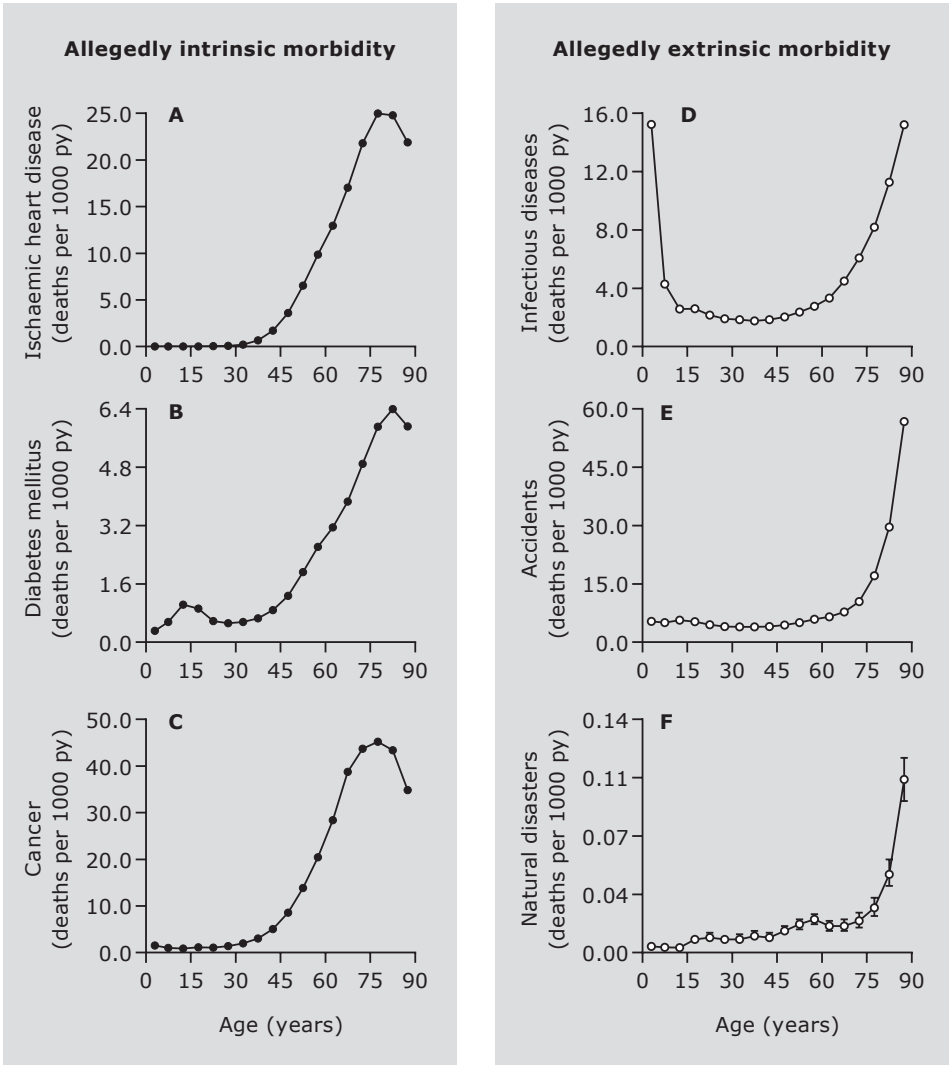

Figure 5.3 • Age patterns of incidence rates for typical examples of allegedly intrinsic disorders due to senescence and extrinsic disorders due to the environment. As typical examples of intrinsic disorders, ischæmic heart disease (ICD-10 codes I20–I25), diabetes mellitus (E10–E14), and cancer (C00–C97) have been included. As typical examples of extrinsic disorders, infectious diseases (A00–B99) and disorders due to accidents such as transport accidents, falls, drowning, and exposure to mechanical forces (V01–X29) and due to natural disasters such as excessive heat or cold, lightning, earthquakes, storms, and floods (X30–X39) have been included. Data have been derived from the European Hospital Morbidity Database of the World Health Organization for 26 European countries and Israel in 2008, 2009, or 2010.[44] Incidence rates are given as numbers of hospital discharges per 1000 person-years (py) with 95% confidence intervals.

Other processes than damage accumulation are identified as the cause of senescence by alternative theories. Notably, senescence is attributed to a continuation and hyperfunction of processes that facilitate development early in life.[8] These developmental processes originate within the body and may be regarded as intrinsic stressors, but are inextricably linked with the body's environment. The developmental processes are, as early as their intrauterine onset, modulated by environmental conditions such as nutrition.[9,10] Later in life, the overgrowth of developmental processes only causes senescence in combination with stressors from the environment. For example, cellular senescence is explained by excessive cell growth induced by developmental growth factors, which depend on environmental signals such as nutrient levels. The cell growth only leads to cellular senescence when the cell cycle has been arrested by intrinsic stressors such as oxidative stress and oncogenes or by extrinsic stressors such as chemical toxins and radiation.[8]

Traditional biological theories explain the evolution of senescence by separating intrinsic and extrinsic mortality. They propose that extrinsic mortality due to the environment constrains survival up to high ages, as a result of which selective pressure declines with increasing age. The decline in selective pressure facilitates the occurrence of senescence, which appears as intrinsic mortality.[11,12] However, empirical studies have failed to consistently confirm a relationship between the level of extrinsic mortality and the process of senescence.[3,12] It has been theoretically demonstrated that the level of extrinsic mortality cannot explain the evolution of senescence, unless extrinsic stressors are acknowledged to interact with and to be modulated by the body's vulnerability that declines with age.[13,14]

It is a fundamental theorem in biology that every phenomenon is explained by the interaction of genes and environments. From this point of view, it is a misconception to equate genes with causal factors within the body and the environment with those outside it.[10] Rather, the effects of genes are modulated by the environment and vice versa.[15] Disease and death are not either genetic or environmental, but of mixed genetic and environmental origin.[16] Yet, little attention is given to gene-environment interaction in the context of senescence.10 The intrinsic and extrinsic stressors to which the human body is exposed can be regarded as genetic and environmental stressors that interact to exert detrimental effects. These effects again interact with the body's genetic susceptibility and increase its vulnerability to further detrimental effects, ending in senescence and death. Since senescence and death are the outcomes of both genes and the environment, they cannot be partitioned as either intrinsic or extrinsic. This is reflected by the exponential increasing age patterns that allegedly intrinsic and extrinsic mortality have in common.

Intrinsic and extrinsic stressors interact in epidemiological and biological studies on senescence

Epidemiological and biological data support that senescence is a result of the interaction between intrinsic and extrinsic stressors. Cardiovascular disease, diabetes mellitus, and cancer are typically regarded as determined by intrinsic senescence, but are meanwhile largely attributable to hazards that originate in the environment, including tobacco and alcohol use, sunlight, pollution, an excessive dietary composition, and a minimal necessity of physical activity.[17] These environmental hazards affect the structure and functioning of the genome and are required for the development of disease.[6,7] Even the accelerated bodily deterioration caused by well-defined genetic substrates as in Huntington's and Duchenne's diseases is influenced by the environment.[18,19] As a consequence, environmental interventions can prevent or postpone cardiovascular disease, diabetes mellitus, and cancer.[20-22]

Infectious diseases, accidents, and natural disasters require environmental risk factors, but cannot be uncoupled from the body's vulnerability that increases with age. Senescence of the immune system increases the risk of infectious diseases.[23] The immune system is influenced by microorganisms and other environmental factors, like smoking, sunlight, and dietary components and meanwhile plays an essential role in the pathogenesis of cardiovascular disease and cancer.[6,24] Commensal and infectious microorganisms can induce or prevent diseases attributed to senescence, including autoimmune disease, cardiovascular disease, neuropsychiatric disease, and cancer.[24] Even the risk of being affected by seemingly fully stochastic hazards is age-dependent. Sensory, cognitive, and executive dysfunctions, disability, and multimorbidity that accumulate with age predispose to burns, chokes, falls, traffic accidents, and other environmental hazards.[25-27]

That the senescence process depends on both intrinsic and extrinsic stressors becomes apparent when populations across different environments are compared. For example, both western populations and populations exposed to endemic infections, malnutrition, and physical labour share similar declines in muscle strength and heart rate variability with age (Chapters 8 and 11 of this thesis).[28,29] This indicates that the senescence process contains components that are independent of the environment. On the other hand, while senescence in western populations is accompanied by a predominance of allegedly intrinsic disorders like cardiovascular disease and diabetes mellitus, these disorders are uncommon up to the highest ages in the populations exposed to endemic infections, malnutrition, and physical labour (Chapters 9 and 10 of this thesis).[30,31] This indicates that the senescence process is shaped by the environment.

Relevance to biomedical research, clinical practice, and public health

Senescence is commonly defined to encompass deleterious, progressive, and intrinsic age-related changes that culminate in death. The intrinsic nature of senescence is understood as being independent of modifiable environmental factors.[32] This traditional definition is rooted in a separation of intrinsic mortality due to senescence and extrinsic mortality due to the environment, neglects the tight interaction between intrinsic and extrinsic stressors as causes of senescence, but influences biomedical research, clinical practice, and public health.

Firstly in biomedical research, intrinsic and extrinsic mortality are separated when measuring the senescence process. Mathematical models are used in such a manner that distinct parameters account for intrinsic and extrinsic mortality.[3,33] For example, one parameter of the Gompertz model describes the minimal risk of dying during adolescence and is used as a measure of extrinsic mortality. Another parameter describes the increase in mortality rate over subsequent ages, which is linear when plotted on a logarithmic scale, as shown in Figure 5.1C. The single parameter that determines this linear increase is used as a measure of intrinsic mortality. Models like the Gompertz model are applied to interpret the effects of experimental interventions as affecting either the intrinsic rate of senescence or the extrinsic age-independent risk of dying. A change in the minimal risk of dying is interpreted as a change in the environmental risk of dying, while a change in the linear increase of the logarithmic mortality rate is interpreted as a change in the rate of senescence.[34,35] We have previously criticised such a biological interpretation of the mathematical parameters.[4] We have argued that the rate of senescence should be measured by the increase in mortality rate with age on an absolute scale, as shown in Figure 5.1D (Chapter 3 of this thesis).[36] The absolute increase in mortality rate is determined by both parameters of the Gompertz model. This illustrates that allegedly intrinsic and extrinsic mortality should not be partitioned to correctly measure the rate of senescence, which is essential for research on senescence.

Others have argued similarly that studies on the relationship between environmental hazards and senescence are handicapped as models and experiments do not take into account the interaction between the environmental hazards and the body's increasing vulnerability with age. This interaction is essential to understand how the environment has facilitated the evolution of senescence and how environmental factors continue to shape the senescence process.[12,14] The comparison of populations across different environments is essential for unravelling the intrinsic and extrinsic components of the senescence process. Here, we focus on the senescence process in western human populations. Our argumentation can be further investigated by comparing human populations living

across different environments and by rearing animal models in different environments. Such research on the interaction between the environment and senescence will be valuable and may yield results that are different from those of traditional investigations.[12,36]

Secondly in clinical reasoning, disease and death are classified as intrinsic or extrinsic in an attempt to better understand and target the senescence process. Disorders such as cardiovascular disease, diabetes mellitus, and cancer are considered as degenerative disorders that progress intrinsically with increasing age. Curative and preventive endeavours focus on the senescence process, but the role of environmental factors in their causation is underestimated.[37,38] Conversely, disorders such as infectious diseases, accidents, and natural disasters are considered as environmental, but the effect of age on the risks of these disorders is underestimated. Alternatively, senescence and death are not explained by a single cause, but by intrinsic and extrinsic stressors that congregate with age and each constitute partial causes.[16] Strategies to combat senescence and death need to take into account their different intrinsic and extrinsic causes to be successful, as has been recently pled for infectious diseases.[39]

Underlying pathogenic processes are sorted similarly. In dermatology, for example, the deteriorating synthesis of interstitial proteins is attributed to intrinsic senescence, while sun-induced damage is thought to constitute extrinsic environmental damage.[40] However, also molecular and cellular phenomena of senescence and environmental damage are extensively interconnected.[6] The damage in the skin that is accrued with increasing age is due to both the deteriorating protein synthesis and sunshine.

Thirdly in public health, the distinction between intrinsic and extrinsic mortality is misleading when designing preventive strategies against senescence and environmental hazards. Above, we have described that disorders attributed to senescence can be prevented by environmental interventions and that the old are most vulnerable to be struck by environmental hazards. Consequently, prevention of mortality due to cardiovascular disease, diabetes mellitus, and cancer should not only be sought in a delay of the senescence process, but may be more readily attained by confining the exposure to their environmental risk factors. Prevention of mortality due to infectious diseases, accidents, and natural disasters should particularly aim at protecting the frail elderly. Hygienic precautions and vaccination against contagions are essential, participation in traffic requires safety measures that compensate for disabilities, and the old skin should be protected as it is easily bruised or sunburnt. Especially lifestyle interventions seem effective, such as limiting sun exposure to delay senescence of the skin.

Conclusion

When intrinsic mortality due to senescence and extrinsic mortality due to the environment are separated, senescence is accepted as an intractable side effect of increasing age while environmental hazards are taken as bad luck. In contrast, when intrinsic and extrinsic stressors are acknowledged to interact tightly in the causation of senescence, disease, and death, new perspectives arise with both a bad and a good outlook. The bad news is: all mortality is related to senescence. The risk of allegedly extrinsic mortality increases exponentially with age just like allegedly intrinsic mortality, because they are both attributable to degeneration of the human body's structures and functions. The good news is: all mortality is related to the environment. The risk of allegedly intrinsic mortality increases with age, but is just as well dependent on environmental hazards. A proper understanding of the tight interaction between intrinsic and extrinsic stressors recognises that senescence is not intractable, but malleable through the environment. Knowledge on this interaction leads the way to identify environmental stressors that cause senescence and can be targeted to prevent senescence.[41,42] To reach this goal, intrinsic and extrinsic mortality should be reunited in mathematical models when measuring senescence, in clinical reasoning when explaining senescence, and in public health when allocating prevention and intervention.

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PAR

PART II

MEASURING SENESCENCE THROUGH MORBIDITY

Τά τε γὰρ ὑπερβάλλοντα γυμνάσια καὶ τὰ ἐλλείποντα φθείρει τὴν ἰσχύν, ὁμοίως δὲ καὶ τὰ ποτὰ καὶ τὰ σιτία πλείω καὶ ἐλάττω γινόμενα φθείρει τὴν ὑγίειαν, τὰ δὲ σύμμετρα καὶ ποιεῖ καὶ αὔξει καὶ σῴζει.

Both the exceeding and the shortcoming exercises ruin the strength,
and likewise the drinks and the foods that become too much and too little
ruin the health, while those that are moderate
produce and increase and preserve them.

Aristotle, *Ethica Nicomachea* II: 2: 6 (1104a15)

6

INTRODUCTION TO PART II

Senescence from a global perspective

As a result of senescence, the risks of dysfunction and disease in human populations increase with chronological age. With vast amounts of empirical data and theoretical knowledge, the senescence process has been characterised extensively in western populations. Here, senescence is well known to be predominantly marked by cardiovascular disease, diabetes mellitus, and cancer.[1-3]

By contrast, as illustrated in Figure 6.1, very little research is performed on senescence in non-western populations, such as in Africa.[4-9] The course of senescence and its relation with disease in these populations remains obscure. Meanwhile, the study of senescence in these populations is valuable for both western and non-western populations. It can unveil new causes of senescence-related diseases, facilitate the investigation of their possible causes that are rare or ubiquitous in western populations, enable the unravelling of the link between infectious diseases and senescence-related diseases, and support the design of local research agendas and public health policies to combat these diseases.[4-6,8]

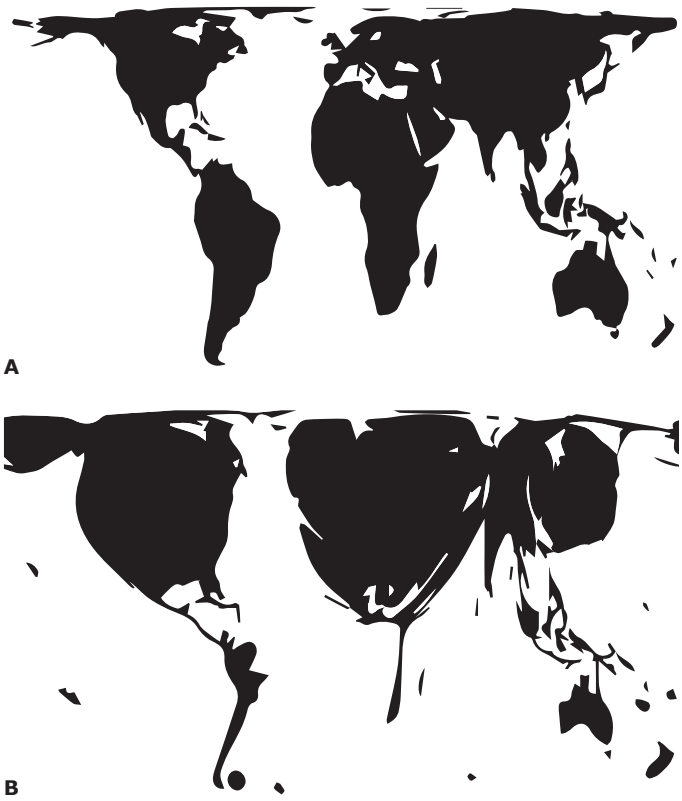

Figure 6.1 • Research is almost exclusively performed in western populations. On map A, each country is displayed according to its actual size. On map B, each country is drawn such that its size reflects the number of scientific articles published in 2001 by authors resident in that country relative to the size of its total population. The maps were obtained from Worldmapper and adjusted by B.W. Florijn. Copyright of the Sasi Group at the University of Sheffield and Mark Newman at the University of Michigan. Reproduced with permission.[10]

Senescence from an environmental perspective

Next to age, lifestyle is the most important risk factor of the diseases that are related to senescence in western populations.[11-15] A western lifestyle is affluent and sedentary. Industrialisation, technological innovation, urbanisation, and economic growth have allowed foods to become abundantly available and enriched with energy and at the same time have greatly reduced the need for exercise in daily life.[16-19] It has been established that a western lifestyle interferes with the body's physiology, for example by causing inflammation, and that it leads to obesity, dyslipidæmia, hypertension, cardiovascular disease, diabetes mellitus, and cancer.[12,20]

Where the aforementioned advancements have not yet taken place, food supply depends on the harvest and climate, hunger is frequent, and physical exertion is indispensable for subsistence. To know how the inhabitants of such regions senesce means to know whether the diseases that are related to senescence in western countries arise largely independent of a western lifestyle or rather as its consequences.

Improvements in hygiene and public health have restrained infectious diseases in western populations, whereas in non-western populations infectious diseases are the main cause of disease and death. In those regions where a western lifestyle is currently emerging, it coexists with local traditional non-western lifestyles. As a consequence, an expansion of obesity, cardiovascular disease, and diabetes mellitus is accompanied by the persistence of malnutrition and infectious diseases. This is referred to as the double burden of disease. While infections induce inflammation, the diseases that are related to senescence in western populations also have an inflammatory nature. It is postulated, therefore, that frequent exposure to infections excites these diseases,[21-24] but, unfortunately, studies on this postulation have seldom been conducted in populations without a western lifestyle.

Senescence from an evolutionary perspective

The history of modern human populations dates back to about 250 000 years ago and those of their hominin predecessors millions of years more. During most of human history, survival was severely comprised by malnutrition and infectious diseases. Only since a few decades and only in western populations, these threats have been largely overcome. These harsh environmental demands have exerted strong selective pressures through many generations. These pressures have promoted qualities that matched the environment, in particular thrifty and proinflammatory qualities.[25,26] Thriftiness refers to the metabolic efficiency to ingest, process, and store nutrients. An immune system is proinflammatory when it aggressively fights pathogens and other potentially injurious agents.

The thrifty and proinflammatory qualities that have been promoted during human evolution are in mismatch with the envi-

ronment in modern western populations. When food is abundant, physical activity is needless, and infectious diseases are uncommon, thriftiness and inflammation lead to obesity, dyslipidæmia, hypertension, cardiovascular disease, diabetes mellitus, and cancer. From this point of view, the diseases that arise during senescence in western populations are, at least partly, attributable to conflicts between out-dated genes in a brand-new environment.[16,20,22,25-27]

In non-western populations where the present environment resembles the harsh environments in which humans have evolved, the selective pressures in the past environments match more closely with those in the present environments. The thrifty and proinflammatory qualities that have long been promoted are still needed for survival in these populations. If the diseases related to senescence in western populations are attributed to a mismatch between genes and the environment, it is expected that these diseases arise less frequently during senescence in non-western populations. However, it has only infrequently been studied whether the thrifty and proinflammatory qualities cause diseases during senescence in non-western as in western populations.

Measuring senescence through morbidity in a traditional African population

With an eye to the three preceding perspectives, we have studied senescence in a traditional rural African population without a western lifestyle in one of the least developed regions of Ghana. Previously, this population has been characterised extensively with regard to its age patterns of mortality and fertility, its lifestyle and culture, the presence of infectious diseases, and the genetic and physiological determinants of inflammation.[28-34]

An impression of the lifestyle, culture, and environment of the Ghanaian study population is given by the pictures on the following pages.

In this population, we have measured senescence through morbidity, focussing on handgrip strength and cardiovascular disease. Loss of handgrip strength is a widely used measure of senescence.[35-39] Cardiovascular disease is the most prevalent disease related to senescence in western populations.[40-42] As described in more detail in the following chapters, we have assessed the age patterns of handgrip strength and various cardiovascular disease, the presence of their determinants, and their relations to mortality in older inhabitants.

By studying senescence in this traditional African population without a western lifestyle, we aim to clarify whether the diseases related to senescence in western populations arise largely independent of such a lifestyle, as is suggested when senescence is regarded as a process intrinsic to the human body, or are rather consequences of such a lifestyle. If a decrease in handgrip strength and an increase in cardiovascular disease are observed in this population similarly to western populations, a western lifestyle is not a prerequisite for their occurrence during senescence, inflammation

due to infections may be an alternative cause, and a mismatch between genes and the western environment does not satisfy as an evolutionary explanation of the senescence process. If a decrease in handgrip strength and an increase in cardiovascular disease are absent in this population as opposed to western populations, a western lifestyle is a prerequisite for their occurrence during senescence, inflammation due to infections cannot by itself be a sufficient cause, and a mismatch between genes and the western environment is reinforced as an evolutionary explanation of the senescence process.

Figure 6.2 • Signboard at the office in the Ghanaian research area. By courtesy of A.D. Pieterse. Reproduced with permission.

Figure 6.3 • A typical compound household in the Ghanaian research area. By courtesy of D. van Bodegom. Reproduced with permission.

Figure 6.4 • Clothes and grains being dried in a courtyard in the Ghanaian research area. By courtesy of H. Sanchez-Faddeev. Reproduced with permission.

Figure 6.5 • Transportation across a river in the Ghanaian research area. By courtesy of D. van Bodegom. Reproduced with permission.

Figure 6.6 • A borehole well in the Ghanaian research area. By courtesy of L. May. Reproduced with permission.

Figure 6.7 • Milling of grain in the Ghanaian research area. By courtesy of D. van Bodegom. Reproduced with permission.

Figure 6.8 • A household's kitchen and, in the background, a field of grain in the Ghanaian research area. By courtesy of T. Menger. Reproduced with permission.

Figure 6.9 • A herd of cattle being led through the town of Garu near the Ghanaian research area. By courtesy of A.D. Pieterse. Reproduced with permission.

Figure 6.10 • A herd of cattle being grazed in the Ghanaian research area. By courtesy of L. May. Reproduced with permission.

Figure 6.11 • A market in the Ghanaian research area. By courtesy of D. van Bodegom. Reproduced with permission.

Figure 6.12 • Different foods sold at a market in the Ghanaian research area. By courtesy of H. Sanchez-Faddeev. Reproduced with permission.

Figure 6.13 • Children washing in a tropical rain shower in the Ghanaian research area. By courtesy of T. Egberts. Reproduced with permission.

Figure 6.14 • A brewery of pito beer in the Ghanaian research area. By courtesy of T. Egberts. Reproduced with permission.

Figure 6.15 • A health care clinic in the Ghanaian research area. By courtesy of T. Egberts. Reproduced with permission.

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AN INFLAMMATORY GENETIC DETERMINANT OF HANDGRIP STRENGTH

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K. G. M. Beenakker*, J. J. E. Koopman*, D. van Bodegom, M. Kuningas, P. E. Slagboom, J. J. Meij, A. B. Maier, R. G. J. Westendorp.
* Both authors have contributed equally.
Variants of the *IL10* gene associate with muscle strength in elderly from rural Africa: a candidate gene study.
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Background • It has recently been shown that the capacity of the innate immune system to produce cytokines relates to skeletal muscle mass and muscle strength in older persons. The *interleukin-10* (*IL10*) gene regulates the production capacities of IL10 and tumour necrosis factor α (TNFα). In rural Ghana, *IL10* gene variants associated with different production capacities of IL10 and TNFα are enriched compared with European populations. In this setting, we explored the association between these gene variants and muscle strength.

Methods • Among 554 Ghanaians aged 50 years and older, we determined 20 single nucleotide polymorphisms (SNPs) in the *IL10* gene, production capacities of IL10 and TNFα in whole blood upon stimulation with lipopolysaccharide (LPS), and handgrip strength as a proxy for skeletal muscle strength. We distinguished proinflammatory haplotypes associated with low IL10 production capacity and anti-inflammatory haplotypes with high IL10 production capacity.

Results • We found that distinct haplotypes of the *IL10* gene associated with handgrip strength. A proinflammatory haplotype with a population frequency of 43.2% was associated with higher handgrip strength (p = 0.015). An anti-inflammatory haplotype with a population frequency of 7.9% was associated with lower handgrip strength (p = 0.006).

Conclusion • In conclusion, variants of the *IL10* gene contributing to a proinflammatory cytokine response associate with higher muscle strength, whereas those contributing to an anti-inflammatory response associate with lower muscle strength. Future research needs to elucidate whether these effects of variation in the *IL10* gene are exerted directly through its role in the repair of muscle tissue or indirectly through its role in the defence against infectious diseases.

Interleukin-10 (IL10) is an anti-inflammatory cytokine with important regulatory effects on inflammatory responses. It downregulates the antigen presenting function and inhibits the production of proinflammatory cytokines like tumour necrosis factor α (TNFα) by various immune cells.[1] In mice, immune cells producing cytokines are crucial for the repair of skeletal muscle tissue.[2-5] We have recently shown that a higher TNFα production capacity of immune cells is positively related to muscle mass and muscle strength in a middle-aged Dutch population.[6]

The capacity to produce IL10 and TNFα upon whole-blood stimulation with lipopolysaccharide (LPS) varies between individuals. This variation is for more than 50% attributable to genetic determinants.[7-9] The *IL10* gene is highly polymorphic[10-12] and its haplotypes are transcribed differently.[13] This interindividual variation is extended by variation in the *IL10* hap-

lotype structure and distribution between ethnicities.[10,14] We have earlier reported that specific *IL10* gene variants are enriched in Ghanaian elderly living under adverse conditions.[12] These variants have functional significance: some are related to a proinflammatory cytokine production capacity, with lower IL10 and higher TNFα levels upon whole-blood stimulation with LPS, while others are related to an inverse anti-inflammatory response.[12,15,16] Interestingly, this *IL10* haplotype structure is less present in European populations living under affluent conditions,[12,14] possibly because balanced selection has conserved this haplotype structure in populations under adverse conditions. The functional variation in the genetic determinants of cytokine production capacity forms a meaningful instrument to study the effects of different cytokine production capacities largely free from confounding and reverse causality.[17]

For this study, we had the unique opportunity to study handgrip strength of individuals aged 50 years and older in the Ghanaian population of which we have characterised the *IL10* gene variants and their effects on cytokine production capacity.[12,15,16] This study aims to investigate the relation between the pro- and anti-inflammatory *IL10* gene variants, which are not present in European populations, and handgrip strength as a proxy of overall muscle strength. To account for the possible effects of ill health on handgrip strength, the analyses were also performed after exclusion of individuals with underweight.

Methods

Research area

This study was performed in a remote, rural, and underdeveloped area in the Upper East Region in Ghana in West Africa. The vast majority of the inhabitants are involved in non-commercial agriculture performed by manual labour. Infectious diseases are the main causes of death.[18] The prevalence of human immunodeficiency virus (HIV) is low (< 4%) compared with other sub-Saharan regions.[18] Since 2002, we have followed a horticultural population in the Garu-Tempane District in the Upper East Region, comprising approximately 25 000 inhabitants living in 32 villages. For each household, we determined the household property value and the socioeconomic status in 2007 according to the Demographic and Health Survey method.[19] Elaborate descriptions of the research population have been given elsewhere.[12,19-21]

Ethical approval was given by the Ethical Review Committee of Ghana Health Services, the Committee Medical Ethics of Leiden University Medical Center, and by the local chiefs and elders. Because of illiteracy, informed consent was obtained orally from the participants in the local language. A consent form was read out to each participant with an explanation on the purpose and conduction of this research project.

DNA collection and genotyping

We collected buccal swabs between 2002 and 2006 of 4336 individuals.[12] Common genetic variation (minor allele frequency

≥ 5%) in the *IL10* gene region was determined by genotyping 20 SNPs, selected from the Yoruba population in the HapMap database (release #21, r^2 = 0.8) and genotyped using mass spectrometry (Seqeunom, San Diego, CA, USA). All SNPs were in Hardy-Weinberg equilibrium, with one exception where a minor deviation was observed.[12] We have recently reported that population stratification is unlikely to influence associations with genetic variation in autosomal genes, as analysis of autosomal DNA, mtDNA and y-chromosomal genetic variation patterns in the research area revealed that women-mediated gene flow is nearly fully random, whereas men-mediated gene flow is highly reduced.[22] This genetic substructure is an immediate result of the patrilocal society. We addressed residual population stratification by adjusting all analyses for tribe. Familial relatedness among individuals was addressed by adjusting all analyses for household.

Handgrip strength and BMI

Handgrip strength and body mass index (BMI) were measured in 2009 and 2010 in 923 individuals aged 50 years and older, recruited independently from the genetic samples. Data on the *IL10* gene were available for 554 of them. We consecutively visited all villages in the research area, in which we set up a mobile fieldwork station. We approached all individuals aged 50 years and older and brought less mobile participants by car. Inclusion was limited by the duration of both field visits. Individuals did not participate if they were unable to leave the house, were absent from the research area for a longer period or refused participation. Handgrip strength was measured using a calibrated Jamar hand dynamometer (Sammons Preston, Bolingbrook, IL, USA) with the participant in an upright position and the arm of the measured hand unsupported parallel to the body. The width of the dynamometer's handle was adjusted to each participant's hand size so that the middle phalanges rested on the inner handle. The participants were instructed to exert maximal force once by each hand. We used the highest measurement of both hands in our analyses. Body height and weight were measured by a calibrated length scale and weighing scale. A BMI of 18.5 kg/m^2 or lower was defined as underweight and a BMI above 18.5 kg/m^2 as a normal BMI, according to the classification of the Food and Agriculture Organization and World Health Organization.[23,24]

Cytokine production capacity

The production capacities of IL10 and TNFα were measured in blood samples that were taken in 2005, 2006, or 2008. Previous publications, studying measurements from these years separately, have reported the procedure by which the blood samples were processed.[15,16,25] Venous blood was locally incubated with *Escherichia coli* LPS, the supernatants frozen and shipped for measurement of cytokine concentrations in the Netherlands by enzyme-linked immunosorbent assay (ELISA). The procedure has been reported to have a small intraindividual compared with the interindividual variation[9] and to be replicable with an interval of two years in this research

area.[16,25] We combined the measurements from all three years of 1177 individuals of whom data on the *IL10* gene were also available.

Analyses

The program Haploview (Broad, Cambridge, MA, USA)[26] was used to test for Hardy-Weinberg equilibrium. Statistical analyses were performed with SPSS Statistics 20 (IBM, Armonk, NY, USA) and Stata/SE 12.0 (StataCorp, College Station, TX, USA). The relations between *IL10* gene SNPs and haplotype copies, cytokine production capacities, and handgrip strength were assessed by linear mixed models. These analyses were adjusted for age, sex, and tribe as fixed factors and household as a random factor. Analyses with handgrip strength were additionally adjusted for height as a fixed factor. Cytokine concentrations were natural-logarithmically transformed due to skewedness and standardised as *z* scores per sex[27] within each year of measurement. The *z* scores of 2005, 2006, and 2008 were averaged into one *z* score per individual. Haplotypes were defined as proinflammatory if associated with lower levels of IL10 and higher levels of TNFα upon stimulation with LPS. Haplotypes were defined as anti-inflammatory if associated with higher levels of IL10 and lower levels of TNFα upon stimulation with LPS.[12] In all haplotype analyses, the posterior probabilities of pairs of haplotypes per individual, as estimated by PHASE,[28] were used as weights.

Table 7.1 • General characteristics of the Ghanaian study population

	Men	Women
Number of individuals	196	358
Age, years	73.0 (9.2)	63.4 (9.2)
Tribe, *n* (%)		
Bimoba	142 (72.4)	252 (70.4)
Kusasi	44 (22.4)	84 (23.5)
Mamprusi	2 (1.0)	12 (3.4)
Busanga	2 (1.0)	8 (2.2)
Fulani	2 (1.0)	1 (0.3)
other	4 (2.0)	1 (0.3)
Number of households	190	299
Household property value, median (iqr) US$	1028 (580–1782)	1183 (585–2055)
Height, cm	166.0 (6.8)	157.9 (6.7)
Weight, kg	49.4 (7.8)	45.4 (7.5)
Body mass index, kg/m^2	17.9 (2.3)	18.2 (2.5)
Body mass index ≤ 18.5 kg/m^2, *n* (%)	113 (57.6)	204 (57.0)
Handgrip strength, kg	29.2 (8.1)	23.4 (5.9)

Data are presented as means with standard deviations unless specified otherwise. Iqr: interquartile range.

Results

Table 7.1 displays the characteristics of 554 individuals aged 50 years and older of whom *IL10* gene variants and handgrip strength were known. Their characteristics were similar as compared with all 4336 individuals of whom *IL10* gene variants were measured and with all 923 individuals of whom handgrip strength was measured (data not shown). Approximately half of them had a BMI of 18.5 kg/m^2 or lower, which is regarded as underweight.[23,24] Mean handgrip strength (with standard deviation) was 27.3 (7.6) kg for those with a normal BMI and 24.1 (6.7) kg for those with underweight. Table 7.2 shows that handgrip strength did not differ between tribes.

Table 7.2 • Associations between tribe and handgrip strength

	n	Handgrip strength, kg
Bimoba	394	26.6 (0.3)
Kusasi	128	26.8 (0.6)
Mamprusi	14	26.3 (1.7)
Busanga	10	30.7 (2.0)
Fulani	3	21.9 (3.6)
Other	3	27.7 (2.8)
p value		0.30

Handgrip strength is presented as means with standard errors, adjusted for age and sex. Differences in handgrip strength between tribes were tested with ANCOVA.

IL10 gene variants and cytokine production capacity

It has been previously shown that several SNPs in the *IL10* gene region influence the production capacities of IL10 and TNFα measured in two independent groups in 2006 and 2008 in this research area.[12,16] First, we confirmed that these relations were present in 1177 individuals of whom *IL10* gene variants were known and of whom measurements of cytokine production capacities were combined from 2005, 2006, and 2008 (Figure 7.1A). When restricting this group to the individuals of whom handgrip strength was also known (n = 457), a similar pattern was present (Figure 7.1B).

It has been previously shown that the SNPs in the *IL10* gene region constitute two haplotypes that influence the production capacities of IL10 and TNFα: a proinflammatory haplotype 1 and an anti-inflammatory haplotype 3.[12,16] We reanalysed the relations between the haplotypes of the *IL10* gene and cytokine production capacities in the 1177 individuals of whom *IL10* gene variants were known and of whom measurements of cytokine production capacities were combined from 2005, 2006, and 2008. We confirmed an additive genetic effect for both haplotypes. With each additional copy of the proinflammatory haplotype 1, z scores of IL10 production capacity were 0.08 lower (standard error (SE) = 0.04; p = 0.028) and z scores of TNFα production capacity were 0.11 higher (SE = 0.04; p = 0.001). With each additional copy of the anti-inflammatory haplotype 3, z scores of IL10 production capacity were 0.19 higher (SE = 0.07; p = 0.005) and z scores of TNFα were 0.10 lower (SE = 0.07; p = 0.167). When restricting this group to the individuals of whom handgrip strength was also known (n = 457), simi-

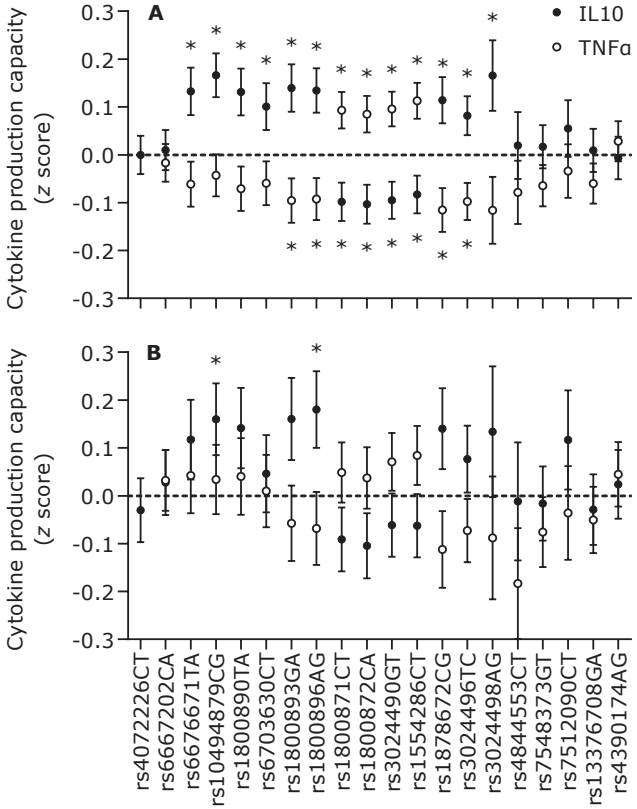

Figure 7.1 • Associations of *IL10* gene SNPs with cytokine production capacities. The relations are shown between the minor allele of each *IL10* gene SNP and the production capacities of IL10 and TNFα for (A) individuals of whom *IL10* gene variants and cytokine production capacities were known (n = 1177) and (B) individuals of whom *IL10* gene variants, cytokine production capacities, and handgrip strength were known (n = 457). Cytokine production capacities are expressed as z scores with standard errors for carriers of at least one copy of the minor allele, adjusted for age, sex, tribe, and household. * $p < 0.05$.

lar patterns were found (data not shown). Haplotype 2 was not related with the production capacities of IL10 (p = 0.813) or TNFα (p = 0.364) and was used in this study as a negative control. Results were not different between men and women.

IL10 gene variants and handgrip strength

Figure 7.2A shows the 20 genotyped SNPs in the *IL10* gene region. Most of the SNPs tag a single linkage disequilibrium (LD) block. The haplotype structure and the frequencies of these haplotypes were previously calculated for all individuals of whom *IL10* gene variants were measured (n = 4336).[12] Haplotype frequencies and Hardy-Weinberg equilibria of the SNPs were not materially different when restricting this group to the individuals of whom handgrip strength was also known (Tables 7.3 and 7.4). Furthermore, allelic frequencies were not different between tribes, with a few exceptions between small and large tribes (Table 7.5).

Figure 7.2B shows that in the individuals of whom *IL10* gene variants and handgrip strength were known, carriers of distinct SNPs had higher or lower handgrip strength when compared with non-carriers. The pattern followed the predefined

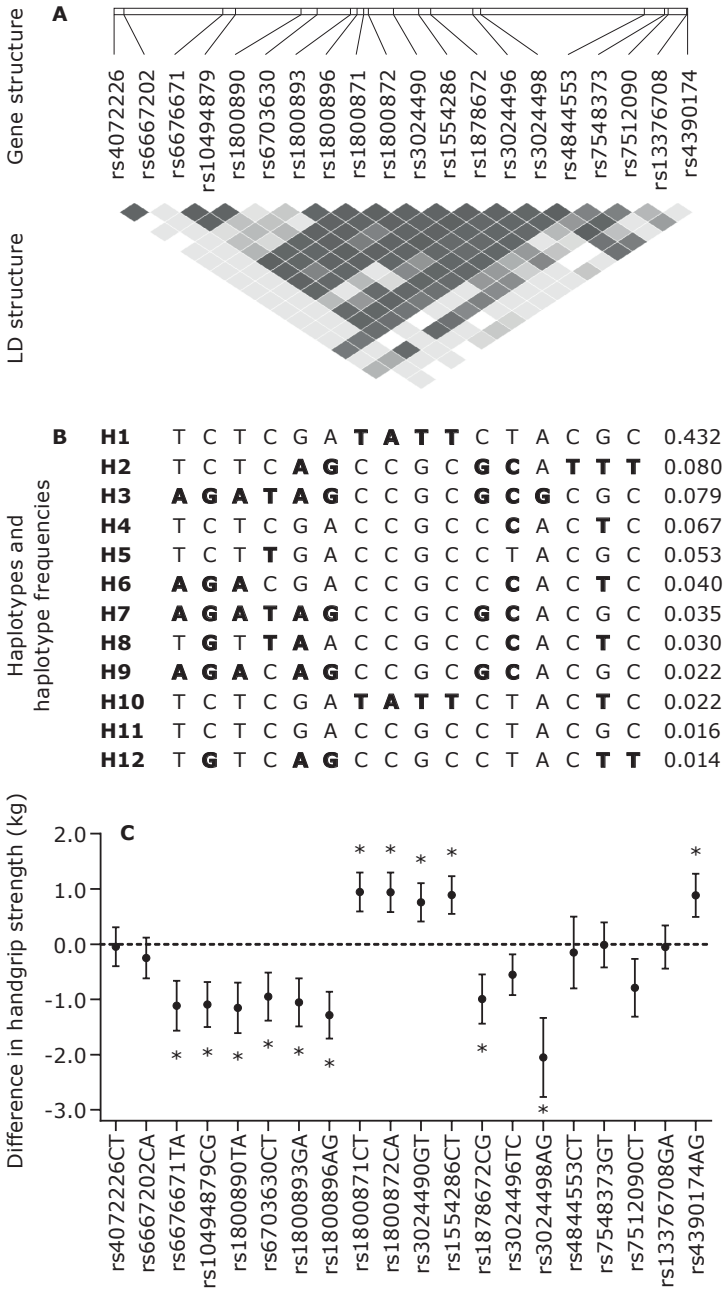

Figure 7.2 • Associations of *IL10* gene SNPs with handgrip strength. Schematic overview of the *IL10* gene region with the locations of the genotyped SNPs indicated by vertical lines (A). Pairwise linkage disequilibrium (LD) as observed in the entire genotyped population (n = 4336) is depicted in greyscale. Population frequencies of the different haplotypes (if > 1%) are presented with the minor alleles of each SNP indicated in bold (B).[12] The relation between the minor allele of each *IL10* gene SNP and handgrip strength for individuals of whom *IL10* gene variants and handgrip were known (C; n = 554). Handgrip strength is expressed as the deviance from the population's mean in kilograms (kg) with standard errors for carriers of at least one copy of the minor allele, adjusted for age, sex, tribe, household, and height. * $p < 0.05$.

Table 7.3 • Haplotype frequencies of the *IL10* gene in the Ghanaian study population and in the entire Ghanaian genotyped population

Haplotype	Study population	Entire genotyped population
n	554	4336
H1	0.457	0.432
H2	0.066	0.080
H3	0.063	0.079
H4	0.069	0.067
H5	0.048	0.053
H6	0.040	0.040
H7	0.048	0.035
H8	0.021	0.030
H9	0.026	0.022
H10	0.021	0.022
H11	0.012	0.016
H12	0.015	0.014
p value	0.79	

Differences in haplotype frequencies between both populations were tested with the Pearson chi-squared test.

anti-inflammatory and proinflammatory haplotype structures shown in Figure 7.2A and Figure 7.1. Results were not different between men and women.

Figure 7.3 shows handgrip strength for carriers and non-carriers of the proinflammatory haplotype 1 and the anti-inflammatory haplotype 3. We observed an additive genetic effect on handgrip strength, which increased with each additional copy of the proinflammatory haplotype 1 and decreased with each additional copy of the anti-inflammatory haplotype 3. Among individuals with a normal BMI, the positive association between the proinflammatory haplotype 1 and handgrip strength was equally strong (p for interaction = 0.398) and the negative association between the anti-inflammatory haplotype 3 and handgrip strength was stronger (p for interaction = 0.009). Both haplotypes were not associated with BMI, neither in all individuals nor in those with BMI above 18.5 kg/m^2 ($p > 0.200$). Contrary to haplotypes 1 and 3, haplotype 2 was not associated with handgrip strength ($p = 0.922$). Results were not different between men and women.

Cytokine production capacity and handgrip strength

Figure 7.4 shows that IL10 and TNFα production capacities were not related to handgrip strength, although an increase in IL10 production capacity concurred with a declining trend in handgrip strength among individuals with a normal BMI. When stratifying by sex, IL10 production capacity was not associated with handgrip strength in either men or women ($p > 0.800$), while TNFα production capacity was positively associated with handgrip strength in men ($n = 128$; $p = 0.007$) but not in women ($n = 329$; $p = 0.819$).

Discussion

We found that distinct haplotypes of the *IL10* gene, associated with variation in the cytokine production capacities of immune cells, were related to handgrip strength in rural Ghana. A proinflammatory haplotype with a population frequency of 43.2% was associated with higher hand-

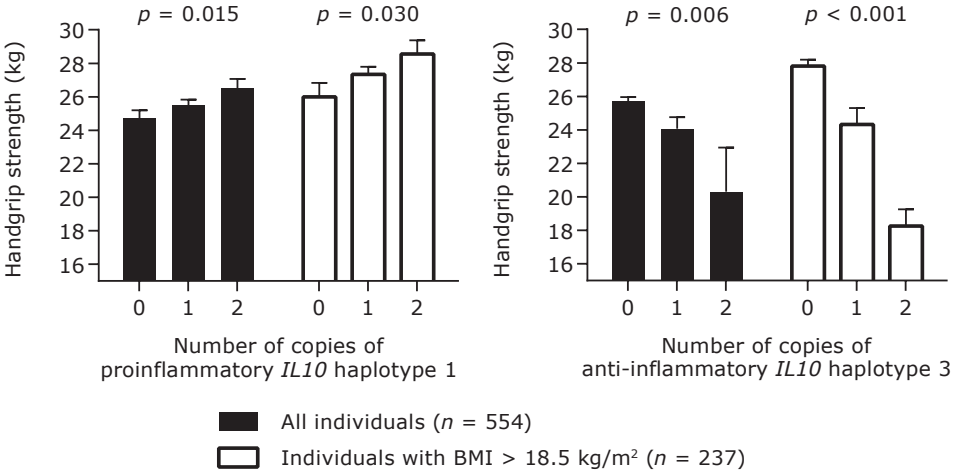

Figure 7.3 • Associations of *IL10* gene haplotypes with handgrip strength in all individuals and in individuals with a normal BMI. Handgrip strength for individuals of whom *IL10* gene variants and handgrip were known (n = 554) presented as means with standard errors, adjusted for age, sex, tribe, household, and height (p values for trends). A BMI of 18.5 kg/m^2 or lower is regarded as underweight.[23,24] For the haplotype structures and frequencies, see Figure 7.2A.

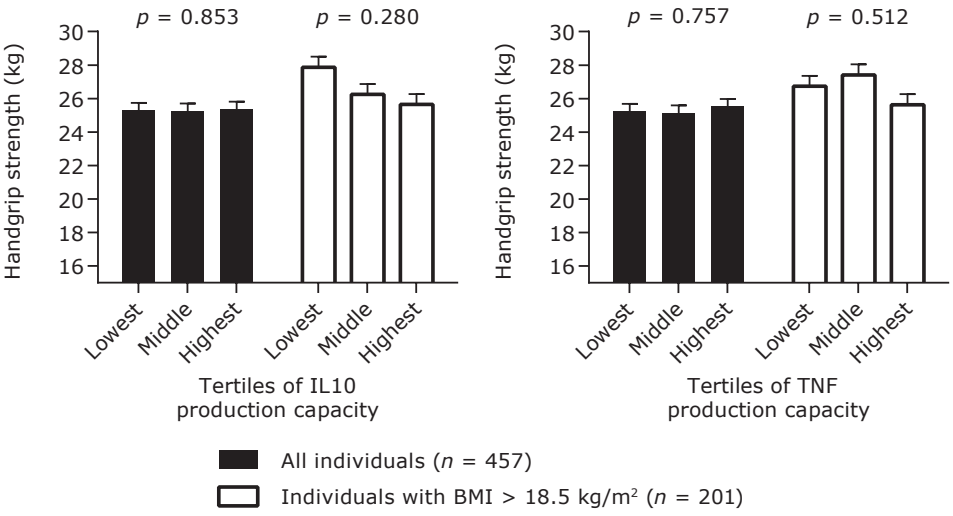

Figure 7.4 • Association of cytokine production capacities with handgrip strength in all individuals and in individuals with a normal BMI. Handgrip strength for individuals of whom *IL10* gene variants, cytokine production capacities, and handgrip strength were known (n = 457) presented as means with standard errors, adjusted for age, sex, tribe, household, and height (p values for trends). A BMI of 18.5 kg/m^2 or lower is regarded as underweight.[23,24]

Table 7.4 • Locations and minor allele frequencies of the *IL10* gene SNPs in the Ghanaian study population and in the entire genotyped population

IL10 SNP	Alleles	Location	Study population		Entire genotyped population	
			MAF	HWE	MAF	HWE
rs4072226	C/T	promotor	0.463	0.936	0.456	0.865
rs6667202	C/A	promotor	0.499	0.851	0.484	0.602
rs6676671	T/A	promotor	0.195	0.870	0.200	0.832
rs10494879	C/G	promotor	0.269	0.823	0.284	0.025
rs1800890	T/A	promotor	0.192	0.822	0.201	0.865
rs6703630	C/T	promotor	0.206	0.579	0.220	0.066
rs1800893	G/A	promotor	0.256	0.005	0.284	0.196
rs1800896	A/G	promotor	0.260	0.064	0.284	0.401
rs1800871	C/T	promotor	0.509	0.984	0.470	0.302
rs1800872	C/A	promotor	0.506	0.811	0.472	0.034
rs3024490	G/T	intron	0.523	0.695	0.484	0.013
rs1554286	C/T	e/i boundary	0.500	0.433	0.468	0.157
rs1878672	C/G	intron	0.228	0.072	0.244	0.612
rs3024496	T/C	exon	0.393	0.928	0.425	0.043
rs3024498	A/G	exon	0.066	0.904	0.083	0.129
rs4844553	C/T	3′ UTR	0.080	0.963	0.096	0.084
rs7548373	G/T	3′ UTR	0.282	0.790	0.297	0.190
rs7512090	C/T	3′ UTR	0.126	0.681	0.132	0.084
rs13376708	G/A	3′ UTR	0.320	0.784	0.327	0.273
rs4390174	A/G	3′ UTR	0.290	0.981	0.282	0.630

Alleles indicate major/minor alleles. MAF: minor allele frequency. HWE: *p* values for Hardy-Weinberg equilibrium. E/i boundary: boundary between an exon and an intron. 3′ UTR: three prime untranslated region.

Table 7.5 • Minor allele frequencies of the *IL10* gene SNPs per tribe

***IL10* SNP**	**Bimoba**	**Kusasi**	**Mamprusi**	**Busanga**	**Fulani**	**Other**	***p* value**
n	394	128	14	10	3	3	
rs4072226	0.443	0.516	0.536	0.400	0.500	0.500	0.29
rs6667202	0.524	0.429	0.464	0.550	0.250	0.400	0.10
rs6676671	0.186	0.213	0.250	0.300	0.000	0.200	0.25
rs10494879	0.367	0.276	0.286	0.350	0.167	0.300	0.48
rs1800890	0.183	0.211	0.250	0.300	0.000	0.125	0.33
rs6703630	0.194	0.238	0.250	0.300	0.000	0.300	0.12
rs1800893	0.261	0.262	0.154	0.222	0.167	0.000	0.20
rs1800896	0.260	0.264	0.231	0.300	0.177	0.200	0.87
rs1800871	0.509	0.508	0.429	0.550	0.833	0.500	0.79
rs1800872	0.506	0.504	0.429	0.550	0.833	0.500	0.77
rs3024490	0.527	0.512	0.429	0.556	0.833	0.500	0.92
rs1554286	0.503	0.500	0.364	0.500	0.833	0.333	0.71
rs1878672	0.227	0.238	0.154	0.462	0.167	0.100	0.80
rs3024496	0.398	0.399	0.423	0.350	0.177	0.250	0.34
rs3024498	0.059	0.089	0.071	0.001	0.000	0.000	0.56
rs4844553	0.087	0.075	0.036	0.000	0.000	0.000	0.044
rs7548373	0.300	0.258	0.250	0.056	0.000	0.200	0.009
rs7512090	0.141	0.105	0.105	0.000	0.038	0.000	0.003
rs13376708	0.308	0.352	0.357	0.250	0.167	0.600	0.23
rs4390174	0.275	0.321	0.321	0.350	0.667	0.200	0.19

Differences in minor allele frequencies between tribes were tested with the linear-by-linear association test.

grip strength, while an anti-inflammatory haplotype with a population frequency of 7.9% was associated with lower handgrip strength. These associations were most outspoken after exclusion of individuals with underweight.

We investigated the effect of *IL10* gene variants as genetic determinants of the *ex vivo* production capacities of IL10 and TNFα by various immune cells in blood.[12,16] The impact of genetic variants on the cytokine production capacity by specific immune cells is not precisely known, but whole blood stimulation with LPS has been shown to particularly reflect the variance in cytokine production by monocytes.[9] Using genetic determinants, the analyses of the influence of cytokine production capacity on muscle strength are largely free from confounding and reverse causality.[17] This is an advantage compared with earlier research in which cytokine production capacity was only measured on the phenotypic level.[6]

Mice studies have shown that repair and maintenance of skeletal muscle tissue are dependent on the innate immune system.[2-5] Critical for this role of the innate immune system are monocytes infiltrating muscle tissue after injury[29,30] and producing proinflammatory cytokines, of which most notably TNFα.[31,32] As a counterbalance, the anti-inflammatory cytokine IL10 downregulates the proinflammatory functioning of monocytes and the production of proinflammatory cytokines such as TNFα[33] and is associated with deceleration of skeletal muscle regeneration.[5] In humans, we have recently reported that a higher capacity to produce proinflammatory cytokines, including TNFα, coexists with higher muscle strength and muscle mass.[6] We now show that the same associations exist between genetic determinants of the cytokine production capacity and handgrip strength. These findings support that cytokine production capacity might be important for human muscle repair and maintenance as well.

As an alternative explanation of the association between the *IL10* gene variants and muscle strength, proinflammatory *IL10* gene variants might yield a better resistance to infectious diseases and thereby a better resistance to muscle wasting due to disease. Cytokine production capacity is related to the incidence and severity of infectious diseases.[34] In rural Ghana, infectious diseases are the main causes of death[18] and we have earlier observed in this research area that carriers of a proinflammatory *IL10* gene haplotype have a survival advantage when drinking from pathogen-rich sources like open wells and rivers.[12] Others have found another anti-inflammatory genetic variant associated with a higher IL10 production capacity to be more prevalent among tuberculosis patients compared with healthy controls in Gambia.[35] Such a mechanism could explain why no relation between a SNP on the *IL10* gene and handgrip strength was found in a European population living in an environment where the pathogenic burden is relatively low.[36]

Infectious diseases and malnutrition, which are common in this research area,[18,21,37-39] are closely associated with

underweight.[23] In an attempt to account for the possible effects of ill health on handgrip strength, we repeated the analyses after exclusion of individuals with underweight. Among individuals with a normal BMI, we found an equal relation between the proinflammatory haplotype and a stronger relation between the anti-inflammatory haplotype and muscle strength. Moreover, we found that the haplotypes that were associated with handgrip strength were not associated with BMI. These findings indicate that the relation between *IL10* gene variants and handgrip strength is unlikely to be largely shaped by differences in health.

Although *IL10* gene variants were related to handgrip strength, the production capacity of IL10 was not associated with handgrip strength. As a possible explanation, monocytes activated by a proinflammatory stimulus like LPS migrate into injured muscle tissue and change only after two days into macrophages with an anti-inflammatory phenotype.[2] We measured the cytokine production capacity of IL10 24 hours after stimulation with LPS, which could have been too early to measure the maximum IL10 production capacity. In addition, IL10 is known to have autoregulatory effects, as it strongly inhibits IL10 mRNA synthesis in LPS-activated monocytes.[40] This could have diluted the IL10 production capacity measurement. Another explanation is that the relation might be confounded. Firstly, depletion of muscle tissue by malnutrition or disease could have disrupted the beneficial role of IL10 production capacity in muscle repair and maintenance. As BMI is likely to reflect muscle mass in this lean population, the stronger relation between *IL10* gene variants and handgrip strength in the higher BMI stratum points at this possibility. Secondly, infectious diseases might have interfered with our measurements of IL10 cytokine production capacity. Earlier, we have shown that infectious diseases are highly endemic in the research area and induce a proinflammatory immune response.[39] The relation between the *IL10* gene variants and cytokine production capacity was less outspoken in the smaller group with available data on handgrip strength, possibly due to such environmental factors.[25] Thirdly, physical activity attenuates the production capacity of monocytes[41] but meanwhile improves muscle strength.[42] In this population, physical activity is of vital importance due to the manual labour in farming and housekeeping that is necessary for subsistence up to the highest ages. Fourthly, while we measured cytokine production in whole blood, muscle tissue is recognised to be a cytokine producing organ itself. Although little has been reported about the muscle-specific production of IL10, the *IL10* gene might exert its effects in muscle tissue in an autocrine and paracrine manner.[43] Such a mechanism would not be revealed by the analysis of cytokine production capacity in whole-blood samples.

While we observed no relation between IL10 production capacity and handgrip strength, we observed a positive relation between TNFα production capacity and handgrip strength in men, but not in women. This finding is in agreement with previous research in Europeans.[6] A men-specific positive relation has been found between

TNFa production capacity upon stimulation with LPS, a toll-like receptor 4 (TLR4) agonist, and muscle mass and strength. A women-specific positive relation has been found between TNFα production capacity upon stimulation with Pam3Cys-SK4, a TLR2/1 receptor agonist, and muscle mass. These findings indicate that in skeletal muscle tissue, the TLR4 pathway is predominant in men and the TLR2/1 pathway is predominant in women.

Our study has some limitations. Firstly, as in all genetic association studies, we cannot exclude that the *IL10* gene variants are in linkage disequilibrium with variants of other genes that affect handgrip strength. However, we have previously described that this is unlikely, because resequencing of the *IL10* gene region and its surroundings did not result in any variants additional to the SNPs that were genotyped in the *IL10* gene.[12] Secondly, we did not document possible epigenetic variation in the *IL10* gene. There is growing evidence that caloric intake and dietary composition modify epigenetic marks,[44,45] which can influence transcription of the *IL10* gene in immune cells.[46] Malnutrition, being common in the research area,[18,21,37,38] could thereby affect the relation between the *IL10* gene and muscle strength. Lastly, this is a cross-sectional study, while it would be valuable to associate *IL10* gene variants with longitudinal decline in handgrip strength with age.

In conclusion, this study shows that *IL10* gene variants associate with the production capacities of IL10 and TNFα and strongly relate to handgrip strength in rural Africa. A haplotype reflecting a proinflammatory immune response associates with higher muscle strength, while a haplotype reflecting an anti-inflammatory immune response associates with lower muscle strength, especially after exclusion of individuals with underweight. Future studies are needed to elucidate whether variants of the *IL10* gene determine handgrip strength through their role in the repair of skeletal muscle tissue directly or indirectly through their role in the defence against infectious diseases.

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MEASURING SENESCENCE THROUGH HANDGRIP STRENGTH

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Handgrip strength, ageing and mortality in rural Africa.
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Background • Muscle strength measured as handgrip strength declines with increasing age and predicts mortality. While handgrip strength is determined by lifestyle through nutrition and physical activity, it has almost exclusively been studied in western populations with a sedentary lifestyle. This study aims to investigate the age pattern of handgrip strength and its relation with mortality in a population characterised by a predominance of malnutrition and manual labour.

Methods • From a traditional African rural population in Ghana, 923 community-dwelling individuals aged 50 years and older were included. Demographic characteristics were registered. At baseline, height, body mass index (BMI), and handgrip strength were measured and compared with those in a western reference population. Survival of the participants was documented during a period of up to two years.

Results • Handgrip strength was dependent on age, sex, height, and BMI. Compared with the western reference population, handgrip strength was lower due to a lower height and BMI, but declined with age similarly. Risk of mortality was lower in participants having higher handgrip strength, with a hazard ratio of 0.94 per kg increase (p = 0.002). After adjustment for age, sex, tribe, socioeconomic status, drinking water source, height, and BMI, only handgrip strength remained predictive of mortality.

Conclusion • In a traditional rural African population characterised by malnutrition and manual labour, handgrip strength declines with age and independently predicts mortality similarly to western populations. Handgrip strength can be used as a universal marker of biological age.

Muscle strength measured as handgrip strength is widely used as a simple and robust marker of biological age. Handgrip strength declines with increasing age in different ethnicities, especially after the age of 50 years.[1-7] At both middle and high ages, low handgrip strength is associated with increased risks of future disability;[8-14] of age-related diseases such as the metabolic syndrome,[15] cardiovascular disease,[16,17] diabetes mellitus,[18] and cognitive impairment,[12,19] of hospitalisation,[13,20] and of treatment-related complications.[13] Moreover, low handgrip strength predicts all-cause mortality[13-15,21-23] as well as mortality due to cardiovascular disease[6,24] and cancer.[15,23,24] Consequently, low handgrip strength is considered as an accurate indicator of frailty.[25]

Apart from age, sex, and ethnicity, handgrip strength is dependent on height, body mass index (BMI), nutritional status, and physical exercise.[11,26-29] While these determinants are closely related to lifestyle, research on handgrip strength has almost exclusively been conducted in western societies where an affluent and sedentary lifestyle is omnipresent.[3,30,31] In societies characterised by a predominance of mal-

nutrition and manual labour, handgrip strength may be a reflection of dietary composition and muscle training rather than senescence. In addition, the association between age, handgrip strength, and mortality may be mediated by age-related diseases and be attenuated when these are uncommon.[27,32]

This study investigates the age pattern of handgrip strength and its relation with mortality in a traditional rural African population where a sedentary lifestyle is absent and age-related diseases are uncommon.[32-34] We show the age pattern of handgrip strength and it with that in a western reference population, we assess the individual characteristics that determine handgrip strength, and we assess whether handgrip strength predicts mortality in this population.

Methods

Research area and participants

This study was conducted in the Garu-Tempane District in the Upper East Region in Ghana. The area is rural, remote, and one of the least developed in the country. The vast majority of the inhabitants are involved in non-commercial agriculture performed by manual labour without proper means of transportation or mechanised farming. Hospital care is absent. Infectious diseases are highly endemic and constitute the main causes of death, although the prevalence of human immunodeficiency virus (HIV) is low (< 4%) compared with other African regions.[35]

Since 2002, we have kept a demographic registry of the population within a research area of 375 km² comprising 32 villages. During yearly visits, we registered the name, age, sex, tribe, and location of living of each inhabitant. In 2007, we determined the property value of each household. From this value, an index of the socioeconomic status with a standard normal distribution was calculated according to the Demographic and Health Survey method.[36] In addition, we registered the main drinking water source of each household. Water from boreholes was classified as safe and water from open wells and rivers as unsafe, based on their pathogen contents.[37] Annual migration relative to the study population's size was 2% into and 1% out of the research area. An elaborate description of this study population has been given elsewhere.[32,34,36,37]

Ethical approval was given by the Ethical Review Committee of Ghana Health Services, the Committee Medical Ethics of Leiden University Medical Center, and the local chiefs and elders. Because of illiteracy, informed consent was obtained orally from the participants after explanation of the purpose and conduction of this research project. Participation was only proceeded after verbal consent in the participant's own language.

Measurements

In 2009 and 2010, we measured handgrip strength among 923 inhabitants aged 50 and older, who were recruited in villages visited consecutively. To ensure maximal participation, we set up a mobile field work

station in the villages and, if necessary, brought less mobile participants by car. Reasons of exclusion included death of the individual since the last registration (n = 48), refusal of participation (n = 35), absence from the research area during our visits because of migration or travelling (n = 30), and other reasons (n = 46).

Handgrip strength in kilograms was measured using a calibrated Jamar hand dynamometer (Sammons Preston, Bolingbrook, IL, USA), while the participant was standing in an upright position with the arms unsupported parallel to the body. The width of the dynamometer's handle was adjusted to each participant's hand size. Participants were instructed to exert maximal force with each hand once. The handgrip strength of the hand with the highest measurement was registered. Body height and weight were measured with a calibrated length scale and weighing scale. BMI was calculated as body weight in kilograms divided by squared body height in metres.

After the measurements in 2009 and 2010, follow-up data on 915 individuals (99.1%) were available in our demographic registry. Follow-up lasted until death, migration out of the research area, loss to follow-up or our last visit to the research area in 2011.

Reference population

To compare the Ghanaian study population with a western population, we retrieved data from the Leiden Longevity Study. This study included offspring of long-lived native Dutch siblings and the partners of the offspring without selection criteria on health or demographic characteristics. The design of the study has been previously described in more detail.[38] We used data on age, sex, height, BMI and handgrip strength measured in 316 offspring and 311 partners aged 50 to 80 years. Handgrip strength did not differ between offspring and partners. The measurements were performed with the same hand dynamometer and in the same position as described for the Ghanaian study population.[39]

Analyses

Differences between both populations in mean values of height, BMI, and handgrip strength and in the decline in handgrip strength per year of age were determined by linear regression with age as an independent variable and were restricted to participants aged 50 to 80 years. Determinants of handgrip strength in the Ghanaian study population were assessed by linear regression including all participants aged 50 to 97 years. Handgrip strength in the Ghanaian study population was standardised according to the age-group- and sex-specific mean height and BMI in the Dutch reference population, using the regression coefficients obtained for these determinants in the Ghanaian study population. To investigate whether handgrip strength predicted mortality, we constructed Kaplan-Meier survival curves with left truncation to account for different ages at baseline. Survival curves were separated between individuals classified as having low or high handgrip strength according to the age-group- and sex-specific medians. Hazard ratios were deter-

mined by Cox regression with follow-up starting at the time of the measurements of handgrip strength.

Results

Table 8.1 shows the characteristics of the Ghanaian study population at the moment of handgrip strength measurement in 2009 or 2010. For comparison, we used data from a Dutch reference population including 316 men and 311 women aged 50 to 80 years. As described previously for this population,[39] mean height (with standard deviation) was 177.9 (7.7) cm in men and 165.7 (5.9) cm in women; mean BMI was 27.1 (4.1) kg/m^2 in men and 26.4 (4.6) kg/m^2 in women and mean handgrip strength was 46.9 (8.1) kg in men and 29.3 (5.5) kg in women. These values of height and BMI were higher than those in the Ghanaian study population adjusted for age (both $p < 0.001$).

Figure 8.1A shows that mean handgrip strength was lower in the Ghanaian study population compared with the Dutch reference population. Overall, the difference (with 95% confidence interval) was 14.7 (13.6 to 15.8) kg in men and 5.7 (4.9 to 6.4) kg in women (both $p < 0.001$). In the Ghanaian study population, handgrip strength declined with 0.4 (0.3 to 0.5) kg per year of age in men and with 0.3 (0.2 to 0.4) kg per year of age in women (both $p < 0.001$). For comparison, handgrip strength in the Dutch reference population declined with a slightly higher rate of 0.6 (0.5 to 0.7) kg per year of age in men up to the age of 80 years ($p = 0.046$), with a similar rate in men up to the age of 75 years ($p = 0.384$) and with a similar rate in women ($p = 0.687$).

Determinants of handgrip strength in the Ghanaian study population are described in Table 8.2. In a multivariate analysis of

Table 8.1 • General characteristics of the Ghanaian study population

	Men	Women
Number of individuals	480	443
Age, median (iqr) years	67 (58–76)	61 (56–70)
Tribe, %		
Bimoba	69.5	68.6
Kusasi	22.5	25.5
other	8.1	5.9
Household property value, median (iqr) US$	1008 (500–1700)	1196 (583–2108)
Access to safe drinking water, %	86.7	88.5
Weight, kg	50.6 (7.9)	45.5 (7.6)
Height, cm	167.5 (6.8)	157.9 (6.8)
BMI, kg/m^2	18.0 (2.3)	18.2 (2.6)
Handgrip strength, kg	31.3 (8.7)	23.6 (5.9)

Data are presented as means with standard deviations unless specified otherwise. Iqr: interquartile range. BMI: body mass index.

demographic and anthropometric characteristics, handgrip strength in both sexes was higher in individuals with a higher age, with a higher height and with a higher BMI. When this analysis was not stratified by sex, handgrip strength was 6.0 (5.0 to 7.0) kg higher in men ($p < 0.001$).

Figure 8.1B shows that the differences in handgrip strength between the Ghanaian study population and the Dutch reference population were attenuated when handgrip strength in the Ghanaian study population was standardised according to the age-group- and sex-specific mean height and BMI of the Dutch reference population. Hereby accounting for the differences in height and BMI between both populations, handgrip strength was similar in men ($p = 0.350$) and 1.7 (0.9 to 2.4) kg higher in Ghanaian women ($p < 0.001$). Standardised handgrip strength declined with age with similar rates in men ($p = 0.067$) and women ($p = 0.233$) in both populations.

Figure 8.2 shows how mortality is predicted by handgrip strength in the Ghanaian study population. Data on follow-up were available for 476 men and 439 women. From the baseline measurements in 2009 and 2010 through the end of follow-up in 2011, we recorded 1492 person-years and 46 deaths. Mean individual follow-up was 20 (6) months. Individuals were classified as having low or high handgrip strength according to the age-group- and sex-specific medians. Risk of mortality was lower in individuals with high handgrip strength, with a hazard ratio of 0.45 adjusted for age and sex ($p = 0.010$).

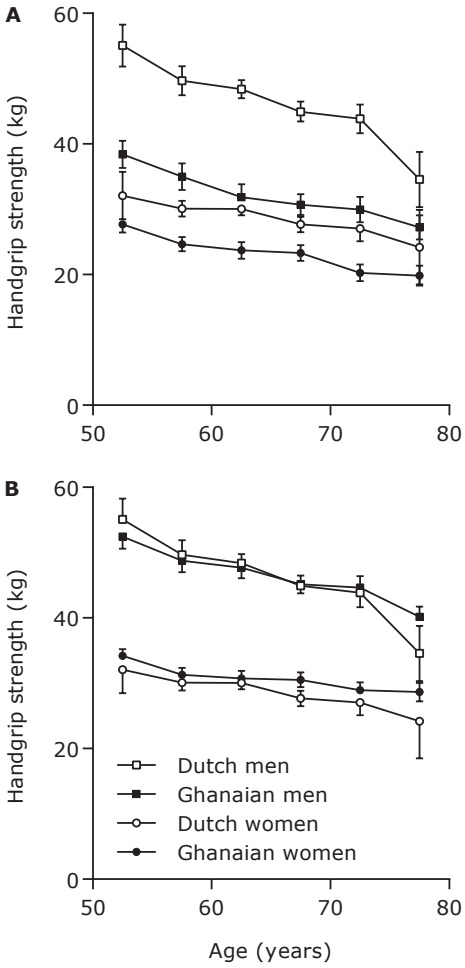

Figure 8.1 • Handgrip strength per sex and per age group in the Ghanaian study population compared with the Dutch reference population. A comparison of mean handgrip strength with 95% confidence intervals per 5-year age category and per sex as observed in the Ghanaian study population and the Dutch reference population (A).[39] Idem after standardisation of the individual handgrip strength measurements in the Ghanaian study population according to the age-group- and sex-specific height and BMI of the Dutch reference population (B).[39]

Table 8.2 • Determinants of handgrip strength in the Ghanaian study population

	Men	Women
	Difference in handgrip strength (95% CI), kg	**Difference in handgrip strength (95% CI), kg**
Age, per year	-0.3 (-0.3 to -0.2) **	-0.2 (-0.2 to -0.1) **
Tribe, Bimoba vs other	+1.3 (-0.1 to +2.6)	+0.1 (-0.9 to +1.1)
Socioeconomic status, per SD	+0.1 (-0.4 to +0.6)	-0.1 (-0.4 to +0.3)
Safe drinking water, vs unsafe	+1.1 (-0.8 to +2.9)	+1.1 (-0.3 to +2.5)
Height, per cm	+0.3 (+0.2 to +0.4) **	+0.3 (+0.2 to +0.3) **
BMI, per kg/m^2	+1.3 (+1.0 to +1.6) **	+0.6 (+0.4 to +0.8) **

Data are presented as kilograms difference in handgrip strength per unit increase for continuous variables or between categories for categorical variables. Differences are shown with 95% confidence intervals (95%CI) and are adjusted for all other variables. SD: standard deviation. ** $p < 0.001$.

Determinants of mortality in the Ghanaian study population are described in Table 8.3. While handgrip strength, age, and BMI determined mortality in the univariate analysis, only handgrip strength determined mortality in the multivariate analysis with a hazard ratio of 0.94 per kg increase ($p = 0.016$). The association between handgrip strength and mortality in the univariate analysis remained unchanged after the adjustments in the multivariate analysis. In the multivariate analysis, the association of handgrip strength with mortality was not different between

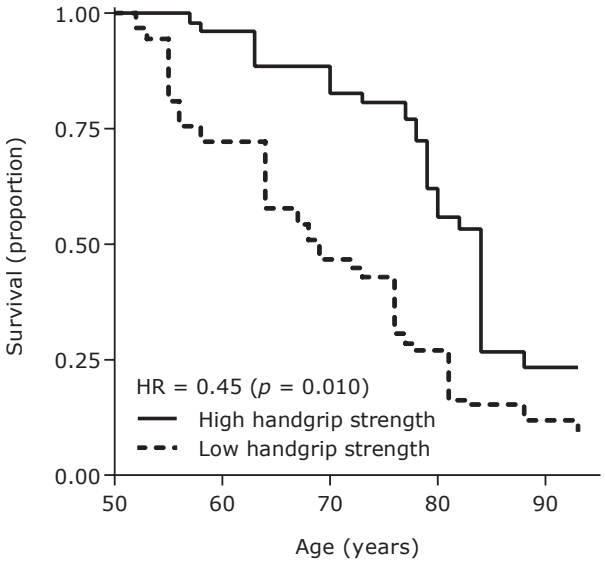

Figure 8.2 • Handgrip strength as a predictor of mortality in the Ghanaian study population. Age-specific survival is dependent on handgrip strength in the Ghanaian study population. Handgrip strength is classified as low or high according to the age-group- and sex-specific medians. The hazard ratio (HR) is given for individuals with high handgrip strength relative to those with low handgrip strength, adjusted for age and sex.

Table 8.3 • Determinants of mortality in the Ghanaian study population

	Univariate	Multivariate
	Difference in handgrip strength (95% CI), kg	**Difference in handgrip strength (95% CI), kg**
Handgrip strength, per kg	0.94 (0.90 to 0.98) *	0.94 (0.89 to 0.99) *
Age, per year	1.05 (1.02 to 1.08) *	1.02 (0.99 to 1.06)
Sex, men vs women	1.44 (0.79 to 2.62)	1.71 (0.76 to 3.84)
Tribe, Bimoba vs other	1.61 (0.80 to 3.25)	1.62 (0.79 to 3.31)
Socioeconomic status, per SD	0.81 (0.63 to 1.06)	0.88 (0.68 to 1.14)
Safe drinking water, vs unsafe	0.92 (0.39 to 2.18)	1.09 (0.46 to 2.57)
Height, per cm	0.99 (0.95 to 1.02)	1.00 (0.95 to 1.05)
BMI, per kg/m^2	0.81 (0.72 to 0.92) *	0.90 (0.79 to 1.03)

Data are presented as hazard ratio per unit increase for continuous variables or comparing categories for categorical variables. Differences are shown with 95% confidence intervals (95%CI) and are adjusted in the multivariate analysis for all other variables. SD: standard deviation. * $p < 0.05$.

individuals below or above the age of 65 ($p = 0.920$), between men and women ($p = 0.380$), between individuals with a low or high BMI ($p = 0.188$), or between individuals with a low or high socioeconomic status ($p = 0.890$).

Additional adjustment for family relations by clustering on the household level did not materially change the results.

Discussion

This study aims to study the age pattern of handgrip strength and its relation with mortality in a traditional rural African population with a non-western lifestyle. Handgrip strength was lower compared with a western reference population due to a lower height and BMI, but it declined with age with a similar rate. Lower levels of handgrip strength predicted mortality independent of its other determinants related to nutritional and socioeconomic status. Its predictive value was comparable with that known for western populations.[6,13,21,24]

The Ghanaian study population contrasts sharply with western populations, as a sedentary lifestyle is absent and age-related diseases are uncommon.[32-34] Because handgrip strength is dependent on nutritional status,[29] this contrast is most relevantly characterised by a low BMI and near absence of obesity (Chapter 9 of this thesis).[32] In line with this, handgrip strength was closely related to BMI and low compared with a Dutch reference population due to a lower BMI. Besides nutrition, handgrip strength is associated with physical activity and socioeconomic status.[26,27,40-42] Unlike western populations, almost all inhabitants in the research area engage in lifelong physical exercise. Man-

ual labour in farming and housekeeping is necessary for subsistence up to the highest ages. Meanwhile, mechanical means of farming and transportation are lacking. Most inhabitants live in poverty[33] and common property is confined to cattle, fertiliser, and iron roofing.[36] Despite these differences, the variation in handgrip strength in the Ghanaian study population was similar to that in the Dutch reference population and as reported for other western populations.[2,43,44] Moreover, handgrip strength declined with age in these populations similarly.

Few other studies have described handgrip strength in traditional lean populations in Africa. Absolute levels of handgrip strength have been reported to be up to 4 kg lower in rural Kenya, rural Malawi, and among refugees from Rwanda compared with those found at similar ages in the Ghanaian study population.[31,45,46] Handgrip strength in these populations was also, though less, dependent on BMI. The decline in handgrip strength with age was similar to that in the Ghanaian study population. In a population-wide study in South Africa, handgrip strength did not differ between ethnicities or between rural and urban areas, but it was associated with age, anthropometry, and health.[30] None of these studies related handgrip strength with mortality.

As a western reference population, we used the Leiden Longevity Study.[39] Handgrip strength in this study is slightly higher compared with other western populations. This difference can be a result of international variations in the level of handgrip strength, while the declines with age are similar.[4] Alternatively, this difference can be a result of variations in body position during the measurements. Body position influences the estimation of handgrip strength, although it is not likely to influence its decline with age or its relation with mortality.[47-50] When using reference data from a meta-analysis of handgrip strength in twelve western study populations with a body position different from the Leiden Longevity Study, the decline in handgrip strength with age was similar to that in the Ghanaian study population.[7] Suitably, the body position during the measurements in the Ghanaian study population was identical to that in the Leiden Longevity Study.

This study has the following limitations. First, handgrip strength was measured only once, while it might have been valuable to relate individual age-related changes in handgrip strength with anthropometry and mortality. Second, nutritional status was documented by BMI, while it might have been valuable to relate dietary composition and physical activity with the level of handgrip strength as well as its predictive value of mortality, but these determinants were not formally documented. Lastly, because diseases were not registered, the possible effects of diseases on handgrip strength could not be studied and neither could handgrip strength be assessed as a predictor of morbidity.

In conclusion, this study shows that handgrip strength declines with age with a similar rate and functions equally well as an independent predictor of mortality in a traditional rural African population com-

pared with western populations. Across divergent environments, in different populations, and despite variations in lifestyle, handgrip strength can be easily and universally used to identify frail people at increased risk of mortality.

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9 MEASURING SENESCENCE THROUGH CARDIOVASCULAR DISEASE

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Background • To test the inflammatory origin of cardiovascular disease, as opposed to its origin in a western lifestyle, we conducted a population-based assessment of the prevalences of cardiovascular risk factors and cardiovascular disease in an inflammation-prone African population, including electrocardiography and ankle-arm index measurement.

Methods • In a traditional population in rural Ghana, characterised by adverse environmental conditions and a high infectious load, we studied a population-based sample of 924 individuals aged 50 years and older. We registered median values of cardiovascular risk factors, including waist circumference, body mass index (BMI), blood pressure, and markers of glucose and lipid metabolism and inflammation. We assessed the prevalences of myocardial infarction detected by electrocardiography and prevalence of peripheral arterial disease detected by ankle-arm index. We compared the results with known prevalences in American and European populations.

Results • As compared with western populations, we found that Ghanaians had more proinflammatory profiles and less cardiovascular risk factors, including obesity, dysglycæmia, dyslipidæmia, and hypertension. The prevalence of cardiovascular disease was also lower. Definite myocardial infarction was present in 1.2% (95% CI: 0.6 to 2.4). Peripheral arterial disease was present in 2.8% (95% CI: 1.9 to 4.1).

Conclusion • Taken together, our data indicate that for the pathogenesis of cardiovascular disease inflammatory processes alone do not suffice and additional factors, probably lifestyle-related, are mandatory.

The pathogenesis of cardiovascular disease has long been described as an accumulation of lipids in a dysfunctional endothelial wall, driven by lifestyle-related factors such as smoking, dyslipidæmia, dysglycæmia, obesity, and hypertension. Since the end of the millennium, it has been commonly accepted that inflammatory processes play a prominent role in atherosclerosis, reclassifying cardiovascular disease as a chronic inflammatory disorder.[1-3] Still, the exact balance between lifestyle and inflammation in its causality has remained unclear. There are both indications of subendothelial lipid retention[4] and inflammation[1] as being the primary causative factor.

The lifestyle-related risk factors are mainly present in western societies, which have experienced an epidemiologic and demographic transition. In these countries, cardiovascular disease is one of the major causes of morbidity and the most common cause of mortality. In developing countries, where these risk factors are absent, infectious diseases prevail.[5] Without the public health care of western societies, infectious diseases are highly lethal. Selectively those individuals that resist the

high infectious pressure by a strong inflammatory response will survive, resulting in a population expressing a selectively proinflammatory immune system.[6-10] If cardiovascular disease is an inflammatory disease, it can be expected to be provoked by such a proinflammatory status. Indeed, with the progressing control of lethal infectious diseases, cardiovascular pathology is becoming more frequent in developing countries, especially in semideveloped environments where a western lifestyle concurs with a high infectious load.[5,11,12] Within western countries, the cardiovascular risk of migrants from developing countries remains elevated.[12-14] Inflammatory markers are used to predict cardiovascular risk.[2,3] These figures reinforce the dominant role of inflammation in cardiovascular pathogenesis. However, the occurrence of cardiovascular disease in the remote and rural regions of developing countries, where a high infectious load induces proinflammatory phenotypes but a western lifestyle is absent, has infrequently been studied.[15-17] Knowledge on these data can further unravel the roles of lifestyle and inflammation as the origins of cardiovascular disease.

We have conducted a large population study in rural Ghana in West Africa. This study population has only recently started to experience an epidemiologic transition; the elderly form not only a subgroup that has survived the pretransitional past, but have also been exposed to a pretransitional environment during the largest part of their lives.[18] Previous immunologic and genetic studies have shown this population to exhibit a proinflammatory immune status.[8-10] A previous study in a comparable population of forager-horticulturalists in Bolivia provided a first indication of a low prevalence of cardiovascular disease in populations where a western lifestyle is uncommon.[7] Our study population concerns a purely horticultural population where lifestyle-related risk factors are even more exceptional. This study firstly extends previous observations with a detailed assessment of cardiovascular health in a traditional African population, including electrocardiography and ultrasound measurement of ankle-arm indexes.

Methods

Research area

The Upper East Region in Ghana is remote, rural, and one of the least developed regions of the country. The vast majority of the inhabitants are involved in non-commercial agriculture performed by manual labour.[19] The yearly per capita income averages US$ 135;[20] 88% of the households lives in poverty.[21] Infectious diseases are the main causes of death.[22]

Since 2002, we have registered and followed a traditional horticultural population in the Garu-Tempane District in the Upper East Region occupying a research area of 375 km^2 with approximately 25 000 inhabitants living in 32 villages. Families are polygamous and live as extended families in compounds. Annual migration relative to the study population's size was 2% into and 1% out of the research area.[18] Hospital care is absent; the nearest physician is at 40 kilometres' distance. Vaccination

of children has recently been introduced. Sewage disposal systems are non-existent.

Life expectancy at birth is 59 years. Based on changes in age-specific mortality rates over time, we have shown epidemiologic transition in this population to have started only recently with declines in mortality rates at low ages. Generations aged 50 years or more are unaffected by these changes and conform to a pretransitional pattern of survival.[18]

All inhabitants have been registered and of each are known the name, age, sex, tribe, and location of living. We determined the lifetime fertility, defined as the median number of born children per postreproductive woman, based on fertility data gathered in the research area in 2003.[23] For each household, we determined the household property value in 2007 according to the Demographic and Health Survey method.[24] In 2008, we determined the prevalences of infections by malaria species by PCR of blood samples and those of infections by helminths and protozoa by PCR of stool samples.[25] A more elaborate description of this cohort has been given elsewhere.[18,23,24] This study was conducted within this cohort.

Ethical approval was given by the Ethical Review Committee of Ghana Health Services, the Committee Medical Ethics of Leiden University Medical Center, and by the local chiefs and elders. Because of illiteracy, informed consent was obtained orally from the participants. A consent form was designed with an explanation on the purpose and conduction of this research project. This form was to be read out to each participant in his own language; consent was given verbally in the local language. Participation was only proceeded after verbal consent of the participant. The full text of the form was approved by the Ethical Review Committee of Ghana Health Services.

Participants

Based on our demographic cohort registration up to 2009, we targeted all inhabitants aged 50 years and older, counting 2684 individuals. We consecutively visited the villages in our research area during two field visits in 2009 and 2010 to approach the targeted inhabitants. Inclusion was restricted by the duration of both field visits. To ensure maximal participation, we set up a mobile field work station in different villages and, if necessary, brought less mobile participants by car. We included 924 individuals, of whom 610 also underwent electrocardiographic measurement. Of the approached inhabitants, 4.4% could not participate due to death of the individual since the last registration, 3.2% refused participation, 2.8% was absent from the research area during our visit because of migration or travelling, and 4.2% did not participate for other reasons. At the station, the identity of the participant was confirmed, the personal data in the cohort registration was checked, and clinical and electrocardiographic investigations were performed. In addition, blood plasma samples of 266 individuals were available from 2008. On these, biochemical investigations were performed.

Clinical measurements

Height and weight were measured by a calibrated length scale and weighing scale. Body mass index (BMI) was calculated as weight divided by squared height. Waist circumference was measured while standing by a measure tape at umbilical level. In a lying and resting position, blood pressure was measured at one arm using a calibrated sphygmomanometer and a Littman stethoscope. Hypertension was determined by these blood pressures and classified as stage I, stage II, or isolated systolic hypertension. Hypertension was defined as stage I for systolic blood pressures of 140 to 160 mmHg and/or diastolic blood pressures of 90 to 100 mmHg, as stage II for systolic blood pressures of 160 mmHg onward and/or diastolic blood pressures of 100 mmHg onward, and as isolated systolic hypertension for systolic blood pressures of 140 mmHg onward with diastolic blood pressures below 90 mmHg.[26] Systolic blood pressures were measured on the dorsalis pedis artery and the posterior tibial artery at both ankles using a calibrated sphygmomanometer and a Doppler ultrasound machine (ImexDOP CT+, Natus-Nicolet, Golden, CO, USA). As a measure of peripheral arterial disease, the ankle-arm index was calculated by dividing the average systolic pressure of both arteries of each ankle by the systolic arm pressure. An index below 0.9 of either ankle indicates peripheral arterial disease.[27] Glucose whole blood concentration was measured on a capillary blood sample from a finger (Accutrend Plus, Roche, Rotkreuz, Switzerland). Whole blood concentrations are circa 11% lower than plasma concentrations.

Biochemical measurements

In 2008, venous blood samples were collected in the morning, carried on ice, centrifuged within four hours, after which plasma was kept frozen during storage and transportation at minimally -20 °C.[9] Blood samples were analysed in our institution's Central Laboratory for Clinical Chemistry. Circulating plasma levels were determined for triglycerides, total cholesterol, apolipoprotein-B100 (apoB100), apolipoprotein-A1 (apoA1), C-reactive protein (CRP), and interleukin-6 (IL6). Measurements of triglycerides and total cholesterol were performed with colorimetric assays (Modular P800, Roche, Rotkreuz, Switzerland), of apoB100, apoA1, and CRP with high-sensitivity immunoturbidimetric assays (Cobas Integra, Roche, Rotkreuz, Switzerland), and of IL6 with sandwich chemiluminescent immunoassay (Evidence Investigator Biochip, Randox, Crumlin, UK). All biochemical markers were measured in plasma; these concentrations are circa 3% lower than serum concentrations.[27] ApoB100 is associated with low-density lipoprotein (LDL) cholesterol, apoA1 with high-density lipoprotein (HDL) cholesterol. The apoB100 concentration and the ratio of the concentrations of apoB100 over apoA1 are strongly predictive risk factors of cardiovascular disease.[27] CRP and IL6 are proinflammatory markers.

Cutoff values for cardiovascular risk factors were derived from the standardly used guidelines of the Adult Treatment Panel III[27] and the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure.[26] Other guidelines were used for criteria not in-

cluded herein, including the concentration of apoB100 and the ratio of the concentrations of apoB100 over apoA1[28] and the concentration of CRP.[29] No commonly accepted cutoff value is available for IL6.

Electrocardiographic measurements

A twelve-lead electrocardiogram (ECG) was recorded for twenty seconds in a lying and resting position (AT-104 PC, Schiller, Baar, Switzerland). All ECGs were assessed by an experienced cardiologist according to the Minnesota criteria.[30] The cardiologist was blinded for the participants' characteristics other than age and sex. The ECGs were classified for indications of myocardial infarction, being absent, possibly, or definitively present and for myocardial ischæmia-like changes, being absent, minor, or major. For both, the localisations were classified, being anterior, septal, inferior, and/or lateral. Merely anteriorly localised ischæmia-like changes were excluded from the analyses (6.4%), as these are reported to be little specific in African ethnicities.[31-33] Indications of definite infarction included major and moderate pathological Q waves and QS patterns (codes 1-1 and 1-2). Indications of possible infarction included minor pathological Q waves and QS patterns (code 1-3). Definite ischæmia-like changes included major ST depression, T wave inversion, and ST elevation (codes 4-1, 4-2, 5-1, 5-2, and 9-2). Minor ischæmia-like changes included minor ST depression, T wave inversion, and complete left bundle branch block (codes 4-3, 4-4, 5-3, 5-4, and 7-1).

Reference populations

We compared the findings from our traditional African study population with those of western populations. As reference populations, we used three population-based studies in the United States and Europe for individuals at ages from 45 or 50 years. Data on the general population in the United States were derived from the National Health and Nutrition Examination Survey (NHANES) for the prevalences of electrocardiographically measured coronary arterial disease (n = 6201, median age 63 years, 45.3% men),[34] the prevalence of peripheral arterial disease measured by ankle-arm index (n = 1868, median age 61 years, 48.4% men), the prevalence of hypertension (n = 2135, median age 62 years, 46.6% men), and the distributions of cardiovascular risk factors and inflammatory markers (n = 2323, median age 62 years, 46.1% men).[35] Data on the general European population were derived from a study in Belgium for the prevalences of electrocardiographically measured coronary arterial disease (n = 29 419, median age 52 years, 77.7% men)[36] and from the CoLaus Study in Switzerland for the prevalence of hypertension and the distributions of cardiovascular risk factors and inflammatory markers (n = 3515, median age 61 years, 45.4% men).[37,38] In the cases of Belgian prevalences of electrocardiographic abnormalities and the Swiss distribution of IL6, only sex-split data were available. For comparability, both were graphically displayed as averages weighed by the number of individuals per sex. For the reference populations, the use of antihypertensive medication was included in the definition of hypertension, the use of antidiabetic

medication was included in the definition of diabetes, and the use of antidyslipidæmic medication was included in within the definition of elevated levels of total cholesterol or LDL cholesterol. Otherwise, applied definitions and cutoff values were identical as described for our study.

Analyses

Confidence intervals were calculated by Wilson's formula for prevalences and using the binomial distribution for medians. Individual associations of presence of cardiovascular disease with cardiovascular risk factors, inflammation, and socioeconomic status were tested by logistic regression analysis, including age and sex as dependent variables. Statistical analyses were performed with PASW Statistics 18 (SPSS, Chicago, IL, USA).

Results

Table 9.1 provides a description of the demographic, socioeconomic, and infectious characteristics of the study population that participated in the electrocardiographic investigations. The overrepresentation of women and a heightened socioeconomic status compared with the total cohort population are results of the selection on age. Otherwise, the baseline characteristics of the study population that participated in the electrocardiographic investigations were similar to those of the study populations that participated in the clinical and biochemical investigations as well as to those throughout the total cohort population.

Table 9.1 • General characteristics of the Ghanaian study population

Number of individuals	610
Men, *n* (%)	169 (27.5)
Age, years	66 (56–74)
Lifetime fertility, children per woman	7 (6–9)
Number of households	422
Household property value, US$	1050 (500–1936)
Indidivuals infected by, %	
malaria species	77.5
helminths	21.4
protozoa	100.0

Data are presented as medians with interquartile ranges unless specified otherwise. The characteristics are given for the study population aged 50 years and older that participated in the electrocardiographic investigations. Lifetime fertility is expressed as the number of born children per woman aged 45 years or more.

Cardiovascular risk factors

Table 9.2 summarises the clinical and biochemical investigations for the detection of cardiovascular risk factors concerning body size, blood pressure, glucose metabolism, lipid metabolism, and inflammation. Hypertension was classified as stage I, stage II, or isolated systolic hypertension. Plasma glucose concentrations were used to detect elevated cardiovascular risk from 5.6 mmol/l onward and to detect diabetes mellitus from 7.0 mmol/l onward.[27] The balance between HDL and LDL cholesterol was represented by the ratio of the plasma concentrations of apoB100 over apoA1. As markers of the inflammatory status, plasma concentrations of CRP and IL6, both proinflammatory cytokines, were used. In our study population, median levels of cardiovascular risk factors were low and prevalences of obesity, hypertension, dysglycæmia or diabetes, and dyslipidæmia were low. The prevalence of moderately elevated levels of CRP was low.

In Figure 9.1, the distributions of cardiovascular risk factors in our study population are compared with those in the western reference populations. Over the age of 50 years in Ghana, lower median values or prevalences were found for all cardiovascular risk factors describing body size, blood pressure, and glucose and lipid metabolism compared with the United States and Europe. For the inflammatory markers, median levels of CRP were lower, but levels of IL6 were higher.

Cardiovascular disease

Table 9.3 shows the prevalences of cardiovascular disease in our study population. We used electrocardiography to detect coronary arterial disease, classified as possible or definite myocardial infarction and minor or definite myocardial ischæmia-like changes. Prevalences of myocardial infarction and myocardial ischæmia-like changes were low. We used measurement of ankle-arm index to detect peripheral arterial disease, defined as an ankle-arm index below 0.9 of either leg. The prevalence of peripheral arterial disease was low; median ankle-arm indexes were high.

As shown in Figure 9.2, the prevalences of both coronary and peripheral arterial disease in Ghana were lower compared with the western reference populations, using identical clinical and electrocardiographic criteria. A similar pattern was found for myocardial ischæmia-like changes. Moreover, Ghanaian prevalences of coronary and peripheral arterial disease increased less with age.

Individual associations

For the individuals affected by cardiovascular disease, no associations could be found with cardiovascular risk factors, inflammation, or socioeconomic status.

Table 9.2 • Cardiovascular risk factors in the Ghanaian study population

	Men	Women
Clinical measurements	(*n* = 480)	(*n* = 444)
Waist circumference, cm	77 (73–81)	76 (72–80)
≥ 102 (♂) or ≥ 88 (♀) cm	0.0 (0.0 to 0.8)	7.4 (5.3 to 10.3)
Body mass index, kg/m2	18.1 (16.5–19.4)	18.1 (16.6–19.7)
≥ 25.0 kg/m2	0.2 (0.0 to 1.2)	1.4 (0.6 to 2.9)
Diastolic blood pressure, mmHg	75 (70–80)	70 (65–75)
Systolic blood pressure, mmHg	120 (110–135)	120 (110–135)
Hypertension		
Stage I	16.7 (13.6 to 20.3)	13.7 (10.8 to 17.3)
Stage II	9.0 (6.7 to 11.9)	8.8 (6.5 to 11.8)
Isolated systolic	15.7 (12.7 to 19.2)	16.2 (13.1 to 19.9)
Glucose, mmol/l	3.8 (3.3–4.4)	4.0 (3.6–4.5)
≥ 5.6 mmol/l	6.0 (4.3 to 8.6)	6.3 (4.5 to 9.1)
≥ 7.0 mmol/l	1.0 (0.4 to 2.4)	1.4 (0.6 to 3.0)
Biochemical measurements	(*n* = 94)	(*n* = 172)
Triglycerides, mmol/l	0.80 (0.68–0.95)	0.95 (0.76–1.21)
≥ 1.7 mmol/l	4.3 (1.7 to 10.4)	6.4 (3.6 to 11.1)
Total cholesterol, mmol/l	3.04 (2.49–3.51)	3.24 (2.81–3.74)
≥ 5.2 mmol/l	1.1 (0.2 to 5.8)	1.7 (0.6 to 5.0)
ApoB100, g/l	0.54 (0.47–0.64)	0.58 (0.49–0.69)
≥ 0.9 g/l	2.1 (0.6 to 7.4)	2.9 (1.3 to 6.7)
Ratio of apoB100 over apoA1	0.58 (0.45–0.68)	0.52 (0.43–0.66)
≥ 0.9 (♂) or ≥ 0.8 (♀)	5.3 (2.3 to 11.9)	12.8 (8.7 to 18.7)
C-reactive protein, mg/l	1.06 (0.34–3.77)	0.95 (0.44–2.35)
3.0–10.0 mg/l	19.1 (12.5 to 28.3)	11.6 (7.7 to 17.3)
Interleukin-6, ng/l	2.28 (1.47–3.23)	1.84 (1.37–2.42)

Distributions are given as medians with interquartile ranges, prevalences are given as percentages with 95% confidence intervals describing the proportion meeting the indicated cutoff value, all for those aged 50 years and older. Hypertension was classified as stage I for diastolic blood pressures of 90 to 100 mmHg and/or systolic blood pressures of 140 to 160 mmHg, as stage II for diastolic blood pressures from 100 mmHg onward and/or systolic blood pressures from 160 mmHg onward, and as isolated systolic for systolic blood pressures from 140 mmHg onward with diastolic blood pressures lower than 90 mmHg. ApoA1: apolipoprotein-A1. ApoB100: apolipoprotein-B100.

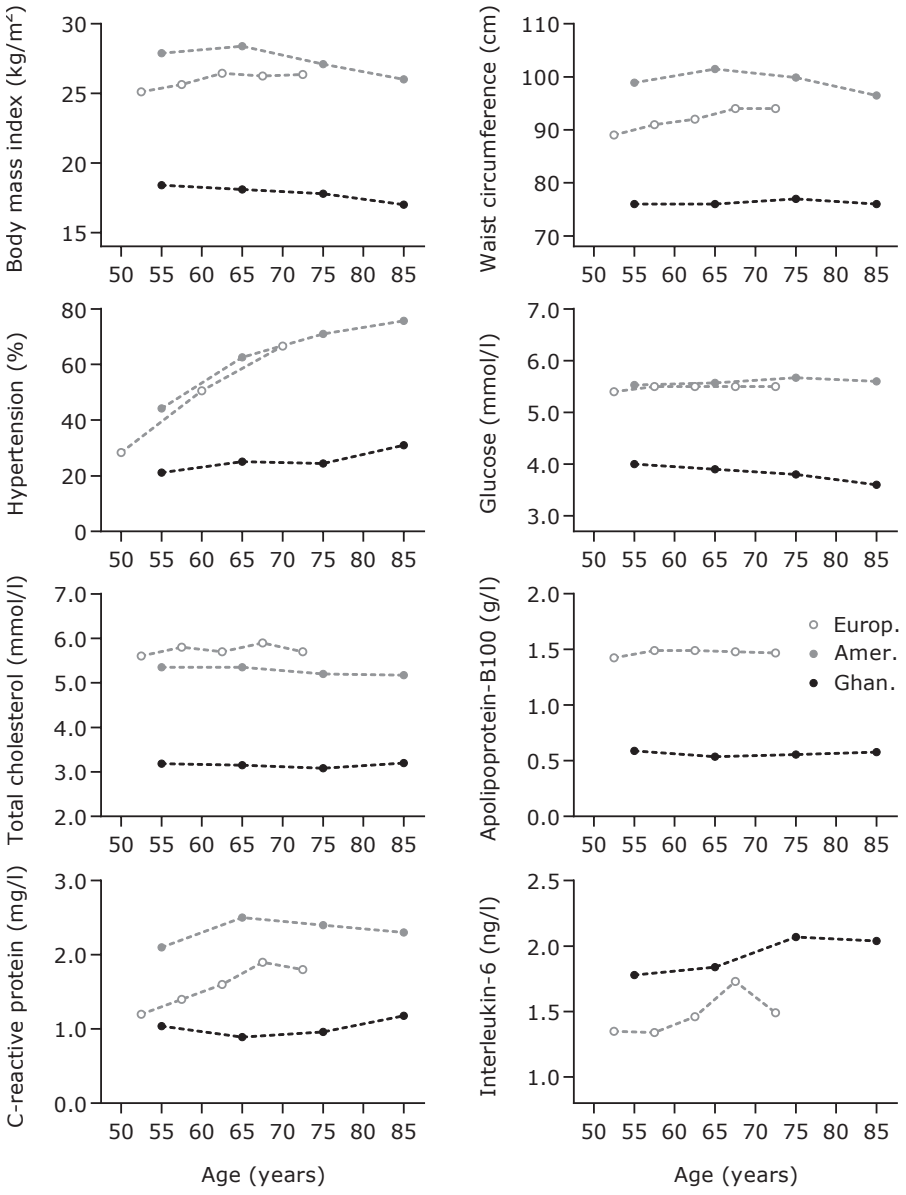

Figure 9.1 • Age patterns of cardiovascular risk factors in the Ghanaian study population compared with those in the American and European reference populations. Distributions are given as medians. Prevalences of hypertension are given as percentages, including stage I, stage II, and isolated systolic hypertension. Values for age represent midpoints of age intervals, because of different age groups used in the reference populations. Europ.: Europeans. Amer.: Americans. Ghan.: Ghanaians.

Table 9.3 • Cardiovascular disease in the Ghanaian study population

	Men	Women
Electrocardiographic measurements	(*n* = 168)	(*n* = 439)
Myocardial infarction		
definite	1.8 (0.6 to 5.1)	0.9 (0.4 to 2.3)
possible	4.2 (2.0 to 8.4)	2.1 (1.1 to 3.8)
Myocardial ischæmia-like changes		
definite	13.7 (9.3 to 19.7)	10.0 (7.6 to 13.2)
minor	2.4 (0.9 to 6.0)	3.2 (1.9 to 4.6)
Clinical measurements	(*n* = 480)	(*n* = 444)
Ankle-arm index	1.16 (1.10–1.24)	1.14 (1.06–1.22)
Peripheral arterial disease	2.3 (1.3 to 4.1)	3.4 (2.1 to 5.5)

Prevalences are given as percentages with 95% confidence intervals) and distributions are given as medians with interquartile ranges, all for those aged 50 years and older. Peripheral arterial disease was defined as an ankle-arm index below 0.9.

Discussion

This study aims to determine cardiovascular health in a traditional rural population in Ghana in West Africa from the age of 50 years onward. The study population is demographically and economically characterised by an underdeveloped pretransitory status relative to western countries. In this adverse environment, individuals are exposed to a high infectious load, favouring survival of those with a proinflammatory immune status. Compared with American and European populations, we found common proinflammatory profiles, but low prevalences of cardiovascular risk factors associated with a western lifestyle, including obesity, hypertension, dysglycæmia or diabetes, and dyslipidæmia. In this population, prevalences of coronary and peripheral arterial disease as measured by electrocardiography and ankle-arm index were correspondingly low.

For some cardiovascular risk factors no formal determination was possible, including smoking behaviour, alcohol use, diet composition, and lack of physical exercise. Tobacco is self-grown and beer is self-brewed. The availability of both is dependent on the harvest, as commercial distribution of cigarettes and alcoholic beverages is locally absent. Their regular use is more or less a social taboo. Based on our long-lasting experiences, regular smoking and drinking among men is common, estimated to concern one third and two thirds of the population, respectively, but the quantities are likely to be limited when compared with developed countries. Women do not publicly smoke or drink, but we presume women to exhibit these behaviours privately in smaller proportions. Physical inactivity is exceptional and western diet is absent. It should be noted that most inhabitants in the research area provide for their own living by non-com-

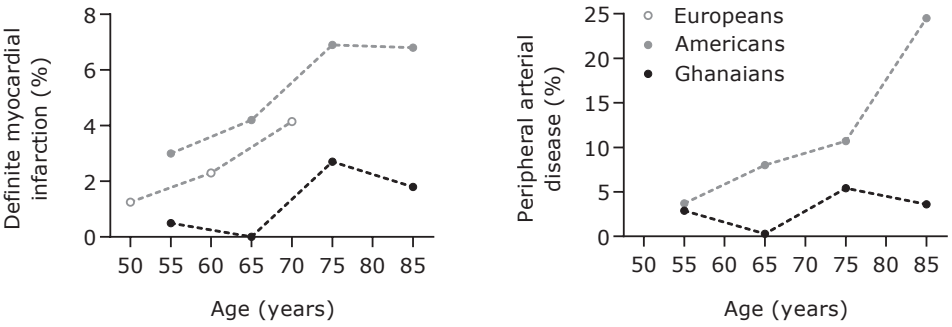

Figure 9.2 • Age patterns of cardiovascular disease in the Ghanaian study sample compared with those in the American and European reference populations. Prevalences are given as percentages. Peripheral arterial disease was defined as an ankle-arm index below 0.9. Definite myocardial infarction was detected by electrocardiography. Values for age represent midpoints of age intervals, because of different age groups used for the reference populations.

mercial agriculture performed by manual labour. The scarcity of electricity and machinery necessitates physical exercise for farming and housekeeping. Transportation is almost always by foot or bicycle. The savannah climate is dry with only one rainy season of variable duration, hindering agriculture. The limited variety of crops that can be grown in this environment — such as millet, corn, okra, beans, onions, and tomatoes — and the omnipresent low social economic standard restrict the ingestion of fat and glucose. Commercial distribution of conserved food has remained financially unattainable for most.

Although the prevalence of cardiovascular disease was low, cardiovascular disease was not absent in our study population. This indicates that low prevalences of obesity, dysglycæmia or diabetes, and dyslipidæmia do not entirely prevent the occurrence of cardiovascular disease. The presence of hypertension is relatively uncommon, but still present in about one fifth of the population. It may be relatively important for cardiovascular pathogenesis in this population. Moreover, some do smoke and drink, but the consumption and attribution are difficult to estimate. Other factors independent of lifestyle, such as genotype, may also render individuals susceptible. In line with this, for those few who are affected, we have been unable to find any association with the cardiovascular risk factors as measured in this study. In this study, genotypes have not been assessed. Recently, we have collected mouth swabs in our study population for genetic analyses in future research.

Previous research on risk factors of cardiovascular disease has shown that in Africa, equal to western populations, cardiovascular disease can be mainly attributed to obesity, dysglycæmia, dyslipidæmia, smoking, and hypertension.[11-13] Pathogenetic mechanisms have been described by

which lifestyle-related risk factors drive cardiovascular disease.[4,40] However, these studies are based on urban regions with westernised risk profiles. Data from populations without a western lifestyle, as our study population, are needed to confirm that these lifestyle-related risk factors are primarily responsible for cardiovascular disease to develop.

In our study population, all individuals were infected by protozoa, while infections by helminths and malaria species were highly endemic. This high infectious load, together with little public and personal hygiene and little medical care, renders infectious diseases as the main cause of death. Previously, we have shown that selectively those individuals that express a proinflammatory immune system survive up to older ages.[8-10] This selection of a proinflammatory status is confirmed by the higher levels of the proinflammatory cytokine IL6 in our study population compared with western populations. Different from IL6, we found lower levels of the inflammatory marker CRP in our study population compared with western populations. Plasma levels of CRP are highly elevated during acute infections. During chronic, subclinical infections, CRP levels may not be elevated. In populations endemic for infections by malaria and helminth species, these levels can be rather within the normal range.[41-43] Probably, in these instances, a systemic inflammatory response is not elicited, as the immune system has become effectively able to restrain the pathogens or has become tolerant. Distinctive to chronic infections, however, chronic non-infectious inflammatory disorders are associated with moderately elevated levels of CRP.[29] Likely, instead of being a risk factor, these moderately elevated levels are an effect of the pathophysiological inflammatory processes and thus a marker of cardiovascular disease.[44-46] In line with this, we found less moderately elevated levels in our study population compared with western populations. Similar results have been obtained in other developing populations with high rates of infectious diseases in Southern America.[47,48] The comparisons of CRP and IL6 levels with those in western populations are consistent when using other reports from western populations.[49,50]

Although we show that inflammation alone is not sufficient to cause cardiovascular disease, inflammation still plays a major role in its pathogenesis. This is reflected by the high prevalences of cardiovascular disease in regions where a western lifestyle coincides with a high infectious load.[5,11-13] In addition, migrants from developing regions are affected by cardiovascular disease in western populations at younger ages and with higher mortality.[11-14] These populations are likely to have developed a proinflammatory immune status and are thereby probably more susceptible to develop inflammatory disease, such as cardiovascular disease. Even in developed countries, such predisposing processes can be effectuated through adverse environmental conditions.[51,52] These notions are supported by the observation of higher levels of CRP in African Americans compared with European Americans[50,53] and the beneficial effects of antidyslipidæmic therapy in individuals with elevated CRP.[54]

This study has several limitations. First, for the registration of glucose and lipid parameters, blood samples have been taken in the morning. Although breakfast is uncommon, participants have not been requested to fast. Observed glucose and triglyceride levels could have been overestimated in this population and fasted values could be even lower than reported here. This reinforces the present interpretation of the study. Second, we have not been able to cover all cardiovascular risk factors, leaving smoking, alcohol use, diet composition, physical exercise, and genetic factors. Third, because of selection on age, women are overrepresented, demanding some prudence in the interpretation of the data for men.

Electrocardiographic criteria are reliable for the distinction of myocardial infarction. However, myocardial ischæmia-like changes are less distinctive.[36] Apart from coronary arterial disease, other disorders can effectuate these changes in repolarisation.[33] Moreover, in African ethnicities, these changes in repolarisation are little specific for coronary arterial disease.[31,32,55] Therefore, true prevalences of cardiovascular disease might be even lower than reported in this study. Unknown as well remain possible confounding selective processes. In our study population, the mortality due to cardiovascular disease is undetermined. If high, those affected could have deceased before being detected. When a selection exists for physically fit individuals to survive, those prone to develop cardiovascular disease might not live to older ages. However, these suppositions are contradicted by our observation of the few cases that were found with a slightly increasing trend with age.

This study determines the population-wide prevalences of peripheral and coronary arterial disease in a traditional African population by measurement of ankle-arm index and electrocardiography. These prevalences have not been described earlier for developing populations without a western lifestyle in relation to lifestyle and inflammation. First, this knowledge is of importance for underdeveloped populations themselves to guide preventive and curative policies. Worldwide, the prevalence of cardiovascular disease is rapidly increasing, especially in developing regions parallel to urbanisation and westernisation.[11,12] As we show, also in these regions, it can be worthwhile to emphasise on the research and prevention of lifestyle-related risk factors for the control of cardiovascular disease. Second, this knowledge is of equal importance for western societies. Of today's African Americans, a large majority is genetically related to traditional populations in West Africa.[39] Predominantly these and other migrant populations suffer from a burden of cardiovascular disease, even after lifelong residence in western society.[11-14] It remains unclear to what extent this disparity can be explained by biological, socioeconomic, and cultural differences. Possibly, these ethnicities have been programmed through selection or through development to be more proinflammatory. Nonetheless, our study confirms that preventive and curative policies may be expected to be successful when addressing particular aspects of lifestyle.

In conclusion, we have shown that in a traditional non-western African population, despite promotion of inflammation, cardiovascular disease infrequently develops because of low prevalences of lifestyle-related cardiovascular risk factors compared with American and European populations. Together, these results indicate that cardiovascular disease is not primarily caused by inflammatory processes, but a western lifestyle is a prerequisite for its development. This provides even further arguments to prioritise the promotion of a healthy lifestyle, both in developed and in developing countries.

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10 MEASURING SENESCENCE THROUGH ATRIAL FIBRILLATION

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Scarcity of atrial fibrillation in a traditional African population:
a community-based study.
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Background • In western societies, atrial fibrillation is an increasingly common finding among the elderly. Established risk factors of atrial fibrillation include obesity, diabetes, hypertension, and cardiovascular disease. Atrial fibrillation has almost exclusively been studied in western populations where these risk factors are widely present. Therefore, we studied the epidemiology of atrial fibrillation in a traditional African population.

Methods • In rural Ghana, among 924 individuals aged 50 years and older, we recorded electrocardiograms to detect atrial fibrillation. As established risk factors, we documented waist circumference, body mass index (BMI), capillary glucose level, blood pressure, and electrocardiographic myocardial infarction. In addition, we determined circulating levels of interleukin-6 (IL6), a proinflammatory cytokine, and C-reactive protein (CRP), a marker of systemic inflammation. We compared the risk factors with reference data from the general population of the USA.

Results • Atrial fibrillation was detected in only three cases, equalling 0.3% (95% CI: 0.1 to 1.0). Waist circumference, BMI, and capillary glucose levels were very low. Hypertension and myocardial infarction were uncommon. Circulating levels of IL6 were similar, but those of CRP were lower compared with the USA.

Conclusion • Atrial fibrillation is very scarce in this traditional African population. Its low prevalence compared with western societies can be explained by the rareness of its established risk factors, which are closely related to lifestyle, and by possible unmeasured differences in other risk factors or genetic factors.

Atrial fibrillation is a common finding among elderly in western societies. Its prevalence increases with age and mounts to 10 to 20% after the age of 80 years.[1,2] Due to ageing of western populations and improved survival from other cardiovascular disorders, the prevalence of atrial fibrillation has grown over time and is expected to continue growing. As it leads to a heightened risk of thromboembolism, cerebrovascular accidents, and congestive heart failure, its public health burden has grown concurrently.[3,4]

The majority of the cases of atrial fibrillation can be attributed to the established risk factors obesity, diabetes, hypertension, prior cardiac disease, and smoking. Hypertension is the most important of these, responsible for at least one fifth of the cases.[5,6] Still, questions remain about the pathogenesis of atrial fibrillation. It is postulated that, next to these risk factors, inflammation plays an important role in the pathogenesis of atrial fibrillation, but this has not yet been confirmed.[1,7] Moreover, it is not clearly understood why atrial fibrillation is less commonly detected in African Americans, while they are more

often affected by obesity, diabetes, and hypertension than European Americans.[5,8,9] However, the epidemiology of atrial fibrillation has been studied almost exclusively in western societies,[1,3] where obesity, diabetes, hypertension, cardiovascular disease, and systemic inflammation are widely present among the elderly.[10] Little is known about the prevalence of atrial fibrillation in non-western societies, such as in rural Africa.[11,12] Knowledge about the risk of atrial fibrillation in the context of different environmental and genetic influences may provide more insight in its pathogenesis.[13]

This study investigates the epidemiology of atrial fibrillation in a traditional rural African population where a sedentary lifestyle is absent. We used electrocardiography to detect atrial fibrillation among inhabitants aged 50 years and older. Established risk factors of atrial fibrillation have been documented, such as obesity, dysglycæmia, hypertension, and myocardial infarction. Circulating levels of interleukin-6 were measured as a marker of proinflammatory immune activation and circulating levels of C-reactive protein as a marker of systemic inflammation.

Methods

Research area

The Upper East Region is remote, rural, and one of the least developed regions of Ghana. The vast majority of the inhabitants is involved in non-commercial agriculture performed by manual labour.[14] The yearly per capita income averages US$ 135;[15] 88% of the households lives in poverty.[16] Infectious diseases are the main causes of death.[17]

Since 2002, we have registered and followed a traditional horticultural population in the Garu-Tempane District in the Upper East Region. This population occupies a research area of 375 km^2 with approximately 25 000 inhabitants living in 32 villages. Annual migration relative to the study population's size was 2% into and 1% out of the research area.[18,19] Hospital care is absent; the nearest physician is at 40 kilometres' distance. Sewage disposal systems are non-existent.

All inhabitants have been registered in a demographic database, including the name, age, sex, tribe, and household. We have determined the lifetime fertility, defined as the total number of children born per postreproductive woman aged 45 years or more, based on fertility data gathered in the research area in 2003.[19] For each household, we have determined the household property value in 2007 according to the Demographic and Health Survey method.[14] In 2008, we have determined the prevalences of infections by malaria species by PCR of blood samples and those by helminths and protozoa by PCR of stool samples.[20] A more elaborate description of this cohort has been given elsewhere.[14,18,19]

Ethical approval was given by the Ethical Review Committee of Ghana Health Service, the Committee Medical Ethics of Leiden University Medical Center, and by the local chiefs and elders. Because of illiteracy, informed consent was obtained orally from the participants. A consent

form with an explanation on the purpose and conduction of this research project was read out to each participant in his own language. The full text of the form was approved by the Ethical Review Committee of Ghana Health Service.

Participants

Within the registered population, we aimed to estimate the prevalences of atrial fibrillation and its risk factors among individuals aged 50 years and older. For this, we set up a mobile field station in different villages during two field visits in 2009 and 2010. From here, all eligible inhabitants were approached. Inclusion was limited by the duration of the field visits. To ensure maximal participation and to avoid selective inclusion of healthy elderly, we brought less mobile participants by car. Of the approached inhabitants, 4.4% could not participate due to death since the last registration, 3.2% refused participation, 2.8% was absent from the research area during our visit because of migration or travelling, and 4.2% did not participate for other reasons.

At the field work station, the identity of the participant was confirmed, the personal data in our registration were checked, and clinical and electrocardiographic measurements were performed. In addition, blood plasma samples were available of 266 individuals randomly selected across age groups in 2008. On these samples biochemical measurements were performed.

Electrocardiographic measurements

A twelve-lead electrocardiogram was recorded twice for ten seconds in a lying and resting position (AT-104 PC, Schiller, Baar, Switzerland). All electrocardiograms were assessed by an experienced cardiologist according to the Minnesota criteria. The cardiologist was blinded for the participants' characteristics other than age and sex. A subset of the electrocardiograms (n = 610) was assessed for myocardial infarction.[21] The heart rate was described using both recordings. Bradycardia was defined as a sinus rhythm below 60/min on either recording. Tachycardia was defined as a sinus rhythm over 100/min on either recording. The electrocardiograms were classified as whether or not displaying atrial fibrillation or atrial flutter (codes 8-3-1, 8-3-2, 8-3-3, and 8-3-4).

Clinical and biochemical measurements

We performed clinical measurements on those participating in the electrocardiographic measurements. We measured height, weight, waist circumference, glucose capillary blood concentration, and blood pressure. Body mass index (BMI) was calculated as weight divided by squared height (kg/m^2). Hypertension was defined as a systolic blood pressure of 140 mmHg or higher and/or a diastolic blood pressure of 90 mmHg or higher.[22] We performed biochemical measurements on venous blood samples to measure circulating plasma levels of interleukin-6 (IL6) and C-reactive protein (CRP).[23]

Reference population

To compare our results with a western population, we derived data on the prevalences of the risk factors of atrial fibrillation from studies performed in the general population of the USA. Age and ethnicity-specific distributions of BMI and CRP and the prevalence of hypertension were derived from the National Health and Nutrition Examination Survey (NHANES) performed in 1999-2000.[24] For the USA, the definition of hypertension was extended to include individuals using antihypertensive medication. Distributions of IL6 for European and African Americans of 65 years of age or older were derived from a publication by Cohen and colleagues.[25]

Analyses

Prevalences of atrial fibrillation were calculated as the number of cases divided by the total number of inhabitants per 10-year age group and given as percentages. Confidence intervals were calculated using Wilson's formula for prevalences and using the

Number of individuals	924
Men, *n* (%)	480 (51.9)
Age, years	66 (56–73)
Age groups, *n* (%)	
50–59 years	307 (33.2)
60–69 years	291 (31.5)
70–79 years	242 (26.2)
80+ years	84 (9.1)
Lifetime fertility, children per woman	7 (6–9)
Number of households	636
Household property value, US$	1077 (533–1942)
Waist circumference, cm	76 (72–81)
Body mass index, kg/m^2	18.1 (16.5–19.5)
Capillary glucose, mmol/l	3.9 (3.4–4.4)
Blood pressure, mmHg	
diastolic	70 (65–80)
systolic	120 (110–135)
Hypertension, %	24.2
Myocardial infarction, %	1.2
Individuals infected by, %	
malaria species	77.7
helminths	21.5
protozoa	100.0
Interleukin-6, ng/l	1.9 (1.4–2.7)
C-reactive protein, mg/l	1.0 (0.4–2.7)

Table 10.1 • General characteristics of the Ghanaian study population. Data are presented as medians with interquartile ranges unless specified otherwise. Lifetime fertility was determined in women aged 45 years or more. Hypertension was defined as a systolic blood pressure of 140 mmHg or higher and/or a diastolic blood pressure of 90 mmHg or higher. Myocardial infarction represents electrocardiographically detected definite myocardial infarction determined in 610 individuals. Infections were determined in 261 individuals of whom blood and stool samples were available. Circulating levels of interleukin-6 (IL6) and C-reactive protein (CRP) were measured in venous plasma samples of 266 individuals.

binomial distribution for medians. Statistical analyses were performed with SPSS Statistics 20 (IBM, Armonk, NY, USA).

Results

Table 10.1 provides a description of the demographic characteristics, the established risk factors of atrial fibrillation, and infectious and inflammatory markers for the Ghanaian study population aged 50 years and older. In the study population, the prevalences and levels of the established risk factors, including obesity, dysglycæmia, hypertension, and myocardial infarction, were very low. The levels of the inflammatory markers IL6 and CRP are described. Infectious diseases were highly prevalent. These characteristics were similar between the entire registered population, the study population selected for the electrocardiographic and clinical measurements, and the subpopulation in which the biochemical measurements were performed, except for minor differences in the distributions of age and sex. The similarities remained after stratification by sex.

Electrocardiograms were obtained from 921 participants, of whom 479 were men (52.0%). The median (with interquartile range) heart rate was 71 (63–80) per minute. Sinus bradycardia and tachycardia were present in 159 (17.3%) and 32 (3.5%) individuals.

Figure 10.1 compares the age-specific prevalences of atrial fibrillation with those in the USA. Of 921 participants, three individuals had atrial fibrillation, equalling 0.3% (95% CI: 0.1 to 1.0). Compared with

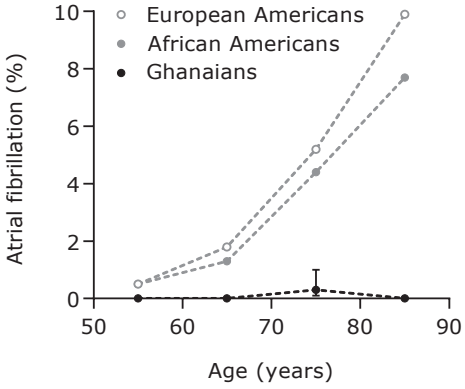

Figure 10.1 • Atrial fibrillation in the Ghanaian study population and the USA. Age-specific prevalences are given as percentages with 95% confidence intervals. Ages represent midpoints of age intervals. As references, prevalences are given for European and African Americans in the general population of the USA.

both European and African Americans, prevalences of atrial fibrillation were very low in the Ghanaian study population.

Table 10.2 shows characteristics of the three cases with atrial fibrillation in the Ghanaian study population. Of these three, one was woman and two were men. The first two were affected by hypertension. The third case was a smoker. Only for the latter case inflammatory markers were known; compared with the entire study population as well as the USA, these levels were low for both IL6 and CRP.

Figure 10.2 compares the distributions over age of the most important established risk factors of atrial fibrillation and compares the levels of the inflammatory markers with known distributions and levels

Table 10.2 • Characteristics of the three cases with atrial fibrillation in the Ghanaian study population

Case	Sex	Age	HR	RR	BMI	Gluc	Smok	Isch	Infa	SES
1	♀	76	104	140/85	21.8	3.2	–	–	–	420
2	♂	76	80	165/80	17.5	3.1	–	–	–	530
3	♂	76	104	125/80	19.1	3.8	+	–	–	1030

HR: ventricular heart rate (/min). RR: systolic/diastolic blood pressure (mmHg). BMI: body mass index (kg/m2). Smok: smoking. Gluc: capillary blood glucose concentration (mmol/l). Isch: electrocardiographic myocardial ischæmia-like repolarisation abnormalities. Infa: electrocardiographic infarction. SES: household value (US$). Smok, Isch, and Infa, are designated as present (+) or absent (–).

in the USA, separated for European and African Americans. The levels of BMI and hypertension in the Ghanaian study population were lower than those of both European and African Americans. The levels of IL6 were comparable with, but the levels of CRP were lower than those of both European and African Americans.

Discussion

In this study we showed that atrial fibrillation was very scarce after the age of 50 years in a traditional rural population in Africa. The near absence of atrial fibrillation in the Ghanaian study population confirms the low prevalences that have been found by a few studies in other traditional African populations. In rural Tanzanians aged 70 years and older, its prevalence was 0.7%.[11] In the South African Bantu population, atrial fibrillation was detected in 0.2% of patients attending a cardiac clinic but not diagnosed with cardiac disease.[26] In patients from the Bantu population hospitalised because of cardiac failure, it was present in 12%.[27]

The prevalence of atrial fibrillation in urban African populations is higher than those in rural African populations. In a South-African study covering both urban and rural communities, atrial fibrillation was detected in 2% of Africans over the age of 30 years.[28] In two large cardiologic hospitals, 4.6% and 5.5% of the admitted patients had atrial fibrillation at relatively low ages.[29,30] Among cardiologic hospitals across several Sub-Saharan African countries, atrial fibrillation was found in 18% of cases with acute heart failure.[31]

The prevalence of atrial fibrillation in western populations is higher than those in rural African populations. Several studies in patient populations and the general populations of the USA and Western Europe have reported its prevalence to rise from less than 2% around the age of 50 years up to 10 to 20% after the age of 80 years.[1] In the general population of the USA, similar increases with age have been described for both European and African Americans.[2]

The low prevalence of atrial fibrillation in rural African populations compared

with urban African and western populations can be explained by a similarly lower prevalence of its established risk factors, including obesity, diabetes, hypertension, and cardiovascular disease. These risk factors are closely related to a sedentary lifestyle.[5,10] With the transition, urbanisation, and ageing of African populations, a sedentary lifestyle is adopted and the prevalence of atrial fibrillation rises.[12,30]

Interestingly, cases of atrial fibrillation described in African populations are accompanied by underlying cardiac disorders in

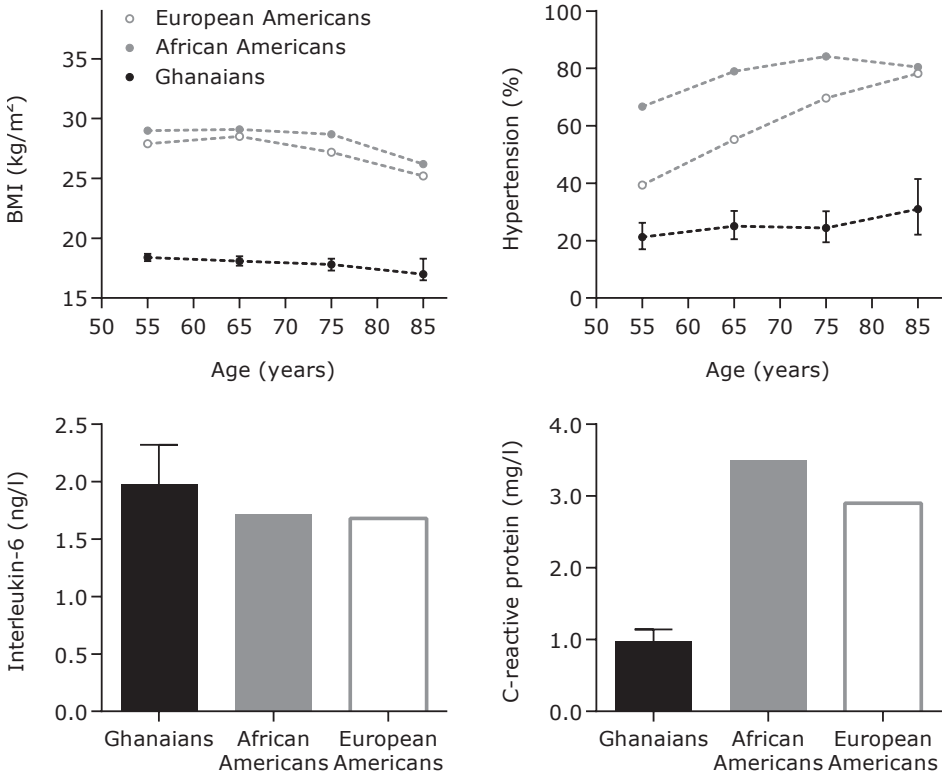

Figure 10.2 • Risk factors of atrial fibrillation in the Ghanaian study population and the USA. As established risk factors, the distribution of body mass index (BMI) is given as age-specific median levels and prevalences of hypertension are given as age-specific percentages. Hypertension was defined as a systolic blood pressure of 140 mmHg or higher and/or a diastolic blood pressure of 90 mmHg or higher, and, for the USA, treatment with antihypertensive medication. As proinflammatory markers, distributions of interleukin-6 (IL6) and C-reactive protein (CRP) are given as median levels for individuals aged 65 or 50 years and older, respectively. For the Ghanaian study population, 95% confidence intervals are given. As references, prevalences are given for European and African Americans in the general population of the USA. These data were derived from NHANES 1999-2000[24] and from Cohen and colleagues.[25]

proportions up to 90%, which contrasts with the large proportion of idiopathic cases described in western populations.[3,4] Mostly, these cardiac disorders concern hypertensive cardiopathy and rheumatic valvular heart disease.[12,27,29,30] In the Ghanaian study population, two of the three cases suffered from hypertension. This relationship between cardiac disease and atrial fibrillation supports that atrial fibrillation may be mainly propagated by obesity, diabetes, hypertension, and cardiovascular disease.

Recently, inflammation has been postulated to play an important role in the pathogenesis of atrial fibrillation.[7] In the Ghanaian study population the level of IL6, an instigator of a proinflammatory response, was similar to that in the general population of the USA. Earlier we have shown that the study population is biochemically and genetically enriched with proinflammatory markers, probably due to the endemic high infectious load.[23,32,33] On the other hand, the level of CRP, a marker of systemic inflammation, was lower compared with the USA. Similarly, we have previously reported that the median level of CRP as well as the prevalence of mildly elevated levels of CRP was lower in the study population compared with the general population in the Netherlands. This difference was attributable to a lower BMI in the Ghanaian study population.[34] Together, these findings may indicate that, while the capacity to generate an inflammatory response is preserved, systemic inflammation is uncommon in the Ghanaian study population.

Mendelian randomisation has shown that an elevation of CRP is rather an effect than a cause of atrial fibrillation.[35] This interpretation is supported by observations that deny an association between CRP level and history of atrial fibrillation, but confirm that CRP is elevated during episodes of atrial fibrillation or due to coexistence of hypertension or obesity.[36-38] Inflammatory processes related to atrial fibrillation seem to be caused by ischæmic or oxidative injury of atrial myocytes, which is again caused by obesity, diabetes, hypertension, and cardiac disease.[39,40] In the Ghanaian study population, hypertension is present in only about a quarter and obesity, dysglycæmia, and cardiovascular disease are rare. The low levels of CRP match with the close relation between these risk factors and inflammation. We have observed a similar pattern when studying inflammation in relation to peripheral and coronary arterial disease in this study population (Chapter 9 of this thesis).[21]

While we found almost no atrial fibrillation in the Ghanaian study population, hypertension was present in a considerable proportion. Similarly, in western populations, African ethnicities are more affected by obesity, diabetes, and hypertension, but less often develop atrial fibrillation compared with European ethnicities.[5,8,11] Meanwhile, the associations between these risk factors and atrial fibrillation are similar in both ethnicities.[5,9,41,42] A solution of this paradox may be provided by the higher sensitivity that seems required for methods to detect atrial fibrillation in Africans compared with Europeans.[43] However, more research in different populations is needed

to unravel the interactions between environmental and genetic risk factors.[13]

The low prevalence of atrial fibrillation in the Ghanaian study population may also be a result of a lack of risk factors other than those measured in this study. No data was available on the prevalence of rheumatic heart disease, which is a common cause of atrial fibrillation in African populations.[12,29,30] Furthermore, we had no information on thyroid disease, smoking, and alcohol use, which are risk factors of atrial fibrillation in western populations.[3,4]

Differences between ethnicities in the risks of atrial fibrillation are possibly caused by genetic factors. Multiple genetic polymorphisms have been associated with an elevated risk of atrial fibrillation, mainly in studies on populations from European decent. Yet, the effects of these associations are modest and differ between ethnicities.[44,45] The lower prevalence of atrial fibrillation among African Americans compared with European Americans may be a result of a lower frequency of genetic variants that predispose to atrial fibrillation among Africans. As African Americans show great genetic similarity with populations in West Africa, where the Ghanaian study population is located,[46] a lower frequency of such genetic variants may likewise explain why atrial fibrillation was scarce in the Ghanaian study population. However, this remains speculative: one report states that European genetic admixture does not explain the differences in prevalence of atrial fibrillation between European and African Americans,[47] while another report contradicts this.[48] Moreover, if the frequency of genetic variants that predispose to atrial fibrillation would be lower among African Americans, the greater prevalence of atrial fibrillation among African Americans compared with the Ghanaian study population reinforces the essential role of lifestyle-related factors rather than such genetic variants.

The scarcity of atrial fibrillation in our study population can also be explained by selective survival of unaffected individuals. When sufficient medical care is absent, patients with atrial fibrillation may decease early from the underlying disorders or complications, such as cardiac disease or stroke. A study on patients with atrial fibrillation in rural Tanzania reports that 8 out of 15 died within a year after detection.[11] In a larger group in Cameroon one-year mortality was 30%, of which more than half was of cardiovascular origin. Of the survivors, 18% experienced cerebrovascular accidents.[12] On the other hand, with relatively low risk scores[12,30] atrial fibrillation in these populations seems unlikely to be so severely lethal to render infinitesimal prevalence estimates. In line with this, we have determined in another study,[49] by means of verbal autopsy on 1263 of the 1406 deaths that were registered in our cohort population, that only 2.7% died from cardiovascular causes. For those who died at the age of 50 years or older, this was 4.5%.

This study on atrial fibrillation in a traditional rural African population has limitations. First, the use of two subsequent electrocardiographic recordings of ten seconds may be insufficient to detect all

cases of atrial fibrillation. More elaborate screening techniques used in western populations yield more reliable estimates of its prevalence; this difference in methodology hampers the comparison of our results with data from western populations. Second, the documentation of the cardiovascular risk factors lacks information on history and family history of cardiovascular disease. Third, due to different life expectancies, the number of elderly aged 50 years and older is lower in our population than in western populations. Fourth, due to the cross-sectional nature of this study, presence or absence of causality in the relationships between the established risk factors, systemic inflammation, and atrial fibrillation cannot be demonstrated.

The data on atrial fibrillation and its risk factors in the Ghanaian study population are not necessarily generalisable to other African populations, as they vary genetically and culturally.[46] In the same manner, the comparison between the Ghanaian study population and the general population of the USA may not reflect a universal difference between African and western populations. Comparisons between divergent populations are informative, but also complicated. As described above, some possible differences between the Ghanaian study population and the general population of the USA remain largely unknown. More research in different non-western populations is needed to overcome the limitations of this study and to extend the scarce knowledge on atrial fibrillation in such populations.[13]

In conclusion, we have shown that atrial fibrillation is nearly absent in a traditional rural population in Ghana. The low prevalence compared with western societies can be explained by the rareness of its established risk factors, including obesity, diabetes, hypertension, and cardiac disease, as well as systemic inflammation, which are closely related to a sedentary lifestyle and uncommon in this population. Future research is needed to elucidate the roles of other risk factors and genetic factors.

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11

MEASURING SENESCENCE THROUGH HEART RATE AND HEART RATE VARIABILITY

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Heart rate variability, but not heart rate, is associated with handgrip strength and mortality in older Africans at very low cardiovascular risk: a population-based study.
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Background • Heart rate and heart rate variability are associated with functional impairment, morbidity, and mortality in western populations. It is unclear whether these associations are mediated by lifestyle-related cardiovascular risk factors. Therefore, we studied these associations in a population where an affluent sedentary lifestyle is absent and risk factors of cardiovascular disease and diabetes are rare.

Methods • Among 822 community-dwelling inhabitants aged 50 years and older in a traditional rural African population in Northeast Ghana, we measured cardiovascular risk factors, including body mass index (BMI), waist circumference, glucose level, diastolic and systolic blood pressure, and ankle-arm index. Heart rate and heart rate variability, measured as SDNN and RMSSD, were derived from electrocardiograms (ECGs) recorded at rest. Physical function was determined by handgrip strength. Mortality was documented during a follow-up period of two years.

Results • Heart rate increased slightly with age and was dependent on BMI, glucose level, and diastolic blood pressure. Heart rate variability decreased with age and was not dependent on cardiovascular risk factors. Heart rate was associated with neither handgrip strength nor mortality. A lower heart rate variability was associated with lower handgrip strength and a higher risk of mortality, independent of age, sex, tribe, and cardiovascular risk factors.

Conclusion • Heart rate variability, but not heart rate, is associated with handgrip strength and mortality in a traditional rural African population, which indicates that this association is independent of lifestyle-related cardiovascular risk factors and probably a reflection of a universal deterioration of the body's autonomic regulation during ageing.

Heart rate and heart rate variability at rest are established risk factors of various forms of morbidity and mortality. In the general population, a high heart rate and a low heart rate variability are associated with an accelerated progression of atherosclerosis[1-3] and predict hypertension, coronary heart disease, heart failure, stroke, and cardiovascular death.[1-8] In addition to cardiovascular disease, a high heart rate and a low heart rate variability are associated with insulin resistance and diabetes mellitus[6,9-11] and predict mortality due to cancer[12] and all-cause mortality.[3,10] A low heart rate variability not only marks morbidity and mortality, but is also associated with measures of functional impairment, such as a weak handgrip strength.[13,14]

Two explanations can be given for the associations of heart rate and heart rate variability with functional impairment, morbidity, and mortality. On one hand, these associations are attributed to the deterioration of the autonomic nervous system during ageing.[13,14] The autonomic nervous

system continuously adjusts the heart rate in response to changes in physiologic conditions to maintain hæmodynamic stability. A high heart rate and a low heart rate variability are thought to reflect dysfunction of the flexible autonomic regulation of the heart rate in particular and of the body's functioning in general.[6,10] On the other hand, a high heart rate and a low heart rate variability can be brought about by the risk factors of age-related diseases such as cardiovascular disease and diabetes. Heart rate and heart rate variability depend on body mass index (BMI),[2,6,10,15-18] lipid levels,[2,6,10,15,17] glucose levels,[6,10,15] blood pressure,[2,6-8,10,15,16,18] inflammation,[10,19] nutrition,[20] and physical inactivity.[6,10,16,18] While these risk factors are closely related to lifestyle, research on heart rate and heart rate variability has almost exclusively been conducted in western societies with an affluent sedentary lifestyle and high prevalences of these risk factors. It has therefore been difficult to determine whether or not heart rate and heart rate variability are associated with functional impairment, morbidity, and mortality independently of cardiovascular risk factors.[1-4] To disentangle the effects of ageing and the lifestyle-related cardiovascular risk factors, the associations of heart rate and heart rate variability with functional impairment, morbidity, and mortality should also be studied in societies where an affluent sedentary lifestyle is absent and these risk factors are rare.

We investigated the associations of heart rate and heart rate variability with handgrip strength and mortality among older persons in a traditional rural African population. In this population, contrary to western populations, food is scarce, lifelong manual labour is necessary for subsistence, and obesity, hyperlipidæmia, diabetes, hypertension, and cardiovascular diseases are rare (Chapters 9 and 10 of this thesis).[21-23] We have recently shown that handgrip strength strongly and independently predicts mortality in this population (Chapter 8 of this thesis).[24]

Methods

Research area

This study was conducted in the Garu-Tempane District in the Upper East Region in Ghana. The area is rural, remote, and one of the least developed in the country. The vast majority of the inhabitants are involved in non-commercial agriculture performed by manual labour without proper means of transportation or mechanised farming. Hospital care is absent. Infectious diseases are highly endemic and constitute the main causes of death, although the prevalence of human immunodeficiency virus (HIV) is low (< 4%) compared with other African regions.[25]

Since 2002, we have kept a demographic registry of the population within a research area of 375 km^2 comprising 32 villages. During yearly visits we registered the name, age, sex, tribe, and location of living of each inhabitant. In 2007 we determined the property value of each household. From this value, an index of the socioeconomic status with a standard normal distribution was calculated according to the Demographic and Health Survey method.[26]

In addition, we registered the main drinking water source of each household. Water from boreholes was classified as safe and water from open wells and rivers as unsafe, based on their pathogen contents and their effects on survival.[27] Annual migration relative to the study population's size was 2% into and 1% out of the research area. Elaborate descriptions of this study population have been given elsewhere.[21-24,26]

Ethical approval was given by the Ethical Review Committee of Ghana Health Services, the Committee Medical Ethics of Leiden University Medical Center, and the local chiefs and elders. Because of illiteracy, informed consent was obtained orally in the participant's own language after explanation of the purpose and conduction of this research project. The study conforms to the ethical guidelines of the Declaration of Helsinki.

Participants and measurements

In 2009 and 2010 we conducted measurements in the morning among 924 inhabitants aged 50 years and older, who were recruited in villages visited consecutively. To ensure maximal participation, we set up a mobile field work station in the villages and, if necessary, brought less mobile participants by car. Reasons of non-participation were death of the individual since the last registration (n = 48), refusal of participation (n = 35), absence from the research area during our visits because of migration or travelling (n = 30), and other reasons (n = 46).

Height, weight, and waist circumference were measured and BMI was calculated as weight in kilograms divided by squared height in metres. Glucose level was measured in a random capillary blood sample from a finger (Accutrend Plus, Roche, Rotkreuz, Switzerland). Blood pressure was measured on one arm in a lying and resting position. Systolic blood pressures were measured on the dorsalis pedis artery and the posterior tibial artery of both ankles with a Doppler ultrasound machine (ImexDOP CT+, Natus-Nicolet, Golden, CO, USA). As a measure of peripheral arterial disease, the ankle-arm index was calculated by dividing the average systolic pressure at the ankles by the systolic arm pressure.[23]

Twelve-lead electrocardiograms (ECGs) were obtained as two sequential recordings of ten seconds in a lying and resting position (AT-104 PC, Schiller, Baar, Switzerland). Three participants with atrial fibrillation, described in Chapter 10 of this thesis,[22] were excluded from the analyses. The recordings of the other participants displayed sinus rhythm with positive P waves in leads I and II and uniform QRS complexes. The timing of the QRS complexes was identified automatically. All recordings were manually reviewed in detail to verify the automatic identification of QRS complexes and to ensure that only normal RR intervals were included in the analyses. Recordings with ectopic complexes were excluded from the analyses, except for twelve participants with an ectopic complex in the last two seconds of the recording, in which cases all complexes from the ectopic complex onward

were excluded. Heart rate in beats per minute (bpm) and two measures of heart rate variability in milliseconds (ms) were determined based on standard methods.[28] Heart rate variability was calculated as the standard deviation of normal RR intervals (SDNN) and as the root mean square of the differences between successive normal RR intervals (RMSSD).

Handgrip strength was measured using a calibrated Jamar hand dynamometer (Sammons Preston, Bolingbrook, IL, USA) while the participant was standing in an upright position with the arms unsupported parallel to the body. Participants were instructed to exert maximal force with each hand once. The handgrip strength of the hand with the highest measurement was registered.[29]

Data on mortality after the measurements in 2009 and 2010 were available from the demographic registry. Follow-up started at the time of the measurements and lasted until death, migration out of the research area, loss to follow-up, or our last visit to the research area in 2011.

Analyses

Because of their skewed distributions, SDNN and RMSSD were transformed logarithmically. Changes in heart rate and heart rate variability with age were assessed using linear regression with age and sex as independent variables. Changes in the variances of heart rate and heart rate variability with age were assessed using Levene's test comparing 5-year age groups. Associations of heart rate and heart rate

Number of individuals	822
Men, *n* (%)	421 (51.2)
Age, years	65 (56–72)
Tribe, *n* (%)	
Bimoba	572 (69.6)
Kusasi	195 (23.7)
other	55 (6.7)
Household property value, US$	1085 (516–1944)
Safe drinking water, *n* (%)	721 (87.7)
Waist circumference, cm	77 (72–81)
Body mass index, kg/m^2	18.1 (16.6–19.6)
Blood pressure, mmHg	
diastolic	70 (65–80)
systolic	120 (110–135)
Ankle-arm index	1.15 (1.08–1.23)
Heart rate, bpm	70 (62–78)
Heart rate variability, ms	
SDNN	19.6 (13.6–29.3)
RMSSD	18.0 (11.4–28.4)

Table 11.1 • General characteristics of the Ghanaian study population. Data are presented as medians with interquartile ranges unless specified otherwise. Heart rate variability was calculated as the standard deviation of normal RR intervals (SDNN) and as the root mean square of the differences between successive normal RR intervals (RMSSD). Bpm: beats per minute.

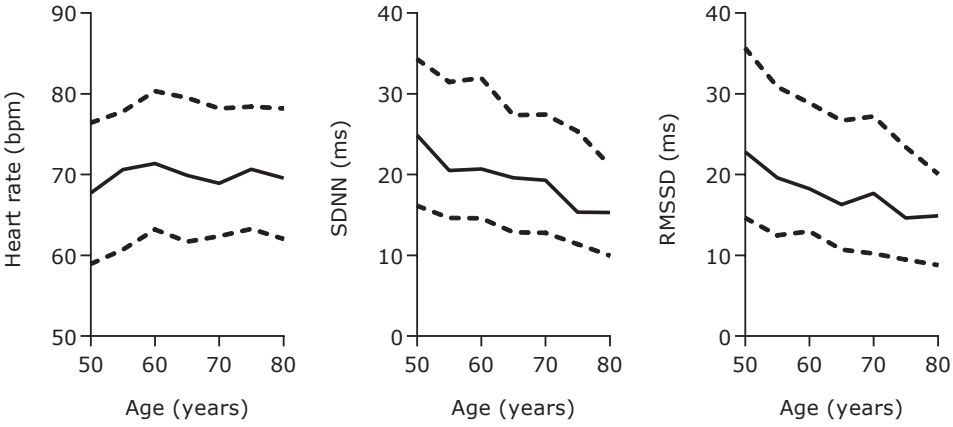

Figure 11.1 • Distributions of heart rate and heart rate variability over age in the Ghanaian study population. The data represent age-specific medians (continuous lines) and interquartile ranges (dashed lines) of heart rate and heart rate variability. Heart rate variability was calculated as the standard deviation of normal RR intervals (SDNN) and as the root mean square of the differences between successive normal RR intervals (RMSSD). Bpm: beats per minute.

variability with handgrip strength were tested with three linear regression models: one without adjustments; one minimally adjusted for age, sex, tribe, and height; and one fully adjusted for height and all demographic and cardiovascular characteristics shown in Table 11.1. Hazard ratios were determined by three Cox regression models: one without adjustments; one minimally adjusted for age, sex, and tribe; and one fully adjusted for all demographic and cardiovascular characteristics shown in Table 11.1. In the fully adjusted models, the associations of heart rate with handgrip strength and mortality were adjusted for SDNN, but the results were similar when adjusting for RMSSD instead. The analyses were performed with SPSS Statistics 20 (IBM, Armonk, NY, USA).

Results

Table 11.1 provides the demographic and cardiovascular characteristics of the Ghanaian study population. Of the 924 individuals who participated in the measurements, 822 could be included with data on heart rate, heart rate variability, and handgrip strength. Both measures of heart rate variability, SDNN and RMSSD, correlated with Spearman's $\rho = 0.83$ ($p < 0.001$). After logarithmic transformation, median (with interquartile range) SDNN was 2.98 ln ms (2.61–3.38), median RMSSD was 2.89 ln ms (2.43–3.35), and their correlation remained similar with Pearson's $r = 0.84$ ($p < 0.001$).

Figure 11.1 shows the distributions of heart rate and heart rate variability over age. As age increased one year, heart rate increased with 0.15 bpm, SDNN decreased

Table 11.2 • Determinants of heart rate and heart rate variability in the Ghanaian study population

	Difference in heart rate in bpm (95% CI)	Difference in SDNN in ln ms (95% CI)	Difference in RMSSD in ln ms (95% CI)
Age, years	+0.16 (+0.08 to +0.25) **	-0.010 (-0.014 to -0.005) **	-0.011 (-0.016 to -0.006) **
Men	-9.45 (-11.13 to -7.78) **	-0.023 (-0.109 to +0.063)	-0.060 (-0.161 to +0.034)
Tribe			
Bimoba	Ref.	Ref.	Ref.
Kusasi	+1.13 (-0.74 to +3.00)	+0.014 (-0.075 to +0.104)	+0.024 (-0.078 to +0.125)
other	-1.78 (-5.15 to +1.59)	-0.156 (-0.317 to +0.006)	-0.157 (-0.339 to +0.026)
Wealth index	-0.26 (-0.86 to +0.33)	+0.038 (+0.010 to +0.067) *	+0.050 (+0.018 to +0.083) *
Safe drinking water	-0.26 (-2.64 to +2.11)	-0.103 (-0.217 to +0.010)	-0.049 (-0.178 to +0.079)
Body mass index, kg/m^2	-0.50 (-0.99 to -0.01) *	+0.010 (-0.013 to +0.033)	0.000 (-0.027 to +0.026)
Waist circumference, cm	0.00 (-0.18 to +0.18)	-0.008 (-0.017 to 0.000)	-0.004 (-0.014 to +0.005)
Capillary glucose, mmol/l	+2.11 (+1.31 to +2.91) **	-0.004 (-0.042 to +0.035)	-0.010 (-0.054 to +0.034)
Diastolic blood pressure, mmHg	+0.24 (+0.14 to +0.35) **	-0.001 (-0.006 to +0.004)	0.000 (-0.005 to +0.006)
Systolic blood pressure, mmHg	+0.01 (-0.04 to +0.06)	+0.001 (-0.001 to +0.003)	+0.002 (-0.001 to +0.004)
Ankle-arm index	+1.96 (-1.70 to +5.61)	-0.153 (-0.328 to +0.022)	-0.081 (-0.280 to +0.117)
Heart rate, bpm	—	-0.019 (-0.022 to -0.016) **	-0.030 (-0.034 to -0.026) **

The data represent the differences in heart rate or heart rate variability per unit change in each characteristic. Tribes are compared with the Bimoba tribe as reference (Ref.). Men and safe drinking water source are compared with women and unsafe drinking water source as references. Heart rate variability was calculated as the standard deviation of normal RR intervals (SDNN) and as the root mean square of the differences between successive normal RR intervals (RMSSD). Each association of each characteristic with heart rate or heart rate variability was adjusted for those of the other characteristics. Bpm: beats per minute. * $p < 0.05$; ** $p < 0.001$.

Table 11.3 • Associations of heart rate and heart rate variability with handgrip strength in the Ghanaian study population

	Difference in handgrip strength in kg (95% CI)		
	Without adjustments	**Adjusted for age, sex, tribe, and height**	**Fully adjusted**
Heart rate, bpm	-0.16 (-0.21 to -0.12) **	-0.03 (-0.06 to +0.01)	-0.01 (-0.05 to +0.03)
Heart rate variability, ln ms			
SDNN	+2.53 (+1.58 to +3.48) **	+1.01 (+0.26 to +1.77) *	+0.91 (+0.15 to +1.67) *
RMSSD	+2.23 (+1.44 to +3.01) **	+0.80 (+0.17 to +1.42) *	+0.78 (+0.11 to +1.46) *

The data represent the difference in handgrip strength per unit change of each measure of heart rate and heart rate variability. Heart rate variability was calculated as the standard deviation of normal RR intervals (SDNN) and as the root mean square of the differences between successive normal RR intervals (RMSSD). The fully adjusted effects were adjusted for age, sex, tribe, height, socioeconomic status, drinking water source, body mass index, waist circumference, glucose level, diastolic and systolic blood pressure, ankle-arm index, and heart rate or heart rate variability. Bpm: beats per minute. * $p<0.05$; ** $p<0.001$.

Table 11.4 • Associations of heart rate and heart rate variability with mortality in the Ghanaian study population

	Hazard ratio (95% CI)		
	Without adjustments	**Adjusted for age, sex, and tribe**	**Fully adjusted**
Heart rate, bpm	1.02 (0.99 to 1.04)	1.02 (0.99 to 1.04)	1.00 (0.97 to 1.03)
Heart rate variability, ln ms			
SDNN	0.49 (0.29 to 0.82) *	0.53 (0.31 to 0.91) *	0.55 (0.29 to 1.01)
RMSSD	0.65 (0.43 to 1.00) *	0.71 (0.46 to 1.10)	0.75 (0.45 to 1.25)

The data represent the difference in handgrip strength per unit change of each measure of heart rate and heart rate variability. Heart rate variability was calculated as the standard deviation of normal RR intervals (SDNN) and as the root mean square of the differences between successive normal RR intervals (RMSSD). The fully adjusted effects were adjusted for age, sex, tribe, socioeconomic status, drinking water source, body mass index, waist circumference, glucose level, diastolic and systolic blood pressure, ankle-arm index, and heart rate or heart rate variability. Bpm: beats per minute. * $p<0.05$; ** $p<0.001$.

with 0.013 ln ms, and RMSSD decreased with 0.016 ln ms (all $p < 0.001$). The variances of heart rate and heart rate variability were constant with age.

As shown in Table 11.2, we explored the determinants of heart rate and heart rate variability in a multivariate model including all demographic and cardiovascular characteristics. Heart rate was higher in older individuals, in women, in individuals with a lower BMI, in individuals with a higher glucose level, and in individuals with a higher diastolic blood pressure. Both SDNN and RMSSD were lower in older individuals, higher in individuals with a higher wealth index, and lower in individuals with a higher heart rate.

Heart rate, heart rate variability, and handgrip strength

Table 11.3 describes the associations of heart rate and heart rate variability with handgrip strength. Handgrip strength was lower in individuals with a higher heart rate and higher in individuals with a higher SDNN or RMSSD (all $p < 0.001$). After adjustment for age, sex, tribe, and height, heart rate was not associated with handgrip strength, but handgrip strength remained higher in individuals with a higher SDNN ($p = 0.009$) or RMSSD ($p = 0.013$). After additional adjustment for other demographic and cardiovascular characteristics, these associations did not change ($p < 0.025$). When stratifying the fully ad-

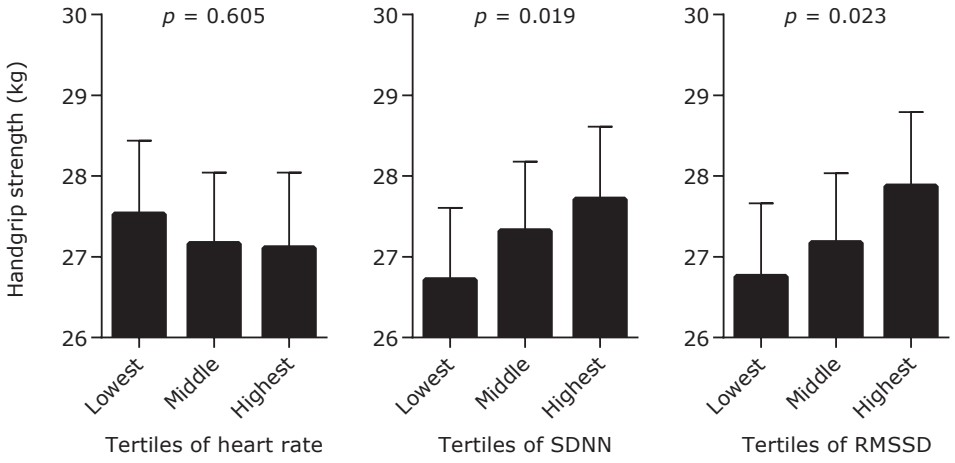

Figure 11.2 • Estimates of handgrip strength per tertiles of heart rate and heart rate variability in the Ghanaian study population. The data represent estimated means of handgrip strength per tertiles of heart rate and heart rate variability. Heart rate variability was calculated as the standard deviation of normal RR intervals (SDNN) and as the root mean square of the differences between successive normal RR intervals (RMSSD). The estimated means were fully adjusted for age, sex, tribe, height, drinking water source, body mass index, waist circumference, glucose level, diastolic and systolic blood pressure, ankle-arm index, and heart rate or heart rate variability (p values for trends). Bpm: beats per minute.

justed model, the association of heart rate variability with handgrip strength was not different between men and women, individuals younger and older than 65 years, or individuals with and without underweight, defined as a BMI below 18.5 kg/m^2.

Figure 11.2 provides estimates of handgrip strength per tertiles of heart rate and heart rate variability, adjusted for all demographic and cardiovascular characteristics. From the lowest to the highest tertile of heart rate, handgrip strength declined non-significantly with 0.57 kg. From the lowest to the highest tertile of SDNN, handgrip strength increased with 1.00 kg. From the lowest to the highest tertile of RMSSD, handgrip strength increased with 1.12 kg.

Heart rate, heart rate variability, and mortality

Table 11.4 describes the associations of heart rate and heart rate variability with mortality. Data on follow-up were available for 814 (99.0%) participants and comprised 1396 person-years and 42 deaths. Heart rate was not associated with mortality. Heart rate variability was inversely associated with mortality: risk of mortality was lower in individuals with a higher SDNN (p = 0.006) or RMSSD (p = 0.048). After adjustment for age, sex, and tribe, these associations remained similar and remained significant for SDNN (p = 0.021), but lost significance for RMSSD (p = 0.121). After additional adjustment for other demographic and cardiovascular characteristics, the associations remained similar.

Sensitivity analyses

To examine the consistency of the results, we repeated the analyses in restricted groups of participants. Firstly, we excluded twelve participants who had shown ectopic complexes in the last two seconds of the ECG. The associations of heart rate with handgrip strength and mortality remained absent in the minimally and fully adjusted models. The associations of heart rate variability with handgrip strength and mortality remained similar. Secondly, we excluded 28 participants with sinus arrhythmia, defined as the presence of consecutive normal RR intervals differing by more than 120 ms. The associations of heart rate with handgrip strength and mortality remained absent in the minimally and fully adjusted models. The associations of heart rate variability with handgrip strength were strengthened; their associations with mortality remained similar (data not shown).

Discussion

This study aims to assess whether heart rate and heart rate variability at rest are associated with handgrip strength and mortality among older persons in a traditional rural African population where obesity, hyperlipidæmia, diabetes, hypertension, and cardiovascular diseases are rare (Chapters 9 and 10 of this thesis).[21-23] Heart rate increased slightly with age and was dependent on BMI, glucose level, and diastolic blood pressure. Heart rate variability decreased with age and was not dependent on cardiovascular risk factors. Heart rate was associated with neither handgrip strength nor mortality. A lower

heart rate variability was associated with lower handgrip strength and, corresponding with our previous report that low handgrip strength predicts mortality,[24] a higher risk of mortality. These associations were independent of age, sex, tribe, and cardiovascular risk factors.

This study is the first to investigate heart rate and heart rate variability in relation to physical function and mortality in traditional Africa. Few studies have assessed heart rate at rest and its associations with cardiovascular risk factors in rural African populations and have reported results that are in accordance with our results. Heart rate was, compared with the Ghanaian study population, similar in rural Tanzanian populations[8,30] and among hypertensive patients attending hospitals in different Sub-Saharan countries,[31] but higher in a population close to our research area with younger participants and higher prevalences of cardiovascular risk factors.[32] In these populations, heart rate was constant with age, higher in women, and positively related with blood pressure and lipid levels, although less strongly than in western populations.[8,16,30,32] Heart rate was found to be higher in Congolese children and adolescents with a lower socioeconomic status and with malnutrition or obesity.[33] Heart rate variability has been measured only in hospitals in several Sub-Saharan countries and without assessing its associations with physical function, morbidity, or mortality. In these studies, heart rate variability declined with age and was higher in women.[30,31]

Heart rate and heart rate variability at rest vary across populations and ethnicities due to both genetic and environmental factors,[16,30,34] which complicates comparisons between populations. Still, the study of heart rate and heart rate variability in populations without an affluent sedentary lifestyle aids the solution of questions that remain despite the research conducted in western populations. Heart rate in western populations is higher in women and dependent on various cardiovascular risk factors, as in the Ghanaian study population, but it has been inconsistently described to decrease, be constant, or increase with age.[7,8,15-18] Conflicting results have also been reported on differences in heart rate between European and African Americans.[11,35-37] A study comparing Americans of European origin, Americans of African origin, and American immigrants from Ghana without hypertension, cardiovascular disease, and diabetes found no differences in heart rate.[38] Other studies showed that differences in heart rate between European and African Americans can be attributed to cardiovascular risk factors.[37,39] The findings from traditional African populations reinforce the possibility that heart rate is dependent on cardiovascular risk factors rather than age and that it is only associated with functional impairment, morbidity, and mortality when these risk factors are present. As a biological explanation, cardiovascular risk factors such as obesity, hyperlipidæmia, hypertension, and diabetes can increase heart rate by inducing hæmodynamic alterations, cardiac conduction abnormalities, and sympathetic hyperactivation.[6,10,15,40]

Heart rate variability decreases with age in western populations as in the Ghanaian study population and other African populations.[2,16,17,41-43] Heart rate variability in the Ghanaian study population was similar to that in different western populations investigated with comparable methodologies, despite differences in cardiovascular risk factors.[44-47] Although conflicting results have been reported on differences in heart rate variability between European and African Americans, these differences seem independent of cardiovascular risk factors.[34,41,48] Not only in the Ghanaian study population, but also in western populations heart rate variability has been reported to be unaffected by cardiovascular risk factors.[18,34,42,49] These findings, combined with those from traditional African populations, suggest that heart rate variability is associated with functional impairment, morbidity, and mortality through mechanisms independent of cardiovascular risk factors. Most likely, heart rate variability declines during ageing as a result of a deteriorating autonomic regulation of the heart rate that occurs across populations with different lifestyles.[13,14,20,50] More research in populations without an affluent sedentary lifestyle is required to substantiate our interpretations.

Since a high heart rate is a long-established predictor of morbidity and mortality, it is regarded as a potential therapeutic target of cardiovascular disease.[1,3,7] While pharmacological lowering of the heart rate has been found to benefit patients with heart failure, it fails to do so in patients without heart failure.[51] In line with our study, it is posited that the heart rate is accelerated not as a cause, but as an effect of cardiovascular disease.[1,51] It could be that in the detection and prevention of cardiovascular disease the role of heart rate is overrated, while that of heart rate variability has remained underrated.

This study has the following limitations. Firstly, ECGs were recorded during 20 seconds, while longer recordings are often preferred.[28] Heart rate variability in short recordings cannot be measured by frequency-domain methods, cannot be compared with longer recordings used in most studies, and is determined with less precision. However, SDNN and RMSSD as measures of heart rate variability can be used to investigate short recordings[28] and several studies of similarly short recordings have demonstrated that these measures are associated with morbidity and mortality.[44-47] Moreover, the less precise determination of heart rate variability in short recordings is thought to result in underestimation rather than overestimation of the true associations.[45,47] Secondly, mortality was registered during only a short follow-up period, which attenuates the statistical power of the mortality analyses. Thirdly, associations of heart rate and heart rate variability with incidence of morbidity could not be assessed, since diseases were not registered. Lastly, the effects of some lifestyle-related risk factors on heart rate and heart rate variability could not be studied. Although nutritional status was reflected by BMI, dietary composition, physical activity, and smoking were not formally documented.

In conclusion, this study shows that a high heart rate is not, but a low heart rate variability is associated with handgrip strength and mortality among older persons in a traditional rural African population as it is in western populations. This suggests that the association of heart rate with physical function and mortality as described in western populations is predominantly mediated by lifestyle-related risk factors of cardiovascular disease and diabetes. By contrast, the association of heart rate variability with physical function and mortality probably reflects a universal deterioration of the body's autonomic regulation during ageing. Across various environments, heart rate variability can be measured to predict functional impairment and mortality.

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PAR

GENERAL DISCUSSION AND SUMMARY

12 GENERAL DISCUSSION

In this thesis, we measure senescence in human populations, defined as an increase in the risks of dysfunction, disease, and death with chronological age. In Part I of this thesis we investigate how a population's senescence rate can be measured through the increase in mortality rate with age. In Part II of this thesis we investigate how senescence can be measured through the increase in morbidity with age in a non-western population and thus be compared with the senescence process in western populations.

The rate of senescence

A population's senescence rate is classically measured as the increase in mortality rate with age on a logarithmic scale, which is described by the Gompertz model's parameter γ. This measure has been criticised and, as an alternative, it has been proposed to measure a population's senescence rate as the increase in mortality rate with age on an absolute scale.[1]

Some who have criticised the classical measure of a population's senescence rate have fallen back to measures of senescence that summarise the varying levels of mortality throughout the entire life in a single age-independent constant, such as the mean lifespan.[2] As such measures are not informative about the age pattern of mortality, they cannot improve on the classical measure.[3]

Others have designed new approaches other than measuring the increase in mortality rate on an absolute scale. Notably, it has been put forward that age patterns of mortality rates can be separated into two dimensions: the pace of senescence and the shape of senescence. The pace of senescence captures the time during which mortality occurs and can be described by life expectancy or maximum lifespan. The shape of senescence does not depend on time, but captures the extent to which mortality changes independently of the pace of senescence.[4] Although this approach provides a thoughtful conceptual view on the measurement of senescence[5] and is already applied to characterise senescence processes,[6,7] the assumptions underlying the separation of both dimensions of senescence have never been empirically tested.

We are the first to empirically verify an alternative measure of a population's senescence rate, derived from the increase in its mortality rate with age on an absolute scale (Chapter 3 of this thesis).[8] We have tested this measure by applying it to populations that are known to have different senescence rates. We have confirmed that this alternative measure yields valid senescence rates, whereas the classical measure yields invalid senescence rates. We have demonstrated how senescence rates can be determined according to the alternative measure by using the derivative function of the Gompertz model. This principle can be applied to any model of the age pattern of a mortality rate other than the Gompertz model. Furthermore, to overcome the limitations of such models, we have demonstrated how senescence rates can be determined according to the same principle by using a non-parametric method that does not require any modelling of mortal-

ity rates (Chapter 4 of this thesis).[9] This method can be applied to any age pattern of a mortality rate.

The validity of the increase in mortality rate on a logarithmic scale as a measure of the senescence rate can also be questioned on mathematical grounds. According to the Gompertz model, the increase in mortality rate m with age t equals $m'(t) = \gamma$ and has the unit per year of age.[10] Non-parametrically, this increase equals:

$$\ln(m'(t)) = m'(t) \,/\, \mathrm{m}(t) \qquad (12.1)$$

which likewise has the unit per year of age. By contrast, the senescence rate measured as the increase in mortality rate on an absolute scale — which is according to the Gompertz model:

$$m'(t) = \alpha\, \gamma\, e^{\gamma t} \qquad (12.2)$$

and is non-parametrically described as:

$$m'(t) = dm \,/\, dt \qquad (12.3)$$

— is expressed with the unit deaths per person-year per year of age, thus mortality rate per year of age. The latter unit matches best with the definition of senescence as the increase in mortality rate with age.

The Gompertz model's parameter γ is not by itself a measure of a population's senescence rate, but the senescence rate is described by the model's derivative function that is dependent on both parameter α and γ. Moreover, it is not necessary to model the age pattern of a mortality rate to measure the senescence rate. It follows that the parameters of the Gompertz model — or of any other model of the age pattern of a mortality rate — cannot be interpreted to have specific biological meanings. Such an interpretation assumes that mortality can be partitioned in intrinsic mortality due to senescence and extrinsic mortality independent of senescence. We have substantiated in Chapter 5 of this thesis[11] why this assumption is false. Accordingly, the function of the parameters is limited to their original function, that is to fit and describe the age pattern of a mortality rate merely mathematically.

Figure 12.1 illustrates the mathematical meanings of both parameters of the Gompertz model. Variation in α as well as in γ affects the decrease in survival with age, the distribution of deaths over age, and the increase in mortality rate with age and, thus, affects the senescence rate. Conversely, the differences in these age patterns that are brought about by variation in either parameter cannot be distinguished as either reflecting a difference in the senescence rate or not.

The way of senescence

Because much is unknown about senescence in populations without a western lifestyle, we have studied senescence in a traditional rural African population in one of the least developed regions of Ghana (Chapters 7 through 11 of this thesis). This population contrasts sharply with western populations: its environment is not affluent and sedentary, but typified by poverty, limited nutrition, regular hunger, continuous physical activity, and many endemic infectious diseases.

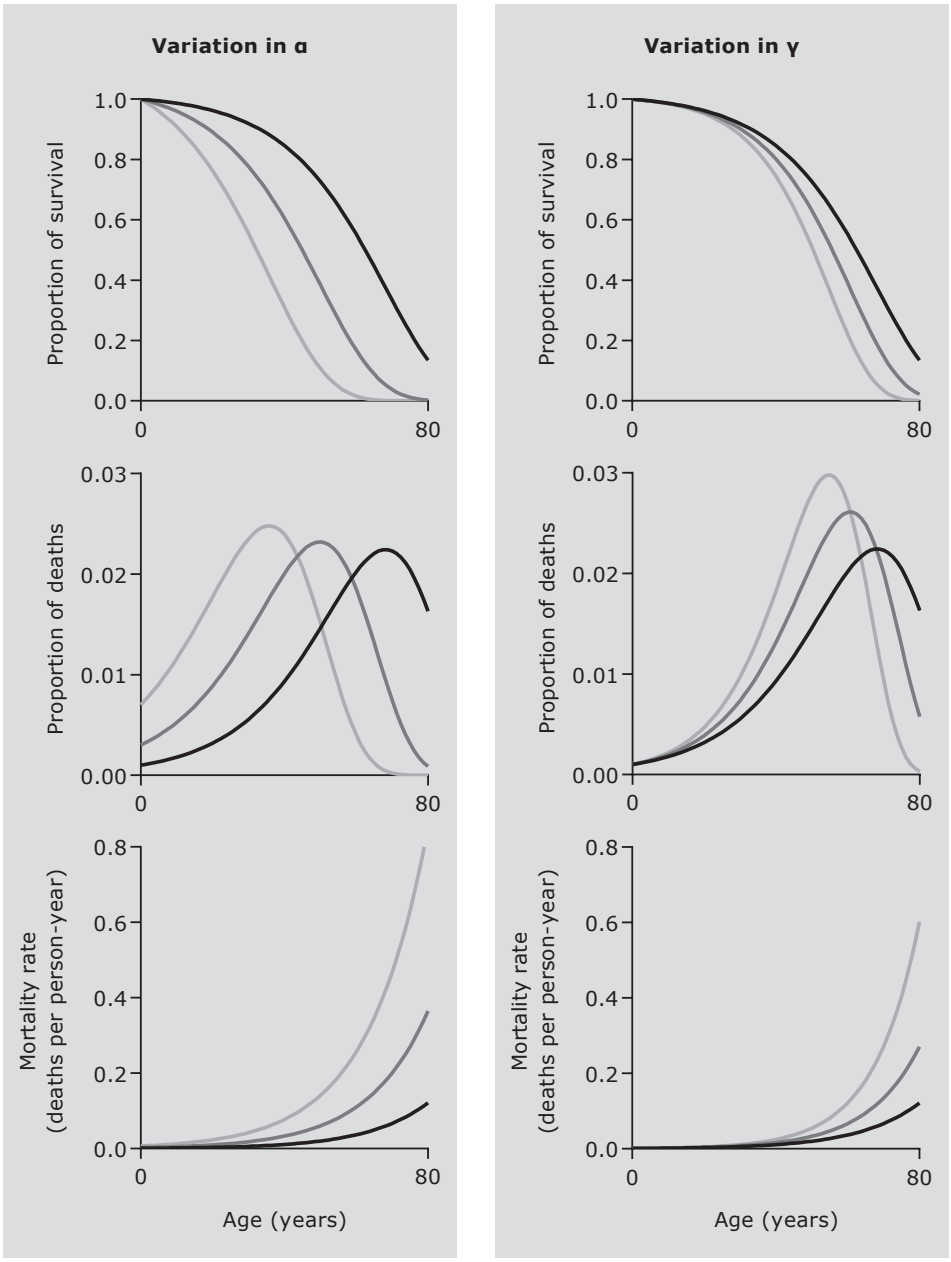

Figure 12.1 • The effects of variation in the Gompertz model's parameters α and γ on the age patterns of a population's survival, deaths, and mortality rate. In the left panels, α is 0.001, 0.003, and 0.007 from black to grey and γ is constantly 0.06. In the right panels, γ is 0.06, 0.07, 0.08 from black to grey and α is constantly 0.001. Others have assessed these effects similarly.[12]

In some respects, the way of senescence is similar in the Ghanaian study population and western populations. We have shown that handgrip strength declines with age and predicts mortality similarly in both types of populations (Chapter 8 of this thesis).[13] Heart rate variability at rest also declines with age and is associated with handgrip strength and mortality similarly in both types of populations (Chapter 11 of this thesis).[14] Handgrip strength and heart rate variability apparently reflect biological age and senescence across different environments and different lifestyles.

In other respects, our studies show that the way of senescence is different in this non-western population as compared with western populations (Chapters 9 and 10 of this thesis).[15,16] Senescence does not manifest as sharp increases with age in the prevalences of cardiovascular disease — including coronary arterial disease, peripheral arterial disease, and atrial fibrillation — and diabetes mellitus. It neither manifests as an increase in heart rate at rest, which is associated with disease and death in western populations. Meanwhile, risk factors of cardiovascular disease, diabetes mellitus, and an increased heart rate — including obesity, dyslipidæmia, and hypertension — are absent or uncommon. Because their risk factors are closely related to a western lifestyle, the development of diseases such as cardiovascular disease and diabetes mellitus and the increase in heart rate during senescence are likely consequences of a western lifestyle.

In western populations, chronic smouldering inflammation arises during senescence and is associated with the diseases that are related to senescence, among which cardiovascular disease and diabetes mellitus. It has been proposed that inflammation plays an essential role in the causation of senescence and senescence-related diseases.[17-22] In the Ghanaian study population, a high burden of infectious diseases and an enrichment of a proinflammatory immune system[23,24] have not resulted in chronic smouldering inflammation, cardiovascular disease, or diabetes mellitus.[15,16,25] Other studies have confirmed that chronic smouldering inflammation in western populations is rather an effect than a cause of cardiovascular disease, diabetes mellitus, and their risk factors, of which most notably obesity.[26-29] In the Ghanaian study population and other similar non-western populations, inflammation is rather acute and attributable to infectious diseases.[25,30-32]

Cardiovascular disease is closely related to senescence in western populations. Its pathogenesis consists of an accumulation of damage in the vascular wall and the heart during ageing due to a disturbance of the blood flow, a deposition of lipids, and a maladaptive inflammatory response.[33,34] These processes are associated with markers of senescence at the molecular and cellular level.[35,36] During life, these processes lead to dysfunction and disease of the vessels and heart[37-39] and are associated with the occurrence of other age-related disorders.[40,41] By contrast, the environment and lifestyle in non-western populations expose their inhabitants to other kinds of bodily damage. Rather than high levels of lipids and chronic smouldering inflam-

mation, damage accumulates during their lives as a consequence of malnutrition and acute inflammation evoked by infectious diseases. The different kinds of damage lead to different kinds of dysfunction and disease. Senescence in non-western populations is not characterised by cardiovascular disease, but by diseases that are unfamiliar from a western point of view.

The rate and way of senescence

Our investigations of the rate of senescence and the way of senescence in human populations converge when we apply our method of measuring a population's senescence rate in order to compare the senescence rates of the Ghanaian study population with those of a western population.

Figure 12.2 shows the age patterns of the causes of death in the general population of the USA and the Ghanaian study population. As is well known for western populations, most deaths during adolescence and young adulthood are due to traumata, while most deaths at higher ages are due to non-infectious diseases such as cardiovascular disease, diabetes mellitus, and cancer (Figure 12.2A). By contrast, in the Ghanaian study population, most deaths are due to infectious diseases at any age, while deaths due to non-infectious diseases account for a minority of deaths (Figure 12.2B). The way of senescence differs between this non-western population and western populations.

Figure 12.3A shows the age patterns of the mortality rates of the general population of the USA and the Ghanaian study popu-

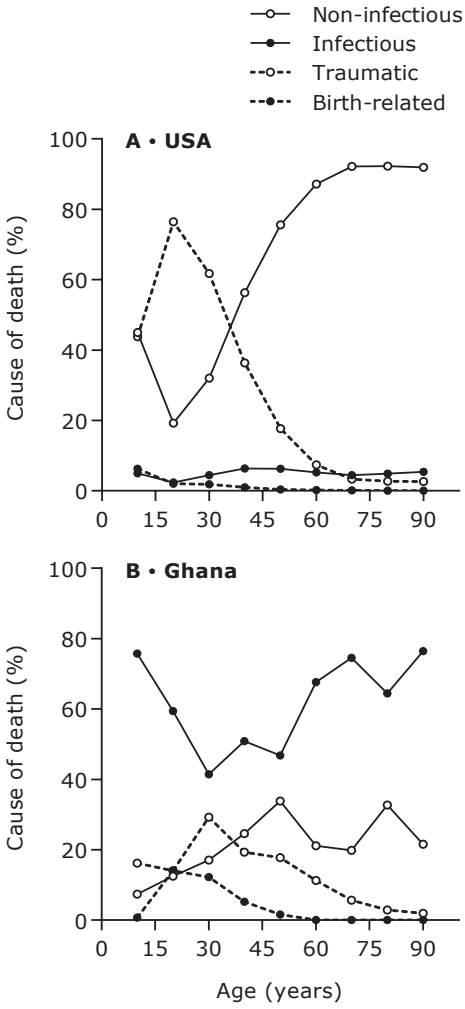

Figure 12.2 • Age patterns of causes of death in the general population of the USA (A) and in the traditional rural Ghanaian study population (B). Data on the general population of the USA are from 1990 through 2010 and were derived from CDC WONDER.[49] Data on the Ghanaian study population are from 2003 through 2011 and were obtained by verbal autopsy.[50,51] Differences in the coding between both sources have been equalised.

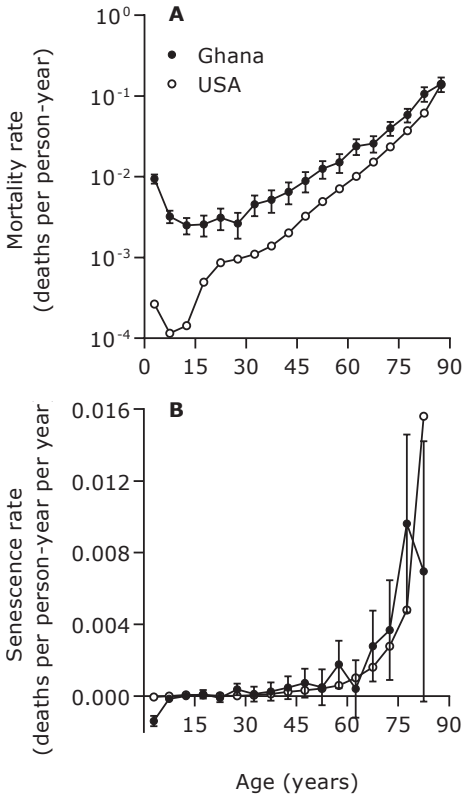

Figure 12.3 • Age patterns of mortality rates (A) and senescence rates (B) in the general population of the USA and in the traditional rural Ghanaian study population. Data on the general population of the USA are from 1990 through 2010 and were derived from CDC WONDER.[49] Data on the Ghanaian study population are from 2003 through 2011 and were registered as described elsewhere.[52,53] The mortality rates and senescence rates are shown with 95% confidence intervals. The senescence rates were calculated from the increase in the mortality rates with age on an absolute scale, as described in Chapter 4 of this thesis.[9]

lation on a logarithmic scale. Conforming to the Gompertz model, these mortality rates increase linearly during adulthood. If these linear increases would be interpreted according to the classical method, the senescence rate of the general American population would be estimated higher than that of the Ghanaian study population. However, if we calculate their senescence rates from the increase in their mortality rates on an absolute scale, another conclusion is drawn. Figure 12.3B shows the senescence rates calculated in this manner. Despite the different ways of senescence, the rates of senescence are similar in both populations.

As discussed in Chapters 1 and 5 of this thesis,[11] senescence is the result of intrinsic and extrinsic stressors that damage the human body in interaction. The comparison of populations with different environments and lifestyles discloses the effects of extrinsic stressors from the environment: the way of senescence differs between the Ghanaian study population and western populations. Meanwhile, our findings of similar declines in handgrip strength and heart rate variability with age and of similar senescence rates in these different environments hint at causes of senescence that are intrinsic to the human body and render it susceptible to damage and deterioration.

It has been hypothesised that the human species exhibits a specific fixed senescence rate.[42] This hypothesis is enfeebled by our observation that senescence rates can vary within a population, as described in Chapters 3 and 4 of this thesis.[8,9] Yet, the

mortality rates of both non-western and western populations and of both individuals with and without end-stage renal disease increase exponentially with age on an absolute scale and thereby conform to the Gompertz model. The same holds for the mortality of animal models and the decay of inanimate objects.[43] Even more, an exponential age pattern is observed in the performance of sportsmen[44] and in markers of cellular senescence.[45-48] The Gompertzian age pattern seems to be universal. Different interactions of intrinsic and extrinsic stressors lead to different senescence processes and different causes of death, but apparently underlie similar exponential increases in mortality rate with age.

Limitations

When measuring a population's senescence rate from the increase in mortality rate, we do not account for the potential effects of population heterogeneity. As explained in Chapter 4 of this thesis,[9] differences in the age patterns of mortality rates between subgroups of a population can produce an age pattern of the mortality rate for the population as a whole that does not reflect the age patterns of the subgroups. To verify that our findings are not distorted by population heterogeneity, our studies can be repeated in homogeneous populations, which is practically possible by studying the mortality of inbred animals housed under standardised conditions. We have tried to approach this goal by stratifying the study population into known subgroups; this has not refuted our conclusions. More sophisticated statistical methods have been developed to investigate the potential effects of population heterogeneity, which merit integration in our future studies.

It is sometimes objected that the measurement of a population's senescence rate from the increase in mortality rate is hampered by the level of the mortality rate. For example, patients with end-stage renal disease who receive dialysis treatment have mortality rates that greatly exceed those of the general population. It is reasoned that high mortality rates cannot increase as much as low mortality rates. However, this line of reasoning is often based on the mortality rates as shown on a logarithmic scale. When the mortality rates are shown on an absolute scale, they increase exponentially in the patients on dialysis as well as in the general population (Figure 3.1). Furthermore, we have not been able to discover any effect of the level of the mortality rate on the increase in the mortality rate by adjusting for follow-up and by longitudinally examining the age patterns of the mortality rates of subgroups of patients with end-stage renal disease.[54] To further assess this objection, it is worthwhile to test our method in cohort studies with lifelong follow-up.

A distinction must be made between the level of a mortality rate and the increase in mortality rate with age. We base the measurement of the senescence rate on the latter only. One may want to correct the increase in mortality rate on an absolute scale for the absolute level of the mortality rate, for example by $m'(t) / m(t)$. However, as mentioned above, $m'(t) / m(t) = [\ln m(t)]'$. Thus, such a correction renders a measure that is

equal to the increase in mortality rate on a logarithmic scale, equally relative, and equally invalid. Moreover, theoretically, if it is accepted that senescence at the population level is defined as an increase in the risks of disease and death with increasing age and that a population does not senesce if its mortality rate is constant with age,[55,56] it follows that senescence is equally absent in populations with mortality rates that are constant with age, but differ from each other. Nonetheless, when comparing populations, for example the Ghanaian study population and the general population of the USA (Figure 12.3), it remains valuable to compare the increases in their mortality rates with age as well as their mortality rates *per se*.

Others have objected that our method of measuring a population's senescence rate is scientifically incomplete, because it does not link the changes in the population's mortality rate with the biological changes in the senescing bodies of the individuals.[57] This is true for any measure of a population's senescence rate. While senescence at the individual level determines senescence at the population level, the measurement of senescence at the population level cannot always be related directly to senescence at the individual level, for example due to population heterogeneity as explained above. These limitations can be largely overcome with data from cohorts with lifelong follow-up, in homogeneous populations such as animal models, or by means of statistical methods that take into account population heterogeneity. Moreover, it is worthwhile to investigate whether and how the methods for measuring senescence rates from the increase in mortality rate, as proposed here, can also be used to measure senescence in individuals, for example from the decrease in handgrip strength with age.

When comparing the senescence process in different populations — for example between patients on dialysis and patients with a functioning transplant or between western and non-western populations — it seems that one compares chalk and cheese. Only the fittest patients on dialysis are eligible for transplantation. Western and non-western populations differ not only in their lifestyles, but also in their health care systems, climates, and genetics. Still, it is our aim to compare these populations precisely because they differ.[58,59] We make use of the facts that patients on dialysis senesce faster than patients with a functioning transplant and that western populations have developed into more affluent and sedentary societies than non-western populations. To be able to discern which of the differences exactly contribute to the differences in the senescence process, it is necessary to make similar comparisons between other populations.

One of the differences that is inherent to the comparison of western and non-western populations is the great inequality in health care facilities. Consequently, studies of the prevalence of disease in non-western populations are at risk of a survival bias: individuals may die from the disease before it can be detected. Have we found little cardiovascular disease and diabetes mellitus because these diseases are highly lethal in the Ghanaian study population?

Causes of death in this population have been determined by means of verbal autopsy.[50,51] Infectious diseases are the main cause of death and deaths due to cardiovascular disease and endocrine disease are uncommon, accounting for 3% of all deaths and 5% of the deaths with a known cause (Figure 12.2B).[50] In other non-western populations, physical autopsy studies have as well shown that cardiovascular disease is uncommon up to high ages.[60-62] These figures support that cardiovascular disease is verily uncommon in non-western populations.

Implications for biomedical research

For gerontological research it is of quintessential importance to determine and compare senescence rates of populations. The classical measure of the senescence rate, derived from the increase in mortality rate on a logarithmic scale, is extensively used to study the process of senescence. It is employed to compare senescence rates between species and environments and to explain the evolutionary underpinnings of senescence.[63-66] It is applied to animal models to assess whether genetic and environmental interventions, such as dietary restriction, affect the rate of senescence or delay the onset of senescence.[67-73] It is used in human populations to compare senescence rates across calendar years,[42,65,66,74,75] birth cohorts,[66,75,76] and ethnicities.[77] We have, however, demonstrated that senescence rates should not be measured as the increase in mortality rate on a logarithmic, but rather on an absolute scale.[1,8,9]

Our studies have shown that the results and interpretations of studies can be radically different when senescence rates are measured as the increase in mortality rate on an absolute instead of a logarithmic scale. A population with a higher senescence rate compared with another population according to the increase in their mortality rates on a logarithmic scale, can have a lower senescence rate according to the increase in their mortality rates on an absolute scale. Two populations with different senescence rates according to the increase in their mortality rates on a logarithmic scale, can have similar senescence rates according to the increase in their mortality rates on an absolute scale. It is necessary to examine whether and to what extent this is true for previous studies and to reevaluate the interpretations of these studies. For example, dietary restriction is thought to decrease the senescence rate in rodents, since it slows the increase in their mortality rates on a logarithmic scale.[73] However, the effect of dietary restriction on the increase in mortality rate on an absolute scale has not been studied, while it may lead to another conclusion.

Although a logarithmic scale serves the visualisation of age patterns of mortality rates, the widely adopted habit to interpret the slope of their straight lines as the senescence rate should be abandoned. In addition, the closely related assumption that intrinsic mortality due to senescence and extrinsic mortality independent of senescence can be separated should be left. Rather, senescence rates should be presented and judged in their relation to age separately from the age patterns of mortality rates.

Implications for western populations

In western populations, senescence is accompanied by sharp increases in the prevalences of non-infectious diseases such as cardiovascular disease and diabetes. These senescence-related diseases are generally regarded as inevitable consequences of a senescence process that is intrinsic to the human body. Indeed, atherosclerosis is already present in young adults,[78-80] has been found in ancient mummies,[81] and cardiovascular disease and diabetes mellitus are not entirely absent in non-western populations.[60-62]

Still, senescence-related diseases can better be considered as lifestyle-related diseases. Our studies support the notion that the senescence process is not intrinsic and intractable, but malleable and much dependent on the environment.[11,15,16] The essential role of the environment in the causation of senescence-related diseases has been confirmed by others and for other diseases that are attributed to senescence.[82] In the absence of a western lifestyle, cardiovascular disease and diabetes mellitus are uncommon even in the eldest.

From an evolutionary perspective, the diseases that arise during senescence in western populations are, at least partly, attributable to a mismatch between, on one hand, the genetic variants that have been selected during a long history in harsh environments and, on the other hand, the modern affluent environment that has been experienced by only very few generations.[83,84] Prolonged selection of thrifty and proinflammatory genetic variants have bestowed humans with qualities, desires, and impulses that predispose to these diseases, but are not so simply suppressed. Prevention and treatment of senescence-related diseases such as cardiovascular disease and diabetes mellitus will be more successful when they are aimed at improvements of the environment rather than the genetic predisposition.[82,85] If food is less readily available and less energy-rich, physical activity is an everyday habit, and infections may once in a while evoke an inflammatory response, our thrifty and proinflammatory natures are well suited and our senescence process is bettered.

Implications for non-western populations

In non-western populations, senescence is not accompanied by sharp increases with age in the prevalences of non-infectious diseases such as cardiovascular disease and diabetes mellitus because of the absence of a western lifestyle. Instead, infectious diseases dominate non-infectious diseases up to the highest ages. Differences in the environment explain why the inhabitants of these populations senesce in a different way compared with western populations. The question how they senesce remains, however, open to further scrutiny.

Meanwhile, western lifestyles are quickly spreading over the world and only few populations have not yet been pervaded. The emergence of western lifestyles goes hand in hand with the emergence of obesity, dyslipidæmia, hypertension, cardiovascular disease, and diabetes mellitus.[86-88]

Whereas western lifestyles have developed over a few generations in western popula-

tions, it is introduced within a generation in non-western populations. This renders non-western populations at an increased risk of suffering from the diseases that are caused by a western lifestyle because of three reasons. Firstly, these populations have experienced the selective pressures exerted by harsh environments until very recently. Thrifty and proinflammatory gene variants that predispose to these diseases are present in these populations.[89] Secondly, these populations have experienced a harsh environment at low age, when genes are epigenetically tuned to the environment. It has been established that exposure to hunger and infectious diseases early in life increases the risk of cardiovascular disease and diabetes mellitus when these conditions have improved later in life, at least partly through epigenetic mechanisms.[90-93] Thirdly, because of the poor environment, ideals of unhealthy foods, corpulence, and physical inactivity are culturally preserved.[94-97] Accordingly, a western lifestyle is eagerly adopted and its consequences are liked.

The prevention of lifestyle-related diseases by environmental interventions is, therefore, especially urgent in non-western populations where a western lifestyle has recently been or is currently introduced. Unfortunately, most public health policies concerning these regions have remained nearsighted and focus on hunger, infectious diseases, and maternal and child mortality. This is meaningfully exemplified by the current endeavours of the United Nations to formulate Sustainable Development Goals. Out of seventeen proposed goals, only one addresses health directly with the intention to „ensure healthy lives and promote well-being for all at all ages". Out of the thirteen statements, in which this goal is subdivided, only one specifically addresses „non-communicable diseases", together with „mental health and well-being".[98]

Conclusions

In this thesis, we show that a population's senescence rate should not be measured, following the classical method, as the increase in mortality rate with age on a logarithmic scale, but rather as its increase on an absolute scale. We present how this novel method can be applied to both modelled and non-modelled mortality rates. The novel method yields conclusions that are radically different from those provided by the classical method. The novel method also acknowledges that senescence results from an interaction between bodily and environmental factors. This interaction becomes apparent when we compare the senescence process in a non-western population with that in western populations. In some respects, the way of senescence is similar, as handgrip strength and heart rate variability at rest decline similarly with age. The rate of senescence is similar too. In other respects, the way of senescence is different, as cardiovascular disease and diabetes mellitus are uncommon up to the highest ages. This indicates that the senescence process can be modulated through the environment and lifestyle. We suggest that the senescence process can be ameliorated when environmental interventions unite the best of both worlds: a non-western lifestyle, which is nutritionally thrifty and physically active, together with western standards of public health.

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13

SUMMARY

As humans grow older, the structures and functions of their bodies deteriorate. This process is referred to as senescence. In human populations, senescence leads to an increase in the risks of dysfunction, disease, and death with chronological age. In this thesis, we measure senescence in human populations from the increase in these risks.

In Part I of this thesis we investigate how a population's senescence rate can be measured through the increase in mortality rate with age. Classically, it is measured as the increase in mortality rate with age on a logarithmic scale. In Chapter 3 we test this measurement by applying it to patients with end-stage renal disease on dialysis treatment, who are clinically known to suffer from accelerated senescence, and we compare them with patients with a functioning kidney transplant and the general population from which these patients originate. We demonstrate that the classical method to measure a population's senescence rate is inaccurate, since it attributes lower senescence rates to the patients on dialysis than the patients with a functioning transplant and the general population. We demonstrate that a novel method, which measures a population's senescence rate through the increase in mortality rate on an absolute scale, is valid, since it attributes the highest senescence rates to the patients on dialysis. We explain how senescence rates can be calculated according to the novel method using the derivative function of the Gompertz model. In Chapter 4 we demonstrate how the same method can be applied non-parametrically without modelling mortality rates. This overcomes the disadvantages of the Gompertz model and other models of the age pattern of a mortality rate. Since it is of quintessential importance for gerontological research to accurately determine and compare senescence rates of populations, senescence rates should not be measured as the increase in mortality rate on a logarithmic, but rather on an absolute scale. As we show, both methods can yield radically different results and interpretations.

The classical method to measure a population's senescence rate through the increase in mortality rate on a logarithmic scale is based on the assumption that mortality is caused by two independent mechanisms: intrinsic mortality would result from the body's senescence unrelated to the environment, while extrinsic mortality would result from environmental hazards unrelated to the body's senescence. In Chapter 5 we explain that the novel method to measure a population's senescence rate through the increase in mortality rate on an absolute scale is inconsistent with this assumption. Moreover, we show that allegedly intrinsic and extrinsic mortality have an exponentially increasing age pattern in common. Theories of senescence as well as epidemiological and biological data indicate that stressors from within the body and from its environment cause senescence and death in interaction. Together, we conclude that the roles of the body and its environment in the causation of senescence cannot be separated. Reversely, the senescence process depends on the characteristics of both the body and its environment.

In Part II of this thesis we investigate how senescence can be measured through the increase in morbidity with age in a traditional rural African population without a western lifestyle and thus be compared with the senescence process in western populations. In Chapter 7 we describe that this population is enriched with genetic variants associated with a proinflammatory immune response, probably due to the high burden of infectious diseases. It has been proposed that inflammation plays an essential role in the causation of senescence and senescence-related diseases. In this population, these genetic variants are associated with a high handgrip strength, which is regarded to indicate a low biological age.

In Chapter 8 we investigate whether handgrip strength predicts mortality in the traditional African population as it does in western populations. Although handgrip strength depends on body mass index, it declines with age and predicts mortality independently of socioeconomic, nutritional, and cardiovascular status. Handgrip strength functions as a measure of biological age and senescence in this non-western as in western populations.

In Chapters 9 and 10 we document that cardiovascular disease — including coronary arterial disease, peripheral arterial disease, and atrial fibrillation — and diabetes mellitus are rare up to the highest ages, whereas these diseases dominate the senescence process in western populations. Meanwhile, risk factors of cardiovascular disease and diabetes mellitus — including obesity, dyslipidæmia, and hypertension — are absent or uncommon. Inflammation alone appears to be insufficient for these diseases to occur. Because the risk factors are closely related to a western lifestyle, diseases such as cardiovascular disease and diabetes mellitus are likely to develop during senescence as a consequence of a western lifestyle.

In Chapter 11 we study the associations of heart rate and heart rate variability at rest with handgrip strength and mortality in the traditional African population. While heart rate is dependent on cardiovascular risk factors and is associated with neither, heart rate variability declines with age and is associated with handgrip strength and mortality independently of these risk factors and similarly to western populations. Heart rate variability, but not heart rate, reflects biological age and senescence across different environments with different lifestyles.

The way of senescence in this non-western population is partly similar and partly very different as compared with western populations. At the same time, the rate of senescence, as measured as the increases in mortality rate with age, is similar in both types of populations. This exemplifies that senescence depends on the interaction between bodily and environmental factors and indicates that the senescence process can be modulated through the environment and lifestyle. We suggest that the senescence process can be ameliorated when environmental interventions unite the best of both worlds: a non-western lifestyle together with western standards of public health.

14 SAMENVATTING

Met het ouder worden verslechteren de structuren en functies van het menselijke lichaam. Dit proces wordt veroudering genoemd. In populaties van mensen leidt veroudering tot een toename in de risico's op ziekte en sterfte met het toenemen van de chronologische leeftijd. In dit proefschrift meten we veroudering in populaties van mensen aan de hand van de toename in deze risico's.

In Deel I van dit proefschrift onderzoeken we hoe de verouderingssnelheid van een populatie kan worden afgemeten aan de toename in het sterftecijfer over de leeftijd. Traditioneel wordt de verouderingssnelheid afgemeten aan de toename in het sterftecijfer over de leeftijd op een logarithmische schaal. In Hoofdstuk 3 testen we deze meetwijze door haar toe te passen op patiënten met eindstadiumnierfalen die worden behandeld met dialyse. Het is bekend dat deze patiënten een klinisch beeld vertonen van versnelde veroudering. We vergelijken hen met patiënten met een functionerend niertransplantaat en de algemene bevolking waaruit deze patiënten afkomstig zijn. We tonen aan dat de traditionele methode om de verouderingssnelheid van een populatie te meten foutief is, aangezien zij een lagere verouderingssnelheid toekent aan dialysepatiënten dan aan patiënten met een functionerend transplantaat en de algemene bevolking. We tonen aan dat een nieuwe methode, die de verouderingssnelheid van een populatie afmeet aan de toename in het sterftecijfer op een absolute schaal, juist is, aangezien zij de hoogste verouderingssnelheid toekent aan de dialysepatiënten. We leggen uit hoe de verouderingssnelheid kan worden berekend volgens de nieuwe methode door gebruik te maken van de afgeleide functie van het Gompertz-model. In Hoofdstuk 4 laten we zien hoe dezelfde methode niet-parametrisch kan worden toegepast zonder modellering van de sterftecijfers. Zo kunnen de nadelen worden omzeild van het Gompertz-model en andere modellen die het verloop van het sterftecijfer over de leeftijd beschrijven. Omdat het van wezenlijk belang is voor het onderzoek naar veroudering dat verouderingssnelheden van populaties correct kunnen worden vastgesteld en vergeleken, dienen verouderingssnelheden niet te worden afgemeten aan de toename in het sterftecijfer op een logarithmische schaal, maar op een absolute schaal. Zoals we laten zien, kunnen beide methoden radicaal verschillende uitkomsten en interpretaties opleveren.

De traditionele methode om de verouderingssnelheid van een populatie af te meten aan de toename in het mortaliteitscijfer op een logarithmische schaal is gebaseerd op de aanname dat sterfte veroorzaakt wordt door twee onafhankelijke mechanismen: intrinsieke sterfte zou voortkomen uit veroudering van het lichaam onafhankelijk van diens omgeving, extrinsieke sterfte zou voortkomen uit de omgeving onafhankelijk van de veroudering van het lichaam. In Hoofdstuk 5 leggen we uit dat de nieuwe methode om de verouderingssnelheid van een populatie af te meten aan de toename in het mortaliteitscijfer op een absolute schaal onverenigbaar is met deze aanname. Bovendien laten we zien dat zowel de vermeende intrinsieke als de vermeende extrinsieke sterfte exponentieel toenemen over de leeftijd. Theorieën over veroudering

alsook epidemiologische en biologische gegevens wijzen aan dat stressoren vanuit het lichaam en vanuit diens omgeving in wisselwerking veroudering en sterfte veroorzaken. Op basis hiervan concluderen we dat het lichaam en diens omgeving niet kunnen worden gescheiden als oorzaken van veroudering. Omgedraaid: het verouderingsproces hangt af van de eigenschappen van zowel het lichaam als diens omgeving.

In Deel II van dit proefschrift onderzoeken we hoe veroudering afgemeten kan worden aan de toename in ziekte over de leeftijd in een traditionele Afrikaanse plattelandspopulatie zonder westerse leefstijl en hoe het verouderingsproces van deze populatie aldus kan worden vergeleken met dat in westerse populaties. In Hoofdstuk 7 beschrijven we dat deze populatie is verrijkt met genetische varianten die zijn geassocieerd met een pro-inflammatoire immuunrespons, waarschijnlijk als gevolg van de hoge infectiedruk. Er is geopperd dat ontsteking een essentiële rol speelt in het ontstaan van veroudering en van ziekten gerelateerd aan veroudering. We tonen echter aan dat deze genetische varianten geassocieerd zijn met een hoge knijpkracht, wat wordt beschouwd als een teken van een lage biologische leeftijd.

In Hoofdstuk 8 onderzoeken we of knijpkracht een voorspeller is van sterfte in de traditionele Afrikaanse populatie zoals in westerse populaties. Hoewel knijpkracht afhankelijk is van de lichaamsomvang, neemt zij af over de leeftijd en voorspelt zij sterfte onafhankelijk van de socio-economische positie, voedingsstatus en cardiovasculaire gezondheid. Knijpkracht functioneert als een maat van biologische leeftijd en veroudering in deze niet-westerse zoals in westerse populaties.

In Hoofdstukken 9 en 10 documenteren we dat hart- en vaatziekten — waaronder coronair vaatlijden, perifeer vaatlijden en boezemfibrillatie — en diabetes mellitus zeldzaam zijn tot op de hoogste leeftijden, terwijl deze ziekten het verouderingsproces in westerse populaties overheersen. Tegelijkertijd zijn risicofactoren van hart- en vaatziekten en diabetes mellitus — waaronder obesitas, dyslipidæmie en hypertensie — afwezig of zeldzaam. Ontsteking op zichzelf blijkt niet voldoende voor het teweegbrengen van deze ziekten. Aangezien de risicofactoren nauw gerelateerd zijn aan een westerse leefstijl, ontstaan ziekten zoals hart- en vaatziekten en diabetes mellitus tijdens veroudering waarschijnlijk als een gevolg van een westerse leefstijl.

In Hoofdstuk 11 bestuderen we de associaties van de hartfrequentie en de variabiliteit van de hartfrequentie met knijpkracht en sterfte in de traditionele Afrikaanse populatie. Terwijl de hartfrequentie afhankelijk is van cardiovasculaire risicofactoren en geassocieerd is met geen van beide uitkomsten, neemt de variabiliteit van de hartfrequentie af over de leeftijd en is zij geassocieerd met knijpkracht en sterfte zoals in westerse populaties. De variabiliteit van de hartfrequentie, maar niet de hartfrequentie zelf, weerspiegelt de biologische leeftijd en veroudering in verschillende omgevingen met verschillende leefstijlen.

De wijze van veroudering is deels vergelijkbaar en deels verschillend in deze niet-westerse populatie vergeleken met westerse populaties. Tegelijkertijd is de snelheid van veroudering, gemeten aan de toename in het mortaliteitscijfer over de leeftijd, vergelijkbaar in beide typen populaties. Dit illustreert dat het verouderingsproces afhangt van de wisselwerking tussen lichamelijke en omgevingsfactoren en toont aan dat het verouderingsproces kan worden beïnvloed door de omgeving en leefstijl. Wij stellen voor dat het verouderingsproces kan worden verbeterd wanneer aanpassingen van de omgeving het beste van beide werelden verenigen: een niet-westerse leefstijl in combinatie met volksgezondheidszorg van westerse kwaliteit.

PAR

APPENDICES

CONTRIBUTING AUTHORS

J. M. Abad MD PhD: Renal Registry of Aragon, Zaragoza, Spain; Department of Preventive Medicine and Public Health, University of Zaragoza, Zaragoza, Spain; Aragon Health Sciences Institute, Zaragoza, Spain.

D. Ansell MD: UK Renal Registry, Southmead Hospital, Bristol, United Kingdom (previously).

K. G. M. Beenakker MD: Department of Gerontology and Geriatrics, Leiden University Medical Center, Leiden, the Netherlands (previously); Department of Psychiatry, Leiden University Medical Center, Leiden, the Netherlands (currently).

D. van Bodegom MD PhD: Department of Gerontology and Geriatrics, Leiden University Medical Center, Leiden, the Netherlands; Leyden Academy on Vitality and Ageing, Leiden, the Netherlands.

Prof. F. W. Dekker MD PhD: Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, the Netherlands; ERA-EDTA Registry, Department of Medical Informatics, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands.

F. M. Engelaer MD PhD: Department of Gerontology and Geriatrics, Leiden University Medical Center, Leiden, the Netherlands; Leyden Academy on Vitality and Ageing, Leiden, the Netherlands (previously).

P. Finne MD PhD: Finnish Registry for Kidney Diseases, Helsinki, Finland; Department of Nephrology, Helsinki University Central Hospital, Helsinki, Finland.

B. W. Florijn MD: Department of Nephrology, Leiden University Medical Center, Leiden, the Netherlands.

J. G. Heaf MD DMSc: Department of Nephrology B, Herlev Hospital, University of Copenhagen, Herlev, Denmark.

D. van Heemst PhD: Department of Gerontology and Geriatrics, Leiden University Medical Center, Leiden, the Netherlands; Leyden Academy on Vitality and Ageing, Leiden, the Netherlands.

Prof. A. J. Hoitsma MD PhD: Department of Nephrology, Radboud University Nijmegen Medical Center, Nijmegen, the Netherlands.

D. J. de Jager PhD: Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, the Netherlands.

K. J. Jager MD PhD: ERA-EDTA Registry, Department of Medical Informatics, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands.

Prof. J. W. Jukema MD PhD: Department of Cardiology, Leiden University Medical Center, Leiden, the Netherlands; Durrer Center for Cardiogenetic Research, Amsterdam, the Netherlands; Interuniversity Cardiology Institute of the Netherlands, Utrecht, the Netherlands.

R. Kramar MD: Austrian Dialysis and Transplant Registry, Kematen an der Krems, Austria.

A. Kramer PhD: ERA-EDTA Registry, Department of Medical Informatics, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands.

M. Kuningas PhD: Department of Gerontology and Geriatrics, Leiden University Medical Center, Leiden, the Netherlands; Department of Epidemiology, Erasmus Medical Center, Rotterdam, the Netherlands (previously).

Z. Li MD MSc: Leyden Academy on Vitality and Ageing, Leiden, the Netherlands (previously).

A. C. Maan PhD: Department of Cardiology, Leiden University Medical Center, Leiden, the Netherlands.

Prof. A. B. Maier MD PhD: Department of Gerontology and Geriatrics, Leiden University Medical Center, Leiden, the Netherlands (previously); Section of Gerontology and Geriatrics, Department of Internal Medicine, VU University Medical Center, Amsterdam, the Netherlands (currently).

J. M. J. De Meester MD PhD: Department of Nephrology, Dialysis, and Hypertension, AZ Nikolaas, Sint-Niklaas, Belgium.

J. J. Meij PhD: Department of Gerontology and Geriatrics, Leiden University Medical Center, Leiden, the Netherlands; Leyden Academy on Vitality and Ageing, Leiden, the Netherlands (previously); Department of Medical Innovation, Amphia Hospitals, Breda, the Netherlands (currently).

R. Palsson MD: Division of Nephrology, Landspitali–National University Hospital and Faculty of Medicine, School of Health Sciences, University of Iceland, Reykjavik, Iceland.

M. Postorino MD: Nephrology, Dialysis, and Transplantation Unit, Azienda Ospedaliera di Reggio Calabria, Reggio Calabria, Italy.

K. G. Prütz MD: Department of Nephrology and Transplantation, Skåne University Hospital, Malmö, Sweden (previously); Swedish Renal Registry, Jönköping, Sweden; Department of Internal Medicine, Hospital of Helsingborg, Helsingborg, Sweden (currently).

P. Ravani MD MSc PhD: Division of Nephrology, Department of Medicine, University of Calgary, Calgary, Canada.

M. P. Rozing MD PhD: Department of Gerontology and Geriatrics, Leiden University Medical Center, Leiden, the Netherlands (previously); Department of Old Age Psychiatry and Neuropsychiatry, GGZ inGeest, Amsterdam, the Netherlands (currently).

F. H. Schalkwijk MSc: Leyden Academy on Vitality and Ageing, Leiden, the Netherlands (previously).

Prof. P. E. Slagboom PhD: Section of Molecular Epidemiology, Department of Medical Statistics and Bioinformatics, Leiden University Medical Center, Leiden, the Netherlands.

Prof. C. Wanner MD PhD: Division of Nephrology, Department of Internal Medicine I, University Hospital of Würzburg, Würzburg, Germany.

M. J. Wensink MD PhD: Max Planck Institute for Demographic Research, Rostock, Germany; Leyden Academy on Vitality and Ageing, Leiden, the Netherlands (previously); Max Planck Odense Center on the Biodemography of Aging, Department of Epidemiology, Biostatistics, and Biodemography, Institute of Public Health, University of Southern Denmark, Odense, Denmark (currently).

Prof. R. G. J. Westendorp MD PhD: Leyden Academy on Vitality and Ageing, Leiden, the Netherlands (previously); Department of Gerontology and Geriatrics, Leiden University Medical Center, Leiden, the Netherlands; Department of Public Health, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; Center for Healthy Aging, University of Copenhagen, Copenhagen, Denmark (currently).

J. B. Ziem MD PhD: Department of Clinical Laboratory Sciences, School of Medicine and Health Sciences, University for Development Studies, Tamale, Ghana.

CURRICULUM VITÆ

The author of this doctoral thesis completed gymnasium secondary school *cum laude* in 2004 at Collegium Marianum in Venlo, the Netherlands. He studied medicine at Leiden University Medical Center (LUMC) in Leiden, the Netherlands, and graduated *cum laude* in 2012. During his studies, he participated in an exchange programme with Karolinska Institute in Stockholm, Sweden and in two honours classes — „Medicine and Literature" and „Biomedicine, Law, and Religion" — of LUMC and Leiden University. He served as an intern at the Academic Hospital Paramaribo and the Medical Mission in Suriname. Parallel to his medical studies, he pursued a master in biomedical sciences, which was obtained *cum laude* in 2012. As part of the master's programme, he was on a laboratory internship at the Department of Neurobiology of Ruprecht Karl University in Heidelberg, Germany.

The author's first introduction into research took place during his studies at the Department of Anatomy and Embryology of LUMC, after being selected for the Excellent Student Trajectory. He also practised scientific skills as a member of the Young Excellence Class of Leyden Academy on Vitality and Ageing in Leiden from 2006 through 2012. In 2009, he started statistical-epidemiological research on the modelling of senescence in patients with end-stage renal disease at the Department of Gerontology and Geriatrics and the Department of Clinical Epidemiology of LUMC and the Department of Medical Informatics of the Academic Medical Center in Amsterdam, the Netherlands. This research was awarded by the Hippocrates Study Fund Foundation. In 2010, he participated in animal-experimental and laboratory research on the neural regulation of hepatic lipid metabolism in mice at the Department of Endocrinology of LUMC. In 2009 and 2010, he performed measurements on the prevalence of ageing-related diseases in rural Ghana for the Department of Gerontology and Geriatrics of LUMC, supported with a personal grant of the Jo Keur Fund.

The doctoral research described in this thesis was conducted from 2012 through 2015 at the Department of Gerontology and Geriatrics of LUMC. This research was supported by LUMC with a personal MD/PhD Grant. In this period, he was a member of the finalist team „Oud of the Box" for the Academic Year Price, organised by the Netherlands Organisation for Scientific Research (NWO) and the Royal Netherlands Academy of Arts and Sciences (KNAW). He was engaged in the development and organisation of the new minor programme „Evolution of Ageing and Disease" of Leiden Institute of Biology, Leyden Academy on Vitality and Ageing, the Faculty of Archæology of Leiden University, and LUMC.

Encouraged by his stay in rural Ghana, the author founded the charitable Professor Gbas Foundation in 2011. Information about the foundation is available at profgbas.nl.

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festina lente, cauta fac omnia mente;

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ver van de regels en de kouwe drukte,
waar mij een pauw van wijsheid heeft verteld
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(G. J. Komrij, *De Schoolverlater*, 2000);

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suguru nan Yenu nsom;

and those in Den Haag, where I learnt:

of making many books there is no end and much study is a weariness of the flesh. Let us hear the conclusion of the whole matter: Fear God, and keep his commandments: for this is the whole duty of man (*Ecclesiastes* 12-13).

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